Coffee and Pregnancy: Attitudes, Consumption and Maternal Vascular Function

'A thesis submitted to the University of Manchester for the degree of PhD in the Faculty of Medical and Human Sciences'

2015

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Abstract

Since the introduction of coffee to Europe in the early 17th century its popularity has steadily increased and, water apart, is the most widely consumed beverage globally. Being derived from a plant, a cup of coffee represents a complex mixture of naturally occurring chemicals such as caffeine, coffee oils and chlorogenic acid. As the popularity for coffee has grown so too has the interest surrounding its possible biological and pharmacological effects. A small number of studies suggest potential risks and benefits associated with coffee consumption in pregnancy; however these have yet to provide definitive conclusions. Furthermore government advice does not directly address coffee intake during pregnancy and there is no information regarding womens' and midwives' views and opinions on this increasingly important issue.

This was a mixed-method research study, with both a qualitative and quantitative components. Firstly, I aimed to gain insight into womens' and midwives' views and opinions on coffee consumption during pregnancy. Informed written consent was obtained from women attending the hospital for their antenatal care. These women were of varying gestational age, ethnic background, socioeconomic status and age. Informed written consent was also obtained from midwives from varying disciplines and experience levels; those from academia, research and practicing midwives included. I recruited twenty participants in each group. Information and perspectives were gathered through semi-structured face-to-face or telephone interviews. Interviews were analysed with the Framework method of analysis.

The second quantitative arm of my study investigated the effect of specific coffee chemicals on placental and myometrial vascular function. Human chorionic plate arteries, isolated from placental biopsies, and maternal myometrial arteries isolated from myometrial biopsies, were assessed by wire myography. Contraction and relaxation were determined to incremental doses of caffeic acid, chlorogenic acid and caffeine. The antioxidative properties of these chemicals were also assessed in response to application of reactive oxygen species.

My findings indicated that women and midwives' were unsure of the information surrounding coffee consumption. Pregnant women and midwives' discussed the provision of information, sources of information and supplementing information. Findings also indicated that the relationship between health care professional and

pregnant women can influence willingness to accept information and womens' level of pregnancy stress. My laboratory studies indicated that the active coffee chemicals did not significantly impact on placental or myometrial arterial function. The chemicals investigated did not elicit any significant protective antioxidative effects.

Combining methods allowed for a more comprehensive primary study to be completed. My literature search indicated that there was a clear gap in the knowledge surrounding coffee and its consumption during pregnancy. There is a clear lack of evidence-based information accessible to women regarding consumption. Midwives feel ill-equipped to provide women with information on coffee but err on the side of caution with their advice. Laboratory studies indicate that the coffee chemicals investigated did not induce an effect and cast doubts on the potential antioxidative effects that have been previously quoted in the literature.

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Acknowledgement

Abbreviations

Acetylcholine (ACH)

Active effective pressure (kPa)

Acute lymphoblastic leukaemia (ALL)

Adenosine Triphosphate (ATP)

Angiotensin II (AII)

Angiotensin Converting Enzyme (ACE)

Basic Fibroblast Growth Factor (bFGF)

Bradykinin (BK)

Calcium (Ca^{2+})

Caffeic Acid (CFA)

Caffeine (CAF)

Chlorogenic Acid (CHA)

Critical Appraisal Skills Programme (CASP)

Cyclic Guanosine Monophosphate (cGMP)

Cytochrome (CYP)

Extracellular Matrix (ECM)

Extravillous Trophoblast (EVT)

Fetal Growth Restriction (FGR)

Food Standards Agency (FSA)

Hepatocyte Growth Factor (HGF)

High Performance Liquid Chromatography (HPLC)

Homocysteine (Hyc)

Hydrogen Peroxide (H_2O_2)

Inositol -1,4,5 triphosphate (IP3)

International Birth Ratio (IBR)

Intrauterine Growth Restriction (IUGR)

In Vitro Fertilization (IVF)

Low Density Lipoprotein (LDL)

Molar (M)

Muscarinic 3 receptors (M3 receptors)

Myosin Light Chains (MLC)

Myosin Light Chain Kinase (MLCK)

National Health Service (NHS)

Nicotinamide adenine dinucleotide (NAD^+)

Nicotinamide adenine dinucleotide phosphate (NADPH)

Phospholipase C (PLC)

Physiological Saline Solution (PSS)

Placenta Growth Factor (PlGF)

Potassium Physiological Saline Solution (KPSS)

Population Intervention Comparison Outcome (PICO)

Potassium (K^+)

Prostacyclin (PGI_2)

Randomised controlled trials (RCT's)

Reactive Oxygen Species (ROS)

Renin-Angiotensin System (RAS)

Rho-associated Kinase (ROK)

Small for Gestational Age (SGA)

Spontaneous Abortion (SA)

Teens Eating for Energy and Nutrition at School (TEENS)

Thromboxane- A_2 (TXA$_2$)

Total Homocysteine Concentration (tHcy)

Uterine natural killer (uNK)

Uterine Placental Blood Flow (UPBF)

Vascular Endothelial Growth Factor (VEGF)

Voltage gated Ca^{2+} Channels (VGCC)

Xanthine (XA)

Xanthine Oxidase (XO)

Total Word Count: 130,133

Chapter 1 Introduction to Thesis and Systematic Review

1.1 Introduction to Thesis

This thesis aims to provide further information on the concept of coffee consumption during the gestational period. Over the past four years I have identified a very distinct gap in the knowledge and with the development of this study, I aimed to address these uncertainties and contribute to body of knowledge. By utilising a mixed methods approach it was possible to investigate the views, attitudes and opinions of pregnant women and midwives' on coffee consumption during pregnancy. These qualitative techniques were combined with the quantitative analysis of active coffee chemicals and their effect on placental and myometrial / systemic vasculature.

This thesis is set out in the traditional format. Chapter 1 is an introduction to my thesis and a systematic review considering coffee and its potential causative effect on poor pregnancy outcome. I focused predominantly on miscarriage, however, other pregnancy complications were considered. This review clearly highlights the gap in the knowledge and informs my aims and objectives. Similarly, I conducted a systematic review into womens' and midwives' views and opinions on coffee consumption during pregnancy. This search indicated that there was no literature surrounding this topic, further confirming the need for research around this subject.

Chapter 2 is the quantitative introduction. This considers the chemical and biological effects of coffee and its active substances. Fetal and placental development is considered, with particular emphasis being placed on vasculature.

Chapter 3 discusses the qualitative methodology. The paradigm which informed this study was pragmatism. Chapter 4 sets out the Qualitative Methods; I employed semi-structured telephone and face-to-face interviews to obtain data from women and midwives. Chapter 5 is comprised of the results or qualitative interpretations. Themes were extracted from the raw data and scrutinized using framework analysis. Following this, Chapter 6 is a qualitative discussion of the interpretations.

Chapter 7 introduces the quantitative methods employed. In my study I assessed placental and myometrial vasculature using wire myography. This was an ideal

method for investigating the effects of caffeic acid, chlorogenic acid and caffeine on the chorionic plate arteries and myometrial vessels. Chapter 8 consists of the quantitative results. I have divided the results section into 2 sub-headings; dealing firstly with the chorionic plate artery findings, and secondly with with the myometrial vasculature results. I subsequently collated the placental and myometrial vasculature to determine if effects of active coffee chemicals were comparable. Chapter 9 is the quantitative discussion of my findings.

Chapter 10 amalgamates both qualitative and quantitative results into a combined discussion. Both sections are pulled together to allow for a more thorough discussion section. The benefits, which will be discussed in greater depth below, are predominantly centred on the fact that this is a 'bench to bedside' study. I have combined two investigatory techniques in order to answer the aims of the study. I discussed the advantages and limitations of the methods utilised, the restrictions of the study and my overall contribution to research. This section includes the final remarks of the study and it explicitly details my unique contribution to knowledge. In addition it includes a section on recommendations and potential future work.

1.2 Introduction to Coffee and the Cafe Culture

Caffeine is the most commonly used psychoactive substance in the world. It is present in a range of foodstuffs, including coffee, tea, carbonated beverages, energy drinks, chocolate and also in some medications, particularly cold and flu preparations (Carrillo & Benitez 2000; Cano-Marquina et al. 2013). Caffeine, which will be discussed in further detail below, is a commonly consumed drug during pregnancy with the potential to affect the developing fetus *in utero*. Many studies have suggested that approximately 75% of women consume some form of caffeinated product during their pregnancy (Aldridge et al. 1979; McKim 1991; Hughes & Beveridge 1991; Wendler et al. 2009; Kuczkowski 2009). However, studies investigating antenatal caffeine intake and pregnancy outcome have produced conflicting results. Some studies have discussed the potential detrimental effects of caffeine on pregnancy, including miscarriage and stillbirth; others have reported no measurable effect or even beneficial effects connected with consuming coffee during pregnancy, especially in relation to the reducing the risk of developing gestational diabetes (Adeney et al. 2007).

Caffeine is the most abundant active chemical found in coffee. Understanding the physiological effects of coffee is a difficult process and investigators are very limited due to the fact that the brewed product contains a vast array of compounds as well as in varying quantities. Although this is the case the current knowledge surrounding coffee allows for a basic understanding of coffee's effect on reproductive health.

1.3 Coffee Popularity and the Cafe Culture / Cafe Culture Past and Present

Since its discovery, coffee has become increasingly popular worldwide, and is now the most commonly consumed pharmacologically active beverage in the UK, above alcohol (Nurminen et al. 1999). According to 'The British Coffee Association' an estimated 70 million cups of coffee are consumed in Britain on a daily basis. It is coffee's rich aroma and biological activity that contributes to its popularity amongst the British population (Dórea & da Costa 2007). The Cafe culture is a prominent topic in the media. Café culture has been defined by the Oxford dictionary as a lifestyle characterized by regular socialising in cafés and coffee houses; it includes the social atmosphere or series of associated social behaviours that are connected to the establishments and the habit. Coffee houses are common place in urban centres and contribute to public sociability and an areas vibrancy (Montgomery 2007). Coffee consumption is associated with adult status and is commonly consumed in work and social environments (Montgomery 2007).

Although traditionally tea consumption is considered more common, coffee has been readily available in Britain since the 17th century. The first coffee house was opened between 1660 and 1670 in London, however within 50 years this had grown to in excess of 3000. According to government statistics, there has been an increasing number of Cafe and coffee shops opening within the UK in 15 years / www.ons.gov.uk). There is a gradual upward trend from approximately 6000 establishments to approximately 20,000 establishments. This trend has continued throughout the economic crisis, with the market thriving in comparison to others. So confident are the authors with this trend that they have forecasted its progress until year 2015. Very few markets have the luxury of such optimistic future progression.

Other examples of the increasing popularity of coffee are demonstrated by its prominence in reports in recent years in the media. Articles published by the BBC on

the rising popularity and presence of coffee houses in Asia (BBC News, 9th February 2012) and on the varying caffeine levels present in coffee house coffees (BBC News, 1st December 2011) are just two examples of the many which can easily be sourced on the internet. See Appendix 8 for example of headlines.

Coffee's growing popularity has, however, been considered to be a growing social issue (Troyer et al. 1984). It is because of this, as well as its ubiquity and its stimulatory effect that caffeine has long been subject to medical scrutiny. Unlike alcohol and tobacco consumption, there does not seem to be a social stigma attached to coffee consumption. As the popularity of coffee consumption increases, so too does the media attention surrounding it. The media often reports the current literature surrounding coffee; this is a very common and effective method of health care information dissemination. Many of the headlines utilised in the media are sensationalist, and could be defined as scaremongering. These headlines are often targeted at pregnant women, advising them to reduce or remove coffee from their diet. The evidence behind these articles is unclear. These articles may cause unnecessary anxiety to pregnant women during an already stressful period of time in their life.

1.4 Active Compounds Found in Coffee / Active Coffee Chemicals (ACC's)

As stated in Section 1.3 a cup of coffee constitutes a pharmacologically active mixture of substances. The preparative roasting steps to which coffee beans are exposed gives coffee its distinctive aroma and flavour, thanks to its content of volatiles and phenols (MacCornack 1977). Aldehydes (50%), ketones (20%), esters and heterocyclics are the primary volatiles found in coffee (MacCornack 1977).

Caffeine (1,3,7-trimethylxanthine) is the world's most widely used psychoactive substance, with approximately 80% being consumed through coffee (Lucas et al. 2011; Frary et al. 2005). Caffeine is readily absorbed in the gastrointestinal tract through the mucosa (Weathersbee & Lodge 1977; Grosso & Bracken 2005) and passes freely through the placenta to the fetus (Cnattingius et al. 2000; Bech et al. 2007). One cup of coffee (200 mls) contains approximately 100 mg of caffeine (Minamisawa et al. 2004; Umemura et al. 1998). Cytochrome P450 is responsible for the demethylation of caffeine to form paraxanthine, theobromine and theophylline

(Grosso & Bracken 2005). Caffeine and its metabolism and metabolites will be further discussed in Chapter 2, Section 2.14.1.

Traditionally two types of beans are used for brewing coffee; *Arabica* and *Robusta*. *Arabica* beans contain Cafestol and kahweol whereas *Robusta* beans only contain about half as much Cafestol and hardly any kahweol (Boekschoten et al. 2004). Cafestol and Kahweol are diterpenes, often described as coffee oils, and are responsible for raising serum levels of the liver enzyme alanine aminotransferase and increasing LDL levels in healthy subjects (Yukawa et al. 2004; Bonita et al. 2007; De Lucia et al. 2009). Cafestol and kahweol are found in higher concentrations in unfiltered coffee (Majer et al. 2005) and consist of about 15% of the total lipid content of the roasted bean (Naidoo et al. 2011).

Chlorogenic acid is a phenolic compound present in many fruit and vegetables, and is found in varying quantities in coffee (Arion et al. 1997; Bouayed et al. 2007; Chang et al. 2010). It is said to have antioxidant properties, such as free radical scavenging and metal ion chelating, as well as inhibiting LDL and DNA oxidative damage (Kasai et al. 2000; Kweon et al. 2001; Chang et al. 2010). There are many who believe that chlorogenic acid could potentially exhibit health benefits through its antioxidant (Bouayed et al. 2007), anti-carcinogenic (Tahanian & Lord-dufour 2010), estrogenic (Zhu et al. 2009) and anti-inflammatory properties (Loke et al. 2010). This is still unclear and *in vivo* experimentation needs further clarification (Zhang et al. 2001). Chlorogenic acid, along with other dietary phenols, are rapidly and extensively metabolized *in vivo* and investigations are inconclusive on whether these metabolites have any antioxidant activity in comparison to their parent compound (Olthof, Hollman & Katan 2001).

1.5 Health in the General Population

Healthy eating is widely discussed amongst the general public and is at the forefront of government policies in modern times. There is a growing concern around the link between 'negative' health behaviours and ill health. The WHO conducted a report (Guilbert 2003) which identified a number of important health and lifestyle factors, including diet, that are linked to chronic illness and poor quality of life (Jepson et al. 2010). Social psychological theories are often used in the development of health

interventions and these often focus on core elements such as knowledge of risks, perceived self-efficacy, goals and motivations (Jepson et al. 2010). Most health promotion interventions include some aspects of education and knowledge building, motivation and health care professional / community-based support (Van Teijlingen et al. 1998; Kelley et al. 2001; Jepson et al. 2010). Public health and health interventions, whether they focus on the individual or a population, aim to change health behaviours through improving health-related knowledge and attitudes (Bandura 2004; Jepson et al. 2010). Health foods and antioxidants are often considered when discussing healthy eating and campaigns. Antioxidants are considered to have preventative properties with regard chronic diseases and positive effects on general health (Verzelloni et al. 2011; Quesada & Medina 2011). As discussed above health promotion is a priority of governments, either through the media or health care professionals. It is therefore essential that those who provide the support and information are well informed on the current body of knowledge and are providing evidence based advice.

1.6 Pregnancy and Coffee

Many studies now consider the *in utero* environment and prenatal exposure to particular compounds and maternal stress as a major risk factor for developing chronic disease in adult life (e.g. atherosclerosis; hypertension; diabetes) (Mongraw-Chaffin et al. 2009; Albrecht 2010). Following from this, studies have shown that approximately 70-80% of women consume some form of caffeinated beverage during their pregnancy (Fenster et al. 1997; Bracken et al. 2003). It is of interest to note that women who suffer from nausea or 'morning sickness' tend to avoid caffeinated beverages as they may exaggerate these symptoms (Fenster et al. 1997). The Food Standards Agency (FSA), UK are a government body responsible for the dissemination of current food and nutrition research to the general public. They are also responsible for labelling and developing nutrition policy across the UK. The FSA have issued guidelines on caffeine consumption during the gestational period. They adcise pregnant women to consume no more than 200 mg caffeine per day. These guidelines were amended in 2008, with previous guidelines setting the maximum daily intake at 300 mg per day.

Methylxanthines consumed by mother cross the placenta and can enter the fetal blood. These compounds can act as CNS and heart muscle stimulants and smooth muscle relaxants (Valero De Bernabé et al. 2004). Paraxanthine, a breakdown product of caffeine, antagonises adenosine A1 receptors in maternal-fetal brain and heart inhibit glutamate release in peripheral tissues, which may have a dose-dependent and cumulative adverse effect on the metabolic activity of both the mother and the fetus (Gaytan & Saadani-Makki 2006; Grosso et al. 2006; Iglesias et al. 2006). Animal studies have shown that chronic caffeine exposure during pregnancy promotes a decrease in adenosine A1 receptors in both maternal and fetal whole brain, which in turn increases stimulatory activities, making the brain and other tissues vulnerable to the harmful effect of caffeine because there is no blood-brain barrier or placental barrier to caffeine (Jahanfar & Jaafar 2013).

The pharmacokinetics of caffeine are altered during pregnancy, thus resulting in an increased half-life and prolonging its presence in the maternal system (Little 1997; Fenster et al. 1997; Bech et al. 2007). Several epidemiologic studies have linked relatively high caffeine consumption (>300 mg / day) to poor pregnancy outcomes. Other studies have suggested that caffeine is neither a reproductive hazard not affects fetal growth, especially after controlling for the confounding effects of smoking (Grosso et al. 2001). Clearance of caffeine from the mother's blood decelerates during pregnancy and studies have shown that the half-life can be doubled, or even tripled, during the second and third trimester of pregnancy (Knutti et al. 1981). During this period of the pregnancy the fetus has insufficient amounts of enzyme needed to metabolise caffeine (Aldridge et al. 1979; Jahanfar & Jaafar 2013).

Caffeine is known to increase the amount of circulating catecholamines, which could potentially cause uteroplacental vasoconstriction and fetal hypoxia (Kirkinen et al. 1983; Bech et al. 2007). These could both contribute to reduced fetal growth, and more severely, spontaneous abortion (SA). Excessive maternal caffeine consumption (which is considered to be in excess of eight cups per day) may also cause an increase in fetal heart rate and arrhythmias (Resch & Papp 1983; Jahanfar & Jaafar 2013).

There is very little or no research on the effect of cafestol and chlorogenic during pregnancy. As coffee consumption is associated with increased plasma total

homocysteine concentration (tHcy), research has been conducted on its potential impact on pregnancy outcome (Motulsky 1996; El-Khairy et al. 2003; Di Simone et al. 2003; Di Simone et al. 2004). Within these studies, there were correlations between coffee consumption, increased tHcy and poor pregnancy outcome however other potential cardiovascular risk factors (smoking, lack of exercise) could have also had an impact.

1.7 Coffee Consumption and Poor Pregnancy Outcome

Poor pregnancy outcome is defined in the literature as a miscarriage / SA, small for gestational age, preterm birth or stillbirth (Kramer 2003). In most developed countries pregnancies are planned, complications are rare and outcomes are usually favourable for mother and infant. When given adverse prenatal diagnosis parents are often shocked and experience acute grief (Statham et al. 2000). Miscarriage and stillbirth are also very traumatic events for mother and partner and can put much emotional strain on the individual and relationship.

In order to maximise the chance of a positive pregnancy outcome, a symbiotic relationship between medical team and woman must be adopted. Health care professionals and their relationship play a vital role in determining positive pregnancy outcome. This will be discussed further in Chapter 6, Section 6.3, and Section 6.15. Open communication is essential; women need to feel comfortable asking questions and to feel that they can disclose information in a non-judgemental environment (Johnson et al. 2003; Wright & Walker 2007; Winklbaur et al. 2008). This is particularly relevant for women who are drug dependent or suffer domestic abuse. Similarly, midwives need to be able to educate women their health, and feel reassured that the women will take their suggestions on board (Renkert & Nutbeam 2001; Nilsen 2009).

Coffee consumption and its potential impact on pregnancy outcome is constantly being discussed in the media. Although this is the case, there is still much conflicting information surrounding its consumption with great discrepancies within the literature. This further alludes to the need for more accurate and in depth research on its potential mechanism of action during the gestational period.

1.7.1 Miscarriage

Miscarriage / SA is defined as any non-viable pregnancy or the spontaneous loss of fetus before the 20th week of pregnancy. The medical term, SA, is often found to be distressing to women and as a result the lay term miscarriage is used interchangeably (Gentzkow 1985). SA is said to affect 1 in 7 women in the UK, however as many as 1 in 4 pregnancies may be affected (Miller & Williamson 1980). According to the NICE Guidelines (2012) miscarriage occurs in about 20% of pregnancies, which can adversely affect the quality of life for many women and accounts for approximately 50,000 hospital admissions annually. A poor pregnancy outcome can cause emotional and financial strain on a mother. It is a traumatic event in the lives of families and can have subsequent effects on future pregnancies (Armstrong 2002). Miscarriage is considered to be a significant psychosocial stressor, resulting in anxiety and grief (Brier 2004). Prenatal loss, as well as being a source of emotional turmoil, is burden on already thinning government resources (Linn et al. 1982; El-Khairy et al. 2003).

Public health surveillance is vital in the ongoing collection, analysis and interpretation of poor pregnancy outcome data. According to the Royal College of Obstetricians and Gynaecologists and the Office of National Statistics (2011 being the most recent) there were 909,109 conceptions recorded in the UK. Women are now more aware of their menstrual cycles and have access to sensitive pregnancy tests, thus allowing for very early detection of pregnancy. The noticing of spotting or bleeding during this early stage of pregnancy, which would have previously been ruled out as a normal cycle, results in women being investigated and hospitalised with SA (Mills et al. 1993; Rasch 2003).

There are still uncertainties as to whether there is a direct link between coffee consumption and miscarriage. As stated above, women who suffer from nausea during pregnancy tend to avoid drinking coffee. Nausea is associated with a viable pregnancy and thus it is fair to conclude that women who do not suffer from 'morning sickness' may be more likely to drink coffee and to suffer from a poor pregnancy outcome (Fenster, Eskenazi, Windham & S. H. Swan 1991; Fenster et al. 1997).

1.7.2 Intrauterine Growth Restriction / Small for Gestational Age / Fetal Growth Restriction

Maternal caffeine consumption during pregnancy has been studied for decades but evidence on coffee intake and impaired fetal growth remains unclear. Caffeine increases the levels of cyclic adenosine monophosphate which may influence cellular development and block specific adenosine receptors (Gaytan & Saadani-Makki 2006; Buscariollo et al. 2011). As adenosine is involved in maintaining the balance between the availability and the utilization of tissue oxygen it is possible that caffeine may block these receptors and thus increase cell susceptibility to hypoxia (Fortier et al. 1993; Buscariollo et al. 2011). As well as this, a study has shown that consumption of as little as 2 cups of coffee per day can cause an increase in maternal adrenaline concentrations and thus decrease intervillous placental blood flow (Kirkinen et al. 1983) which may also lead to the development of a hypoxic environment within the placental tissues.

There is a growing body of evidence suggesting that there is a causal relationship between caffeine intake and IUGR (Mills et al. 1993; Grosso et al. 2001; Grosso et al. 2006). The association has been found in several epidemiologic studies, which will be discussed in further detail below (Section 1.12.3). None of these studies have used a standardized form (i.e. the reported studies have used varying populations and definitions for IUGR). Recent research suggests that the definition of IUGR is failure of the fetus to reach its genetic growth potential (Rodeck & Whittle 2009). Customised growth charts represent ideal growth, however recent research suggests that they may no be feasible given the limitations associated with them. One of the major limitations includes the inclusion of maternal and fetal characteristics based on birth weight data does not take disproportional effects of these characteristics during pregnancy into consideration; growth charts assume that the proportionality equation that links fetal growth to birth weight is valid for each fetus. The current model continues to use ultrasound data, which although clinically convenient, has yet tp be validated. Furthermore, it remains unclear whether characteristics included in customised models are truly physiological (Gaillard & Jaddoe 2014).

Confounders, as discussed above (Section 1.6), are also relevant in fetal growth studies. A possible confounder is that the definition for small for gestational age

covers all neonates with a birth weight below the 10th percentile for gestational age (Wollmann 1998). According to Wollmann (1998), this definition overestimates the percentage of growth restricted new-borns as it is improbable that 10% of neonates suffer from IUGR; for example, some babies will have reached their growth potential but are "small" compared to the rest of the population (i.e. that are not "pathologically small"). Using small for gestational age may therefore lead to inappropriate conclusions being drawn.

Another example by which erroneous conclusion may be drawn is that nearly all studies rely on maternal self-reported caffeine consumption to estimate exposure; this can be problematic as there is considerable heterogeneity. Recall diaries, although convenient, are more often than not inaccurate. A woman's compliance with recall is necessary; if information is not entered very shortly after its occurrence it is more likely to be omitted. With this method of data collection it is also impossible to gauge coffee strength, volume and caffeine concentration. However, unless conducted in a laboratory setting this information cannot be obtained. This can often be seen in alcohol studies when participants have issues recalling or estimating the alcohol unit consumption (Gill 2002; Hughes et al. 2008).

Other confounding factors will be discussed in greater detail in Chapter 2, Section 2.17 and Chapter 10, Section 10.2, along with their implications to my study.

1.7.3 Preterm Birth

A large body of literature already exists on the deleterious effects of cigarette smoking and diet on fetal developments. The information regarding the effects of caffeine on pregnancy outcome is much more limited. A few studies have reported that coffee consumption may be associated with an increased risk of perinatal complications including congenital malformations and preterm birth (Berkowitz et al. 1982; Rasch 2003).

Preterm birth is defined as spontaneous labour or delivery prior to the completion of 37 weeks gestation (Savitz 2008). Despite screening and advancements in medical interventions, preterm birth continues to be a public health issue. It is a leading cause of infant mortality and significantly increases risk of neurodevelopmental,

respiratory and gastrointestinal complication and development of adult disease (Maslova et al. 2010).

Preterm birth results from a series of disorders, implicating maternal and fetal disease, some of which are explained and inter-related, and others of which are of unknown cause (Slattery & Morrison 2002). There has been much research into preterm birth over the last few years however this has not resulted in improvements in prediction and prevention in the pathology (Chiaffarino et al. 2006; Maslova et al. 2010). For complex reasons, the overall frequency of preterm births seems to be increasing and there is a general poor understanding of the normal physiology of human parturition (Slattery & Morrison 2002).

The lack of any significant association between coffee and tea consumption and preterm birth was once considered to be comforting (Berkowitz et al. 1982) however this was preliminary data and should be regarded as so, since studies have indicated opposite trends.

1.7.4 Congenital and Behavioural Anomalies

Evidence has shown that caffeine consumption is associated with some teratogenic properties which could possibly cause some adverse effects in pregnancy or result in a poor pregnancy outcome (Christian & Brent 2001; Browne 2006). Articles surrounding caffeine's ability to cause adverse effects on embryoneogenesis are still relatively unclear, yet experimentation in animal models indicates increased angiotensin II type 2 expression but decreased angiotensin II type 1 gene expression (Tanuma 2003). This could potentially lead to adverse events in the placenta and disruption to the renin-angiotensin system (RAS) in the placenta (Nurminen et al. 1999; Tanuma 2003).

Raised serum homocysteine levels are associated with neural tube defects and talipes equinovarus (also known as congenital clubfoot) (El-Khairy et al. 2003). In population studies, high intakes of coffee are associated with raised concentrations of plasma homocysteine; this is generally a predictor of risk of cardiovascular disease. The compounds in coffee that are responsible for this effect are not yet known however research has suggested that the most likely causative agents are caffeine and chlorogenic acid (Olthof, Hollman, Zock, et al. 2001; Verhoef et al. 2002;

Selhub 2008). These increased levels of homocysteine are also responsible for the development of congenital heart conditions (Eskes 2001). Homocysteine and its potential mechanism of action will be further clarified in Chapter 2, Section 2.18. Further human studies are required as animal models indicate behavioural anomalies such as reduced movement, poor concentration and decreased grooming time when exposed to coffee during gestation (Groisser et al. 1982).

The majority of congenital anomalies are associated with smoking and alcohol consumption (Gleich 1954; Krsnjavi & Mimica 1987; Parazzini et al. 1996) and it is important to remember that there is a positive association between coffee consumption and alcohol and smoking consumption (Alderete et al. 1995; Grewal et al. 2008).

1.7.5 Adverse Effects in Adulthood / Development of Adult Disease

Many studies published in the scientific literature are now concerned with the idea that diseases in adult life are as a direct result of their *in utero* environment (Wendler et al. 2009). This hypothesis was first cultivated in the early 20th century using European birth registries, namely the Dutch Famine study (which will be discussed in greater depth below). The hypothesis holds that events during early development have a profound impact on one's risk for development of future adult disease. Today, large and diverse human cohorts and murine models have extensively replicated these original observations. For example, it has been suggested that fetal development in a potentially hostile environment can affect the hypothalamo-pituitary-adrenal axis' function and activity (Tsubouchi et al. 2006), coronary heart disease, hypertension, obesity and insulin resistance (Lucas et al. 1999; Calkins & Devaskar 2011).

A1 adenosine receptors, found primarily on the heart, play an important role in protecting the embryo from hypoxic conditions and regulating heart rate (Fredholm 1995; Elmenhorst et al. 2011). Animal studies have indicated that when these receptors are deleted cardiac anatomy remains the same, however, protection is greatly reduced (Schulte et al. 2004; Lankford et al. 2006; Wendler et al. 2009). Caffeine exposure during embryogenesis was linked with significantly decreased fetal growth and poor cardiac function during adulthood (Wendler et al. 2009).

An Australian study suggested that there was an association between maternal coffee consumption and development of childhood acute lymphoblastic leukaemia (ALL), primarily in mothers that do not smoke, however the results were inconclusive and speculative (Milne et al. 2011). Genetic factors have also been considered in the development of childhood leukaemia in relation to coffee consumption (Clavel et al. 2005). The genotype *NQO1* is not associated with childhood leukaemia and there was a negative interaction between maternal coffee consumption and this polymorphism (Clavel et al. 2005). There was, however, a potential risk to children who carry the polymorphism CYP1A1, which is involved in caffeine metabolism and maternal smoking (Clavel et al. 2005; Mongraw-Chaffin et al. 2009).

Maternal coffee consumption is associated with an increased risk of cryptorchidism, or the absence of one or more testes from the scrotum, but a decreased risk of developing testicular cancer in adulthood (Mongraw-Chaffin et al. 2009).

1.7.6 Dutch famine study

The Dutch Famine study was a longitudinal study conducted from 1944 onwards. Around this time there was famine throughout most of Europe, including Holland, and the famine had a profound effect on the general health on the population. The mortality rate had almost doubled in 6 years and it was thought that this was attributable to malnutrition (Roseboom et al. 2006). Although this was a time of great suffering women continued to conceive and deliver, without significant, obvious complications. It was then suggested that the effects of maternal malnutrition during different stages in the gestational period on the off springs health in adult life could be investigated.

This study was the first of its kind and because of the unique experimental characteristics it is no surprise that many investigators have studies the individuals born around the time of the Dutch famine. Some of the long-term consequences investigated included glucose tolerance, blood pressure, renal function, airway disease, lipids clotting and coronary heart disease, hypothalamic-pituitary-adrenal response and breast cancer. The results of this study supported the concept that intrauterine conditions during distinct, organ-specific periods of sensitivity may permanently determine outcome in later life (Mongraw-Chaffin et al. 2009;

Roseboom et al. 2006; Lucas et al. 1999). There were certain limitations to the study. The reduced fertility of the women during this period must be considered; women who did conceive during this period of famine, whose offspring were exposed to reduced nutrient intake in the first trimester / early gestation, may have had a different constitution. Their health, constitution, (weight / height), age and parity were confounded for and did not seem to impact the results. Another potential limitation was the fact that women were required to self-report on many aspects of their diet- similar to my investigations. Of course, recall bias is a very real possibility. However, this study paved the way for much future research. Besides providing an insight into the role of prenatal factors in the origins of chronic disease, this information may also help identify susceptible patient groups and be useful in the development of more appropriate therapies from common chronic diseases in the future. More importantly, it has contributed to the prevention of chronic diseases through the development of adequate dietary advice to women before and during pregnancy.

Although the Dutch Famine Study is not specifically related to my study it is important to consider; this study lays the foundation for my investigations into the effect of specific nutrition and *in utero* environment. As mentioned above, this was this first study of its kind and it has paved the way for many studies that focus on nutrition and the gestational period. The majority of studies conducted within Maternal and Fetal Health centres of excellence in the United Kingdom have links to this study, highlighting the importance of its groundwork.

1.7.8 Conclusion

From above, one can see the clear need for further clarification on the potential harmful or beneficial effects of coffee during pregnancy. An accurate portrayal of womens' consumption needs to be ascertained along with their opinions on coffee intake during pregnancy. Investigating womens' knowledge will also provide a good foundation for further research and policy / education creation. This is also true for midwives; investigating health care professionals knowledge on coffee consumption will provide a clearly illustrate the gaps in the education and dissemination stages. Secondary outcomes will include details on the pregnant woman-midwife relationship and the opinions on education and provision of information within the

National Health Service (NHS). For this reason it was decided to conduct a Systematic Literature Review. This review would include two major research questions; (1) what are the physiological effects of coffee consumption on pregnancy, with a dominating focus on poor pregnancy outcome and (2) what are women and midwives' view, attitudes and opinions on coffee consumption during pregnancy. A systematic literature review possesses many advantages over the more traditional narrative review. The protocol and results of this review will be discussed below, Section 1.10 and Section 1.11.

1.9 Conducting a Systematic Literature Review

There is considerable emphasis and growing interest in systematic reviews, particularly with regard to health and social science literature. The primary aim of conducting a systematic review is to dispel any uncertainties surrounding a particular topic and to strengthen the current evidence base (Petticrew 2003). According to many academics (Petticrew 2003; Sandelowski 2008; Hemingway 2009) systematic reviews are becoming the cornerstone of evidence based practice. It is further appealing because of its promise to permit valid, although provisional, conclusions on clinical problems from an ever increasing number of research findings addressing certain problems (Sandelowski 2008). Summarizing evidence or knowledge is difficult, particularly in reproductive medicine as for each question asked there is the possibility that there are multiple studies, using various models and designs (Collins & Fauser 2005).

What makes a review systematic, as opposed to narrative, is the use of an explicit and concise protocol for review. This protocol sets out the problem that is to be reviewed, the main research purposes or the questions that are being addressed (Sandelowski 2008). The protocol also sets out the methods that will be used to search for, select and locate the included research and the techniques that will be used to appraise these reports and to analyse these findings (Hawker et al. 2002; Sandelowski 2008; Hemingway 2009). Systematic reviews differ from traditional narrative reviews by adopting the reproducible methods. This transparent process aims to minimize bias through exhaustive literature searches (Tranfield et al. 2003). Narrative reviews have been widely criticized for being singular, descriptive accounts of contributions in a particular field or lacking critical assessment (Hart

1998; Fink 1998; Tranfield et al. 2003; Green et al. 2006). A comprehensive, unbiased search is one of the fundamental differences between the more traditional narrative review and the systematic review, which although time consuming is argued to be more efficient and a higher quality method for identifying and evaluating current research (Mulrow 1994; Tranfield et al. 2003).

1.10 Aims and Objectives of the Review

As discussed above, a systematic review aims to identify, appraise and synthesize evidence from multiple studies of the same research question. In this review two separate questions are considered, which will be discussed separately in the following section.

The initial objective of this review was to determine if a relationship exists between coffee consumption and poor pregnancy outcome. The main focus was on miscarriage / SA, with considerations to fetal growth, preterm birth, congenital anomalies and development of adult diseases.

1.11 Methods

1.11.1 Data Sources and Search Strategy

A predefined search strategy was used to generate information that would meet the objective of determining whether a relationship exists between coffee consumption and the risk of women suffering miscarriage.

1.11.2 Exclusion / Inclusion Criteria

Primary research papers were included if published in peer reviewed journals and reported in English between 1980 and 2014. This time period was chosen to reflect the changes in patterns of consumption and incidence of miscarriage and other poor pregnancy outcomes. Though the search was not restricted to English language publications, lack of translation services excluded these from the review. Sample size is not significantly important when considering a systematic review, more the quality of the paper. Papers and reviews with a clearly defined explanation of how coffee ingestion / caffeine exposure was measured were reviewed, including both

qualitative and quantitative methods of measuring consumption. As murine models can be less sensitive to teratogenic effects (e.g. thalidomide) animal studies were not included in the data synthesis (Nehlig et al. 1994).

Studies that defined fetal growth restriction as less than the 10th percentile of birth weight for gestational age were used. This involved using an external standard of birth weight for gestational age; this standard is adjusted for gender and ethnicity that was developed from singleton births.

1.11.3 Parameters

Strict inclusion and exclusion criteria highlighted many studies which were considered unsuitable. Studies were excluded if they included poor pregnancy outcome of abnormal karyotype, utilised a murine model or did not satisfy the criteria of the systematic review paper. The maximum number of participants considered was 56,000, with which 21.6% experienced a miscarriage.

As stated by Mulrow *et al* (1994) researchers use systematic reviews to recognise and avoid errors of previous work, especially with regard to sample size (Mulrow 1994). It was felt that a small sample size would not affect the outcomes, particularily as this was a preliminary study.

A concise search of all relevant databases (detailed in table 1) was conducted.

Electronic Database	
Biological Abstracts	Web of Science
Bioinformatics Harvester	American Psychological Association
Bio-one	PsycInfo
VALDO	TRIP (Turning Research Into Practice
EMBASE	British Nursing Index
Merck Index	HMIC (Health Management Information Consortium)
Food Science and Technology Abstracts	National Research Register Archive
HubMed	United Kingdom Clinical Research Network
GoPubMed	Zetoc
Medline Plus	Scirus
Cochrane Library	CINAHL
Google Scholar	FADE: The North West Grey Literature
Mendeley	

Table 1 : Concise list of all databases utilised in the review.

Public health databases were also included as well as any unpublished and grey literature. This was considered to be non-commercial printed works, theses and proceedings. Internal reports and commissioned documents, which are not usually intended for general circulation, were also included.

Search terms that were used in the initial discovery phase are detailed in table 2.

Search Terms		
Coffee Caffeine Chlorogenic Acid Cafestol / Kahweol Caffeic Acid Homocysteine Paraxanthine AND Reproductive Health Infertility AND Pregnancy (UK / Europe / Global) Prenatal Maternal Fetal AND Pregnancy complications	Miscarriage Fetal loss (early) Spontaneous Abortion (normal karyotype) Fetal Growth Restriction (FGR) Small for Gestational Age (SGA) Poor pregnancy outcome Previous poor pregnancy outcome	Recurrent miscarriage /spontaneous abortion AND Chronic Illness / adverse effects in adulthood Parkinson disease Diabetes Cardiovascular disease Hypertension Vascular disease Behavioural Anomalies Congenital anomalies Liver disease Cancer (testicular/hepatic)

Table 2: Concise list of all search terms included in the review.

Synonyms and Boolean terms were included; terms with different spellings were also included (for example; fetal / foetal). Bias was considered under the CASP evaluation system and analysed under the four headings; selection bias, performance bias, detection bias and attrition bias. CASP will be discussed in further detail below.

1.11.4 Study Selection (PICO)

A search of the above databases did not produce any reviews that explored the relationship between coffee consumption during pregnancy and fetal loss. Initially databases were searched to identify studies related to the objective, considering the exclusion criteria. Once abstracts were identified these were reviewed taking into consideration the inclusion criteria. At this stage it was unclear whether the papers which I had chosen were eligible or whether they lacked sufficient criteria.

The PICO (Population, Intervention, Comparison and Outcome) approach was adopted in order to translate the clinical problems into a structured question and identify the key concepts(Huang et al. 2006). The paradigm of evidence based medicine recommends the use of the PICO template when addressing clinical questions. Searches performed on utilising PICO are shown to retrieve a higher percentage of relevant citations than performing random searches of databases (Schardt et al. 2007).

1.11.4.1 Population

The population investigated in this review was pregnant women. The table below illustrates the varying participants included in the review.

Varying age	Varying Gravida/Parity
Varying gestational age	Varying coffee consumption (chronic consumption to nil consumption)
Varying ethnicity	Varying confounding factors (Smoking / Alcohol consumption)
Multiple Births	

Table 3: Table illustrates inclusion criteria, more specifically relating to pregnant participants.

Studies investigating congenital anomalies and chronic diseases were also considered during the initial stages of review.

Populations that were excluded;

Pregnant women with a known complication

Miscarriage / SA with abnormal karyotype

1.11.4.2 Intervention

The intervention is coffee. Coffee consumption pre-conception and post-conception was considered, including chronic, minimal and nil use. All brands and types of coffee were considered, including studies focusing on decaffeinated coffee.

1.11.4.3 Comparison

Comparisons were made of the similarities and differences between participant groups and study design. Comparison of the similarities and differences between findings were made, including those reporting no significance or inaccuracies.

1.11.4.4 Outcome

The primary outcome focused on was SA / miscarriage, irrespective of gestational age. Secondary outcomes reviewed include:

Congenital anomalies

Intrauterine Growth Restriction / Fetal Growth Restriction / Small for Gestational Age

Preterm birth

Chronic diseases in later life

Stillbirth

Gestational diabetes

Pre-eclampsia

1.11.5 Data Extraction (Critical Appraisal Skills Programme)

Assessing quality and susceptibility to bias is essential when interpreting primary research and conducting a systematic review. A methodological approach paired with

the correct appraisal tool that matches the methods is essential for interpreting meaningful results (Singh 2013).

Quality was assessed using the Critical Appraisal Skills Program (CASP) tools (CASP 2006). The CASP tools can be used to teach critical appraisal skills in a wide variety of settings, including public health (National Collaborating Centre for Methods and Tools 2006). The CASP system has aided in the development of an evidence-based approach in health and social care (CASP 2006; National Collaborating Centre for Methods and Tools 2006). The primary aim of the CASP system is to help individuals develop skills to search for and understand research findings, and thus enabling them to apply evidence to practice (Ciliska et al. 2008; Jones et al. 2011).

There are seven tools available, each developed to assist with the critical appraisals of articles presenting different research types. Each CASP tool asks three broad questions; is the study valid; what are the results; will the results help locally / our population. These appraisal tools consist of approximately 10 - 12 questions. Screening questions ensure the efficient and rapid review of the paper in question. For this review I focused primarily on two questions; I initially examined whether the study asked a clearly focused question. The second question asked whether the authors used an appropriate method(s) to answer their question. The characteristics of the studies chosen and how they answered these questions are listed in Appendix 10.

As stated above the CASP guidelines were used to appraise studies identified using searches of the databases outlined in table 1. The CASP system encourages a systematic method by which we sieve through literature, identifying strengths and weaknesses of particular studies. Following the strict protocol followed by the CASP tool enhances the worth of a study and its results (Singh 2013). The system which I used did not utilise a grading or scoring system, but more assessed whether the literature reviewed answered our specific questions. I manually assessed each paper, using the tools provided by CASP, and systematically worked through each requirement. These tools are forms which were developed to assess each type of study included in the review. An example of the CASP tool for Cohort studies can be found in Appendix 11.

The idea of a systematic review is to bring together material which has some commonalities but elements of diversity. The strength and value of a systematic review is particularly evident when results from literature indicate that clinically significant results exist (Higgins & Thompson 2002; Higgins et al. 2003). Methodological and clinical heterogeneity existed in this systematic review which resulted in a meta-analysis being impossible to conduct. In some cases even if the studies considered were clinically homogeneous there were still statistical heterogeneities.

As well as utilising the CASP tool, literature was reviewed by other members of the team to ensure rigour and validity of studies. Manual analysis of the studies was conducted. Papers were read and separated into groups depending on their research question and then by their study design and participant groups. Notes were made on each paper briefly outlining the key points within that paper. Prior knowledge of analysis influenced the thematic extraction; a similar method to that adopted in the familiarization process was utilized. Previous literature searches also influenced the narrative analysis and thematic synthesis. The most prevalent themes will be discussed below (Section 1.12).

Quality is an amorphous concept within a systematic review. One interpretation is 'suceptibility to bias' however other aspects of the literature not directly associated with bias can be included in a quality assessment. It is, however, more important to distinguish between quality of reporting and quality of design, conduct and analysis (Sanderson et al. 2007). Although a large amount of heterogeneity amongst the studies included in the review, all articles decided upon contributed to the growing body of knowledge and answered the specific question within the systematic review.

1.12 Results

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┌──────────────────────────────┐          ┌──────────────────────────────┐
│ Total number of references   │          │ Titles/ abstracts excluded   │
│ retrieved from electronic/   │          │ as not relevant/ duplicate   │
│ hand searches: 1402          │─────────▶│ references removed: 1049     │
└──────────────────────────────┘          │ removed                      │
               │                          │                              │
               │                          │   • Non-English articles     │
               ▼                          │   • Identified as being      │
┌──────────────────────────────┐          │     prior to 1980            │
│ Unique references identified │          └──────────────────────────────┘
│ as potentially relevant and  │
│ full text obtained: 353      │          ┌──────────────────────────────┐
└──────────────────────────────┘          │ Full text references         │
               │                          │ excluded as not relevant:    │
               │─────────────────────────▶│ 165 removed                  │
               │                          │                              │
               ▼                          │   • Articles that did not    │
┌──────────────────────────────┐          │     focusing specifically    │
│ Relevant full text           │          │     on coffee AND the        │
│ publications: 188            │          │     gestational period.      │
└──────────────────────────────┘          └──────────────────────────────┘
               │
               │                          ┌──────────────────────────────┐
               │                          │ Relevant References excluded │
               │─────────────────────────▶│ from the review: 135 removed │
               ▼                          │                              │
┌──────────────────────────────┐          │   • Utilised animal models.  │
│ References included in the   │          │   • Did not confound for     │
│ review: 53 which focused     │          │     maternal age,            │
│ on Coffee/caffeine and the   │          │     gestational age or       │
│ specific poor outcomes       │          │     ethnicity; key           │
│ outlined above.              │          │     confounders.             │
└──────────────────────────────┘          └──────────────────────────────┘
       │         │          │
       ▼         ▼          ▼
┌──────────┐ ┌──────────┐ ┌──────────┐
│Miscarriage│ │IUGR/ FGR/│ │Preterm   │
│: 35       │ │SGA: 13   │ │Delivery: │
│           │ │          │ │6         │
└──────────┘ └──────────┘ └──────────┘
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Figure 1: Flow diagram outlining the process of selection of papers and articles revised during the Systematic review process.

A total of 53 articles were identified that considered the relationship between coffee consumption and poor pregnancy outcomes and their abstracts reviewed; from this 33 papers were selected considering miscarriage, 4 papers focusing predominantly on nausea and the pregnancy signal, 13 articles focusing on IUGR / SGA /FGR and finally 6 articles focusing on preterm delivery. No theses, dissertations or reports were identified.

Below I will address the primary outcomes and relevance of the literature reviewed. I will discuss the key points of the studies assessed, their impact on the current body of knowledge and any bias identified.

1.13 Primary Outcomes

1.13.1 Miscarriage

The complexity and difficulty with research into the aetiology of miscarriage in relation to coffee consumption are reflected in many studies. The inconsistency in results can be attributed to one or more sources of bias including, for example, imprecise estimation of caffeine intake and difficulty in recalling food / drink intake. For example assuming coffee and tea are the only sources of caffeine, retrospective measurement of assessment of caffeine consumption or inadequate control for confounding factors, particularly alcohol consumption and smoking, all contribute to bias and uncertainties. My literature search identified 29 unique publications investigating the association between coffee consumption and pregnancy.

This initial finding is supported by Greenwood *et al* (2010) who stated that there are no large well conducted effectiveness studies (Greenwood et al. 2010). Greenwood *et al* (2010) investigated caffeine exposure with regard to late miscarriage and stillbirth. The population was 2643, aged between 18 and 45 years old and between 8 and 12 weeks gestation. Caffeine was estimated using a validated consumption questionnaire. The authors concluded that caffeine intake should be limited, however did not control for 'pregnancy signal'. The pregnancy signal is defined as nausea and

vomiting during the gestational period. It is often referred to as 'morning sickness' but modern literature is moving from this term. The term pregnancy hormonal embryonic signal has also been utilised in some of the literature, acknowledging role an increased level of hormones plays in nausea and vomiting during pregnancy. The potential for caffeine consumption during the first trimester of pregnancy to be a significant risk factor for a poor outcome may be stronger than initially assumed however there are still many uncertainties. This was further verified with their review paper in 2014 which concluded that there was a small but consistently increasing incidence of miscarriage associated with increased daily caffeine intake (Greenwood et al. 2014). A similar study, which was excluded from this review also corroborated these findings, concluding that caffeine intake before and during pregnancy was associated with an increased risk of fetal loss (Infante-Rivard et al. 1993).

One of the difficulties with an attempt to correlate an increased incidence of SA / miscarriage with increasing coffee consumption is that exposure is determined by estimating the amount of coffee or caffeine-containing beverages consumed and estimating the amount of caffeine in these beverages. In order to reduce this methodological error, Klebanoff *et al* (1998) measured serum caffeine and paraxanthine to determine actual caffeine consumption rather than relying on self-assessment / historical data (Klebanoff et al. 1998) which was succeeded with an epidemiological study (Klebanoff et al. 1999). Paraxanthine, a caffeine metabolite, could be considered a risk factor in SA if concentrations reached a very high level (Klebanoff et al. 1999). Klebanoff *et al* (1999) stated that, although the metabolite could potentially cause SA it would be very unlikely that these high concentrations that they utilised would be common today. Karypidis *et al* (2006) conducted a case-controlled study investigated the risk of miscarriage with CYP1B1 polymorphisms. CYP1B1 enzymes are involved in the oxidative metabolism of xenobiotics, carcinogens and endogenous substrates such as steroids, fatty acids, vitamins and caffeine (Karypidis et al. 2006). Pregnancy signal (nausea) was controlled for as well as other lifestyle and dietary factors. The concept of the pregnancy signal / nausea will be discussed in greater depth below (Section 1.12.2). The Karypidis *et al* (2006) study included 507 women who suffered a miscarriage and 908 controls. They concluded that the presence of these enzymes was associated with first-trimester miscarriage, and this risk increased amongst coffee drinkers (Karypidis et al. 2006).

Signorello *et al* (2001) investigated whether the rate of which caffeine is metabolised potentially influences miscarriage risk (Signorello et al. 2001). Caffeine is primarily metabolized by P4501A2 (CYP1A2) however other enzymes, such as N-acetyltransferase 2, participate further in the metabolism of caffeine. This will be more thoroughly discussed in Chapter 2, Section 2.14.3, and Section 2.14.4. This study focused on 101 women who suffered a miscarriage of normal karyotype and 953 pregnant women between 6 - 12 weeks gestation. A combination method was used; patients were asked to recall and report their caffeine intake as well as providing a urine and blood sample. A urine sample was utilised for phenotyping for cytochrome P4501A2 and a blood sample was used to genotype for N-acetylation / N-acetyltransferase 2 status. Signorello *et al* (2001) found that high CYP1A2 activity may increase the risk of miscarriage independently or by modifying the effect of caffeine. The effect of N-acetyltransferase 2 was less conclusive but the authors erred on the side of caution and suggested that those with slow acetylators may be at an elevated risk of miscarriage (Signorello et al. 2001). Similarly, Sata *et al* (2005) investigated the CYP1A2 polymorphism and the risk of recurrent miscarriage. This was a case controlled study and the authors reported that there was no association between caffeine intake (below 300 mg per day) and miscarriage (Sata et al. 2005). The hypothesis behind their study was that a polymorphism of the enzyme could result in the ability to rapidly metabolise caffeine and therefore be able to tolerate larger quantitie / a higher exposure. However, pregnancy signal was not controlled for and the small sample size could be considered a limitation of the study, with the authors stating that there was no risk associated with caffeine consumption.

Cnattingius *et al* (2000) and Maconochie *et al (*2007) both attempted to control for confounding factors by evaluating cigarette smoking and exposure to caffeine by measuring the metabolic by-products of caffeine (Cnattingius et al. 2000; Maconochie et al. 2007). Coffee and caffeine consumption has been correlated with smoking and similar poor lifestyle habits in other literature (Olsen 1991). Cnattingius *et al* (2000) interviewed 562 women who suffered a miscarriage. Their study concluded that there may be an increased risk in non-smoking women but only with very high concentrations (Cnattingius et al. 2000). Maconochie *et al* (2007) considered biological, behavioural and lifestyle risk factors and found that there was a positive risk associated with lifestyle (Maconochie et al. 2007). By studying first

trimester miscarriages the investigators were able to determine that the women who experienced nausea were less likely to suffer from a miscarriage. The authors stated that if you did not control for the 'pregnancy signal' the study would demonstrate a positive association between caffeine ingestion and miscarriage, and thus not be valid. Similar studies by Armstrong (1991) and Rasch (2003) also investigated lifestyle and dietary components with miscarriage (Armstrong et al. 1991; Rasch 2003). Armstrong focused more on cigarette and alcohol consumption over coffee intake, interviewing 56000 women who had a successful delivery or suffered a miscarriage. However in both studies, inability to control for the pregnancy signal suggests that the results are inaccurate and it was not possible to verify a causal relationship to caffeine exposure. In his defence he raised some issues with recording caffeine exposure in the fetus post miscarriage. It is important to consider caffeine metabolites when discussing their potential link to miscarriage and other poor pregnancy outcome. These metabolites and their biological effects will be discussed in greater depth throughout my research (Chapter 2, Section 2.14) however it is important to note that levels have been recorded in neonatal serum (McGowan 1988; Grosso & Bracken 2005). This implies that caffeine and its metabolites pass freely through the placental barrier.

Zusterzeel *et al* (2000) performed a case controlled study, focusing on recurrent miscarriage associated with polymorphisms in glutathione *S*-transferase and cytochrome P450 genes. Recurrent miscarriage is defined as three or more consecutive pregnancy losses (Gentzkow 1985). It is a common pregnancy complication with both a physiological and psychological impact on the woman. Recurrent miscarriage is certainly important to consider when we discuss coffee and caffeine intake as its incidence in the UK increases each year (Clifford et al. 1997; Rai et al. 1997). The authors above hypothesized that genetic polymorphisms would impair drug metabolism and thus increase susceptibility to drug exposure, in particular caffeine. The studied cases included women who had experienced at least two unexplained consecutive miscarriages. The limited data presented in this paper offered no evidence to suggest that coffee plays a specific role in miscarriage (Zusterzeel et al. 2000).

Di Cintio *et al* (2001) focused specifically on dietary factors and reproductive risk. Hospitalization and pathological examination identified 912 women who suffered a

miscarriage before 12 weeks of gestation. Interviews were conducted using a standardised questionnaire. Nausea as a confounding factor was considered as it was noted that it can impact dietary habits and recall. Results found that cases consumed more coffee before pregnancy. Naturally there would be a recall bias and the fact that data was collected at different times between cases and controls could impact results.

George *et al* (2006) performed a case controlled study, identifying 562 women who suffered a miscarriage, 108 of them presenting with two or more consecutive miscarriages. Controls were identified from women who sought prenatal care. The authors noted that high caffeine intake was a potential risk factor, particularly non-smoking women (George et al. 2006). However this study did fail to control for the pregnancy signal which could have potentially exaggerated their results or concluding risks. Another case controlled study, performed by Parazzini *et al* (1998) studied women who suffered a miscarriage before the 12th week of gestation; this study also duration of coffee consumption and preconception caffeine exposure (Parazzini et al. 1998). This study noted cigarette and alcohol consumption and nausea intensity however did not seem to apply this to the final results. The conclusions drawn suggested that coffee drinking in pregnancy was associated with an increased miscarriage risk.

Tolstrup *et al* (2003) conducted a nested case control study, defined as a study where only a subset of controls from the cohort are compared to the incident cases, focusing on miscarriage with young, non-pregnant women. Participants were interviewed however information on the miscarriage was not obtained from hospital records which would have validated the study. The authors concluded that only high exposure (>900 mg per day caffeine) was associated with miscarriage (Tolstrup et al. 2003). A more recent study conducted by Savitz (2008) also considered caffeine consumption amongst a cohort of 2407 pregnant women. Daily consumption was recorded preconception and post-miscarriage and the results of this study suggest that there was no association between moderate coffee consumption (before or during pregnancy) and miscarriage. A possible limitation of the study was the potential for recall bias.

Pollack *et al* (2010) conducted a prospective cohort study focusing on time of exposure to caffeine and its link with miscarriage (Pollack et al. 2010). This study

measured caffeine consumption during what they considered were the 'sensitive windows'. Their findings, although having methodological limitations such as measurement errors and recall bias, indicated that there was no evidence to support the link between caffeine consumption and miscarriage. Wen *et al* (2001) also investigated the association between maternal coffee consumption and miscarriage with caffeine ingestion being quantified using a food intake questionnaire. Initial results suggest that there was an increased risk of suffering a miscarriage with exposure to between 100 – 300 mg caffeine daily. Yet, when considering the pregnancy signal, there was an increased risk of miscarriage amongst women who did not suffer from nausea and vomiting in the first trimester. It is therefore accurate to assume that there was incomplete evaluation of confounding factors and the results were not statistically significant (Wen et al. 2001). This study will be further discussed below when considering the pregnancy signal, miscarriage and coffee consumption (Section 1.12.2).

Weng *et al* (2008) performed a prospective cohort study with data which had been previously collected from studies surrounding miscarriage. Face to face interviews were conducted to determine caffeine exposure once pregnancy had been confirmed. A wide range of hazards were controlled for including the pregnancy signal. This study found that the group exposed to less than 200 mg caffeine per day had no increased risk of miscarriage (Weng et al. 2008). Similarly to this Mills *et al* (1993) found that despite their intensive surveillance of women there was no evidence that moderate caffeine consumption increased the risk of SA, IUGR or microcephaly, when confounding factors are accounted for (Mills et al. 1993). Within this study, a cohort of 431 women were monitored and pregnancy outcome noted. First trimester caffeine consumption was not significantly higher in women who aborted versus women who delivered live-born infants.

It was important within this review to recognise the need to include studies that did not just focus on normal, healthy pregnancies. The incidences of pregnancy complications increases every year as too does the age at which women are considering having children. For that reason I also sourced research that focused on already complicated pregnancies and those pregnancies that resulted from *In Vitro* Fertilization (IVF). Khoury *et al* (2004), focusing on women with Type 1 Diabetes, investigated prenatal smoking and caffeine consumption during pregnancy. Their

study indicated that caffeine consumption during early pregnancy, regardless of participant's glycaemic control has the potential to increase the risk of miscarriage. A further outcome of their study indicated that participants who continued with their caffeine and tobacco intake had a reduced risk of developing pre-eclampsia (Khoury et al. 2004). Klonoff-Cohen *et al* (2002) investigated the risk of miscarriage amongst participants who were undergoing IVF treatment. An increased risk of miscarriage was observed, however, it was concluded that this was unlikely to be a result of caffeine intake as caffeine has minimal mutagenic potential preconception (Klonoff-Cohen et al. 2002).

During this systematic review several review articles were considered and many of these have been discussed above. Christian and Brent (2001) both evaluated the reproductive and teratogenic effect of caffeine consumption during pregnancy (Christian & Brent 2001). The initial review stated that caffeine may be a potential risk and could cause harm to the embryo if used in excess. The findings in the second review claimed that caffeine, in moderate and high amounts, did not increase the risk of congenital malformations or miscarriage. Dlugosz *et al* (1992) also conducted a review into the reproductive effects of caffeine, considering a number of epidemiologic studies. In this review they felt that interview data alone was not sufficient to measure caffeine consumption but that moderate caffeine consumption may not adversely harm the fetus (Dlugosz & Bracken 1992). They conducted a second review in 1996 assessing the relationship between caffeine beverage consumption and miscarriage in 2967 pregnant women. This review concluded that caffeine consumption was more strongly associated with miscarriage than alcohol or cigarette smoking in the first trimester (Dlugosz et al. 1996). Fernandes *et al* (1998) was a considerably poorer review as it was unable to control for confounding factors however they found that there was a statistically significantly risk with moderate caffeine (>150 mg) consumption (Fernandes et al. 1998). Methodological issues are always an issue when considering the effect of particular chemicals and pregnancy. Golding (1995) conducted a review highlighting the methodological problems mounting in appropriate studies that assess coffee and caffeine consumption during pregnancy (Golding 1995). This review indicated that there was some evidence that suggested caffeine consumption was associated with miscarriage however further work was needed to ensure that this was not as a result of the increased likelihood of

mothers without nausea to suffer a SA. McKim (1991) considered epidemiologic studies which focused on the neonate and suggested that there was evidence that indicated that caffeine consumption had potential adverse effects (McKim 1991). This review was relatively basic and paved the way for more thorough literature analyses. Peck *et al* (2010) conducted a thorough literature review including epidemiologic studies between the years of 2000 - 2009 (David et al. 2010). These focused specifically on the reproductive health effects of caffeine, from infertility to a poor pregnancy outcome. Confounding factors were considered, with particular emphasis being put on the pregnancy signal and nausea. Results from this review stated that there was insufficient evidence and alternative plausible explanations for poor outcomes other than caffeine consumption; there was insufficient evidence to suggest a positive correlation between miscarriage and moderate caffeine consumption. Another recent review by Signorello and McLaughlin (2004) stated that the evidence overestimated the risk associated with caffeine consumption and poor pregnancy outcome. They stated that until the limitations which are present in many studies are overcome then the results will remain inconclusive (Signorello & McLaughlin 2004). A very detailed review by Leviton and Cowan (2002) summed up the current literature precisely. They concluded that many of the articles that found as association between caffeine and a reproductive hazard were more likely to be seen as lower quality studies (Leviton & Cowan 2002). This study also concluded that the pregnancy signal should be considered as a potential confounding factor; their specific findings suggest that caffeine consumption does not increase the risk of any reproductive adversity when nausea is incorporated.

1.13.2 Pregnancy Signal / Nausea and Miscarriage

The concept of pregnancy signal and nausea has been mentioned several times above. This term was coined in the 1980's and is defined as the association of early pregnancy nausea and vomiting, often abbreviated to NVP (Weigel & Weigel 1989).

The propensity for women with nausea to decrease or avoid caffeine results in bias. Participant selection is therefore impacted, particularly in randomised control trials, as patients who are more likely reduce or eliminate caffeine in their diet due to nausea will not be included. Another point, and potential limitation, is that as researchers we must be particularly critical of a study that finds an increased risk of

reproductive effect from both preconception and post conception exposures to caffeine or other coffee chemicals because the effects would have to be operating by two separate and distinct mechanisms (Scialli et al. 1995).

Wen *et al* (2001), which was discussed briefly above, concluded that risk of abortion was only increased in women without nausea who were exposed to caffeine and that the exposure had to be >300 mg per day (Wen et al. 2001). In some studies authors gained information on these vomiting / nausea symptoms, also known as the 'pregnancy signal'. A similar study conducted by Giannelli *et al* (2003) studied the effect of caffeine consumption and nausea on the risk of miscarriage (Giannelli et al. 2003). This case controlled study concluded that high caffeine consumption is an independent risk factor, especially exposure to >300 mg caffeine per day. Nausea was identified as an independent protective factor for lower risk of miscarriage. Participants were identified if they were diagnosed were diagnosed with miscarriage and interviewed 3 weeks after their pregnancy loss and controls were interviewed at their first prenatal visit, usually in the third trimester. Similarly, Signorello *et al* (2001) investigated the effect of caffeine consumption and nausea on the risk of miscarriage (Signorello et al. 2001). Utilizing the same case control study population reported in Cnattingius *et al* (2000), chromosomally normal miscarriages were identified (101 participants) and compared with 953 controls. The results of this study were discussed in greater depth above (Section 1.12.2).

Studies have observed that women who smoke are less likely to experience nausea and vomiting during pregnancy than non-smokers (Weigel & Weigel 1989; Gadsby et al. 1997; Wen et al. 2001; Källén et al. 2003). As discussed, nausea during the early stages of pregnancy is considered a good sign; these are a sign that hormonal levels are high and this is more likely to result in a viable pregnancy (Weigel & Weigel 1989). A review article by Stein and Susser (1991) considered what they described as the 'epiphenomenon' that accompanied pregnancy in relation to caffeine and miscarriage (Stein & Susser 1991). This review further highlighted the need to consider nausea when considering caffeine use during pregnancy.

1.13.3 Intrauterine Growth Restriction / Small for Gestational Age / Fetal Growth Restriction

As a perinatal outcome, fetal growth is considered a marker of healthy intrauterine development and a predictor of postnatal morbidity and mortality (David et al. 2010). Most studies of caffeine and fetal growth have assessed Intra Uterine Growth Restriction (IUGR), also referred to Fetal Growth Restriction (FGR) / Small for Gestational Age (SGA). Although there are many studies investigating the potential effects of coffee / caffeine on *in utero* growth, some of these studies state that there is insufficient evidence to evaluate the effect accurately. Fetal growth restriction is defined as birth weight $<10^{th}$ centile on a customised centile chart which takes into account maternal height, weight and sex (www.gestation.net).

I identified 13 papers investigating the association between coffee, caffeine and IUGR / SGA / FGR. Maternal caffeine intake was assessed in association with birth weight. The timing of exposure assessment is vital when investigating fetal growth restriction, but the exact window of susceptibility is unknown. Although fetal size was previously believed to be determined in the third trimester of pregnancy, when weight gain is most rapid, there is now evidence that suggests that growth can be determined by first trimester *in utero* environment (Cnattingius et al. 2000; Grosso et al. 2001; Parazzini et al. 2005; David et al. 2010). These uncertainties highlight the importance of assessing caffeine exposure throughout all stages of pregnancy when investigating possible associations with fetal growth (David et al. 2010).

Although I did not consider them for this review, many animal studies have found that caffeine can alter fetal growth and this effect seems to be dose dependent (Gilbert et al. 1988). Murine models, although helpful, are not always representative of systemic effects in human models (Worp et al. 2010). This is particularly true when considering coffee and caffeine consumption and exposure; animals tend to have far more striking results with regard caffeine withdrawal symptoms (Griffiths & Woodson 1988). It was for this reason that I disregarded these studies for my systematic review.

The potential causal effect of caffeine and IUGR persists when known confounders, including smoking, are taken into consideration (Martin & Bracken 1987; Fenster, Eskenazi, Windham & S. H. H. Swan 1991). Fenster *et al* (1991) conducted a study

with questionnaires administered over the telephone, relying on women to recall their caffeine consumption. Following this, analyses were conducted on 1230 singleton, live births. Their results concluded that caffeine consumption of ≥300 mg was associated with low birth weight but not preterm delivery (Fenster, Eskenazi, Windham & S. H. H. Swan 1991). Bracken *et al* (1987) found that there was a significant dose response relation between caffeine consumption during pregnancy and risk of delivering a reduced birth weight fetus post 36 weeks gestation (Martin & Bracken 1987). They followed this paper with one in 2003 investigating the association of maternal caffeine consumption with decrements in fetal growth. A small decrease in birth weight was noted amongst 2291 mothers with singleton live births. Although a slight decrease was noted the authors suggested that it was unlikely to be clinically significant apart from in those women consuming ≥600 mg caffeine per day (Bracken et al. 2003). Olsen *et al* (1991) found that maternal coffee consumption (>4 cups / day) was associated with a moderate decrease in birth weight, especially amongst smokers (Olsen et al. 1991). Within this study, the association between coffee consumption and preterm births or congenital malformation was very weak.

As coffee features more prominently in today's media, women are more aware of its potential harmful effects. These women may search for alternatives to coffee, for instance, decaffeinated beverages. Fortier *et al* (1993) found that decaffeinated coffee was not associated with IUGR while caffeinated coffee had an effect (Fortier et al. 1993). They found that caffeine intake during pregnancy increases the risk of fetal growth restriction, with risk increasing gradually as the amount consumed is increased. This study provided additional an argument for the role of caffeine and its potential detrimental effects on fetal growth. This study suggested that the pharmacologic properties of caffeine, its pharmacokinetics during pregnancy and the substantiated dose-effect of caffeine on fetal growth in animals (Groisser et al. 1982; Fortier et al. 1993; Nehlig et al. 1994; Christian & Brent 2001; Ricketts et al. 2007) all support the biologic plausibility of the association.

In studies where women reduced the caffeine intake significantly in the first weeks of pregnancy (from 300 mg / day to <50 mg / day), mean birth weight was higher than that of those who maintained high caffeine consumption (Konje 2008). Bang *et al* (2009), a similar paper which focused on fetal growth, did not focus solely on coffee

consumption but more dealt with an array of health and lifestyle factors (Bang & Lee 2009). There is much literature surrounding the importance of diet and lifestyle and its impact on *in utero* environment; it is important to note that *in utero* environment plays a major role in fetal growth and development. In the paper mentioned above by Bang *et al* (2009) coffee consumption was considered as a health risk and lifestyle habit, which was surveyed amongst 403 pregnant women in their second trimester (20 - 36 week) of pregnancy. With approximately 10% of women in the study stating that they consumed >5 cups of coffee pre day Bang *et al* suggest that women be advised to reduce their intake. There were several limitations to this study, with only 234 pregnant women being able to recall and provide information on birth weight and gestational age. Sample selection was geographically based and not random and not all confounding factors were catered for. Mills *et al* (1993) found that early fetal growth was not impacted by moderate caffeine consumption. In saying this, mothers who consumed >300 mg caffeine per day had a significantly higher proportion of babies with birth weights and head circumferences below the 10th centile. Once confounding factors, particularly tobacco consumption, was taken into consideration this was no longer significant (Mills et al. 1993)

One study found that higher caffeine levels were associated with a decreased risk of IUGR however higher serum paraxanthine concentrations were associated with an increased risk of IUGR (Grosso et al. 2006). Paraxanthine, a caffeine metabolite which crosses the placental barrier easily, was briefly mentioned above and has links to miscarriage and other pregnancy complications. It will be discussed in greater detail in Chapter 2, Section 2.14. The paraxanthine association was only seen when caffeine was also included in the model. This implies that it may be CYP1A2 enzymatic activation that influences fetal growth.

In addition, Bech *et al* (2005) conducted a randomized trial on caffeine intake in the second and third trimester of pregnancy. They investigated the outcomes of birth weight and gestational age and found no effect (Bech et al. 2005). Similarly Godel *et al* (1992) found that caffeine intake, which was prevalent in the majority of groups investigated, appeared to be relatively innocuous when smoking was controlled for (Godel & Pabst 1992). This verified the findings of many precious and future studies.

As mentioned above, it was initially thought that the third trimester was the most significant when considering fetal growth. Vik *et al* (2003) found that high caffeine intake in the third trimester may be a risk factor for fetal growth restriction, in particular if the fetus is a boy (Vik et al. 2003). Boys have higher mean weight, length and head circumference at birth than girls and this suggests a different growth pattern *in utero*. Gender specific studies are particularly popular in recent years; gender differences become particularly pronounced in the third trimester and there are many gender-specific risk factors for IUGR. A biological explanation for the results found by Vik *et al* (2003) remains obscure.

The relationship between total caffeine intake during pregnancy and FGR showed a significant trend with increasing caffeine consumption. This relationship was consistent across all three trimesters (Konje 2008). This was a more thorough study as it considered coffee and caffeine consumption and exposure from conception to birth. Konje (2008) noted that caffeine consumption greater than 200 mg per day was associated with a reduction in birth weight of 60 - 70 g, with a more significant trend with higher caffeine intake (Martin & Bracken 1987; Fenster, Eskenazi, Windham & S. H. H. Swan 1991; Peacock et al. 1991; Konje 2008). It has been said that a reduction in fetal weight of 60 - 70g, although seeming insignificant, could increase perinatal morbidity and mortality in an already compromised fetus (Regnault et al. 2002; Grosso et al. 2006; Konje 2008).

Review articles of caffeine and fetal growth restriction are equivocal. Many studies report weak associations with IUGR or reduced birth weight and other studies observing no effects. The strengths of the evidence for a potential effect of caffeine on fetal growth restrictions are diminished by the inability to rule out alternative, credible explanations for the observed associations, namely confounding factors. In conclusion, the evidence does not support a positive relationship between caffeine consumption and poor reproductive or perinatal outcome.

1.13.4 Preterm Birth

Preterm birth is defined as the spontaneous birth of a fetus at less than 37 weeks gestation. Some of the causal pathways to preterm birth may be related to infectious or immune dysfunction aetiology and premature contraction may be mediated by an

imbalance in inflammatory eicosanoids. Therefore diets that may decrease the inflammatory eicosanoids or have other beneficial health effects may help (Barger 2010).

I identified 6 unique publications investigating the effect of coffee and caffeine exposure and preterm delivery. Early retrospective studies into caffeine and preterm births found that results were not statistically significant when confounding factors were taken into account (Berkowitz et al. 1982). Later prospective and methodologically more accurate studies have also failed to show and relationship with preterm birth. The positive association disappeared once confounders, such as smoking and alcohol consumption, were taken into account.

Bech *et al* (2007) a randomized double-blind controlled trial conducted in women who consumed >3 cups coffee / day found that there was no significant difference in mean length of gestation (Bech et al. 2007). Similarly, Chiaffarino *et al* (2006) found that there was no clear association between coffee and risk of preterm birth however there was a more marked inverse association for coffee consumption in the third trimester of pregnancy in SGA cases compared to NGA (Normal Gestational Age)(Chiaffarino et al. 2006). This may be as a result of a small increase in consumption amongst the control group, probably related to the decrease in nausea. Women in the third trimester of pregnancy with non-physiological fetal growth may pay greater attention to their health and therefore tend not to drink the same levels of coffee. The concept of the pregnancy signal was discussed above (Section 1.12.2).

Wisborg *et al* (1996) observed that women who consumed caffeine and tobacco were three times more likely to experience preterm birth than those who did not consume caffeine (Wisborg et al. 1996). An earlier study, briefly mentioned above, conducted by Berkowitz (1982) found that there was no association between coffee drinking and shortened gestation however tea consumption (>4 cups/ day) was more frequent among women with a preterm birth (Berkowitz et al. 1982). Once again, when confounding factors were controlled for, there was no significant relationship associated with heavy tea consumption.

Fortier *et al* (1993) investigated preterm birth as well as IUGR. This study found that caffeine intake was not related to preterm birth or low birth weight, but was associated with an increased risk for IUGR (Fortier et al. 1993). Once again, there is

a potentially for recall bias as this study relied on women disclosing specific information on their consumption during a telephone interview.

A review article, more specifically a meta-analysis, by Maslova *et al* (2010) found that there was no significant adverse effect between caffeine intake during pregnancy and the risk of preterm birth (Maslova et al. 2010). Many of the review articles assessed could not find a substantial link between caffeine consumption and preterm delivery.

1.13.5 Confounders and Conflicting Evidence

With any study, confounding factors must be taken into consideration prior to formulation of conclusions from the presented data. Confounding factors have the potential to exaggerate or attenuate the results, and thus mask underlying issues.

There is much conflicting evidence surrounding the potential benefits and risks of coffee consumption. Some of these will be discussed in greater depth in Chapter 2 and Chapter 10. There are many suggestions as to why conflicting results exist regarding coffee consumption and pregnancy. Suggested explanations for these conflicting findings include variation in study design and population, inaccurate classification of caffeine before and during the pregnancy, inaccurate assessment of poor pregnancy outcomes and disregard for potential confounding factors (Fenster et al. 1997).

In this review I aimed to address the uncertainties regarding the association of coffee consumption with poor pregnancy outcome, undertaking a systematic review of published literature to assess whether there is a significant link between coffee consumption and miscarriage, FGR / SGA, preterm birth and congenital anomalies and to consider the quality of the published studies.

There were many confounding factors which were not evaluated that prevented the studies from definitively concluding that caffeine was causally related the occurrence of miscarriage. One of the most prominent confounding factor was failure to control for the pregnancy signal; evaluating the subjects with regard to the pregnancy signal would permit the investigator(s) to identify subjects with high and low reproductive risks (Christian & Brent 2001; Brent et al. 2011). It is possible that a reduction in

caffeine is not the cause of the adverse outcomes and more a signal or marker for a healthier pregnancy.

Similarly, positive associations between maternal coffee consumption and miscarriage have been reported in many different epidemiological studies and reviews; however these observations may be attributed to the confounding effects of maternal cigarette smoking or other nutritional factors.

Epidemiological studies have also been inconsistent; no epidemiological study cited the "non-human" mammalian literature dealing with caffeine exposure and miscarriage. These animal studies, although not primarily considered in this review, reveal that when animals are exposed to the wide range of caffeine intakes associated with human studies, increased pregnancy loss did not result.

Although studies suggest a positive relationship between caffeine consumption and preterm birth, modern methods of multivariate adjustments were not used. Failure to adjust for confounding factors led to the observed significant positive results and heterogeneity. As discussed the current recommendation is to either limit or eliminate caffeine intake during pregnancy. Although these recommendations may be sensible with respect to other pregnancy outcomes such as, low birth weight, the risk of preterm birth does not seem to be affected by caffeine consumption (Maslova et al. 2010). However, in saying this, it is difficult to draw conclusion regarding caffeine intake >300 - 400 mg / day during pregnancy as many studies use these levels as their upper limit. Higher caffeine intake is of particular interest in recent years as there are many new caffeine sources (bottled water, energy drinks, herbal supplements) which often do not report their caffeine content and as a result may covertly increase caffeine consumption during pregnancy (Frary et al. 2005).

Coffee and its effects are often discussed in the media with articles being found in local papers, national tabloids and broadsheets; women are thus exposed to all forms of information. The Financial Times suggest that the relationship people have with their coffee is similar to a 'romance'; individuals are very eager to discover the secrets behind a cup of coffee' (www.ft.com, 2014). The paucity of information in the health arena is therefore surprizing. Women are now obtaining their information from many different sources. Electronic formats are becoming more and more popular, especially with the increased presence of 'smart phones'. The internet is a

primary source of information. According to the Office of National Statistics, 99% of 16 - 24 year olds had access to the internet. It is estimated that approximately 43.8 million adults have internet access in the UK (Office for National Statistics, 2013) and although men are more likely to use the internet than women (88% v's 84%) women are more likely to search for health related articles and information (Larsson 2007). As discussed above, pregnancy is a transition period in a woman's life into motherhood; she can be easily influenced by the information she receives. Media can impact a woman's choice, especially those of a childbearing age, as they can access electronic sources more readily. Studies have shown that the medial information provided on the internet is not always evidence based, reliable or current (Impicciatore et al. 1997; Kunst et al. 2002; Weiss & Moore 2003).

Of the studies that have been published, the heterogeneity with regards to their design, coffee consumption and caffeine exposure measurements and definitions of miscarriage / SA make data pooling very difficult. This limits the usefulness of the findings. These conflicting results call for properly designed double-blind randomised controlled trials to establish any possibility of poor maternal outcomes or secondary fetal outcomes.

Given the observational nature of much of the evidence stated above, we cannot confidently draw inferences on the causal nature of the associations identified within this review. As well as this, one particular issue common to the majority of studies is the lack of consideration or objective measurement to the exposure of tobacco smoke. Smoking is an established risk factor when discussing pregnancy complications and poor pregnancy outcome. It is a potentially very strong confounder as there is literature that suggests that smokers both consume more caffeine than non-smokers because smokers altered CYP1A2 activity leads to faster caffeine clearance.

If the observed association between coffee or caffeine exposure and poor pregnancy outcome is causal, it is possible that it may be due to caffeine itself, to its metabolites, or a combination of them. Of the four primary routes of caffeine metabolism, 3-demethylation is the more important where by the caffeine is converted into paraxanthine by CYP1A2. This process of metabolism will be discussed in further detail below (Chapter 2, Section 2.14.3). There is much inter-

individual variation in caffeine metabolism with varying levels of CYP1A2 present in humans. It is therefore fair to say that accurately measuring coffee or caffeine intake does not accurately indicate the levels of metabolites or by-products in maternal or fetal circulation.

In summation, the strengths and limitations of systematic reviews and meta-analyses have been well established and thus proved the perfect method. These reviews should follow a protocol in order to minimize bias and ensure that the findings are reproducible. As well as this, blood caffeine / paraxanthine concentrations should be measured and careful consideration of alternative dietary sources could potentially resolve the discrepancies outlined.

1.14 Conclusion

There is much debate surrounding coffee and its potential beneficial or harmful effects on human health. In normal, non-pregnant individuals, consumption of approximately 2 - 3 cups per day has been associated with improved cognitive function, improved sense of sensation and digestion as well as being protective against coronary heart disease, type II diabetes and neurological disorders. In contrast, excessive consumption (>3 cups per day) is associated with insomnia, heart disease and other pathologies and it is of interest to note that current advice for pregnant and postmenopausal women is to avoid excessive coffee consumption.

Assessing the quality of evidence from studies requires tools that are designed and developed with a specific purpose in mind. It is for this reason that the CASP method was adopted as it was considered to be the most comprehensive method of assessing quality to date.

Health disparities related to coffee are often attributed to the consumption of excessive amounts of caffeine or the active components in the lipid fraction (Butt & Sultan 2011). Conclusions can be drawn that 1) without controlling for nausea and vomiting symptoms it is not possible to verify a causal relationship to caffeine exposure and 2) there is insufficient evidence to evaluate the effect of caffeine and coffee on fetal, neonatal and maternal outcomes. Comparative observational studies often suggest that caffeine can have a negative effect on the *in utero* growth of the fetus and result in miscarriage. However, in contrast, observational studies with

cohort study design and large sample size fail to reflect any association between caffeine consumption and poor pregnancy outcome (Cnattingius et al. 2000; Bracken et al. 2003; Jahanfar & Jaafar 2013).

The above review indicated that there was a small but quantifiable association between caffeine intake during the gestational period and incidence of miscarriage, stillbirth and decreased birth weight. There was no evidence of an association between caffeine intake and preterm delivery. Heterogeneity within and between the studies was generally high. In addition, the size of associations were small relative to some established risk factors, for instance maternal tobacco and alcohol consumption. It is therefore important to interpret any public health implications regarding caffeine consumption in the context of known lifestyle risk factors. Caffeine consumption is associated with other poor lifestyle factors such as alcohol and tobacco intake (Bech et al. 2007). On the whole, the weight of evidence does not support a positive relationship between caffeine consumption and adverse reproductive or perinatal outcomes. Associations with decreased fertility, congenital malformations and preterm birth are not routinely observed. Studies investigating fetal loss and IUGR / FGR / SGA are more prominent because of the frequency with which adverse effects are reported in connection with caffeine consumption (Golding 1994; Signorello & McLaughlin 2004; Jahanfar & Jaafar 2013).

It is important that pregnant women make informed decisions on their diet during pregnancy. This will be discussed in greater depth within my thesis. Diet can influence *in utero* environment which can impact fetal growth and development. There is much conflicting advice on coffee consumption available, both from healthcare professionals, online and a wide variety of written material. An example of the vast quantity of material found in the media surrounding coffee consumption during pregnancy can be found in appendix 8. When we interpret the results of this review, or any review of this type, we must consider the clinical implications of the outcomes. For example, whilst the consequences of SGA infants are less severe than miscarriage or stillbirth, SGA has been associated with an increased risk of perinatal mortality and morbidity. If shown to be causal, a small association could still be of great importance from a public health perspective.

In summary, combining results from a large number of studies has allowed me to make associations between coffee and caffeine exposure with poor pregnancy outcome. Whilst the issues are still unresolved, my analysis confirms that precautionary guidance should be offered during pregnancy. There is a clear gap in the knowledge with regard investigations into the effect of coffee and caffeine to the developing fetus during the gestational period and maternal and fetal vasculature. My review has also identified many significant methodological weaknesses common in studies regarding poor pregnancy outcome and pathologies, which would limit the confidence in causal interpretation.

1.15 Recommendations

To contribute to the understanding of the role of caffeine during pregnancy the application of improved methodology should be considered when examining the association of maternal caffeine / coffee intake with miscarriage. Development of a highly detailed caffeine assessment tool and repeat biomarker examination (salivary caffeine and paraxanthine) should be developed to prospectively quantify total caffeine intake.

These conflicting results call for more effective, properly controlled double-blind randomised controlled trials (RCT's) to establish the possibility of confidently advising women about avoiding caffeine during pregnancy. Further studies, incorporating long-term outcome measures in evaluating caffeine intake during pregnancy may prove difficult and unethical (Jahanfar & Jaafar 2013). In order to actually assess our primary outcome, miscarriage, an intervention would need to be applied potentially preconception or in the first trimester of pregnancy. This would be unethical as well as being difficult to design as there are still uncertainties surrounding how much caffeine could cause fetal or maternal debilitating effects, if any.

Future studies addressing the methodological limitations of the current research may alter the conclusions which I have drawn. In particular, quantitative methods are available for adjustment for measurement error on the absence of a gold standard and would be useful when estimating the impact of errors in the assessment of maternal caffeine exposure (David et al. 2010).

1.16 Systematic Review: Womens' and Midwives' views and Opinions on Coffee Consumption during Pregnancy

Guidelines recommend limiting caffeine intake immediately before and during pregnancy as a precaution (Greenwood et al. 2010) whilst recognising that the strength of any association is still unclear. Maternal care aims for positive pregnancy outcomes for bother mother and child and this includes both physical well-being as well as mental and social well-being (www.who.int).

Pregnancy is a transitional time for many women into motherhood. It is a period of time which can be full of emotional turmoil and physical discomfort. Many women experience changes that influence their well-being (Sjöström et al. 2004). Many women describe the experience as exciting but also a time for concern. These changes can bring confusion and many women report that their whole lives change with the pregnancy (Rogan et al. 1997; Barclay et al. 1997). It is therefore accurate to say that the transition into motherhood is considered to be a major life event. Though the transition is a unique experience to each individual, for most women it is a vulnerable period in their lives (Darvill et al. 2010). It is therefore essential that women are considered when providing health care information and that their views and opinions are taken into consideration; thus they should be clearly investigated.

Many studies have concluded that the optimum time to encourage health and lifestyle changes is during pregnancy as women are more motivated and receptive (Bryce & Enkin 1984; Van Teijlingen et al. 1998; Dodd et al. 2008). Midwives are in an optimum position to promote these changes and have a duty to guide women during their pregnancy (Lavender et al. 2001). The Department of Health (1999) encourages midwives to adopt a more proactive role providing information; this information should be of a high standard, evidence based and cater to the patient (Lavender et al. 2001; Melnyk & Fineout-Overholt 2011; Seefat-van Teeffelen et al. 2011).

As discussed above, there is much uncertainty surrounding coffee consumption and pregnancy. It is associated with many poor pregnancy outcomes yet there is very little definitive literature.

1.17 Aims and Objectives

The main aim of this review was to investigate women and midwives' views and opinions on coffee consumption during pregnancy. The information women were given by their midwives was also assessed and discussed.

1.18 Methods

1.18.1 Data Sources and Search Strategy

As described above, a predefined search strategy was used to generate information that would meet the objective of determining whether literature exists investigating women and midwives' opinions and attitudes on coffee consumption during the gestational period.

1.18.2 Exclusion / Inclusion Criteria and Parameters

There were no restrictions on the type of study, year of publication or parameters included.

A concise search of all relevant databases (detailed in table 4) was conducted. Public health databases were also included as well as any unpublished and grey literature; as above, non-commercial printed works, theses and proceedings. Internal reports and commissioned documents, which are not usually intended for general circulation, were also included.

Synonyms and Boolean terms were included; terms with different spellings were also included (for example; fetal / foetal). Bias was considered under the CASP evaluation system and analysed under the four headings; selection bias, performance bias, detection bias and attrition bias. CASP will be discussed in further detail below.

Electronic Databases	
AMED	Motherisk
British Nursing Index	TRIP Database (Turning Evidence into Practice)
Cinahl	Hardin. MD
Cochrane Library	Maternity & Infant Care (Ovid)
PubMed MEDLINE	Medline- EBSCO
Psycinfo	Nursing and Midwifery Council
CAMBase	ASSIA NET
DrugData	SCOPUS
POPLINE	Web of Knowledge
SciFinder Scholar	Embase
Social Care Online	UKPressOnline
DARE (Database of Abstract of Reviews of Effects)	ProQuest Historical Newspapers (The Guardian / The Observer)
MIDIRS	Times Literary Supplement

Table 4: the table below illustrates an extensive list of databases utilised for the purpose of this review

Search Terms			
Coffee Caffeine Chlorogenic Acid Cafestol / kahweol Caffeic Acid Homocysteine Paraxanthine AND Reproductive Health Infertility AND Pregnancy (UK/Europe/Global) Prenatal Maternal Fetal AND Pregnancy complications Miscarriage	Spontaneous Abortion (normal karyotype) Fetal Growth Restriction (FGR) Small for Gestational Age (SGA) Poor pregnancy outcome Previous poor pregnancy outcome	Recurrent miscarriage / spontaneous abortion AND Chronic Illness / adverse effects in adulthood Parkinson disease Diabetes Cardiovascular disease Hypertension Vascular disease Behavioural Anomalies Congenital anomalies Liver disease Cancer (testicular)	Fetal loss (early) AND Midwifery Impact of Information Providing Information Views and Opinions Care during pregnancy Education Attitudes Practice AND Women Impact of information Amount consumed Views / Opinions Education Attitudes Anxiety

Table 5: the below table includes an extensive list of terms searched for during this review.

1.18.3 Study Selection (PICO)

The PICO method was utilised; the PICO approach translates clinical problems into a structured question and thus identifies the key concepts.

1.18.3.1 Population

The population investigated in this review were pregnant women and midwives. The table below illustrates the varying participants included in the review.

Varying age	Varying Gravida / Parity
Varying gestational age	Varying coffee consumption (chronic consumption to nil consumption)
Varying ethnicity	Varying confounding factors (Smoking / Alcohol consumption)
Multiple Births	

Table 6: Table illustrates inclusion criteria, more specifically relating to pregnant participants.

Studies investigating congenital anomalies and chronic diseases were also considered during the initial stages of review. No populations were excluded.

1.18.3.2 Intervention

The intervention is views and opinions on coffee. Coffee consumption pre-conception and post-conception was considered, including chronic, minimal and nil use. All brands and types of coffee were considered, including studies focusing on decaffeinated coffee. All types of information available on coffee were considered.

1.18.3.3 Comparison

Comparisons of the varying views and opinions on coffee consumption during the gestational period of women and midwives' would be drawn.

1.18.3.4 Outcome

The primary outcome focused on was the views and opinions of women on coffee consumption during their current or previous pregnancy. Furthermore, midwives' opinions and views on coffee consumption during the gestational period would be investigated.

1.18.3.5 Data Extraction (Critical Appraisal Skills Programme)

Quality was assessed using the Critical Appraisal Skills Program (CASP) tools (CASP 2006). The CASP tools can be used to teach critical appraisal skills in a wide variety of settings, including public health (National Collaborating Centre for Methods and Tools 2006).

As stated above the CASP guidelines were used to appraise studies identified using searches of the databases outlined in table 4. Search terms utilised can be found in table 5 above. I assessed the validity of each study using the CASP scoring system; each study was individually scored and given a mark to assess its quality. The CASP system encourages a systematic method by which we sieve through literature, identifying strengths and weaknesses of particular studies. Following the strict protocol followed by the CASP tool enhances the worth of a study and its results (Singh 2013).

As well as utilising the CASP tool, literature was reviewed by other members of the team to ensure rigour and validity of studies. Manual analysis of the studies was conducted. Papers were read and separated into groups depending on their research question and then by their study design and participant groups. Notes were made on each paper briefly outlining the key points within that paper. Prior knowledge of analysis influenced the thematic extraction; a similar method to that adopted in the familiarization process was utilized.

1.19 Results

The initial examination revealed that there was no empirical literature surrounding women and midwives' views and opinions on coffee consumption during pregnancy.

It is clear from this that there is a gap in the knowledge and justification into further research into this niche topic.

1.20 Discussion and Recommendations

As stated above, further research needs to be conducted in order for health care professionals to accurately advise women on caffeine and coffee consumption. When assessing the available literature in this review, we were unable to find any studies investigating womens' and midwives' views and opinions on coffee consumption during pregnancy. The lack of literature may be as a result of the lack of evidence surrounding coffee during the gestational period. This lack of evidence was apparent from both my qualitative and quantitative literature searches and there was much uncertainty surrounding the literature which was obtained. It is possible that in the future there will be further studies conducted as the wealth of evidence increases and as coffee's popularity continues to increase. I am also confident that qualitative studies will be conducted; these are essential for obtaining a more comprehensive picture on the impact of coffee on pregnancy, health and society.

In summary, the above review identified that there is a distinctive gap in the literature. It is clear that there is an opportunity to research this niche area and the potential to influence and impact guidelines and public health.

Chapter 2 Quantitative Introduction

2.1 The Placenta

The placenta is a specialised, pregnancy specific structure; a multifaceted organ, that develops concurrently with the development of the embryo and fetus (Soares & Hunt 2006). A common and simple conceptualisation of the placenta is as a structure consisting of a villus tree, perfused by the fetus in a homogenous intervillous space, perfused by maternal blood (Sebire & Talbert 2001). An early definition of the placenta was a fusion of fetal membranes with the uterine wall allowing for the exchange of nutrients and waste products, as well as gas transfer (Mossman 1937). The placenta plays critical roles in maintaining and protecting the developing fetus. These roles include transferring nutrients from the mother to fetus, waste secretions from fetus to mother, acting as a barrier for the fetus against pathogens and maternal immune system, serving as an active endocrine organ capable of synthesising and secreting a plethora of hormones, growth factors, cytokines and other bioactive substances (Regnault et al. 2002; Reynolds et al. 2010)

From an evolutionary perspective, the placenta is the essential factor in permitting viviparity. This is a reproductive strategy in which fetal development proceeds within the female reproductive tract (Trevathan 2011). Viviparity can also be defined as the retention and growth of a fertilized egg within the maternal body until the offspring is capable of independent existence (Zeh & Zeh 2000; Soares & Hunt 2006; Trevathan 2011; Renfree 2013). Viviparous species are able to provide greater protection from environmental risks and can more precisely control the development of their progeny while they reside *in utero* (Zeh & Zeh 2000; Soares & Hunt 2006; Trevathan 2011). Trophoblast cells, which will be discussed in further detail (Section 2.7), play a fundamental role in enabling viviparous development (Paulesu et al. 2005).

Although placental functions are highly conserved, species specific elements and variations of placental organisation and activity are evident (Soares & Hunt 2006). Consequently, placental research has greatly benefited, and will continue to benefit from, a comparative approach; each species presents experimentally valuable attributes that can be exploited to better understand the physiology of the placenta

and viviparity (Zeh & Zeh 2000; Soares & Hunt 2006; Trevathan 2011; Renfree 2013).

The placenta can also be defined as a haemochorial structure; maternal blood is in direct contact with the apical syncytiotrophoblast, and thus there is perfusion with both maternal and fetal blood (Medawar 1953; Fisher & Damsky 1993). Avoiding prolonged contact of the trophoblast with unmodified maternal connective tissue is achieved by the rapid migration of trophoblast into the deciduas (previously known as endometrium) and rapid invasion of maternal vessels to establish a haemochorial environment (Enders & Welsh 1993). The complete placental structure includes the fetal placenta, placental bed and the uteroplacental vessels (Sebire & Talbert 2002).
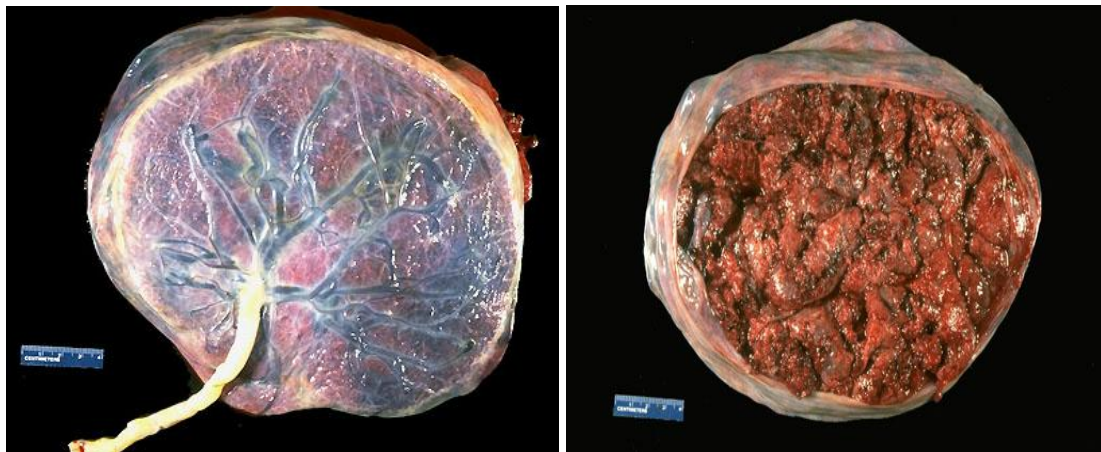
Figure 2: Gross structure of placenta. The left panel displays chorionic plate or fetal surface; umbilical cord and fetal membranes are clearly visible. The right panel displays basal plate or maternal surface; cotyledons present (pathlogyoutlines.com/ www.pathologyoutlines.com/topic/placentanormalanatomy).
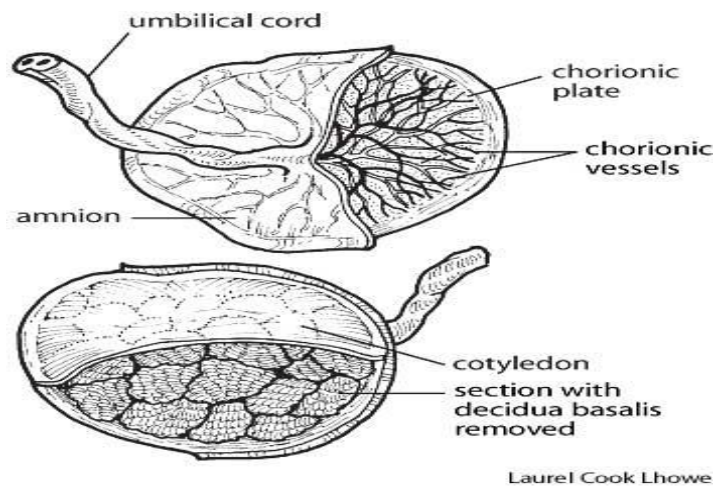
Figure 3: Illustration of placental vasculature and basal structures (pathlogyoutlines.com/ www.pathologyoutlines.com/topic/placentanormalanatomy).

2.2 Placental Development, Structure and Function

The process of implantation and placentation requires the production of numerous angiogenic growth factors, cell adhesion molecules, cytokines, growth factors, extracellular matrix metalloproteinases, hormones and transcription factors (Regnault et al. 2002). During normal human pregnancy, extravillous trophoblast (EVT) cells migrate and invade the spiral artery vessel walls within the endometrium, now known as the deciduas, and the myometrium (Brosens et al. 1967; Regnault et al. 2002).

The placenta undergoes much remodelling after the 9th week of development in order to facilitate the growing nutritional demands of the fetus; at term, the placenta receives approximately 70% of uterine blood flow (Castellucci et al. 1990; McNanley & Woods 2004). Foremost among these changes is an increase in surface area between maternal and fetal components to facilitate exchange (Sadler 2011).

A number of angiogenic growth factors have been identified in human placenta including: basic fibroblast growth factor (bFGF) (Shams & Ahmed 1994; Crescimanno et al. 1995), hepatocyte growth factor (HGF) (Kilby et al. 1996), placenta growth factor (PIGF) (Shore et al. 1997; Vuorela & Hatva 1997) and vascular endothelial growth factor (VEGF) (Charnock-Jones 1993; Shams & Ahmed 1994; Shore et al. 1997; Vuorela & Hatva 1997). While VEGF expression occurs in the villous trophoblast (Charnock-Jones 1993; Ahmed et al. 1995; Vuorela & Hatva

1997), PIGF expression appears confined to villous syncytiotrophoblast (Shore et al. 1997; Vuorela & Hatva 1997). The presence of functional VEGFR-1 receptors on isolated human trophoblast suggests that VEGF and PIGF binding to trophoblast VEGFR-1 may play a role in EVT invasion and differentiation. This revelation supports the hypothesis that early placental development occurs in a hypoxic environment (Shweiki et al. 1992; Shweiki & Itin 1993; Stavri et al. 1995), which is a known stimulator of VEGF expression (Regnault et al. 2002).

The placenta is comprised of many different cell types. Among these cell types are specialised epithelioid cells called trophoblasts which as noted above play a fundamental role in viviparity. Trophoblastic cells protect the embryo and developing fetus from noxious substances by programming maternal support and preventing maternal immune rejection while at the same time ensuring appropriate bidirectional nutrient / waste flow (Soares & Hunt 2006). These functions are required for adequate growth and maturation of the embryo and progress through the gestational period. The unique anatomy of the human placenta is a result of its epithelial stem cells, termed cytotrophoblasts; how these cells differentiate determines whether chorionic villi will float in maternal blood or anchor the fetus to the uterine wall (see below) (Genbacev 1997). The basal surface of the placenta is covered in approximately 10 - 40 protruding structures called maternal cotyledons (Myren et al. 2007). The fetal circulation that extends through the placenta ends within these protrusions (Wigglesworth 1969; Myren et al. 2007). In floating villi, cytotrophoblasts differentiate by fusing to form a multinucleated syncytiotrophoblast. The primary function of the syncytiotrophoblast is transport and therefore its ideal location is the villus surface (Genbacev 1997). In anchoring villi, cytotrophoblasts form cell columns and remain single (mononucleated). At the distal end of these columns the trophoblast cells attach to and deeply invade the uterus and its arterioles and this process is known as endovascular invasion (Reynolds & Redmer 1995; Genbacev 1997).

Endovascular invasion involves the cytotrophoblast cells replacing the endothelial and muscular linings of the uterine arterioles. This process initiates maternal blood flow to the placenta and greatly enlarges the vessel diameter (Reynolds & Redmer 1995; Genbacev 1997; Khong 2004). Endovascular invasion also involves the cells invading superficial portions of uterine venules, however, the mechanism of this is

yet unknown (Genbacev 1997; Regnault et al. 2002). During much of the first trimester endovascular invasion is minimal and thus maternal flow to the placenta is also minimal (Osol & Mandala 2009). After week 10 of placental development, endovascular invasion occurs much more rapidly and extensively allowing for direct contact with maternal blood from spiral arterioles, increasing mean oxygen pressure (Genbacev 1997). This implies that as cytotrophoblasts continuously invade through the uterine wall they encounter a steep positive gradient of oxygen tension (Genbacev 1997; Pittman 2011). It is therefore suggested that oxygen tension plays a key role in cytotrophoblast proliferation and differentiation (Fisher & Damsky 1993; McMaster et al. 1995; Genbacev 1997). Cytotrophoblast cells differentiate in stages. The single nucleated columnar cells react with an antibody against Ki67 antigen (anti-Ki67) which is indicative of DNA synthesis (Schwarting 1993). Following this, cytotrophoblasts intricately modulate their expression of stage-specific antigens, including integrins cell adhesion molecules (Damsky et al. 1992), matrix metalloproteinase-9 (Librach et al. 1991), HLA-G (cytotrophoblast class Ib major histocompatibility complex molecule) (McMaster et al. 1995) and human placental lactogen (Kurman et al. 1984).

The anatomical configuration of the placenta prevents direct contact between maternal and fetal blood. It is therefore essential that transport proteins, electrochemical gradients and diffusion channels are present to allow for exchange across the placental interface (Brett et al. 2014). Fetal growth is largely dictated by maternal nutrient availability and their ability to be transported into fetal circulation via the placenta (Brett et al. 2014). The transfer of essential nutrients and gases is typically divided into three stages: (1) the delivery of nutrients and gases via maternal circulation, (2) transfer of these substances across the trophoblast tissue and, finally, (3) uptake by fetal circulation (McNanley & Woods 2004). There are many factors involved in the delivery of oxygen and nutrients to the developing fetus. Maternal delivery involves blood entering the intervillous space via spiral arteries and bathing the syncytiotrophoblast (outer lining of the villi) in nutrient rich blood. Fetal blood enters the basal surface of the placenta which further branches into villus capillaries and nutrient rich blood travels towards the fetus via the umbilical vein. Maternal delivery of these essential nutrients is limited by the concentration, rate of uterine blood flow and the orientation of maternal and fetal

blood flow (McNanley & Woods 2004). The mechanism by which the gases and nutrients are transferred includes diffusion, active transport and receptor mediated endocytosis (Reynolds et al. 2010). Further to this, the fetus and placenta interact constantly in order to modify the transfer of these nutrients (McNanley & Woods 2004).

The placental barrier, which separates maternal and fetal circulations, is composed of the syncytiotrophoblast, the cytotrophoblast, the trophoblastic basal lamina, connective tissue and the fetal endothelium. As discussed above, the syncytiotrophoblast covers the chorionic villi and lines the intervillous spaces; it is the site of solute exchange and limits the transfer of macromolecules across the placental barrier by acting as a passive filter (Pierce & Midgley 1963; Genazzani et al. 1975; Cockell et al. 1997).

A number of placental structural abnormalities are associated with pregnancy complications. Abnormalities can adversely affect placental function, and ultimately deprive the developing fetus of the nutrients required for optimal growth (Regnault et al. 2002; Khong 2004). The placenta plays a pivotal role in offspring growth and is essential for nutrient transfer. Examples of abnormalities within the placenta include decreased arterial number and branching, decreased lumen size as well as decreased villous number, diameter and surface area (Giles 1985; Krebs et al. 1996; Regnault et al. 2002).

2.3 Placental Vasculature

The placental vasculature consists of two circulatory systems working in tandem; the uteroplacental (maternal) circulation and the fetoplacental (fetal) circulation (Reynolds & Redmer 1995). The placenta is supplied with maternal blood from the spiral arteries which arise from the myometrial radial arteries; these originate from the left and right uterine arteries (Zygmunt et al. 2003). Adaptations which occur in the spiral arteries during pregnancy, will be discussed in further detail below (Section 2.6, Section 2.7).

The fetoplacental vasculature comprises of two umbilical arteries; these divide to form the chorionic plate arteries and stem villous arteries, which eventually reach down to form the intermediate villi.

Venous drainage, or blood return to the fetus, is possible due to the complex network of veins and venules, which supply the umbilical vein (Poston et al. 1995). Within the placenta, similar to pulmonary vasculature, fetoplacental veins carry oxygenated blood towards the fetus whilst the fetoplacental arteries carry deoxygenated blood from the fetus to the placenta (Rudolph & Heymann 1967).

2.4 Fetoplacental Vascular Development

Placental development occurs as the blastocyst invades the maternal endometrium (now known as the deciduas). This occurs approximately day 5- 8 post-conception. The placenta and its primary structures are formed from the outer trophoblastic cellular layer, as described above, while the umbilical cord and fetal tissue are derived from the embryonic disc. As discussed above, following implantation, cytotrophoblast cells proliferate and differentiate in order to form the syncytiotrophoblast layer. This further develops into lacunae, which allows for maternal blood pooling, and eventually the formation of intervillous spaces (Sadler 2011). It is the development of the villi and anchoring villi into the basal plate that give rise to the maternal-blood filled space. Development of a villous tree is stimulated by further proliferation of the cytotrophoblasts in the trabecula. These primary villi are invaded internally by extra embryonic mesenchyme and this gives rise to secondary villi (Castellucci et al. 1990; Sadler 2011).

Fetoplacental vasculogenesis begins after day 20 *in utero* with the formation of tertiary villi. From this stage, up to approximately week 25 of gestation, profound vasculogenesis occurs. During this period VEGF is highly expressed, stimulating branching and the development of a low resistance capillary bed (Shweiki & Itin 1993). PIGF, which antagonises VEGF, is moderately expressed (Demir 2009).

Tertiary villi continue to develop throughout gestation. Immature intermediate villi form from tertiary villi and contain arterioles, venules and capillaries (Kingdom et al. 2000). These immature villi are the precursors to stem villi which anchor the villous tree to the chorionic plate. Stem villi are composed of fibrous stroma surrounding central arteries (Kohnen & Kertschanska 1996); these arteries are covered in a layer of vascular smooth muscle and a further outer layer of myofibroblasts (Demir &

Kosanke 1997). These contractile cells are thought to be involved in placental homeostasis as well as regulation of blood volume and resistance (Graf et al. 1994).

Following this, villous development is directed more towards non-branching angiogenesis with the emphasis being on the expansion of gas exchanging villi; these develop at the tip of the existing villous tree (Castellucci et al. 1990; Kingdom et al. 2000). This is achieved through looping and elongation of the villi, along with decreased trophoblastic proliferation, resulting in the formation of terminal villi which are covered in a thin layer of syncytiotrophoblasts. This makes them the ideal site for nutrient and gas exchange.

As the pregnancy progresses the terminal capillaries dilate and form sinusoids. These are important as they reduce overall vascular resistance and ensures even blood flow throughout the placental bed (Kingdom et al. 2000). VEGF and PIGF levels remain constant indicating their importance in fetoplacental development (Demir 2009).

2.5 Umbilical and Chorionic Plate Vascular Development

It is also important to note at this stage that this developing villous tree is connected to the fetus by vessels which push down the developing cord and branch out across the chorionic plate; these are the umbilical vessels within the umbilical cord. The umbilical cord attaches the developing fetus to the placenta, more specifically to chorionic plate vessels. The chorionic plate represents the cap of the intervillous space as well as serving as a basement membrane from which the villous tree is suspended into the intervillous space.

The development of the chorionic plate begins as soon as the first lacunae appear in the syncytiotrophoblast of the embryo at approximately day 8; this process begins with the formation of the primary chorionic plate. At this stage the chorionic plate is composed primarily of syncytiotrophoblastic and cytotrophoblastic cells, with its primary function being to separate the early lacunar system from the blastocyst cavity. This mono-layered plate becomes a triple-layered tissue as soon as the extraembryonic mesenchyme develops and spreads around the cytotrophoblastic surface of the blastocyst cavity. This layering process will be maintained until term (Benirschke et al. 1995; Benirschke & Kaufmann 2000). As this occurs, the trophoblastic trabeculae begin to proliferate and form the outgrowth of the villous

tissue; this proliferation is discussed in greater detail above (Section 2.2, Section 2.4).

Langhans' Fibrinoid layer is a layer of fibrinoid deposits along the chorionic plate which alter its architecture, namely structure and thickness. The functional importance of these plaques is still unknown however, it is thought that they play a role in the immunologic barrier between maternal and fetal tissue as well as anchoring the placenta (Sutcliffe et al. 1982; Myren et al. 2007).

The umbilical cord is formed and lengthened as the embryo prolapses backwards into the amniotic sac. During this process of expansion, the amniotic mesenchyme touches and eventually fuses with the chorionic mesoderm, occluding the exocoelomic cavity (Lacro et al. 1987; Heifetz 1996; Carter 1997). During the third week of pregnancy, the extraembryonic yolk sac, vitelline duct (that connects the embryonic gut) and the allantois become supplied with fetal blood vessels. Following this the fetal blood vessels derive from the allantois via the connecting stalk; at this point they establish contact with the chorionic plate around day 28 post-conception. These vessels are derived from allantoic vessels; two allantoic arteries originating from the internal iliac arteries and one allantoic vein that enters the hepatic vein (Wigglesworth 1969; Benirschke & Kaufmann 2000; Chaddha et al. 2004). These vessels are spread across the chorionic mesoderm and following this enter the early stem villi. These vessels come into contact with locally formed fetal villous capillaries, thus resulting in the completed fetoplacental circulatory system (Hamilton et al. 1945; Demir et al. 1989; Benirschke & Kaufmann 2000). Capillaries are essential for the nutrition of the various placental tissues however, they are rare and localized on the plate (Benirschke & Kaufmann 2000).

As stated above, the umbilical cord is the communication between fetus and placenta. The rudiment of the umbilical cord is represented by the tissue which connects the rapidly growing embryo with the extraembryonic area or yolk sack of the fertilized ovum. Included in this tissue are the body-stalk and the vitelline duct (Hamilton et al. 1945; Sadler 2011). The body stalk contains the allantoic diverticulum and the umbilical vessels, whilst the vitelline duct forms the communication between the digestive tube and the yolk-sac (Sadler 2011). The body-stalk is the posterior segment of the embryonic area, and is attached directly to

the chorion; this consists of a plate of mesoderm covered by thickened ectoderm on which a trace of the neural groove can be seen, indicating its continuity with the embryo (Lacro et al. 1987; Castellucci et al. 1990; Sadler 2011). Running through this embryonic mesoderm are the two umbilical arteries and the two umbilical veins, along with the canal of the allantois. Its dorsal surface is covered by the amnion, while its ventral surface is bounded by the extra-embryonic coelom, and is in contact with the vitelline duct and yolk-sac. With the rapid elongation of the embryo and the formation of the tail fold, the body stalk comes to lie on the ventral surface of the embryo where its mesoderm blends with that of the yolk-sac and the vitelline duct. The somatopleure or somatopleuric mesenchyme can be defined as a complex sheet of embryonic cells that fold and grow round on or within the embryo. These folds meet on the ventral aspect of the allantois, and enclose the vitelline duct, vessels and part of the chorionic cavity. The umbilical cord is covered in a layer of ectoderm which is continuous with that of the amnion and its various constituents are enveloped by embryonic gelatinous tissue, myxoid paucicellular matrix or Wharton's Jelly (Sadler 2011). The vitelline vessels and duct, together with the right umbilical vein, undergo atrophy and disappear; and thus the cord, at birth, contains a pair of umbilical arteries and one (the left) umbilical vein (Castellucci et al. 1990; Regnault et al. 2002; Sadler 2011).

Finally we consider the ultrastructure of the umbilical and chorionic tissue. A single layer of cuboidal amniotic epithelium covers the outer surface of the umbilical cord. The chorionic plate consists of a single layer of cuboidal amniotic epithelium overlying a paucicellular collagenous matrix (chorion) containing numerous large vessels of fetal origin. Interestingly, studies have shown that chorionic plate arteries demonstrate similarities to cerebral cortical vessels, with regard to vascular branch patterns, histological cross sections and angiographic appearances (Kwok et al. 2014). Histologically, umbilical arteries and veins contain two layers of smooth muscle orientated longitudinally and circularly (Altura et al. 1972; Lacro et al. 1987; Heifetz 1996; Benirschke & Kaufmann 2000). *In vitro* studies have shown that administered vasoactive substances have an effect on umbilical vascular tone (Ritter et al. 1982; Mak & Gude 1984; Toyofuku & Nishimura 1995). Histological studies of the term chorionic plate have also identified several layers, namely, amniotic epithelium, compact layer, amniotic mesoderm, spongy later (separating the amnion

and chorion), chorionic mesoderm, proliferating cytotrophoblast and Langhans' fibrinoid layer (Benirschke & Kaufmann 2000).

2.6 Maternal Uterine Vascular Adaptations during Pregnancy

The placenta is a unique vascular organ that is continually perfused with blood from both the maternal and fetal systems. It is said to have two separate circulatory systems; the uteroplacental circulatory system and the fetoplacental system (both circulations have been discussed extensively above). The uteroplacental or maternal circulation begins with the maternal blood flow into the intervillous space through the modified decidual spiral arteries. The maternal blood pools in the lacunae and flows around terminal villi in the intervillous space allowing for nutrient transfer. The in-flow of maternal arterial blood forces deoxygenated blood back into the decidua and finally uterine veins to complete the journey back into the maternal systemic vascular system (Reynolds & Redmer 1995; Kliman 2000; Wang 2010; Reynolds et al. 2010).

Maternal blood volume increases but blood pressure can remain unchanged or decrease during pregnancy (Longo 1983). We can therefore ascertain that uterine hemodynamic changes are primarily affected by a profound decrease in uterine vascular resistance (Thaler et al. 1990; Osol & Mandala 2009). This is achieved though several different, yet complimentary mechanisms, including circumferential structural enlargement of the entire uterine vascular tree, reduction in vascular tone and, most importantly, development of the placenta (Carbillon et al. 2000; Osol & Mandala 2009). In humans, unlike some murine models, the increase in uterine placental blood flow (UPBF) is gradual and fairly linear, with the proportion of blood being directed to the placenta increasing as the third trimester is reached (Khong 2004; Osol & Mandala 2009; Reynolds et al. 2010).

Spiral arteries were first defined as vessels 'passing between womb and placenta' and it has taken more than a century to clarify their detailed anatomy (Pijnenborg et al. 2006). The convoluted structure of the spiral artery is as a result of arterial growth exceeding the increase in endometrial thickness during the menstrual cycle and during pregnancy. Their tortuous anatomy has haemodynamical repercussions, diminishing the force of the circulation as is approaches the placenta (Pijnenborg et

al. 2006). Not only does it result in a progressive decrease in the pressure along the length of vessel but it ensures a decreased pulse, essential for maintaining a constant and consistent flow to the intervillous space of the placenta (Meekins & Pijnenborg 1994; Wolf 1973; Pijnenborg et al. 2006). Uterine spiral artery remodelling is essential for normal human pregnancy. The mechanisms underlying the trophoblast-independent remodelling are unclear however, are most likely to be pregnancy specific. Uterine natural killer (uNK) cells are present in large quantities and represent approximately 70% of the decidual leukocyte population (Robson et al. 2012) and it is thought that these play a role in limiting the depth of trophoblast invasion as well as preparing myometrial spiral arteries for trophoblast invasion (Robson et al. 2012).

In order to support placental function and to further cater to the needs of the developing fetus the placental bed spiral arteries (100 - 150 total; diameter 200 – 300 µm) are altered to form non-compliant, low resistance vessels (Pijnenborg et al. 1983; Meekins & Pijnenborg 1994). The placental bed is considered to be the area of the uterus underlying the placenta, and plays a fundamental role in supporting placental function by supplying the key nutrients and oxygenated blood to the intervillous space via spiral arteries (Lyall 2005). One potential mechanism of action for this is the interactions between maternal tissue and fetal-derived trophoblastic cells (Pijnenborg et al. 1983).

Absolute blood flow to the myometrium increases in proportion to uterine mass, whereas relative uterine blood flow (mm / min per 100g of tissue excluding the placenta) may fluctuate and decrease somewhat, or remain fairly constant (Osol & Mandala 2009). During pregnancy, the diameter of the main uterine artery roughly doubles in size but there is no thickening of the vascular wall (Brosens et al. 1967). This increase in lumen diameter results in an increased cross sectional area; this is also known as outward hypertrophic remodelling (Mandala & Osol 2012; Heijden 2005). As well as uterine arteries, small arcuate and radial arteries remodel in this fashion, with an increase in vascular diameter of between 25% - 220% (Thaler et al. 1990; Heijden 2005; Osol & Mandala 2009; Mandala & Osol 2012). In addition to this cellular hypertrophy, there is strong evidence from many murine models that hyperplasia within the vascular wall occurs since increased rates of smooth muscle

cell divisions have been reported in uterine vessels (Cipolla & Osol 1994; Cipolla et al. 1997; Hammer & Cipolla 2005; Osol & Mandala 2009)

Pregnancy induced vascular adaptations are essential for a successful pregnancy. Maternal uterine vascular adaptations during pregnancy, and its significance to fetal health, are highlighted by the fact that its absence is associated with several common pregnancy pathologies, for example, intrauterine growth restriction and preeclampsia (Osol & Mandala 2009; Samangaya et al. 2011).

2.7 Trophoblast Invasion and Uterine Blood Flow

Two populations of invasive trophoblast have been identified; interstitial and endovascular trophoblasts (Wolf 1973). By 8 weeks interstitial trophoblasts migrate into the decidual stroma and myometrium and cluster around the spiral arteries (Pijnenborg et al. 1981; Pijnenborg et al. 1983). Endovascular trophoblasts invade the lumen of the spiral arteries and progress downward (away from the placenta) from about 6 - 8 weeks gestation (Wolf 1973). The trophoblasts ablate the endothelium and smooth muscle of the arterial wall and reorganize the matrix elements; trophoblasts adopt an endothelial cell-like phenotype to mimic the cells they replace which reduces the possibility of the maternal immune system rejecting the placental tissue (Zhou et al. 1997; Janatpour et al. 2000). The arterial wall muscles become more fibrous and thus lose their vasoactive properties. This invasion leads to the development of a low resistance uterine circulation.

Vascular remodelling during pregnancy is thought to extend to the majority of the spiral arteries; invasion also occurs towards the inner third of the myometrium within the central region of the placental bed and to a lesser extent towards the peripheries of the placental bed (Meekins & Pijnenborg 1994).

2.8 Blood Flow to the Placenta

Humans, as well as mice, rats, guinea pigs and rabbits, utilise a haemochorial type of placentation (Section 2.1); intraplacental pressure, created by the maternal blood occupying the intervillous space must be kept low enough to avoid compression of the intravillous fetal vessels, demanding the contribution of significant resistance by upstream vessels (Moll et al. 1975; Osol & Mandala 2009). With haemochorial

placentation, only the fetal cellular bilayer needs to be crossed (trophoblast and intravillous endothelium) in order for a molecule to pass from the maternal to fetal compartments, (Enders & Welsh 1993; Shore et al. 1997; Osol & Mandala 2009).

During the first weeks of fetal life evidence has shown that maternal spiral arteries are plugged with endovascular trophoblasts. This mechanism limits maternal blood flow to the placenta although plasma may be able to leak into the intervillous spaces (Hustin & Schaaps 1987; Burton et al. 1999; Jauniaux et al. 2000). This research suggests that blood flow to the placenta is extremely restricted during the first trimester. These plugs disintegrate during weeks 10 - 12 allowing for maternal flow to the placental bed / syncytiotrophoblast, increased oxygen tension and nutrient availability (Jauniaux et al. 2000).

It has been suggested that maintaining a low flow and hypoxic *in utero* environment may protect the fetal tissue from physical damage due to the angiogenesis occurring all around, or the potential oxidative injury (Rodesch & Simon 1992; Jauniaux et al. 2000; Myatt & Cui 2004). Determining the mechanisms by which this low flow is maintained through the vasculature within the placental unit is therefore an important area for study.

2.9 Studies of Isolated Fetoplacental Arteries

By isolating fetoplacental vessels we can assess their structure and function *in vitro*. As with all experimentation the methodology has advantages and disadvantages. A key strength is that by studying isolated vessels *in vitro* experimental conditions can be manipulated allowing for greater investigatory scope (Halpern & Kelley 1991).

Large umbilical vessels were amongst the first fetoplacental vessels to be investigated. These large cord arteries were examined using isometric ring preparations (Tulenko 1979). It was later found in animal studies that investigation with smaller (<500 μm) "resistance" arteries was more appropriate as they contribute most to the resistance generated within a vascular bed (Mulvany & Aalkjær 1990). It was through this that the myography technique was born (Section 2.10).

One of the major disadvantages, and a potential problematic experimental factor, is the isolation of resistance arteries. Identification relies heavily on anatomic location,

smooth muscle content and size. Studies conducted to identify and characterise resistance arteries have always been conducted in small mammals under anaesthesia, which may not accurately reflect the conscious physiological state of larger mammals (Davis et al. 1986; DeLano & Schmid-Schönbein 1991; Christensen & Mulvany 2001).

2.10 Myography

Wire myography is a technique used to assess the pharmacological and physical responses of vessels in controlled conditions. Ideally these vessels should be between 100µm - 500µm as these most accurately reflect a resistance artery (Mulvany & Halpern 1976). A brief overview of the method employed will be given below; however, a more comprehensive explanation will be given in Chapter 7, Section 7.5.

Dissected vessels are mounted on two steel wires (40 µm diameter); these wires are guided through the lumen of the vessel allowing for isometric tension measurements to be made. Temperature and gas concentrations are constants permitting comparisons between *in vitro* and *in vivo* models. Its viability with regards to smooth muscle contractility, perivascular nerve and endothelial function have been further verified in the last 30 years (Aalkjær & Mulvany 1981; Nyborg et al. 1987; Mulvany & Aalkjær 1990; Ashworth & Warren 1997; Wareing & Baker 2004; Wareing, Myers, et al. 2005; Mills et al. 2007). Wire myography is a reproducible and robust method of investigating small resistance artery function with a high throughput. A number of studies have utilised this method to assess fetoplacental vasculature and have successfully demonstrated its application within the field of maternal and fetal health (McCarthy & Woolfson 1994; Sabry & Mondon 1995; Kwek et al. 2001; Wareing et al. 2002; Wareing & Baker 2004; Wareing, Myers, et al. 2005).

As with every investigatory method there are several limitations associated with the technique wire myography. The dissection and mounting of the vessel dissociates the vessel from neuronal input and may result in endothelial damage thereby affecting the endothelial-dependent responses; distension of the vessel walls by the wires has also been associated with reduced sensitivity to certain agonists in comparison to pressurised vessels (Falloon & Stephens 1995). This method of pressurised, cannulated artery preparation maintains the physiological morphology of the vessel,

reduces the likelihood of damaging the endothelium as a result of the mounting of vessels and imitates certain *in vivo* conditions such as intraluminal flow (Halpern & Kelley 1991). Although the advantages seem to outweigh those of wire myography, pressure myography is more technically challenging, requires a larger number of observations (patients) to determine statistical significant results and therefore is inappropriate for the majority of studies involving fetoplacental vasculature.

2.11 Substances the Effect Vascular Tone

As discussed above (Section 2.10) the fetoplacental circulation functions with low vascular resistance to blood flow, together with high cardiac output. As a result, those substances which would have a potent vasoconstrictory effect *in vivo* (catecholamines / oxytocin / vasopressin) seem to respond poorly *in vitro* (Mak & Gude 1984; Maigaard 1986). The placental vessels seem to be non-innervated and the mechanisms involved in the control of vascular tone are not yet understood. There are very few endogenous vasoconstrictors that play a role in the regulation of fetoplacental vasculature; some of the more predominant ones will be discussed in further detail below (Section 2.11.1 – Section 2.11.3).

Vascular smooth muscle cells display at least two distinct phenotypic states *in vivo* - a contractile phenotype in which the cells are quiescent and in which a relatively high percentage of the cell volume contains longitudinally organised microfilaments composed of smooth muscle specific contractile proteins; and a synthetic phenotype in which the micro filaments are replaced by extensive rough endoplasmic reticulum and a large Golgi complex.

As plasma membranes have high input resistance, only a small augmentation in steady state current is required to have a significant effect on membrane potential. Membrane hyperpolarisation occurs when K^+ channels are opened and an efflux of K^+ from the cell results. This efflux is due to the electrochemical K^+ gradient. Depolarisation of the smooth muscle cell activates voltage gated Ca^{2+} channels (VGCC); this in turn causes an influx of Ca^{2+} thus increasing intracellular concentrations of Ca^{2+}. Studies have indicated the presence of K^+ channels in placental arteries (e.g. Wareing et al 2006; Jewsbury et al 2007; Kiernan et al 2010; Brereton et al 2013); more specifically, voltage gated K^+ (K_V) channels, large

conductance Ca^{2+} activated K^+ (K_{CA}) channels and Adenosine Triphopshate (ATP) sensitive K^+ (K_{ATP}) channels. Further investigations have identified that, not only do vascular smooth muscle cells exhibit these channels but endothelial cells also; K_V, K_{CA}, and K_{ATP} channels as well. The presence and function of these channels, within the endothelial cells of the fetoplacental vasculature, have yet to be assessed; data from systemic vessels suggests that these endothelial K^+ channels may regulate cell membrane potential and Ca^{2+} influx (see (Jackson 2005) for review).

Within any vascular bed there is a fine balance between constrictory and relaxatory mediators; this is crucial for determining and maintaining vascular resistance and blood flow through vessels. It is therefore natural that we discuss active vasodilators with regard to fetoplacental vasculature and its ability to alter vascular tone. In systemic vasculature nitric oxide (NO) and prostacyclin (PGI_2) play a major role in vascular smooth muscle relaxation. Activation of the receptors on the endothelium or mechanical forces exerted on the endothelial cells releases these potent vasodilators (Quiñones & Guerrero 2014). Endothelium derived hyperpolarising factor (EDHF) also contributes to vasorelaxation in systemic vessels (Dautzenberg & Just 2013). Studies suggest that EDHF contributes to bradykinin induced relaxation of fetoplacental vasculature in normal pregnancy, although further studies are needed due to the lack of reproducibility and the difficulty in achieving a sustained agonist-induced endothelium-dependent vasodilation in this vascular bed (Kenny et al. 2002; Kenny & Baker 2002).

2.11.1 Thromboxane (TXA_2)

Thromboxane (TXA_2), a cyclooxygenase product of arachidonic acid, is formed largely in platelets and is known for its potent vasoconstrictory properties and as a potent platelet activator (Mitchell & Bibby 1978; Fitzgerald et al. 1990). TXA_2 is also routinely produced by the placental trophoblasts, the fetoplacental vasculature and is present in amniotic fluid (Mitchell & Bibby 1978; Ritter et al. 1982; Walsh & Wang 1995; Tulppala & Marttunen 1997; Benassayag et al. 1997). Analogues of TXA_2 have been shown to cause constriction in human umbilical artery strips and raise perfusion pressure in isolated human placental cotyledons (Hedberg et al. 1989). TXA_2 is also thought to regulate fetoplacental vasculature in both normal and pathological pregnancies (Walsh & Wang 1995). Oxygen (O_2) metabolites (reactive

oxygen species) stimulate arachidonate metabolism and the production and release of the potent vasoactive arachidonate product thromboxane (Tate et al. 1984). These O_2 metabolites will be discussed in further detail in Section 2.14.

U46619, a TXA_2 mimetic has demonstrated its ability to increase perfusion pressure in a perfused placental cotyledon and is a potent vasoconstrictor in isolated chorionic plate arteries and veins (McCarthy & Woolfson 1994; Kwek et al. 2001; Wareing et al. 2002; Wareing et al. 2003). These investigatory studies have confirmed the presence of functional thromboxane receptors in fetal vascular tissues (Hedberg et al. 1989) however, there is still much scope for its investigation through immunohistochemistry.

TXA_2 causes constriction of vascular smooth muscle by increasing intracellular calcium (Ca^{2+}) or by increasing the sensitivity of the myofilaments of smooth muscle to Ca^{2+}. There can be an increase in Ca^{2+} concentration due to an influx of Ca^{2+} into the cell through Ca^{2+} channels or by the release of Ca^{2+} from internal stores. This free Ca^{2+} binds to a specific Ca^{2+} binding protein, calmodulin; Ca^{2+}-calmodulin activates myosin light chain kinase (MLCK), an enzyme that has the ability to phosphorylate myosin light chains (MLCs) in the presence of ATP. MLCs are 20kD regulatory subunits found on the myosin heads. MLC phosphorylation leads to cross-bridge formation between the myosin heads and the actin filaments which further leads to smooth muscle contraction.

TXA_2 increases intracellular Ca^{2+} in several ways; TXA_2 binds to specific G protein receptors (TP receptors) which are closely associated with membrane-bound phospholipase C (PLC). As noted above, studies have indicated that PLC stimulates the release of intracellular Ca^{2+} from cellular stores, (Tosun et al. 1998). TXA_2 also acts by increasing the influx of Ca^{2+} into smooth muscle cells through activation of PLC; by opening the opening receptor operated Ca^{2+} channels in response to diacylglycerol which is produced by PLC (Toyofuku & Nishimura 1995; Read et al. 1999).

U46619, a thromboxane / TXA2 mimetic, acts as a contractile agent in chorionic plate arteries by increasing Ca^{2+} concentrations from intracellular stores as well as extracellular stores (receptor operated calcium channels / store operated calcium channels). The presence of functional thromboxane receptors within the fetoplacental

vasculature has been confirmed since a reduction of U46619-induced constriction, in the presence of specific TXA_2 receptor agonists (SQ29548), within a perfused placenta was noted by (Hedberg et al. 1989).

TXA_2 has also been shown to exhibit activity on ion channels inducing receptor-mediated contraction of the vascular smooth muscle. The dominant ion channels, that play a major role in the regulation of membrane potential, are potassium (K^+) channels. These are predominantly found in the plasma membrane of arteriolar smooth muscle and have a role in regulating the concentration of intracellular concentrations of Ca^{2+} (Jackson 2005).

Finally, TXA_2 is also associated with sensitisation of the contractile intracellular filaments to Ca^{2+}, suggesting that there is an increase in the contractile response independent of the intracellular Ca^{2+} concentration. This may be a similar mechanism utilised by and observed in chorionic plate resistance arteries (Wareing, O'Hara, et al. 2005). This additional mechanism is complex; phosphorylation and inhibition of MLCP by Rho-kinase (serine / threonine protein kinase) activated by the G protein Rho A, and subsequent activation of its downstream effector Rho-associated kinase (ROK). ROK_α is expressed in both myometrial and placental blood vessels of pregnant women; its stimulation has been suggested to contribute to the agonist induced Ca^{2+} sensitization of force in vascular smooth muscle (Wareing, O'Hara, et al. 2005).

2.11.2 Arginine Vasopressin (AVP)

Arginine vasopressin (AVP) is an endogenous nanopeptide, essential for cardiovascular homeostasis. It is synthesised as a large pro-hormone in the hypothalamus (magnocellular neurons / supraoptic nuclei) and transported to the posterior pituitary gland where it is released into the systemic circulation. Vasopressin is stored in the granules of the pars nervosa of the posterior pituitary until it is needed. AVP has many systemic effects, with particular emphasis on renal and cardiac systems. Its release *in vivo* is associated with blood pressure control, gluconeogenesis, neurotransmission and platelet aggregation depending on cell and receptor types (Jagger et al. 1998; Holmes et al. 2004).

Three subtypes of AVP receptors have been identified and investigated; V1a, V2 and V1b. The V1a receptor is predominantly expressed in vascular smooth muscle and hepatocytes, and thus is of particular interest within this study. Within these cells, AVP plays an important role in vasoconstriction, hepatic gluconeogenesis and platelet aggregation through the V1a receptor (Holmes et al. 2004). V2 receptors are predominantly found in the kidney, more specifically the collecting duct principal cells of the medullary nephrons (Imai 1980; Nonoguchi & Owada 1995). V1b receptors are expressed in the pituitary gland (Kjær 1993; Aguilera & Rabadan, Diehl 2000).

AVP is a potent vasoconstrictor in systemic vessels and has some therapeutic benefits, for example in the treatment of shock (Holmes & Patel 2001; Patel et al. 2002; Russell et al. 2008). AVP acts on several Ca^{2+} channels; L- type and T-type and receptor operated cation-channels (Brueggemann & Martin 2005; Shi et al. 2007). Opening these channels contributes to the rise in intracellular Ca^{2+} and resulting in constriction of the vascular smooth muscle. Those channels that are voltage dependent require early cellular depolarization (Brueggemann & Martin 2005; Wareing, X Bai, Seghier, et al. 2006).

As briefly mentioned above, K_{ATP} channels are targeted by many vasoactive hormones and neurotransmitters (Shi et al. 2007; Yang et al. 2008); however, modulation of these receptors by AVP is still unclear (Wakatsuki et al. 1992; Dumont & Lamontagne 1995).

2.11.3 Oxytocin, Angiotensin II, Eicosanoids and Endothelin and other vasoconstrictors in Fetoplacental Vasculature

Oxytocin is predominantly known for its role as a potent stimulator of uterine smooth muscle, however, studies of isolated umbilical vessels suggest that there is an additional role for the compound in maintenance of vascular tone (Kommunehospital & Western 1986).

Angiotensin II (AII) is a potent vasoconstrictor that is produced in placental tissue provided that there is an intact renin-angiotensin system (RAS). The RAS is a complex system that operates through interactions between several proteins and peptides. The primary role of the RAS is regulation of arterial blood pressure and

sodium and water homeostasis. Local and circulating vasoactive substances may function independently or collectively; they participate in a paracrine and autocrine regulation of various aspects of tissue function (Nielsen et al. 2000).

Angiotensin (AI) is formed by the proteolytic cleaving of antiotensinogen (renin substrate) by renin. Angiotensin converting enzyme (ACE) converts the decapeptide AI to the biologically active octapeptide AII. AII has a relatively short half-life as it is cleaved to biologically active and inactive fragments by angiotensinases (Nielsen et al. 2000). AII interacts with specific cell membrane bound receptors (AT_1 and AT_2) (Inagami 1999; Nielsen et al. 2000). AII receptors have been identified in the umbilical artery and the small placental arteries, (Tencé & Petit 1989; McQueen & Jardine 1990) however, the concentrations of AII that were needed to cause a significant contraction far exceeded the concentrations detected in fetoplacental serum (Pipkin & Symonds 1977; Mak & Gude 1984).

There are several vasoactive prostaglandins present during gestation. Concentrations of vasoconstrictor prostaglandins, for example PGF_α and PGE_2, are thought to remain in steady concentrations during the gestational period (Ritter et al. 1982; Walters & Boura 1991).

A final vasoconstrictor, Endothelin, is a 21 amino acid peptide that is produced by the vascular endothelium from a 39 amino acid precursor. It has three major isoforms; ET-1, ET-2 and ET-3 (Yanagisawa et al. 1988). ET-1 is produced by the placenta and has been identified in the endothelium of placental vessels and trophoblasts (Yanagisawa et al. 1988; Wilkes et al. 1990; Robaut et al. 1991; Myatt & Brewer 1992). It is a potent vasoconstrictor that is thought to regulate fetoplacental vascular resistance in pregnancy, although the mechanism of action is still unknown. Cord serum concentrations of ET-1 have been shown to be three times greater than in maternal serum (Tsukahara et al. 2002).

2.11.4 Endothelium Dependent Vasodilators: Acetylcholine and Bradykinin

Acetylcholine (ACH) and Bradykinin (BK) are good examples endothelium-dependent vasodilators of many non-placental vascular beds (Melmon & Cline 1968; Sastry et al. 1984); both agonists are present in fetoplacental circulation where they have been shown to stimulate NO from the umbilical artery and vein (Voorde 1997).

ACH synthesis is facilitated by choline acetyltransferase and is present systemically. Its vesicular release depends on depolarisation of nerve terminals resulting in the influx of Ca^{2+}. This influx causes simultaneous exocytosis of many other vesicles, however, the mechanism and physiological significance of these is still under investigation. ACH has many systemic effects mediated by two major classes of receptors; nicotinic and muscarinic. Nicotinic receptors are ligand gated ion channels located on the neuromuscular junction at the motor end plate. The mechanism of muscarinic receptor stimulation is much more complex, and relevant to this work. Muscarinic 3 (M3) receptors are coupled with the enzyme phospholipase C through a G protein and are located on smooth muscle, glands and endothelial cells in vasculature (Furchgott & Zawadzki 1980; Macklin & Maus 1998; Lu et al. 2015). When this enzyme stimulates the M3 receptor, is stimulated by this enzyme it causes an increase in fragmentation of phosphatidylinositol polyphosphates of the cell membrane. Inositol -1,4,5 triphosphate (IP3) is produced and released intracellularly and acts on the surface of the sarcoplasmic reticulum (Morel et al. 2003). This in turn increases Ca^{2+} release from the sarcoplasmic reticulum of the smooth muscle cell and also increases cytosolic Ca^{2+} (Lu et al. 2015).

The peptide mediator BK is also another potent systemic vasodilator. BK is a relatively short lived, biologically active substance that is predominantly generated by the enzymatic action of kallikreins on kininogen precursors (Golias et al. 2007). These kinins are peptide hormones that exert their biological effects via G protein-coupled receptors. This protein exerts its vascular relaxatory action through direct stimulation of specific endothelial B_2 receptors thereby causing the release of prostacyclin, nitric oxide (NO) and EDHF. BK is also known for its pro-inflammatory and neuromediator properties as well as its vascular regulatory abilities (Hornig et al. 1997; Golias et al. 2007).

Inconsistent responses to endothelium-dependent vasodilators on fetoplacental vasculature have been reported *in vitro*. For example, ACH had no effect on perfusion pressure in a pre-constricted perfused placental lobule (Myatt & Brewer 1992) and there is still little known about B receptors in the fetoplacental vasculature (Golias et al. 2007; Moyes et al. 2014).

2.11.5 Vasodilator Peptides, Prostacyclin and Nitric Oxide

Corticotrophin releasing hormone (CRH) is a peptide hormone, most commonly produced by the parvocellular neuroendocrine cells within the paraventricular nucleus of the hypothalamus, but also found in the syncytiotrophoblast of the human placenta. It is a 41 amino acid sequence that plays a central role in the adaptations of the organism to stress, and has been reported to induce significant relaxation at physiological concentrations (Hatzoglou & Margioris 1996; Clifton & Challis 1997; Claes 2004). Studies suggest that CRH acts via the NO / cyclic guanosine monophosphate (cGMP) – dependent pathway reduced relaxation in the presence of NO and guanylate cyclase inhibitors (Clifton & Challis 1997). The peptides urocortin and urotensin-1 are also members of the corticosteroid family that exhibit these vasorelaxatory properties. These peptides are expressed on the placental and fetal membranes and are thought to be more potent vasodilators than CRH itself (Petraglia & Florio 1996).

Neurokinin B, a neuropeptide and member of the kinin family, has also demonstrated vasodilatory properties in the syncytiotrophoblast (Wareing 2003). In general, it plays more significant roles in olfactory, gustatory, visceral and neuroendocrine processing with significant bronchoconstrictory and vascular regulatory effects. In human pregnancy the expression of neurokinin B is identified on the outer syncytiotrophoblast layer of the placenta and concentrations have been detected in plasma at 9 weeks gestation. Grossly elevated levels of neurokinin have been detected in pathological pregnancies, such as pre-eclampsia and pregnancy-induced hypertension, suggesting that it may play a significant role in fetoplacental vascular tone (Page et al. 2000).

Vascular endothelial growth factors (VEGF's) which were discussed briefly above (Section 2.2, Section 2.4) are known to affect endothelial cell function. These peptides increase vascular permeability, angiogenesis and endothelial cell growth, and exhibit vasodilatory effects in specific vascular beds (Shweiki & Itin 1993; Brownbill et al. 2008; Demir 2009). More specifically, VEGF is expressed within the syncytiotrophoblast, fetal membranes and umbilical smooth muscle (Brownbill et al. 2008). There is a substantial body of evidence to suggest that there is a tandem effect

between neurokinin B and VEGF that contributes to vascular tone in normal pregnancy.

Prostacyclin is an endogenous vasodilator, synthesized by cyclooxygenase and released by the endothelium (Okatani et al. 1997; Quiñones & Guerrero 2014). Its release and synthesis can be potentiated by dilatory agents such as bradykinin (Whorton et al. 1982) and carbon dioxide (hypoxia / hypercapnic states) (McCalden et al. 1984); it is thought to work through smooth muscle relaxation by stimulation of adenylyl cyclase and the generation of cAMP (Quiñones & Guerrero 2014; Christman et al. 1992). Prostacyclin is synthesized in the placenta and thought to modulate NO synthesis in conjunction with CRH; however, it has a particularly short half-life and may be inactivated by placental enzymes (O'Brien et al. 1988; Donoghuea et al. 2000).

NO is a widely known as a physiological vasodilator, synthesized from L-arginine by the enzyme nitric oxide synthase (NOS) (Kublickiene et al. 1997; Quiñones & Guerrero 2014). Bradykinin, acetylcholine, increased intracellular calcium and stress all contribute to the production, and subsequent release, of NO. NO acts directly on the underlying smooth muscle by diffusion and increasing levels of cGMP, via the activation of a soluble guanylate cyclase. cGMP and its activation of the cGMP-dependent kinase pathway relaxes the vascular smooth muscle through the reabsorption of cytosolic calcium (Garg & Hassid 1991; Blatter & Wier 1994). Endothelial NOS has been located in the umbilical, chorionic plate and villous tree vascular endothelium, suggesting that NO plays a significant role in the regulation of fetoplacental vascular tone (Myatt et al. 1997; Rutherford 1995).

2.12 Oxygen Tension

Oxygen tension is fundamental to the regulation of vascular tone, particularly within the fetoplacental circulatory system. Maternal blood flow within uterine arteries has a pO_2 of approximately 95 - 100 mmHg (Kliman 2000), similar to levels in systemic vasculature. As mentioned above, placental development begins in a hypoxic or low oxygen environment (Section 2.2). Measurement of oxygen tension in the intervillous space indicates that there is a dramatic rise at approximately 10 - 12 weeks; this correlates with the time maternal blood flows through the placenta

(Castellucci et al. 1990; Sadler 2011). An intervillous pO_2 of 55 mmHg has been recorded at 16 weeks gestation (Jauniaux et al. 2000) but thus far no investigations have been conducted on term placenta. Investigations have shown that pO_2 concentrations within umbilical vessels and the systemic venous system reach levels of approximately 40 mmHg (Benson 1980; Sandoo et al. 2010). Amniotic fluid surrounds the chorionic plate and thus has the potential to influence chorionic plate vasculature tone; amniotic fluid pO_2 declines as the size of the cavity decreases.

Evidence suggests that the placental vasculature is exposed to low oxygen tensions *in vivo* compared with systemic vasculature. Studies have been conducted to assess the effect of varying levels of oxygen tension and their potential association with pregnancy pathologies. Acute hypoxic vasoconstriction is associated with an increase in intracellular Ca^{2+}, which can arise from the opening of specific channels. Oxygen-sensitive K^+ currents, identified in the chorionic plate arteries, are said to contribute to the inhibition of these effects through depolarisation and thereby suppress the hypoxic crisis in the fetoplacental vasculature occurring. One particular study indicated that hypoxic vasoconstriction in the placenta involved the inhibition of K^+ channels causing an increase in intracellular Ca^{2+} concentration and depolarisation of vascular smooth muscle which further caused an increase in basal vasomotor tone (Cooper et al. 2005; Hampl & Jakoubek 2009). Cooper *et al.* (2005) investigated the effect of varying oxygen tension on fetoplacental resistance vessels and concluded that the fetoplacental vascular response, namely vasoconstriction, can be modulated by oxygen tension but not at levels present in physiological conditions (Cooper et al. 2005). Another study by this group demonstrated that by reducing oxygen tension three major physiological observations were identified. By reducing oxygen tension, an increase in vasodilation of human chorionic plate veins was observed. Reduced oxygen tension also increased tone of chorionic plate arteries when exposed to U46619 (Section 2.11.1), increasing their maximal constriction to this thromboxane memetic. It did not, however, have an effect on chorionic plate vein constriction or sensitivity (Wareing, S. L. Greenwood, et al. 2006).

2.13 Reactive Oxygen Species

Pregnancy is a period of time when the human body experiences an increased production of reactive oxygen species (ROS). ROS are defined as highly reactive

molecules containing an unpaired electron and other non-radical intermediates (Wisdom et al. 1991). The most common ROS formed in the placenta is the superoxide anion (O_2^-). Superoxide is generated in all living cells by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, flavin enzymes and enzymes in the mitochondrial electron transport chain (Myatt & Cui 2004). The process of formation will be discussed in greater detail below (Section 2.14.1).

Increased production of ROS or impaired antioxidant capacity, due to enzymes, other molecules, dietary products that neutralize the reactivity of pro-oxidant species, can all cause significant damage during the gestational period. This imbalance is often noted in pathological pregnancies and leads to oxidative stress (Myatt & Cui 2004). Excess ROS can cause cellular damage and impact tissue function as a result of lipid peroxidation, amino acid modification and DNA oxidation. Burton (2009) investigated the effects of altered placental oxygenation with increasing gestational age (Burton 2009). Fluctuating oxygen conditions can contribute to increased ROS production particularly in tissues with a high energy demand or those with large amounts of mitochondria, namely the placenta (Pereira et al. 2015). Typically, there is a balance between the production of ROS and antioxidants; this balance is essential for placental function, angiogenesis, many cellular processes and signalling (Pereira et al. 2015).

Oxidative stress has been clearly shown in placental tissue and this can often impair placental function. There is a growing body of evidence suggesting that ROS have a major role in regulating vascular function *in vivo*, particularly in pathologies such as diabetes, hypertension and atherosclerosis. We can therefore infer that it may contribute to the regulation of fetoplacental vascular reactivity in normal and compromised pregnancies (Mills et al. 2009).

2.13.1 Formation of Reactive Oxygen Species

ROS are formed through the univalent reduction of molecular oxygen which produces the free radical superoxide (O_2^-). ROS are highly reactive molecules and complex pathways have evolved in order to maintain health levels and prevent the accumulation of these potentially harmful molecules. O_2^- is generally membrane

impermeable however it has the ability to cross through cell membranes via anion channels.

The superoxide anion, O_2^-, is detoxified by superoxide dismutase (SOD) to H_2O_2 and water. Vascular tissues contain three forms of these detoxifying SOD's; a cytosolic copper-zinc SOD, a mitochondrial manganese SOD and an extracellular copper-zinc SOD. When considering the placenta, particularly the villous vascular endothelium, both copper-zinc and mitochondrial manganese SOD have been identified (Watson et al. 1997; Fattman et al. 2003). Investigations have been conducted in order to measure vascular concentrations of O_2^-; in the presence of normal SOD activity O_2^- is estimated to be in the picogram range (Li et al. 1997). Although such low concentrations would not be associated with a harmful effect, the by-product of detoxification H_2O_2 could potentially interrupt vascular signalling pathways. H_2O_2 which is mainly produced from the SOD-catylisation or spontaneous dismutation of O_2^- is a more stable species with a greater half-life than the superoxide anion itself. H_2O_2 is scavenged by catalase and glutathione peroxidase; both of these enzymes are found in the placenta and their activity is increased in the central region in normal pregnancy. These concentrations reflect the perfusion of maternal oxygenated blood (Jauniaux et al. 2000; Hempstock et al. 2003).

The formation of peroxynitrite ($ONOO^-$) also occurs when NO competes for O_2^- *in vivo.* This reaction occurs when O_2^- binds with NO at a rate of 6.7 ($\pm$ 0.9) x $10^{-9}\,M^{-1}s^{-1}$ and this reaction occurs at least three times faster than the reaction of O_2^- and SOD (Huie & Padmaja 1993). $ONOO^-$ is a powerful vasoactive oxidant with the potential to cause vascular dysfunction particularly when concentrations are increased in pathophysiological conditions. $OONO^-$ has a relatively short half-life with the ability to diffuse easily through cell membranes; it reacts slowly and selectively only interacting with certain functional groups on proteins such as thiols, iron / sulphur centres, zinc and tyrosine (Beckman & Koppenol 1996; Denicola et al. 1998). Nitrotyrosine is a biomarker of $ONOO^-$ and has been detected in placental villous vasculature (Myatt & Cui 2004) suggesting that it has a role in normal vascular function.

Excessive concentrations of H_2O_2 in the presence of transition metals (such as Fe^{2+}) may result in a further reduction via what is known as Fenton or Haber-Weiss

reactions, and the generation of hydroxyl radicals (OH^-). OH^- is an extremely reactive molecule and is considered to be the most likely culprit for oxidative injury *in vivo*, especially when considering its involvement in radiation induced damage (Dizdaroglu & Jaruga 2002). Formation of OH^- is a relatively slow process, requiring chelated Fe^{2+} to be reduced by the superoxide anion, and then to react with H_2O_2. In normal physiological conditions antioxidants prevent this reaction from occurring. However, OH^- is highly reactive with most biological molecules in close proximity therefore its diffusion is limited to the diameter of small proteins (Hutchinson 1957). Conclusions can therefore be drawn regarding the effects of OH^- on tissues; these may be limited by a relatively slow rate of synthesis and high reactivity which confines toxicity to a relatively small area.

2.13.2 Sources of Reactive Oxygen Species in the Placenta

There are several potential mechanisms of ROS formation in the placenta, but under physiological conditions the major sources of ROS are likely to be enzymes in the mitochondrial electron chain and NADPH oxidase. In pathological conditions other enzymes such as xanthine oxidase may have a greater role. The mitochondria consume the majority of oxygen in the production of adenosine triphosphate (ATP) through the action of the mitochondrial electron transport chain enzymes (Liu et al. 2002). The role of mitochondria electron transport chain enzymes in generating ROS in the placenta has not been extensively investigated, however, it is important to note that mitochondrial mass increases over gestation.

The majority of vascular O_2^- is likely to derive from the vessel wall, where smooth muscle cells and fibroblasts have been identified as the most important sources (Pagano 1993; Pagano & Ito 1995). In non-placental vessel, NADPH oxidases have been identified in endothelial, smooth muscle and fibroblast cells (Mohazzab-H & Wolin 1994; Mohazzab-H 1994; Wolin et al. 1999).

Xanthine oxidoreductase has two functionally distinctive forms; xanthine dehydrogenase and xanthine oxidase. Under normal physiological conditions the greatest portion of this enzyme exists in the dehydrogenase form; this enzyme converts hypoxanthine to xanthine, and xanthine to uric acid, with reduction of Nicotinamide adenine dinucleotide (NAD^+) to NADH (Stirpe & Corte 1969; Chan et

al. 1994). In pathological conditions, the oxidase form is more common, which further results in the production of O_2^-. Low levels of xanthine oxidase have been reported in placental villous trophoblasts, small vessels and stroma (Many & Westerhausen-Larson 1996).

2.13.3 Effect of Reactive Oxygen Species on Vascular Tone

ROS are generated in the systemic vasculature and are important signalling molecules, however, they also have the potential for deleterious effects. Endothelial and smooth muscle cells are the source of ROS as well as being the site at which they exhibit an effect (Wilcox 2002; Suvorava & Kojda 2009). There are several different enzymes that regulate the homeostasis of vascular ROS production. Formation and its elimination are tightly regulated within the vascular wall in order to maintain desirable effects (Yung et al. 2006; Suvorava & Kojda 2009).

Increased bioavailability of vascular ROS may result in several pathologies. ROS stimulates vascular smooth muscle cell proliferation, migration, collagen deposition and altered metalloproteinases activity (Touyz 2004). In endothelial cells, oxidative radicals induce apoptosis and anoikis thereby impairing endothelial cell function (Suvorava & Kojda 2009). Oxidative stress stimulates activation of pro-inflammatory transcription factors (NFκB / AP-1) and pro-inflammatory genes (cytokines, interleukins), upregulation of adhesion molecules (ICAM, VCAM, PECAM), stimulation of chemokine production (MCP-1) and recruitment of pro-inflammatory cells (monocytes, macrophages). These processes are fundamental in vascular inflammation and injury (Fishbein et al. 1980; Griendling & FitzGerald 2003; Touyz 2004).

ROS have been associated with effects on vascular function by modification of vasoactive pathways or by direct effects on vascular endothelial or smooth muscle cells. The majority of investigations into the effects of ROS have been conducted *in vitro* through the direct application of species (H_2O_2), or using varying generating systems of ROS (Xanthine / xanthine oxidase). The vascular effects of ROS vary greatly depending on the vascular bed, species, concentration and presence of the endothelium (Ellis & Triggle 2003; Stone & Yang 2006).

2.13.4 Reactive Oxygen Species and the Nitric Oxide / Cyclic Guanosine Monophosphate pathway

Investigations into ROS activity indicate that they are modulators of NO availability in vascular systems. NO has been demonstrated as a potent vasodilator and this is a determinant of low resistance in the placental and systemic vasculature. The reaction between NO and O_2^{2-} results in reduced availability of NO. Impaired endothelium-dependent relaxation, a phenomenon demonstrated in many pathological states, is often associated with decreased NO availability and increased O_2^{2-}. ROS have been shown to influence the soluble guanylate cyclase activity; this molecule interacts with NO to promote smooth muscle relaxation by stimulating production of cGMP. cGMP is a messenger molecule, vital for the formation of protein kinase G, followed by the formation of myosin phosphatase and thus smooth muscle relaxation (Wareing, Jennifer E Myers, O'Hara, et al. 2006; Sandoo et al. 2010).

2.13.5 Reactive Oxygen Species and Calcium Signalling

ROS ability to disrupt Ca^{2+} signalling and ion channel function has also been shown to influence vascular tone. ROS have been shown to modulate intracellular Ca^{2+} concentration; it is important to note that normal physiological concentrations regulate NO and other autacoid production. ROS have also been shown to affect Ca^{2+} handling in vascular smooth muscle. This mechanism involves the inactivation of the sarcoplasmic or endoplasmic reticulum Ca^{2+}-ATPases (which pump / transport Ca^{2+} against its concentration gradient). This mechanism is predominantly found in small, more active arteries rather than larger ones (Grover et al. 1999).

In summation, ROS have been demonstrated to significantly affect Ca^{2+} signalling in the vasculature, with the main site of influence being the endothelial and smooth muscle cells. ROS have the potential to enhance agonist-induced Ca^{2+} signalling in endothelial cells and may increase production of NO. The effect of NO on placental arteries has been assessed using the NO donor, sodium nitroprusside. This exhibited a similar, vasorelaxation effect to that observed in systemic vessels.

2.13.6 Reactive Oxygen Species and Potassium Channels

Voltage gated K^+ channels are located on smooth muscle cells and are known to play a vital role in determining vascular tone. The opening of these channels is associated with vasodilation, independent of NO (Jackson 2005). These K^+ channels have been identified on small placental vessels and it is thought that these contribute to regulation of fetoplacental tone. A hyperpolarizing mechanism is associated with systemic vasculature. Evidence suggests that K^+ channels are susceptible to modulation by ROS, namely activity on Ca^+ / K^+ channels, voltage gated K^+ channels and K_{ATP} channels (Krenz et al. 2002; Sesti et al. 2010).

2.14 Active Coffee Chemicals

Active coffee chemicals are defined as the functional substances within the popular beverage. As noted in Chapter 1, there are thousands of active ingredients present in coffee, both the parent compound and the finished beverage. There is much literature surrounding the biologically active compounds present in coffee, focusing on both their physiological effect and their association with pathologies, however, very few are conclusive. The studies that have considered coffee, its active chemicals and their relationship with pregnancy are also inconclusive. Furthermore, to my knowledge, there are no studies concerned with active coffee chemicals and the placental vasculature. As stated above, adequate placental function is essential for intrauterine growth and development of the fetus; there is a clear gap in the knowledge as to whether its consumption can be linked with placental compromise or improved antioxidant abilities. My study focused particularly on caffeine, caffeic acid and chlorogenic acid which, by their concentration or known activity, were likely to exert significant biological effects on placental and myometrial vascular beds.

As stated, caffeine is considered to be the most biologically active substance within the finished coffee beverage. Because of this the majority of research is conducted on its effects and thus the Department of Health suggest that it should be limited, particularly during the gestational period. I have included a table of the approximate caffeine content within common beverages in Appendix 9.

2.14.1 Caffeine

Caffeine (1,3,7-trimethylxanthine) is the world's most widely consumed psychoactive substance, which can be found in beverages, food and many medications (Nurminen et al. 1999; Nawrot et al. 2003). The most well-known caffeinated beverages include tea (20 - 40 mg per 150 mls), carbonated soft drinks (15 - 24 mg per 180 mls) and energy drinks (200 - 500 mg per 100 mls) (Nurminen et al. 1999). However, coffee is the main dietary source of caffeine (approximately 75% of all exposure to caffeine is from coffee) with caffeine levels ranging from 70 mg to 400 mg per 100 ml (Grosso & Bracken 2005). Although these are the standard quoted figures, there is much variability between brands and types, which will be discussed in Chapter 9, Section 9.4.1 and Chapter 10, Section 10.3.
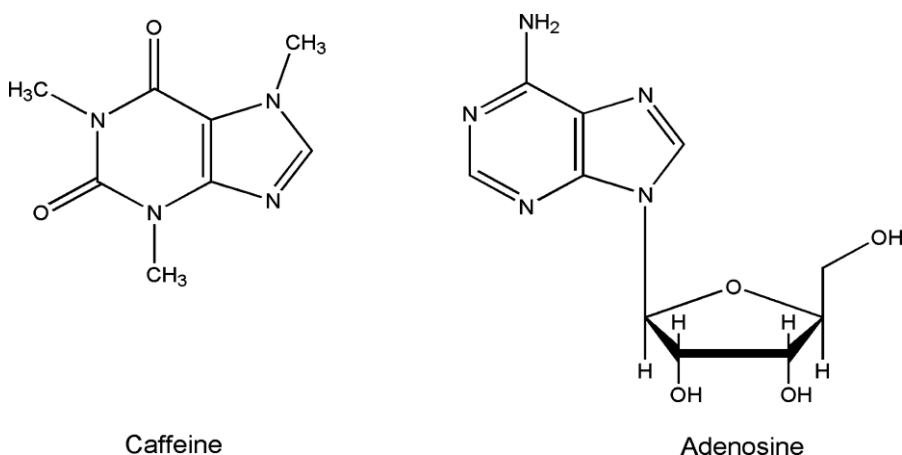
Figure 4: Structure of caffeine and adenosine (Higdon & Frei 2006)

2.14.2 Effect of Caffeine

Caffeine has potential therapeutic applications; these include the treatment of apnoea in preterm infants, a somnolytic for postprandial hypotension, prolongation of seizures in electroconvulsive therapy, increase in body weight loss in obese patients and as an adjuvant analgesic (Carrillo & Benitez 2000). Caffeine is known to have interactions with other medicinal preparations. Studies have shown that caffeine may increase the side effects of β-adrenergic stimulating drugs (Carrillo & Benitez 2000) as well as inhibition of anti-psychotic medication (Higdon & Frei 2006). Caffeine is also known to decrease the elimination of theophylline and acetaminophen (Knutti et al. 1981; Carrillo & Benitez 2000). Caffeine has also been shown to enhance the bioavailability of aspirin thus improving its analgesic effect, and this explains why

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many preparations (e.g. cold remedies) now include it (Carrillo & Benitez 2000; Higdon & Frei 2006; George et al. 2008).

Caffeine is a purine alkaloid similar to that in DNA (Martin & Bracken 1987). An article by Martin and Bracken (1987) suggested that caffeine, because of this similarity in structure to adenosine (see Figure 2), may have the potential to impact cell metabolism and proliferation, however, this has not been experimentally validated (Martin & Bracken 1987).

Caffeine exerts a stimulatory effect on the central nervous system (Nurminen et al. 1999). Its main mechanism of action is as an antagonist of all types of adenosine receptors, as adenosine is structurally similar to caffeine (James 2004). Adenosine, a molecule found in the neuronal extracellular tissue, is linked with metabolism and ATP breakdown (Higdon & Frei 2006; Elmenhorst et al. 2011). This endogenous compound exerts its influence on neuronal communication via synchronizing and desynchronizing receptor activation (Ribeiro & Sebastio 2010). As adenosine primarily exerts inhibitory effects, antagonism of the A1 and the A2a subsets of the adenosine receptor by caffeine usually results in stimulatory effects (Fredholm 1995; James 2004; Higdon & Frei 2006). Thus, when acting as an adenosine receptor agonist, caffeine when administered acutely has the opposite effect of activation due to the removal of the adenosinergic tonus. As a pharmacological tool, caffeine is a poor adenosine receptor antagonist as affinity for these receptors is weak (Ribeiro & Sebastio 2010).

Caffeine also has the ability to effect endothelial and smooth muscle cells. Caffeine has the potential to upregulate the expression of the vasodilator nitric oxide (NO), which has an autocrine effect, increasing intracellular Ca^{2+} and causing vasodilation (Echeverri et al. 2010). Caffeine may also exert its vascular mechanisms of action through the direct and indirect stimulation of vascular smooth muscle cells (Echeverri et al. 2010). Studies have been conducted on caffeine and its effect on umbilical vasculature; these did not indicate any significant effect on tone stating that embryonic and umbilical artery flows were insensitive to peak maternal caffeine serum concentrations (Momoi et al. 2008). Another study by Matsuoka *et al.* (2006) indicated that high serum caffeine concentrations were capable of inhibiting the growth of umbilical vein endothelial cells and induce apoptosis via the caspase-9

pathway (Matsuoka et al. 2006). As stated above, umbilical and chorionic tissue differs histologically and therefore the effect of caffeine may also differ (Wigglesworth 1969; Benirschke & Kaufmann 2000).

The pharmacology of a drug relies heavily on it absorption and metabolism. Caffeine is primarily absorbed in mucosa of the upper intestinal tract. Studies have shown that it can easily pass through the placenta allowing for concentrations of caffeine to reach similar levels observed in maternal serum. This was substantiated when neonatal serum levels were sampled and confirmed relatively high concentrations of caffeine (James 2004; Kuczkowski 2009). Caffeine is said to have a half- life of approximately 2.5 to 4.5 hours however, this is prolonged in pregnancy (Bech et al. 2007). Drug half-life is calculated from a plasma concentration versus time curve and its prolongation can result in potentially harmful events. This is one example of the pregnancy induced changes in pharmacokinetics (Anderson 2005).

A number of studies have documented caffeine withdrawal symptoms which include headaches, fatigue, drowsiness, irritability, difficulty concentrating and depression (Liguori et al. 1997; Nawrot et al. 2003; Higdon & Frei 2006). There is little definitive evidence to verify this experimentally.

2.14.3 Caffeine Metabolism

As stated above, the half-life of caffeine is between 2.5 and 4.5 hours (Grosso & Bracken 2005; Kuczkowski 2009) but this can be as long as 12 hours, with peak concentrations varying due to delays in gastric emptying (Grosso & Bracken 2005). Studies of pharmacokinetics of xenobiotics are usually done in non-pregnant, healthy volunteers and therefore there is much still unknown regarding caffeine and its metabolism during the gestational period.

Cytochrome P450 is primarily responsible for the metabolism, or demethylation and oxidation, of caffeine to paraxanthine, theobromine and theophylline in the liver (Aldridge et al. 1979; Knutti et al. 1981; Nurminen et al. 1999; Grosso & Bracken 2005). The cytochromes are a super family of mono-oxygenases which carry out the oxidation of carbon and nitrogen groups usually resulting in the addition of an alcohol. Many of the metabolites generated by the P450's are further subjected to conjugation with either glucuronic acid, sulfate, glutathione or acetyl groups during

phase II metabolism. Practically all lipid-soluble xenobiotics pass through the placental barrier, and thus have the potential to cause harmful effects. The cytochrome enzymes of the human conceptus are relatively well developed and thus the fetus has extrahepatic abilities to metabolise xenobiotics. The placenta is, however, devoid of many cytochrome enzymes. Due to the small size of the fetus and low abundance of cytochrome enzymes in the placenta, the contribution to fetoplacental metabolism is minor (Hakkola et al. 1998). Although its ability to metabolise many xenobiotics, studies have shown that the fetal tissue is unable to metabolise caffeine as well as adult tissue (Aldridge et al. 1979). Smokers are known to metabolise caffeine faster as smoking induces the cytochrome pathway thus giving way to greater clearance (Parsons & Neims 1978; Bech et al. 2007; Plichart et al. 2008).

The metabolism of caffeine differs between species and consequently it is difficult to translate conclusions from animal studies to humans (Kline et al. 1991). In addition, caffeine metabolism varies between specific groups in the population. Caffeine metabolism is increased in smokers (Parsons & Neims 1978) and reduced in those with genetic disorders which inhibit or limit caffeine metabolism (Grosso & Bracken 2005). As stated above, smoking decreases the half-life of theophylline and caffeine due to polycyclic hydrocarbons present in the smoke which increase the activity of liver enzymes (Parsons & Neims 1978; Knutti et al. 1981; Grosso & Bracken 2005). Therefore, to some extent tobacco consumption may be beneficial in the removal of the potentially harmful effects of caffeine during pregnancy yet smoking during the gestational period is a prime culprit in many pregnancy pathologies (Alderete et al. 1995).

Caffeine has also been associated with pharmacological tolerance. Behavioural, electrophysiological and neurochemical tolerance to caffeine has been investigated in both murine and human models, indicating that prolonged exposure may reduce the physiological effect of caffeine (Holtzman & Finn 1988; Evans & Griffiths 1992; Shi & Benowitz 1993; Kendler & Prescott 1999). Habitual coffee consumers also discuss the effects of caffeine withdrawal, stating that headache, nausea and irritability are all side effects; these further substantiate the evidence that caffeine ellicit a physiological effect (Phillips-Bute & Lane 1997; Nehlig 1999; Kendler & Prescott 1999; Dews et al. 2002).

2.14.4 Caffeine Metabolism in Pregnancy

The half-life of caffeine is increased during pregnancy from approximately 4 hours in early pregnancy to 10 hours after 17 weeks gestation (Knutti et al. 1981; Grosso & Bracken 2005). It is suggested that the reduction of CYP1A2 activity as the pregnancy progresses is responsible for the reduction in the clearance rate of caffeine (Knutti et al. 1981; Tsutsumi & Kotegawa 2001; Grosso & Bracken 2005). Increased plasma volume during pregnancy affects the volume distribution of caffeine (Knutti et al. 1981). As the concentration of some plasma proteins are decreased during pregnancy, due to the two fold increase in the total body water volume, there is an increase in the unbound fraction of caffeine in the circulation (Little 1997). As stated above, and verified by many articles, caffeine easily crosses the placental membranes reaching the fetus and amniotic fluid (Parsons & Neims 1978; Grosso & Bracken 2005). Neither the placenta nor the fetus can metabolize caffeine effectively due to the lack of CYP1A2, which is only produced after 8 months of age, and so length of exposure to caffeine is greatly increased (Aldridge et al. 1979; Grosso & Bracken 2005).

2.14.5 Chlorogenic Acid

Chlorogenic acid is a dietary phenol present in coffee and is a metabolite of quinic acid metabolism (Clifford 2000; Olthof, Hollman & Katan 2001). The structure of chlorogenic acid is shown in Figure 3. It is found in other foods and beverages to a much lesser extent (Olthof, Hollman, Zock, et al. 2001; Olthof et al. 2003).
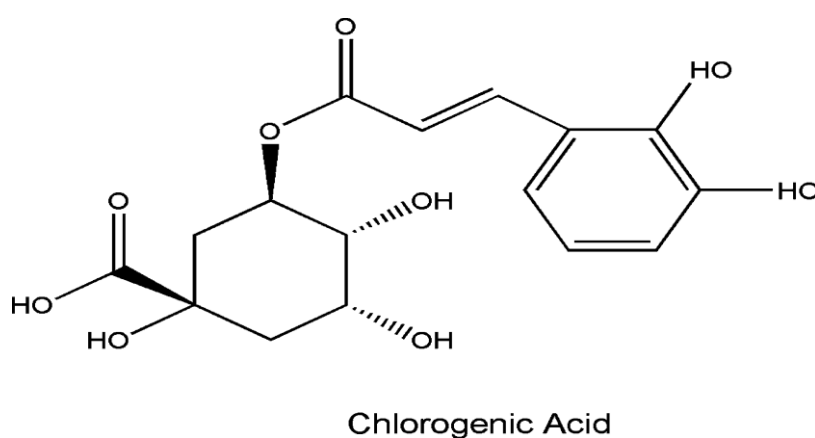
Chlorogenic Acid

Figure 5: Structure of chlorogenic acid (Higdon & Frei 2006)

2.14.6 Effect of Chlorogenic Acid

Dietary phenols are often shown to exhibit antioxidant (Bouayed et al. 2007), anti-inflammatory (Loke et al. 2010), anti-carcinogenic (Tahanian & Lord-dufour 2010) and oestrogenic (Zhu et al. 2009) activities *in vitro* and therefore can potentially have beneficial health effects (Wang & Mazza 2002; Olthof et al. 2003). Although exhibiting strong antioxidant effects *in vitro*, the effects of chlorogenic acid *in vivo* remain uncertain (Zhang et al. 2001). Dietary phenols, including chlorogenic acid, are extensively metabolized in humans and there are inconclusive results on whether these metabolites have potentially lower antioxidant activity when compared with their parent compound (Olthof, Hollman & Katan 2001).

Magnesium and chlorogenic acid, both of which are found in coffee, are said to have beneficial effects on insulin sensitivity and thereby could have an effect on the development or progression of diabetes (Salmeron & Manson 1997; Khan et al. 1998; Agardh et al. 2004). Some animal studies have indicated chlorogenic acid may have the ability to decrease serum triglyceride and total cholesterol levels along with liver lipid peroxide, thus reducing the likelihood of developing secondary cardiovascular diseases (Okuda et al. 1983; Chang & Hsu 1992). Chlorogenic acid is also present in cigarette smoke in the form of a pigment complex, yet the direct effects are unknown (Becker & Hajjar 1985). Much research has been carried out on chlorogenic acid (Clifford 2000) however, the pathways by which it exhibits their effect remains inconclusive or unidentified.

2.14.7 Chlorogenic Acid Metabolism

Chlorogenic acid is metabolized in the colon to form Hippuric acid through bacterial degradation (Clifford 2000) with approximately 33% of chlorogenic acid being absorbed in the ileum (Olthof et al. 2003). It is important to know the mechanism by which chlorogenic acid is metabolized so that it is possible to induce its biological effects when it enters the circulation as these may have a potential beneficial heath effect (Olthof, Hollman & Katan 2001).

The mechanism of absorption of chlorogenic acid is still unknown, however, there are two generally accepted hypotheses regarding this. There is a possibility that chlorogenic acid is absorbed as a whole compound with traces (0.3% of ingested

material) being detected in the urine as a result of the intensive and thorough metabolism (Olthof et al. 2003). The second potential mechanism suggests that hydrolysis of chlorogenic acid in the upper gastrointestinal tract allows for easier absorption (Olthof, Hollman & Katan 2001; Granado-Serrano et al. 2007). Studies, similar to that of Cafestol and kahweol, have been done using healthy ileostomy subjects to measure absorption of chlorogenic acid and similar findings have been deduced (Clifford 2000; Olthof, Hollman & Katan 2001).

Animal studies have suggested that chlorogenic acid has the ability to decrease serum triglyceride and total cholesterol levels as well as liver lipid peroxide (Okuda et al. 1983; Chang & Hsu 1992).

2.14.8 Caffeic Acid

Phenolic compounds are secondary plant metabolites and are present in almost all plant products; these are thought to be an integral part of the human diet. Hydroxycinnamic acid is the major subgroup of phenolic compounds (Gülçin & Gulcin 2006); hydroxycinnamates and their derivatives are thought to exhibit *in vitro* antioxidant activity which may have a beneficial health impact *in vivo* (Kroon & Williamson 1999).
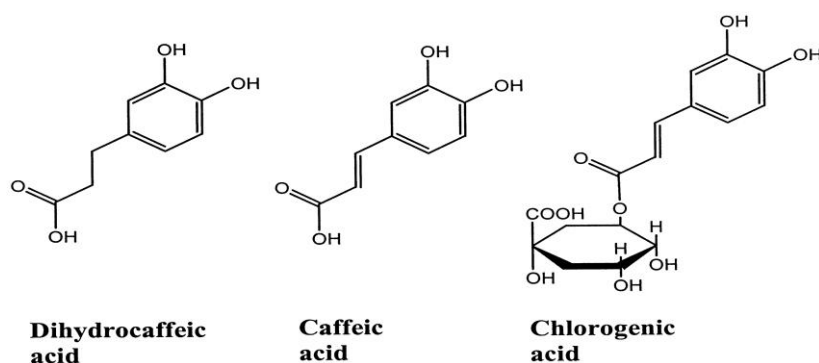
Figure 6: Structure of caffeic acid and its derivatives (Moridani et al. 2001).

Caffeic acid (3,4-dihydroxycinnamic acid; see Figure 4) has been shown to be an α-tocopherol protectant in LDL. In many studies its conjugates (chlorogenic acid, caftaric acid) are considered to be more powerful antioxidants (Kono et al. 1997; Kweon et al. 2001; Bouayed et al. 2007). Caffeic acid and its substrates undergo

oxidation under certain conditions, and similar to other coffee chemicals, there are uncertainties regarding the biological activity of these metabolites (Vieira et al. 1998; Gülçin & Gulcin 2006).

Passive absorption in the stomach and active absorption in the small intestine play a role in the uptake of caffeic acid in humans. The absorbed metabolites may induce biological effects in the blood; serum studies have concluded that caffeic acid is present in systemic circulation (Olthof, Hollman & Katan 2001). Caffeic acid's ability to inhibit oxidation of LDL *in vitro* suggests that it may have some vasoprotective effects *in vivo* (Chen & Ho 1997; Gülçin & Gulcin 2006).

Early studies on caffeic acid demonstrated its ability to inhibit platelet aggregation and stimulate the biosynthesis of thromboxane. This may act as a mediator causing bronchoconstriction in allergic reactions; increased production of the cyclooxygenases and thus stimulation of prostaglandin synthesis (Koshihara 1984). Contradictory studies have suggested that caffeic acid has the ability to inhibit certain enzyme activities such as Lipoxygenase, cyclooxygenases, glutathione S-transferase and Xanthine oxidase, suggesting that it may exhibit some anti-inflammatory properties (Koshihara 1984; Ploemen et al. 1993; Chan et al. 1994; Mirzoeva et al. 1996; Michaluart et al. 1999). Other studies have suggested that caffeic acid has the potential to inhibit HIV replication (Fesen et al. 1993; Kashiwada & Nishizawa 1995) as well as having anti-inflammatory properties (Chan et al. 1994; Fernandez et al. 1998) and anti-tumour properties (Tanaka et al. 1993; Frenkel et al. 1993). Caffeic acid efficiently inhibits ceramide-induced NFκB binding activity, a complex that controls DNA transcription, thus potentially playing an important role in cancer invasion and metastasis (Chung et al. 2004).

2.14.9 Cafestol and Kahweol

Cafestol and kahweol, otherwise known as coffee oils, are heterocyclic diterpenes that are present in both coffee beans and the end beverage (Gross et al. 1997; Young et al. 2004; Huber et al. 2004; S T J Van Cruchten et al. 2010). They are associated with much of coffee's pharmacological activity (Kim et al. 2004; Hwan et al. 2010) and their concentrations are high in unfiltered coffee, such as Scandinavian and Turkish press, and low in filtered coffee (Majer et al. 2005).
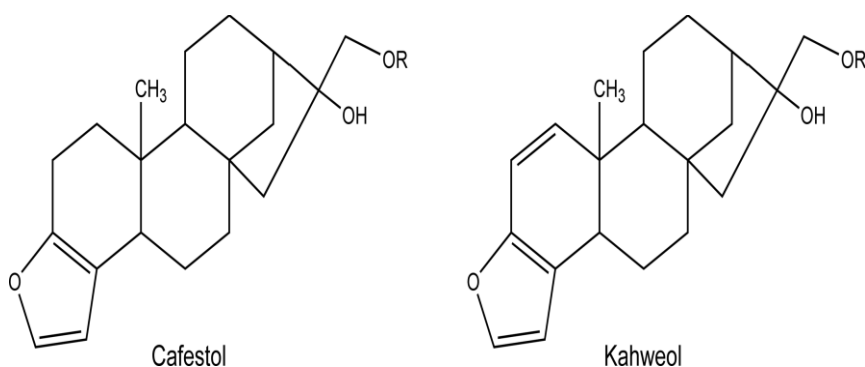
Figure 7: Structure of Cafestol and kahweol (Higdon & Frei 2006)

2.14.10 Effect of Cafestol and Kahweol

Cafestol and kahweol (see Figure 5 for structures) have been linked with many health benefits, more specifically anti-mutagenic and anti-carcinogenic properties (Cavin et al. 2001; Huber et al. 2002; Cavin et al. 2002; Kim et al. 2004). Cafestol and kahweol exhibit inhibitory effects on cytochrome P450 and induce the production of detoxifying enzyme, for example, glutathione-S-transferase and UDP-glucuronosyl transferase (Huber et al. 2004; Huber et al. 2008). They have also been linked with anti-inflammatory properties (Jung et al. 2010; Quesada & Medina 2011).

Cafestol has a characteristic furan group and pharmacologically displays both positive and negative effects (Hofman et al. 2004). It is hypothesized that the epoxidation of this furan group is responsible for the induction of many pathways (S T J Van Cruchten et al. 2010; Saskia T J Van Cruchten et al. 2010). Studies using isolated Cafestol have displayed anti-tumour and chemoprotective properties, primarily in the liver, through the inhibition of particular enzymes and their pathways (Hofman et al. 2004; Lee et al. 2007; Hwan et al. 2010; Choi et al. 2011).

Kahweol is very difficult to isolate from Cafestol and when isolated is highly unstable (Muriel & Arauz 2010) yet it is known to have some pharmacological activity on its own (Gyun et al. 2006; Quesada & Medina 2011). Studies have shown kahweol's ability to induce A549 cells (lung carcinoma) and leukemia U937 cell apoptosis exhibiting its anti-carcinogenic effects (Kim et al. 2009; Hwa et al. 2009).

Cafestol and kahweol are found in the highest concentrations in boiled, unfiltered coffee (Gross et al. 1997; Cavin et al. 2001). They are exceptionally difficult to isolate as kahweol is highly unstable when purified (Muriel & Arauz 2010). These diterpenes comprise of approximately 15% of the lipid content of the roasted bean (Naidoo et al. 2011). As a result of this, Cafestol and kahweol are both responsible for the increase in low density lipoprotein serum concentrations observed in those who regularly consume coffee (Yukawa et al. 2004; Bonita et al. 2007). Again, with the difficulty in isolating these compounds, conflicting outcomes from previous research and lack of human epidemiological studies result in difficulty disseminating accurate information (Bonita et al. 2007).

2.14.11 Cafestol and Kahweol Metabolism

Studies on participants who have undergone bowel surgery and have an ileostomy have shown that almost 70% of Cafestol and kahweol is absorbed in the intestine (De Roos et al. 1998; Higdon & Frei 2006; S T J Van Cruchten et al. 2010). Cholesterol 7α-hydroxylase, which is a bile acid homeostatic gene, is shown to specifically target and encourage the uptake of the cholesterol inducing Cafestol (Ricketts et al. 2007). Cafestol has the ability to down-regulate this gene, further causing hypercholesterolemia (Umemura et al. 1998; Ricketts et al. 2007).

It is important to note that the brewing style can also have an effect on the pharmacological activity of coffee. It is advised by most health organisations to switch to filtered coffee which can reduce low density lipoprotein cholesterol levels significantly reducing the likelihood of developing any secondary cardiovascular diseases (van Dam & Hu 2005; Higdon & Frei 2006).

2.15 Processes and Preparation of Coffee

The method of coffee production and preparation has a significant impact on the pharmacological activity of the end product. There are two methods of processing the coffee bean, dry and wet, and these are combined with grading, cleaning and polishing the bean (Ramalakshmi & Raghavan 2010). Roasting is the next process, where the bean is exposed to temperatures greater than 200 ºC which results in alterations to the size, shape and colour of the bean (Rubayiza & Meurens 2005;

Ramalakshmi & Raghavan 2010; Keidel et al. 2010). The roasting process results in pyrolysis, oxidation, reduction, hydrolysis, polymerisation and decarboxylation of the chemical components within the bean (Pugalendhi & Ramakrishnan 1990).

Cafestol, kahweol and other esters can be easily removed through the use of filter paper, thereby removing the cholesterol increasing factor associated with coffee consumption (Cheung et al. 2005). Unfiltered coffee, such as Scandinavian and Turkish press, contain higher concentrations of the coffee oils Cafestol and kahweol within the finished beverage (Majer et al. 2005). Although this advice is clear from the literature very little is available to the general public and so informed decisions cannot be made regarding coffee brewing and consumption.

According to the International Food Information Council Foundation (IFIC) one cup (8 oz / ~225 mls) of coffee contains 60 – 85 mg of caffeine and a 1 oz / ~28 ml serving of espresso contains 30 - 50 mg of caffeine (IFIC 2007). Although these are approximate figures, Crozier *et al* (2012) has shown that there are varying levels of caffeine present in many commercially available preparations (Crozier et al. 2012). This study investigated the caffeine content in 20 commercially prepared coffees through the use of high performance liquid chromatography (HPLC) and found that, in one serving, caffeine content could vary from 51 to 322 mg. This is a worrying discovery, as although women are advised to significantly reduce their caffeine intake during pregnancy, many do not reduce their consumption during the gestational period. This study also mentions chlorogenic acid to a much lesser extent and does not consider the other micronutrients present in coffee. It does, however, give an interesting snap shot of a potential problem.

Although Crozier *et al* (2012) did not consider other coffee constituents they did highlight interesting points on the impact of coffee preparation on caffeine concentration. They suggested that different batches of beans, roasting procedure, grinding conditions, temperature of water used in barista procedure, water in extraction vessel and, finally, coffee to water / steam ratio may all play an important role in caffeine concentration (Crozier et al. 2012).

2.16 Coffee and the Placental Barrier

Caffeine is primarily absorbed in the mucosa of the upper intestinal tract. Studies have shown that it can easily pass through the placental membranes allowing for fetal and umbilical plasma concentrations of caffeine to reach similar levels to that of maternal serum. This has been further verified; serum was sampled from neonates and confirmed that active levels of caffeine were present (James 2004; Kuczkowski 2009). Caffeine is said to have a half-life of approximately 2.5 to 4.5 hours, however this is prolonged in pregnancy (Bech et al. 2007)

Caffeine has been associated with deficiencies in reproduction as far back as 1977 with the suggestion that disruption of the cyclic AMP molecule by caffeine affects cell proliferation and hormone levels (Weathersbee & Lodge 1977; Martin & Bracken 1987; Alderete et al. 1995). Caffeine has been detected in rabbit blastocysts before implantation and in human embryos as young as 7 weeks old (Kline et al. 1991). The ability of caffeine to pass easily through tissues, as shown in this study, indicates that caffeine exposure to the ovum and zygote does occur. There are other studies which consider caffeine and conception and the general consensus is that women who do not smoke and consume moderate amounts of coffee do not suffer from infertility (Dlugosz & Bracken 1992; Alderete et al. 1995).

Caffeine is shown to cause chromosomal breaks in sperm cells *in vitro* and cause other complications in male reproductive gametes, including abnormalities in function, numbers, structure and motility (Kline et al. 1991; Olsen 1991). It is noted that caffeine is deposited in gonadal tissue and can also be detected in seminal fluid (Dlugosz & Bracken 1992; Ramlau-Hansen et al. 2008).

2.17 Coffee and Maternal Vasculature

Maternal vasculature undergoes much transformation during pregnancy including an increase in both cardiac output and blood volume, as well as certain pregnancy conditions increasing vessel sensitivity to constricting agents (Wareing, O'Hara, et al. 2005). Caffeine is a known vasoconstrictor and can potentially cause adverse events in reproduction as a result (Weathersbee & Lodge 1977; Ochiai et al. 2004). Mild to moderate caffeine exposure reversibly and competitively binds to the adenosine receptors. Higher levels of exposure are associated with the inhibition of

cyclic nucleotide phosphodiesterases and induce intracellular calcium release via ryanodine receptors (Momoi et al. 2008)

Although there have been positive links identified between coffee consumption and poor pregnancy outcome, confounding factors have very rarely been accounted for. Confounding factors that need to be considered include maternal alcohol consumption, smoking and drug use (Parazzini et al. 1998); there is therefore much scope for research. Caffeine metabolism becomes slower in pregnancy and ingested caffeine easily crosses the placenta (Picard et al. 2008). The half-life in the first trimester of pregnancy is similar to that of a non-pregnant state, but increases two fold during the second trimester and triples in the third (Klebanoff et al. 1998). Although it has been suggested that the risk of fetal toxicity associated with coffee and its metabolites is low, several studies have confirmed that moderate to heavy exposure may increase the risk of developing pathologies (Wendler et al. 2009; Santos et al. 2012). Caffeine elimination and metabolism can be influenced by tobacco use, prior exposure and inter-person variability (Brown et al. 1988; Swanson et al. 1994; Carrillo & Benitez 2000).

To my knowledge, there have been no studies conducted on the effect of coffee on maternal vasculature. Several studies have measured the paraxanthine levels in pregnant women caffeine metabolites that are not representative of the complete product. Serum homocysteine levels, which are associated with coffee consumption, are also associated with pregnancy pathologies, and will be discussed in greater detail below (Section 2.19).

2.18 Coffee consumption and increased serum Homocysteine concentration

There is a strong, dose dependent relationship between coffee consumption and total plasma homocysteine (Nygård et al. 1997). Homocysteine is a sulphur amino acid that is strongly linked pathologies, cardiovascular disease in particular (Olthof, Hollman, Zock, et al. 2001; Verhoef et al. 2002). It is non-protein forming and its metabolism is at the intersection of two metabolic pathways; methylation and transulfuration (Selhub 2008).

There has been much debate in the academic world on whether it is the caffeine or chlorogenic acid present in coffee that increases serum levels of homocysteine.

Pasman *et al* (2002) found that pure solutions of caffeine increased homocysteine levels however this was significantly reduced when paper-filtered coffee was used (Verhoef et al. 2002) suggesting that other factors are responsible for the concentration increase. Olthof *et al* (2001) suggest that chlorogenic acid, present in both tea and coffee, results in increased total plasma homocysteine levels (Olthof, Hollman, Zock, et al. 2001). The results, similarly to Pasman *et* al (2002), were inconclusive suggesting that chlorogenic acid was only partly responsible. There is a risk of a high coffee consumption resulting in hyperhomocysteinemia, which is defined as a fasting plasma homocysteine (Hyc) >100 µmol / L (Nygård et al. 1997). Fluctuations in homocysteine levels are common in normal physiological conditions however increased levels are usually associated with oxidant stress-induced cellular toxicity (Maron & Loscalzo 2009). Hyperhomocysteinemia is an independent risk factor for many pathologies including cardiovascular disease, cerebrovascular disease, osteoporotic fractures and dementia-like disorders (Motulsky 1996; Selhub 2008; Maron & Loscalzo 2009).

As well as the above mentioned list of potential pathologies, there have been links to obstetrical disease and poor pregnancy outcome associated with increased homocysteine plasma concentrations. Raised levels of homocysteine have been linked with trophoblast apoptosis and DNA degradation (Di Simone et al. 2003). However this study was *in vitro* and did not represent the actual processes that occur *in vivo*. It was, however, a good basis and raises many questions regarding the effect *in utero*. Folic acid supplementation is encouraged prior to and during pregnancy to reduce the risk of neural tube defects (Milunsky et al. 1989; Motulsky 1996; De Wals et al. 2007); this has been shown to impact serum homocysteine levels. Thus homocysteine plasma concentrations are also a good indicator of maternal folate status (Jacques et al. 1999). *In vitro* studies have shown that folic acid prevents trophoblast inhibition by increased homocysteine levels (Di Simone et al. 2004). Although this study was considered to be one of the first of its kind and presented a good model for future work it only measured the *in vitro,* short term effects of the compound on trophoblast activity and is not reflective of *in vivo* conditions where the tissue is exposed for longer periods. There is also the possibility that several other pathogenic mechanisms may be present as well as confounding factors associated with all pregnancy research.
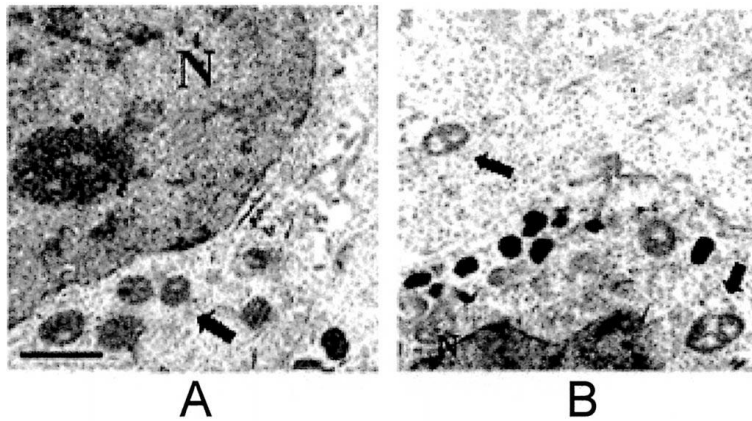
Figure 8: the above figure from Di Simone *et al* (2003) is an electron microscope image of homocysteine treated placental cells. Image A illustrates untreated trophoblast cells with the arrow pointing to mitochondria with normal structural integrity. Image B illustrates cells that have been treated with 20µmol / L homocysteine with the arrow pointing to mitochondria that are swollen and vacuolated (Di Simone et al. 2003)
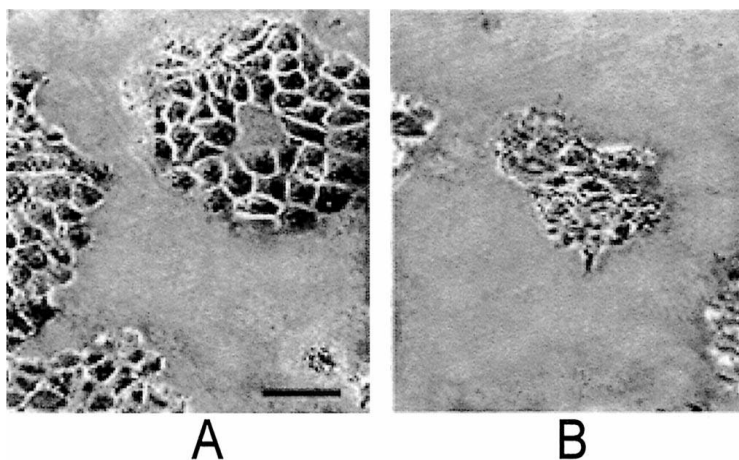
Figure 9: the above figure from Di Simone *et al* (2003) illustrates the altered morphology of trophoblast cells treated with 20µmol / L homocysteine for 48 hours (Image B) in comparison to untreated trophoblast cells (Image A) (Di Simone et al. 2003).

2.19 Summary

Coffee is a complex mixture of many pharmacologically active substances. It has been extensively researched in murine models and in human, non-pregnant states.

The physiological effects vary greatly and are difficult to investigate in any population due to the wide range of confounding factors. The effect of active coffee chemicals on vasculature has been limited to caffeine specifically and, again, the evidence is conflicting.

The fetoplacental and uteroplacental vasculature are fundamental to a positive pregnancy outcome. From the information provided above, it is clear that placental development, angiogenesis and nutrient transport are complex processes. It is therefore rational to conclude that placental vascular compromise is often associated with poor pregnancy outcome. Whether caffeine and / or coffee metabolites impact the placental vasculature is still under debate. Also, whether the effects noted in non-pregnant or non-placental blood vessels are transferable to the chorionic plate arteries specifically is unknown; this could only be determined through extensive experimentation but may be important with regards to development and functionality of these resistance vessels.

Although pregnancy is a physiological state, it is not a benign condition. Pregnancy is defined by the medical world as a condition associated with high morbidity levels and thus continuous research is pertinent to improving outcome. Coffee has been associated with many pregnancy pathologies however a definitive link has yet to be made. These studies, which do consider these pathologies are often plagued by limitations, such as, recall bias and inability to measure coffee metabolites, as well as failure to take into account relevant confounding factors.

Following an extensive literature search it was clear that there was a definite gap in the knowledge with regard to coffee consumption during the gestational period. Coffee, as a whole, was very rarely investigated however it metabolite caffeine was regularly considered. The majority of studies have failed to consider the cumulative effects of the active coffee chemicals on pregnancy outcome.

As a result of these uncertainties I feel the area of coffee consumption and pregnancy is still an area for much research. More specifically, I wished to investigate the effect of active coffee chemicals on maternal and placental vasculature. Using wire myography as an *in vitro* assessment of vascular reactivity I aimed to:

1) Determine the effects of active coffee chemicals caffeic acid, chlorogenic acid and caffeine on placental chorionic plate artery function;

2) Determine the effects of active coffee chemicals caffeic acid, chlorogenic acid and caffeine on maternal (myometrial) arteries and finally;

3) Compare / contrast placental with maternal effects of these active coffee chemicals.

By extracting the most abundant substances within the complete coffee product and investigating their effects on the fetoplacental and maternal / systemic vasculature I aimed to determine whether there was evidence to suggest a definitive risk associated with coffee consumption during gestation. These types of investigations into vascular tone would be the most representative of function as compromised vascular tone *in vitro* is associated with pregnancy pathologies and maternal morbidity.

Chapter 3 Qualitative Methodology

3.1 Brief Introduction

In this chapter I will discuss the research methodology which has been used to inform the methods of this study. This study adopted a pragmatic method of recording and organizing human expertise and experiences. It was important to choose an appropriate methodology and the appropriate methods to be used for investigating the problems and constructing the theories in that domain (Weaver & Olson 2006).

Research has been defined as a systematic investigation or inquiry where data is collected, analysed and interpreted in an effort to 'understand, describe, predict or control an educational or psychological phenomenon' (Burns 1997; Mertens 2005; Mackenzie & Knipe 2006). Although there are many differing routes to knowledge it is important to ensure that these paths build a systematic body of evidence for, in our research, clinical practice. Ultimately, our aim is that this research should lead to practical clinical knowledge (Kennedy & Lowe 2001). There is a broad spectrum of approaches to knowledge development that use both qualitative and quantitative methods to gather and analyse data (Creswell et al. 2006). I aim to briefly discuss these and rationalise my decision to adopt a pragmatic paradigm and mixed-methods approach to data collection.

3.2 Qualitative Methodology

Qualitative inquiry was used to explore womens' and midwives' views and opinions on coffee consumption during pregnancy. I adopted a pragmatic paradigm as the methodological approach. Qualitative research can be said to be rooted in philosophical assumptions of interpretive and naturalistic enquiry. In contrast quantitative research focuses on hypothesis testing, numerical data and deductive analysis (Verhoef & Casebeer 1997; Mertens 2005; Hanson 2008). Mixed-methods, a combination of both qualitative and quantitative methods, is a growing area of methodological choice for many in the world of scientific and social research (Bryman 2006; Kelle 2008; Cameron 2011). This will be described in further detail below.

3.3 Theoretical background and theoretical framework

There is much worth in a theoretical framework; it is essential in guiding the design and implementation of mixed-methods research. The qualitative researcher must have a clear idea about their theoretical perspective in order to begin their study; this process of designing a theoretical frame work is developmental and experiential (Lavender et al. 2004; Sinclair 2007).

The term mixed-methods is a relatively new one and there are much uncertainties surrounding the definitions, language, nomenclature and typologies (Evans et al. 2012). The most accepted definition is that mixed-methods research combines qualitative and quantitative research in viewpoints, data collection, analysis and conjecture. Recent research has described mixed-methods as a 'third methodological movement' that is composed of distinctive combination of practices from both qualitative and quantitative methods (Greene 2006; Doyle et al. 2009; Evans et al. 2012).

Morgan (2007) summed up the need for a theoretical framework in order 'to connect issues in epistemology with issues in research design, rather than separating our thoughts about the nature of knowledge from our efforts to produce it' (Morgan 2007). Frameworks provide an orderly, efficient scheme for bringing together observations and facts from separate investigations. Summarizing and linking findings into an accessible, coherent structure and a further understanding of phenomena (what and why of occurrence) that allow for prediction are also possible if a structured framework is adopted (Polit & Beck 2004). The theoretical framework allows for clear navigation within a study consisting of concurrent or sequential investigations, similar to this study.

There is continuing emphasis being put on the need to develop more efficient ways of implementing mixed-methods research; theoretical frameworks are a potential answer to this. There is a need for logical guidance through this fundamentally new style of research. Theoretical frameworks can provide navigational tools through the practices in studies involving complex human behaviours that require multiple, relevant complementary perspectives and methods of investigations (Greene 2006; Armitage 2007; Evans et al. 2012).

3.4 Paradigm/ Pragmatism/ Pragmatic paradigm

Paradigms are systems of beliefs and practices that regulate inquiry within a discipline; they enable the development of a framework and, in turn, the processes through which investigations are accomplished (Weaver & Olson 2006). Paradigms have often been described as 'disciplinary matrices', 'research traditions' and 'world views' (Guba & Lincon 2000; Morgan 2007; Walker & Bucher 2009). Kuhn (1970 / 2012) first coined the term 'paradigm' to describe a heuristic framework for examining the natural sciences and disciplinary matrix for the social sciences (Kuhn 2012). Another term that is often used when discussing qualitative methodologies is "research tradition'; this term, research tradition, was used to describe a set of general assumptions about the entities and processes within a study (Laudan 1970). Although the term paradigm is considered to be ambiguous and inconsistent (Allen et al. 1986) it is appropriate for midwifery and nursing research and thus was suitable for my study.

Paradigms are essential for interpreting significant fundamental issues within healthcare. They are a set of philosophical foundations for any study and include the specific research approaches and how these should flow (Allen et al. 1986; Denscombe 2008). The paradigms that are used in health related research include positivist, post-positivist, interpretive and critical social theory.

A positivist paradigm is appropriate for quantitative research. It is often referred to as the scientific method or science research and is considered to be based on the 'rationalistic, empiricist philosophy' the originated with Aristotle, Frances Bacon and some other noteworthy names through history (Mertens 2005; Mackenzie & Knipe 2006). There is a reliance on controlled experimentation and there is only one correct interpretation of the results (Spratt et al. 2004). Events can be explained in terms of cause and effect however there is a lack of regard for the subjective states of the individual (Dykes & Williams 1999; Guba & Lincon 2000). Although there is value in the quantification of data, people and the complexity of social interactions cannot be reduced to clearly defined variables and it is often impossible to produce matched groups of people (Spratt et al. 2004; Creswell et al. 2006).

Positivism was replaced after World War II by post-positivism, which works under the assumption that research is influenced by a several well-developed theories,

alongside the theory being investigated (Cook & Campbell 1979). In simpler terms, what could be interpreted as 'truth' for one individual or cultural group may not necessarily be 'truth' for another. This is a more holistic approach than positivism yet is not fully applicable to all research (O'Leary 2004; Mackenzie & Knipe 2006). Post-positivism research aims to reach the full understanding based on experimentation and observation and its concepts and knowledge are held to be the product of straightforward experiences, interpreted through rational deduction (Ryan 2006). This was a more robust paradigm, underpinning contemporary empirical research activity (Ford-Gilboe 1995; Clark 1998). Post-positivist research does not exclude qualitative data or 'truths' found outside the quantitative method, contrary to the positivist approach. Its acceptance of this is crucial to rejecting the strict dichotomy between qualitative and quantitative paradigms (Khun 1962; Clark 1998).

A constructivist or interpretivist paradigm focuses on the dynamics of interactions with the emphasis on the world as a socially constructed reality that involves multiple perspectives (Gage 1989). This paradigm was developed from the philosophy of Edmund Husserl's phenomenology and other German philosophers study of interpretive understandings called 'hermeneutics' (Gage 1989; Mertens 2005; Mackenzie & Knipe 2006). The interpretivist researcher acknowledges the impact that both the participants and their own background and experiences has on the research (Creswell 2003). The perceptions and values of each participant is considered in order to explore the various possible interpretations (Spratt et al. 2004). As well as this, the interpretivist or constructivist researcher does not begin with a theory but generates or inductively develops a theory throughout the research process (Creswell 2003; Mackenzie & Knipe 2006). Although this paradigm enriches the understanding of social situations it does not necessarily focus on the areas which may need change and often does not attempt to find appropriate ways to improve situations or rectify social issues, which may be the objective of the research (Spratt et al. 2004; Creswell et al. 2006).

It was decided that these paradigms would be rejected in favour of the pragmatic paradigm and mixed-methods approach to data collection. Studies have explored the rationale behind choosing a mixed-methods approach (Bryman 1988) considering the role of theory in relation to the research, the epistemological orientation and the ontological orientation. The pragmatic paradigm is distinct from the positivist

perspective of quantitative research and the constructivist perspective of qualitative research (Tashakkori & Teddlie 2002). Pragmatism, when regarded as an alternative paradigm, sidesteps the contentious issues of truth and reality and accepts that there are singular and multiple realities that are open to inquiry so as to address the practical problems of the real world (Creswell 2003; Creswell et al. 2006; Morgan 2007; Yvonne Feilzer & Feilzer 2009). It can be said that pragmatism frees the researcher of the mental and practical constraints imposed by positivism, postpositivism and constructivism (Creswell & Clarke 2007; Creswell & Hanson 2007).

At a level of translating epistemological and ontological concerns into research methodology and finally the decision of the methods, a pragmatic paradigm poses some methodological questions. Mixed-methods research offers to plug this gap by using quantitative methods to measure some aspects of the phenomenon in question and qualitative methods for others (Johnson & Onwuegbuzie 2004; Yvonne Feilzer & Feilzer 2009).

In social science no single paradigm dominates and thus competing paradigms co-exist. It is also important to note that the research paradigm and the methodology work together to form a research study. Many researchers now view qualitative and quantitative methods as complimentary, and thus choose the most appropriate methods based on the research question. Paradigms which explicitly recommend a mixed method approach allow the question to determine the data collection and analysis method which will be applied thus integrating the data at different stages of inquiry (Creswell 2003). In my study I adopted a mixed-methods approach, the methods, advantages and disadvantages of which will be discussed in further detail in Chapter 10.

Pragmatism offers an alternative worldview to those of positivism, post positivism and constructionism and focuses on the problem to be researched and the consequences of the research (Brewer & Hunter 1989; Miller 2006). Using a pragmatic paradigm allowed me to investigate particular areas of interest using the methods which, as a research team, we felt were appropriate without compromising our value system (Armitage 2007).

An eclectic mixed-methods approach or pragmatic paradigm was chosen for the current study. Pragmatism is not committed to any one system of philosophy or reality (Mackenzie & Knipe 2006). This approach is more capable of handling the complexity of modern society and technology. It focuses on the practical issues that arise rather than the realities and theories of society whilst acknowledging the weakness of current evaluation tools (Gage 1989; Guba & Lincon 2000; Spratt et al. 2004). It is more concerned with the 'what' and 'how' of the research problem (Creswell 2003). Pragmatism is seen as the paradigm that provides the underlying philosophical framework for mixed-methods research (Tashakkori & Teddlie 2002) even though some researchers believe that mixed-methods can be used with any paradigm. There is no 'correct' approach within the pragmatic paradigm and the researcher is encouraged to maintain an open mind with regard their approach (Spratt et al. 2004).

Pragmatism (often referred to as the alternative paradigm) was developed as an alternative to the dominant paradigms, with the aim of resolving any anomalies in the existing system (Morgan 2007). The pragmatic paradigm seeks to balance and improve communications between researchers who adopt different paradigms yet strive to advance knowledge (Maxcy 2003). The pragmatic paradigm also helps to shed light on how, through combining research approaches, we are offered the best opportunity for answering important research questions (Michell 2003; Hoshmand 2003; Johnson & Onwuegbuzie 2004).

The decision to use a pragmatic paradigm for my study was based on the general objectives of the research, the research question and, finally, the skill and preference of the researcher (Spratt et al. 2004). Considering the paradigm, as discussed above, is essential in making decisions within research. It is important to note that it is the paradigm and research question determine the methods by which research data is collected and analysed. Therefore, with the pragmatic paradigm, both qualitative and / or quantitative methods may be employed and these methods will be matched to the specific questions and the overall purpose of the research (Mertens 2005; Mackenzie & Knipe 2006). As discussed above the pragmatic paradigm is often utilised with studies interested in common observations or research which attempt to investigate common assumptions and practices amongst the general public or in social science (Spratt et al. 2004). The pragmatic paradigm can be applied to thinking, practical

experiences and experimentation (Johnson & Onwuegbuzie 2004) which made it a useful tool for my study.

Pragmatism has often been reviewed as a general belief system (Maxcy 2003) and as a justification for combining qualitative and quantitative methods (Johnson & Onwuegbuzie 2004). These reviews suggest several ways which pragmatism provides new options for addressing methodological issues in social science (Morgan 2007). Some other justifications for adopting this approach included; the ability to fill in the gaps left when using one dominant approach, the use of qualitative research to facilitate and further justify quantitative research and vice versa, gaining the perspective of the researcher and the participant, to address the issue of generality and finally to study different aspects of phenomena (Hardy & Bryman 2004; Bryman 2006; Armitage 2007). It was for these reasons that it was decided to adopt the pragmatic paradigm and mixed-methods approach for my study.

The pragmatic paradigm does not inhibit the researcher from investigating the emerging issues, but actively encourages them to include the findings in the overall results. The advantages of the pragmatic paradigm make it an ideal approach for this study. The combination of qualitative and quantitative research gives a more detailed results section, a thorough examination of coffee and its physiological and social impact. It allows for a holistic study to be conducted whereby results and recommendations can be easily disseminated and put into practice. This follows the key ideas of the pragmatic paradigm, where an intuitive appeal, permission to study areas that are of interest and embracing methods that are appropriate lead to obtaining insightful results which also agree with the researchers belief system (Tashakkori & Teddlie 2002; Creswell 2003; Armitage 2007). A mixed-methods approach enables the researcher to answer questions that other approaches cannot. A mixed-methods approach can answer questions simultaneously, both confirmatory and exploratory questions. It also provides stronger inferences through depth and breadth when answering complex social phenomena and allows for differing viewpoints through divergent findings (Armitage 2007). Although a mixed-methods research approach or pragmatic paradigm does not provide a perfect solution, it does however, at this time, attempt to fit together the insights provided by qualitative and quantitative research, and the positivist and constructivist paradigms, into a workable solution (Johnson & Onwuegbuzie 2004).

Pragmatism does not, however, excuse for negligent research. A pragmatist should never be confused with expedient but requires a good understanding of quantitative and qualitative methods and analyses, which is transparent and replicable (Denscombe 2008; Yvonne Feilzer & Feilzer 2009). The combination of qualitative and quantitative methods, that is mixed-methods, should form what constitutes good quality social research (Hammersley 2008; Yvonne Feilzer & Feilzer 2009). Pragmatism offers the researcher the chance to combine these approaches and produce properly integrated methodology for the social sciences in acknowledging the value of both quantitative and qualitative research methods and the knowledge produced by such research in further understanding of society and social life (Morgan 2007; Morgan & Winship 2007; Yvonne Feilzer & Feilzer 2009). Pragmatism, in its simplest form, is a practical approach to problem solving and has strong associations with mixed-methods research. It is often considered to be the bridge between paradigm and methodology (Greene & Caracelli 1997; Cameron 2011). Patton (2002) stated the pragmatic paradigm sensitises the researcher and evaluators to methodological biases that are often associated in research which adopts a dominant paradigm (Patton 2001). He suggests that pragmatism promotes methodological appropriateness which encourages the research to be flexible and adaptable (Patton 2001).

However, bearing this in mind adopting pragmatism is a commitment to uncertainty. The researcher must acknowledge that any knowledge produced through research is relative and not absolute; even if there are causal relationships these are transitory and difficult to specify and identify (Tashakkori & Teddlie 2002; Yvonne Feilzer & Feilzer 2009). The researcher is not being sceptical but merely appreciating that relationships, structures and events are open to shifts and changes depending on precarious and unpredictable occurrences (Mounce 1997). By acknowledging this, as a pragmatic researcher, it is necessary to be more flexible and open to the emergence of unexpected data; it is the researchers 'duty' to remain curious and adaptable (Khun 1962; Pope & Mays 1995; Yvonne Feilzer & Feilzer 2009; Fishman 1991).

It is for these reasons, along with the advantages listed above, that the use of a pragmatic paradigm would be the appropriate and most beneficial paradigm to base my study on. The pragmatic paradigm and mixed-methods research has much to offer, especially in a clinical setting. It ultimately brushes aside the quantitative /

qualitative divide and ends the paradigm war by suggesting that the most important question is whether the research has helped to answer our question (O'Leary 2004; Hanson 2008). It focuses on problem solving and action orientated inquiry along with encouraging the researcher to ask better and more precise questions (Cameron 2011). As a pragmatist, the emphasis is not on the methods but on whether the methods chosen have the potential to answer particular questions and generate useful data.

3.5 Qualitative Versus Quantitative

Qualitative methodology is inductively driven, implying that observations are made and the researcher seeks the perspectives of participants and uses these perspectives to gather data on a certain population, obtain a greater understanding of social settings, or develop a hypothesis on why something occurs the way it does. Denzin and Lincon (1994) described qualitative research as 'the study of things in their natural settings, attempting to make sense of, or interpret , phenomena in terms of the meanings people bring to them' (Denzin & Lincoln 2005). Qualitative research focuses on the 'how' and 'why' rather than the 'how much'. The data obtained can come in many formats; in the case of my study I obtained rich verbal descriptions and interpretations from semi-structured interviews. Although ideal for social research, qualitative research often lacks the large numbers associated with quantitative research. As the researcher is heavily involved in the, often time consuming, research it could possibly lead to bias and skewed data. This was considered and minimised by ensuring reflexivity and rigour, which will be discussed in further detail in Chapter 4, Section 4.3.

Quantitative research methodology is driven by deductive reasoning implying that the generated hypothesis will be tested so that the evidence supports the theory (Kennedy & Lowe 2001). This method follows the evidence-based practice paradigm since the variables are measured using strict criteria which are usually standardized through the development of a protocol. The evidence gathered can either show whether an intervention has had an effect or outcome, has generated a further hypothesis or disregarded a hypothesis. This is not always a practical method of data generation in a clinical setting as there are ethical aspects which need to be taken into consideration, for instance whether potential treatments should be withheld or

whether participants should be exposed to potentially harmful substances (Gostin 1991; Kennedy & Lowe 2001).

Quantitative research also has its limitations. This method of obtaining knowledge within traditional scientific and positivist approaches is characterised by reductionism and quantifiability (Watson 1981) and is linked to the natural and physical sciences (Holloway 2001). Although this rationalistic methodology is appropriate for research with the medical model, it is often unsuitable and cannot be applied to research in nursing and midwifery (Verhoef & Casebeer 1997; Holloway 2001; Creswell 2003; Michell 2003). Quantitative research does not focus on personal experience and personal knowledge but is based on the assumption that all knowledge is objective, factual and acontextual. In social research this is untrue; knowledge is interpretive, socially constructed and context-dependent (Bryman 1988; Holloway 2001; Creswell et al. 2006). It was essential in my study that I was aware that if a singular quantitative approach was adopted we would miss the rich data which would assist our understanding of the way people interpret and give meaning to 'what happens them and which enables them to justify their actions' (Holloway 2001).

3.6 Mixed-methods research

There are many advantages to conducting mixed-methods research; it conveys a sense of rigour and by combining the two distinct methodologies it compensates for their mutual and overlapping weaknesses (Johnson & Turner 2003; Bryman 2006; Kelle 2008). In essence, mixed-methods designs provide important tools to overcome limitations of both quantitative and qualitative 'mono-method' research (Kelle 2008).

Mixed-methods research is now considered to be a more appealing method of data collection. It moves beyond the arguments of qualitative versus quantitative and recognises that both methodologies are important and useful (Johnson & Onwuegbuzie 2004). The aim of mixed-methods research is not to replace either approach but rather to use the strengths and eliminate the weaknesses of each in order to develop a more rigorous method of evidence generation and data analysis (Greene & Caracelli 1997; Johnson & Onwuegbuzie 2004). This method of research is a more practical approach as it is more applicable to clinical practice. Over the past

20 years mixed-methods research has grown in popularity, so much so that there have been journals developed which are specifically devoted to mapping mixed-methods research studies and the extent to which they integrate different methodologies (Tashakkori & Teddlie 2002; Yvonne Feilzer & Feilzer 2009).

Mixed-methods studies need to be reported in a clear and transparent fashion, emphasising explicitly the research design decision making and clearly outlining the rationale behind them (Bliss et al. 2003; Spratt et al. 2004). This is also applicable in the decision to adopt a framework approach of analysis (discussed in further detail below) (Chapter 4, Section 4.5). Similar to the systematic review, maintaining transparency is essential so that others can easily mimic the process and methods utilized in the study (Furber 2010).

Mixed-methods or multi-strategy research, as it is often referred to, attempts to merge methods from differing paradigms. Rocco *et al* (2003) suggest that some of the most useful research involves mixing methods and paradigms (Bliss et al. 2003). Within this paper the authors suggest that research paradigms, design and analysis strategies ought to be appropriate to the research question at a technical, philosophical and political level (Bliss et al. 2003). It has also been suggested that it is possible for any and all paradigms to employ a mixed-methods approach rather than being restricted to a singular method thus potentially diminishing the limitations on the depths and richness of the research.

There are many different approaches to mixed-methods. Creswell (2009) suggest that a researcher question all aspects of their investigation before deciding on a method (Creswell 2003). A useful review in Creswell (2003) summarizes and classifies some mixed-methods approaches briefly (Creswell 2003). According to Creswell there are six mixed-methods approaches; sequential explanatory design, sequential exploratory design, sequential transformative design, concurrent triangulation, concurrent nested and finally concurrent transformative (Creswell 2003). Although this is the case I utilised a parallel mixed methods approach. Parallel mixed methods design has been defined as a methods that allows for qualitative and quantitative data to be collected at the same time; one does not dominate over the other (Graff 2014). Creswell recommended the 6 methodologies and I drew on aspects of all; combining the qualitative and quantitative components post analysis provides a more holistic

approach. The priority was equal between the qualitative and quantitative components. The amalgamation was conducted once the analysis and interpretation phases were complete. This method was the most suitable as my research involves the combination of in-depth interviews along with the laboratory investigation of the effects of active coffee chemicals on maternal and placental vasculature.

The analysis of the qualitative data was conducted separately at first. I then moved back and forth between each data set with the knowledge being produced by each step and then finally grouped together which enabled the interpretation of the data from a multidimensional perspective; each data set was informed, questioned and enhanced by the others. The process by which I analysed the raw data will be discussed in greater detail in the methods section.

3.7 Ethics

Health care ethics is defined as the moral conduct and principles that govern members of the health care profession. According to Hope *et al* (2003), medical ethics is dominated by consequestialism and deontology (Tanner et al. 2008; Szeremeta et al. 2001) which considers the consequences of the researchers study as well as their duties and actions within the investigation.

'Good' science is defined as adopting a comprehensive approach to ethical requirements, going above and beyond what is expected to ensure that, as researchers, we are ethically responsible. Ethical guidelines have been structured and reviewed by many international bodies and governments. The Declaration of Helsinki outlines principles proposed by the World Medical Association initially in 1964 and progressively through to 2000. These principals allow the researcher to deal with ethical issues that arise in medical research as well as ensuring the researcher is ethically responsible (Touitou et al. 2004) .

The International Ethical Guidelines for Biomedical Research Involving Human Subjects provides good recommendations on the principles which should be core to any research. For every study a set of foundation principles must be adhered to as well as developing specific principles to conquer any unique issues that may arise.

A principle can be defined as any rule or belief system which governs a researcher's behaviour and actions. Similarly to ethics, it guides an investigator to achieve a morally correct attitude in the hope of achieving sound results whilst causing minimal disruption to the participants way of life or daily activities (Touitou et al. 2004).

Some ethical issues can be anticipated and thus I designed my study accordingly; however 'everyday dilemmas' due to their unpredictability were dealt with situationally (Fluehr-Lobban 1998). Whilst it is possible to construct a set of ethical principles, it is important to acknowledge that the application of these rules and which actions constitute following these rules, may not be particularly clear in the complexity of the field setting (Maurice 1986; Burgess 1989). However, in general, the foundation principles (see below) which were applied to my study were easily applicable and should were consistently maintained.

Foundation principles include; (a) protecting the health and well-being of the individual participant as well as the population, (b) the right of populations to self-determination including the right to refuse participation in the research, (c) protection of vulnerable individuals and populations, (d) protecting the individual's right to privacy and confidentiality and finally (e) the fair distribution of benefit and burdens of research. Some of the specific considerations related to my research would include the impact that the interview and recruitment process has on pregnant womens' dietary decisions and concerns. The interviewing process could also influence midwives teaching practices and advice provision.

The Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organisation (WHO) has set out specific ethical principles for research involving pregnant women (CIOMS & WHO 2002). This states that pregnant women can be presumed eligible for participation in research providing they are adequately informed about the potential benefits and risks to themselves, their unborn child and their fertility. These guidelines provide a good base for any research as they place a significant amount of emphasis on the core principles, such as beneficence and informed consent. They recognise that, whilst working with pregnant women, the researcher must be aware that the participant is not only making a decision for herself but for the fetus and this can be further

complicated by the need to involve the father into the decision making process or the potential for unknown or ambiguous risks to be present (Regan et al. 1989).

The favourable benefit to harm ratio is considered when applying the principal of beneficence (Gostin 1991). This model / paradigm states that the potential benefit to the participant must outweigh the potential harm. The benefit must be well understood, the risks must be reasonable and the adverse effects must be carefully monitored and controlled. Beneficence is the core principle in any study involving human participants. We must take into consideration not only the physical health of our participants but the implications that our study has on the specific group or greater population. Ethical research always places the desires and needs of the participant over those of the researcher (Maurice 1986; Hope et al. 2003).

The research should be relevant to public health issues. For my research study, examining the views and opinions of women and midwives' towards coffee consumption is an emerging social concern for the developing world. This would be irrelevant in developing countries where a more pressing concern may be maternal and fetal health. CIOMS (2002) also states that, when conduction research with pregnant women as the specific population, there must be a great public health need and when necessary and if possible animal experimentation should be conducted (CIOMS & WHO 2002). This is fundamental in the case when considering teratogenic substances and there is still little known about caffeine (or other active chemical's contained in coffee) and its potential mutagenic possibilities.

Sometimes the researcher's lack of experience can impact their ability to obtain fully informed consent from a potential participant in a study. The inadequate experience of the investigator can undermine the ability of the participant to withhold or withdraw their consent (Gostin 1991; Patel 2003; Lepping 2007). If the researcher does not disclose all potential risks to the participant then fully informed consent cannot be achieved. Practical realities and obstacles, such as poor communication, can impact the ability of the participant to make an informed decision. Language barriers and illiteracy can also impede the participant's ability to give informed consent and thus a great focus is put on the ensuring that the principle of informed consent is strictly adhered to. By providing the information in a comprehensible and consistent manner though we can overcome these issues and ensure that informed

consent is achieved. Limitations, such as these mentioned above, will be discussed in greater detail in Chapter 10, Section 10.2.

Respect for a person as an individual is a key principle within our study. One of the primary ways to respect an individual is to accept and abide by their choices whether or not the researcher or others believe them or feel that they are wise or beneficial (Gostin 1991). Stemming from this ethical principle is the participants right to privacy and confidentiality. Maintaining privacy and confidentiality, although not always easy, is vital within any research. Any sensitive information disclosed by the participant must be treated accordingly and participants have the right to choose who has access to this information and under what circumstances.

It is important to note that research participants can often come to think of investigators as clinicians and thus they anticipate that there will be strict confidence between both parties. It can therefore be stated that the researcher makes a promise to the participant, whether implicitly or explicitly, to keep any information or observations strictly confidential (Gostin 1991; Hope et al. 2003; Touitou et al. 2004). This, however, is not always a possibility. It is often necessary for the researcher to disseminate their findings to other members of the research team or to the wider scientific community. In the case of our study, one of the aims was to gain an insight into views, opinions and attitudes of our participants and therefore necessary to directly quote those who have taken part. We overcame this by ensuring that the participant was fully aware that the information they had provided would be disclosed and to whom (Smith 1999; Lepping 2007). Within my study, all participants were given a pseudonym and all details were anonymized. This will be discussed in further detail (Chapter 4, Section 4.2).

A final ethical principle which we apply to our research is that of justice. It is a less well defined principle which essentially states that the benefits and burdens of research should be evenly distributed (Gostin 1991). Investigators have a duty to not only prevent harm to their participants but to protect their health and well-being by providing, within reason, prevention and health services (Gostin 1991; Smith 1999; Hope et al. 2003). In our study, as the chief investigator is not a clinician it is essential that should an issue arise that requires medical attention then the researcher is obligated to contact a member of the health profession / member of the

participant's clinical care team. It is true to say that it is the researchers' ethical responsibility to safeguard their participants' health and welfare. Equally, if there is any benefit or new knowledge that could potentially benefit the health of the specific population or future populations it is the researchers' duty to distribute this information accordingly.

Ethical approval for my study was gained in February 2012. This approval was obtained from the NRES Committee North West- Greater Manchester West; REC Reference 12 / NW / 0079, please see Appendix 1 for copies of documentation and forms.

Chapter 4 Qualitative Methods

4.1 Aims and Objectives

The aims of this study were

1. To investigate womens' views, attitudes and opinions on coffee consumption during pregnancy.
2. To investigate midwives' views, opinions and knowledge on coffee consumption during pregnancy.

4.2 Methods

I decided that using a semi-structured interview technique would be the best method for gaining information. There were several reasons as to why the use of a semi-structured interview technique was the most appropriate. Firstly, I had a very specific research question and this required a method of data collection that had some focus, however also enabled free responses from participants. Use of semi-structured interviews is ideal for the novice researcher; it guides the researcher along the right path until they become accustomed and comfortable with using this method of research. There is also scope to add questions and follow a line of enquiry in more depth, should the need arise. A script was developed to aid this; this included prompts, to ease the flow of conversation, and to ensure that all questions were asked. Literature searches, as well as discussions with expert researchers, helped to inform the script (Appendix 4).

Interviews were held in a soundproof, private room. Interviews were digitally recorded, with the fully informed consent of the participant. During the transcription phase each participant was given a pseudonym to permit use of direct anonymized quotes to be used to support assertions made following framework analysis of the interviews.

Semi-structured interviews with midwifery professionals and pregnant women allowed me to obtain rich, qualitative data. From initial examination of the data, by my supervisory team and I, it was decided that a framework approach of analysis would be used; this is consistent with the pragmatic approach, enabling the research

question to inform analysis. This consisted of transcribing the interviews and identifying the key themes. These themes could then be indexed and charted, essentially merging the data and identifying the emerging themes from all interviews, which will be discussed in greater depth further (Chapter 6). Interpretations would have to be made from some of the interview data, and mood of participant and environment would also be noted. With qualitative research a question, rather than a hypothesis, is considered; this is investigated with the semi-structured interviews. What the general views, opinions and attitudes of pregnant women and health care professionals to coffee consumption during pregnancy was assessed. There were no right or wrong answers and after some initial interviews it was noted that many participants did not have a developed opinion on coffee consumption due to the lack of information present on its benefits and risks. As a result participants were more concerned about information provision and education and coffee's presence in the media.

4.2.1 Ethical Approval

Ethical approval was obtained in February / March 2012. This process involved developing Standard Operating Procedures and Protocols, along with completing the required National Research Ethics Service (NRES) / Integrated Research Application System (IRAS) documentation. Once these were submitted, NRES Committee North West- Greater Manchester West reviewed the documentation and set conditions to obtain a favourable opinion. Following this, I was invited to attend a meeting to discuss any further issues with the forms, process by which I would be obtaining consent, recruitment, or conducting the study. All issues were clarified, necessary amendments were made and once a favourable opinion was obtained the recruitment process began.

4.2.2 Sample Selection and Recruitment

The target population included pregnant women and midwives. Women recruited were receiving their antenatal care in a large NHS Trust in North West England, tertiary referral hospital in the North West of England (Care Quality Commission (CQC) Maternity Services Survey 2013). This was an ideal setting for my research for many reasons. Firstly, as the hospital is a teaching and research hospital it was

ideal for approaching midwives from all experience levels. This benefited the study greatly as midwives were able to contribute to the formation of the study design as well as taking part in the study. It was also a convenient choice; being a large referral hospital meant that it was ideal for approaching many women, from many different geographical and socioeconomic areas. Women were approached regardless of their gestation, age and ethnicity. Women were not approached if there was a known fetal abnormality, were having a multiple pregnancy or were under the age of 18.

In order to ensure that the potential recruit was suitable for the study, the midwife in charge was approached and asked about the woman's suitability. The health care professional indicated which women would be appropriate for the study and these were approached accordingly. Women were approached about the study in the antenatal clinic whilst they were waiting for their appointments. A brief description of the study was given and verbal informed consent was obtained from the woman. Initial consent forms were signed; consent to contact and general consent forms. Information sheets were given and the women were given a chance to ask any questions. A time and date for the telephone interview was then organised.

Midwives were approached in their working environment, and similarly a time and date to conduct the face-to-face interview was organised. All midwives were considered; these ranged in years of experience and area of expertise.

4.2.3 Interviews

A total of 41 interviews (21 midwives and 20 womens' interviews) were conducted ranging from 5 to 25 minutes in length. There were many factors impacting the varied range, which will be discussed in further detail (Chapter 6 and Chapter 10). Interviews were digitally recorded and transcribed verbatim. Data saturation was achieved; this was highlighted by the fact that no new knowledge was being brought forward from the interviews.

Telephone interviews were conducted with the women. This was the most convenient method for the women and the researcher. The wide geographical area covered and length of time between antenatal appointments would have limited the ability to conduct face-to-face interview with women. Women were much more responsive to the idea of telephone interview as it could conveniently be done at a

time and place that suited them. Women were called at a date and time previously specified. Further oral consent was obtained; I ensured that it was an appropriate time for the interview and that she could dedicate a minimum of 20 minutes time for the interview. Although the majority of interviews did not last this long it was essential to ensure that the woman was given the maximum amount of time needed and that the interview process was not rushed. To ensure that women could dedicate this time, interviews were often conducted after standard working hours (5pm) or in the evenings. I conducted the interviews in a soundproof room using a teleconference phone; practice interviews found that this was the best method and optimised sound quality. A digital recording device was used to record the interviews (Model Olympus VN-712PC Digital Voice Recorder Dictaphone). Women were informed that interviews were recorded and consent was obtained to do so.

At the beginning of the interview I reintroduced myself, gave another brief outline of the study and explained what would be required of the woman during the interview. I discussed the implications of the study, anonymity and her right to refuse to answer questions. Women were offered the option to pick a pseudonym, and those who declined had one assigned to them. Initial identifier questions were asked including name, date of birth, ethnicity, parity, smoking status and employment. An interview script was developed, but as these were semi-structured interviews, it was used more as a prompt rather than a strict series of questions to follow. Please see appendix 4 and appendix 5 for example of interview schedule and interview transcript.

Face-to-face interviews were conducted with the midwives. A snowballing (Atkinson & Flint 2004; Babbie 2010) and convenience method (Kam et al. 2007) of recruitment was adopted. Midwives were approached in the antenatal clinic or on the research floor where they worked. In some instances midwives suggested other members of staff based on their experience or interest in the study. Similarly to the women, midwives were approached and initial consent was obtained. A date, time and place was organised to conduct the interviews. The majority of interviews were conducted on the research floor, in a private, soundproof room. This was not always convenient for the midwives, especially those based in other buildings and thus I booked rooms that were easily accessible to them without compromising sound quality or privacy. Interviews were recorded using a digital recording device.

Midwives were informed that interviews were to be recorded and consent was obtained to do this.

At the beginning of the interview I once again introduced myself, gave a brief introduction of the study and obtained further consent. Again, it was essential to ensure that the midwife could dedicate enough time to the interview and that she would not be rushed. Midwives often gave up their lunch break in order to conduct the interview. The initial stages of the interview involved me discussing the implications of the study, their role in the study, their anonymity and right to refuse to answer any question. Midwives were offered the option to decide a pseudonym and those who refused had one allocated to them. Baseline questions included name, years of experience and area of experience. Similarly to the women, an interview script or prompt was used but in general the interviews flowed smoothly. Please see appendix 4 and appendix 5 for example of interview schedule and interview transcript.

A reflection diary was kept and utilised during and following every interview. This diary included information that may not have been initially obvious from the recorded interview. The reflection diary contained detailed information on environment and impressions. Questions and statements that were made during the initial meeting / consenting time were noted and also when the recording device was not turned on. It is important to note that many women and midwives' felt more relaxed when the recording device was turned off and discussed the thoughts, views and opinions more freely. The reflection diary also included any difficulties or problems encountered and allowed for modifications to the interview style to be made.

Many interviews, with both women and midwives, had to be rescheduled due to other commitments by the participants or participants forgetting times and dates of the interviews. These interviews were rescheduled for more convenient times. All midwives who were approached agreed to be interviewed.

Interviews were transcribed verbatim. It was at this stage that all identifiable data was removed and a study number and pseudonym were allocated. This was a lengthy process, involving hours of listening and re-listening to interview transcripts. It is also the stage at which many emerging themes are noted.

4.3 Rigor and Reflexivity

One of the biggest challenges facing qualitative researchers is ensuring the quality and trustworthiness of their research. The value and quality of qualitative research needs to be argues for and justified against established criteria.

Internal validity and credibility are essential in all research. These concepts have since moved on, with many qualitative researchers rejecting the framework for validity that was commonly accepted within social science. Credibility, transferability, dependability and confirmability are all terms that are not widely accepted amongst the qualitative research community (Sandelowski 1986). This can be ensured through the use of confirmability and extensive training in the field (Freshwater 2005). Confirmability can be defined as and achieved when auditability, truth value and applicability are established within the research (Sandelowski 1986). Although member checks of the data were not conducted, all information post interview was recapped at regular intervals with the research team. This involved written, aural and verbal data. Confirmability was achieved through detailed documentation throughout the study; the research team acknowledged and supervised the documentation of the data and audit the results and their quality regularly. More specifically, transcripts of the interviews were inspected by the research team, key points and themes were brought to their attention and this further ensured that there was agreement when interpretations and conclusion were constructed. Furthermore the credibility of the research was achieved by ensuring that every member of the research team had complete access to the data. This was achieved by ensuring all members of the research team had access to the interview recordings, interview transcripts, charted data and interpretations. An extensive paper trail was kept, with all materials reviewed at regular intervals by my supervisory team. Training and mentoring was given before I began any actual data collection. Extensive descriptions of setting and participants (field notes) assured external validity and transferability, as discussed above for example via completion of my reflection diary. Constant data and documentation audits ensured that data capture was transparent. These 'criteria' outlined for ensuring rigour and reflexivity not only validate findings but address the special qualities of qualitative research and encourage the researcher and readers to explore the broader impact and social relevance of the project (Guillemin & Gillam 2004; Freshwater 2005; Jootun et al. 2009). Rigour and ethical

integrity and the criteria that qualitative researchers apply to achieve these can often vary in order to meet the requirements of the context (Ryan-Nicholls & Will 2009). What is important, and what was applied during this project, was that as a researcher I strived to be transparent with my research.

4.4 Data Analysis and Framework analysis

Analysis of qualitative data can seem overwhelming to an inexperienced investigator because of the sheer quantity of data or the inability to distinguish clear strategies of analysis (Fitzpatrick & Boulton 1996). The vast amount of data that was obtained was unstructured and text based. Verbatim transcriptions and field notes were very detailed and in micro form (accounts, descriptions, observations etc.) (Ritchie & Spencer 1994). As the main researcher, it was my aim, through qualitative analysis, to structure this large data set whilst maintaining the original accounts and ideas that came through (Ritchie & Spencer 1994; Fereday & Muir-Cochrane 2006; Bradley et al. 2007). According to Ritchie and Spencer (1994), the overall objective of qualitative analysis is 'detection', which is achieved by defining concepts, categorizing, theorizing, explaining, exploring and finally mapping.

I decided that framework analysis was an ideal method of data analysis. This can be broken into 5 stages of analysis; familiarization, identifying a thematic framework, indexing, charting, mapping and interpretation. Framework analysis led me to work systematically through the raw data. It is a method that guarantees transparency throughout the analysis process, facilitated by the fact that framework analysis follows distinct phases (Smith & Firth 2011). Because the method was developed for applied policy research I felt that it would also be an appropriate method of analysis for my research. The framework approach also has certain key features which make it an attractive method of analysis. Framework analysis allowed me to understand and interpret the data set as a whole, further verifying the statement that framework analysis is a rigorous and methodical data analysis process (Srivastava & Thomson 2009; Cameron 2011; Smith & Firth 2011).

4.5 Framework Analysis

Framework analysis was developed by an independent social research institute, the Social and Community Planning Research (SCPR). Throughout the years it has been developed and refined to achieve the aims and objectives of qualitative analysis but the fundamental principles have been maintained (Ritchie & Spencer 1994; Pope et al. 2000; Jacelon & O'Dell 2005). It is a robust and comprehensive method of raw data analysis which allows for the researcher to work systematically thought a bulk of material and draw conclusions on social interactions and behaviours (Furber 2010; Black 1994).

Framework analysis is based on or driven by original accounts and observations. It is a dynamic approach which allows for amendments or additions throughout the analytical process. It is systematic and comprehensive as all data is dealt with or analysed fully and methodically. Finally, it is a method which is easily retrievable in that each stage can be accounted for and referred back to. This makes it easier for inter- and multi- case analysis as well as being accessible to people other than the primary researcher / analyst to reproduce or examine (Ritchie & Spencer 1994; Srivastava & Thomson 2009; Furber 2010).

As well as framework analysis having certain key features, it also involves a number of specific stages. It is important to note that although these stages are presented in a logical fashion below, it is not a steadfast rule and framework analysis is not a mechanical process with a guaranteed outcome (Ritchie & Spencer 1994; Srivastava & Thomson 2009; Furber 2010). Quite conversely, the framework approach requires the analyst to be creative and imaginative so as to interpret meanings and seek connections between cases (Fereday & Muir-Cochrane 2006). However by following the general procedure laid out below there is potential to reconsider and rework ideas whilst systematically analysing the data according to the key issues and emerging (Pope et al. 2000; Jacelon & O'Dell 2005).

Framework is an ideal method of analysis that allows for robust results to be obtained yet is highly adaptable to many forms of qualitative research (Fereday & Muir-Cochrane 2006; Lu & Shulman 2008; Furber 2010). The approach can be adapted and amended to suit specific aims and objectives and has proved to be very flexible in a range of studies (Ritchie & Spencer 1994; Furber 2010). It is a rigorous

and methodical approach to qualitative analysis which best suits our aims and objectives (Verhoef & Casebeer 1997; Guba & Lincon 2000). Although some aspects of the process can be difficult to verbalize emphasis must be put on the fact that the researcher must document each step of their methods and techniques in order to allow for replication and examination (Srivastava & Thomson 2009) and potentially contributing to the ever growing pool of knowledge (Ritchie & Spencer 1994).

I will now describe the five stages (familiarization, identifying a thematic framework, indexing, charting, mapping and interpretation) in greater detail and how they were applicable to my study.

4.5.1 Familiarization

Once the data had been collected, verbatim interview transcripts in our case, it was essential as the analyst to become totally emerged in the body of material; this is extensively discussed in the literature (Ritchie & Spencer 1994; Furber 2010). As the primary researcher I was aware of the diversity and scope of the data collected as well as having an overview of the material as a whole. This immersion into the data was an extremely time consuming task; as the analyst I listened to recordings and read transcripts several times in order to be completely familiar with each individual interview.

Framework analysis stresses that the analyst must be aware that the field notes are a format of qualitative research and must also be included in this familiarization process. These provide information on atmosphere, environment, behaviours of the participant and ease at which data was collected and are of great value (Tausig & Freeman 1988; Holloway 2001; Rabiee 2007). If there is a large data set or a strict time limit it may be essential for several analyst's to pool together and finally share their overview and interpretations, however this did not occur in my study. At this stage the analyst should be noting down emerging themes and ideas for review in the latter stages. In my study, this involved the reading and re-reading of the verbatim transcripts and becoming completely familiar with all details in the reflection diary and field notes. Potential themes were highlighted or underlined and notes were made on each interview.

4.5.2 Identifying a thematic framework

As stated above, during the familiarization process the analyst will be making notes on the emerging themes and recurring ideas. It is at this point that the researcher must be their most creative as the process of abstraction and conceptualization begins. By making notes and documenting responses, recurrent themes and issues can easily be unearthed. This allowed the data to be further examined within the thematic framework (Fereday & Muir-Cochrane 2006; Braun & Clarke 2006). There are two main stages in thematic framework analysis. The initial analysis, or first stage, was primarily descriptive and focused mainly on the priori issues. Within my study this included the issues and questions considered in the research proposal such as coffee consumption, information and advice provision and education. A more creative and interpretivist approach is adopted in the second stage of analysis where by the analyst will consider underlying and emerging themes. This stage involved making judgements about meanings, distinguishing importance and relevance and uncovering issues and connections (Ritchie & Spencer 1994; Jacelon & O'Dell 2005; Furber 2010). No matter what, it was essential that the original research question was constantly referred to and was fully addressed, and that the complete transcripts are reviewed in order to get a greater and in depth understanding of the data set as a whole.

4.5.3 Indexing

Following thematic framework analysis, the themes were indexed. This involved noting the themes and sub-themes and then applying these to the data in its textual form. Interview transcriptions were indexed using a numerical format according to the thematic framework, based on the indexing headings which will be discussed within the results section. Within my research I considered two separate populations, pregnant women and midwives, and it was therefore necessary to develop separate indexes for each group or more ideally develop a common index but account for the additional themes using sub-categories. Indexing requires the analyst to interpret meanings and make judgements, which is a subjective method of analysis. However by clearly annotating and referring back to the thematic index, the process was clearly visible and interpretations could be easily justified (Ritchie & Spencer 1994; Braun & Clarke 2006; Srivastava & Thomson 2009). Another advantage of clearly

indexed and ensuring clarity of the indexing process was the ability of colleagues and other analysts to copy the methods and further justify my rational. Patterns and connections were easily identified at the indexing stage and it is often considered an 'early clue' for the subsequent stages of framework analysis (Ritchie & Spencer 1994).

There was the potential to use computer software at this point in the analysis stage however, as a research team, we determined that the data was in a format that was more suited to using a manual approach. There are arguments for and against the use of computer programmes as has been discussed in detail elsewhere (Mangabeira 2004; Duff & Séror 2005; Lu & Shulman 2008). However, it is important to note that these programmes would not analyse the data but merely facilitate the indexing process. Our rationale behind using a more traditional method of data analysis over computer facilitated was briefly based on Lacey and Luff (2009) (Lacey & Luff 2009). There was no need to use valuable time and monetary resources on these techniques. The programme would not be suitable as I had less than 10 hours of interview to analyse. There is also the fundamental belief that qualitative studies are not designed to be representative in terms of statistical generalisability (Pope et al. 1995; Pope et al. 2000). Furthermore, although the using these packages makes the process more systematic, I was aware that using a computer package may not always make the analysis less time consuming (Pope et al. 2000).

4.5.4 Charting

Once the transcriptions and field notes were indexed, as the primary analyst I rearranged the data according to theme. This was a more organised approach and achieved a more complete picture of the dataset as a whole. There were many considerations when debating the best method to set out a chart, however, I opted for a more general style including headings and sub-headings from the thematic framework and the priori research question (Ritchie & Spencer 1994). An example of this is provided in Appendix 6. I decided to adopt a thematic approach rather than a case-by-case analysis. A chart was devised including the key headings and under each heading the data was entered based on its relevance. I produced a number of different versions of the charts to not only ensure accuracy of the analysis process but also to ensure that I included the relevant information. This step involved heavy

influence from members of the research team. It was necessary that consistency was maintained across the charting process in order for a clear assessment to be made of the ease of analysis, replication and examining (Ritchie & Spencer 1994). This charting phase required more than just a 'cut and paste' approach. Each excerpt had to be thoroughly analysed and correctly indexed; this involved including the identified experiences, behaviours and environments (Verhoef & Casebeer 1997; Srivastava & Thomson 2009; Furber 2010).

4.5.5 Interpretation

The final stage of framework analysis, the interpretation phase, involves the systematic processing of the charted data. This is the point where the analyst returns to the key aims and objectives and analyses the data accordingly (Ritchie & Spencer 1994). Defining concepts involved searching for the main themes of the data. For my study, these themes included elements on present coffee consumption and advice and information. It also uncovered information on the recruitment process and protocols. Mapping the range and nature of phenomena is defined according to Ritchie (1994) as identifying 'the form and nature of a phenomenon and where appropriate, to map polarities' (Ritchie & Spencer 1994). Here I discussed attitudes and experiences of the target population and further reviewed these whilst considering our central objective. The creation of typologies includes the analyst conducting a multidimensional analysis and attempting to link these accordingly (Jacelon & O'Dell 2005; Bradley et al. 2007). An interesting discovery at this stage was the links and patterns which emerged, by which particular views and responses are linked to particular behaviours or characteristics (Daly et al. 2007). As with all qualitative research, the overall aim was explain a certain population's views or experiences, in our case pregnant womens' and midwives' views, opinions and attitudes on coffee consumption. The explanation was essential to answer the initial research question. Although there is much to consider in the mapping and interpretation stage of framework analysis, as long as the analyst considered reviewing the charts and notes, comparing and contrasting the perceptions and searches for patterns the data was easily pieced together to form an overall picture of social behaviour (Bryman 1988; Pope & Mays 1995; Jacelon & O'Dell 2005).

Chapter 5 Qualitative Interpretations

The interpretations were constructed from the analysed and charted data. This was the final step in the extensive analysis of data. It was the most time consuming of steps within Framework analysis, requiring months of reading and re-reading the charted information. The interpretation stage is often considered to be the most difficult; it involves both a systematic and a creative interpretation of the data as a whole.

The interpretation step involved combining the charted data into logical paragraphs that would highlight the emerging themes. These themes were then discussed in further detail throughout the section, including quotes and relevant material to back up my interpretation. Charted data were comprehensively studied using both hand written copies and electronic documents. Combining both methods ensured rigour; using both methods resulted in more accurate interpretations being extracted. Interpretations were discussed with the supervisory team, to confirm the accuracy of my interpretations; evidence, in the form of quotes, were included to ensure others can verify the interpretations.

The main themes identified surrounded women and midwives, and focused on coffee consumption and knowledge / information. These themes were;

- Reduced coffee during pregnancy
- Women lacking specific information on coffee
- Variation in advice given to women by midwives
- Advice on coffee is marginalised / no risk perceived
- Sources of information
- Women discussing their concerns
- Midwives lacking the specific information
- Sources of midwifery information
- Inadequate information
- Conflicting opinions on womens' sources of information
- Recommending sources of information to women
- Lack of emphasis placed on coffee information by midwives

– Midwives wish more information was available to them / more research is needed

The interpretation section is laid out in such a way that both womens' and midwives' opinions are presented alongside each other. This is the first time in the analysis phase that we can effectively visualize the raw data and quotes and thus form logical conclusions based on the findings. It is at this stage that we can see how both parties responded to similar questions, how these questions were addressed and the opinions and views of women and midwives' on particular topics, mainly coffee consumption and information provision. The interpretation phase of analysis lets us draw conclusions on the analysed data; it is a particularly personal step with no definitive end point. The interpretations, as mentioned above, will be set out in headings but will include both women and midwifery responses under each thematic segment.

5.1 Demographics

The demographic details of the women and midwives' interviewed have been tabulated below. Women varied in age, socioeconomic status and gestational age. Midwives varied in area of expertise and years of experience. The demographic details are particularly useful during the interpretation phase of analysis. One can clearly identify how participants replied to particular questions and follow this by making assumptions as to whether her particular credentials may have impacted her response. Similarly, trends may appear amongst sub-populations. This was not completely true amongst our pregnant women participants, however when comparing responses with experience levels of midwives certain interesting facts were highlighted, particularly regarding information provision. This will be discussed in greater detail (Chapter 6).

5.1.1 Women

Pseudonym	Age	G.A	Children	Smoke	Ethnicity	Occupation	Coffee / Caffeine
Sandra	35	39	1	No	White British	Teacher	Continue as normal, not a big drinker
Kerry	29	13	1	No	White British	Police Officer	Caffeine free coke, not much coffee / caffeine
Lucy	43	14.5	0	No	Irish	Manager	5-6 cups daily preconception. None during pregnancy
Catherine	28	24	1	No	Polish	Waitress	3 decaf coffee daily
Rachel	33	13	0	No	White British	Restaurant Manager	Nil
Phoebe	25	14	1	No	White British	Teacher	Nil
Niamh	35	20	0	No	White British	Librarian-University	Back to normal consumption after first 13 weeks

Maise	23	13	0	Yes	White British	Care Support Worker	4 - 5cups daily preconception. Switched to decaf coffee during pregnancy
Libbie	25	21	1	No	White British	Bank	Nil
Samantha	37	14	0	No	Mixed-White British / Black Caribbean	Commercial Director	2 - 3 cups daily preconception. Switched to decaf during pregnancy
Chloe	22	29	1	Yes	White British	No	5 cups daily preconception. 1-2 cups weekly during pregnancy
Joanna	30	21	1	No	White British	Teaching Assistant	Drinks more tea than coffee
Katie	26	37	1	No	Indian	No	Nil

					Sikh		
Tanya	39	37	3	No	White British	Mother	Only drank coffee preconception. 1 a day during pregnancy
Stephanie	41	20	1	No	White European	Disability Coordinator	No coffee consumption
Deirdre	27	24.5	1	No	White British	Legal executive	Nil
Eleanor	32	36	0	No	White British	Manager-GP Surgery	3 cups of coffee daily preconception. Cut out coffee during pregnancy
Amanda	29	21	0	No	White British	Translator	6 - 8 cups of tea daily preconception. 2-3 cups of decaf coffee during pregnancy
Daisy	40	34	1	No	North African	No	3 - 4 cups of coffee

							daily
Nicola	28	30	2	No	White British	No	1 - 2 cups of coffee daily, normal tea consumption

Table 7: The above table lists the demographics for pregnant women recruited and interviewed in my study. The table illustrates the pseudonym allocated to the woman, age, parity, ethnicity and maternal occupation. Womens' smoking and tobacco status is also listed.

5.1.2 Midwives

Pseudonym	Experience	Area
Amy	17 years	Clinical / Research
Sarah	4 years	Research
Fiona	17 years	Clinical / Research / Teaching
Claire	5 years	Rotational
Jenny	24 years	Rotational
Kirsty	6 months	Clinical / Research
Patricia	16 years	Research
Gemma	11 years	Clinical / Research
Emma	30 years	Academia / Lecturing
Mary	6 years	Clinical / Research
Anna	7 years	Rotational / Hospital / Antenatal
Amelia	26 years	Clinical/Research
Ashley	10 years	Rotational
Rita	5 years	Clinical / Research
Monica	10.5 years	Clinical / Research
Emily	8 years	Research
Lexi	10 years	Clinical / Research
Freya	15 years	Clinical / Research
Hannah	8 years	Clinical / Delivery
Charlotte	7 years	Clinical/Research
Rebecca	16 years	Clinical / Research / Rotational

Table 8: The above table lists the demographics for midwives recruited and interviewed in my study. The table lists the pseudonym allocated to the midwives as well as their years of experience and area of experience/employment.

5.2 Reduced Coffee Consumption during Pregnancy

Preconception health and diet during pregnancy can have a great impact on pregnancy, with many studies indicating that *in utero* environment plays a significant role in the health of the infant (Anderson et al. 1993; Anderson et al. 1995; Anderson 2007). Therefore it is often desirable for women to alter unhealthy behaviour from a public health perspective and for the health of their unborn child. It is widely recognised that current dietary behaviour is often not in line with health recommendations. Many studies indicate that it is often very difficult to change habitual behaviours unless there is a stimulator or a motivator. Fortunately, women are often very motivated to alter their behaviours and habits during pregnancy hoping to provide the best chance for their unborn child. In line with this statement one of the primary recurring themes was womens' likelihood to reduce their coffee and caffeine intake during pregnancy.

'*Well in the first 4 -5 months I stopped taking coffee*' Daisy

'*I went on to decaf pretty much as soon as I was trying to conceive*' Tanya

'*I drink some tea and I cut down on coffee*' Nicola

Many of the women discussed continuing on as normal with their coffee / caffeine consumption. This implies that women are either unaware of the information surrounding caffeine and its effects or do not believe the potential negative effects associated with coffee consumption.

'*I continued on as normal. I am aware that you're not meant to drink too much tea and coffee*' Sandra

Caffeine was first claimed to be of potential harm to pregnant women around 1980's (Giannelli et al. 2003). It crosses the placental and blood-brain barrier and the human fetus does not have the specific enzymes required for detoxification of caffeine, via demethylation (Morris & Weinstein 1981; Eteng et al. 1997; Wendler et al. 2009) which was discussed in further detail a previously (Chapter 2, Section 2.16). We considered those women who drink more coffee than most as many studies suggest that they nearly always differ from other pregnant women; these women are more likely to smoke and / or have poor lifestyle habits (Godel & Pabst 1992; Menegaux et

al. 2005; Grewal et al. 2008). This relationship, between coffee and poor lifestyle habits was important to consider when constructing the interpretation stage, however, when asked none of the women, in this study, stated that they consumed tobacco. Women are potentially less likely to consume tobacco as they are more motivated during pregnancy to make healthier lifestyle choices (Renkert & Nutbeam 2001; Szwajcer et al. 2007; Nilsen 2009) or they may be ashamed to disclose their actual consumption due to the stigma attached to tobacco during pregnancy (Chan et al. 2004). The literature surrounding coffee and womens' motivation will be discussed in greater depth below (Chapter 6).

As discussed above, *in utero* environment plays a vital part in fetal development. The benefits of a balanced diet are associated with a positive pregnancy outcome. It is therefore necessary that all health care professionals and nutritional specialists increase the mothers' knowledge and improve the dietary practice to promote their general and reproductive health as well as aiming to reduce maternal and neonatal mortalities (Fouda et al. 2012). This is also important considering that women are more motivated to improve health and dietary habits during the gestational period. The psychology surrounding womens' motivations will be discussed in further detail below.

Women discussed their desire for further information about coffee and many midwives felt inadequately prepared to answer these questions.

'*Do you feel adequately equipped to answer a question?* [Interviewer]

'*I suppose I wouldn't. If somebody specifically said well how much caffeine is in* a [cup of coffee] *I don't know...* [I] *doubt that maybe even the information from the coffee companies is right*' Fiona

The above statement highlights that midwives, when asked, felt inadequately prepared to answer questions specifically related to coffee. This statement also illustrates the doubt surrounding the information that is available. Reliability of information is a recurring concept within this study with both women and midwives' discussing their thoughts, feelings and concerns regarding the issue. This will be further discussed below (Section 6.2, Section 6.8, Section 6.9, Section 6.12).

'Do you feel adequately equipped to give a women advice on coffee consumption during pregnancy? [Interviewer]

Probably not. Well having said that I suppose to some extent I do but I don't know the underlying... I'm not informed enough about the underlying reasons why we're discouraging caffeine use so although I know we're discouraging it I don't really understand why we're discouraging it' Claire

The above statement highlights that there is more of an issue with information in general than with information surrounding coffee. The midwife is saying that she knows what to say but does not understand why she is saying it. This links back to evidence based practice, being a knowledgeable practitioner and professionalism.

As discussed, the general consensus amongst midwives was that they did not feel adequately equipped or prepared to answer specific questions surrounding coffee consumption during pregnancy. Midwives also felt that more clarity was needed on the information that they were providing.

Some of the women interviewed felt that if they had been 'big coffee drinkers' they would have been more concerned and thus put more consideration into reducing the amount that they consumed during pregnancy.

'I know the allowance is more than sort of two cups a day I think..... So I never really sort of worried about it too much but if I had been a big caffeine drinker I would have cut down'. Sandra

The amount consumed by the pregnant women interviewed varied. Some of the women consumed more than 5-6 cups of coffee per day, however the majority of these women stated that they switched to decaffeinated coffee once they found out they were pregnant. Women were conscious of the stigma attached to coffee and of the information surrounding coffee; there was awareness that coffee potentially has more of an effect in the first trimester of pregnancy. As a result women were more likely to return to their initial coffee habits after the first 6 months of pregnancy.

'I don't think I would have drank coffee in the early stages because I know it's more dangerous in the early stages' Niamh

The term stigma is defined as a strong feeling of disapproval that most people in a society have about something. Although women did not use the term 'stigma', it was clear that they were aware of the reported harmful effects associated with consumption. My interviews indicated that women are nervous about consumption and, similarly, midwives err on the side of caution and advise limited consumption.

Preconception consumption was discussed by a small number of women. Some of the expectant women consumed large quantities of coffee (4 - 5 cups) daily before they found out about their pregnancy. It was interesting to note that those that mentioned this stated that they changed their habits immediately however continued to drink tea regularly.

'I would have drunk quite a lot of coffee before [my] *pregnancy, particularly in the work, and I could have easily about 5 or 6 coffees a day…. originally when I found stuff out, which was at three weeks of being pregnant… I went to completely no caffeine but actually felt horrendous'* Lucy

This is particularly interesting as women perceived a potential harmful effect associated with coffee but not with tea, which also contains concentrations of caffeine. This could be because coffee and its potential physiological effects are discussed more frequently in the media. This may also be reflective of traditional and modern consumer practices; tea is traditionally consumed in the UK but the Cafe culture and coffee consumption is becoming more popular.

Only one woman stated that she could no longer tolerate tea and switched to coffee consumption during her pregnancy.

'I never drank coffee at all before but I started drinking it in this pregnancy, but decaf [decaffeinated coffee]*'* Amanda

This statement suggests that it was something other than caffeine causing the nausea. We could also conclude that some women were unaware of the potential effects of coffee or caffeine consumption. This could be the reasoning behind this woman's change in consumption from coffee to tea.

Morning sickness and nausea were also key factors in reducing coffee intake during the first trimester of pregnancy. Very few of the women returned to their original

habits once the nausea had subsided and some women even stated that they maintained their 'decaffeinated' habit for a time after their pregnancy. Tolerance to coffee, regular and decaffeinated, was affected significantly. Nausea and vomiting is a common symptom associated with the first trimester of pregnancy. It has been suggested that the presence of nausea is a predictor of a viable pregnancy (Weigel & Weigel 1989). Aversion to tastes and smells that are ordinarily well tolerated is often seen among women who report nausea but is absent among those who do not (Fenster, Eskenazi, Windham & S. H. H. Swan 1991; Fenster et al. 1997).

'Things tasted bad as well, everything tasted strange. Food didn't taste the same, it was very difficult eating… but I'm back on drinking tea and coffee again but at first I couldn't drink tea or coffee, it made me sick' Niamh

'I've actually not drank as much coffee, I drank mostly tea with this pregnancy, I weren't that keen.... it didn't agree with me' Joanna

Maintaining tea consumption during pregnancy suggests that some women were unaware of the caffeine content within tea. We could also conclude that women may also be aware that there is less caffeine in tea than in coffee and this may be their way of reducing intake. Unfortunately this was not probed in great depth during the interview process; this is a significant limitation to the study as a result of researcher inexperience. This could potentially be an area for further scope in the future.

The absence of the pregnancy signal can be a sign that women were at higher risk of miscarriage to begin with, making it difficult to detect any added risk associated with caffeine exposure. However, the increased risk in heavy caffeine consumers who report nausea support the hypothesis that caffeine may be harmful to a potentially viable fetus (Fenster et al. 1997; Wen et al. 2001).

Some of the women discussed the times at which they drank coffee and their 'need' to drink coffee. Coffee was consumed socially by some of the women, however the majority of women consumed coffee in their home or work environment. This was not unusual to hear as Cafes and coffee houses are becoming increasingly popular in the social scene, especially for young professionals (Montgomery 2007).

'the one I miss the most now[coffee], *is the one first thing in the morning, so to make me feel a bit more alert'* Lucy

Coffee has a special place in the lives of the general public. It is a social custom associated with adult status, as it is assumed that children are not allowed to drink it (Troyer et al. 1984; Temple 2009). Coffee is present in many social and business situations and despite its popularity, as discussed above, coffee has come under attack (Troyer et al. 1984). Some women considered coffee a 'treat' and stated that they would, on occasion, consume a caffeinated beverage. Coffee consumption was described as a 'pick me up'. Although being advised to reduce their intake women felt the 'need' to consume coffee.

'I started drinking coffee once in a day ....when I need a pick up' Catherine

'For me it wasn't that I needed the coffee, just I liked to drink it, a social thing' Daisy

'*I have the odd treat very occasionally and it's usually decaf*' Samantha

The above statements suggest that women have different motivators for consuming coffee. My study corroborates with the literature in that coffee plays a major role in modern society. Women either consume coffee for its stimulatory effects, particularly in the morning or when needing a 'perk'. Other women discussed enjoying a coffee in social setting or as a 'treat'. Many of the women interviewed reaffirmed the notion of the Cafe culture and its prominence in society. Some women discussed the types of coffee that they preferred and the occasion in which they drink it.

'No regular coffee, just decaf latte and then recently, from 5 month onwards [of pregnancy] *I started taking one to 2 coffee's a day… for me, it wasn't that I needed the coffee, just I liked to drink it, a social thing'* Daisy

Caffeine withdrawal symptoms can include headache and fatigue (Salín-Pascual et al. 2006; Butt & Sultan 2011) and were experienced by some of the women who had reduced their coffee intake significantly. Caffeine withdrawal is a well-documented phenomenon featuring more and more frequently in literature. Abrupt coffee cessation resulted in some of the women feeling jittery, disruption to sleeping patterns and general feelings of being unwell.

'It made me jittery..... it messed my sleeping patterns' Stephanie

As stated above, caffeine dependency, tolerance and withdrawal has been documented in literature for more than a century (Griffiths & Woodson 1988), the majority of which have been investigated in the last twenty years (Reissig et al. 2009). In addition to headache and fatigue, other withdrawal symptoms include dysphoric mood, difficulty with concentration, decreased cognitive performance, irritability, nausea and muscle ache (Griffiths et al. 1990a; Griffiths et al. 1990b; Juliano & Griffiths 2004). These withdrawal symptoms can vary in intensity and incidence and caffeine withdrawal is recognized as an official diagnosis (Juliano & Griffiths 2004). Tolerance, defined as a decreased responsiveness to a drug, is also associated with chronic caffeine exposure.

There are certain genetic factors associated with caffeine vulnerability and intoxication. For example, studies that compare monozygotic versus dizygotic twins have shown higher concordance rates for monozygotic twins for caffeine intoxication, total caffeine consumption, heavy use, caffeine tolerance and caffeine withdrawal (Swan et al. 1997; Kendler & Prescott 1999).

In conclusion, the majority of women interviewed decreased their coffee and caffeine ingestion. The reasons behind their decisions varied, however the guidelines provided and morning sickness impacted their decisions greatly, as might be expected. There were particular triggers for coffee consumption, for example the social setting and the presence of a 'physiological 'caffeine dependency in some women. Although this was the case, women were still greatly influenced by their pregnancy and acted cautiously with regard coffee consumption.

5.3 Women Lack Specific Information on Coffee

Coffee and its consumption are well documented in modern day literature and are commonly featured in international, national and local media. Speculation around the health risks and benefits of coffee are the reasons for it featuring so frequently in the media. As a result of this it was interesting to question women about their specific knowledge of coffee consumption and compare this against the evidence based information. I questioned women on their knowledge about the specific physiological and psychological effects of coffee, whether related to them or in general. As discussed in chapter 1, coffee is a biologically active beverage which is known to

have a physiological effect. This effect can vary between individuals but pregnancy can impact its direct effects on the body systems significantly.

There was a general awareness of the harmful effects of coffee consumption but there was a lot of confusion and conflicting statements surrounding the specific risks. Women stated that coffee consumption was associated with increased energy release, more specifically linked with high caffeine intake.

'[coffee] *increases your heart rate and...it gives you a quick rush of energy, and that's it. I wouldn't have said there was any* [risks]' Joanna

The above statement suggests that some women did not perceive the increased heart rate and stimulatory effect as harmful, to them or their unborn baby. Some women were unaware of the potential harmful effects associated with coffee and caffeine consumption. These women may have even seen these effects as a potential advantage of coffee consumption, reducing tiredness and stimulating the individual.

Those women who discussed coffee's potential harmful properties only associated the caffeine in coffee with the effects; women did not discuss the other compounds within the beverage. Caffeine is often regarded as the key component in studies (Wisborg et al. 2003). One woman stated that she could not see any benefit to drinking coffee whatsoever but perceived a potential harmful effect. This suggests that many women are aware of harmful effects, but as mentioned briefly above associate these with the caffeine in coffee and do not consider the other compounds within the beverage.

'I don't know what the effects are of drinking lots of caffeine are... but I'd imagine it's not good' Sandra

In general women were unsure of the effects of coffee on health. Many women stated that they knew coffee 'was not good' for their health or the health of their unborn child. Some women stated that they were aware that there were certain times that coffee should not be consumed, specifically during the first few weeks of pregnancy or the first trimester of pregnancy. The first trimester is a crucial time in a pregnancy and women are constantly reminded of this on the internet and in pregnancy books (reference internet sites; BabyCenter, NHS, Boots, WebMD).

'...the baby can't break it down and that it can bring on miscarriage... And I think it said a little bit of caffeine, in like diet coke and things like that is ok but try to stay away from it if you can, especially in the first 12 weeks' Rachel

Some of the women stated that coffee had the potential to increase fetal movements *in utero*; *w*omen stated this when asked what they perceived as the negative effects of coffee. When discussing the specific effects of coffee some women discussed miscarriage, fetal growth restriction, reduced fetal weight and fetal developmental abnormalities. Also, some of the women discussed the possibility of experiencing a poor pregnancy outcome or delays and complications with conceiving. Conversely, when asked about the potential positive health benefits associated with coffee consumption women were unable to state anything definitive apart from its ability to act as a stimulant.

'I like the sort of, stimulant effect from it [coffee]*, so you know, the one I miss the most now, is the one first thing in the morning, so to make me feel a bit more alert'* Lucy

'The risk?! It increases your heart rate...I wouldn't have said there was any [benefit]*'* Joanna

Other women stated that they had no specific knowledge about coffee and its potential effects.

In conclusion the majority of women lacked specific knowledge on the potentially harmful or beneficial effects of coffee. Women did not consider or were not aware of the other active chemicals present in coffee. However, in saying this, they were aware of the contradictory advice circulating its consumption and the information surrounding this topical issue. Women also discussed some of their personal concerns, which will be dealt with in greater depth below (Chapter 6, Section 6.1, Section 6.14, Section 6.15).

'Just 'cause different people were saying that you can't have this and you can't have that and the other people were saying you can' Maise

'The conflicting advice is out there with everything to do with pregnancy. I mean about breast feeding, about screening ..... about anything really' Stephanie

5.4 Variation in Advice given to women by midwives

Women are provided with a plethora of information during pregnancy, focusing all aspects of the upcoming nine months. The accuracy of this information and the format in which this information was provided was one of the research questions I was interested in investigating. The information women are provided with and the lifestyle changes they make during their pregnancy can impact the *in utero* environment, the health of the fetus and the outcome of the pregnancy. It is therefore for these reasons that I investigated the variations in advice provided to women and their opinion on this advice.

Women were questioned on the information and advice they were provided with, specifically the exact information they were given on coffee consumption and caffeine intake. Pregnancy is a time when women are provided with enormous amounts of information from health care professionals as well as family and friends. Many women also sourced information for themselves.

'I think I had just done a Google search and obviously I'm aware of which sites are worth reading and which ones aren't. So I did a bit of research online' Samantha

'When I've been looking for advice and stuff… I've searched on Google, the NHS one's come up, and I've been on that a few times' Kerry

It is a period of great excitement and worry for many women, especially those who are experiencing their first pregnancy or for those who experienced a poor outcome in the past (Deutsch & Ruble 1988; Deave et al. 2008). According to Rising (1998) pregnancy is a time of great transition for women, filled with many physical discomforts and emotional fluctuations (Rising 1998). Women are open to learning and are eager to know more about all aspects of pregnancy and childbearing therefore emphasis is put on the information that they receive; it uniformity and womens' perception of its reliability.

In general, women were told by their midwives that they should reduce their caffeine intake. There was a mantra of sorts amongst midwives, 'everything in moderation', that was provided to women. This will be discussed further when we compare the advice that women stated they were given against the information that midwives

state they provide. Women indicated that they were given general advice on caffeine rather than specific amounts of intake or specific evidence-based advice.

'Because my midwife told me to... just not to have caffeine and stuff as much' Maise

Some women were told to cut coffee and caffeinated products out of their diets completely. Coffee has long been thought to be a risk and detriment to a successful pregnancy. It was, however, more likely that they received this specific information from the internet or applications (Apps). It is interesting to note that women are also receiving this type of information from mobile phone applications. This will be discussed further in chapter 6.

'Not to have too much caffeine in the first place, but I didn't realise at all that caffeine wouldn't have been am recommended in pregnancy... that bit of information, that first bit of information I actually got from an application, you know an app' Lucy

'I've got nothing to compare them [forums] *to... from what I've read it seems to be reliable'* Kerry

These contemporary methods, although not always rooted in medicine / evidence-based, are very popular sources amongst women. They are easy to access, more personal as they are on private held-hand devices and provide information instantaneously at the touch of a button. As a result, women trust these methods and follow the advice provided by them.

'if I had it [Informative Application] *on my phone I'd probably use it'* Stephanie

Often, even if women were informed about coffee or caffeine from their midwife they could not remember the exact advice or information. Some women could remember advice, but this was often incorrect information or variations of the correct advice.

'I can't think of anything specific that she [the midwife] *went into regarding coffee'* Stephanie

Online sources, such as the internet and 'Apps', often recommend that women reduce or completely cut out their coffee intake. Women often questioned their

midwife about the online sources of information, and midwives verified this information and encouraged women to cut caffeine out of their diet.

'[I] can't remember exactly what she said but I think it was along the lines of keep it within a limited consumption amount, sort of thing, don't overdo it' Deirdre

If women had a previous successful pregnancy they were offered a 'refresher' on diet, and women stated that the midwives did not mention coffee or caffeine consumption during this. Women were 'less panicked' about searching for information the second time around.

'Just a refresher on what they recommend not to eat' Libbie

It is therefore clear from the above statements that women are provided with a significant amount of conflicting information. It is unclear what the impact of this variation will have on women and their unborn baby, however some women were concerned. Further on in the chapter we will compare the womens' responses with those of the midwife; comparisons should hopefully identify or indicate how health care professionals could better approach information provision.

5.5 No risk perceived and the Marginilization of Advice on Coffee

The marginalization of information was a common subtheme that ran through the majority of interviews. The concept of marginalization is putting less emphasis on certain aspects or information points than others. Women marginalised information if they felt it was unnecessary, if they had a previous successful pregnancy or did not have the time to deal with every aspect. Midwives also marginalised the information, which will be discussed in further depth later in the chapter. In general, midwives did not discuss coffee and caffeine consumption with women. This could have potentially led to women being less concerned about certain aspects or risks. In some incidences it was other health care professionals, General Practitioners in particular, that provided information on diet, preconception and during pregnancy, and what they needed to know in the early stages of pregnancy.

'My GP did make recommendation... because he's the first person that I went to see' Lucy

For some women their first pregnancy was experienced in other areas of the world, for example Australia. This particular woman stated that she was surprised with the lack of information in this pregnancy but did not state that she was anxious or concerned at the reduced amount of information. This agrees with the notion that women are less worried after their first pregnancy, providing the previous pregnancies were successful.

'They did with my first one but not this one. But with the first one I got pregnant in Australia so maybe they see it different there' Libbie

Pregnant women often marginalise information if they do not perceive that there is a genuine risk. Some of the women said that they threw leaflets away as they felt they had enough information from their previous pregnancy. Many women discussed obtaining information from friends and family, especially if they had had a previous successful pregnancy.

'To be honest I chucked most of them away because I've already been through pregnancy twice before and I just didn't see the point' Nicola

Other women felt that more information was needed in the media, not just on coffee, but on many different aspects of pregnancy and diet.

'If there was to be more... like headlines.... if you need like specific..... If you had a book, like specific chapters, or a table of contents, if it had everything in the contents in bullet points and then if you knew what you were looking for you could just go to it straight away' Rachel

When discussing the need for further information women discussed this desire as this would allow them to make more informed choices.

'I don't think it's as bad as alcohol or smoking but I think more research should be done' Daisy

There is a growing consensus that people should be clearly informed about all aspects surrounding their health care and thus this will influence their lifestyle choices (O'Cathain et al. 2002). In recent years there has been a lot of emphasis put on the necessity of information that is evidence based, clear and concise in maternity care. Groups like Midwives Information and Resources Service (MIDIRS) and the

NHS Centre for Reviews and Dissemination have produced many leaflets on informed choice (Stapleton et al. 2002; O'Cathain et al. 2002; van Teijlingen et al. 2003).

'It should be uniformed and harmonised all the advice given to a pregnant women, that would help' Stephanie

Preconception information and awareness was another area that women were concerned about. Some women even discussed the fact that receiving information at 14 weeks or later was too late, and the crucial stage of the pregnancy was missed; any harm was already done. Similarly education on food safety and practices is a concept that is appearing more frequently in medical literature (Bondarianzadeh 2008).

'I've not had my booking appointment yet but from my first appointment I've not had any information of caffeine yet' Samantha

Women preferred the idea of information being discussed with them rather than being given information verbally or physically.

'The coffee wasn't the most complicated one but I think it would be good if somebody... talked me through the alcohol and medication, quickly talk you through a bit more about the food maybe' Eleanor

On the other hand, some women did not like the idea of being told what to do. Some women felt that midwives held an authoritarian role when providing maternity information. One woman in particular resented this authoritative role, referring to the midwives as 'pregnancy police'.

'With so many rules and regulations and the pregnancy police are everywhere but there's not actually that much information' Samantha

This term 'pregnancy police' was coined in the 1980's, and some literature investigates extremes cases of control, medically and legally, with the rights of the fetus being considered more frequently. Medical and legal attempts to control and limit womens' behaviour are becoming more frequent in today's modern culture (Woods 1985; McNulty 1987; Shakespeare & Issues 2004). However this is an extreme case and does not refer directly to my study or the view expressed by the

women in my study. The concept of 'pregnancy police' and the authoritarian role of the midwife will be discussed in further detail in the Discussion chapter.

Woman controlled health care was a concept that shone through when discussing women and their information. Women sourced information using more contemporary methods. As well as this, women either stated or implied that they wanted more information surrounding the risks to their pregnancy. Women are now more aware of their pregnancies. There is an increasing number of hospital admissions, especially with regard to miscarriage, which may reflect the increased focus on the first stages of pregnancy (Rasch 2003). Womens' access to sensitive pregnancy tests and increased knowledge of the early stages of their pregnancy impact their focus and interest, especially in recent years (Gentzkow 1985; Rasch 2003).

Leaflets were favoured by some women as they felt that they were user friendly and easy to access. Leaflets, providing that they are evidence based, are an effective method of providing information.

'having the leaflet that you can take home and read again and again and just have it there in your bed or on your table it's quite handy' Stephanie

Some of the women discussed the impact that conflicting advice and information had on their pregnancy, and the potential impact it could have on young, influential mothers. Providing support for this vulnerable group of women is vital and much literature surrounds this concept (Sagrestano et al. 1999)

'[In] your teens or even early 20's or something you're quite influencible so whatever the last person said goes, you know, so I can understand why it is so important' Stephanie

Marginalisation of information was a recurring subtheme throughout the interviews.

5.6 Sources of Womens' Information

Interviewing women has provided me with a vast amount of qualitative data. The analysis process has allowed me to condense this data down, develop a thematic framework and begin the interpretation phase. It is important to note that this data would not be considered rich; this could be seen as a limitation to the study, however

it potentially highlights an area where further information and research is needed. This will be discussed in greater depth below (Section 5.14). The analysis process is a lengthy process which has led me to the initial interpretation of my findings. Many recurring themes have been brought to my attention, one of them being where women source their information and their opinion on these sources. This is a significant theme; where women get their information will dictate their practices during pregnancy, especially with regard diet and lifestyle (Anderson et al. 1995). Poor decision-making and ill advice at this time can have significant repercussions on the woman, her baby and the wider family for the rest of their lives (Soltani & Dickinson 2005).

Womens' awareness of the availability of information was questioned as part of this study; this was discussed in further detail in the methods section. Many women discussed their varying levels of awareness and what their thoughts on the information which is provided were. Although some women were aware of the advice and information on coffee, this information was not prioritised; coffee is rarely on the hierarchy of womens' concerns during pregnancy. There are many factors that influence this. Midwives marginalize some of the information that they give, paying particular attention to alcohol and smoking, over other potential risks. Both women and midwives' felt the need to balance this information. Prioritising information is a very personal process as what one woman may deem a concern, others may feel that it does not relate to them or is a trivial risk.

The method by which women get their information varies greatly in the interviews conducted. I have categorised these sources of information into two groups, traditional methods and contemporary methods.

5.7 Traditional Methods

Some women opted to use more traditional methods of gathering information. These tried and tested methods include leaflets, pregnancy books and, of course, direct information from their health care professional. Traditional methods are often considered to be more reliable and appropriate means of gathering information (Rothman & Kiviniemi 1999; Kelley et al. 2001; O'Cathain et al. 2002). They are

often the methods advocated by health care professionals but not used by women (Soltani & Dickinson 2005).

'I've only been to the midwife 3 times now and the first 2 times I got quite a lot of information, booklets and stuff like that to read through, which was very, very helpful' Rachel

During the interview I asked women about leaflets; whether they used them and what their opinion on them was.

'It was more when I got pregnant I found out a lot more... like through the leaflets and from the midwife' Katie

There were mixed opinions on the usefulness of leaflets. Some women felt that leaflets were tedious and tiring, that they were bombarded with leaflets and had no time to read them.

'I read them... I can remember reading them and I found them useful. I also bought a few books and I read them as well' Tanya

Some women stated that other commitments, such as having a young family and work, prevented them from dedicating large quantities of time reading the leaflets that were provided.

'When are you going to have time to read that when you have a baby, no way' Catherine

Also, some felt that 'real' pregnancy was not dealt with in the leaflets, acknowledging the fact that each pregnancy is unique. An interpretation of the concept 'real pregnancy' is the woman's own personal pregnancy and all the aspects of pregnancy that make it unique. This will be considered further in the discussion, Section 6.7. Women were aware that each pregnancy is different and many like a more personal approach to advice.

'I didn't find them helpful because I don't think they tell you anything, to be honest. I think it's better just to get the updates and then they tell you word for word exactly what's going on with your baby...but the leaflets don't really tell you... well nothing to do with real pregnancy anyway' Chloe

Specificity was discussed and most women felt that it was only the bare minimum was dealt with; leaflets did not deal with the many concerns that women had. These concerns will be discussed further below. The below statement, although vague, highlight that many women require supplemental information. This was, however, the general consensus amongst the pregnant women.

'I would say more information about most things' Lucy

Conversely others found that leaflets were useful and a good source of information. The fact that leaflets were factual and specific enough was appealing to women. They are a very accessible method as they could be given leaflets at every appointment with a health care professional and are readily available in waiting areas. The ease at which information can be obtained was a recurring theme throughout. Leaflets were considered an easy method as they could be picked up and put down whenever the woman wanted.

'Would you have preferred a different format of information provision? [Interviewer]

Having the leaflet that you can take home and read again and again and just have it there in your bed or on your table it's quite handy really. But for other things, like, maybe the diet thing or how to keep a healthy lifestyle or how to meet other mums or you know.... maybe I would cut down on those [leaflets] *and give to people who ask for them. I think the essential ones* [leaflets]*, it's quite nice to have them on paper, because you can keep it. Mind you if I had it on my phone I'd probably use it just the same'* Stephanie

5.8 Alternative Knowledge

One woman mentioned that she had received advice from her GP. What was interesting was that the information he provided was based on personal experience rather than factual, evidence-based information. The GP directed the woman to books and other methods which his wife, who had recently had a baby, used. The woman herself found the information that she was given was useful, but also liked the fact that the information was discussed with her. This highlights the importance

of personal experience on information, for both professionals and pregnant women. This also leads us to question the role of the health care professional.

'and he [the GP] *did tell me about, two books that he recommended, but they were more all about pregnancy... that probably would have been a useful'* Lucy

The Health Care Professional plays a vital role in information provision as they are usually the first point of contact during pregnancy. Studies have indicated that women are particularly motivated during the gestational period and thus accurate information should be provided. This information should be evidence based as women perceive those in a medical profession to be a reliable source of information. We can conclude that not only is it the role of the health care professional to maintain general health and wellbeing but they have a responsibility to the woman and her unborn child to provide the most current and accurate information available. Early *in utero* environment has a major impact on fetal development and thus the health care professional is obligated to ensure that the woman is given the correct information in order to make an informed decision and potentially modify her health or lifestyle behaviours.

The general feeling surrounding leaflets was that they were an old-fashioned method of information provision. Unappealing information was less likely to be utilised (Lagan et al. 2010). Women liked the idea of fast, specific information that was 'at their finger tips'. As a profession it is important for midwifery academics and staff to consider why women feel that the traditional methods are not meeting their needs and how these methods can be re-vamped. Health care professionals need to be aware that women are not encouraged to use these sources and are looking elsewhere for advice.

The information that the midwife provides could potentially have a huge impact on a woman's pregnancy.

5.9 Contemporary Methods

As technology progresses, so too do the ways and means by which women get information. More modern methods of research are becoming the norm for women as

they search for additional information. Internet searches, internet forums and mobile applications are some of the methods utilised by expectant women today.

Media influences decision making during pregnancy. Women are exposed to many formats of information provision and are given advice on every aspect of diet and lifestyle. It is therefore no surprise that women communicated with me their concerns surrounding coffee and pregnancy. Traditional methods did not satisfy their hunger for knowledge and so more unconventional methods were adopted.

'Google' and 'Yahoo' were the search engines that women used for their information. They stated that they would do a 'general' search, looking for more information on general aspects on pregnancy, not just on diet.

'I've been looking for advice and stuff I think that's come up when I've searched on google, the NHS' Kerry

Women stated they liked having the information at their fingertips [this was mentioned in the field notes, part of a conversation with a participant]. Although a basic method of searching for information, it was easy and women felt that it was trustworthy. As stated above, ease of information provision is very important.

Medical sites, such as NHS Choices, were considered a more 'appropriate' source. The NHS and Government run sites were felt to be more reliable as they were factual and medical based. They were seen to be more straight-forward and 'no-nonsense'. Reliability is a huge concern of women. Although they may have decided to source information alternatively, reliable and trustworthy information is very important to them.

'I probably have a mixed view really... you have to be careful really, outside of say maybe factual things anyway, so you know... NHS advice, I tend to more think yes I'm sure that's accurate' Lucy

Forums are a more controversial method of information provision. These are websites where lay people can post their opinions and advice on pregnancy. These sites are usually not monitored by governing bodies or health care professionals. It could be said that these are more similar to social networks than information sites, but they are still very relevant and are regularly used by women.

Again, as with all forms of information, there were mixed reviews on forums. Some women felt that forums were a reliable source of information. They liked the intimacy of forums, as a source of information but also as a method of communication. Email alerts and updates produced by forums were enjoyable to read, according to women. The idea of following the babies' progress felt more personal and women liked this.

'You know what's going on in the pregnancy and how the baby is progressing' Chloe

Mobile phone Applications or 'Apps' are a fairly new method of information provision. With the introduction of smart phones women now have easy access to information, in a very user friendly fashion. Overall, women expressed very positive views with regard to Apps. Again, they felt this method was very personal and intimate method and liked the idea of updates, tips and advice.

'I have an App on my phone, a Babycentre App called My Pregnancy and everyday it brings up something to do with the pregnancy... I find it quite reliable' Amanda

'Because they're ['apps'/ forums] *more like intimate.... they tell you more.... like me, I love to read up about what's going with the baby, how she's progressing'* Chloe

The above statement, although not directly relating to coffee consumption, indicates how pregnant women source their information. An application is a convenient method of information provision, providing women have access to internet and a mobile phone. The major limitation to this method regards its accessibility. Some women find these methods reliable; this method is similar to sourcing information online. The reliability of this information is questionable and thus more should be done to vet the content.

During the initial stages of analysis we are noticing that women feel that information is lacking. Women are aware that information can be incorrect or that most of the information online is non-factual but pregnant women were left to their own devices in searching for information and felt at a loss when it came to sourcing accurate information. Women expressed a need to supplement the basic or non-specific information that they were given.

'I felt like I had to supplement it [the information] myself online… I'd been kind of left to my own devices' Niamh

Their midwives or healthcare professional may not have mentioned anything, or not dealt with their particular concerns. One woman felt that the information she was provided with was scaremongering and that the leaflet was limited. For this reason she used the internet for a further search to clear up her particular issues.

'I found that a lot of it [information] *was just very, very limited and I found there was a lot of scare mongering'* Deirdre

This powerful statement suggests that many women are confused and anxious with the information being provided. The quantity of information provided, particularly during the first trimester, is vast. Women are requested to read literature and are not provided with much time to ask questions. Similarly, there in much information and advice on the internet and certain topics, such as coffee consumption, are frequently featured in the media. Women are bombarded with information and following this required to extract the essential points within. If women do not perceive coffee and caffeine as a potential harmful substance they may feel that its presence in the literature / media is scaremongering. If this is the case, further research needs to be conducted on how to approach diet during pregnancy in a less intimidating and condensed format so that women are provided with accurate information and allowed to make informed decision. Scaremongering is defined as the use of fear to influence opinions and actions; women should feel relaxed and comfortable during this already stressful time of their life and should be encouraged to make certain necessary modifications to diet rather the forced or bullied.

5.10 Family and Friends

Family and friends have an immense impact on women during pregnancy. Support, particularly familial support, is important for the pregnant woman and studies show that women are most likely to turn to their own mother for practical advice and information on pregnancy, labour and child rearing (Bunting & McAuley 2004). Overall, research indicates that the provision of support and advice improves maternal well-being more so than any other independent variable, confirming the importance, particularly for young, new or vulnerable mothers (Aaronson 1989;

Thompson et al. 2004; Bunting & McAuley 2004; Darvill et al. 2010) Partner support has been an area that has been neglected by British research yet US studies have found that it does play a major role in womens' decisions (Thompson et al. 2004; Bunting & McAuley 2004).

'I think a lot of friends do give you advice… which is quite useful… if they have been pregnant' Lucy

Pregnancy is a time when women are very motivated to do the right thing and not harm their unborn child. In saying this, it is also a time when women can be very vulnerable and as a result those who are closest to women are very influential. Some of the pregnant women were aware of the impact that friends and family have, especially the impact on young pregnant women who can be very impressionable.

5.11 Women Discuss their Concerns

Pregnancy is a period in a woman's life that is characterized by dramatic biological, physiological and social change (Lederman 1990; Gupton 2001; Ohman et al. 2003). A woman's first pregnancy is often described as a transition phase; a developmental stage with implications for a woman's self-image, values, behaviour and relationships with others (Lederman 1990; Ohman et al. 2003). More importantly it is a transition for women into motherhood.

During the interview process many women discussed the concerns they had surrounding diet during their pregnancy. These concerns varied greatly from general concerns about their diet to specific concerns about certain foods and drinks. General concerns included their uncertainties about cheese, seafood and eggs. Nutrient intake, specifically folic acid and iron, were discussed. Food substitutions and improving lifestyle were considered by many of the women interviewed.

'*I was concerned about making sure I get the right things* [food / nutrients]' Kerry

Pregnancy is a time in a woman's life when they are often motivated to improve health and lifestyle so as to give their unborn child the best chance (Szwajcer et al. 2007). Pregnancy as a motivator will be discussed in further detail in Chapter 6. Women are anxious that they do the best for the growing fetus; this involves ensuring that they are eating right and obtaining all the correct nutrients. The

uncertainties within the information and advice and the limited information further feeds these concerns. Coffee consumption falls into this category; many women were aware of the potential harmful effects but were unsure exactly and midwives did not feel adequately equipped to provide information on such a specific, yet prominent, topic. This situation could potentially be rectified with the provision of accurate information. This information would have to disseminated down through the ranks and finally to the women. As well as this, dedicating more time to women to discuss their concerns, relating to any and all pregnancy issues, would help elevate the stresses associated with this life altering time in a woman's life.

Some women discussed particular topics of diet that they were interested in. Vegetarianism and vegan diet was discussed, but only very briefly. The majority of women were more concerned about not getting enough nutrients because of their morning sickness and nausea. Some women discussed a feeling of guilt if they were not able to consume the right amount of nutrients because of morning sickness and nausea.

'You feel guilty because you want to eat healthily for the baby and you have all these plans that you're going to have a really healthy diet when you're carrying a baby. But really when you feel so ill you just eat what you can' Niamh

The above statement also highlights the fact that pre-pregnancy expectations are not always met. It is difficult for women to plan during such a volatile time and this can further the stress and worry that they are already experiencing. Most women wish to do the best for their unborn baby and pregnancy is often considered one of the most effective motivators to improve diet. It is therefore understandable that women feel guilty if they feel that they are not able to provide the nutritional requirements to their unborn baby. This is an issue that they should feel comfortable discussing with their midwives and hopefully their support and advice would alleviate some of the stress and guilt.

Desire to reduce caffeine intake was a common concept during the interviews. Women were unsure of the information and advice surrounding coffee consumption and were aware that the advice was conflicting. Women stated that they were unsure of the amount of caffeine in a single cup of coffee and even questioned the quantities (how much caffeine is present in a single mug or cup of coffee). Studies have shown

that the amounts of caffeine present in similar amounts of coffee can vary greatly (Crozier et al. 2012). The conclusions of these studies state that coffee connoisseurs are unknowingly consuming over the recommended amounts of caffeine; ingesting large quantities of caffeine could potentially put susceptible individuals at risk such as young children, those who are suffering from liver disease and pregnant women (Crozier et al. 2012). This was not the sole worry of women, who acknowledged that there was conflicting advice surrounding all aspects of pregnancy. Women discussed their knowledge, or lack thereof, on caffeine and coffee. Some women even stated their relief in finding out about coffee and its potential effects in the early stages of their pregnancy. One woman in particular mentioned her specific concerns as she had continued to consume caffeinate products, more than the recommended advised amount.

'I was probably drinking about 5 caffeinated drinks a day. I tended to have a couple cups of tea and then maybe 3 cups of coffee... I would have been less worried if I had more background information as to why I was not meant to be drinking the caffeine'
Eleanor

The above statement is particularly important when we consider womens' desire to give their unborn baby the best chance. Women are unsure and therefore worried about the consequences of their coffee drinking. This was apparent during the initial recruitment meeting; women often questioned me in depth about the specific effects of coffee during pregnancy when they were initially approached. I noted in the reflection diary that many women stated that they had not thought about coffee before and thus were interested in taking part in the study. It was clear that women were interested and possibly slightly concerned regarding their own caffeine intake and were searching for someone to put their minds at rest. Also, as discussed above, as *in utero* environment is important for fetal development more information needs to be available pre-pregnancy. Those who are actively trying to get pregnant should be aware of the potential harmful effects; this information should be easily accessible and reliable.

When the question was posed to women about their specific concerns many discussed how they were aware of the risks surrounding certain beverages, particularly alcohol and caffeinated soft drinks. Although very few women discussed

their coffee consumption or concerns surrounding it some did discuss their worries about their tea consumption. Rather than discussing their particular concerns surrounding their diet women often stated the changes that they made before and during their pregnancy.

'I had to cut out diet coke… I started taking tablets, folic acid and stuff and think about more high iron foods like cabbage, spinach… uhm… broccoli and you know high energy foods because I was anaemic when I was younger. So, you know, just incorporating more fruit and veg really' Rachel

Some women were surprised with the reduction in the amount of information that they received during their second pregnancy. This marginalisation of information will be further discussed in the discussion chapter (chapter 6)

'I was surprised that you see your midwife less' Sandra

Previous poor pregnancy outcome can result in women being overly nervous or particularly concerned about their current pregnancy (RCOG 2004). Experiencing a poor outcome resulted in women describing their current situation as terrifying. One woman described her current pregnancy as being 'hectic'. She was panicked about diet and fetal movements and expressed a particular desire to follow guidelines rather than listen to those who were constantly 'going on about babies'. It was clear that women who experienced a poor outcome were deeply interested in receiving evidence based information.

'I had a miscarriage like just before getting pregnant again so this pregnancy....has been like pretty hectic anyway, I've just been terrified all the way through really' Chloe

Preconception awareness of coffee, caffeine and its risks was also talked about, if only briefly, during the interviews. Similarly to women who experienced a poor pregnancy outcome, women with pre-planned pregnancies had many dietary concerns. Healthy eating and lifestyle changes were womens' top priority, discussing how they switched to decaffeinated coffee as they were unsure of the amount of caffeine they could consume.

'I've been trying to conceive for about two years so I've been very aware of diet and health… I've sort of been healthy eating and I've been conscious of nutrition for quite a while before the pregnancy' Samantha

A small number of women discussed general concerns surrounding breastfeeding and screening.

As stated above, pregnancy is a transitional period for many women into motherhood and can be filled with emotional turmoil and excitement. Anxiety, although natural, can be distressing for women and it is the role of the midwife and other health care professionals to address womens' concerns and try to relieve this. Both women and midwives' made suggestions, such as further literature or discussions with women that could potentially tackle the uncertainties that women have.

In conclusion women have many varying views and opinions on coffee consumption during gestation. Their sources of information vary with socioeconomic status and age, which will be discussed in further detail in the discussion chapter.

5.12 Midwives Lack the Specifics

As stated above in Section 5.7, midwifery is a profession very much rooted in traditional practices and providing information orally. This makes the role of modern day midwives considerably difficult especially since the amount of information that the midwife must provide has increased greatly in the last 20 years. Women have access to more sensitive pregnancy tests and are becoming more informed about risks and potential poor outcomes. This results in increased pressure being placed on the midwife to provide the most accurate information in order for that woman to make an informed decision about her lifestyle choices during her pregnancy. Midwives were questioned about their personal knowledge on coffee and its potential side effects. Within the clinical setting, similar to the literature there were clearly evident contradictions between the midwives personal and professional knowledge (Fleming 1998).

Some midwives acknowledged that they did not have specific knowledge on coffee and its effects on pregnancy. There was a general consensus that coffee consumption

during pregnancy should be discouraged but the evidence behind this advice was unclear.

'I don't really understand ... why it's not a good idea... I thought there was something a bit funny about it [coffee consumption], I had heard that it wasn't entirely clear' Claire

There could be many reasons for general advice being provided. It was possible that midwives were unaware of the specific knowledge and information surrounding coffee and caffeine consumption and therefore played it safe by providing more traditional, generic advice. Traditional practices, such as using the statement 'everything in moderation' is still very common in the midwifery profession (Kroke et al. 2004; Rosenberg & Donald 1995; Davidoff & Haynes 1995). Midwifery generally remains an oral culture in which midwives come to know through experience and tradition. The extent to which this is done depends heavily on the historical context and socioeconomic setting in which the knowledge is produced (Fleming 1998).

Midwives who believed that coffee could be a risk factor for an unsuccessful pregnancy encouraged women to cut out or cut down on caffeine intake.

Some midwives questioned women on their caffeine consumption and advised women on other sources of caffeine based on this. This is probably the most accurate method or practice as the information and advice is catered to that woman's specific needs.

Some midwives had specific knowledge on coffee and its potential harmful effects during pregnancy. Midwives felt that because coffee is a stimulant and has an effect on the central nervous system then it is only natural to believe that it is harmful to the unborn child. Midwives listed some of the specific poor pregnancy outcomes that they were aware of; they mentioned miscarriage (recurrent / in the first trimester), low birth weight, growth restriction, increased fetal heart rate, increased fetal movements and general poor pregnancy out comes.

'We know that large doses of caffeine are involved in early miscarriage- that there's evidence out there. Although I have to say, I couldn't give you detailed information about the evidence itself' Amy

Midwives also commented on coffee's effect on general health, preconception health and the effect coffee can have on the new born. Coffee is a central nervous system stimulant, increasing energy levels followed by a sharp decrease in energy levels. One of the midwives stated that from her experience drinking coffee resulted in a sugar imbalance and impacted her energy levels. Coffee is a mild diuretic, increasing urine production. Pregnancy also impacts urine production. Midwives stated that they felt reducing coffee consumption would not further increase the need for urination, thus improving womens' quality of life. One midwife felt that coffee consumption during pregnancy could induce preterm labour.

'[Coffee is] a diuretic so the more you're drinking it you're peeing out the same time so… you want with less caffeine in it. That's why I say to women not to drink coca cola because the majority of coca cola has caffeine in it… if they've had a coffee or a cigarette it makes the heart [beat faster].... *because the baby is tachycardic... so you think just don't drink it for at least an hour or a couple of hours before you come in because it does have an impact on the fetal heart rate'* Amelia

Midwives also discussed the potential impact that coffee consumption could have on the neonate. Midwives were aware that caffeine easily passes the placental barrier and therefore may influence the health of the baby. Caffeine cannot be broken down by the neonate, they do not possess the correct or matured enzymes needed for caffeine's demethylation (McKim 1991; Cazeneuve et al. 1994). As a result caffeine could potentially impact infant irritability and sleeping patterns. One midwife was aware that caffeine was expressed in breast milk and could enter the infants system. There is much literature surrounding caffeine secretion during lactation. It is estimated that the half-life of caffeine is 4.9 hours in adults however this is increased to 120 hours in the neonate (Jumonville et al. 2013). These findings suggest that further information and research needs to be conducted.

During the interview some of the midwives discussed their personal views on coffee consumption, including discussing their own personal consumption, either during their own pregnancies or on a day-to-day basis.

'The general increase in energy you get but that's generally followed by a rapid energy slump…. so for me I know if I have a coffee I instantly want a sugar fix' Hannah

Some of the midwives continued with their normal caffeine consumption during their pregnancies and stated that they experienced no complications.

'I wasn't a mad coffee drinker but I would have probably had about 6 cups of tea a day throughout my pregnancies and… my children are fine. So, my personal experience of it is fine' Sarah

Experiences play a major role in informing our decisions and with the advice we provide. The above statement indicates that a midwife uses her own experiences to advise her patients. Although this method sometimes useful, it is not always the most reliable. This is a more personable method; women can easily relate and may find it more appealing if they are talking to another mother (Seefat-van Teeffelen et al. 2011). However, a more accurate method of information provision is needed. This method is a more traditional approach and not necessarily the gold standard method; midwives and other health care professionals should be providing evidence based information to women. In the case of coffee consumption where there is much conflicting evidence and advice midwives must make utilise their best judgement and inform women of all the risks and benefits surrounding the beverage. Ideally, reliable, evidence based information will become available to health care professionals in the near future and this can be tailored to women in such a way that they respond to it as well as they would experiential learning.

One midwife, however, stated that she read up on caffeine before her pregnancy and as a result she altered her caffeine consumption. Since reading up on the recommendations and the literature surrounding coffee consumption the midwife changed the advice that she provided to women; she now encouraged women to significantly reduce their caffeine intake during pregnancy.

'I do know caffeine can cause miscarriage… being pregnant myself I was very conscious to cut out caffeine, but back then then it never crossed my mind to tell them, plus, to be fair, when we booked them, they were about 12 or 13 weeks pregnant' Anna

Other midwives felt that there was no need for further information and that the general public and media were being neurotic about dietary advice and other pregnancy issues.

'I think that a lot of it is quite neurotic anyway, you know, the endless, endless dietary advice it's sort of, probably over the top' Claire

They also felt that coffee was not the issue amongst the majority of women, but more focus should be put on energy drinks and carbonated drinks.

'I've been told a lot of women take fizzy drinks, what's your experience? [Interviewer]

They do come in with them [fizzy / carbonated drinks] *and I'd probably say more than coffee, that would be the main thing'* Rita

'It's not just coffee, it's tea and chocolate and fizzy drinks' Jenny

'I don't know, I think coke and energy drinks and things like that are a cause for concern but coffee, I don't know' Rebecca

I asked midwives what their opinions were on their own knowledge, specifically to reflect on their specific or personal knowledge on coffee consumption. The majority of the midwives felt ill equipped to provide information to women. They were unable to recall information on specific dietary recommendations and were aware that they lacked the relevant knowledge to provide evidence based advice.

'If a woman came to you and asked you a question on coffee, do you think you'd feel adequately equipped to answer, if she asked for more advice or something like that? [Interviewer]

Probably not because I would just tell them to cut down and that it's a stimulant… just give them the general information…. I wouldn't be able to go into the ins and outs of what coffee does to you' Kirsty

Some midwives expressed concern at their lack of knowledge stating that they did not know why they provided the advice that they gave. Those midwives erred on the side of caution and advised women to reduce or completely cut out caffeine and coffee in their diet.

'I'm not informed enough about the underlying reasons why we're discouraging caffeine use so although I know we're discouraging it I don't really understand why we're discouraging it' Claire

Some midwives were also aware that there was much conflicting advice surrounding decaffeinated coffee and thus were reluctant to advise women to substitute their regular coffee with decaffeinated. There was not just conflicting information and advice surrounding coffee and caffeination but surrounding quantities and the discrepancies in the amounts women are advised to reduce their consumption to (2 cups, 200 mg, this can be very subjective).

'No, no really I don't if I'm honest…..I would obviously do as I'm told and give the advice…but really in order to feel fully prepared to speak to one [a pregnant woman] *I would have to do my own research, I would have to look into it myself… I couldn't say hand on heart that I would be particularly happy'* Sarah

'I couldn't say I know straight off hand' Patricia

Some of the midwives mentioned that they had not kept up to date with the most current information and were anxious that their recommendations were out of date and potentially harmful to the pregnancy. One midwife also stated that she was nervous in case she missed something vital during the booking. It was clear from this section of the interviews that midwives had a genuine concern for women and how their pregnancy progressed.

'Whether there's a lack of research out there or I've missed a training session that says why, I've just been told to tell them to reduce their caffeine intake so I do. If they asked me why well I'd have to say this is the thing I've been told to tell you and I haven't got the answer to hand but I will find out for you' Mary

In general the midwives wished that they had more information so that they could provide the most accurate, evidence based advice to women. Some stated that these interview that we actually beneficial as they highlighted areas that were lacking in their knowledge.

5.13 Sources of Midwifery Information

There were many different sources of midwifery information and during the interviews we discussed the areas where midwives went to get their advice. Similarly to the women interviewed, midwives used both traditional and contemporary sources of information. Many midwives used their experience and knowledge from their

experience as sources of information. This will be expanded on further when we discuss midwives ways of knowing (experiential / academic / personal) in the discussion section of the thesis.

More traditional methods of obtaining information were those sources rooted in evidence. Evidence based journals, midwifery journals and published articles were all stated when midwives discussed the sources they used. Midwives also sourced information from reputable sites on the internet. MIDIRS, Antenatal NICE Guidelines, the Food Standards Agency were all methods used by midwives to gain information on dietary recommendations during pregnancy. These are government controlled and monitored sources of information and are considered more reliable than contemporary methods.

Some of the midwives stated that they would use a general internet search, for example Google, to source their information. This was considered an easier method of sourcing information as it was at their fingertips, easy to sieve through and essentially instant.

Many midwives discussed approaching their peers or other health care professionals for further information on specific topics, especially if they had been questioned by a woman on a particular subject. Passing information down from generation to generation of midwives is a common practice. Traditional practice rather than evidence based practices are often utilised in the midwifery profession (Stoner 1986; Choudhry 1997; Carroll & Benoit 2004; Pollard 2011). As stated above, this method could be considered outdated with very little research being conducted in recent years and thus women are more inclined to utilise other sources, such as below, a different health care professional that they perceived as more reliable regarding information provision.

'I went to the nutritionist really' Emily

One midwife stated that she would use a woman's lifestyle magazine, for example Cosmopolitan, for her information. This would be an exceptionally unreliable source of information however it would give a good representation of what information women are being exposed to. As well as this, some midwives utilised parent forums for their information. Forums are also an unreliable source of information; they are

not monitored by government bodies and are not rooted in evidence based research. Forums are simply websites which allow the general public to communicate their thoughts and views on particular subject. Although this is the case many women use, and are encouraged to use forums, as they are a good method of communication and support. Some of the midwives used leaflets and government guidelines for information. These guidelines are often disseminated down from midwifery managers to the midwives on the wards. Emails and updates were often sent to the team members identifying changes in practices or updating current knowledge. Midwives trusted that their superiors would highlight any alterations in practices and keep them informed of any necessary or relevant information.

Midwives who worked for the University stated that they would be more likely to use university resources, such as the library. In saying this midwives who worked in antenatal clinic felt that they were better informed and many midwives who worked in other areas agreed with this statement. This was because midwives in the antenatal clinic were exposed to womens' questions and concerns and thus were aware of any recent information.

5.14 Inadequate Information

The concept of midwives feeling inadequately prepared to answer specific questions was very apparent. Midwives felt they were not knowledgeable enough to provide evidence based information to women. The vast quantity of information that must be provided to a woman during their booking appointment and the limited time frame resulted in many midwives being pressured into answering only the questions that they knew and not having time to research those that they did not. Some of the midwives stated that they felt guilty for not having the specific information and were embarrassed that this was being highlighted during the interview. Being prepared to answer questions is a key part of any health care role. The health care professional is often seen as a point of information, not just for care, and this is especially true in antenatal care. Often women like to discuss certain concerns with their midwives to either gain advice or reaffirm advice and information previously researched. It is therefore essential that midwives can cope with the demands and are provided with the adequate resources in order to ensure a 'gold standard' of care.

During the interviews midwives discussed the reliability of the sources of information. Midwives acknowledged that sources of information that were considered more reliable were more difficult to understand. Some of the midwives stated that the information in the more reliable sources could easily be misinterpreted by younger or more inexperienced midwives. In general midwives felt that more reliable sources of information are better. These traditional, more reliable, evidence based methods can often be outdated, and this is obvious when I personally conducted a literature review. Much of the literature was twenty, or more, years old.

'because it's so engrained the traditional practice, amongst midwives, to actually change it there has to be some gold standard research that makes the commissioners of the service to direct you that you have to change your service' Mary

As stated above, midwives felt that those who worked in an antenatal care setting were better informed. This could be true as midwives that work in the antenatal clinic are exposed to the most current guidelines and are posed with the most recent questions from women. Midwives working in antenatal clinic or community midwives are the first point of call for women. Women come to midwives with their questions and concerns about their pregnancy.

'I'm sure midwives that work in antenatal clinic and book women for their care and give them the initial advice about diet, I'm sure they're better informed than some other people' Fiona

Some of the midwives felt that unless NICE Guidelines changed or enforced new guidelines then traditional practices will be upheld. One of the midwives stated that she was trained to give the advice not to drink coffee during pregnancy and that she upheld this practice throughout her career.

'I've always been trained not to drink coffee and caffeine in pregnancy so it's just the way I've been trained really and truly… I haven't really thought any more into it in a sense' Patricia

Midwives also discussed their opinion on their sources of information. Many felt that traditional methods of sourcing information were the most reliable. However, a few midwives discussed the dissemination of new research and guidelines; they felt that some midwives may have difficulties interpreting the new recommendations.

'It's difficult for midwives to interpret that information' Amy

Many midwives discussed using more modern methods but they did not discuss their rational for using these. It could be assumed that midwives use these methods for the same reason as women do; they are easy to access and provide instant information. Midwives would however be able to sieve through the abundant information that can be found on the internet and extract what is useful and accurate.

'I could you know go on the internet and look back for articles that they've published about it [coffee consumption]*'* Gemma

Midwives discussed the sources of information that they knew or assumed that women used. Midwives considered leaflets, internet and forums to be the most popular methods utilised by women. Health information is one of the most frequently sought topics on the internet (McMullan 2006). Reuters, who are an online consumer / business reporting agency, have found that on average 53% of Americans search the internet for health information. With regards to the EU / UK, health professionals are still by far the main source of health information (45.3%) followed by traditional media (19.8%) and newspapers (7.4%) (McMullan 2006). They also talked about which methods they felt were most reliable. In general midwives felt that forums were unreliable sources of information as they were not monitored or evidence based sources. Forums are predominantly areas for communication, where women can post their view and opinions on their pregnancy.

The internet has become a major source of health information. It can improve womens' understanding of their pregnancy and empower them to make informed health decisions. Communication between the patient and their health care professional is vital for patient-centred care.

In saying this when discussing the sources of information that women used in greater depth, midwives felt that the guidelines were vague and were sceptical about whether they were evidence based.

'There is a lot of information, vague as it might be, the general consensus seems to be just to avoid it, but it's not very specific' Amy

Midwives had conflicting views on the information; some felt that women were not provided with enough facts in order to make an informed decision and others felt that women were bombarded with information. The term bombarded was used in many of the interviews, especially when talking about providing information to women. Although the midwives mentioned that there was conflicting advice and mixed messages they were confident in advising women to 'keep everything in moderation'.

'I think they can get bombarded with information that may not be relevant to their pregnancy' Monica

'In pregnancy women get bombarded with so much information, especially so much information about what they can and can't eat and what they can and can't do' Emily

When advising women on certain sources of information, midwives acknowledged that not every woman was computer literate, spoke English or had access to this 'easily' available information. Many of the midwives felt that the best source of information was the midwife herself; if the midwife advises the woman on dietary habits then this leaves little room for misunderstanding. Midwives were aware that they needed to target their information according to their audience (Athearn et al. 2004; Soltani & Dickinson 2005).

'I think women need to be very careful where they get their information from… so I think the best place is from a health care professional providing they know the evidence' Emma

During the interpretation phase I noted that there were many conflicting statements amongst specific populations. For instance, above, many midwives feel that sourcing their information from the midwife directly is the most appropriate and most reliable. Conversely, many midwives stated that they did not feel adequately equipped to provide information on this topic and thus would have been unable to address any issues women had surrounding coffee consumption. The only possible way to rectify this situation would be for midwives to be provided with the most recent, evidence based information regarding coffee consumption and pregnancy and following this they can confidently provide reliable information to their patients. Midwives and

their feelings of inability to tackle the specific issue of coffee consumption will be discussed in greater depth in Chapter 6.

5.15 Recommending Sources of Information

Another subtheme dealt with is the recommendation of other sources of information. There are many reasons a midwife may signpost women to other points of information provision; lack of time during antenatal clinic, too specific of a question, support from other mothers. As discussed above, women are motivated to make improvements to their diet and other lifestyle habits during pregnancy; recommending women search for further information or encouraging them to utilise other sources can often enhance or increase the likelihood of her succeeding in breaking these behaviours. Support and communication are also vital during the pregnancy period. Reading blogs and entering forums can often provide this support as they are usually written by mothers for mothers. Unfortunately, although midwives regularly encourage the use of other sources of information it is important to remember that women can often be subjected to inaccurate, unreliable information. As well as this, women may interpret information incorrectly and this could pose a risk to her and her unborn child.

When I discussed with midwives where they would recommend women to search for further information the majority suggested the internet, but reputable sources that are monitored or government led. Some of the midwives suggested that women use NHS Choices website or the Food Standards Agency. Only one of the midwives said that she would direct women to the Antenatal NICE Guidelines. Although these are the national guidelines utilised by health care professional they may be too complicated or could easily be misinterpreted.

As well as the government monitored sources stated above a few midwives mentioned the parent education classes. These are classes run by a group of midwives where they address particular aspects of pregnancy and offer women another opportunity to question the advice they have been given or address any aspect of their pregnancy that has been a cause for concern.

'I was on the Health Education team… we covered parent education class… we'd cover coffee consumption and chatting about that quite a lot in the health education

classes… They'd come to our classes at 35 weeks so it's only if they had a query or a question or if something came up in the media or something that we'd chat to them. It wouldn't be part of our agenda' Patricia

A very small number of midwives stated that they would encourage forum use. They acknowledged their unreliability and lack of evidence but were aware that they were easy to use and an excellent source of support and method of communication for pregnant women. As pregnancy is often a time of uncertainty women crave support and tend to compare notes with other women as pregnant progresses' and share their experiences (Rising 1998). Midwives play a vital role in directing women to safe sources of information and areas where they can communicate and gain the support the need without jeopardising the information based information that they have been given.

'[Forums] I think they're good, I mean I've looked on them and there's lots of women on there… discussing things but I've also noticed people asking questions and then it's the women giving them the answers back and therefore it's not always the same information that they're being told or it might be the same information but it's not the information that we would give them' Charlotte

'Babycentre… I used that quite a lot during my pregnancy for different things. That actually was recommended by my GP… he said that it was a really good website and him and his wife had just had their baby' Ashley

A few midwives said that they would research the specific topics themselves and then disseminate that information to the women who requested it. They felt that this was the safest and most reliable method as it eliminated the possibility of women misunderstanding the information and unduly worrying.

'I just tell them don't look online because a lot of time you get led off the research based evidence and look on people's opinions so you can't rely on it… I don't tell them or ask them to look online. If there's anything that I've not got information on I'll sometimes look myself and print off information for them' Rita

'If it was a real need to know exactly how much, I think what I would have to do then is go and do some investigation myself. Go and have a look at the research' Amy

It is important to note at this stage that some midwives also used lay methods of sourcing information; they used these mediums to inform themselves on certain topics and, following this, advise women. Using search tools such as 'Google' was common, with midwives stating that they would use these. These methods are convenient and easily accessible however reliability is questionable. The use of these will be discussed in further detail in Chapter 6, Section 6.9.

5.16 Lack of Emphasis placed on Coffee Information by Midwives

Midwives discussed providing information to women. There are limited resources and thus adequate time cannot always be dedicated to providing information to women on specific dietary information. A clear example of this was when one of the midwives compared her NHS community work with her private clients. She was able to dedicate more time to her private clients and therefore address more of their concerns. The reasons behind this were unclear and further probing was not conducted.

'I only really discuss it in that much depth with my private ladies' Monica

Marginalization of information, which was discussed above, was certainly a recurring theme amongst the midwifery interviews. Many midwives discussed the lack of resources and time; many midwives stated being pressured to cover the more important topics and not having time to cover those which they considered less important. It is often for this reason that midwives direct women to other sources of information. Encouraging women to research their own information can often be beneficial, providing that information is evidence based and reliable. Midwives must also be aware that some women are not computer literate, speak English or have the technology available to them. It is for that reason that the information and sources must be either catered to women individually or accessible to all.

5.17 Midwives want more information and feel more research is needed

When discussing the subtheme 'more information' I considered two aspects; whether more information needs to be provided to women and whether more information needs to be provided to midwives. I posed this question to midwives and obtained a mixed response. In general, many midwives felt that if coffee consumption did pose

a risk then further research was necessary and this information should be disseminated down accordingly. However, many midwives did feel that women experienced information overload and suggested that potentially a different format should be adopted to better provide the necessary information to women. This is especially important since more and more information is being created and provided to women.

The majority of midwives stated that there was a need for further information especially if there was a potential risk associated with coffee. Midwives felt that womens' concerns should be addressed with further information and discussions with their health care professional.

'Certainly if there is some thoughts that it might be related to miscarriage or pre-term labour then I think it's not worth the risk and if people can reduce it then they should' Lexi

Some midwives also felt the need for further midwifery information in the form of leaflets or campaigns specifically for health care professional. It was felt that if this information was rooted in evidence then women were more likely to take it on board. Midwives hoped to provide a 'gold standard' of care and cause an upheaval of the traditional practices. Many of the midwives were content with the methods they obtained their information, which was usually disseminated down from more senior members of the team. Not only did these midwives feel that information should be disseminated down through the ranks but that this information should be passed on to women through discussions and consistent advice.

There was of course those who were unsure of the exact advice and were unsure whether there was a need for any further information. They felt that there was only a need for research if a woman had a particular concern.

'I didn't know there was any different advice… I would always have said it was better to reduce your caffeine intake. I haven't read personally anything that has said otherwise, yea. But if a lady really wanted to carry on [with coffee consumption], *she would find out herself. And if somebody asked we could always direct her to other sites to look it up, couldn't we…'* Jenny

In conclusion, there was a desire for more information. Midwives felt that more research was needed and once the evidence had been collated, conclusions could be distilled and guidelines updated accordingly. The format in which this information was provided to pregnant women was also discussed with a general consensus being that it should be short, concise and easily accessible.

Chapter 6 Qualitative Discussion

6.1 Introduction to Qualitative Discussion

The following chapter will discuss the findings of my interviews with women and midwives' (Chapter 5, Qualitative interpretations) in more detail. It is important to revisit and acknowledge the lack of literature that surrounds coffee and pregnancy. As highlighted in Chapter 1, my literature search suggested that there was no literature surrounding the opinions and views of midwives with regard to coffee consumption during the gestational period. This, therefore, adds to the uniqueness of my work. My research involved interviewing pregnant women and practicing and academic midwives with regard to coffee consumption. The framework analysis of the interviews uncovered a number of themes, which I will now explore and expand on. Framework analysis, which was discussed in greater depth in Chapter 4, Section 4.5, was a flexible analysis process; the structure of this process allows for analysis during and after data collection. The key stages of framework analysis are familiarization, identification of a thematic framework, indexing, charting and finally, mapping and interpretation (Ritchie & Spencer 1994). Below I will discuss the central themes, and their implications, in greater depth.

Sound health behaviour decision-making requires accurate and up-to-date health knowledge. It is therefore necessary that medical professionals understand the pregnant womens' motivations and information seeking behaviour. Pregnancy, especially when it is the mother's first experience of the condition, is a major life event. It is a transitional period when a women is no longer solely responsible for her own health and wellbeing but also for that of her developing baby (Van Teijlingen et al. 1998; Szwajcer et al. 2009). My research corroborated this statement, with many women stating that they changed health behaviours and habits during their pregnancy. The majority of women interviewed were multiparous yet all discussed either their interest or anxiousness in health education during pregnancy.

In-depth analysis of womens' health behaviours, views and opinions permits health care professionals to better understand womens'' needs during their pregnancy; this then allows health care professionals to consider, predict, and influence the sources of information used by the women in their care. Although much literature surrounds

certain behaviours and habits, as stated above, no literature surrounded opinions or views on coffee consumption. There is often a focus on health behaviours and habits that are considered pathological (eg. Tobacco, alcohol) yet the uncertainties surrounding coffee and its effects may excuse the limited research. Providing quality patient information is generally considered as an essential tenet of good health care (Sheard & Garrud 2006). Health literacy and its impact on maternal wellbeing and pregnancy outcome will be discussed in further detail in Section 6.11.

Many studies highlight the limited knowledge of health care professionals regarding certain aspects of womens' health (Smith et al. 1994; Henwood et al. 2003). This is particularly true with regard to technological advances and modern health care practices. This aspect will be discussed in further detail in Section 6.9.

Diet during pregnancy was also addressed within my study. Habit plays an important role in food choice and consumption but we must consider that food habits are also situational (Triandis 1977).Womens' motivations and habitual behaviours influence their dietary choices during pregnancy (Szwajcer et al. 2007; Verbeke & De Bourdeaudhuij 2007). Given the importance of pregnancy in people's life the fact that specific dietary recommendations are issued leads us to assume that different food choices are being made by pregnant women (Anderson 2007; Wen et al. 2010). This will be discussed further in Section 6.4.

Maternity care in the United Kingdom, in contrast to the USA, is mainly provided within the public health-care system (Clark et al. 1991; Ferlie & Shortell 2001). It is free of charge and offers care from pregnancy through to the postnatal period. Midwives are responsible for caring for women with normal, uncomplicated pregnancies as well as for women whose pregnancies are affected by pathologies such as fetal growth restriction, gestational diabetes and pre-eclampsia, to name but a few. Almost all pregnant women attend a maternity hospital or local community-based clinic for their antenatal care, provided by their midwives, and will visit their midwives up to ten times during their pregnancy.

6.2 Health Information during Pregnancy

As discussed, pregnancy is a major life event for all women and therefore the promotion of healthy nutrition and other lifestyle behaviours is vital to ensure a

healthy outcome for mother and her baby (Schneider 2002; Hofberg & Ward 2003; Szwajcer et al. 2009). In many European countries, including the UK, health organisations are increasing midwives responsibility in their role as an intermediary in nutrition promotion (Guilbert 2003; Bandura 2004; Martins & McNeil 2009; Szwajcer et al. 2009). Many studies make the assumption that women are more likely to accept information provided by their midwife than by sources with less authority, expertise and trustworthy on matters surrounding pregnancy (Anderson et al. 1993; Margetts et al. 2001; Szwajcer et al. 2009; Borland et al. 2009). My study indicated that women did take the information they received from their Health Care Provider on board however this information was often not substantial enough and thus had to be supplemented. The need for information supplementation will be discussed in greater depth below.

The mode of health information was discussed in great detail with women and midwives' alike. The quality and delivery were subjects that both groups felt needed to be altered in order to improve its quality. The concept of golden standard or research was considered with the majority of midwives were aware that this was the only way to provide quality information. Unfortunately, dissemination of this information can often become bureaucratic and therefore impact the care that is being provided; midwives were unable to access information unless more senior members of staff shared this.

Traditional practices and technology with regard to health information were highlighted during my study. Many midwives discussed using the 'everything in moderation' statement as a safe recommendation during pregnancy. This concept has been studied within the literature and although effective in some circumstances could prove harmful without sufficient evidence to back it up (Koren 2000). Women, on the other hand, used more modern practices, often using technology to supplement the information given by their health care provider (De Santis et al. 2010). Although one would think that the traditional and modern practices would conflict, it is my understanding that the information gained from both was strikingly similar. Midwives and online sources often agreed, with both sources offering varying information. We could conclude that uncertainties surrounding coffee and its potential physiological effects are present in all sources of information provision.

These uncertainties were highlighted within my work and it was clear that more solid and comprehensive research was needed.

6.3 Midwives are Primary Providers of Care and Information

Midwives are the primary providers of care for childbearing women (Koblinsky, Matthews, et al. 2006; Koblinsky, Filippi, et al. 2006), and this was true of the women I interviewed within my study. Even though this is the case, there are considerable variations in the organisation of midwifery services; this is particularly apparent with regards to education and the role of midwives (WHO 2006). In many countries, for example North America, maternity care is predominantly obstetrician led for the vast majority of pregnant women (Vries et al. 2001). This is not the case for countries such as the UK, Ireland, Netherlands and New Zealand, where primary care is not the sole responsibility of the doctor but a combination of midwifery-led and medical-led (Hatem et al. 2009). The midwifery-led model of care is based on the premise that pregnancy and birth are normal life events and is woman-centred approach (Kirkham 2000; Hatem et al. 2009; Howarth et al. 2012). Continuity of care along with the monitoring of mothers wellbeing are all central concepts within the midwifery-led model. Continuity of care will be elaborated on further detail when we discuss the relationship between midwife and woman and what impact that has on pregnancy (Section 6.15). Within my study, the women interviewed received the majority of their care from midwifery professionals. Although this is standard for NHS care it is important to note that all women were recruited from a large NHS Trust in the North West of England and thus were registered there; no interviewees were receiving their care in a community setting.

Whether midwives are the best source of information has been debated in many studies (Hundley et al. 1994; Glazener & Statistician 1997; Graham 1997). For a woman and her partner enquiring about the safety and reliability of each practice, particularly with regard to information on diet, it is unacceptable to say we are unsure. To the general public, midwives amongst other health care professionals, are the primary source of information and advice. Their uncertainties further fuel the argument that more information is needed. The goal is to engage in a system where both patient and midwife and confident and satisfied in the knowledge provided (Graham 1997; Kirkham 2000; DeJoy 2010). It is for this reason that a more

complementary method should be adopted to ensure that women, and their baby, are being comprehensively cared for. The majority of women I interviewed relied heavily on the midwife, not only as their primary care giver, but for information on their pregnancy and what to expect at each stage. This viewpoint has been consistently backed up with the literature (Howarth et al. 2012). This relationship will be discussed in further detail below in Section 6.15. Although above we state that partners enquire about health during pregnancy, within my study I did not question the supportive role of the partner with regard to education; it may be the case that other parties to the pregnancy research diet and behaviours and may have considered coffee.

My study highlighted that midwives lacked the specific knowledge surrounding coffee consumption and pregnancy. Midwives were unsure of the actual information to provide on coffee consumption and thus erred on the side of caution. Midwives suggested that women reduce their coffee and caffeine intake however this information was not, according to them, evidence based.

Information continuity is also an issue when considering obstetrician-led versus midwifery-led care. Information continuity concerns the timely availability of relevant information. As is often the case, the most recent research and practices are available to more senior members of medical staff and it the disseminated amongst the medical staff on the frontline of maternal care (Smith et al. 1994; Waldenström & Turnbull 1998; Proctor 1998; Soltani & Dickinson 2005). Information dissemination has been the subject of many reviews for the past half century (Trist 1951; Feldman 1966; Connor 1967), with focus in recent years on evidence based practices and methods (Rosenberg & Donald 1995; Melnyk & Fineout-Overholt 2011).

What was clear from my study was the women very rarely questioned the information they were given by their midwife. Health professionals are often perceived as credible sources because of their level of expertise (Almeida & Graca 1997; Dillen & Hiddink 2003; Benoit & Strathman 2004; Szwajcer et al. 2005). It is felt that this expertise is verified by education, training and their experience in the field (Szwajcer et al. 2005). Knowledge through knowing will be discussed in Section 6.10. The women interviewed did not discredit any information they were given, however some did feel that information needed to be supplemented. Many of

the women I interviewed stated that they supplemented their information; sources included the internet and baby books. Although this being said, none of the participants revealed supplementing their current information with reputable sources or from other health care professionals, such as a nutritionist or dietician. We can, however, with a certain degree of confidence conclude that women respond more favourably to information provided by their midwife, especially one with which they have regular contact.

My study has a particular focus on the midwifery-led model as this is how midwifery care is provided to women in St. Mary's Hospital, Manchester. The midwifery-led model was briefly discussed above as a successful model for care in the NHS. It is defined as care covered by midwives during the antenatal, labour and postnatal period (Hatem et al. 2009). Midwives placed a particular emphasis on individualised education, counselling and antenatal care within this model, which was discussed during the interviews. There is a growing demand in our health care system for consumers to be provided with verbal, written, pictorial or recorded information; sources that will help them actively participate in their own health care as well as to make informed health decisions for themselves and their families. This is true in general medicine but is also becoming more relevant to pregnancy information and midwifery. Midwives discussed the concept of individualised care and information provision during the interviews; all women have a one-on-one booking appointment where they discuss the pregnancy and progress. Whilst this is the case, both midwives and women discussed their limited resources and time constraints. There is growing pressure on midwifery staff as birth rates slowly but steadily increase (Office for National Statistics, 2013 - 2014). The lack of time resulted in midwives having to provide what they perceived as being the most vital information (Aaronson et al. 1988; Carolan 2007; Szwajcer et al. 2009) which is very subjective and unreliable. It is important to note at this point that coffee consumption during pregnancy was not seen as a priority by the midwives; the midwives interviewed felt that discussing coffee consumption specifically. This further highlights the uncertainties surrounding coffee and its potential pathological effects.

It is important to acknowledge that many of the midwives I interviewed had a background in research. These midwives, in general, were aware of the most accurate and reliable sources of information however were unaware of the potential impact of

coffee consumption on pregnancy. This implied that these midwives did not perceive a potential harm with coffee consumption during the gestational period. This was further corroborated by the fact that these midwives stated that they were unaware but were intrigued when they researched either directly before or after the interviews. Transferability of findings refers to the degree in which the research can be filtered down to the midwives on the front line, as it were, and finally to the women concerned. Midwives were aware of the need for accurate information and made many viable recommendations and suggestions. The fact that midwives were aware of the most reliable sources of information yet were unsure of the most accurate information further highlights the fact that there is a definite gap in the knowledge. Transferability of results is a very important concept within research; studies suggest that the limitations of mono-method research, for example quantitative research, are the limited transferability of findings (Kelle 2008). The fact that there was a difference between the method by which midwives search for information highlights the need for more complete dissemination of information between all members of midwifery staff. This, however, also highlights that although the research midwives may have been better equipped to research coffee and its potentially harmful or beneficial impact they were unaware of its effects, highlighting the general lack of information.

In response to the demand for further information there has been a growing awareness in the health sector of the need to provide information to patients in a format that both meets their needs and is evidence based (Richardson et al. 1995; Johnson & Sandford 2005; Ciliska et al. 2008). There has been much debate amongst health care professionals on the format that information should be in. Suggestions have included verbal and written instructions, audio and videotapes, email and telephone communication with a health care professional and finally access to websites providing further information. A more modern technique, one which is becoming increasingly popular amongst today's youth, is mobile applications or 'apps'. An estimated 19% of smart phone owners have at least one health app on their phone (Fox 2012). The concept of providing information through this medium is now a widely discussed topic (Cummiskey 2011; Milošević et al. 2011; Buijink et al. 2012; Pandey et al. 2013). Many women interviewed discussed the use of mobile phones and applications for sourcing their information. These participants felt that

this was a convenient and more user-friendly method for accessing information. The women who utilised this method also liked the personable feel to the applications and its tailor made qualities for pregnancy information.

A vast quantity of written information is available on pregnancy and health education. Despite this, there have been very few studies undertaken to assess the effectiveness of this method of information provision (Arthur 1995). For example, brochures and leaflets are considered to be an interesting, useful and accessible method of information provision; this has been suggested to be the case especially with regard to pregnant women (Street 1991; O'Cathain et al. 2002; Szwajcer et al. 2009). In support of this assertion, Den Broeder *et al* (1999) also found that midwives, in a clinical setting, value the use of leaflets and attach high importance to their use for dissemination of information to pregnant women (Broeder et al. 1999). Thus both the service user and provider could potentially benefit from further, good quality, written information (Arthur 1995; Johnson & Sandford 2005; Szwajcer et al. 2009). These corroborate with my findings as some of the women and midwives' alike stated that they utilised and liked the written information provided during their care. However other studies, correspondingly to my findings, expressed doubt regarding the benefits of providing written information to pregnant women. For exampleBroeder *et al* (1999) discussed their concerns with regard to written and verbal nutrition communication in midwifery practice; they concluded that although efforts have been made to improve written information there are still relatively few studies on their effectiveness (Broeder et al. 1999). Health professionals are wary of whether providing written information is viable (Johnson 1999; Johnson & Sandford 2005). What women do with the information is generally unknown. As discussed, and corresponding to my study, many women stated that they did not read the information that they were provided with because they felt they did not need to or they did not have the time to (Savaş & Evcik 2000; Walker et al. 2005). As well as this, we must consider whether women comprehend the written information that they are provided with. Health literacy is defined as 'the genitive and social skills which determine the motivation and ability of individuals to gain access to, understand and use the information in ways which promote and maintain good health' (WHO, 1998). Comprehension of the information means more that reading the leaflets that are

provided; it concerns whether women have the capacity to use this information effectively and thus put it into practice (Renkert & Nutbeam 2001).

There must be a synergistic relationship between verbal and written nutritional information communication to ensure efficacy. Through this method there is an assurance that the weak points of both communication methods should be compensated for and thus information provision is improved (Johnson & Sandford 2005; Szwajcer et al. 2009). Potential methods for enforcing this synergistic approach will be discussed in the recommendations Section 10.3.

6.4 Pregnancy and Diet

There is a general consensus in the literature and amongst health care professionals that that there needs to be further investigations into nutrition and pregnancy (World Health Organisation 1965; Mulliner et al. 1995; Borland et al. 2009). What pregnant women eat, how their diet is impacted by their pregnancy and how a pregnant woman's diet is impacted by their socioeconomic status, cultural patterns, beliefs and advice received can potentially impact the *in utero* environment and thus impact pregnancy outcome (Pearce et al. 1991; Dosman et al. 2001; Athearn et al. 2004). A pregnancy can lead to an internal state of desire to give birth to a healthy child and this can stimulate a woman to be more aware of her nutrition (Szwajcer et al. 2009; Wen et al. 2010; Peadon et al. 2011). This is an area that has received much attention over the past 30 years (Borland et al. 2009; Barger 2010).

Diet considerations and pregnancy has long been a public health issue. In 1965 the World Health Organisation Expert Committee on Nutrition and Pregnancy and Lactation acknowledged that further investigations were needed on food intake, dietary habits and the beliefs of pregnant women (World Health Organisation 1965; Pearce et al. 1991). Two studies of particular interest are the Dutch Famine study, conducted in 1940's and the more recent TEEN's study. The Dutch Famine study was a particularly interesting study as it allowed for the investigation of under nutrition during specific periods of pregnancy; this study was unique in that it imposed nutritional restrictions on an otherwise well-nourished population thus removing the potential confounders, such as infection, specifically associated with famine (Susser & Stein 1994; Painter et al. 2005; Roseboom et al. 2006). The Dutch

Famine birth cohort study generally supported the fetal origins hypothesis; a suggestion the *in utero* environment has a direct impact on development of diseases in adulthood (Roseboom et al. 2006). This hypothesis has also been considered when discussing coffee consumption and the development of certain pathologies in adult life, however this will be discussed in greater detail in section 6.7 (Mongraw-Chaffin et al. 2009). The basic principle is that chronic diseases originate in the womb through adaptations made by the fetus in response to its environment (Ekbom 1998; Roseboom et al. 2006). The Dutch Famine Study also gave the fetal origins hypothesis further dimensions; we must now consider maternal nutrition at specific points in gestation and how the adaptations will impact adverse consequences for health in later life (Painter et al. 2005; Roseboom et al. 2006). As well as this, the study has highlighted the importance of maternal nutrition during the first trimester especially when considering the cardiovascular risk of the offspring (Roseboom et al. 2006). The major outcome was its potential to develop adequate dietary advice for women before and during pregnancy so as to reduce the risk of developing chronic diseases (Susser & Stein 1994). Maternal nutrition features consistently within my study. My interest focused on coffee consumption and there are many studies that link the beverage with pregnancy pathologies. My attempt to ascertain womens' consumption habits highlighted the need for more clarification; women were unsure of the recommended amounts and thus either adopted a precautionary stance and abstained from coffee or were unaware of the information and advice and for those reasons continued on with regular habits.

Despite the recommendations of the WHO the majority of studies report on nutritional composition and calorific content of foods rather than on the specific foods themselves (Pearce et al. 1991; Chen et al. 2009; Wen et al. 2010). This information and knowledge is meaningless if it is not put into context. Ideally, a multi-disciplinary approach needs to be adopted, endeavouring to gather the available literature and developing an evidence-based source for women and health care professionals (Pearce et al. 1991; Margetts et al. 2001; Athearn et al. 2004).

Womens' socioeconomic status, beliefs and practices influence their diet, pre- and post-conception. This was also true within my study; many women believed that coffee consumption was associated with physiological effects and thus abstained from the beverage during their gestation. Similarly, many women were influenced by

their previous pregnancy experience. These women, who had a successful outcome, were content in adopting the same approaches to diet as in previous pregnancies. Although within my work I did not focus specifically on socioeconomic status the majority of women were middle-lower class. Information on socioeconomic status and coffee consumption within this study was limited therefore comparisons could not be made. There is, however, potential for further research on coffee consumption, pregnancy and socioeconomic status. This will be discussed further in future work (Chapter 10, Section 10.3).

There is a limited body of literature surrounding food preference during pregnancy. There are many factors which impact a woman's decision on her diet during pregnancy. Diet is often taken into consideration before pregnancy too. Some women are aware that diet can have an impact on fecundability and thus make significant changes (Jensen et al. 1998; Szwajcer et al. 2007). A review by Higdon *et al* (2006) identified two studies that found consumption of greater than 300 mg caffeine daily was linked with significant delays in conception (Stanton & Gray 1995; Jensen et al. 1998; Higdon & Frei 2006).

Coffee consumption and caffeine intake, as discussed at great length in previous chapters has been linked with poor pregnancy outcome (Chapter 1, Chapter 2, Chapter 6, Chapter 9). The majority of studies focus on coffee / caffeine intake and delayed conception, miscarriage, developmental anomalies, prematurity and low birth weight. Although the focus of my study was on the coffee participants often responded to my questions by relating the questions to other pregnancy issues. Women openly discussed their concerns during the interviews and these will be discussed below.

6.5 Habitual Behaviours and its Influences on Diet

Psychosocial factors are now recognised as profound influences on diet during pregnancy. Food choices and dietary intake can be affected by psychosocial factors and potentially lead to poor nutritional status and health (Hurley et al. 2005; Szwajcer et al. 2005). Previous studies reported that well-educated, middle-class women who reported more weight restrictive dietary habits during pregnancy were more anxious, stressed, depressed, angry and felt less uplifted about their

pregnancies (Dipietro et al. 2003). A further study by Hurley *et al.* (2005) investigated whether further features of psychological stress were associated with variations in dietary patterns and intake during pregnancy (Hurley et al. 2005). Unfortunately, as with many studies, measurement errors were encountered since a questionnaire based approach was adopted (Hurley et al. 2005). A number of studies have investigated social and cultural influences on dietary habits. Many comparison studies have been carried out across the US, Europe, Africa and Asia and there was evidence to suggest that dietary changes during pregnancy were often superimposed upon existing food habits (Taggart 1961; Desai & Tavares 1980; Darwish et al. 1982; Olsen et al. 1989; Fairburn et al. 1992; al-Kanhal & Bani 1994).

Alcohol cessation during pregnancy was a particularly interesting, and relevant, investigation when considering the impact of culture and social setting on a woman's dietary decisions (Nilsen 2009). A particular study of interest was one conducted by Ockene *et al* (2002) who investigated spontaneous cessation of alcohol in a low-income, predominantly unmarried population (Ockene et al. 2002). This particular study found that older women with previous births and women with less support were less likely to abstain from alcohol; this study indicated that young women, women who are experiencing their first pregnancy and those with little social support should be targeted and offered the most encouragement (Ockene et al. 2002). This study and its findings have several implications for interventions and research. Similarly with my work, we must consider its implications on social policy and nutritional advice during the gestational period. The above article suggests that when it comes to providing education and interventions, women need realistic approaches and they are not often able to alter their environment. This study also recognised the importance of health care professionals as a source of social support; information needs to be communicated in a way that is sensitive and relevant within the context of the womens' lives in order to maximize the health benefits to themselves and their unborn (Hoffman & Hatch 1996; Ockene et al. 2002; Nilsen 2009). This study corroborated my findings. Women should be given more realistic advice with regards to coffee consumption during pregnancy; the participants interviewed were aware of the importance of accurate information and as well as the impact of diet on pregnancy outcome. Not only should these guidelines be realistic, but they should be evidence based and disseminated amongst the appropriate health care professional.

Unfortunately, my research highlighted the uncertainties surrounding coffee consumption during pregnancy. Many women were unfamiliar with the information that surrounds coffee and its effects. These participants were often unable to quote the referenced ranges or we unsure of the guideline amounts during pregnancy. This was also true amongst the midwives, who were unsure of the recommended daily allowance of coffee during pregnancy. Fundamental changes need to be made in order for the correct information to be obtained. From my research there is a clear need for entwinement of both quantitative and qualitative research on coffee consumption in order for the most accurate information to be provided. This will be discussed in greater depth in Chapter 10.

Whilst discussing habitual behaviours we can tie in womens' behaviours in previous pregnancies. As discussed above, previous pregnancy experience dictates future behaviours. Many participants interviewed, who experienced positive pregnancy outcomes, were keen to maintain the same or similar diet patterns as they did in previous pregnancy. This was also true for their coffee consumption; women either maintained their coffee consumption or abstained from the beverage completely. This suggests that first-time expectant mothers should be targeted when aiming to improve dietary habits. As we know, women are more motivated to improve health and lifestyle during the pregnancy period. Midwives also referred to their previous experiences, both personal and professional. Although much research has been conducted into the influence of experience on information provision we cannot argue that it is best practice; evidence based information is superior to experience based medicine.

It is likely that stronger associations between psychosocial factors and habitual behaviours would be found in a more heterogeneous population; for example low-income populations that often have poorer diets and experience higher levels of stressful life eventsare more predisposed to pathologies (Dipietro et al. 2003; Hurley et al. 2005; Kolasa 2009). These specific factors may play a role in pregnancy pathologies, and have been briefly considered within this work.

6.6 Stigma Attached to Coffee Consumption

There is an all too real stigma attached to coffee consumption in today's society. Articles are constantly being released in both broadsheet and tabloid newspapers (Appendix 8). Stigmatization of certain foods (e.g. alcohol; tobacco) often presents methodological challenges when hoping to investigate them. An early article in the Lancet (1981) suggested that coffee was potentially linked with detrimental health effects (Anon 1981). This light hearted article, although not the first, paved the way for more accessible methods of journalism to disseminate the information on coffee consumption to the wider public.

In utero environment has been associated with pregnancy outcome, and has previously discussed in Chapter 1 and Chapter 2. My studies did not investigate as to whether this was the case and thus cannot corroborate with the literature. Matching consumption to pregnancy outcome has been investigated previously however confounding factors are often not taken into consideration. These studies are often quoted in the media; this is a common method of dissemination of health information. There is no definitive literature on the stigma surrounding coffee consumption. Although this is the case, there are vast quantities of media literature on the potential effects of coffee consumption. The headlines are particularly sensational and are usually targeted at pregnant women. This tactic of scaremongering was also, somewhat, employed by the midwives when advising them to err on the side of caution with coffee consumption. Again, the lack of evidence based research to back up the media could be unnecessarily worrying women during this already stressful period of their life.

All too frequently, the samples used for studies are those of convenience and thus bias cannot be avoided. A good example of potential recall bias is observed in George *et al* (2006) where coffee and caffeine intake, when combined with smoking, was associated with risk of repeated miscarriage (George et al. 2006). Dews *et al* (2002) hypothesized that often bias and priming of subjects can lead to exaggeration in the literature (Dews et al. 2002; Nawrot et al. 2003). Bias and other limitations, both within the literature and within my study, will be discussed in Chapter 10, Section 10.2. Sample selection within studies should be representative of all pregnant

women and therefore the investigators can generalize, with as little limitations as possible, to the population as a whole (Leviton & Cowan 2002; David et al. 2010).

Coffee is not associated with a social stigma, unlike alcohol and tobacco consumption. However, as the Cafe culture increases in popularity, so too does it link in social interactions and it has been described as the lubricant of many social settings. It may, however, be the case that as more information and research is conducted into coffee and its active chemicals, a more pronounced social opinion will be developed and thus imposed on its consumers.

6.7 Adaptations to Pregnancy

Pregnancy is not only a time of psychological transition for a woman but it also a time when the woman's body must physiologically adapt to the pregnancy state. Major adaptation in maternal anatomy, physiology and metabolism are required for a successful pregnancy (Heidemann & McClure 2003; Gordon 2012). As discussed in greater detail above (Chapter 2, Section 2.6), hormonal changes significantly alter maternal physiology and these changes persist through the pregnancy the initial postpartum period (Gordon 2012; Heidemann & McClure 2003). For example, there is a significant increase in total body water during pregnancy from approximately 6.5 L to 8.5 L by the end of gestation (Heidemann & McClure 2003). Apart from the water content of the developing baby, the placenta and the amniotic fluid within uterine environment, additional water is accounted for by the expansion of maternal blood volume (blood volume increases from 1500 ml to 1600 ml, plasma volume increases from 1200 ml to 1300 ml and the volume of RBC's increases from 300 ml to 400 ml) (Theunissen & Parer 1994). In light of these increases in volume, pregnancy has been defined as a condition of chronic volume overload which results in maternal weight gain, heamodilution, physiologic anaemia of pregnancy, elevated maternal cardiac output (Longo 1983; Elrad & Gleicher 1985; Robson & Hunter 1989; Oppen et al. 1996; Gordon 2012). A number of compensatory changes are made by the mother's body to cope with this increase in volume. For example, altered vascular function leads to venous pressure in the upper extremities remaining unchanged during pregnancy; however pressure rises progressively across gestation in the lower extremities (10 cm H_2O at 10 weeks gestation to 25cm H_2O at 38 weeks gestation) (McLennan 1942; Elrad & Gleicher 1985). Further physiological changes,

such as the diminished pressor response to angiotensin II and vascular remodelling are all specific adaptations to pregnancy and were discussed previously (Chapter 2, Section 2.6). Vascular adaptations were of particular interest within my research; the effect of active coffee chemicals on maternal and fetoplacental vasculature was assessed using myography. The results indicated that, although trends were noted, no significant effect was noted. However, as we can see, vascular adaptations are not the only changes that occur during the gestational period.

Another example that appositely illustrates altered maternal physiology is the common nausea and vomiting (morning sickness) associated with early pregnancy. Nausea / vomiting is present in approximately 70% of pregnancies and is most common in the first trimester, between 4 and 8 weeks gestation (Weigel & Weigel 1989; O'Brien & Zhou 1995; Gadsby et al. 1997; Källén et al. 2003; Giannelli et al. 2003). Whilst the exact cause of nausea during pregnancy is still unknown, a number of putative mechanisms have been suggested; the body's reaction to the presence of high levels of hormone human chorionic gonadotropin, GI disturbances, genetic factors, olfactory, psychosocial and environmental factors (Goodwin 2002; Davis 2004; Wills & Forster 2008). It is because of the unknown nature of the cause of nausea during pregnancy that health care professionals often struggle in treating and managing the symptoms (Wills & Forster 2008). Nausea and vomiting in pregnancy can significantly reduce the quality of life for a pregnant woman and affect her ability to function at home and at work (Wills & Forster 2008).

As many women are often hesitant to take medication during their pregnancy, dietary and lifestyle changes are often the first lines of symptom management (O'Brien & Zhou 1995). Modifications to diet including consuming more carbohydrates, drinking carbonated drinks and increased rest periods have all been reported to ameliorate symptoms of nausea (O'Brien & Naber 1992; O'Brien & Zhou 1995; Lacroix et al. 2000). As is the case with much midwifery advice, there is little scientific evidence to advocate these measures; however they are popular 'tried and tested' suggestions. Traditional methods of practice are often favoured over evidence-based practices within midwifery. This will be discussed in further detail later on (Section 6.8). It has been observed that there are very few clinical trials have been conducted investigating the modern day commonly accepted advice surrounding dietary and lifestyle modifications (Hollyer et al. 2002; Davis 2004;

Jewell & Young 2009). Most evidence is anecdotal or provided by self-reports of pregnant women who have experienced nausea and vomiting in pregnancy (O'Brien & Naber 1992; Lacroix et al. 2000; Goodwin 2002)

It has been suggested that nausea in early pregnancy may explain the association between caffeine intake and miscarriage (Stein & Susser 1991). Nausea is predictor of a viable pregnancy; as discussed above, nausea during pregnancy can result in many women to have aversions to tastes and smells that were ordinarily well tolerated (Fenster et al. 1997). This correlated with my study as many women discussed their aversion to the strong smell associated with coffee.

Investigators have noted that there is an association between heavy caffeine consumption and miscarriage amongst women who reported nausea (Chapter 1, Section 1.12.2) but was absent among those who did not (Weigel & Weigel 1989; Fenster et al. 1997; Wen et al. 2001). As nausea is a predictor of a viable pregnancy the absence of an association in non-nauseated women could be partly explained by the fact that these women were at higher risk of miscarriage to begin with, making it difficult to detect any added risk associated to caffeine exposure (Giannelli et al. 2003).

It is not only the physiological adaptations of pregnancy that we must consider but the psychological must be considered. Pregnancy is a time of considerable change for women and the transition into motherhood is a topic of great debate in the literature. The concept of real pregnancy was mentioned in Chapter 5, the interpretations section. The idea that information should deal with 'real pregnancy' suggests that women desire information that is tailored to their unique needs. It may also highlight the concept that current information is unrealistic and therefore women feel it does not fit their pregnancy. Similarly, the interpretation of 'real pregnancy' may suggest that women want information on the aspects of pregnancy that may surface in day to day life. Information on coffee is very comparable to that on alcohol as it often discussed in the media however there is little to no solid information surrounding the direct effects. There is definite scope in this area to improve the information that women are provided with and this will be discussed in further depth within the recommendation chapter (Chapter 10, Section 10.3).

6.8 Traditional Practice

Traditional practices present within a particular culture, approaches to childbearing and the care of the pregnant woman are still apparent within the NHS System. Studies of evidence-based leaflets have indicated that literature provided for pregnant women did not promote informed choices (Stapleton et al. 2002). Indeed the choices made by women, and decision making processes by which these conclusions were reached, are still heavily circumscribed by the pressures and norms of the local obstetric culture. Studies in the UK have shown that there is little diversity in clinical practice and advice within maternity units (Stapleton et al. 2002; Party 2001). However, the role of the midwife is often reported as 'framing' information and guiding women towards making the right decision, whether the advice is evidence based or just common practice (McKenzie 2009; Freeman & Griew 2007). As a result, there is a possibility that pregnant women are not receiving the correct information, harming them and their unborn baby (Athearn et al. 2004).

As discussed above (Section 6.2) the way in which information is presented influences decision making, for both health care professionals and pregnant women (Bekker et al. 1999). Passive dissemination of information is ineffective in changing the behaviours of midwives (Stapleton et al. 2002). Midwives should be encouraged to actively participate in sourcing information and obtaining evidence based advice (Richardson et al. 1995; Melnyk & Fineout-Overholt 2011). Further recommendations on sourcing evidence based information and advice will be discussed below in Chapter 10, Section 10.3.

Dietary patterns and the basis for individuals and populations to select the foods they choose to eat are more complex. It is important to note this level of complexity when developing guidelines on diet during pregnancy. Many of the midwives discussed the difficulty with completely restricting women from consuming certain foods as often these specific items are essential as part of a varied and balanced diet (Margetts et al. 2001). As well as this, midwives must be aware that nutritional information more often than not has to be tailored to the individual (Verbeke & De Bourdeaudhuij 2007; Fouda et al. 2012; Soltani & Dickinson 2005). This ensures that all women and their unborn baby are given the best possible chance whilst respecting their wishes, be it cultural, religious or personal. This is particularly true for women experiencing

morning sickness, women from specific cultural and religious backgrounds, and women with specific dietary requirements (Choudhry 1997; Schilling 1986; Dindyal 2002; Darwish et al. 1982; al-Kanhal & Bani 1994; Jarvis 1983). According to the literature it is also ill-advised to adopt such an authoritative role with regards to dietary advice as many women do not respond well to this method of information provision. As discussed above, the majority of dietary advice, especially that surrounding coffee and caffeine consumption is broad and non-specific and can often be ambiguous.

As well as the information being ambiguous studies have shown that information and advice that is not actively supported by midwives is rarely undertaken by pregnant women (Stapleton et al. 2002). Childbearing women generally comply with expected norms in their encounters with staff, who they perceive as experts in the field (Howarth et al. 2012).

6.9 Modern Sources of Information

It has been argued that modern information technology has had more of a profound effect on routine life than the industrial revolution (McCartney 2000). A large percentage of the British population (73%) have access to a computer at home, and most have a connection to the internet (42%). As well as this, access to the internet using a mobile phone more than doubled between 2010 and 2013, from 24% to 53% of adults (Office for National Statistics & Britain 2013). The search topics vary between genders, with more women searching for health related information than men (Sarkadi & Bremberg 2005). Several studies have been conducted investigating internet use and have shown that patients frequently use the internet to search for medical information and advice (Tuffrey & Finlay 2002; Dhillon et al. 2003; Bernhardt & Felter 2004). There are many studies investigating internet use amongst pregnant women. The findings of my study, that women frequently use the internet to search for health-related information, have also been suggested by a study by Larsson (Larsson 2007). This study confirmed that the majority of women searched for information on the internet once a month or more, and most often in the beginning of their pregnancy. It is unsurprising that women have a stronger need for information during the first trimester; this is the point at which they enter a new life stage (Deutsch & Ruble 1988).

A qualitative study investigating womens' needs from antenatal care that was conducted in three European countries found that women requested new information to help them feel more confident and gain a more in-depth understanding of their pregnancy, along with gaining information which could be assimilated and applied to their previous pregnancy knowledge (Luyben & Fleming 2005; Larsson 2007). Women often rely on their previous pregnancy experience for information, and this will be explored in further detail below when I discuss the concept 'Knowing from Experience' in Section 6.10.

Studies have shown that patients want their health care professionals to provide them with advice on useful internet sites that are geared to specific health issues (Salo et al. 2004). These results were similar to my findings; women wanted detailed information from their health care professional. Many women discussed the need to supplement the information that they were given or stated their shock at the lack of advice or limited amount of time spent with them.

The internet provides an easily accessible forum to disseminate both accurate and inaccurate health information which could potentially facilitate but also jeopardise healthcare provision (Kunst et al. 2002). According to Kunst *et al* (2002) website credibility have only slight or moderate correlation with accuracy of information. They came to the conclusion that even apparently credible websites may not necessarily provide higher levels of accurate health information (Kunst et al. 2002; Drentea & Moren-Cross 2005; Larsson 2007). It is often the case that on websites references may not be provided to support statements / assertions; accurate referencing, according to many investigators, is an important criterion for the judgement of the quality of information (Impicciatore et al. 1997; Kunst et al. 2002; Weiss & Moore 2003). Although internet forums are considered good sources for some types of information, women were aware that they were personal opinions of forum users and thus an unreliable and overall inappropriate method. We must now consider womens' motivations for using unreliable sources of information, be they web-based or other, which I will discuss below.

6.10 Knowing Through Experience

Womens' experience of previous pregnancies plays a role in the advice and information that they will utilise. In my study I found that many women felt that they had the information that they required from their previous pregnancy, that they remembered what they were told or had researched enough during their first pregnancy and so did not need to read or research anymore. Some women felt that the information that was provided was common sense, advice that everyone would or should know. The idea that the information was common sense ran through many of the interviews yet conflicting views, advice and concerns often illuminated the fact that information was lacking.

Naturally, pregnant women will seek advice and information from those whom they are closest to. It is therefore reasonable to assume that women are encouraged if they see those around them having successful pregnancies. There is an idea or 'notion' in society that, if one person behaves in a certain way and they did not experience any adverse effects then it is fair to assume that nothing 'bad' will happen to them if they mirror that behaviour. This statement is particularly common with regards to smoking and alcohol consumption.

6.11 Health Information Literacy

Whether women understand the information and advice that they receive or not is a common talking point amongst health care professionals and researchers. Information provision is not monitored and this is, potentially, a serious public health issue. Women are often provided with numerical information about the probability that a specific pattern of behaviour will lead to a particular pathology (for example, women with a BMI >30 are three times more likely to suffer from pre-eclampsia (Wolf et al. 2001; O'Brien et al. 2003)). Although this is the case, the dissemination of this information has increased public awareness of potential health problems its effects on the individual (i.e. the personal) risk for these problems has been less dramatic (Weinstein 1998; Dosman et al. 2001; Brewer & Weinstein 2004). A possible explanation is that patients, pregnant women included, routinely misunderstand what can often be complex numerical information (Edwards et al. 2002). Confusion and misunderstanding information leads to misinterpret the

implications and this undermines and effect the information would have had on their beliefs and behaviours (Rothman & Kiviniemi 1999). Miscommunication is particularly apparent when considering the pathology pre-eclampsia, as we discussed above. If the risk of a woman's pregnancy being affected by a complication such as pre-eclampsia is 1 in 100, tripling the risk may sound a highly significant (when considered in those terms) and in this case it is. However, if the actual risk of a pregnancy complication is only 1 in 10,000, then tripling the risk to 3 in 10,000 is only a minor effect. Understanding the actual figures and risk is therefore only possible given all the information (O'Brien et al. 2003).

Miscommunication may not always as a result of insufficient health literacy on the behalf of the woman or the health care professional; miscommunication is often as a result of the language used (Browner & Preloran 2003; Binder & Borné 2012). It is essential that information is communicated in such a way that it is accessible to women in order for them to make an informed decision (Browner & Preloran 2003; Sankar 2004; Binder & Borné 2012).

We must consider language, not only when providing health information but when recruiting women and healthcare professionals to research studies. Data was not gathered on womens' ability to understand the information provided during their pregnancy. There is a possibility that pregnant women are unable to understand the information that is provided to them, verbal or written. The majority of women interviewed were white British and English was the first language of all participants interviewed. This is an important finding; women from ethnic minorities may be less likely to participate in studies and therefore we get unrepresentative findings.

Language is the corner stone of information. The language that is used, either in verbal or written information, can impact uptake. From my research, it was clear that some of the women felt that the information that they received was insufficient however none of them stated that they did not understand the information provided. The role of language proficiency is key to information uptake (English et al. 1996; Eijsden 2006; Binder & Borné 2012).

From a medical point of view, health care professionals need reliable information to establish the right diagnosis and an efficient and accurate treatment plan. However, from the pregnant womens' point of view, two needs need to be met; the need to

know and understand and also the need to feel known and understood (Ong et al. 1995). My findings corroborate with the literature; patients almost always want as much information as possible and health care professionals seem to underestimate this desire (Ong et al. 1995; Seefat-van Teeffelen et al. 2011).

6.12 Supplementing Information

Womens' need for information was clearly demonstrated in my study, with the majority of women stating their desire for further advice. Many of these women expressed a need for this information at their initial antenatal visit. My initial interpretation of the data suggests that many women feel the need to back up the information that they are given. A brief (midwife-led) discussion with women, supplying them with additional information, is probably most desirable and ensures that women understand the information that they are given.

Women stated that they liked the idea of discussing information with their midwife. Being 'told what to do' reduces the likelihood of women misinterpreting the advice and causing undue concern. There was a low level of concern amongst the women regarding coffee consumption, which I believe increased once the interview had concluded. Although every effort was naturally made to ensure that there was no undue worry, many women had stated that they had initially not thought about coffee consumption and thus the interview had highlighted the unclear information. These findings were not necessarily highlighted during the interview process as many women asked questions when initially approached in the antenatal clinic or once the interview concluded. Findings were documented in field notes; women indicated that when participants had time to think or were questioned about their coffee they became more aware of their consumption. Women could potentially elevate some of these concerns by discussing these with their health care professional; as stated, many women enjoy discussing issues with the midwives. Unfortunately, there are time constraints on the midwives time and thus the concept of discussing their concerns is more of an ideal than an actuality.

There is literature, especially surrounding first time mothers / young mothers, that verify my findings (Hoffman & Hatch 1996; DeJoy 2010; Seefat-van Teeffelen et al. 2011). Women seek support and information during this transitory period; their

midwife is a stable and continuous figure during the pregnancy period (Waldenström & Turnbull 1998; Hildingsson et al. 2002; Freeman et al. 2006). Women also discussed preferred methods of information provision. They liked compiling the information into one, accessible and user-friendly method, such as a magazine. However, although this was the majority feeling, the term 'pregnancy police' did arise at least once during the interviews. It is true to say that many felt that coffee was less important than tobacco and alcohol consumption; it is for that reason that both women and midwives' felt emphasis does not need to be put on its consumption during pregnancy. The idea that there is too much dietary information, or general information, features in much research and is subject to varying opinions (Aaronson et al. 1988; Rothman & Kiviniemi 1999; Soltani & Dickinson 2005; Sheard & Garrud 2006).

People are not passive or unbiased when the process the information that they have been given (Taylor & Brown 1988). Women welcome favourable information about their health and the health of their baby and although this is the case, it is also true that participants minimize or disregard unfavourable health information (Ditto & Lopez 1992; Chaiken 1992; Gerrard et al. 1996).

Womens' ability to discern between a reliable source or method was not investigated during my research. This should be taken into consideration as it plays an important role in uptake of information and, potentially, improving health outcome. Midwives ability to discern between accurate information is also uncertain. Their aptitude for discriminating between reliable sources could potentially impact the information that they pass along to their patients; if this information is unreliable it could influence the decisions or dietary choices that women make.

6.13 Willingness to Accept Information

As researchers we must consider womens' motivations behind supplementing the information that they are given by their healthcare providers. There is limited information on pregnant womens' internet habits and whether they use the internet as a source of information or just to supplement their information (Larsson 2007). There was a sense of urgency amongst some women as they felt that the information that they were provided with was not given on time. Women expressed surprise and

concern with the lack of information they received on nutrition, and drew comparisons between their previous experiences and this pregnancy. Women were particularly surprised that coffee and caffeine had not been mentioned preciously; although this was the case, very few women could state a potential risk associated with coffee consumption during pregnancy further confirming the finding that there is a relatively unjustified stigma attached to coffee.

As mentioned above a number of studies have shown that internet users are generally hesitant about the reliability / veracity of information that they seek out on the internet (Dhillon et al. 2003; Bernhardt & Felter 2004). Studies have also shown that experienced internet users tend to avoid commercial internet pages in favour of those web pages governed by Universities and Health care bodies, such as the NHS (Ziebland et al. 2004). Although this is the case in many studies Larsson (2007) found that the majority of her participants considered the information that they obtained on the internet as reliable (Larsson 2007). This too was true within my research as the majority of women said that they preferred to rely on governed websites. Their ability to discern between a reliable method or source was debatable and was discussed in greater detail in section 6.12.

Hart *et al* (2004) suggest that information has more "weight" if it is sourced from a University / medical webpage (Hart et al. 2004). This study found that very few patients challenge medical authority (Hart et al. 2004); this conclusion was also true of my study. Women articulated high levels of trust in their midwife and health practitioners; however it was interesting to note that they did not discuss the information that they had searched for online with their midwife.

It was a surprising finding in my study that so many highly educated women perceived the health information found on the internet trustworthy, as many studies have found that this is not the case (Impicciatore et al. 1997; Kunst et al. 2002; Weiss & Moore 2003). The potential reasoning behind this could be that women who are highly educated may feel that they have the appropriate training in critical thinking and therefore considered their judgement on assessing the reliability of information appropriate. Many women were able to distinguish the important factors that contribute to reliability of information (references; agreement with other sources of

information; etc.); however there is still a very obvious risk that women overestimate, that is the reliability of the information that they find online.

Another particularly interesting finding from my study was the limited amount of women who directly interacted with their midwife about the information that they had found on the internet. Neither women nor midwives had initiated discussions around the sources of information or surrounding the particular information. Studies have shown that participants / patients very rarely discuss information retrieved from the internet with their health-care professional unless that particular care-giver initiates the dialogue (Diaz et al. 2002). One study in particular indicated that if participants are directed to relevant information on particular health conditions they are likely to adhere to this advice (Alessandro et al. 2004).

Many studies state that it is the responsibility of the midwives providing antenatal care to support and direct women to more accurate and evidence-based sources of information (Ciliska et al. 2008; Stapleton et al. 2002; Hunter et al. 2008). This can be achieved by developing a trusting relationship with both the pregnant woman and her partner, this concept will be developed further below (Larsson 2007).

I can therefore confidently conclude that the key to successful information provision is a balance between computer technology and holistic midwifery care, which is a particular challenge for antenatal care in the 21st century (Posmontier 2002). Recommendations will be discussed below (Chapter 10, Section 10.3).

6.14 Pregnancy and Stress

Pregnancy can be a stressful time for many women. With all the conflicting advice on so many aspects of pregnancy, women are prone to worry. Another point to consider is womens' readiness to take all the information that they receive as truth. As discussed above, some women, especially young mothers, can be vulnerable and it is clear that consistent information should be provided. The need for a reliable source cannot be emphasized enough.

Similarly, women discussed many aspects of their pregnancy during the interviews, and although my research focused solely on coffee consumption, many women responded to my questions by drawing comparisons to other aspects that they were

concerned or unsure about. Pregnancy is a time of great change, and the stress, for a woman. The transition into motherhood can raise many questions for the mother and her partner. This is not only true for first time mothers; as research progresses so too does the information that women are receiving and what may have been true for previous pregnancies may not be relevant or accurate for current or future pregnancies.

Benoit and Stratheman (2004) found that women were more likely to communicate their pregnancy concerns with their midwife than with other pregnant women or those who were recently pregnant (Benoit & Strathman 2004). This is not always the case as many studies, including mine, noted that women turn to friends and family for information and support (Aaronson 1989; Hoffman & Hatch 1996; Bunting & McAuley 2004; Thompson et al. 2004).

Preconception information and dietary awareness was also a recurrent theme during the interviews. Women who were planning their pregnancies in advance had researched all potentially harmful substances and had altered their diets accordingly. Some women, however, were unaware of the precautions that expecting mothers are encouraged to take and thus were concerned for the welfare of their developing infant. This was apparent at the initial meeting; many women were curious for information once the title of my study was mentioned. Questions regarding coffee's potentially harmful effects were raised and some women stated that they were anxious as they had not considered coffee and thus had continued on with their normal consumption.

Stress during pregnancy in itself cam have potentially harmful effects. Stress induced pregnancy complications have been shown to be the cause of maternal and perinatal morbidity and there is considerable research being done worldwide on the effects. Some specific complications arising from prenatal stress include preterm labour, low birth weight babies, pregnancy-induced hypertension and developmental delays in the offspring (Mulder & Medina 2002; Cardwell 2013).

6.15 Relationship between Women and Midwives

The relationship between women and their midwife is a complex one. Literature suggests that a good relationship between women and midwives' may have a positive

influence on pregnancy outcome (Glazener & Statistician 1997; Freeman et al. 2006; Hunter et al. 2008). There are concerns surrounding the quality of midwifery care around the world, which have been highlight by the wealth of literature available. According to Hunter *et al* (2008) the quality of relationships are fundamental to the quality of maternity care (Hunter et al. 2008).

The rapid rise in internet use, which has been well documented, has the potential to shift information provision towards more equitable, and patient controlled relationships between practitioner and patient (Ferguson 1997; Hardey 2001; Bessell & McDonald 2002; Hart et al. 2004). Though there have been previous studies analysing the patient-health care professional relationship, research must be directed toward evaluating the impact of electronically obtained knowledge on this relationship. Further analysis of the current models may reveal new, emerging trends are taking place.

From my study, a major concern is the lack of communication between the women and their midwife. The relationship between the woman and the midwife is essential for a positive experience for woman during the childbearing period (Lundgren & Berg 2007). Very few women said that their midwife gave them direct information on coffee. Women rely heavily on their midwife for information about their pregnancy. As well as this, according to many studies, pregnancy is also an ideal time to give women general health information as they are very motivated to improve their lifestyle and do no harm to their unborn child. Interventions and support are key to improving lifestyle during pregnancy (DiClemente 2000; Kelley et al. 2001; O'Connor & Whaley 2007)

It is a fair assumption that a stronger relationship with the midwife could greatly benefit women, reduce adverse pregnancy outcomes and in the future decrease the strain on public health services. As well as, this a deeper understanding of the internet use is needed which could have the potential to advance the current models of practitioner-patient interaction and thus could be used in the educational preparation of new practitioners (Gothill & Armstrong 1999; Little et al. 2001).

In conclusion, the internet age is altering the patient-practitioner relationship. If midwives actively assist their patients in the information-gathering process an improved relationship may result. Gerber *et al* (2001) felt that by acknowledging the

ever evolving role of the health care professional and gaining a deeper understanding of the patients decision making process, the burden of responsibility will be shared (Gerber & Eiser 2001).

My study revealed that some health care professionals were defensive about their own computer literacy and the reliability of the information obtained from the internet. As a result, they tended to assert their medical authority more thus dismissing the potential positive impact of the on-line information.

In my study, and in corroboration with other studies (Scott 1990; Pollock & Grime 2002; Hart et al. 2004), time limitations at the clinical point of contact constrained the possibility of engaging in dialogue with women about the information that they had sourced from the internet about diet and lifestyle choices during pregnancy and how this might affect their pregnancy outcome.

The quality of the relationship developed between women and their midwife is fundamental to the quality of maternity care. There is a plethora of research evidence demonstrating the quality of relationships with health care professionals is fundamental to a woman's experience of child birth (Berg et al. 1996; Halldórsdóttir & Karlsdóttir 1996; Anderson 2000; Kirkham 2000; Lundgren & Berg 2007). It is worth noting that these relationships are rarely identified as causal factors in improved care, particularly in macro-level maternity care (Hunter et al. 2008) even though there is a wealth of evidence suggesting that the relationship between midwife and mother may affect the quality of the woman's childbirth experience (Niven 1994; Hunter 2006). It is for this reason that it is worth investigating this area further in the interviews that I conducted. Women are generally very receptive to information and are highly motivated to improve their lifestyles during their pregnancy (Lavender et al. 2001; Kroke et al. 2004; Dodd et al. 2008). Pregnant women are a particularly favoured and susceptible target group for advice as they are especially concerned not only for their wellbeing but also for that of their unborn child (Bryce & Enkin 1984).

The woman-midwife relationship can develop in three ways; midwife centred, woman centred or where the midwife acknowledges the woman's desire for more information and guides her in her search for knowledge.

There has been much research into the relationship between the midwife and patient / pregnant woman, especially with regard to information provision and responsiveness. Health professionals can respond to the more 'internet informed' patient in several ways. The relationship between midwife and woman can become 'health professional centred'. McMullen *et al* (2006) suggested that those health care professionals who are not trained in the latest technology practices may feel that their medical authority is being threatened by the information the patient brings and could possibly respond defensively by asserting their 'expert opinion' (McMullan 2006). It is possible that the midwives may steer women towards their preferred source of information and assert a more dominant, authoritative role when providing nutritional information (Anderson et al. 1993; Hart et al. 2004). A more desirable relationship, and certainly more beneficial to the woman, would be a 'patient centred' relationship. This method is also encouraged by the Department of Health; the health professional and patient collaborate to ensure all parties concerned are catered for (National Institute for Health and Care Excellence 2008; Chief Nursing Officers of England, Northern Ireland 2011). Many of the women, during the interviews, state that they did not have the time or motivation to search for information regarding nutrition. It was of interest to note that health professionals similarly stated that they did not have time to search for answers or solutions to every clinical situation that they may encounter, as well as not having time to analyse and inspect all the information relevant to a certain topic (McNulty 1987; McMullan 2006; Lagan et al. 2011a). This was particularly relevant to coffee consumption during pregnancy as many midwives felt that there were more pressing issues, such as drug, alcohol and tobacco use during pregnancy.

Despite the body of evidence suggesting the importance of a good midwife-woman relationship it is known that womens' experiences are often far from positive; for example woman often highlight their preconception concerns, and as the pregnancy progresses, increased fears surrounding pain management during delivery (Hunter et al. 2008). Women can be intimidated by information overload and it is possible that health care professionals do not acknowledge or recognise this problem. Woman-centred information provision encourages women to change their concepts on obtaining information and accept responsibility for 'owning' the information. The internet has also contributed to a shift in the role of patients from passive recipients

to more active consumers of information (McMullan 2006; Lagan et al. 2011b; Gold et al. 2012).

The third possibility is where midwives take a 'back seat' approach to providing information to women; i.e. the midwife simply recommends sources of information to the women the care for. Although not completely the case within my study, there were aspects of this which were present. Women were guided briefly to sources of information and 'left to their own devices'. This idea has been further discussed when we consider women feeling the need to supplement the information (Section 6.12). This has sometimes been referred to as 'internet prescription', where by midwives guide their patient to reliable and accurate information. It is important therefore that midwives know what is the current advice or accurate information on a particular topic and also to be able to direct women to reputable sites that provide this information. In addition to providing information much of the academic literature suggests that it is the role of health care professional to educate women and also to train them to filter / distil the relevant information effectively (Mulliner et al. 1995; Renkert & Nutbeam 2001; McMullan 2006). This is, however, assuming that health professionals themselves have been sufficient well-trained to pass on this skill. It has also been suggested that by combining the two above scenarios could create an ideal situation of woman-centred, professional-guided scenario.

During the interviews we discussed the ease at which women approached midwives with questions regarding any aspect of their pregnancy. Some midwives felt that in the past women may have been less likely to question their health care professional; they may have felt intimidated by the position of power held by the midwife or for personal reasons such as their weight or lifestyle choices. One midwife stated that women who had a high BMI may be more reluctant to ask for advice on lifestyle changes as they may feel embarrassed or ashamed if they to struggle to change their poor habits. In more recent times however women are more informed and empowered to question the advice they are receiving. Women were also more likely to question the advice and to ask questions about specific concerns if their midwife was more approachable and friendly; if the midwife developed a good rapport with the woman she was more likely to discuss any problems or worries she may have.

Although this is true some of the midwives were aware that women could often find information provision intimidating. The idea that midwives were there to make womens' lives 'miserable' was discussed along with their reluctance to take information on board unless provided in a positive manner. As discussed previously, some women considered midwives to be the 'pregnancy police', telling them what they should and should not do. This idea was touched on when we considered the language the women used when describing their experience of information provision. Many women stated that they were 'told to' abstain or adhere to certain things. The use of very authoritarian terms suggests that many women see their midwives in a dominant role, as an expert in their field.

Developing a strong relationship with the midwife was also associated with continuity of care in much of the literature (Fleming 1998; Hunter 2006; Lundgren & Berg 2007). Continuity of care is a fundamental principle underpinning woman-centred care. Continuity of care has been defined as a shared philosophy through which the provision of care by a known care giver or a small group of care givers is delivered throughout the childbirth experience (Freeman et al. 2006). If the women had one midwife throughout her pregnancy, that midwife was better able to advise her, as she was more aware of her lifestyle and habits. Similarly, in meeting with the same midwife, women would feel more comfortable if a familiar face greeted them. Establishing a relationship may encourage women to disclose more information on their lifestyle habits or concerns. When it is possible to develop meaningful relationships work is also fulfilling for midwives (Curtis et al. 2002; Hunter et al. 2008). Midwives acknowledged that this method of providing care is not always possible (community care versus hospital antenatal care). Resources are stretched and in order to provide efficient and accurate care sometimes it is not always possible to dedicate as much time as women want to them.

In essence, without the existence of strong relationships between women and their midwife; any disruption threatens the integrity of the system as a whole. One would recommend, particularly with regards to my work, that we develop collaborative research to further explore the complex issues of relationships and communication skills.

6.16 Summary

In summation, the above points highlight that my study was uniquely different from those previously conducted. The literature reviews conducted suggest that there was a clear gap in the knowledge with regards to women and midwives' opinions on coffee consumption during the gestational period. More specifically, when women and midwives' were interviewed it was clear that there was very little definitive information provided. Many of the women were aware of the stigma attached to coffee consumption and thus removed coffee and caffeine from their prenatal diet. This, although a recommended precautionary measure, may not be necessary as there is still little knowledge surrounding the physiological effects of coffee, particularly in the pregnant state. Midwives, similarly, were unsure of the recommended quantities allowed during pregnancy however erred on the side of caution and advised women to reduce or remove intake during pregnancy. Again, this information is precautionary as there is still little to no evidence that coffee consumption results in poor pregnancy outcome when consumed in moderate amounts. Women and midwives' conflicted when questioned about sources and modes of information. Women felt the need to supplement information that they were given by their health care professional with more modern sources. Types discussed included internet and mobile phone applications. This suggests that health information is not meeting the needs of women and providers need to be aware of this for future production. Midwives however felt that written information was the most appropriate as it was reliable and easily accessible. This was true however did not reflect the opinions of the women interviewed. There was a clear division between the more traditional practices and modern methods. From the data gathered it is clear to see that a more desirable method of information provision is needed; this should be evidence based, easily accessible and cater to the needs of the individual. Although the internet seems like an obvious choice the fundamental issue is that the information provided will be evidence based and finally disseminated to those providing the front line care. Finally, when considering the relationship between the midwife and the pregnant women we must acknowledge the ever changing role during the 9 months. Midwives are a source of support and advice during the early stages of pregnancy for many women. All women interviewed had had appointments with their midwives and discussed relatively positive experiences. As pregnancy progresses midwives roles

evolve to more support and health care provision, as well as care from the woman and baby during labour and after. This ever changing role places pressure on the midwife to cover all aspects of pregnancy, particularly in a short amount of time. Midwives are expected to prioritise during antenatal appointments; placing importance on issues and subjects which they feel are more relevant to the woman and her individual pregnancy. None of the midwives singled out coffee consumption suggesting that it was not a subject of high priority. This could be due to the absence of knowledge surrounding coffee consumption or the lack of time during the appointments.

This study advances our knowledge because we now have a greater understanding of women and midwives' views and opinions on coffee consumption during the gestational period. As well as this, we have an appreciation of the opinions of women and midwives' on prenatal advice and information. This study has paved the way for future work; a more in depth analysis on the sources, mode and quality of information. Whether the information sourced by women and midwives' is evidence based is still unclear.

Chapter 7 Quantitative Methods

7.1 Aims and Objectives

The three aims to my study:

1. Determine the effects of active coffee chemicals caffeic acid, chlorogenic acid and caffeine on placental chorionic plate artery function;

2. Determine the effects of active coffee chemicals caffeic acid, chlorogenic acid and caffeine on maternal (myometrial) arteries and finally;

3. Compare / contrast placental with maternal effects of these active coffee chemicals.

7.2. Ethical Approval

This study was conducted with the approval of the North West (Haydock Park) research ethics committee (Reference Numbers: TB 08 / H1010 / 55 - CTB 08 / H1010 / 55(+5)) and informed consent was obtained from all women prior to participation. An example of the Tissue Bank Information Sheet and Tissue Bank Consent form can be found in Appendix 7. All specimens were therefore obtained ethically and with appropriate consent; the Biobank is licensed by the Human Tissue Authority. The investigation conforms to the principles outlined in the declaration of Helsinki (WMA 2013).

At the local level, the research and innovation office at St. Mary's Hospital, Manchester, gave approval for the above study.

7.3 Study Groups and Inclusion Criteria

Women were recruited for inclusion in my study if they were undergoing elective Caesarean section at term (37 + 0 to 42 + 0 weeks gestation) with otherwise uncomplicated pregnancy (i.e. no evidence of gestational hypertension; fetal growth restriction; gestational diabetes). Individualized Birth Ratios (IBRs) relate to a predicted birth weight calculated using independent coefficients for gestation at delivery; fetal sex, parity, ethnicity, maternal height and booking weight (De Jong et

al. 1999; Wilcox et al. 1993; Gardosi & Mongelli 1995). If the IBR was ≤ 5th centile for gestational age it was not included in the study as babies that fall within this group are likely to indicate underlying pathology. Women with pre-existing medical disorders (e.g. hypertension; infection) and pregnancies with identified fetal anomalies were also excluded.

7.4 Sample Collection

Whole placentas from non–complicated, term pregnancies (37 + 0 to 42 + 0 weeks gestation) were collected within 30 minutes post Caesarean section delivery. Samples were collected from the theatre using a double containment method; this involved using a sealed containment bag and a plastic, sealed bucket.

Chorionic plate arteries were identified directly from the umbilical artery where the umbilical cord inserts to the placental disc. Once a suitable area of the placental chorionic plate was identified (an area rich in branches of artery on the surface of the chorionic plate) a biopsy was taken using surgical scissors. These biopsies were taken and placed into a small dissecting dish containing ice-cold physiological saline solution (PSS; for composition see Section 7.12). The sample was pinned to the Sylgard® (Sigma Aldrich, Poole UK) surface using small dissecting needles. Small chorionic plate arteries (100 – 500 μm) were dissected from the surrounding connective tissue with the aid of a stereomicroscope. Great care was taken so as not to damage the vessel wall; using forceps vessels were cleaned of blood and excessive connective tissue. This was done using dissecting scissors and forceps and cut into four 2 mm lengths and placed in a myograph bath ready for mounting on wires.

Myometrial tissue biopsies were harvested during the elective caesarean sections. Myometrial samples were taken from the upper lip of the uterine incision. Samples were collected in Tissue Collection Buffer (XM) and transported on ice. Myometrial biopsies were placed in ice-cold PSS whilst vessels were being dissected. These biopsies were taken and placed into a small dissecting dish containing ice-cold PSS. The sample was pinned to the Sylgard® (Sigma Aldrich, Poole, UK) surface using small dissecting needles. Small myometrial arteries (100 – 700 μm) were dissected from the surrounding connective tissue under a stereomicroscope. Great care was taken so as not to damage the vessel wall; using forceps vessels were cleaned of

blood and excessive connective tissue. This was done using dissecting scissors and forceps and cut into four 2 mm lengths and placed in a myograph bath ready for mounting on wires.

7.5 Wire Myography

7.5.1 Chorionic Plate Artery

Lengths of arteries (~2 mm) were dissected out very promptly, as described above. These were then mounted onto a Danish Myotechnologies 610 wire myograph (Danish Myotechnology, Aärhus, Denmark). Two 40 µm steel wires were inserted into the lumen of each vessel. These wires were then attached to the jaws of the bath and secured under the screws. Four vessels were mounted in this fashion. Vessel length was measured using a calibrated eyepiece micrometer attached to the microscope. Vessels were bathed in ~6 ml of gassed PSS solution. For chorionic plate arteries solutions were gassed with 5% oxygen, 5% carbon dioxide balanced with nitrogen. Solutions used for myometrial artery experimentation were gassed with 5% carbon dioxide balanced with air.

A micrometer is attached to one of the jaws of the myograph bath. This allows for movement of the wires and permit vessel normalization (see Section 7.5.2). A strain gauge is connected via a transducer to the opposing jaw. In response to contraction of the mounted vessel segment, the myograph is able to measure force generation (mN/mm) under isometric conditions.

7.5.2 Normalization and Steady State Conditions

Determination of the blood vessel resting luminal diameter is important as it is necessary to set arteries to an internal circumference which achieves either 1) optimal responses from the artery or 2) sets the artery at a baseline similar to that expected to be present *in vivo* (Mulvany & Halpern 1977; Mulvany & Aalkjær 1990). Classical normalisation is a method of determining optimal diameter for contraction under which to place an arterial segment in vitro. The method aims to calculate the internal circumference of the vessel when fully relaxed and under a transmural pressure of 13.3 kPa (for a systemic vessel). The method was original

described for use with rat mesenteric arteries (Mulvany & Halpern 1977; Mulvany & Aalkjær 1990). Data by Wareing et al (2002) suggested that placental arteries can normalized in a similar fashion but that a lower value of 5.1 kPa should be used for normalization purposes. This places the arteries at a more appropriate pressure similar to that which they would experience *in vivo* (i.e. fulfils criterion 2 above) (Wareing et al. 2002).

In order to mimic *in utero / in vivo* conditions the vessel mounted on the wire support was allowed to equilibrate at 37°C for approximately 30 minutes. Zeroing the vessels involved slowly turning the micrometer, decreasing the values on the micrometer barrel, until there was a sudden negative deflection. Once this was done, the micrometer was adjusted slightly to ensure that the jaws were not touching and I then began the normalization process. This involved stretching the vessel in steps, raising passive force. Vessels were stretched by approximately 100 µm by moving the micrometer and allowed to equilibrate for approximately 2 minutes. Following this recovery period the passive force was recorded and wall tension was calculated. As the change in tension was measured and the internal radius calculated from knowing the micrometer readings and the wire diameters (40 µm), the equivalent increase in pressure was generated. Finally, the artery was partially relaxed by micrometer adjustments to reduce the circumference to 0.9 of $L_{5.1\ kPa}$. This process can take approximately 4 to 6 stretches. Data was collected and analysed using the Myodaq normalization software package (Danish Myotechnology, Aärhus, Denmark).

For myometrial arteries, a similar zeroing and normalization protocol is followed. Transmural pressure for myometrial arteries is greater, at 13.3 kPa. This process can take approximately 4 - 6 stretches.

$$\text{Effective pressure} = \frac{\text{Wall Tension x } 2\pi}{\text{Internal Circumference}}$$

Figure 10: Laplace relationship to calculate the effective pressure of the vessel

7.5.3 Measuring Chorionic Plate Artery Viability

Chorionic plate arteries were assessed for viability with a potassium physiological saline solution (KPSS) (120 mM; equimolar substitution of KCl for NaCl) solution. KPSS is a depolarising agent; two exposures separated by 10 minutes were performed and vessels were excluded if the constriction was less than 1.0 kPa. Post-exposure, the bath was drained and replaced with the "standard" PSS. Vessels which failed to constrict sufficiently were eliminated from the study. Once a stable baseline was achieved chorionic plate arteries were constricted to a maximal dose of the Thromboxane- A_2 (TXA$_2$) mimetic U46619 (10^{-6} M). Acetylcholine (10^{-6} M) was added to pre-constricted vessels so as to assess vessel relaxation. Once satisfactory results were achieved the arteries were repeatedly washed with PSS to achieve a stable baseline.

7.6 Effect of Coffee Phenols on Chorionic Plate Artery Function

7.6.1 Caffeic Acid Dose Response Curve

In order to assess the effect of caffeic acid on chorionic plate arteries, a dose response curve was constructed. I produced a 10^{-2} M stock solution of caffeic acid by adding 19.2 mg of caffeic acid to 10.657 ml DMSO (Dimethyl Sulfoxide) followed by gentle mixing. DMSO is an inorganic solvent, as well as dissolving polar and non-polar compounds. This solution was then stored at -20 ℃ until required. The stock solution was then made up into three solutions; A, B and C, in order to complete a serial dilution of 10^{-10} M to 10^{-5} M. This was made up fresh on the day of experimentation.

Solution	Method
Solution A (10^{-6} M)	25 µl of 10^{-2} M caffeic acid stock solution in 225 µl DMSO
Solution B (10^{-8} M)	10 µl of A in 990 µl DMSO
Solution C (10^{-10} M)	10 µl of B in 990 µl DMSO

Table 9 Protocol for creating desired concentration for serial dilutions of caffeic acid from stock solution (10^{-2} M).

Once a stable baseline was achieved incremental doses of caffeic acid (10^{-10} M-10^{-5} M) were added at 2 minute intervals.

µL	Concentration (M)	Solution
6	10^{-10}	C
54	10^{-9}	C
5.4	10^{-8}	B
54	10^{-7}	B
5.4	10^{-6}	A
54	10^{-5}	A

Table 10: Illustration of serial dilutions used to construct dose response curve to caffeic acid including final bath concentration and amount of stock solution that was added to each bath.

To assess the potential relaxatory effect of caffeic acid on chorionic plate arteries were stimulated with a maximal dose of the TXA_2 mimetic U46619 (10^{-6} M). Similarly to the previous dose response, once a stable constriction was achieved,

incremental doses of caffeic acid (10^{-10} M - 10^{-5} M; Table 10) were added at 2 minute intervals.

Observations will be discussed in Chapter 8 and Chapter 9.

7.6.2 Chlorogenic Acid Dose Response Curve

In order to assess the effect of chlorogenic acid on chorionic plate arteries, a dose response curve was constructed. I produced a 10^{-2} M stock solution of chlorogenic acid by adding 13.2 mg of caffeic acid to 3.726 ml DMSO (Dimethyl Sulfoxide). This solution was then stored at -20 ºC until required. The stock was then made up into three solutions; A, B and C, in order to complete a serial dilution of 10^{-10} M to 10^{-5} M. This was made up fresh on the day of experimentation.

Solution	Method
Solution A (10^{-6} M)	25 µl of 10^{-2} M chlorogenic acid stock solution in 225 µl DMSO
Solution B (10^{-8} M)	10 µl of A in 990 µl DMSO
Solution C (10^{-10} M)	10 µl of B in 990 µl DMSO

Table 11: Protocol for creating desired concentration for serial dilutions of chlorogenic acid from stock solution (10^{-2} M).

Once a stable baseline was achieved incremental doses of chlorogenic acid (10^{-10} M - 10^{-5} M) were added at 2 minute intervals.

µL	Concentration (M)	Solution
6	10^{-10}	C
54	10^{-9}	C
5.4	10^{-8}	B
54	10^{-7}	B
5.4	10^{-6}	A
54	10^{-5}	A

Table 12: Illustration of serial dilutions used to construct dose response curve to chlorogenic acid including final bath concentration and amount of stock solution that was added to each bath.

To assess the potential relaxatory effect of chlorogenic acid on chorionic plate arteries were stimulated with a maximal dose of the TXA_2 mimetic U46619 (10^{-6} M). Similarly to the previous dose response, once a stable constriction was achieved, incremental doses of chlorogenic acid (10^{-10} M - 10^{-5} M; Table 12) were added at 2 minute intervals.

Observations will be discussed in Chapter 8 and Chapter 9.

7.7 Effect of Exogenous Reactive Oxygen Species (ROS) on Chorionic Plate Artery Function

The effect of reactive oxygen species (ROS) on chorionic plate artery reactivity were assessed using direct application of H_2O_2 and a generating system, xanthine and xanthine oxidase (XA / XO).

7.7.1 H_2O_2

Chorionic plate arteries were normalized and assessed for viability as described in Section 7.5.2 and Section 7.5. Vessels were incubated with caffeic acid or

chlorogenic acid (10^{-5} M) for a period of 15 minutes. Control vessels were incubated with DMSO for same length of time. Vessels were then incubated with H_2O_2 (10^{-4} M) for a further 15 minutes. Finally all vessels were washed repeatedly with PSS to stable baseline.

7.7.2 XA / XO

Chorionic plate arteries were normalized and assessed for viability as described in Section 7.5.2 and Section 7.5.3. Vessels were incubated with caffeic acid or chlorogenic acid (10^{-5} M) for a period of 15 minutes. Control vessels were incubated with DMSO for same length of time. Vessels were then incubated with xanthine (XA; 10^{-4} M) plus xanthine oxidase (XO; 10 mU / ml) for a further 15 minutes. Finally all vessels were washed repeatedly with PSS to stable baseline.

7.8 Caffeine

7.8.1 Chronic Exposure

Chorionic plate arteries were normalized and assessed for viability as described in Section 7.5.2 and Section 7.5.3. In order to assess the chronic effect of caffeine on chorionic plate artery, vessels were incubated with caffeine (10^{-5} M) from the beginning of the experiment. Control vessels were incubated with PSS. Standard start protocol (KPSS x2; U46619; Acetylcholine) was followed as stated above (Section 7.5.2 and Section 7.5.3). Vessels were then incubated with a single dose of H_2O_2 (10^{-4} M) for a period of 15 minutes to assess antioxidant properties. Finally all vessels were washed repeatedly with PSS to stable baseline.

7.8.2 Acute Exposure

Chorionic plate arteries were normalized and assessed for viability as described in Section 7.5.2 and Section 7.5.3. To assess the acute effect of caffeine on chorionic plate arteries vessels were incubated for 15 minutes with caffeine (10^{-5} M). Control vessels were incubated with PSS. Vessels were then incubated with a single dose of H_2O_2 (10^{-4} M) for a period of 15 minutes to assess antioxidant properties. Finally all vessels were washed repeatedly with PSS to stable baseline.

7.9 End Vessel Viability and Termination of Experiment

To complete the experimental protocol all chorionic plate arteries were washed with PSS to a stable baseline. A further 120 mM KPSS was conducted to ensure that vessel viability was not compromised during experimentation.

7.8 Wire Myography

7.8.1 Myometrial Arteries

Myometrial arteries were dissected out from biopsies, as described above (Section 7.4). These vessels were cut to similar lengths as chorionic plate arteries and mounted in a similar fashion. These were gassed in 20% oxygen balanced with nitrogen; bath temperature was maintained at 37°C.

7.8.2 Measuring Myometrial Vessel Viability

Myometrial arteries were assessed for viability with a KPSS (120 mM; equimolar substitution of KCl for NaCl) solution. KPSS is a depolarising agent; two exposures separated by 10 minutes were performed and vessels were excluded if the constriction was less than 1.0 kPa. Post-exposure, the bath was drained and replaced with the "standard" PSS. Vessels which failed to constrict sufficiently were eliminated from the study. Once a stable baseline was achieved myometrial arteries were constricted to a maximal dose of the Arginine Vasopressin (AVP; 10^{-8} M). Bradykinin (BK; 10^{-6} M) was added to pre-constricted vessels so as to assess vessel relaxation. Once satisfactory results were achieved the arteries were repeatedly washed with PSS to achieve a stable baseline.

7.9 Effect of Coffee Phenols on Myometrial Vessel Function

7.9.1 Caffeic Acid Dose Response Curve

In order to assess the effect of caffeic acid on chorionic plate arteries, a dose response curve was constructed. A 10^{-2} M stock solution of caffeic acid was prepared as described above and in Section 7.6.1.This was then made up into three solutions;

A, B and C, in order to complete a serial dilution of 10^{-10} M to 10^{-5} M. This was made up fresh on the day of experimentation.

Solution	Method
Solution A (10^{-6} M)	25 µl of 10^{-2} M caffeic acid stock solution in 225 µl DMSO
Solution B (10^{-8} M)	10 µl of A in 990 µl DMSO
Solution C (10^{-10} M)	10 µl of B in 990 µl DMSO

Table 13: Protocol for creating desired concentration for serial dilutions of caffeic acid from stock solution (10^{-2} M).

Once a stable baseline was achieved incremental doses of caffeic acid (10^{-10} M -10^{-5} M) were added at 2 minute intervals.

µL	Concentration (M)	Solution
6	10^{-10}	C
54	10^{-9}	C
5.4	10^{-8}	B
54	10^{-7}	B
5.4	10^{-6}	A
54	10^{-5}	A

Table 14: Illustration of serial dilutions used to construct dose response curve to caffeic acid including final bath concentration and amount of stock solution that was added to each bath.

To assess the potential relaxatory effect of caffeic acid on chorionic plate arteries vessels were stimulated with a maximal dose AVP (10^{-8} M). As with placenta (Section 7.6.1), once a stable constriction was achieved, incremental caffeic acid doses (10^{-10} M -10^{-5} M; Table 14) were added at 2 minute intervals.

Observations will be discussed in Chapter 8 and Chapter 9.

7.9.2 Chlorogenic Acid Dose Response Curve

In order to assess the effect of chlorogenic acid on myometrial arteries, a dose response curve was constructed. I produced a 10^{-2} M stock solution of chlorogenic acid as described above and in Section 7.6.2. This was then made up into three solutions; A, B and C, in order to complete a serial dilution of 10^{-10} M to10^{-5} M. This was made up fresh on the day of experimentation.

Solution	Method
Solution A (10^{-6} M)	25 µl of 10^{-2} M chlorogenic acid stock solution in 225 µl DMSO
Solution B (10^{-8} M)	10 µl of A in 990 µl DMSO
Solution C (10^{-10} M)	10 µl of B in 990 µl DMSO

Table 15: Protocol for creating desired concentration for serial dilutions of chlorogenic acid from stock solution (10^{-2} M).

Once a stable baseline was achieved incremental doses of chlorogenic acid (10^{-10} M - 10^{-5} M) were added at 2 minute intervals.

µL	Concentration (M)	Solution
6	10^{-10}	C
54	10^{-9}	C
5.4	10^{-8}	B
54	10^{-7}	B
5.4	10^{-6}	A
54	10^{-5}	A

Table 16: Illustration of serial dilutions used to construct dose response curve to chlorogenic acid including final bath concentration and amount of stock solution that was added to each bath.

To assess the potential relaxatory effect of chlorogenic acid on myometrial arteries vessels were stimulated with a maximal dose of the AVP (10^{-8} M). Similarly to the previous dose response, once a stable constriction was achieved, incremental doses of chlorogenic acid (10^{-10} M - 10^{-5} M; Table 16) were added at 2 minute intervals.

Observations will be discussed in Chapter 8 and Chapter 9.

7.10 Effect of Exogenous Reactive Oxygen Species (ROS) on Myometrial Vessels

The effect of reactive oxygen species (ROS) on myometrial artery reactivity were assessed using direct application of H_2O_2 and a generating system, xanthine and xanthine oxidase (XA / XO).

7.10.1 H_2O_2

Myometrial arteries were normalized and assessed for viability as described in Section 7.8.2. Vessels were incubated with caffeic acid or chlorogenic acid (10^{-5} M) for a period of 15 minutes. Control vessels were incubated with DMSO for same length of time. Post-incubation vessels were stimulated with a maximal dose of AVP

(10^{-8} M) to produce a constriction. Vessels were then incubated with H_2O_2 (10^{-4} M) for a further 15 minutes. Finally all vessels were washed repeatedly with PSS to stable baseline.

7.10.2 XA / XO

Myometrial arteries were normalized and assessed for viability as described in Section 7.8.2. Vessels were incubated with caffeic acid or chlorogenic acid (10^{-5} M) for a period of 15 minutes. Control vessels were incubated with DMSO for same length of time. Post incubation vessels were stimulated with a maximal dose of AVP (10^{-8} M) to produce a constriction. Vessels were then incubated with xanthine (XA; 10^{-4} M) plus xanthine oxidase (XO; 10 mU / ml) for a further 15 minutes. Finally all vessels were washed repeatedly with PSS to stable baseline.

7.11 End Vessel Viability and Termination of Experiment

To complete the experimental protocol all myometrial arteries were washed with PSS to a stable baseline. A further 120 mM KPSS was conducted to ensure that vessel viability was not compromised during experimentation.

7.12 Solutions and drugs

Chemicals and solutions were obtained from Sigma-Aldrich (Gillingham, Kent, United Kingdom).

mM/L	PSS	120 mM KPSS
NaCl	119	12.45
$NaHCO_3$	25	25
KCl	4.69	120
$MgSO_4$	2.4	2.4
$CaCl_2$	1.6	1.6
KH_2PO_4	1.18	1.18
Glucose	6.05	6.05
EDTA	0.034	0.034

Table 17: the above table illustrates the general composition of PSS and KPSS. Solutions were gassed with 95% air / 5% CO_2; pH 7.4.

Drug	Molarity	Dilution
U46619	10^{-6} M	Stock solution 10^{-2} M; diluted in PSS; stored in freezer
Acetylcholine	10^{-6} M	Stock solution 10^{-2} M; diluted in PSS; stored in freezer
ArginineVasopressin	10^{-8} M	1μM stock solution; diluted in dH_2O
Bradykinin	10^{-6} M	Stock solution 10^{-2} M; diluted in PSS; stored in freezer
Caffeic Acid	10^{-2} M	Stock solution 10^{-2} M; diluted in PSS; stored in freezer
Chlorogenic Acid	10^{-2} M	Stock solution 10^{-2} M; diluted in PSS; stored in freezer
Caffeine	10^{-2} M	Stock solution 10^{-2} M; diluted in PSS; stored in freezer
H_2O_2 (SIGMA-ALDRICH)	10^{-4} M	Stock solution 10^{-2} M; diluted in dH_2O; made as required
Xanthine	10^{-4} M	Stock solution 10^{-2} M; diluted in PSS; stored in freezer
Xanthine Oxidase	10 mU / ml	Used directly; stored in freezer

Table 18: Table of chemicals utilised, their concentrations and storage requirements. All chemicals in the table were purchased from Sigma Aldrich (Poole, UK).

7.13 Statistical Analysis

Prior to analysis all data were tested for conformation to a normal distribution. Normal distribution is defined as the theoretical curve and aims to identify how often an experiment will produce a particular result (Daly & Bourke 2000). The curve is symmetrical and bell shaped; this indicates that results often fall near the average but can potentially deviate by large amounts. The width of the bell indicates how much confidence one can have in the result (mean) of an experiment (Daly & Bourke 2000). The data was assessed for normality using the Kolmogov-Smirnov test, the D'Agostino and Pearson omnibus normality test and the Shapiro-Wilk normality test All three methods were used as these formal tests alone are not unlikely to be able to discriminate between Gaussian and non-Gaussian distributions when sample size is <12. Following analysis for normality, the appropriate parametric or non-parametric statistical test was used. Clinical and demographic data were compared using a Mann-Whitney U test.

Vessel tension was expressed as ΔT (active wall tension) in mN / mm was transformed to active effective pressure (kPa) to standardize for variations in diameter between individual vessels. Active effective pressure was calculated from the active wall tension (ΔT) divided by the normalized internal radius (mm) of the vessel. The contractile effects of KPSS and U46619 on vessel was analysed using a Wilcoxon matched pairs signed rank non-parametric t-test. The potential relaxatory effects of Acetylcholine and Bradykinin were examined using a one sample t-test, again assuming a hypothetical mean of 100.

The potential contractile ability of our active coffee chemicals, caffeic acid and chlorogenic acid, were assessed using 2-way repeated measures ANOVA. This test was also used for comparisons between relaxation curves. Assessing the potential antioxidative effects of our drug with reactive oxygen species (H_2O_2 and XA / XO) was compared using a Kruskal-Wallis test, with a Dunn's multiple comparisons post hoc test. This was used for both maximum contraction and residual effect of the species. The effect of caffeine on a vessel when exposed to a ROS used a Mann-Whitney U test.

All data were expressed as mean ± SEM, where n indicates individual vessels studied per placenta and N indicating the number of placentas / myometrial biopsies.

Statistical analyses were performed on individual vessels. P<0.05 indicated statistical significance.

Chapter 8 Quantitative Results

8.1 Quantitative Results

Firstly, I will present in detail the results from my placental chorionic plate artery experiments followed by similar experiments using maternal myometrial arteries. This will be followed by a section contrasting the effects of active coffee chemicals on these vascular beds.

8.2 Clinical Demographic Data

Demographic details / clinical data for the women from whom samples were collected are detailed in Table 19. All women had normal, uncomplicated pregnancies (N = 83) and delivered at term (37 + 0 to 42 + 0 weeks gestation) with no sign of maternal diabetes, hypertension or other pathology. The majority of samples were obtained from women delivering by Caesarean sections (40 out of 43). Reasons for Caesarean section included maternal request, previous section and breech presentation.

	Placenta	**Myometrium**
N = Placentas/ women **n = vessels**	N = 43 / n = 169	N = 39/ n = 136
Maternal Age (Range)	33 (23 - 42)	33 (25 - 42)
Gestation	39+0 (37.2 - 40)	39+0 (36.3 - 41.9)
BMI (Range)	25.5 (17.9 – 38.1)	27.9 (20.7 - 40.7)
Weight	68.1 (48.4 – 96.4)	74.1 (53 - 102)
Height	163.3 (150- 182)	162.9 (149.5 – 180)
Smoking	Yes: 2 No: 41	Yes: 5 No: 31
Mode of delivery / birth	NVD: 3 ELCS: 40	NVD: 0 ELCS: 39
Gravidity	3 (0 – 8)	3
Parity	0 – 4 (0 – 4+0)	0 - 4
Birthweight (g)	3340 (2664 – 4356)	3359 (2760 - 4680)
Fetal Sex	Male: 15 Female: 22 Unreported / unknown: 6	Male: 17 Female: 19 Unreported / unknown: 3
Individualised Birthweight Ratio	48.4 (7.1 – 99.7)	46.0 (9.9 – 99.0)

(IBR)		

Table 19: Demographic and clinical data for women recruited to the study. Data are median / mean and range; N = number of participants and n = number of arteries.

Samples were collected either from normal vaginal delivery or elective Caesarean section; a total of 3 NVD were collected, with the remaining samples collected through ELCS. Ethnicity of women who donated placental samples was 51% Caucasian with the remainder including Pakistani, Indian, Black African, Middle Eastern and Black Caribbean. Ethnicity of women who donated myometrial samples was 81% Caucasian with the remainder including Black African, Arabian, Pakistani, South American and Mixed Race. Average maternal age was 33 years old with average BMI calculated to be 25.5 in the placental group and 27.9 in the myometrial group. BMI is calculated as weight (kg) divided by height squared (m^2). The ideal BMI range for adults is 18.5 - 24.9 kg.m^{-2}. If BMI is 25 or greater it is considered to overweight and a BMI of 30 - 39.9 is considered to be obese. Our samples fell in the overweight range. Smoking status was identified; 6 participants indicated that they consumed tobacco. There were no known pathologies reported; all samples were normal. Samples were excluded if fetal weight was below 2500 g as these babies are likely to be IUGR; this was confirmed by calculation of the individualised birthweight ratio (IBR). The average IBR was 48.4 (placental) and 46.0 (myometrial) suggesting that samples were collected from normal pregnancies with appropriately grown babies. I attempted to collect all data for all samples collected, however there were some missing data which could not be accounted for (gender / fetal weight / IBR); this data was not present on hospital database.

8.3 Chorionic Plate Arteries

8.3.1 Optimal Steady State for Chorionic Plate Arteries dissected from Placental Biopsies

Once chorionic plate arteries were mounted on the wire myograph, optimal steady state for investigations into vascular function was determined by completion of the classical normalization method as outlined in Chapter 7, Section 7.5.2 and Section

7.5.3. Placental chorionic plate arteries were allowed to equilibrate for 20 minutes post normalization to 0.9 of $L_{5.1\ kPa}$.

8.3.2 Chorionic Plate Artery Response to KPSS (Depolarisation-induced contraction)

Chorionic plate artery diameter was $282.3 \pm 8.0\mu m$ (n = 169). Post-normalization basal tone was 0.6 ± 0.03 kPa equivalent to 4.35 ± 0.2 mmHg (n = 169). Vessel viability was assessed using known concentrations of a contractile agonist. Depolarization-induced constrictions following addition of 120 mM K^+ containing physiological salt solution (KPSS) were observed in all experiments. Vessels were not used for analysis of vascular function if the contraction to KPSS (as active effective pressure (Ki) in kPa) was less than 1 kPa. An example of KPSS-induced contraction is demonstrated in figure 10.

8.3.3 Chorionic Plate Artery Response to U46619 and Acetylcholine (Agonist-induced contraction and relaxation respectively)

Following normalization, equilibration and the initial contraction assessment with KPSS, a constriction response to the thromboxane (TXA_2) mimetic U46619 (10^{-6} M) was performed. U46619 consistently produced a significant concentration-dependent constriction of chorionic plate arteries. Acetylcholine (10^{-6} M) did not induce a significant sustained relaxation in pre-constricted chorionic plate arteries. An example trace is demonstrated in figure 10.
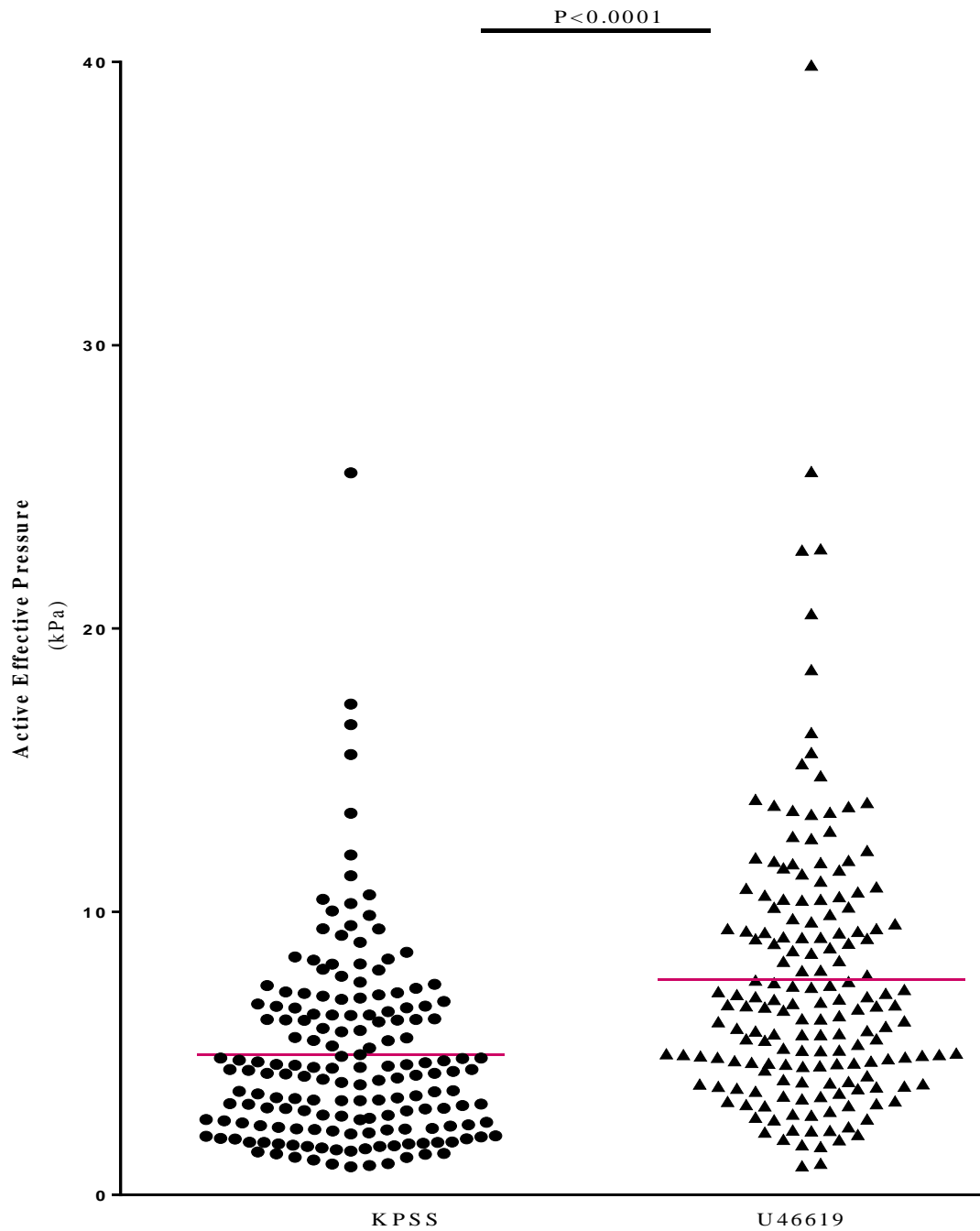
Figure 11: Effects of contractile agents (KPSS; U46619) on chorionic plate arteries. Maximal contraction was significantly higher in U46619 compared to KPSS contracted arteries (Mean ± SEM; n = 169; P <0.0001; Wilcoxon matched pairs signed rank non-parametric t-test; theoretical mean of 100).
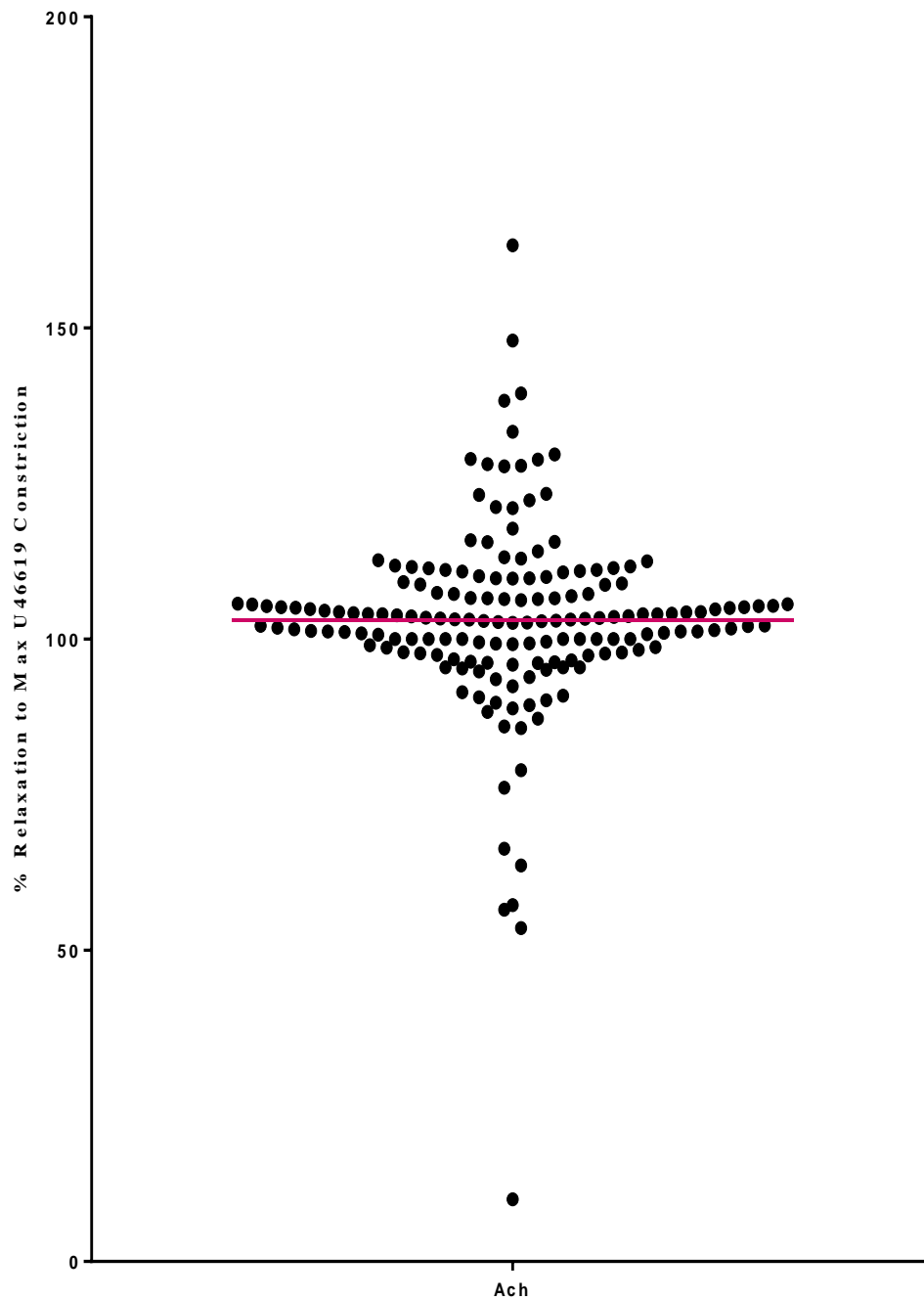
Figure 12: Effect of endothelium-dependant relaxatory agent (ACH) on chorionic plate arteries. Acetylcholine did not exhibit a significant relaxatory effect on chorionic plate arteries (one sample t-test).

Experiment Trace Placental Vessels
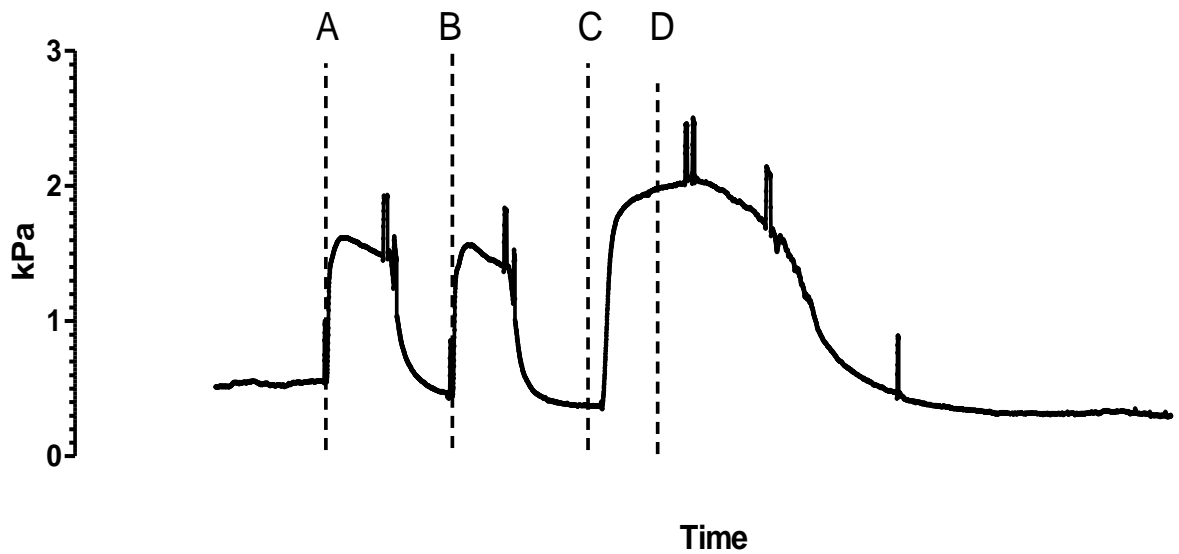
Figure 13: Example trace showing the effects of contractile agents (KPSS; U46619) and the endothelium-dependant relaxatory agent (ACH) on chorionic plate arteries. 'A' is point of first KPSS wash and 'B' is point of the second KPSS wash; from the above trace we can see that vessel viability is intact. Following washes in PSS to baseline, 'C' is point of U46619 (10^{-6} M) addition. Maximal constriction is achieved after approximately 6 - 8 minutes. 'D' is point of addition of ACH (10^{-6} M); minimal alteration in tone was noted in this chorionic plate artery.

8.3.4 Effect of Caffeic Acid on Chorionic Plate Arterial Function

Vessel diameters of those vessels exposed to caffeic acid were 326.9 ± 23.7 µm (n = 30; Mean $\pm$ SEM). Chorionic plate arteries were exposed to incremental doses of caffeic acid to investigate its possible contractile properties. Addition of caffeic acid (10^{-10} M to 10^{-5} M; Figure 11) produced no significant alteration to vascular tone (0.5 ± 0.2 kPa (caffeic) versus 0.8 ± 0.5 kPa (control); P>0.05; 2-way RM –ANOVA, n = 30).

Vessels were pre-constricted with a maximal dose of U46619 (10^{-6} M). Once a stable contraction was achieved, addition of caffeic acid (10^{-10} M to 10^{-5} M; Figure 11) had no significant relaxatory effect on the level of pre-contraction of chorionic plate arteries (P>0.05; 2-way RM –ANOVA, n = 30).
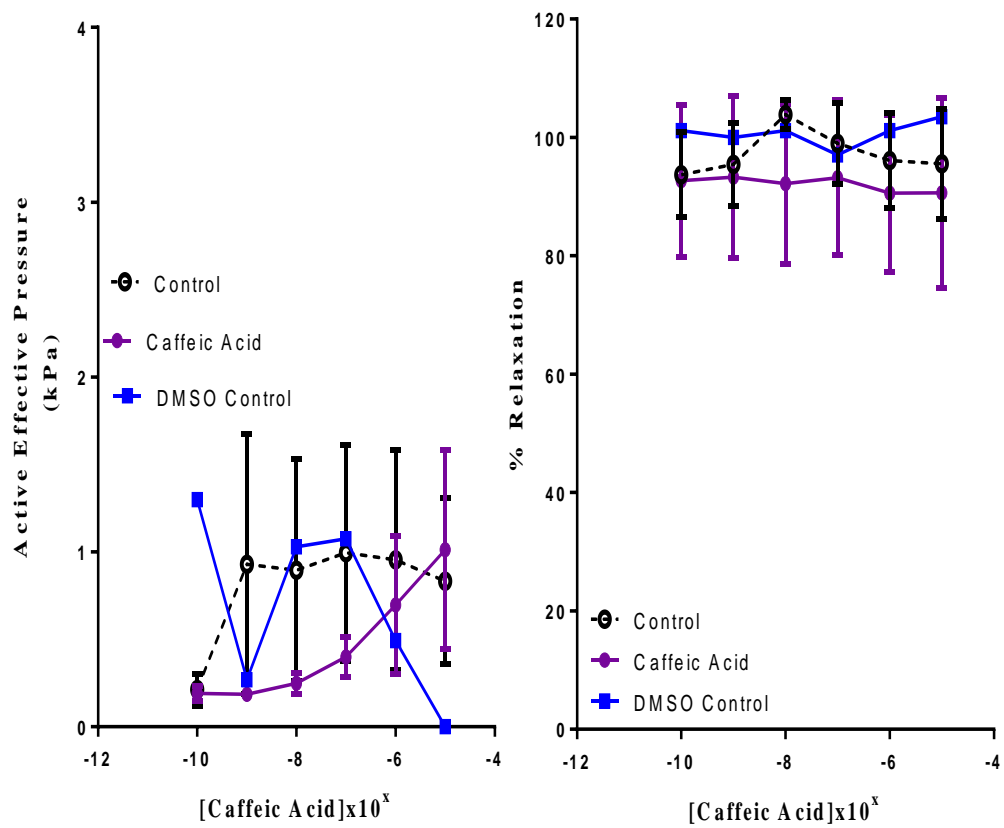
Figure 14: Effect of caffeic acid on chorionic plate artery vascular function. Dose response data for / to caffeic acid (10^{-10} M to 10^{-5} M) in comparison to diluent control (DMSO). Contractile (left panel) and relaxatory (right panel) effects are shown. Data were not significantly different compared to control and diluent (DMSO) groups (P>0.05; 2-way RM −ANOVA, n = 30; 0.5 ± 0.2 kPa versus 0.8 ± 0.5 kPa). All data are mean ± SEM.

8.3.5 Effect of Chlorogenic Acid on Chorionic Plate Arterial Function

Vessels exposed to chlorogenic acid were 270.2 ± 22.8 µm (n = 31; Mean $\pm$ SEM) in diameter. Chorionic plate arteries were exposed to incremental doses of chlorogenic acid to investigate its possible contractile properties. Addition of chlorogenic (10^{-10} M to 10^{-5} M; Figure 12) acid produced no significant alteration to vascular tone (0.2 ± 0.1 kPa (control) versus 0.2 ± 0.05 kPa; P>0.05; 2-way RM –ANOVA, n = 31).

Vessels were pre-constricted with a maximal dose of U46619 (10^{-6} M). Addition of chlorogenic acid (10^{-10} M to 10^{-5} M) had no significant relaxatory effect on the level of pre-contraction of chorionic plate arteries (P>0.05; 2-way RM –ANOVA, n = 31).
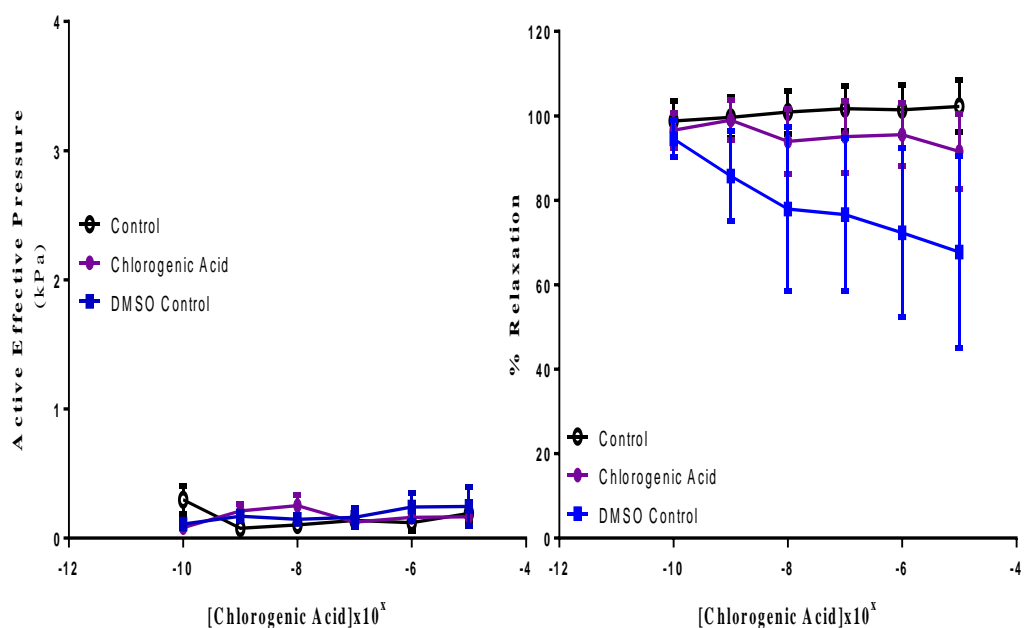
Figure 15: Effect of chlorogenic acid on chorionic plate artery vascular function. Dose response data for / to chlorogenic acid (10^{-10} M to 10^{-5} M) in comparison to diluent control (DMSO). Contractile (left panel) and relaxatory (right panel) effects are shown. Data were not significantly different compared to control or diluent (DMSO) groups (P>0.05; 2-way RM –ANOVA, n = 31; 0.2 ± 0.1 kPa versus 0.2 ± 0.05 kPa). All data are mean ± SEM.

8.3.6 Chorionic Plate Artery Response to Reactive Oxygen Species

Chorionic plate arteries were pre-incubated for 15 minutes with a maximal dose of caffeic acid or chlorogenic acid (10^{-4} M). This concentration was decided upon based on a search of the scientific literature (Kono et al. 1997; Cremin et al. 2001; Manach et al. 2005; Farah et al. 2008). The bioavailability of caffeic acid and chlorogenic acid largely depends on its metabolism by the gut microflora (Gonthier et al. 2003). Much of the research has been conducted in murine models (Azuma et al. 2000; Gonthier et al. 2003; Frank et al. 2003; Lafay et al. 2006; Karthikesan et al. 2010) however the few studies investigating its bioavailability found that these phenols are highly absorbed and metabolized in humans (Manach et al. 2005; Farah et al. 2008). Caffeic and chlorogenic acid absorption is discussed in more detail in Chapter 2.

8.3.7 Hydrogen Peroxide

Control vessels were incubated in PSS. Post incubation vessels were exposed to the reactive oxygen species hydrogen peroxide (H_2O_2; 10^{-4} M).

Vessels exposed to 10^{-4} M H_2O_2 were 259.3 ± 14.1μm (mean ± SEM). The response of chorionic arteries to direct application of H_2O_2 was assessed in unstimulated arteries. 10^{-4} M H_2O_2 induced a rapid constriction of chorionic plate arteries; a raw data trace is shown in Figure 13. Addition of H_2O_2 caused a significant increase in arterial tone (1.9 ± 0.4 kPa; n = 10). As can be seen from Figure 13, the contraction was not maintained but was of a transient nature; at 15 minutes post-addition the residual contraction was decreased to 43.3 ± 9.7 % (n = 10) of the initial peak.

8.3.8 Caffeic Acid and Chlorogenic Acid

As stated above, vessels were pre-incubated with caffeic or chlorogenic acid (10^{-6} M) for 15 minutes. This did not cause a significant constriction or relaxation in the vessel.

There was no significant difference in peak vessel constriction to H_2O_2 in vessels pre-incubated for 15 minutes with caffeic acid (2.7 ± 0.6 kPa; n = 10) or chlorogenic acid (2.7 ± 0.5 kPa; n = 10) versus control maximal constriction (1.9 ± 0.4 kPa; n = 10; Figure 13).

The longevity of the H_2O_2 –induced response was not attenuated by pre-incubation with caffeic acid (37.8% ± 6.6%) versus H_2O_2 control (43.3 ± 9.7 %; n = 34; mean ± SEM; P<0.05; Mann-Whitney U test with Dunn's *post hoc* test). Similarly, the longevity of the H_2O_2 –induced response was not significantly attenuated by pre-incubation with chlorogenic acid; residual constriction was 18.7 ± 10.8 % with chlorogenic acid pre-incubation compared to 43.3 ± 9.7 % in control vessels (P = 0.31 chlorogenic acid versus H_2O_2 control; n = 34; mean ± SEM; Kruskal-Wallis test with Dunn's *post hoc* test).
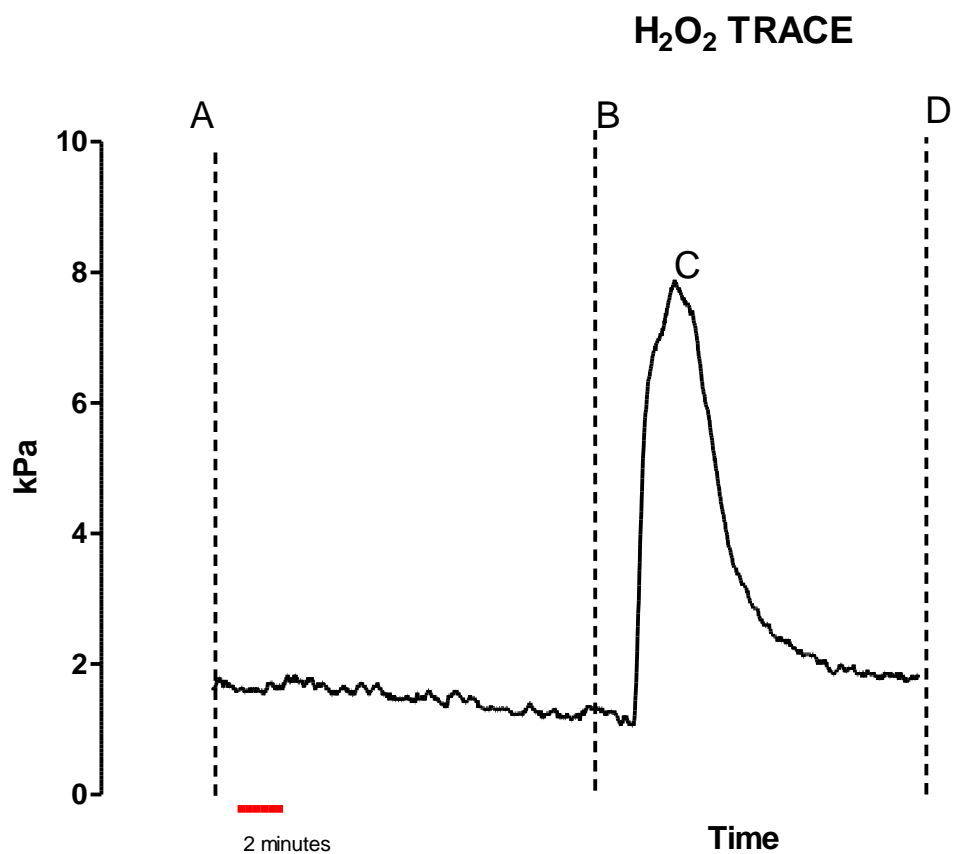
Figure 16: Chorionic plate artery / tension tracing taken from "standard" H_2O_2 experiment. 'A' is point of pre-incubation with drug (caffeic acid / chlorogenic) or control for 15 minutes. 'B' is point of addition of 10^{-4} M H_2O_2. 'C' is maximum achieved constriction. 'D' is the point at which residual constriction was measured (after 15 minutes).
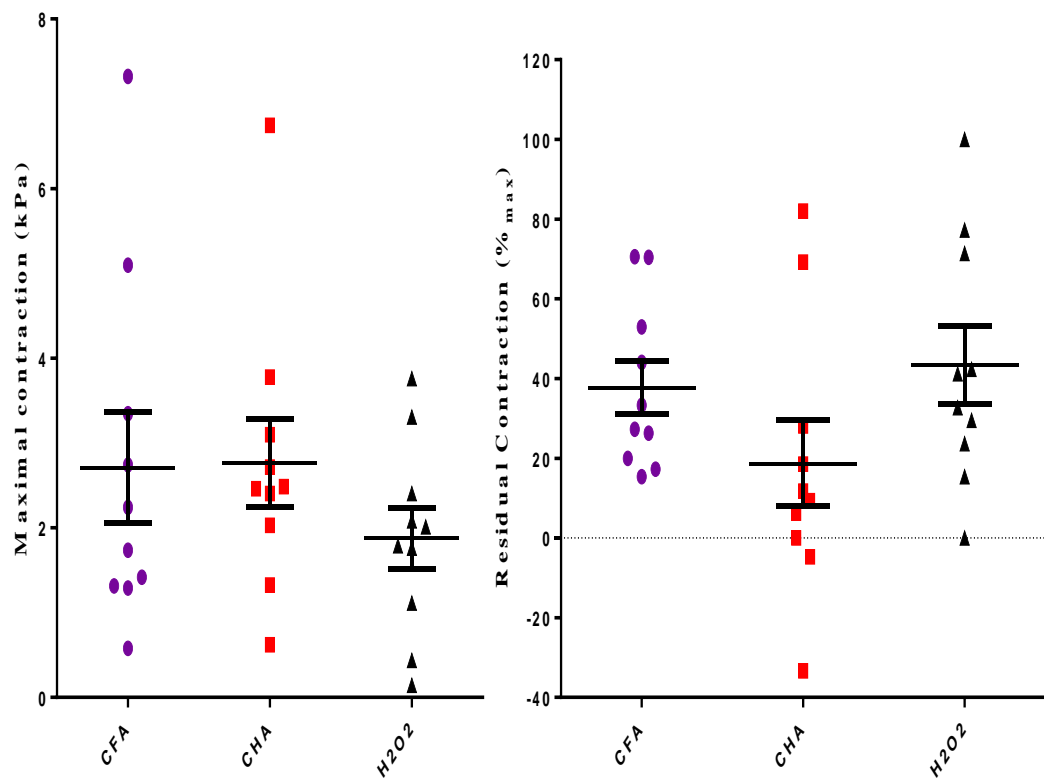
Figure 17: Peak H_2O_2 induced contraction in chorionic plate arteries was unchanged (n = 34; Mean +/- SEM; P<0.05; Kruskal-Wallis test with Dunns *post hoc* test). Residual H_2O_2 contraction as % maximum constriction indicated that the longevity of response was not reduced by pre-incubation with CHA or CFA of maximal H_2O_2 induced contraction at 15 minutes (n = 34; Mean +/- SEM; P>0.05; Kruskal-Wallis test with Dunn's *post hoc* test).

8.3.9 Caffeine

As stated above for experiments with caffeic acid and chlorogenic acid, vessels were pre-incubated with caffeine (10^{-6} M) for a period 15 minutes (acute incubation) or additionally for the duration of the experiment (chronic incubation). This did not cause a significant constriction or relaxation in the vessel. This verified my previous investigations / experimentation on placental / maternal vasculature.

Vessels exposed to 10^{-4} M H_2O_2 were 304.9 ± 20.0 µm (mean $\pm$ SEM). The response of chorionic arteries to direct application of H_2O_2 was assessed in unstimulated arteries. 10^{-4} M H_2O_2 induced a rapid constriction of chorionic plate arteries. Addition of H_2O_2 caused a significant increase in arterial tone (2.2 ± 0.6 kPa; n = 8). The contraction was not maintained but was of a transient nature; at 15 minutes post-addition the residual contraction was decreased to 28.5 ± 9.0 % (n = 8) of the initial peak.

There was no significant difference in peak vessel constriction to H_2O_2 in vessels chronically incubated with caffeine (1.6 ± 0.4 kPa; n = 8) or acutely incubated with caffeine (1.2 ± 0.3 kPa; n = 8) versus control maximal constriction (2.2 ± 0.6 kPa; n = 10; Figure 15).

The longevity of the H_2O_2 –induced response was not attenuated by chronic incubation of caffeine ($30.4\% \pm 14.5\%$) versus H_2O_2 control (28.5 ± 9.0 %; n = 8; mean $\pm$ SEM; P<0.5; Mann Whitney U test with Dunn's *post hoc* test). The longevity of the H_2O_2 –induced response was not significantly attenuated by acute incubation with caffeine (36.6 ± 14.7 %) compared to H_2O_2 control (28.5 ± 9.0 %; n = 8; mean $\pm$ SEM; P<0.5; Mann Whitney U test with Dunn's *post hoc* test).
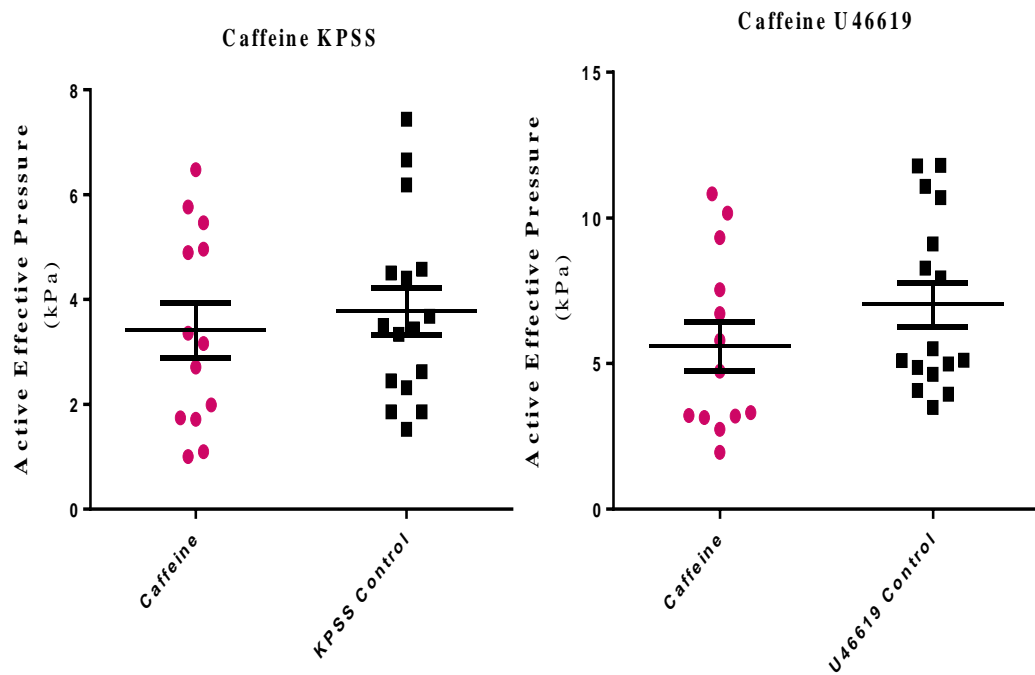
Figure 18: Effect of chronic caffeine incubation on initial KPSS wash (left panel). Vessels incubated with caffeine from start of experiment did not significantly impact KPSS contraction. Effect of chronic caffeine incubation on initial U46619 induced constriction (right panel). Vessels incubated from start of experiment did not significantly alter maximal U46619 induced constriction. (n = 29; Mean +/- SEM; P<0.05; Mann-Whitney U test).
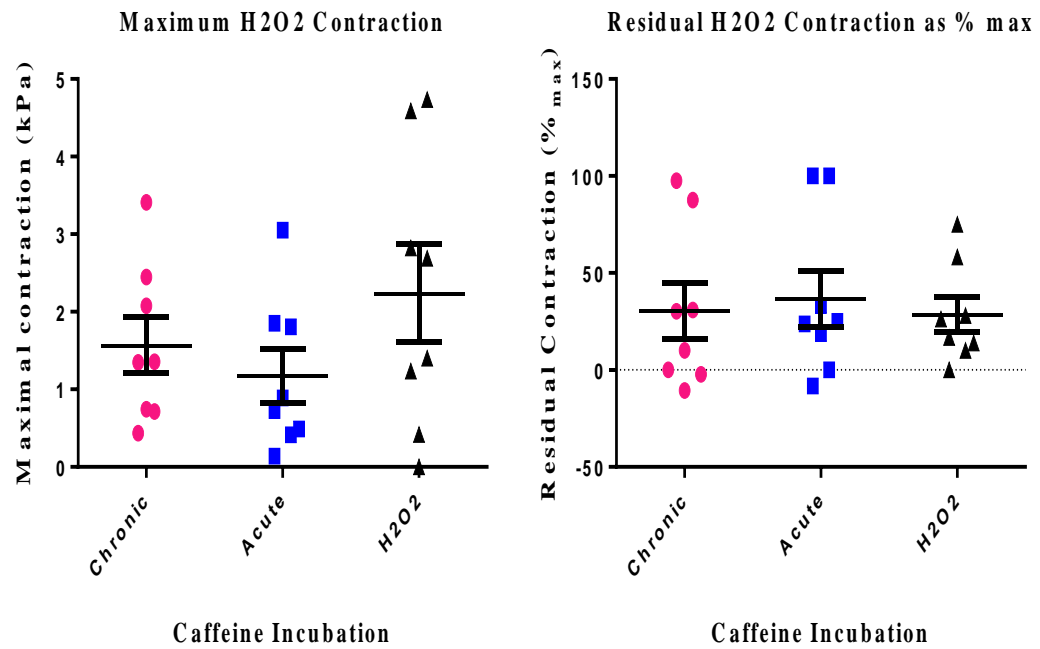
Figure 19: Peak H_2O_2 induced contraction in chorionic plate arteries was unchanged (n = 29; Mean +/- SEM; P<0.05; Mann-Whitney U test). Residual H_2O_2 contraction as % maximum constriction indicated that the longevity of response was not reduced by pre-incubation with caffeine, either acute or chronic, of maximal H_2O_2 induced contraction (n = 29; Mean +/- SEM; P<0.05; Kruskal-Wallis test with Dunns *post hoc* test).

8.3.10 Xanthine- Xanthine Oxidase

Chorionic plate arteries were normalized and assessed for viability as described in 7.5.2. Vessels exposed to XA / XO were 263.6 ± 9.7 μm (mean ± SEM). The response of chorionic arteries to direct application of Xanthine / Xanthine Oxidase (XA / XO) was assessed in unstimulated arteries. Vessels were incubated with XA (10^{-4} M) plus XO (10 mU/ ml); incubation caused a sharp / rapid constriction (a raw data trace is shown in Figure 17). Addition of XA / XO caused a significant increase in arterial tone (4.6 ± 0.8 kPa). As can be seen from Figure 17, the XA / XO induced-contraction was not maintained, so that at 15 minutes post-addition the residual contraction was 74.6 ± 6.6% (n = 11) of the maximum achieved contraction.

Similar to H_2O_2 investigations, vessels were pre-incubated with caffeic or chlorogenic acid (10^{-6} M) for 15 minutes. This pre-incubation did not cause a significant constriction or relaxation in the vessel. This verified my previous investigations / experimentation on placental / maternal vasculature.

There was no significant difference in peak vessel constriction to XA / XO in vessels pre-incubated for 15 minutes with caffeic acid (3.5 ± 0.5 kPa; n = 11) or chlorogenic acid (6.2 ± 1.6 kPa; n = 11) versus control maximal constriction (4.6 ± 0.8 kPa; n = 12) (Figure 18).

The longevity of the XA / XO–induced response was not attenuated by pre-incubation with caffeic acid (82.9 ± 4.7%) versus XA / XO control (74.6 ± 6.6%). Similarly, the longevity of the XA / XO –induced response was not attenuated by pre-incubation with chlorogenic acid (89.9 ± 3.9 %) versus XA / XO control (mean ± SEM; Kruskal-Wallis test with Dunn's p*ost hoc* test).
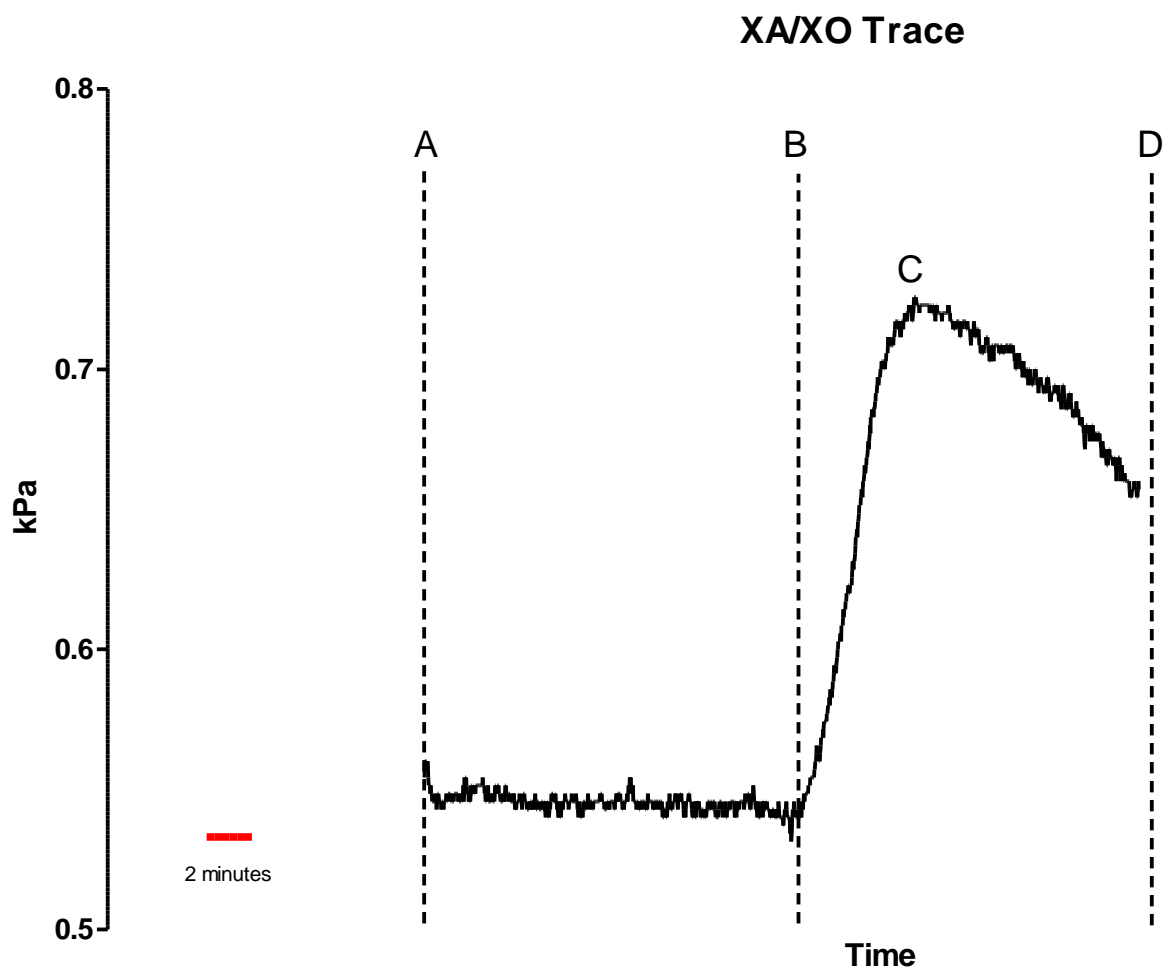
Figure 20: Chorionic plate artery/ tension tracing taken from standard XA / XO experiment. 'A' is point of pre-incubation with drug (caffeic acid/ chlorogenic acid) or control for 15 minutes. 'B' is point of addition of XA (10^{-4} M) plus XO (10 mU / ml). 'C' is maximum achieved constriction. 'D' is the point at which the residual constriction was measured (after 15 minutes).
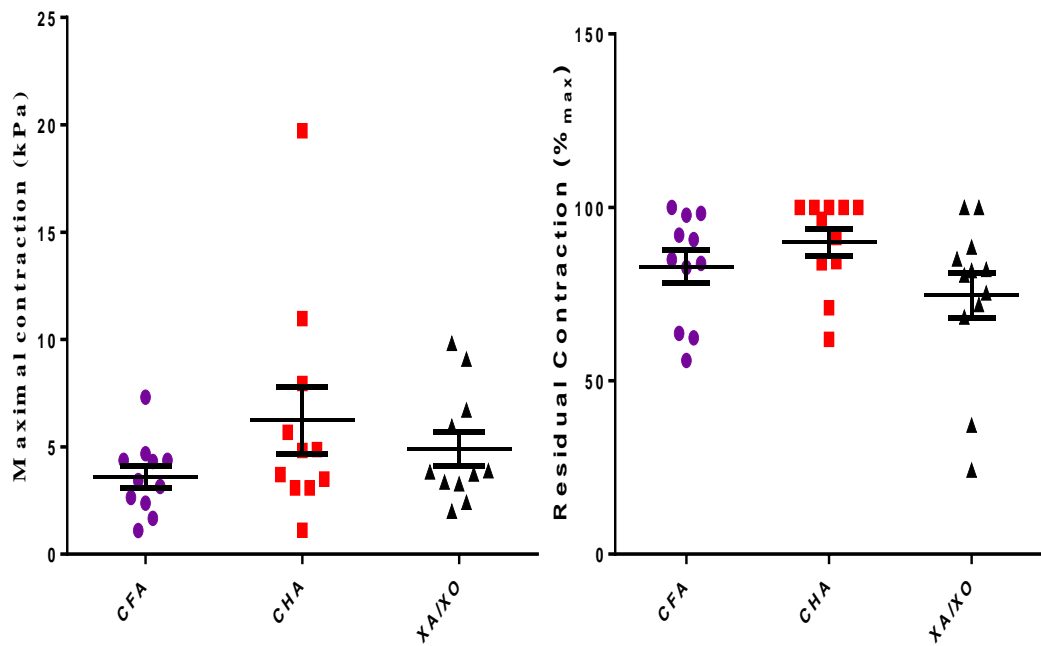
Figure 21: Peak XA / XO induced contraction was similar in control (XA / XO only) or pre-incubated (CFA; CHA) in chorionic plate arteries (n = 45; Mean +/- SEM; P>0.05; Kruskal-Wallis test with Dunns *post hoc* test). Residual XA / XO contraction as % maximum constriction indicated that the longevity of response was not significant (P<0.05; CFA vs. XA / XO; n = 45; Mean +/- SEM).

As both the reactive oxygen species we use, H_2O_2 and XA / XO, induce a concentration dependent constriction we compared them to ascertain whether one had a more profound effect on chorionic plate arteries than the other. Control vessels were stimulated with H_2O_2 (n = 10) and XA / XO (n = 12). A greater constriction was noted in vessels incubated with XA / XO (4.6 ± 0.8 kPa) in comparison to those incubated with H_2O_2 (1.9 ± 0.4 kPa). Similarly, the contraction induced by XA / XO was significantly prolonged in comparison to H_2O_2. The longevity of the contractile response was reduced in vessels incubated with H_2O_2 (43.3 ± 9.7 %; n = 10) versus those incubated with XA / XO (74.6 ± 6.6 %; n = 12).
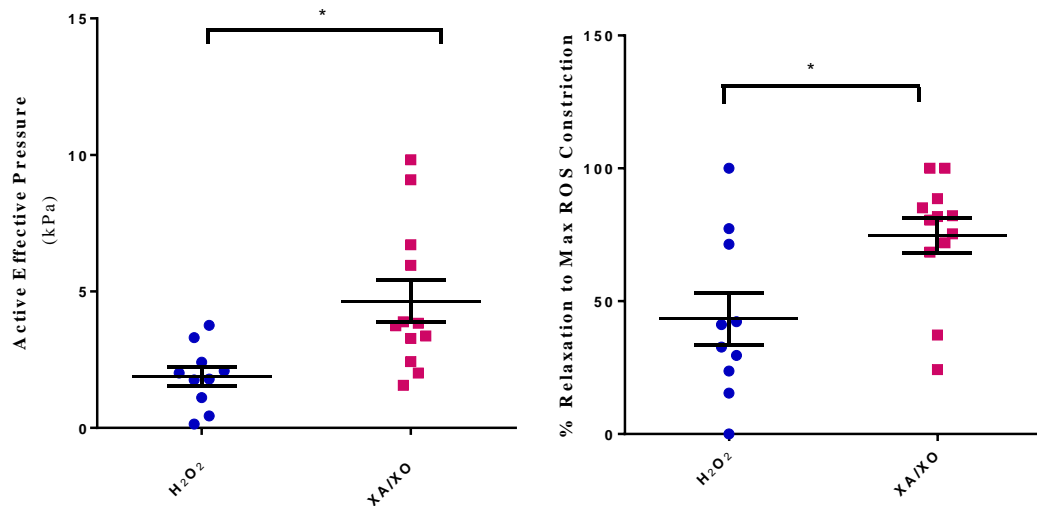
Figure 22: A comparison of maximum contraction and residual contraction of H_2O_2 to XA / XO. The left panel illustrates a significantly greater constriction associated with XA / XO than H_2O_2 (1.9 kPa ± 0.4 kPa versus 4.6 kPa ± 0.8 kPa; n = 10 vs. n = 12; Mean +/- SEM; Unpaired t-test; P = 0.0068, *). The right panel illustrates the residual contraction after 15 minutes. XA / XO had a significantly prolonged contraction in comparison to H_2O_2 (43.3 ± 9.7 % versus 74.6 ± 6.6 %; n = 10 versus n = 12; Mean +/- SEM; Unpaired t-test; P =0.0126, *).

8.4 Summary

Vessel viability was assessed using a standard start; two KPSS washes and addition of U46619 to assess contractibility and the addition of ACH to assess relaxation. Caffeic acid and chlorogenic acid did not have a significant effect on chorionic plate artery basal tone. Vessels were then stimulated with U46619; similarly caffeic acid and chlorogenic acid did not exhibit any relaxatory effects.

The potential antioxidative effects were assessed through 15 minute incubation with coffee chemical and addition of ROS. XA / XO achieved a significantly greater, and longer lasting, effect on chorionic plate arteries compared to H_2O_2. There was no significant effect on maximum contraction or residual contraction indicating that caffeic acid and chlorogenic are not viable antioxidants / are not eliciting a protective effect against ROS challenge in chorionic plate arteries.

Effect	Caffeic Acid	Chlorogenic Acid	Caffeine
Constriction	↔	↔	↔
Relaxation	↔	↔	↔
H_2O_2 Maximal Constrict	↔	↔	↔
H_2O_2 Residual	↔	↔	↔
XA / XO Maximal Constrict	↔	↔	N / A
XA / XO Residual	↔	↔	N / A

Table 20: Summary of the effect of caffeic acid/ chlorogenic acid/ caffeine on chorionic plate artery function and basal tone.

8.5 Myometrial Arteries

8.5.1 Optimal Steady State for Systemic/ Maternal Arteries dissected from Myometrial Biopsies

Once myometrial arteries were mounted on the wire myograph, optimal steady state for investigations into vascular function was determined by completion of the classical normalization method as outlined in Chapter 7, Section 7.8.2. Myometrial arteries were allowed to equilibrate for 20 minutes post normalization to 0.9 of $L_{13.3\ kPa}$.

8.5.2 Myometrial Artery Response to KPSS (Depolarisation-induced contraction)

Myometrial artery diameter was 350.1 ± 10.8 µm (n = 136). Post-normalization basal tone was 0.6 ± 0.03 kPa equivalent to 4.5 ± 0.2 mmHg (n = 136). Vessel viability was assessed using known concentrations of a contractile agonist. Depolarization-induced constrictions following addition of 120 mM KCl containing physiological salt solution (KPSS) were observed in all experiments, as described in Chapter 7, Section 7.8.2. Vessels were not used for analysis of vascular function if the contraction to KPSS (as active effective pressure (Ki) in kPa) was less than 1 kPa. An example of KPSS-induced contraction is demonstrated in figure 10.

8.5.3 Myometrial Artery Response to Arginine Vasopressin and Bradykinin (Agonist-induced contraction and relaxation respectively)

Following normalization, equilibration and the initial contraction assessment with KPSS, a constriction response to Arginine Vasopressin (AVP; 10^{-8} M) was performed. AVP consistently produced a significant concentration-dependent constriction of myometrial arteries. Bradykinin (10^{-6} M; BK) was administered once the arteries reached their maximal constriction potential. BK induced a significant sustained relaxation in pre-constricted myometrial arteries. An example trace is demonstrated in figure 20.
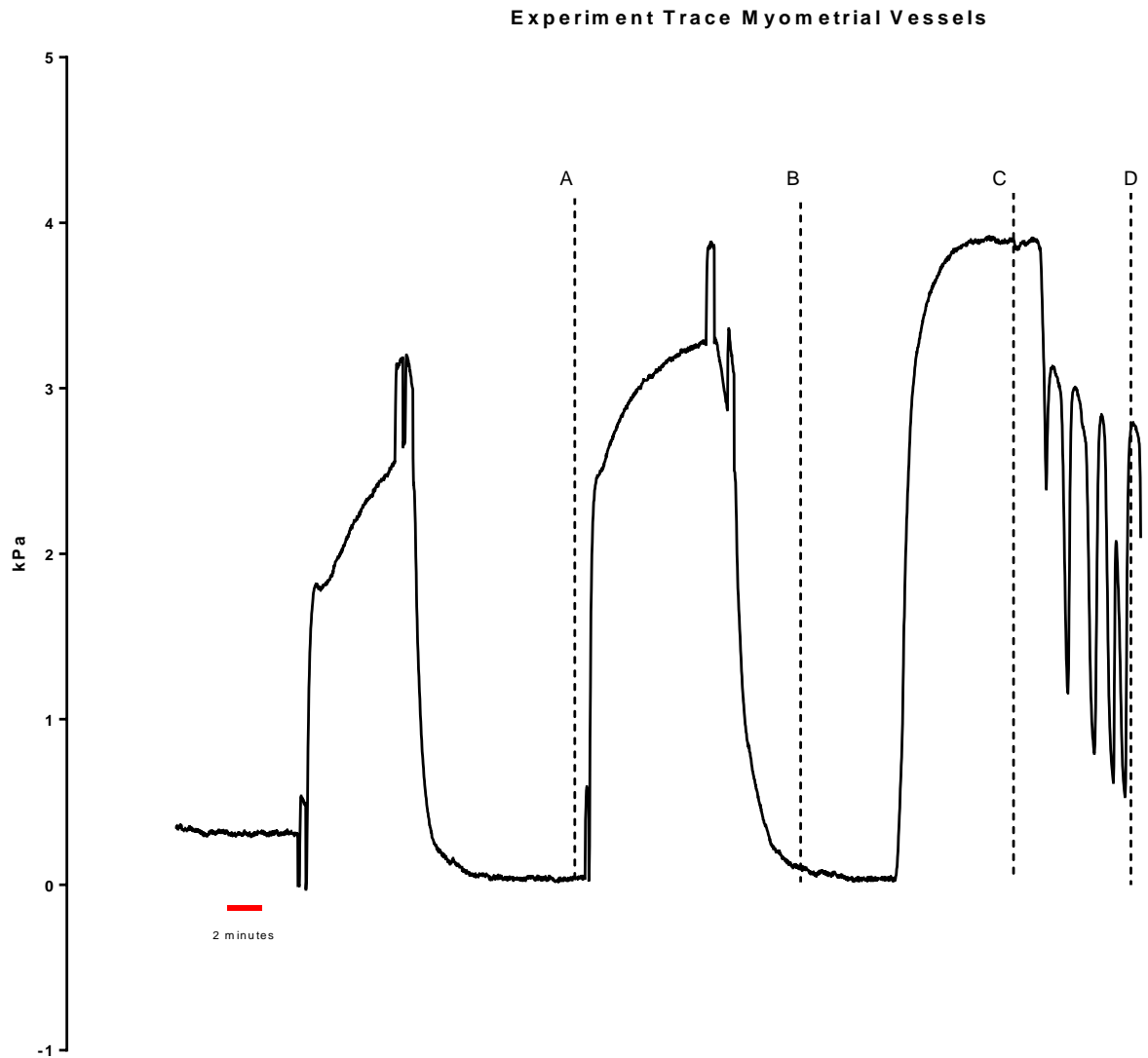
Figure 23: Example trace showing the effects of contractile agents (KPSS; AVP) and the endothelium-dependant relaxatory agent (BK) on myometrial arteries. 'A' is point of the first KPSS wash and 'B' is point of the second KPSS; from the above trace we can see that vessel viability is intact. Once vessels had been washed to baseline, 'C' is point of AVP (10^{-8} M) addition. Maximal constriction is achieved after approximately 6 - 8 minutes. 'D' is point of addition of BK (10^{-6} M), which was followed by a significant relaxation (with some oscillations in tone in this example).
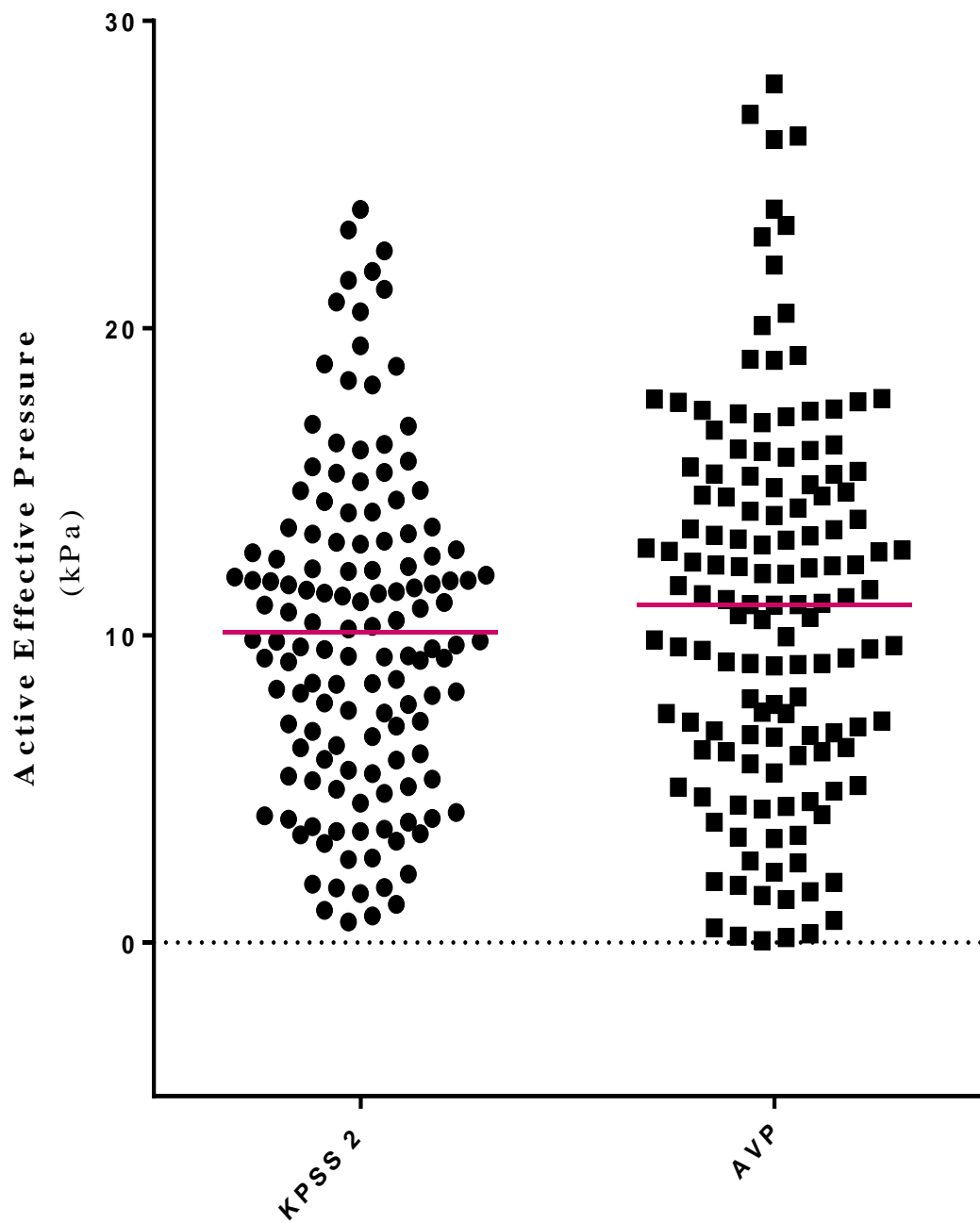
Figure 24: Effects of contractile agents (KPSS; AVP) on myometrial arteries. Maximal contraction was significantly higher in AVP compared to KPSS contracted arteries (Mean ± SEM; n = 136; P <0.0001; Wilcoxon matched pairs signed rank non-parametric t-test).
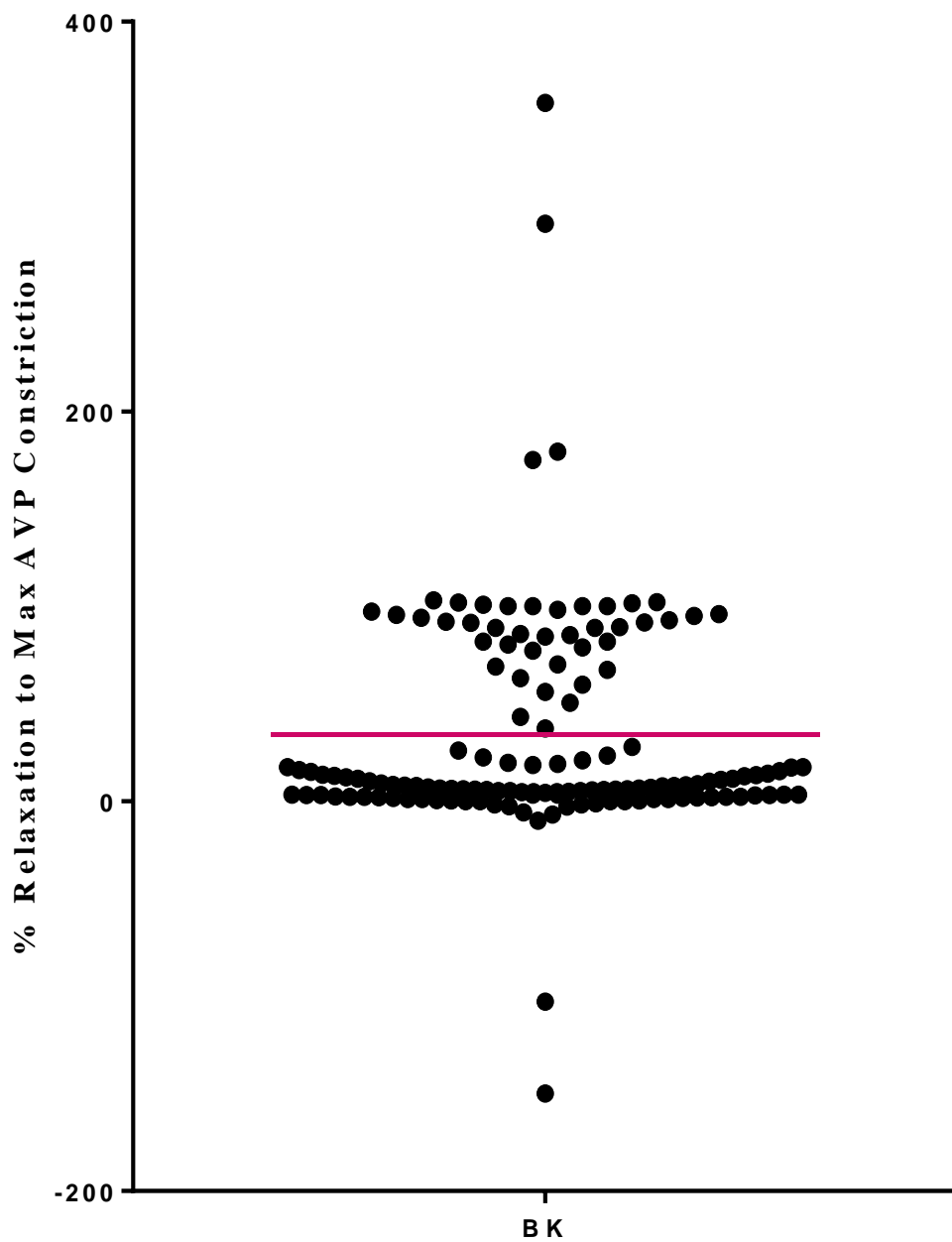
Figure 25: endothelium-dependant relaxatory agent Bradykinin (BK) on myometrial arteries. Bradykinin exhibited a significant relaxatory effect on myometrial vessels (one sample t test with a theoretical mean of 100).

8.5.4 Effect of Caffeic Acid on Myometrial Arterial Function

Vessel diameters of those vessels exposed to caffeic acid were 355.2 ± 19.9 µm (n = 38; Mean $\pm$ SEM). Arteries were exposed to incremental doses of caffeic acid to investigate its possible contractile properties. Addition of caffeic acid (10^{-10} M to 10^{-5} M; Figure 22) produced no significant alteration to vascular tone (0.1 ± 0.06 kPa (caffeic) versus 0.1 ± 0.04 kPa (control); P>0.05; 2-way RM −ANOVA, n = 30).

Vessels were pre-constricted with a maximal dose of AVP (10^{-8} M). Once a stable contraction was achieved, addition of caffeic acid (10^{-10} M to 10^{-5} M; Figure 22) did not have a significant relaxatory effect on the level of pre-contraction of myometrial (P>0.05; 2-way RM −ANOVA, n = 38).
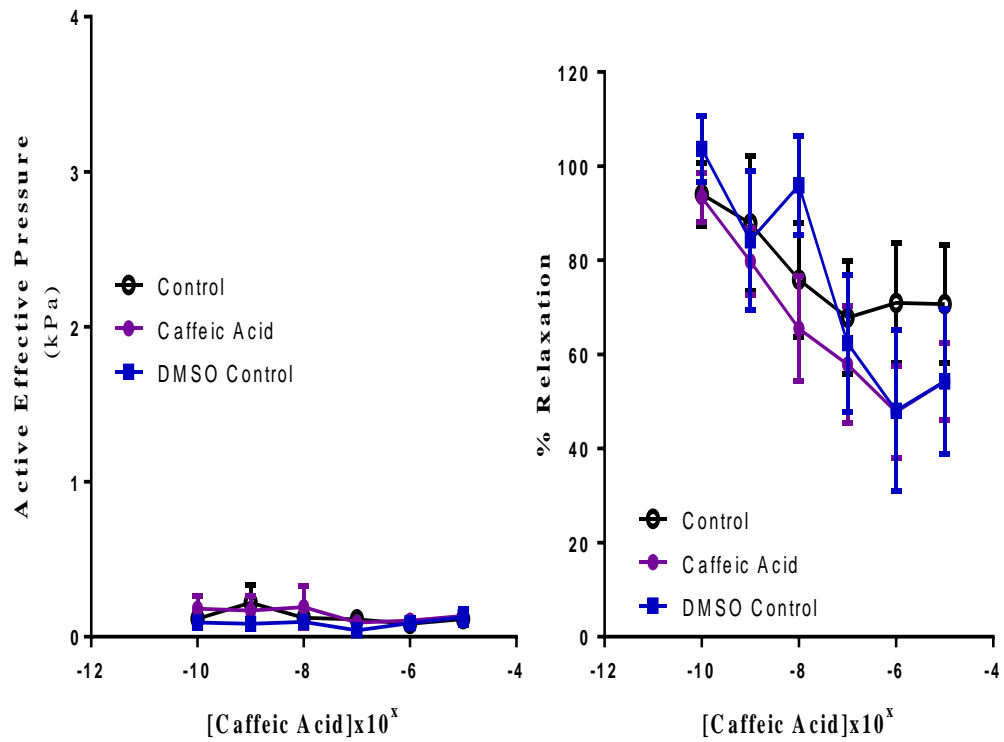
Figure 26: Effect of caffeic acid on myometrial artery vascular function. Dose response data for / to caffeic acid (10^{-10} M to 10^{-5} M) in comparison to diluent control (DMSO). Contractile (left panel) and relaxatory (right panel) effects are shown. Data were not significantly different compared to control and diluent (DMSO) groups (P>0.05; 2-way RM –ANOVA, n = 38; 0.1 ± 0.06 kPa versus 0.1 ± 0.04 kPa). All data are mean ± SEM.

8.5.5 Effect of Chlorogenic Acid on Myometrial Arterial Function

Vessels exposed to chlorogenic acid were 400.8 ± 21.9 µm (n = 40; Mean ± SEM) in diameter. Chorionic plate arteries were exposed to incremental doses of chlorogenic acid to investigate its possible contractile properties. Addition of chlorogenic (10^{-10} M to 10^{-5} M; Figure 23) acid produced no significant alteration to vascular tone (0.1 ± 0.02 kPa (control) versus 0.1 ± 0.05 kPa; P>0.05; 2-way RM –ANOVA, n = 40).

Vessels were pre-constricted with a maximal dose of AVP (10^{-8} M). Addition of chlorogenic (10^{-10} M to 10^{-5} M) acid had no significant relaxatory effect on the level of pre-contraction of myometrial (P>0.05; 2-way RM –ANOVA, n = 40).
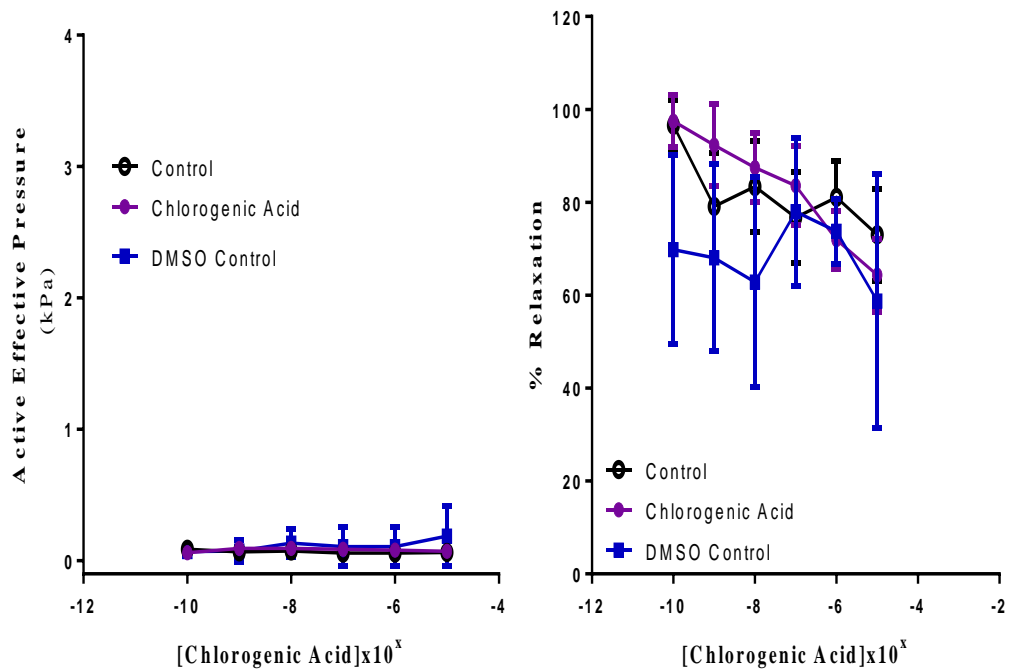
Figure 27: Effect of chlorogenic acid on myometrial artery vascular function. Dose response data for / to chlorogenic acid (10^{-10} M to 10^{-5} M) in comparison to diluent control (DMSO). Contractile (left panel) and relaxatory (right panel) effects are shown. Data were not significantly different compared to control or diluent (DMSO) groups (P>0.05; 2-way RM –ANOVA, n = 40; 0.10 ± 0.02 kPa (control) versus 0.10 ± 0.05 kPa). All data are mean ± SEM.

8.5.6 Myometrial Artery Response to Reactive Oxygen Species

Myometrial arteries were pre-incubated for 15 minutes with a maximal dose of caffeic acid or chlorogenic acid (10^{-6} M). Control vessels were incubated in PSS. The concentrations of caffeic / chlorogenic acid were decided upon based on a search of the scientific literature (Kono et al. 1997; Manach et al. 2005; Farah et al. 2008). Post-incubation with caffeic / chlorogenic acid, vessels were stimulated so as to induce a significant constriction with a maximal dose of AVP (10^{-8} M).

8.5.7 Hydrogen Peroxide and Caffeic Acid / Chlorogenic Acid

As stated above, vessels (312.1 ± 17.4 μm) were pre-incubated with caffeic (n = 10) or chlorogenic acid (n = 10; 10^{-4} M) for 15 minutes. This did not cause a significant alteration in basal tone (constriction or relaxation) in the vessel. As noted above, AVP (10^{-8} M) induced a consistent constriction in vessels. Subsequent addition of H_2O_2 caused a significant relaxatory effect (to 15.5 ± 5.6 % of the maximal contraction to AVP) post 15 minutes incubation.

As demonstrated in the trace (figure 24), there was a rapid relaxation, which usually rebounded or would lead to oscillations. The initial relaxation was measured as the peak to the first trough. Total relaxation was noted as 66.0 ± 13.4 % of the total constriction. More specifically, vessels incubated with caffeic acid relaxed more significantly (82.7 ± 15.6 %) when compared to control vessels (53.0 ± 10.0 %) of maximum constriction. Vessels incubated with chlorogenic acid also relaxed more (61.2 ± 14.7%) than control vessels (53.0 ± 10.0%), however this was not as pronounced.

There was no significant difference in peak vessel relaxation to H_2O_2 in vessels pre-incubated for 15 minutes with caffeic acid (16.0 ± 6.5 % of the maximal contraction to AVP) or chlorogenic acid (10.4 ± 3.2 % of the maximal contraction to AVP) versus that seen in control (20.1 ± 7.0 % of the maximum contraction to AVP; n = 10; Figure 25).
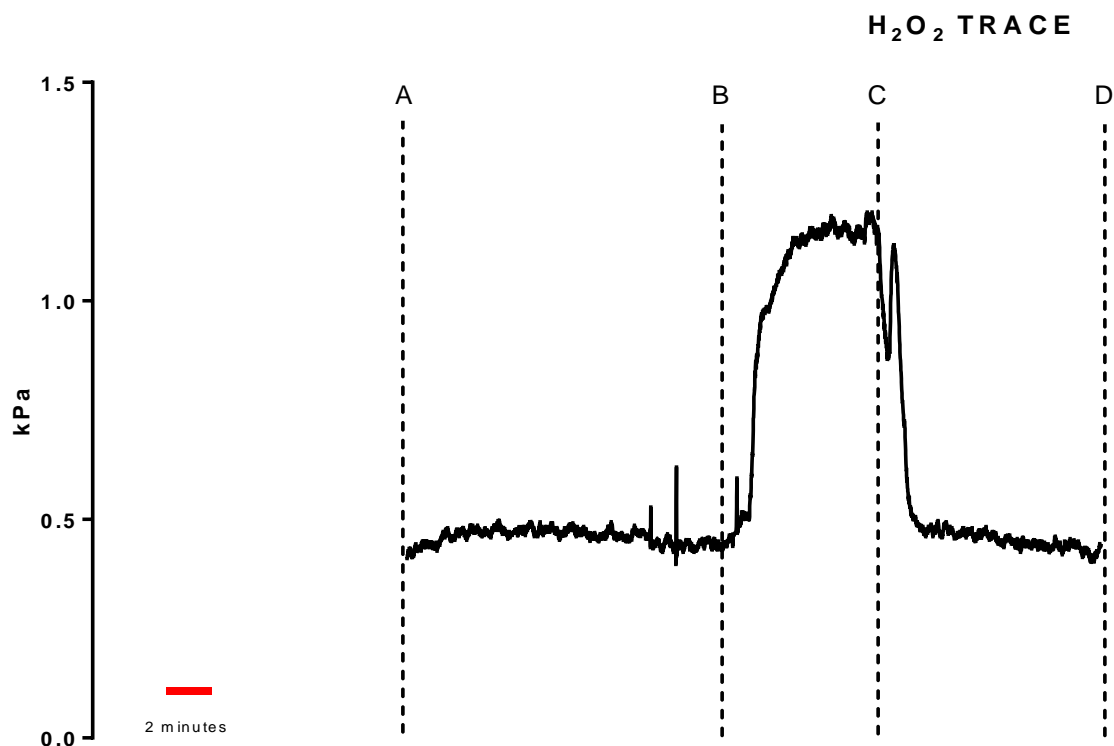
Figure 28: Myometrial artery tracing taken from "standard" ROS experiment. 'A' is point of pre-incubation with drug (caffeic acid / chlorogenic) or control for 15 minutes. 'B' is point of addition of 10^{-8} M Arginine Vasopressin, which elicited a significant contraction of the artery. At 'C' a stable / maximal constriction was noted and H$_2$O$_2$ (10^{-4} M) added; a rapid relaxation was noted. 'D' is the point at which residual constriction was measured (after 15 minutes).

Figure 29: Peak AVP induced contraction in myometrial arteries was unaffected by incubation with caffeic / chlorogenic acid (left panel; n = 10; Mean +/- SEM; P<0.05; Kruskal Wallis with Dunns *post hoc* test). H_2O_2 relaxation as % of maximum constriction indicated that the longevity of response was not reduced by pre-incubation with CHA or CFA of maximal H_2O_2 induced contraction at 15 minutes (n = 10; Mean +/- SEM; P>0.05; Kruskal Wallis with Dunns *post hoc* test).

8.5.8 Xanthine- Xanthine Oxidase

Myometrial arteries (311.8 ± 14.2 µm (mean $\pm$ SEM; n = 20) were normalized and assessed for viability as described in Chapter 7, Section 7.8.2. As above, myometrial arteries were pre-incubated for 15 minutes with a maximal dose of caffeic acid or chlorogenic acid (10^{-6} M). Control vessels were incubated in PSS. Post-incubation with caffeic / chlorogenic acid vessels were stimulated so as to induce a constriction. Vessels exposed to a maximal dose of AVP (10^{-8} M) exhibited a rapid constriction (10.1 ± 2.2 kPa; n = 5) as shown in the raw data trace (Figure 26). There was no significant difference in peak vessel constriction to AVP in vessels pre-incubated for 15 minutes with caffeic acid (13.3 ± 4.2 kPa) or chlorogenic acid (9.3 ± 2.3 kPa).

Vessels exposed to XA / XO were 311.2 ± 25.5 µm (mean $\pm$ SEM; n = 20). The response of myometrial arteries to direct application of Xanthine / Xanthine Oxidase (XA / XO) was assessed in stimulated arteries. Vessels were incubated with XA (10^{-4} M) plus XO (10 mU / ml) post-AVP contraction; incubation caused vessel relaxation (49.4 ± 17.3 %; a raw data trace is shown in Figure 27).

As demonstrated in the trace (figure 26), there was a rapid relaxation, which usually rebounded or would lead to oscillations. The initial relaxation was measured as the peak to the first trough. Total relaxation was noted as 52.6 ± 14.8 % of the total constriction. More specifically, vessels incubated with caffeic acid relaxed more significantly (39.5 ± 16.6 %) when compared to control vessels (58.0 ± 13.3 %) of maximum constriction. Vessels incubated with chlorogenic acid also relaxed more (60.3 ± 14.5%) than control vessels (58.0 ± 13.3 %); unlike previously observed, there was a significantly greater relaxation observed with chlorogenic acid than caffeic acid.

The longevity of the XA / XO–induced response was not affected by pre-incubation with caffeic acid (50.6 ± 14.6 %) versus XA / XO control (49.4 ± 17.3 %). Similarly, the longevity of the XA / XO –induced response was not attenuated by pre-incubation with chlorogenic acid (22.4 ± 4.7 %) versus XA / XO control (mean $\pm$ SEM; Kruskal Wallis with Dunns *post hoc* test).
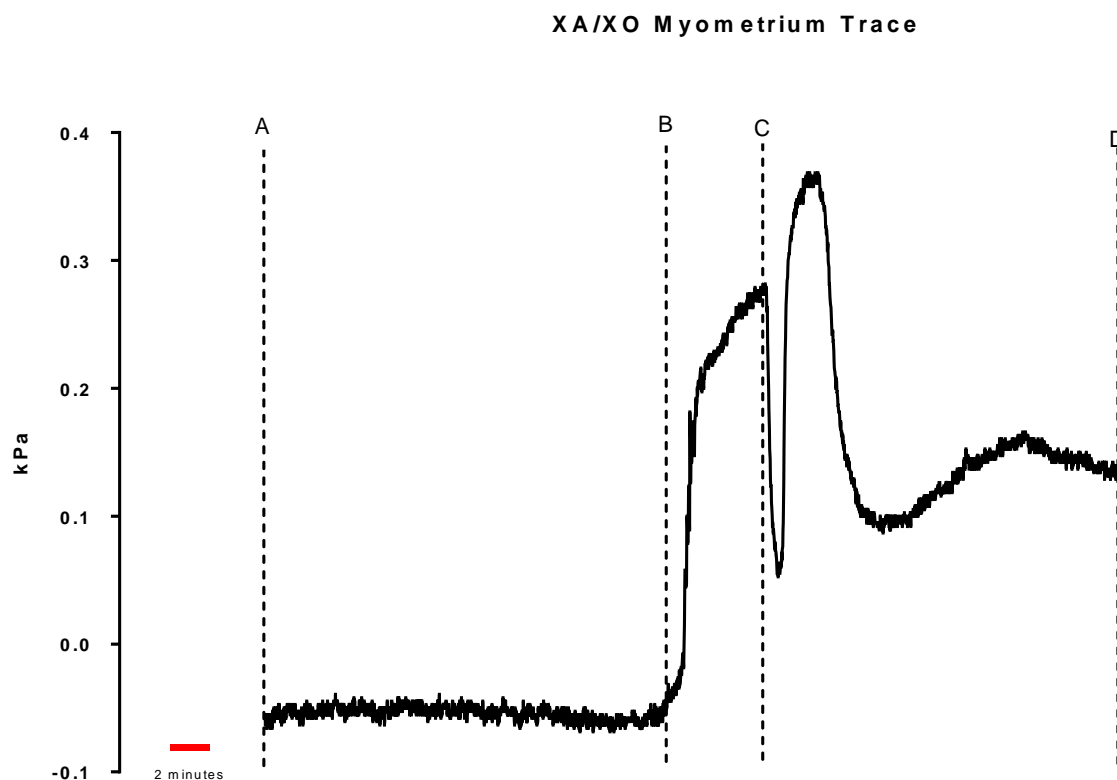
Figure 30: Myometrial artery tracing taken from "standard" ROS experiment. 'A' is point of pre-incubation with drug (caffeic acid / chlorogenic) or control for 15 minutes. 'B' is point of addition of 10^{-8} M Arginine Vasopressin, which elicited a significant contraction of the artery. At 'C' a stable / maximal constriction was noted and XA / XO (XA: 10^{-4} M, XO: 10 mU / ml) added; a rapid relaxation was noted. 'D' is the point at which residual constriction was measured (after 15 minutes). The rebound effect noted between 'C' and 'D' was noted in all studies.
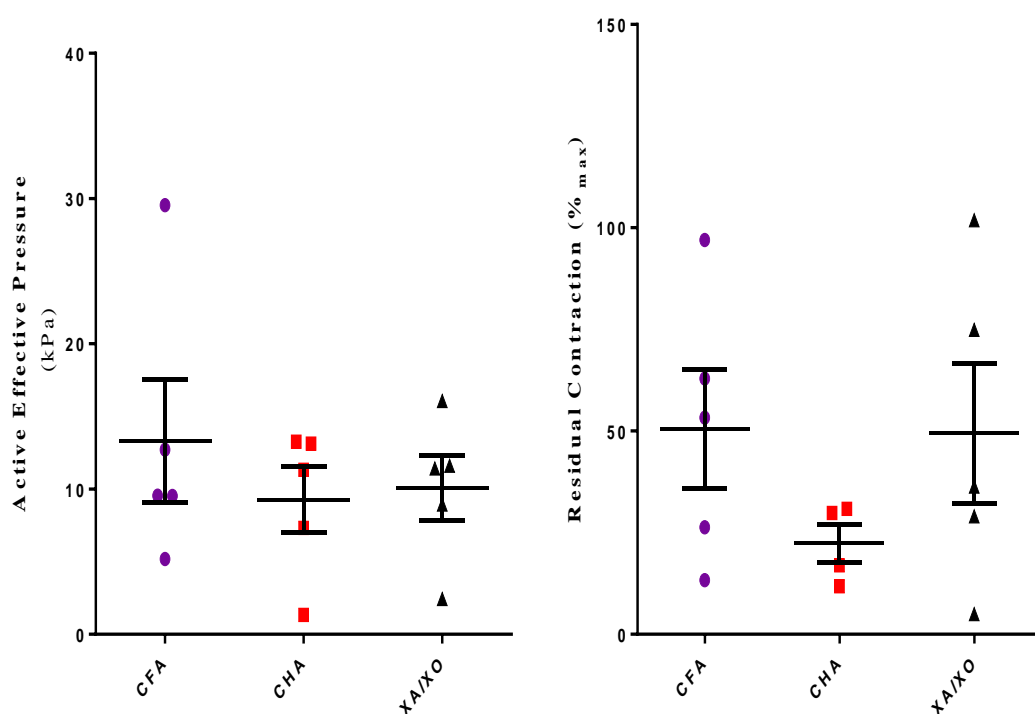
Figure 31: Peak AVP induced contraction in myometrial arteries was unaffected by incubation with caffeic / chlorogenic acid (left panel) (n = 10; Mean +/- SEM; P<0.05; Kruskal Wallis with Dunns *post hoc* test). Residual XA / XO contraction as % maximum constriction indicated that the longevity of response was not significantly affected by incubation with caffeic / chlorogenic acid (P<0.05; n = 4; Mean +/- SEM; Kruskal Wallis with Dunns *post hoc* test).

8.5.9 H_2O_2 versus XA / XO

Myometrial vessels experienced a sharp relaxatory effect after the direct addition of a reactive oxygen species (H_2O_2 or XA / XO). The vessel often returned to their initial peak contraction before gradually relaxing.

There was no significant difference in peak to trough relaxation in vessels incubated with caffeic acid (82.7 ± 15.6 %) or chlorogenic acid (61.2 ± 14.7%) and exposed to H_2O_2 (54.0 ± 10.0 %). There was also no significant difference in peak to trough relaxation in vessels incubated with caffeic acid (39.5 ± 16.6 %) or chlorogenic acid (60.3 ± 14.5 %) and exposed to XA / XO (58.0% ± 13.3%).
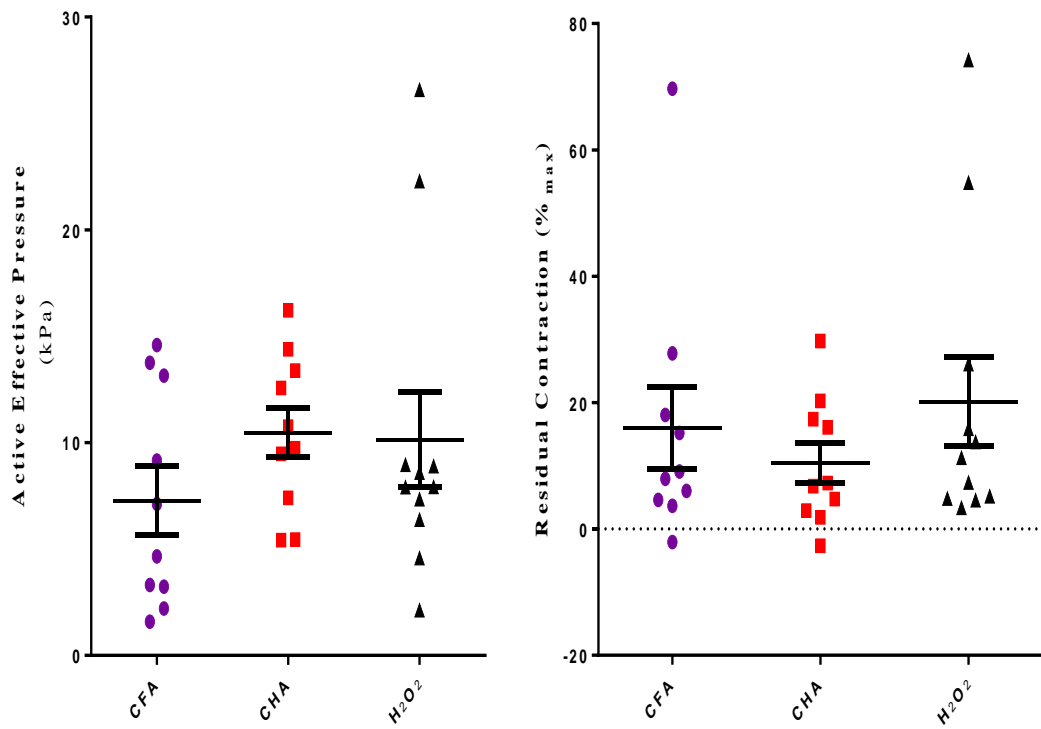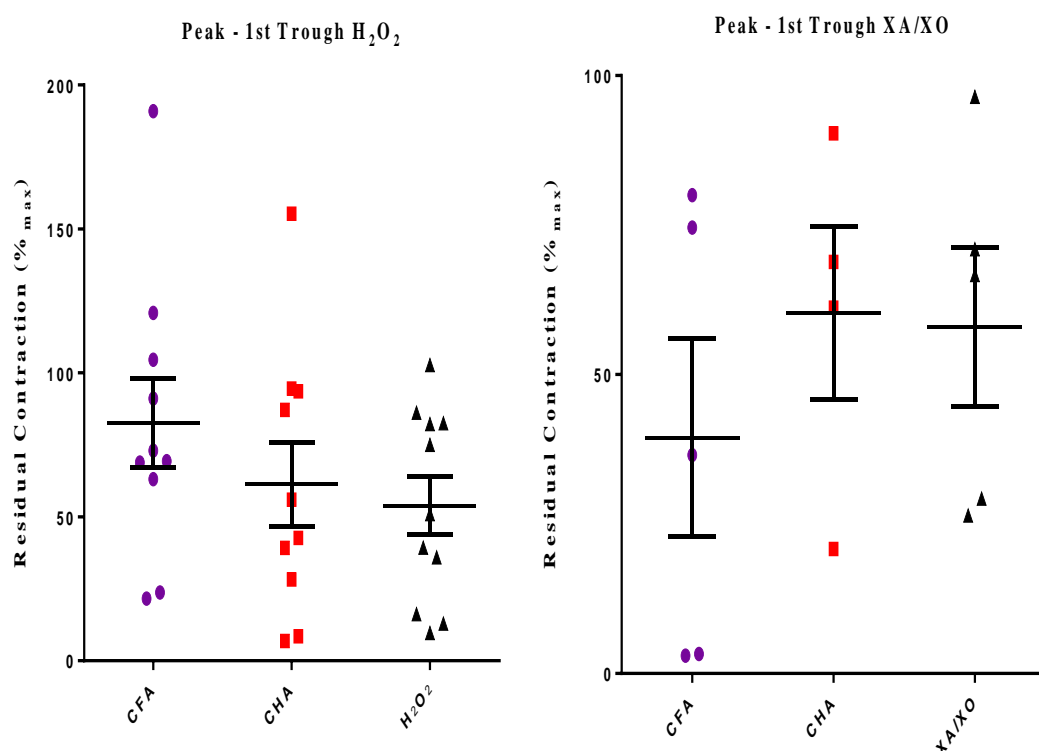
Figure 32: Initial relaxation in myometrial arteries was unaffected by incubation with caffeic / chlorogenic acid (left panel) (n = 10; Mean +/- SEM; P<0.05; Kruskal Wallis with Dunns *post hoc* test). Residual XA / XO contraction as % maximum constriction indicated that the longevity of response was not significantly affected by incubation with caffeic / chlorogenic acid (P<0.05; n = 4; Mean +/- SEM; Kruskal Wallis with Dunns p*ost hoc* test).

As described above, a comparison of residual contraction between H_2O_2 to XA / XO indicated that there was no significant difference in relaxation after 15 minutes incubation in myometrial vessels (P = 0.0779). The example trace indicates that there may be a trend towards significance, similar to the H_2O_2 -XA / XO Chorionic plate artery results. Lack of XA / XO numbers (n = 5) may account for these results.

8.6 Summary

Myometrial vessels assessed for vessel viability with a standard start, including two KPSS washes, an AVP constriction and addition of BK to assess for relaxation. Investigations into the effect of active coffee chemicals on myometrial vessels were insignificant. Caffeic and chlorogenic acid did not significantly alter basal tone. Caffeic and chlorogenic acid did not significantly relax myometrial vessels that were stimulated with AVP.

The potential antioxidative effects of caffeic and chlorogenic acid were also assessed; vessels were incubated with caffeic acid and chlorogenic acid for a period of 15 minutes. A ROS was added post stimulation with AVP. There was no significant protective antioxidative effect on the vessels; maximum constriction was not affected and residual contraction after 15 minutes was similar to that of control vessels. This implies that caffeic acid and chlorogenic acid do not exhibit antioxidative properties with regards to myometrial vessels.

Effect	Caffeic Acid	Chlorogenic Acid
Constriction	↔	↔
Relaxation	↔	↔
H_2O_2 Maximal Constrict	↔	↔
H_2O_2 Residual	↔	↔
XA / XO Maximal Constrict	↔	↔
XA / XO Residual	↔	↔

Table 21: Summary of the effect of caffeic acid/ chlorogenic acid on myometrial function and basal tone.

8.7 Chorionic Plate Arteries versus Myometrial Vessels

Constant and conflicting data was obtained from the fetoplacental and myometrial vasculature. We are restricted to comparing the initial KPSS reaction between chorionic plate arteries and myometrial vessels as we utilised varying agonists during the experiment. Chorionic plate arteries were constricted to 5.0 ± 0.5 kPa. Myometrial vessels were exposed to the same amount and concentration KPSS; these vessels constricted 9.2 ± 0.8 kPa. This was a significantly greater contraction, analysed using a Mann-Whitney Test, $P = 0.0003$.

Chorionic plate arteries that were exposed to U46619 constricted by 7.7 ± 0.8 kPa. Myometrial vasculature was exposed to AVP; this induced an 11.0 ± 0.9 kPa constriction. Vessels were exposed to acetylcholine and bradykinin, both endothelial dependent relaxatory agents. Acetylcholine did not exert a relaxatory effect on chorionic plate arteries, with the vessels actually continuing to constrict post exposure ($103.0 \pm 2.7\%$ of residual contraction); however myometrial vessels relaxed significantly when exposed to bradykinin ($36.1 \pm 7.4\%$ of residual contraction).

Chorionic plate arteries were exposed to ROS (H_2O_2 and XA / XO) post incubation with caffeic acid and chlorogenic acid. Placental arteries constricted on addition of ROS species. This constriction was assessed over a 15 minute period and residual contraction was noted. Myometrial arteries were exposed to a contractile agonist (AVP) post 15 minute incubation with caffeic acid and chlorogenic acid. Addition of ROS induced a relaxatory effect on the maternal vasculature. There were two points of relaxation measured; the initial peak to first trough and the residual contraction. The mechanism of action and this explanation of the contrary effect will be considered below in the Discussion (Chapter 9).

No effect was noted on fetoplacental and maternal vasculature when exposed to active coffee chemicals. Caffeic acid and chlorogenic acid did not demonstrate any constrictor or relaxatory effects on chorionic plate arteries or myometrial vessels.

These coffee compounds did not exhibit any antioxidative properties on the chorionic plate and myometrial vasculature when exposed to ROS, H_2O_2 and XA / XO.

Chronic and acute exposure of caffeine on chorionic plate arteries was assessed. Unfortunately, time and sample constraints did not allow us to assess the effect of caffeine on myometrial vasculature. Caffeine did not exhibit any considerable effect on the vasculature; no constrictor, relaxatory or antioxidative properties were demonstrated.

8.8 Conclusion

In summation, my results indicate that placental and myometrial vasculature behaved as expected with regards to stimulation to agonists and relaxatory agents, however, exhibited some contradictory behaviour when exposed to the isolated coffee chemicals utilised (Mills et al. 2005; Myers et al. 2005; Sweeney et al. 2008; Mills et al. 2009). These findings will be discussed in greater detail in Chapter 9.

As stated above, chorionic plate artery and myometrial vasculature were assessed for viability by administering a 6 ml 120 mM of KPSS. This elicited a significant contraction within both the fetoplacental and maternal vasculature, indicating that these vessels were viable for experimentation, as well as reacting similarly in the presence of a high potassium concentration.

Chapter 9 Quantitative Discussion

9.1 Introduction to Quantitative Discussion

Coffee is one of the most frequently consumed beverages in the world; it represents both culture and economy and plays a major role in our day to day society. Its consumption is generally considered to be associated with disease and poor health behaviours by the general public and media however the majority of epidemiological studies have not yielded clear conclusions. The stigma attached to coffee consumption is very apparent and has been discussed previously (Chapter 1 and Chapter 2). These uncertainties are mainly due to the lack of concrete and consistent information regarding frequency of consumption, the exact composition of the beverage, and factors associated with an unhealthy lifestyle (e.g. smoking). All of these aspects are potential causal factors for disease. I previously discussed my findings regarding the stigma attached to coffee consumption amongst the population I investigated. These women were seeking their pregnancy care at St. Mary's hospital and ranged in gestational ages. My findings correlated with those reported in much of the literature.

Many epidemiologic studies assess the potential relationship between coffee and cardiovascular disease. Over 2000 substances have been isolated from coffee; the major compounds being caffeine, caffeic acid, chlorogenic acid and Cafestol and kahweol as discussed above (Section 2.14). My work focused primarily on caffeic acid, chlorogenic acid and caffeine due to their ease of access. Cafestol and Kahweol, although potentially pharmacologically important, were not easily available and there were particular time constraints with the experimentation.

Much research on coffee and its physiological effects has been conducted; both beneficial and harmful effects have been considered. It has been suggested that the antihypertensive action of the coffee bean is due to the fact that the raw and processed product contains chlorogenic acid as a major phenolic compound. In turn, ferulic acid a metabolite of chlorogenic acid has been shown to act on NO derived from the vascular endothelium. Studies investigating its anti-mutagenic and anti-carcinogenic effects have been conducted and, similarly, results reported were inconclusive. The anti-mutagenic and anti-carcinogenic effects are often grouped

under the heading of coffee's antioxidant effect. The mechanism of action of antioxidants involves the removal of free radicals within the body. It is thought that these free radicals induce cellular injury, amongst other pathological processes. Thus my study aimed to investigate whether incubation with active coffee chemicals could potentially inhibit or reduce the cellular or vascular injury elicited through H_2O_2 administration.

Coffee is a large part of modern society's diet. Coffee shops and Cafes are becoming more common in both urban and suburban areas; they are social hubs that are utilised by all members of the population. Coffee is the most popular caffeinated beverage in the UK, predominantly amongst young adults. Adequate diet and nutrition are fundamental during the gestational period. Studies have been conducted regarding the effect of maternal diet on *in utero* environment; results indicate that what the mother consumes during pregnancy can impact pregnancy outcome and development of adult diseases amongst her offspring.

As gestational age increases, so too does the need for energy to meet the nutritional demands of the fetus. The placenta plays a pivotal role in off spring growth and adequate nutrient transport. It is the primary organ for nutrient transfer and its compromise could lead to development of specific pregnancy pathologies. Fetal growth is directly related to maternal nutrient availability and the placentas ability to transport these nutrients from the maternal circulation to the fetal circulation. Placental vascular compromise is a risk factor for poor pregnancy outcome. It has been associated with pathologies such as preeclampsia, gestational diabetes and intrauterine growth restriction. My work focused on the effect of active coffee chemicals on the chorionic plate arteries and myometrial vessels in normal pregnant samples. The aim was to investigate whether these compounds could impact vascular tone and potentially be associated with vascular compromise.

Wire myography was used to assess the vascular function in normal samples obtained. Wire myography has been demonstrated to be a robust and reproducible technique for the study of vascular function in small placental and myometrial resistance vessels (McCarthy & Woolfson 1994; Wareing et al. 2002). Isolating vessels from the placenta or myometrial sample inevitably leads to the removal of certain *in situ* structure however it is preferable over the placental perfusion model.

One of the main advantages is the increased level of experimental control that is associated with wire myography.

9.2 Placental Chorionic Plate Arteries versus Myometrial Vasculature

It is obvious to any student of maternal and fetal health that multiple factors contribute to the intrauterine environment that permits normal growth and development of the conceptus. The maternal *in utero* environment must provide safety factors for embryonic survival under stressful conditions. Initially we considered the fetoplacental vasculature separate to maternal vasculature. Its development has been discussed above (Chapter 2, Section 2.3 and Section 2.4). There are similarities and differences between both types of vasculature. The fetoplacental vasculature represents what we would expect to see in fetal circulation, myometrial vasculature is representative of the maternal circulation.

As stated, there are many similarities between the two types of vessels. Structurally these vessels are very comparable; using light microscopy it is easy to identify the single layer of endothelial cells, middle layer of smooth muscle cells and an outer adventitia. Smooth muscle cells are orientated in a circular fashion around the lumen of the vessel and the layers are separated by extracellular matrix (ECM). The endothelial cells of the myometrial arteries, similar to systemic arteries, are supported by an elastic lamina. This is absent in placental resistance vessels as the endothelial cells and smooth muscle cells are separated by a basement membrane (Sweeney et al. 2006). The smooth muscle cells in placental arteries were also separated by much connective tissue and contain relatively small numbers of sarcoplasmic reticulum in comparison to myometrial arteries. Smooth muscle cells of placental resistance vessels are separated by much collagen fibres, orientated in many directions; this gives rise to a thick medial layer. In contrast, the smooth muscle layers of myometrial vessels are tightly packed with less ECM.

Non-pregnant uterine blood vessels respond similarly to those of any other muscular organ. The predominant difference is the sensitivity to oestrogenic stimulation, response to local anaesthetic agents and response to prostaglandins. During pregnancy the blood vessels supplying the placenta progressively dilate until a point where no further dilation can occur. It is therefore plausible to conclude that stimuli

which evoke vasodilation in the non-pregnant uterine vessels are ineffective during pregnancy, particularly once complete placentation has occurred. Placental vessels retain their ability to vasoconstrict similar to that in the non-pregnant state. Human placental and myometrial vascular tone is maintained though factors including TXA_2, prostacyclin, NO, angiotensin II and endothelin (Sweeney et al. 2008). It is essential that there is a tight balance between these factors to ensure adequate placental perfusion.

The primary role of the placenta is to provide nutrition to the growing fetus, to remove waste products and finally, protect the developing embryo from harmful substances. Similar to the blood-brain barrier, the placental barrier inhibits certain substances from crossing but freely allows others to pass. As discussed at length above, coffee contains many active substances, some of these known to exact an effect on human vasculature. Caffeine and other coffee chemicals pass this barrier with ease. As discussed above, the formation of the chorionic plate arteries and adaptations of maternal myometrial vessels may occur during the gestational period however their physiological differences are significant. Chorionic plate arteries are considered to be part of the fetoplacental circulation whereas myometrial vessels are considered to be systemic vessels.

My investigations indicated that there was a difference in the contractility of the vessels to the same stimulus. I found that myometrial vessels produced a greater contraction when exposed to 60mM KPSS. This correlates with previous investigations conducted by groups investigating vascular function in both placental and myometrial vascular beds (Myers et al. 2006; Sweeney et al. 2008). KPSS is potassium solution used to assess vascular function for both placental and myometrial vessels.

I cannot directly compare the contraction between chorionic plate arteries and myometrial vessels with respect to receptor-induced activation of smooth muscle cells as I used different contractile agents; U46619 with placental vasculature and AVP with myometrial vessels. Similarly, we cannot directly compare relaxation as we utilised ACH for chorionic plate resistance arteries and BK for myometrial vessels. I can, however, conclude that results obtained mirrored those achieved by

other groups within the field of maternal and fetal health (Ashworth & Warren 1997; Wareing et al. 2002; Wareing & Baker 2004).

U46619, the thromboxane mimetic, induced a maximal contraction in the placental resistance vessels. U46619 is thought to be more physiologically relevant than vasopressin, it is produced locally by release from platelets and provides sustained, reproducible contractions (Wilkes et al. 1990; Myatt & Brewer 1992; Wareing, Susan L Greenwood, et al. 2006). This process is a standard method of assessing placental vascular function and the results I obtained were further verified by previous studies conducted within the field. AVP induced a maximal constriction in myometrial vessels, as expected. I demonstrated that ACH was largely ineffective as a vasodilatory agent within the placental bed. This correlates with the work conducted by McCarthy *et al.* (1994) and Wareing *et al* (2002) (McCarthy & Woolfson 1994; Wareing et al. 2002). AVP-constricted myometrial vessels exhibited a dose-dependent relaxation to increasing concentrations of BK.

Vessels were maximally constricted to assess vascular function. Many other studies utilise this method to ensure endothelium is intact and thus ensure vascular function is representative of an *in vivo* state.

I also noted that a proportion of my myometrial vessels demonstrated the phenomenon of vasomotion. Vasomotion or myometrial oscillations are defined as cyclical variations in the amount of tone demonstrated by arteries. Similar to other studies, vasomotion was detected in vessels that were previously constricted with the agonist AVP. Thromboxane induced oscillations in human chorionic plate arteries were not observed. The main theory behind this observation is rooted in NO bioavailability. Sweeny *et al* (2008) suggested that this might reflect endothelial cell receptor signalling differences between fetoplacental and myometrial vascular beds whereby vascular tone regulation differs. Maternal placental vascular resistance is mainly determined by the diameter of the myometrial vessels. This tissue is susceptible external forces, such as physical influences or neuronal input altering activity of the surrounding smooth muscle tissue. Synchronised oscillations in blood vessel tone are a common feature of myometrial vessels. It is thought that the rhythmic alterations are a physiological necessity to ensure the necessary amounts of O_2 and nutrients are perfused to the developing fetus (Sweeney et al. 2008).

On administration of ROS, chorionic plate arteries and myometrial vessels behaved very differently. In non-placental vascular beds, ROS are important mediators for vascular tone and are present in both physiological as well as pathological conditions. Placental production of ROS increases significantly during gestation as placental mitochondrial mass increases (Myatt & Cui 2004; Mills et al. 2009).

H_2O_2 and XA / XO administration resulted in an induced transient constriction in the placental vascular bed. Peroxides induce vasoconstriction in the placental bed by stimulating thromboxane production. Considerable evidence suggests that the thromboxane generation is stimulated as a result of H_2O_2 interacting at several sites in the pathway of arachidonic acid metabolism. More specifically, the O_2 metabolites cause an increased liberation of substrate arachidonic acid from cell membranes. Two major possibilities seem to exist; ROS attack unsaturated bonds of membrane lipids and this lipid peroxidation can become autocatalytic, thus leading to changes in membrane structure and increased release of membrane bound arachidonic acid (Tate et al. 1984). Another possible, but less likely mechanism, is the stimulation of phospholipases by ROS (Tate et al. 1984).

My research supported previous studies on the effect of ROS on myometrial vessels (Mills et al. 2006). H_2O_2 is thought to induce myometrial relaxation via several mechanisms. One suggested mechanism is the conversion, in the myometrium, of O_2^{2-} to H_2O_2 by superoxide dismutase. As well as this, the expression of H_2O_2 has been shown to up regulate NO synthase expression. The final suggested, and most likely, pathway of vasorelaxation involves the activation of cGMP-dependent protein kinase. This would result in the phosphorylation and activation of voltage dependent K^+ channels (Appiah et al. 2009). Whether the activation of these channels is direct or indirect is still unexplained. H_2O_2 may act via direct activation by targeting protein thiol groups that regulate the K^+ channels. The indirect pathway may involve reactive nitrogen species (Appiah et al. 2009).

As discussed previously, increased ROS production can be a sign of pathology and cause damage to the resistance vasculature of the placenta (Myatt & Cui 2004). We must also be aware that ROS, namely H_2O_2, potentiates vascular tension in the umbilical artery however the mechanism is unknown (Okatani et al. 1997). The administration of an antioxidant, or O_2^- scavenging compound could reduce smooth

muscle cell constriction thereby preventing the potential damage to the tissue and improving placental perfusion. Studies utilising well known antioxidants (Ascorbic Acid / Vitamin C or Vitamin E) have been conducted and have shown any beneficial results (Mills et al. 2009).

We hypothesized that the coffee compounds, caffeic acid and chlorogenic acid, had potential antioxidative activities. These properties had been demonstrated in other tissues however had never been considered within the uteroplacental vasculature. Incubation of both chorionic plate arteries and myometrial vessels with concentrations of caffeic acid and chlorogenic acid did not seem to attenuate the effect of ROS on constriction or relaxation of vessel. Acute and chronic caffeine did not have any significant effect on chorionic plate arteries, suggesting that it did not exhibit antioxidative or vasorelaxatory properties.

9.3 Potential Contractile and Relaxatory Ability of Caffeic Acid, Chlorogenic Acid and Caffeine on Placental and Myometrial Vasculature

Coffee, more specifically, active coffee chemicals are often associated with cardiovascular pathologies. There are also suggestions that coffee consumption is associated fetal cardiovascular function; *in utero* environment is associated with pregnancy pathologies, growth and development of the fetus, and development of adult cardiovascular disease. As stated above, active coffee chemicals and their effects on the placental and myometrial vasculature has not been assessed.

Caffeic acid, a metabolite of chlorogenic acid, is thought to reduce cell proliferation and down-regulates the NADPH oxidase activity in angiotensin II-induced vascular smooth muscle cells (Suzuki et al. 2006) but no work has been identified assessing the effect of direct application of these compounds on vasculature. An extensive literature search indicated that the information surrounding caffeic acid and chlorogenic acid was ambiguous. The work that had been conducted focused mainly on the potential antioxidative properties of these chemicals *in vitro* and *in vivo*. My investigations assessed the effects of multiple concentrations upon dissected vessels. My results indicated that caffeic acid did not significantly effect on placental or myometrial vessels *in vitro*. The concentrations that I used in my study did not stimulate a constriction in the vessel and did not induce a relaxation in those that

were previously stimulated. Chlorogenic acid showed similar results; i.e. no contractile or relaxatory affect noted on the direct application of chlorogenic acid upon placental and myometrial vessels.

Of the alkaloids, the most studied and recognized on is caffeine, which makes up to 1.3 to 2.4% of the bean's weight (Echeverri et al. 2010). Caffeine is also absorbed rapidly and completely form the intestinal tract, making it 100% bioavailable (Manach et al. 2005; Echeverri et al. 2010). When considering caffeine and its systemic effect, we must be aware of the mechanisms of action of caffeine at the endothelial level. Endothelium is one of the most extensive tissues within the human body. It lines the arterial walls and is a highly selective and permeable membrane. Its ability to synthesize and release a wide range of vasoactive substances allow for the regulation of vascular tone (Sandoo et al. 2010). This is true for both systemic and placental vasculature. Caffeine promotes NO synthesis in the endothelium by the release of Ca^{2+} from the endoplasmic reticulum. However, caffeine should be an antagonist of the adenosine receptors. It is well known that adenosine induces vasodilation therefore antagonization of the adenosine receptor could induce vasoconstriction. My study investigated the acute and chronic effects on placental vessels; the results did not indicate any significant effect of caffeine on vascular tone. However, from the results I noted a trend towards increased vascular contraction; this was not a significant one. The rationale for this was unknown however may be due to a limited sample size; thus these variations could be eliminated with increased numbers. One possible justification for my findings could be that the balance between the vasodilatory effect of caffeine as an endothelium-dependent vasodilator and the vasoconstrictive effect of caffeine as an adenosine receptor antagonist may regulate vascular tone.

9.4 Potential Antioxidative Effect of Active Coffee Chemicals

ROS are recognized to be physiological regulators of vascular tone, however, in larger concentrations is associated with vascular dysfunction. Mechanisms underlying impaired endothelial function in various pathologies are likely multifactorial. There is a growing body of evidence suggesting that oxidative stress, defined as an imbalance between endogenous oxidants and antioxidants in favour of the former, contributes to mechanisms of vascular dysfunction. A dominant

mechanism is the oxidative action of superoxide, as mentioned above (Chapter 2, Section 2.13.1). When I assessed the potential antioxidative properties of caffeic acid and chlorogenic acid I did not observe any significant effect. These results were obtained upon direct application of the chemical upon either placental or myometrial vessels. As stated above, direct administration of ROS on chorionic plate arteries and myometrial vasculature induces contrasting effects. As stated above, the administration of H_2O_2 and XA / XO on isolated chorionic plate arteries induces constriction. Conversely, H_2O_2 and XA / XO both relax the stimulated myometrial vasculature.

Elevated concentrations of ROS are associated with fetoplacental compromise and thus pregnancy pathologies such as fetal growth restriction and pre-eclampsia; it is suggested that altered K^+ channel function induced by ROS contributes to fetoplacental dysfunction (Mills et al. 2009).

There is great interest in the potential therapeutic therapies that target ROS production, particularly relating to pregnancy. There have been suggestions that maternal supplementation with potential antioxidants may reduce the incidence of certain pregnancy pathologies. These studies were actually associated with adverse events in pregnancy (Poston et al. 2006; Rumbold & Crowther 2006). These unfavourable results direct investigators to search for alternative substances that may give more encouraging results.

There is much literature surrounding the potential antioxidant properties of coffee chemical. Caffeic and chlorogenic acid have previously been shown to exhibit antioxidative properties *in vitro* (Olthof, Hollman & Katan 2001). The antioxidative activity of polyphenols is associated with their hydroxyl groups; however is not the only factor in determining the potency of their activity. Chlorogenic acid, an ester of caffeic acid with quinic acid, and has been demonstrated to be less effective antioxidant than caffeic acid (Chen & Ho 1997). My results did not concur with the bulk of the literature surrounding caffeic acid and chlorogenic acid and their potential antioxidative properties. There was no reduction in effect of ROS on the isolated vessels suggesting that there was no antioxidant effect. There could be several factors influencing our results though, which will be discussed in further detail below (Chapter 10, Section 10.2).

9.4.1 Caffeine

Within my study I assessed the potential protective effect of caffeine on placental vasculature. My investigation concluded that caffeine did not seem to exhibit any effect on placental vasculature. These were unexpected results as caffeine is a xanthine compound which studies have shown displays several mechanisms of action on the vascular wall. This effect has been extensively studied on the endothelial tissue and vascular smooth muscle cells (Echeverri et al. 2010). As well as this, caffeine is known for its effects on the autonomic nervous system and on arterial pressure. These noted effects are as a result of the inhibition or blocking of receptors, namely adenosine, IP3, NO (Elmenhorst et al. 2011). There also seems to be a contradictory effect depending on the site and cellular structure, as well as the duration and concentration of exposure. Studies have found the there is a mild and transitory constrictor effect on vascular smooth muscle and this is dependent on caffeine concentration (Echeverri et al. 2010). However the most common effect of caffeine on the vasculature is dilatory, acting equally on the vascular smooth muscle both directly and indirectly, and on endothelial cells. During the study I varied length of exposure however these exposures maintained a steady caffeine concentration. The concentration of caffeine utilised was based on literature surrounding caffeine within serum; it is important to remember that serum caffeine levels are often very similar to maternal levels (Klebanoff et al. 1999). Studies which examined serum levels of caffeine in fetal and neonatal serum were considered (Cazeneuve et al. 1994; McGowan 1988; Picard et al. 2008) as well as those studies which linked specific serum caffeine concentrations with a particular poor pregnancy outcome (Klebanoff et al. 1999; Greenwood et al. 2014; Bracken et al. 2003; Grosso et al. 2006; Momoi et al. 2008; Holland et al. 1998).

9.5 Foundational Dietary Studies and Their Impact on Health Care and Research

Health and nutrition have been prominent in research over the last century. These studies have highlighted the impact that diet has on general health, development of disease and pregnancy. Neither of these studies have a direct association with my research however they have paved the way for studies that concern diet and pregnancy. The Dutch Famine Study was fundamental research conducted in the

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early half of the 20th century; it examined the effect of poor nutritional status on pregnancy. The use of interventional studies amongst adolescents has been utilised all over the world in the last decade; these studies aim to improve health by increasing intake of healthy, low fat foods. Many of the interventions suggested were demonstrated in the TEENS and Higgins Intervention study, to name but a few. Aspects of these studies have been applied in more recent research, particularly with regard to teenage pregnancies (Symon & Wrieden 2003; Wrieden & Symon 2003; Nielsen et al. 2006).

9.5.1 Dutch Famine Study Revisited

The Dutch Famine Study as a cornerstone of maternal and fetal nutrition studies was discussed briefly above (Section 1.7.6). The findings of the Dutch famine birth cohort study broadly supports the fetal origins hypothesis. The suggestion that chronic diseases originate in the womb through adaptations made by the fetus in response to a nutritional and non-nutritional stimuli has been further verified in the following 90 years. Similarly, my investigation considered an aspect of maternal diet however has taken a more modern slant. Women during the post-war period, and for the majority of the 1900's, were not exposed to coffee during the gestational the same yet the fundamentals are appropriate in considering the impact of nutrition on pregnancy outcome in our modern population. The Café culture has thrived in the last 25 years and thus it is important to consider coffee during the gestational period of our current population. Little is still known about what an adequate diet for pregnant women may be however the fact that women are more receptive to advice on diet and lifestyle during the gestational period is well known. The importance of adequate dietary advice was highlighted in both the Dutch Famine Study and the work that I have undertaken; this should be exploited to improve the health of future generations.

9.5.2 Nutritional Intervention Studies

Dubois *et al* (1997) applied the Higgins Nutrition Intervention Program, aiming to increase term birth weight and reduce the risk of developing pregnancy pathologies, particularly amongst adolescents (Dubois et al. 1997). The Higgins program has multiple intervention components, but can be broken into four steps; assessment of

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the risks within the pregnancy, determination of individual dietary prescription based on the combination of the normal requirements of pregnancy and rehabilitation allowances for diagnosed risks, nutritional education that meet individual dietary prescriptions, while respecting pre-existing food habits, and follow up and supervision by health care professionals at regular intervals (Dubois et al. 1997). These interventions have been shown on numerous occasions to reduce the likelihood of adverse outcomes occurring (Higgins 1976; Higgins et al. 1989; Dubois & Dougherty 1991).

The TEENS study was another nutritional intervention study, focusing on improving the diet and nutritional status of adolescents, as well as altering social behaviour (Lytle et al. 2004). The interventions within this study have been shown to have beneficial results and thus are being applied in those studies focusing on pregnancy in the adolescent. The outcomes of these studies are uniform in that they suggest that nutrition educators need to provide information to adolescents; this education should improve outcome and ensure pregnant women gain the required amount of weight during gestation (Hunt et al. 2002).

The Great Beginnings study also aimed to improve nutrition in the pregnant adolescent. The experimental group demonstrated marked improvements in health and reduced risk of pregnancy pathologies (Long et al. 2002).

These interventional studies further corroborate with the concept that poor nutrition and pregnancy pathologies link. Furthermore, the converse can also be said, in that improved nutritional status and social and cognitive behavioural therapy can positively affect pregnancy outcome and reduce the likelihood of a pregnancy pathology occurring. This concept is fundamental within my research. Women are more motivated during their pregnancy to improve health and adopt better nutritional habits in order to do 'what's best for baby' (Renkert & Nutbeam 2001; Nilsen 2009; Szwajcer et al. 2007). For this reason, we must examine the effects of coffee on pregnancy, format evidence based on these results and inform women thusly.

9.6 Summary

Coffee and its by-products are thought to have physiological effects. There are paradoxical results, with literature suggesting both positive and negative effects. My

research focused on caffeic acid, chlorogenic acid and caffeine as these are thought to be the most biologically active. The placenta and its vasculature is representative of the fetoplacental circulation; for this reason administration of varying concentration on these resistance vessels may indicate an effect. Myometrial vessels are representative of maternal systemic vasculature. These vessels and, similarly, administration of coffee metabolites may ellicit an effect. Myometrial and fetoplacental vasculature play a fundamental role in fetal growth and development thus any effect may cause inadequate perfusion and poor nutrient transfer. The overall findings from my investigations into the effects of caffeic acid, chlorogenic acid and caffeine on chorionic plate arteries and myometrial vessels were not significant. These metabolites did not demonstrate any constrictory or relaxatory effects on chorionic plate arteries or myometrial vasculature. Similarly, the metabolites caffeic acid, chlorogenic acid and caffeine did not demonstrate any protective effects on placental or myometrial vasculature when incubated with a ROS.

Chapter 10 Final Remark, Strengths, Limitations, Recommendations and Future Work

10.1 Impact of My Study

This chapter will introduce the concept of combining the qualitative and quantitative work and linking their findings to answer our aims and objectives. As with all new research, we must consider the 'so what?' factor; this can be defined as the direct influence or effect that my research has at this very point in time. I will also discuss the potential impact that my study has on the current body of knowledge and what bearing it may have on future work.

As stated previously my researched involved both qualitative and quantitative strands in order to assess and achieve the aims outlined. The qualitative aims were to investigate womens' and midwives' views, attitudes and opinions on coffee consumption during the gestational period. This information was obtained by conducting semi-structured telephone and face-to-face interviews with participants. The quantitative aims were to examine the potential contractile and relaxatory effects of coffee oils on chorionic plate and myometrial vessels. As well as this, I investigated the potential antioxidative properties of coffee oils on chorionic plate and myometrial arteries. These investigations were conducted utilising the technique wire myography.

The aims and objectives of both branches, qualitative and quantitative, were met throughout the research period. With regards the qualitative research, data saturation was achieved and no new concepts or knowledge were obtained i.e. no new knowledge would be collected with the addition of participants. With the quantitative work, the required numbers of experiments were completed, and similarly, data collection ceased when no new results were achieved and we could say, with some degree of certainty, that the data were not significant. Although a wealth of data was gathered, time restraints and limited sample collection impeded some branches of research, which will be discussed further below (Section 10.2)

A concise systematic review, searching all relevant literature, indicated that there was a significant gap in the knowledge surrounding coffee consumption and pregnancy. My search indicated that there was no literature surrounding womens'

and midwives' opinions and viewpoint on coffee consumption during pregnancy. There was much literature surrounding the physiological and potential pathological effects of coffee and its by-products. The findings of these latter studies were conflicting, with some reporting possible positive and negative consequences of coffee consumption but very few conclusive outcomes. It was also noted that confounding factors, such as tobacco and alcohol use, are known to influence results and thus must be taken into consideration.

My unique contribution to knowledge includes both qualitative and quantitative aspects. My work has paved the way with regards to investigating womens' and midwives' views and opinions on coffee consumption. The interviews that I conducted varied in depth and briefly investigated womens' coffee consumption and considered the views on consumption during their pregnancy. The information that I gained could potentially be used to restructure future interviews and look for / at different aspects of coffee consumption and attitudes. I also investigated their opinions on sources and modes of information. With regards to midwives and their interviews, I questioned them on their opinions on coffee consumption during pregnancy and the advice that they provide to pregnant women. My findings suggest that women and midwives' are aware of the stigma attached to coffee; thus women tend to reduce intake pre-conception and during the prenatal period. Midwives advise women to reduce intake, although the rationale behind this counsel is often unsubstantiated. My quantitative research suggests that the active coffee chemicals had no significant effect on chorionic plate arteries and myometrial vessels at physiological concentrations. These findings suggest that the stigma and advice surrounding coffee may not be accurate or evidence based; this could lead the way for more investigations and thus more evidence based advice being provided, which will be discussed further in the recommendations section (Section 10.3).

As stated my research is unique and has, in its own way, contributed to the current body of knowledge. It is the first to my knowledge that considers womens' and midwives' views and opinions on coffee consumption during the gestational period. My investigations into the effects of coffee chemicals on the fetoplacental and systemic vasculature were also novel but unfortunately did not find any highly significant findings. This highlights the current dilemma, which is when there is not sufficiently strong evidence it is difficult for midwives to provide concise and

accurate information. This in turn forces women to search for more evidence which can be unreliable. Women supplement the advice and information they are given with sources that are potentially inaccurate; this could have potential ramifications on the pregnancy. Midwives struggled to source sufficient evidence based information and stated that dissemination of available information down through the ranks was often flawed.

The experiments conducted were basic in nature and there are still so much more to be done so that women can be better informed and midwives will be more confident. Further future potential experimentation will be discussed in Section 10.3.

Abstinence and scaremongering are the current practices when providing information on coffee consumption during pregnancy. These methods are no longer effective as women need more substantial advice that caters to their needs. Women are more aware of their pregnancies and are highly motivated to alter poor life habits to ensure a good pregnancy outcome. Women stated that they supplemented the information obtained but whether this information was accurate is yet to be established. This further validates the concept that abstinence and scaremongering are the only practices utilised with regard to advising women on coffee during their pregnancy.

Contrary to this, women and midwives' would have highlighted if there were definitive information available, either in the recommended literature or the media. No significant findings were unveiled and thus we can assume that there is none available to the general public. If there was solid, evidence based information surrounding coffee consumption we can assume that changes to practice would have already occurred.

10.2 Strengths and Limitations

There are strengths and limitations to my research, in both the qualitative and quantitative components. In general, my research aimed to improve on the research of others, identify the gaps in the knowledge and make logical research recommendations based on these findings. The advantages and disadvantages of mixed method research were briefly discussed however the more research specific benefits and complications will be considered below.

The main strengths of mixing methods, as I have performed in my study, are corroboration and elaboration. Corroboration is defined as deriving the same results from both qualitative and quantitative research (Brannen 2005). My qualitative analysis identified uncertainties surrounding the potential impact coffee consumption during the gestational period has on pregnancy outcome. Similarly, my literature search and quantitative data indicated that the physiological effects of coffee were unclear. My research highlighted this ambiguity and has created a platform for further research to be conducted.

My research aimed to generate insight into an under researched topic; mixing both qualitative and quantitative methods provided greater insight. I was able to design an effective research strategy and thus frame the necessary questions. A combined research process enabled me to put the research into context, make sense of the data and finally re-contextualize the results in relation to the initial hypothesis. Contextualization is a critical part of a multi-method process in order to better understand the data.

When evaluating caffeine and coffee exposure, inaccuracies often occur that could alter the results. For example, exposure to caffeine is often estimated after the occurrence of the event (Leviton & Cowan 2002) with associated recall bias. Total caffeine consumption is also difficult to measure accurately without resorting to collecting maternal blood and accurately measuring the levels of caffeine and its metabolites. As discussed above, women can be exposed to caffeine from many sources (David et al. 2010). As well as the source, caffeine content of individual beverage servings varies considerably by method of preparation, product brand and size of serving (Bracken et al. 2002). This highlighted in a studies by both Crozier *et al* (2012) and Ludwig *et al* (2014), where evidence suggested that there were great variations of caffeine and chlorogenic acid in different coffee preparations (Crozier et al. 2012; Ludwig et al. 2014) Relying on self-reporting of coffee intake alone can therefore result in an underestimation of total caffeine exposure and investigators need to be aware of the importance of measuring exposures during the relevant periods of gestation and capture the changing intake patterns as the pregnancy progresses (David et al. 2010). Self-reporting was not the only limiting factor that I encountered. As discussed above, tolerance can be developed to certain coffee chemicals, such as caffeine. Participants preconception coffee consumption, along

with accurate readings throughout the gestational period, would need to be measured in order to obtain accurate readings.

I utilised a variety of concentrations of caffeic and chlorogenic acid aiming to ellicit an effect from placental chorionic plate arteries and maternal myometrial vessels. With regards caffeine, I utilised a concentration equal to that found in serum, based on previous research (Grosso et al. 2006; Klebanoff et al. 1999). The concentrations I utilised may not have been high enough to induce constriction or relaxation of the vessel. I administered a dose-response of caffeic acid, chlorogenic acid and caffeine to small sections of chorionic plate arteries and myometrial vessels. To our knowledge, this was the first study of its type and thus there was no reference as to what concentrations to use.

I have also mentioned, on several occasions, that confounding factors should be taken into consideration when assessing the effect of coffee consumption on the physiological state. Unfortunately, it is often impossible to assess all confounding factors. The main issue experienced with confounders was with reporting errors; demographic data was either missing or misreported. Information on smoking status is often misreported by expectant women, thus potentially influencing our results. For smoking status, at St. Mary's Hospital this information is now restricted to the following basic information: currently smokes; has smoked in the past; has never smoked. Information on alcohol and drug use was not obtained prior to sample collection; these factors could also affect our findings.

It was impossible, given our current ethical approval, to obtain specific dietary information on the women who were donating the tissue. The information we obtained included weight, height, BMI and smoking status but we were unable to acquire information of the woman's diet or record their consumption of coffee. Studies, as previously mentioned (Chapter 2, Section 2.14.3) have indicated that individuals who consume coffee on a regular basis may build up tolerance and effectively become immune to possible physiological and psychological effects of the compounds present in the beverage; this concept was briefly mentioned in Chapter 2. This could potentially interfere with the results I obtained. An extension of my current study would be to 1) determine womens' coffee drinking habits pre-pregnancy and during pregnancy prior to collection of the placental / myometrial

tissues and / or 2) measure plasma levels of coffee chemical across gestation and at term using a pre-delivery maternal blood sample. These will be expanded on further in Section 10.3.

Smoking and caffeine metabolism are discussed at length in the literature, with many studies linking coffee consumption with tobacco use (Swanson et al. 1994). This accelerated metabolism of caffeine amongst smokers could be an explanation for increased coffee consumption amongst those that consume tobacco (Brown et al. 1988). In support of this conjecture, induction of CYP1A2 activity by tobacco smoke components, is responsible for the increased rate of caffeine clearance (Sachse & Bhambra 2003; Fuhr et al. 1993) possibly leading to a reduced stimulus from drinking coffee.

Coffee is a complex mixture of chemicals. As a result, we must consider that the product as a whole can impact vasculature and not just the administration of a singular high dose of one extracted compound. Combinations of compounds may have a more profound effect on the vasculature. This is particularly true with regards the potential protective effects of coffee consumption (Kono et al. 1997; Lee et al. 2007; Gülçin & Gulcin 2006; Cavin et al. 2002). Chlorogenic acid, and its metabolite have been shown to exhibit some antioxidative properties within the literature; it is possible that both of these play a role in unison (Kasai et al. 2000; Bouayed et al. 2007; Zhao et al. 2012). As well as this we must be aware that there may be vital compounds or enzymes missing from the solution that stimulate the protective effect. Although coffee has been studied extensively over the last 100 years, there are still many uncertainties about its exact effect *in vivo*. Pharmacological preparations of coffee extract are difficult to obtain however preparations of coffee oil can be purchased (Anon 2013). The combination of compounds within these preparations may ellicit the antioxidative properties observed by other research groups.

Pharmacological preparations of active coffee chemicals can now be easily purchased, however, these may not reflect the consumer available concentrations. Previous studies indicated that there are varying concentrations of caffeine in consumer available preparations, even within similar types of coffee. This suggests that concentrations available to the scientific community may not reflect actual consumer available concentrations. It is therefore very difficult to conduct a study

using these concentrations as they are not necessarily reflective of the population as a whole.

Much of the literature surrounding coffee has been conducted in animal models. Although a fairly accurate representation, every species has the potential to metabolize coffee differently. Also, those studies that do utilize human models generalize for the whole population. From our literature search (Chapter 2, Section 2.6) we are aware that pregnancy has a profound effect on the body, including the cardiovascular system. As a result, the half-life of caffeine in maternal serum is significantly increased. We must take these discrepancies into account when considering the study as a whole and its contribution to knowledge. The rate of metabolism will have a profound impact on levels of caffeine and other coffee chemicals present in the circulation. Smoking tobacco increases metabolism and clears caffeine from the circulation considerably quicker than in those women who do not smoke. Assessing whether women smoked prior to their pregnancy, or prior to sample collection, would be beneficial.

Furthermore, the incubation time could have an effect of observed results. I incubated the vessels with the potential antioxidant for 15 minutes prior to addition of ROS; this may not have been sufficient time to ellicit a protective effect. Experimentally, this aspect could be explored in the future by increasing incubation times and by using different levels of antioxidant to titrate possible activity.

Wire myography is a complex and delicate technique that requires many weeks of training to perfect the method. As well as this, myometrial samples are difficult to obtain due to the nature of the harvesting of these. Women are not always as willing to donate a sample of myometrial tissue as it is a more invasive procedure than donating placental tissue. As well as this an obstetric surgeon must biopsy a small section of myometrial tissue once the neonate has been delivered; this is an added pressure and often it is not practical to do so. Myometrial vessels must also be biopsied from pregnant women, as we know that pregnant vessels behave differently to non-pregnant vessels, thus narrowing the sample size further. As a result a large sample size is not always feasible within these studies. A more relaxed or greater time frame would allow the researcher to increase their sample size and potentially experiment using different techniques, for example, pressure myography and whole

placental cotyledon perfusion studies. It is important to note that pressure myography, which is a far more complex and time consuming experiment, would only be conducted if an effect were detected so as to better understand the underlying mechanism behind the effect observed.

My research was beneficial in that it contributed to the limited amount of knowledge surrounding the effect of coffee on vascularity. Furthermore, it was unique in that it was the first study, to our knowledge, that considered the effect of the specific coffee chemicals on chorionic plate arteries and myometrial arteries. Such a bold statement as multi-method or mixed methods research is better cannot be made. The combination of qualitative and quantitative processes is an approach employed to address a variety of questions posed within an investigation, which may give rise to the use of further methods.

Although the mixed methods approach is considered to be a great strength of the study we could consider the limited depth of the interviews as a potential drawback. The interview process was semi-structured, allowing for some scope, but there were time limitations for each individual interview. Similarly, the findings from the interviews with the women and midwives' did not highlight any pivotal or consequential concepts or themes and thus probing was limited. Although rich data was obtained, nothing fundamental or information that would potentially immediately change practice was found. If information like this existed it is likely that it would have been found previously and consequently practices changed. The findings did however highlight the uncertainties surrounding coffee consumption and their views and opinions on the provision and sources of information.

Finally, this was a difficult study to perform as a single person but the qualitative and quantitative branches do go hand in hand. Collecting substantial numbers of participants / samples takes time and effort; unfortunately time restraints, ethical limitations and the PhD framework does not allow for me to conduct further research into this area at this time. Fortunately, it does pave the way for future work and potential changing health recommendations. We can only give good health related information providing there is good, solid scientific based information and evidence behind it. With quantitative questions and hypothesis, what we may initially think is

a simple question may require a considerable amount more experimentation and effort than initially perceived or predicted.

10.3 Recommendations and Future Work

Interactive and repetitive contact between midwives and women makes it possible for them to discuss and care for that individuals specific information needs. In an ideal situation, the midwife would be able to dedicate time and identify specific dietary needs, address nutrition questions and clarify any personal questions that the pregnant woman may have (Szwajcer et al. 2009). Unfortunately, this is not a realistic solution and thus we must consider other options. The most likely solution is to place added emphasis on information quality and provision. We should aim to provide consistent, evidence based information. Midwives are responsible for delivering health and dietary information during the gestational period; they should, ideally, be equipped with multiple sources of information with a constant message surrounding a particular aspect pregnancy.

It is important to note that brochures are useful as a reference tool to provide general information and instructions (Suh 1999; McQuail 2010); however in todays society this method may not be the most suitable. Women are more aware of health and diet, and the media is a driving force behind many of their anxieties. Health care professionals, research teams and the media need to work together to ensure that information that is being disseminated to the general public is accurate and evidence based. Obviously, it is impossible to control what is broadcast across the Internet and therefore it may be beneficial to develop a website or social media / forum for women to access the most reliable information. The development of more modern methods of accessing information should also be considered. As mentioned above, many women use mobile phone 'Apps' to obtain their information. Their intimate nature and convenience appeal to many women. A specific application could be developed with nutrition during pregnancy being its main focus. This could be user friendly as well as providing women with a consistent message. Midwives need to actively communicate with women and discuss the use of these modern methods of obtaining information and clearly explain their unreliability. Furthermore, as the body of evidence grows surrounding coffee and its potential impact on pregnancy,

health care professionals should interact more with the general public and media to ensure that the most reliable information is being disseminated down.

Midwives need to be sensitive to the information that they provide to women. As was the case in my research, it may be that the woman has consumed caffeinated beverages or coffee prior to her first meeting with the midwife. In situations like this it is the midwives responsibility to ensure that the expectant mother not only receives accurate information but reassurance and support from her health care professional. Both midwives and women need to be more aware of the sources of caffeine and there should also be a general consensus on the amounts that can be consumed during pregnancy. Unfortunately, many sources of information contradict each other which can result in maternal distress and loss of confidence in her health care professional.

Preconception information must also be considered. Many women are leaving the journey to motherhood later in life and thus plan for the event well in advance. Women should be provided with increased preconception advice about all aspects of diet and general health. This method of information provision has been highly beneficial to public health with the most obvious example being preconception folic acid consumption and the decreased incidence of neural tube defects.

Coffee and socioeconomic statuses were not considered during this study. I did not specifically consider socioeconomic status and therefore we were limited for comparison from the group of participants enrolled into the study. I did not identify any ethnic minorities during my research however from a brief analysis those that volunteered tended to be white British pregnant women. Future work should take into account cultural diversity and coffee consumption and attempt to delve into this area more thoroughly.

Womens' beliefs regarding coffee consumption was only briefly discussed as well as previous pregnancy experience. Literature supports the concept that previous experiences dictate and influence future behaviours. Some of the women interviewed were multi-parous; these women stated that they adopted their previous dietary habits providing pregnancy outcome was positive. There is scope for future work into beliefs and experiences and how these influence womens' coffee consumption. Similarly, midwives beliefs and experiences may also influence the advice they

provide regarding coffee consumption and pregnancy. More in-depth research could be conducted to shed more light into what influences health care professionals and the advice they provide, particularly with regard to caffeine and coffee consumption.

Partner support and its influence on womens' diet is also a potential consideration. As stated, there is literature suggesting that many factors influence womens' diet. The partner is often the biggest source of support during pregnancy; thus we could assume that their opinions on coffee may influence their pregnant partner's consumption.

Finally further research should be conducted into women and midwives' view and opinions on coffee consumption and pregnancy. A larger sample size could be approached, ensuring that all age ranges and socioeconomic factors are being taken into consideration. Different geographical locations could be considered, comparing those women who live in urban areas and may have easier access to coffee shops versus women who live in the countryside or suburbs. As interviewing and transcription is a time-consuming process, a questionnaire could be developed. This could combine both qualitative and quantitative aspects and would hopefully give rise to a larger, and more varied, sample size. This may also give a more accurate representation of the amount of caffeine / coffee women are consuming during their pregnancy. As well as this, questionnaires are anonymous and women may be more inclined to disclose accurate quantities if the feel safe to do so.

As discussed above, coffee is a beverage containing many active substances, many of which are said to possess antioxidant abilities. To date, no research has been conducted on their effect on placental or myometrial vasculature. Future research could be conducted by further determining / measuring antioxidant activity within the placental vasculature. Within my study I predominantly focused on chorionic plate arteries and myometrial (systemic) vessels. There is potential to assess the antioxidant potential of caffeic and chlorogenic acid on stem villous vessels. Another potential direction that we could take would be placental perfusion. Perfusion experiments would allow us to investigate the effect of active coffee chemicals on the villous tree as a whole rather than on isolated vascularised areas.

As this was a novel study, the potential antioxidant effect of chemicals within coffee was investigated independently. Whilst it is important to understand the effects of

potential individual antioxidants, this is not reflective of a systemic situation; combinations of chemicals may contribute to the total antioxidant effect witnessed. Further studies could be conducted investigating the potential effect of combined antioxidative substances extracted from coffee. Whilst combination effects are often seen *in vitro*, we must also consider the potential effect of the by-products of coffee metabolism. Future research could include investigations into whether coffee metabolites have an effect on placental and myometrial vasculature.

I utilised a range of concentrations of caffeic and chlorogenic acid with chorionic plate and myometrial vessels. These concentrations may not have been sufficient to ellicit a reaction and thus future work should be conducted into the use of higher concentrations of the active coffee chemicals. I also utilised a singular concentration of caffeine with chorionic plate arteries; future investigations into a wide variety of caffeine concentrations may exhibit a reaction with different levels of caffeine exposure. Incubation may also play a role in the antioxidative properties of the chemicals I investigated. Further studies could investigate both acute and chronic incubation with the potential antioxidative substances. These could involve 8 to 24 hour incubations (allowing for a possible antioxidant effect to manifest itself prior to vessel reactivity being compromised by time) with or without regularly changing the media concentrations of the antioxidant.

Similarly, serum levels of antioxidants could be measured in pregnant women. This could be correlated with self-reported coffee consumption. A more accurate experiment could then be conducted to determine if prior exposure had altered the capacity of isolated arteries to cope with a pro-oxidant stressor. With my study women did not report their coffee consumption prior to delivery. This limitation was previously discussed in further detail (see Section 10.2).

To summarise, the strengths of the study greatly outweigh the limitations encountered. This study is a good example of primary research within this under-researched field. The tactic of erring on the side of caution is no longer adequate when advising women on diet during the gestational period. As stated, this study was a good starting point and I obtained a vast quantity of rich data however there is much scope for further, more in-depth research. This was a huge study to undertake as a single person and there is potential for development of the quantitative and

qualitative branches. There is a need for evidence based information to become accessible to midwives so that more accurate advice can be given to women. Health literacy along with the need for accessible information was highlighted; women discussed their need to supplement information. The provision of information that meets the needs of the women, that has moved with the times and is also evidence based is priority and possible policy change.

Appendix 1: Ethical Approval

Health Research Authority

NRES Committee North West - Greater Manchester West

27 February 2012

Miss Siofra McDermott
5th Floor, Research St Mary's Hospital
Oxford Road
Manchester
M13 9WL

Dear Miss McDermott

Study title: **Coffee and Pregnancy- Attitudes, Consumption and Maternal Vascular Function.**
REC reference: **12/NW/0079**

Thank you for your letter of 21 February 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Alternate Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

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Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

The committee would like the Information sheets revised in the following manner:

    a) Under the heading 'Will my taking part in this study be kept confidential?', the last sentence in the first paragraph is incomplete. Please state who else besides the designated researchers will have access to the code.

    b) Under 'What will happen to the results of the research?', the first sentence should refer to coffee consumption and not healthy eating, as mentioned in point 2(a) of the Provisional Opinion letter.

    c) Under 'What would happen if I consent but then loose the capacity...etc' the correct spelling is lose.

    d) Under 'What would happen if I consent but then loose the capacity...etc', please remove the reference to 'tissue collected' as no tissue is being collected.

The committee also suggest that the Questionnaire PIS is revised in the following way:

    i. Under the heading 'What happens to the information we collect?' revise the paragraph to reflect the fact that the questionnaire is anonymous so it will not include names and addresses.

    ii. Under the heading 'Can I change my mind?' rewrite this paragraph to reflect that the fact that data cannot be withdrawn once the questionnaire has been submitted, as it will be impossible to know who has completed each questionnaire.

    iii. Under the heading 'Will my taking part be kept confidential?' please rewrite the paragraph to reflect the anonymous nature of the questionnaire.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Advertisement	1	30 November 2011
Covering Letter		10 January 2012
Covering Letter		20 February 2012
Evidence of insurance or indemnity	University of Manchester	19 December 2011
Interview Schedules/Topic Guides	Midwives - Version 1	01 November 2011
Interview Schedules/Topic Guides	Women - Version 1	01 November 2011

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Investigator CV	Siofra McDermott	
Investigator CV	Tina Lavender	
Investigator CV	Mark Wareing	
Investigator CV	Suzanne Thomas	
Letter from Sponsor	University of Manchester	19 December 2011
Other: Coffee and Pregnancy Distress SOP		
Other: Lone Worker Protocol		
Other: Participant Recruitment Protocol		
Other: Telephone Protocol		
Other: Study Timescale		
Other: SOP - Interview Schedule (Midwives)		
Other: SOP - Interview Schedule (Women)		
Participant Consent Form: Consent to Contact	1	30 November 2011
Participant Consent Form: Questionnaire	1	30 November 2011
Participant Consent Form: Participant Consent Form	2	16 February 2012
Participant Consent Form: Health Care Professional Participant Consent Form	2	16 February 2012
Participant Information Sheet: Questionnaire Participant Information Sheet	2	16 February 2012
Participant Information Sheet: Health Care Professional Participant Information Sheet	2	16 February 2012
Participant Information Sheet: Participant Information Sheet	2	16 February 2012
Protocol	1	01 November 2011
Questionnaire: Baseline Questionnaire	2	16 February 2012
REC application	3.3	13 January 2012
REC application	Revised	
Response to Request for Further Information		21 February 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

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<u>Feedback</u>

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.
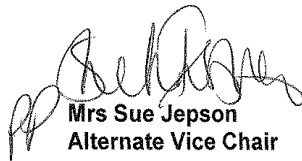
Further information is available at National Research Ethics Service website > After Review

12/NW/0079	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Mrs Sue Jepson
Alternate Vice Chair

Email: shehnaz.ishaq@northwest.nhs.uk

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Lynne Macrae – The University of Manchester

                Dr Lynne Webster - Central Manchester NHS Foundation Trust

                Professor Tina Lavender
                Jean McFarlane Building
                University Place
                Oxford Road
                Manchester
                M13 9PL

                Dr Mark Wareing
                5th Floor (Research), St Mary's Hospital
                Oxford Road
                Manchester
                M13 9WL

                Ms Suzanne Thomas
                5th Floor (Research), St Mary's Hospital
                Oxford Road
                Manchester
                M13 9WL

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<u>Appendix 2: Participant Information Sheets</u>

Coffee and Pregnancy

Coffee Time!

We are performing a study exploring midwives' views on coffee consumption in pregnancy.

We need your help. Would you like to join in?

For more information contact:

Siofra McDermott

0161 701 6960

Siofra.McDermott@postgrad.manchester.ac.uk

Participant Information Sheet

(Version 3: 28/02/12)

> We would like to invite you to take part in the above study. Before you decide you need to understand why the research is being done and what it will involve for you.

Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you would like to take part.

Who is organizing the research?

The research is being organized by scientists and health professionals at The University of Manchester and the Central Manchester University Hospitals NHS Foundation Trust.

We hope that by improving our understanding of coffee consumption and how it affects pregnancy will ultimately benefit all pregnant women worldwide.

What are we trying to find out in this study?

Women want to know about coffee consumption in pregnancy. We want to find out what pregnant women know and think about drinking coffee, if and where they look for advice and what information they obtain from the health care professionals who

look after them during their pregnancy. We aim to do this by obtaining the views of women like you.

Why have I been chosen?

You are being asked because you are attending St Mary's Hospital for your care and for that of your baby during your pregnancy.

Do I have to take part?

No – the choice is yours. Your decision will NOT affect the standard or type of care you will receive from the hospital, or health care professional team who provide your pregnancy care, now or in the future.

What will happen to me if I agree to take part?

If you agree to join the study you will be asked to sign a consent form, and you will be given a copy to keep. You will also be given this information sheet to remind you of what you were asked to do.

You will be asked to complete a short baseline questionnaire which will take approximately ten minutes to fill out. This will include details like name and date of birth, and will be kept strictly confidential.

You will be asked to take part in one interview which will take approximately one hour; this will take place by telephone and will be at a time that is convenient to you. The interview will be carried out by a researcher, from The University of Manchester, who will discuss your views and experiences. There are no right or wrong answers, we just want to try and understand your views.

What happens to the information we collect?

Any identifiable data such as your name and address will be removed to protect your identity. If you agree, the interviews will be recorded where possible and the recordings will be transcribed (word for word) into a written format for analysis by the researcher and research team. Following transcription the digital recordings will be destroyed. The data will be analysed by the researcher and the research team.

Can I change my mind?

Yes. You can change your mind at any time by contacting your original hospital or The Maternal & Fetal Health Research Group directly. **You do not need to tell us why you wish to withdraw.**

If you wish to withdraw:

- All the stored information will be deleted so that it cannot be used again.

If you change your mind after a long time, the information may have already been used by researchers. If, by then, the information has already helped create new knowledge, that new information cannot be undiscovered and will contribute to medical understanding.

Are there disadvantages or risks in taking part?

There are no disadvantages to yourself or your baby if you agree to take part in the study. The standard of care that you receive will not be affected.

What are the possible benefits of taking part?

There is no direct benefit to you of taking part.

The results of research will NOT be put in your health records or told to you, your relatives or your doctors because the researchers will not know who you are; your details will not be given to them.

You will not receive any personal financial reward for taking part in the study.

What if new information becomes available?

Should any relevant new information come to light the researcher will tell you about it and discuss whether or not you want to continue in the study.

Will my taking part in this study be kept confidential?

Yes. Your participation will be strictly confidential. **Your name, address and contact details will be removed so that you cannot be recognized.** Any information about you that we wish to use in publications about the study, including direct quotations, will be referred to using a pseudonym (false name); this ensures that you cannot be identified. The designated researchers who are organizing the study are the only individuals who will have access to this code. In the event of your wishing to withdraw from the research, they will be able to act on your wishes by removing the correct collected data.

All information will remain confidential, unless disclosure is essential to protect you or others from the risk of significant harm, or disclosure is required by law or by order of a court. Should further studies be planned related to you and your involvement in this study, we will contact you to seek your permission.

What will happen to the results of the research?

We hope that the research will lead to new information about the consumption of coffee during human pregnancy. These results would be publicized at scientific meetings and published in scientific and medical journals. **We would emphasize that you will not be identified in person in any report or publication. Your individual results will not be available.**

What would happen if I consent, but then lose the capacity to consent during the study?

The participant and all identifiable data would be withdrawn from the study. Any data which is not identifiable to the research team may be retained.

What if something goes wrong?

In the event that something does go wrong and you are harmed during the research you may have grounds for a legal action for compensation against The University of Manchester or Central Manchester University Hospitals NHS Foundation Trust but you may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

Complaints

If you have a concern about any aspect of this study, you should ask to speak to the researchers, who will do their best to answer your questions. If they are unable to resolve your concern or you wish to make a complaint regarding the study, please contact a University Research Practice and Governance Co-ordinator (0161 275 7583 or 0161 275 8093, or by e-mail (research-governance@manchester.ac.uk).

Who has reviewed this study?

This study has been reviewed by GM West Research Ethics Committee.

Thank you for your time

Contact for Further Information:

Researcher: Siofra McDermott

0161 701 6960

Siofra.McDermott@postgrad.manchester.ac.uk

Research Midwife Co-ordinator: Suzanne Thomas

0161 701 6957

SuzanneL.Thomas@cmft.nhs.uk

Professor of Midwifery: Tina Lavender

0161 306 7744

Tina.Lavender@manchester.ac.uk

Senior Scientist: Mark Wareing

0161 701 6970

Mark.Wareing@manchester.ac.uk

Health Care Professional Participant Information Sheet

(Version 3: 28/02/12)

> We would like to invite you to take part in the above study. Before you decide you need to understand why the research is being done and what it will involve for you.

Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you would like to take part.

Who is organizing the research?

The research is being organized by scientists and health professionals at The University of Manchester and the Central Manchester University Hospitals NHS Foundation Trust.

We hope that by improving our understanding of coffee consumption and how it affects pregnancy will ultimately benefit all pregnant women worldwide.

What are we trying to find out in this study?

Women want to know about coffee consumption in pregnancy. We want to find out what pregnant women know and think about drinking coffee, if and where they look for advice and what information they obtain from the health care professionals who

look after them during their pregnancy. We aim to do this by obtaining views of health care professionals like you.

Why have I been chosen?

You are being asked because you are a health care professional who is actively involved in the health care provision for women who attend St Mary's Hospital, Manchester during their pregnancy.

Do I have to take part?

No – the choice is yours.

What will happen to me if I agree to take part?

If you agree to join the study you will be asked to sign a consent form, and you will be given a copy to keep. You will also be given this information sheet to remind you of what you were asked to do.

You will be asked to complete a short baseline questionnaire which will take approximately ten minutes to fill out. This will include details like name and date of birth, and will be kept strictly confidential.

You will be asked to take part in one interview which will take approximately one hour; this will take place face-to-face or over the telephone and will be at a time that is convenient to you. The interview will be carried out by a researcher from The University of Manchester, who will discuss your views and experiences. There are no right or wrong answers, we just want to try and understand your views.

What happens to the information we collect?

Any identifiable data such as your name and address will be removed to protect your identity. If you agree, the interviews will be recorded where possible and the recordings will be transcribed (word for word) into a written format for analysis by the researcher and research team. Following transcription the digital recordings will be destroyed. The data will be analysed by the researcher and the research team.

Can I change my mind?

Yes. You can change your mind at any time by contacting The Maternal & Fetal Health Research Group directly. **You do not need to tell us why you wish to withdraw.**

If you wish to withdraw:

- All the stored information will be deleted so that it cannot be used again.

If you change your mind after a long time, the information may have already been used by researchers. If, by then, the information has already helped create new knowledge, that new information cannot be undiscovered and will contribute to medical understanding.

Are there disadvantages or risks in taking part?

There are no disadvantages to you if agree to take part in the study.

What are the possible benefits of taking part?

There is no direct benefit to you of taking part.

You will not receive any personal financial reward for taking part in the study.

Will my taking part in this study be kept confidential?

Yes. Your participation will be strictly confidential. **Your name will be removed from any data collected so that you cannot be recognized.** Any information about you that we wish to use in publications about the study, including direct quotations, will be referred to using a pseudonym (false name), so that you cannot be identified. The designated researchers who are organizing the study are the only individuals who will have access to this code. In the event of your wishing to withdraw from the research, they will be able to act on your wishes by removing the correct collected data.

All information will remain confidential, unless disclosure is essential to protect you or others from the risk of significant harm, or disclosure is required by law or by order of a court. Should further studies be planned related to you and your involvement in this study, we will contact you to seek your permission.

What will happen to the results of the research?

We hope that the research will lead to new information about the consumption of coffee during human pregnancy. These results would be publicized at scientific

meetings and published in scientific and medical journals. **We would emphasize that you will not be identified in person in any report or publication. Your individual results will not be available.**

What would happen if I consent, but then lose the capacity to consent during the study?

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Thank you for your time

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0161 701 6960

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Research Midwife Co-ordinator: Suzanne Thomas

0161 701 6957

SuzanneL.Thomas@cmft.nhs.uk

Professor of Midwifery: Tina Lavender

0161 306 7744

Tina.Lavender@manchester.ac.uk

Senior Scientist: Mark Wareing

0161 701 6970

Mark.Wareing@manchester.ac.uk

Central Manchester University Hospitals NHS Foundation Trust

Participant Consent to Contact Form

(Version 1: 30/11/2011)

To give us your consent, please can you confirm 'yes' to the questions below by placing your initials in the box provided. Please then sign your name in the box at the bottom of the form and have this witnessed at the same time as you sign it.

1. Name:

2. Address:

3. Preferred Contact Number:

4. Preferred Contact Time:

Name of Participant	**Date**	**Signature**

MANCHESTER 1824

The University of Manchester

NHS

Participant Consent Form

(Version 2: 16/02/12)

Coffee and Pregnancy

To give us your consent, please can you confirm 'yes' to the questions below by placing your initials in the box provided. Please then sign your name in the box at the bottom of the form and have this witnessed at the same time as you sign it.

Please initial box

1. I confirm that I have read and I understand the information sheet dated 16/02/2012 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation in this study is voluntary and that I am free to withdraw my consent at any time. I do not have to give a reason and my medical treatment or legal rights will unaffected by withdrawal of my consent.

3. I understand that relevant sections of data collected during the study may be looked at by responsible individuals from the University of Manchester, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in the research. I give permission for these individuals to have access to this data.

4. I understand that stored information will be kept confidential at all times; the research data generated by this study will have personal information removed in a way that protects my identity.

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5. I understand that I will not personally benefit, financially or otherwise, from my participation in this study.

6. I understand that if I should lose the capacity to consent during the study, I and any identifiable data, shall be withdrawn from the study and any data which is not identifiable to the research team may be retained.

7. I agree to the use of digital recording of my conversation during interview.

8. I agree to having quotations published using a false name (pseudonym).

9. I agree to take part in the above study.

Name of Participant	**Date**	**Signature**

Name of Researcher	**["Role"]**	**Date**	**Signature**

Health Care Professional Participant Consent Form (Version 2: 16/02/12)

Coffee and Pregnancy

To give us your consent, please can you confirm 'yes' to the questions below by placing your initials in the box provided. Please then sign your name in the box at the bottom of the form and have this witnessed at the same time as you sign it.

Please initial box

1. I confirm that I have read and I understand the information sheet dated 16/02/2011 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation in this study is voluntary and that I am free to withdraw my consent at any time. I do not have to give a reason and my medical treatment or legal rights will unaffected by withdrawal of my consent.

3. I understand that relevant sections of data collected during the study may be looked at by responsible individuals from the University of Manchester, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in the research. I give permission for these individuals to have access to this data.

4. I understand that stored information will be kept confidential at all times; data will only pass to researchers in an anonymous way that protects my identity.

5. I understand that I will not personally benefit, financially or otherwise, from my participation in this study. □

6. I understand that if I should lose the capacity to consent during the study, I and any identifiable data, shall be withdrawn from the study and any data which is not identifiable to the research team may be retained. □

7. I agree to the use of digital recording of my conversation during interview. □

8. I agree to having quotations published using a false name (pseudonym). □

9. I agree to take part in the above study. □

Name of Participant	**Date**	**Signature**

Name of Researcher	**["Role"]**	**Date**	**Signature**

Appendix 4: Interview Schedule

WOMEN

(Version 1, November 2011)

INTRODUCTIONS

Before any contact is made the researcher will confirm that the pregnancy is on-going

The interviewer will thank the participant for agreeing to be interviewed and will attempt to make them feel as relaxed as possible. The interviews will be conducted over the telephone at a time that is most convenient to the participant.

SETTING OF GROUND RULES

- Explain Study
- Explain that matters disclosed will not affect level of care in any way
- Explain tape recording & transcription
- Explain study numbers/confidentiality
- Explain names will not be used or changed if appropriate
- Explain can stop at any time
- Explain that the interview is intended to take no longer than 1 hour
- Explain can refuse to answer question
- Opportunity to ask questions
- Consent
- Check tape
- Opportunity for referral to specialist services if required

INTERVIEW PROMPTS

This interview will be semi structured and respondent led, however, the following areas will be explored and the questions will guide the interview.

Participants will be invited to partake in the telephone interviews whilst at the antenatal clinics. They will be allowed to choose a time that is convenient to them.

Interview:

- Confirm name and date of birth.

- How many children do you have?

- How would you describe your ethnicity?

- Do you drink coffee?
    - Did you drink coffee prior to pregnancy?
- How much coffee would you say you drink on a daily basis, both prior to and during your pregnancy?
- What type of coffee do you drink?
- Do you know of any risk or benefit associated with coffee drinking?
- Do you know of any risk or benefit associated with coffee drinking during pregnancy?
- Where did you get this information?
    - Have you looked online for information on coffee drinking?
    - Have you asked your GP/Midwife for any information of coffee drinking?
- What advice have you been given on coffee drinking during pregnancy?
    - Why have you chosen to accept or disregard this advice?
- Would you find it beneficial if there was advice or information provided on coffee drinking during pregnancy?
    - Would you be likely to take on board this advice? Why?
    - What format would you like this information to be in?

FOLLOWING THE INTERVIEW

- The researcher will then thank the participant and ask if there are any questions which she would like to ask.

MIDWIVES

(Version 1, November 2011)

INTRODUCTIONS

Before the interview will take place the researcher will ensure that this is an appropriate time for the interview and that the midwife understands that the interview could take sixty (60) minutes.

The interviewer will thank the participant for agreeing to be interviewed and will attempt to make them feel as relaxed as possible. The interviews will be conducted in an area that is most convenient to the participant, which is most likely to be on the hospital grounds.

SETTING OF GROUND RULES

- Explain Study
- Explain tape recording & transcription
- Explain study numbers/confidentiality
- Explain names will not be used or changed if appropriate
- Explain can stop at any time
- Explain that the interview is intended to take no longer than 1 hour
- Explain can refuse to answer question
- Opportunity to ask questions
- Consent
- Check dictaphone

INTERVIEW PROMPTS

This interview will be semi structured and respondent led, however, the following areas will be explored and the questions will guide the interview.

Interview:

- Confirm name and date of birth.
- How many years midwifery experience do you have?

- What area of midwifery care do you work?

- How would you describe your ethnicity?

Diet:

- Do many women approach you with regards their diet?

- What areas are they interested in?

- What information do you provide on drinking?

- Do you ever consider coffee drinking and pregnancy?

Coffee Consumption:

- What advice/information do you give women on coffee consumption during pregnancy?

- Why do you give this information?

- Where did you obtain this information?

- Do you think women take this advice on board?

Further Probes:

- What are your personal views on coffee consumption during pregnancy?

- Is it part of your practice to provide information on coffee consumption during pregnancy?

FOLLOWING THE INTERVIEW

- The researcher will then thank the participant and ask if there are any questions which she would like to ask.

Appendix 5: Interview Transcript

WOMAN

Ok, can I get you to confirm your name and your date of birth please?

Yea, my name's xxxxx xxxxxxx and my date of birth is the 29/11/1991

And do you have any children?

I have one and one on the way.

Ok, and how would you describe your ethnicity?

Am, how do you mean?

Are you British......?

Oh yea, white British yea.

Do you smoke?

Yea, I do.

Ok, and do you work?

No.

And can I ask how far along in your pregnancy are you?

Am, coming up to 29 weeks.

Can I ask when you found out you were expecting were there any areas of your diet you were concerned about or questioned your midwife about?

Well, I had a miscarriage like just before getting pregnant again so this pregnancy like ....has been like pretty hectic anyway, I've just been terrified all the way through really.

Sorry to hear that. Did you make any changes to your diet or drinking habits?

Am, well I made sure like I ....cheese, I don't know, I read that that was bad..... Like soft cheese and stuff so I stopped eating that. Am, like eggs...like you can't have a

runny yolk and things like that. Meat has to be well done. Am, caffeine...you're not meant to drink a lot of like coke and coffee and things like that.

Ok, and am, well I suppose my study is focusing on coffee. Did you drink coffee before you found out you were expecting?

Yea.

And did you make any changes?

I don't drink no way near as much of it.

Ok, how much do you drink now in comparison?

I used to drink about say maybe 5 cups a day but now I drink maybe like 1 or 2 cups a week.

Oh wow, that was a massive cut down.

Yea, I know. It's just it's scary because they're always going on about it with babies and pregnancy so I'd rather follow the guideline and not have anything go wrong.

Did you find it difficult to cut down?

Am, all in all yea because you have to change like quite a few things, like when you're pregnant. Yea, so all of it together was quite hard.

Yea, I can imagine.

I know.

Your reason for cutting down? You said you heard a lot of stuff but did you midwife tell you anything about coffee or caffeine?

No.

No, and did you ask her or anything like that?

No.

Can I ask where you got the information, we'll say....?

I get updates online every week, am, you know like 26 weeks, 27 weeks like I get a new update every week from the Bounty club and the SMA club.

And those are good?

Yea, you know what's going on in the pregnancy and how the baby's progressing and like what's going on with their lungs at this point and what's going on with all the major arteries you know.

Yea.... they are nice, I've seen some of them. Have you ever seen MUMSNET or Babycenter?

Am, no, what are they?

Am they're like forums....

Oh, no no I've not seen those.

Am, can I ask where you'd go for information and stuff like that?

Am, on those websites? For some information yea because it's just like.... well that information is like, it's sort of like, it gives you a bit about the baby, about how the baby's progressing and how big it is expected to be at this size and what weight it is expected to be the next week. But it also gives you like hints and tips of like how to help labour, how to keep relaxed so yea, yea I think they're quite good.

Is there any other websites or method you'd use to get information?

No, it's just those 2 I use.

Ok, I know you get leaflets during your pregnancy, did you find them helpful?

I didn't find them helpful because I don't think they tell you anything, to be honest. I think it's better just to get the updates and then they tell you word for word exactly what's going on with your baby and then you know everything word for word and like by the day you know everything, but the leaflets don't really tell you... well nothing to do with real pregnancy anyway.

So if you knew another pregnant woman, would you advise her to use one of.... to get these updates?

Yea, definitely, I think they're really good, because they describe like movements as well like because as the baby gets bigger in your belly the movements like slow down and it tells you like when to expect that you know like.... and it stops you from panicking really as well. If you're a panicky person but you're already expecting the movements to slow down then it doesn't make you panic as much. I suppose when you get information like that you can relax.....it makes you relax a little bit more.

Yea, you can relax.

Yea, and it lets you know what's going on with your baby, it's more intimate like what's happening with your baby inside your belly and that's nice.

Yea, and they're easy to use.

Yea, they are, they just get sent to my email every week and then all I have to do is read through it and it just tells you like everything you need to know, all the questions you have in your head they just get answered.

Yea, I understand. Can I just go back to the coffee for a bit. I know you said you used to drink quite a bit, what kind of drinks do you drink now instead of coffee?

To be honest I just try and like drink like orange juices, apple juices and just normal juice drinks now.

Ok, and I know you said you heard a lot about caffeine and coffee. Can you remember any of the risks that they said? Like what it did?

I can't remember the risks but I remember that it's not.... that you're not necessarily meant to drink a lot of caffeine.

Can you remember the exact wording was or anything, what they said?

No, I don't have a clue you know. I just know it's just not that good, like caffeine products, like to avoid them, I don't really know why.

Ok, that's fine. Would you have..... if your midwife had provided some information... or more information on diet or anything like that or discussed.....

I've not had any information during my pregnancy, I've not had no leaflets or nothing like that.

Really?

Yea....

Would you have found them useful or helpful?

Am, no not really, not the leaflets, no because they don't really tell you anything.... no anything that actually happens in a real pregnancy.... It's like all...am.... I don't know. The way the leaflets describe pregnancy and that sort of stuff it's just not really like that so.... I don't know. See the updates that I get are perfect. They really do describe pregnancy and everything that goes on in it, I don't bother with the leaflets, I don't think they really tell you anything.

Can I ask why do you think they're different?

Because they're more like intimate.... they tell you more.... like me, I love to read up about what's going with the baby, how she's progressing, near enough 29 weeks, how far has she left to go, can she be born at this age... I like to know all that sort of stuff and you get all that sort of information off the web, off my updates that are sent to me. Like when her major arteries are all done and finished preparing and how's the blood pumping and everything you need to know what's going with your baby you know.

Yea, and who told you about this, who recommended it?

You know, I was actually looking for prams online and it was just a link that I went on to and just from there I signed up to the Bounty club and the SMA club and I've just been getting my updates ever since.

Oh ok. Well I think that's pretty much all of my questions. Is there anything you'd like to ask me or anything you'd like to add?

No, no.

Well it's been really interesting talking to you, thank you.

Well I love to know what's going on with the baby. You should know before and after, not just after they're born

No, absolutely. Are you going to keep using Bounty after she's born?

Am, Yea because it gives you like tips and stuff like it says that you keep getting updates after the baby is born and I think it will give me hints and tips about things because it does already. And I rely on it anyway and everything it says is going to happen every week has happened so....

Ok, and would it have been helpful in your first pregnancy?

Yea, probably because I was only 16 in that pregnancy and I didn't know how babies even grow in your belly, I didn't know anything about it at all, so it would have been useful to be able to get something .... as a heads up, a what to expect sort of thing, you know what I mean...... instead of just all these movements coming and then the movements stopping and you get a bit panicked and they keep me sane anyway.

No, it's been really interesting, thank you.

I think they're really good, they're interesting.

I know the NHS Choices have like a little cartoon, or slideshow and it sounds sort of similar.

Yea, I like all that stuff. But like I said this one gives you more in depth detail about it all. I don't know, but if you really want to know detail about exactly what's going on I think those two websites are brilliant.

Ok, and do you know are they written by women or..... do they say anything about it?

To be honest I don't know. I just signed up because I knew it was like a pregnancy thing and that sort of thing so I just signed up and then when I started getting the updates that's when I realised how useful they were. So I don't know who's sending me the updates, or anything, I just know they come from the Bounty club and the SMA club.

Yea, it's just.... information is more intimate if it by mothers for mothers.

Yea, they seem to know exactly what they're talking about.

Thank you so much for talking to me today, I hope I didn't take too much of your time.

No problem, thank you.

MIDWIFE

Can I get you to confirm your actual name please?

My actual name is xxxxx xxxxxxxxxx.

And can I ask how many years midwifery experience you have?

16.

Ok, and can I ask what areas of midwifery you've worked in?

I have worked in all areas of midwifery in those 16 years so wards, delivery unit, community, research, parent ed, antenatal clinic and at the minute I'm in a parent education job.

Well since my study focuses on diet we could talk about that in relation to parent education classes and antenatal. So when you're meeting women like that do they ever approach you about their diet, with concerns about their diet?

Yes, the 2 times that they might bring it up would be at booking, you know very early in pregnancy, what should I be eating and what should I be avoiding. But they very often bring it up, because I do breast feeding workshops with the women and post natal breastfeeding support as well, and they often bring it up in relation to breastfeeding. Like, what can I eat, what can I eat, what should I eat, other foods I should eat to increase milk, other foods I shouldn't eat.

What foods do you discourage when they ask about breastfeeding?

None, the advice is eat to hunger and drink to thirst. And then I usually you know get them to think about it globally and how women eat through out the world and all manage to breastfeed so the babies adapt to whatever you're eating and also I reassure if there's anything you're eating that disagrees with the baby you'll find out about it pretty quickly because it just goes straight through and you'll just find you're changing a lot of nappies a lot and you'll think, hmmm maybe I should avoid eating that. But there's nothing that will harm a baby except certain medications and caffeine.. is something that I would bring up, that they should avoid drinking a lot of caffeine if they're breastfeeding.

Do they ever bring it up? Do they ever ask?

Yes sometimes they do.

And what advice…what's like the specific advice?

I would say to them to avoid caffeine.

Ok.

Both in pregnancy and breastfeeding or certainly lower their intake right down and I never specifically say any cups I just say lower it or reduce it and remind them that caffeine in present in tea and coke and that it just …. In breastfeeding, it just might make the baby wakeful and am… that in pregnancy I think my advice would be less specific really because I don't have that much knowledge about what caffeine can do to a baby in utero but I know it makes the…the fetus more active, doesn't it, or it can do. So yea, I just say to avoid it.

Ok, am, I was going to ask do the women that come to you…. Do they…. Are they often coffee drinkers or a lot of caffeine or have you come across many?

No, I haven't come across many….it doesn't….I haven't come across many women who have said I drink so much coffee, I love coffee, how am I going to cut down. They all see to find cutting down quite easily. I think in pregnancy you know when they're first pregnant a lot of them will start cutting down on coffee and a lot of women go off coffee don't they.

Yea the taste and the morning sickness yea. I suppose the only reason I'm asking is because the women I'm speaking to it has ranged. Some say they found out and they stopped drinking coffee immediately because the midwife said and then I have others who don't drink coffee but drink other caffeinated products. I was wondering do you hear the same thing?

Am, yes I mean, they would bring that up and you know my advice would be the same, that if it's got caffeine in it, and a lot of energy drinks do…

Yea, and they're high sugar…

…. I probably need to mention that more, instead of just saying coke I need to say high energy drinks.

I guess I heard different things from the midwives too, they say people haven't approached them but the women say that they do drink them…

So the women aren't bringing it up with the midwife yet they could be possibly drinking. So we need to be bringing it up. I tend to see, and I'm generalising here, older ladies in my teaching groups who I imagine, and again I'm generalising, wouldn't be big Red Bull drinkers. I think that's a younger thing, I don't know. They tend to be quite educated in terms of food and drink intake.

Do you notice then a difference then with those women in approaching their midwife, asking questions, do you notice a difference? I suppose are the women who are coming to your education classes more willing to ask questions and take on board advice?

I think they would be, yes.

Do you notice differences through out or….?

It's hard to say. I think that would be hard to say but generally they are very receptive to taking advice.

Ok.

And they don't usually challenge it. They ask the question and they seem to take what I say, or what the midwife teaching says as good advice.

Ok. I suppose I might ask a little bit about what you know about coffee and pregnancy or just coffee in general?

Yes, as I said I don't have any specific information, I wish I had looked up some research before I came.

Well what's your personal opinion on coffee?

I drink very little coffee because…. And I'm drinking it this morning because I have this cold and I thought it might just make me feel a little bit pepped up. I drink very little because it really affects my stomach and makes my heart race and can make me

feel really not very well sometimes, so if I do, because I love it, I would drink it with quite a lot of milk, a nice strong coffee with lots of milk, and then I don't seem to feel the effects quite so much. Am, you know like a cappuccino, a good cappuccino or a good latte. And occasionally I would have an espresso after a meal, but usually abroad or after a rich meal and never in the afternoon or the evening or I'll be awake. You know, people laugh at me when I say that but it's true.

They are strong.

I had a coffee at friends a while ago, and it was the afternoon, and I remember saying this is a bit daring for me having a coffee in the afternoon, it must have been about half past two and that night I thought I cant sleep and it's because of that coffee.

Really?

Yea.

Wow. I have heard others say that they've felt that effect.

Mmm, yea, definitely. And more so as I've gotten older. When I was younger we all used to drink loads of instant coffee didn't we………. so yea I would have coffee once a week, at the weekend. So what I would say to pregnant women be that in pregnancy they should avoid it because it can make the fetus am…. It does through to the fetus because they've seen it on scans haven't they, giving a woman coffee and watching the baby on the scan and they're all active and I suppose that could affect growth, I don't think I've ever said that.

Well what I've read, there are some links with reduced growth in some cases, miscarriage but that's in really high doses….

But you would need to be taking an awful lot. And so the advice, like with most things would be to avoid it because we don't have that much information on it.

Well that's another thing, the advice is quite conflicting. It varies in the booklets and the NICE guidelines. What is your opinion on conflicting advice in pregnancy?

We haven't really got that much research on small amounts of alcohol in pregnancy where as there's a lot of research on fetal alcohol syndrome, which is a different

thing entirely, but we have very little research on what one glass a wine a week so…. I, in terms of alcohol wont do you any harm but I know that the NICE guidelines are that you should tell women to avoid it because we don't have enough information on the small amounts of information.

I think there's an element of, when you tell women to cut everything out it's a little harsh….

And because, you know in our society we don't perceive small amounts of alcohol as being harmful, to ourselves so how would it be harmful in pregnancy…. But I know that is not best advice. And I suppose my attitude to coffee would be a bit similar. I don't have full information on it and so I say something a little bit vague like cut down or drink less but I didn't know that there was a guidleing on the amount of caffeine that they should take.

Not, to worry, not many do. Some women look it up and it's 200 mg….

Well what does that mean?

Yea…. It is…. It doesn't say it in the advice but it's about 2 cups of coffee.

But is that 2 cups of espresso, 2 cups of Americano, 2 lattes, 2 instants…. Those are completely different things.

So what do you think of the information that is given to women, and even to midwives on diet? Do you think it's sufficient or do you think improvements can be made?

I think some of the… I think women often misinterpret a lot of the diet advice you know the advice on soft cheeses and blue cheeses… they think that means Philadelphia or Dairylee or something like that. So sometimes I think we should be more specific about why your saying reduce those foods and it's because of the risks of salmonella and listeria whatever so a processed carries nil risk of salmonella and listeria and very few cheeses do, the same with eggs and we have people in a state of anxiety about undercooked eggs and the risk of salmonella in eggs is minute now, as far as I know. So I think some of the advice is quite old and has just been carried on and carried on and carried on and again things like avoiding liver…. Some woman will cut it out all together and the advice is only a certain portion, same with tuna and

…. They're like I can't eat tuna can I? And I say well no….it's just reasonable portions. And I sometimes feel that they're cutting out really healthy foods because of, not misinformation but misinterpretation of the information?

How do you think it could be improved?

I suppose more leaflets. Advice should be more….. specific, a bit more scientific instead of….. really detailing the cheeses that should be avoided because for most women they will think I never eat unpasteurised brie from the cheese shop anyway….

I've heard stories similar, with regard the cheese too.

I think they should be really specific about the cheeses to avoid, about the makes, and the brands.

Yea, yea. Do you like the leaflet format?

Yea…. Yea. And that needs to be consistent so leaflets in bounty packs, tommys leaflets , our leaflets, health authority leaflets all need to be consistent. Magazines… you know they get a lot of information in glossy magazines and it's basically just trying to sell them products.

Am…how about you? Where would you go for your information on coffee?

For my information? For me or for a pregnant woman?

For you…. What's your point of call? Even if it's just Google? Websites…. Books?

On coffee..?

Or diet…. Or anything really?

Am, I don't know, where do I get all my information….? I suppose, I suppose yea, I'd Google it and look for good sources. I don't know how to answer that.

That's ok. I think that's pretty much most of my questions. I was going to ask whether you think coffee consumption is an area for concern?

In pregnant women?

Or in general?

Should we be concerned…. Certainly when I think how odd it makes me feel. Am, …. I don't know, I think coke and energy drinks and things like that are a cause for concern but coffee I don't know…. I don't know.

Ok….

Am….No I can't answer that. I cant decide if it's a cause for concern.

No, that's completely fine. Is there anything you'd like to add or ask?

No, I don't think so.

Appendix 6: Framework Analysis

QUESTIONS Asked

- Do you think I should drink caffeine or not?
- If you have too much, is it not good for you? } (w 15062012)
- Reasons for not having coff during pregnancy?
- What about coffee & breastfeeding?
- Other than miscarriage what are the risks? } (w 27062012)
- Why are you not supposed to have coffee? } (w 29082012)
- What does it do to your baby?
- What are you hoping to find?
- Is the advice/are the risks true?
- Why did you choose coffee specifically?
- Are sugary & fizzy drinks bad for you?
- Are you doing this study specifically on coffee & pregnancy?
- What are the bad effects of drinking more than 2 cups of coffee day?
- What are the guidelines?
- What are the risks & benefits of coffee?
- Do you know are there any papers on the effects of caffeine?

MIDWIVES QUESTIONS

- I would like to know what the recommended safe dose is?
- How long is the study going to be?
- Are you interviewing any women because it'll be interesting to hear what they have to say?
- How many health professionals will you ask & are you going to ask women also?
- I'd like to know about the conflicting advice on caffeine?
- What are the potential effects to the mum & the baby?
- What about tea?
- So what are you hoping to prove or produce?
- What's your opinion on coffee & pregnancy?
- How much is 200ug a day?
- Is it the coffee or caffeine thats bad for you?
- What are womens opinion on caffeine?
- What do women know & dont know?
- Is it the placenta you're focusing on or the acute physiological effects it has on the body?
- Is organic coffee any better?
- What have you heard from other midwives you've interviewed?
- Do the responses seem to be similar to myself?
- So what is it you're looking into?
- So is there an actual recommendation?
- What about cramping - does coffee effect women getting cramps?
- What does 200ug mean?

DESIRE FOR MORE INFORMATION

WOMEN

- Don't get as much advice from your midwife the second time around, surprised that you see your midwife less (w14062012)

- Not massively worried but surprised (w14062012)

- Not want more info because felt she knew enough (w14062012)

- Felt I had enough information (w27062012) but definitely more for those who couldn't have access. Would have continued with normal consumption + not known of links to miscarriage.

- I prefer if someone tells you (w29082012).

- They can give you information in the book but I think the midwife should tell you first (w29082012).

- No time to read the info. with another baby at home (w29082012)

- You can't be ringing up people or on websites looking up every single thing - leaflets are useful (w13092012).

- More info. in the headlines / media on specific aspects of pregnancy (w13092012)

- Specific book with table of contents (w13092012) Bullet points

- Something specific for people who don't have friends / family or may not find info as easy (w13092012).

- Be nice to be given a book - books today are outdated / old fashioned. It's easier to search online too (w26092012)

- Reassuring to presented with something with all the info at the beginning (w26092012).

- I think it should be looked into more deeply, consequences of coffee consumption are not fully understood (w26092012)

- Women should be aware of the risks so as to weigh up whether its worth it (w26092012).

DESIRE FOR MORE INFORMATION (V2) ✓

- Want more information on most things, because of her age & difficulties in getting pregnant (w27062012)
- More information should be provided, especially for those who do not have access (w27062012)
- There should be more information in the headlines/media on specific aspects of pregnancy (w13092012)
- Want a book with table of contents & information in bullet points (w13092012)
- Need something specific for people who don't have friends/family or may not find information as easily (w13092012)
- It would have been nice to be given a book. Books are outdated or old fashioned (w26092012)
- Something more online as that would be easier (w26092012)
- It would be reassuring if you were presented with 'something' that had all the information at the begining of your pregnancy (w26092012)
- I think it should be looked into more deeply as consequences of coffee consumption are not fully understood (w26092012)
- Women should be made more aware so they can make an informed decision, weigh up the risks of returning to her normal coffee consumption (w27062012)
- The results of studies may change her mind on returning to her normal coffee consumption (w27062012)
- You don't know how much caffeine is in one cup, they don't have it written on the sides of cups (w15102012)
- Wasn't sure how much was a safe amount to drink so I just cut it out (w15102012)
- Desire for information earlier than 14 weeks pregnant (w15102012)
- You sit in St. Marys & everyone has a cup of coffee-they are obviously not aware so more information is needed (w15102012)
- Helpful if there was some hard data, clear evidence, case studies or examples (w15102012)
- Information should be uniformed & harmonised, that would help (w16012013)
- Compile it into magazine rather than 100's of leaflets, compact & would be easier (w21012013)
- I think it would be good if somebody talked you through coffee like they do alcohol & medication (w29012013)
- Maybe a poster in the waiting room or given the option to have access to a specific leaflet (w29012013)
- Information before you become pregnant (w29012013)
- They tell you to cut out food without giving an explanation why (w29012013)
- Need to have information in other languages & make sure midwife makes/explains to the women (w30012013-2)
- A video would be nice, I think you can memorize it better (w04022013)
- I don't think its as bad as smoking & alcohol but it should be researched further (w04022013)

DESIRE FOR MORE INFORMATION

MIDWIFE

- We do talk about it but we should really provide that information (M2004 2012)
- It would be nice to know exactly what research/evidence was out there (M2004 2012)
- More information is needed, probably in the pregnancy booklet because they do love their pregnancy booklet (M26042012)
- If it becomes a big issue it probably warrants a leaflet all of it's own (M26042012)
- When we see them it's 10-12 weeks & if there's a risk of miscarriage & if they're shovelling coffee into them it'll be too late (M01052012)
- We need to find out first how effective the advice we give is (M01052012)
- If there is a risk & if women need to reduce caffeine prior to pregnancy then the information should be out there (M01052012)
- Maybe we should put warnings on coffee jars (M01052012)
- Website; aimed at Mums & government run with evidence based health advice (M09052012)
- I like the idea of campaigns, they can't ignore it because they're everywhere (M11052012-2)
- More information but provided in a very clever way (M14052012)
- Issues regarding worries should be highlighted & people informed on it (M14052012)
- Media; text messaging, BBM, Facebook, Twitter for the youth (M14052012)
- Campaigns on the TV, paper, radio - although they only work for 4-6 months & then they are forgotten (M14052012)
- Yes because the only guidance I have is this leaflet & the Tommys one (M15052012)
- Maybe a short concise leaflet (M15052012)
- I think anything that is given should be backed up with a conversation (M16052012)
- I would suggest that the information be given at pre-conception (M16052012)
- I think there should be an opportunity for women / midwives to revisit the information to make sure it's been understood (M16052012)
- I like posters, I like visual so if it was a leaflet it would have to be visual, in different languages (M16052012)

- I don't think there's that much research & that makes it difficult for midwives (M01062012)
- There has to be gold standard research for changing practice, otherwise its just traditional care & advice (M01062012)
- Yes we need more information if this is an issue, we need something to back up what we're saying for women to take notice (M01062012)
- I think information needs to go to the professionals first & then it can be disseminated down to the women (M21062012)
- Any form really; newsletters, leaflets, RMC, emails, hospital newsletters (M21062012)
- Credit card size information cards (M17082012)
- Maybe it should be a question that should be asked at booking (M22082012)
- Give women a better information leaflet, something that tells them what to avoid & why (M05092012)
- U think if you advertised it more they would take it on board (M05092012)
- I don't think women & HCP's are that well informed about coffee so there is a need for research & publications so that we can give women the information & they can make informed decisions (M20092012)
- They're isn't anything new; I don't get emails from anywhere saying look at this new research (M20092012)
- We should know more about what we're advising because pregnant women listen to midwives (M20092012)
- Timing of information needs to be considered (M20092012)
- Credit card size information or something on the internet (M18102012)
- They get loads of leaflets, maybe there are other ways of getting information across (M02112012)

... get loads of leaflets, maybe there are other ways of getting information across (u18102012)
... something on the internet (u02112012)

- Posters around the hospital, something with a drip-drip effect, in their face so they can't read it & bin it (u02112012)

- Texting & internet because everyone's on their phone. Information programmes on the TV, campaigns. Get out in the community & actually raise awareness (u06112012)

- Campaigns in doctors waiting areas, TV screens in the waiting room (u06112012)

- I think we probably need better information (u06112012)

- Limited knowledge, more research because the studies are comparable rather than causual (u06112012)

- I think women need to address drinking cone too (u18122012)

- Maybe some training or a session so we know what we should be telling them so that they're hearing the same thing (u18122012)

- If there's no definite answer & there's people out there & women drinking it & are concerned then we need research (u18122012)

- Leaflets need to be consistent, magazines, glossy magazines (u30012013)

- There's a need for more information, certainly when I think about how odd it makes me feel but I think coke & energy drinks are a cause for concern (u30012013)

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| Desire for more Information (Women) | | | Want more information, especially considering participants difficulty in conceiving and more information for those with access issues. The results of further studies may influence womens decisions | More information needed in the headlines/media. A book with bulletpoints | Something more specific for those who do not have the advice and support from friends and family | Participant would have liked a book or something easy online. Participant would also have liked something at the begining of her pregnancy. Also feels that coffee consumption should be investigated as the risks are unknown- | | | Unsure of the amount of caffeine in one cup, this should be known. Because of the uncertainty women wonder should they just cut it out. This information should be privided earlier than 14 weeks, especially since women are not aware according to participant. Hard data, clear evidence, case studies and examples are needed |

					allow to make informed decisions.		

				Uniform/harmonised information is needed	Magazine, compact and easy to read instead of leaflets	A discussion surrounding coffee, similar to other risks, along with explanations. Posters and campaigns in public areas in the hospital	Wish for MW to discuss the information further, need for leaflets to be in several languages	Video information and further research is needed	

Desire for more Information (MWs)	Talk about info with women but should provide more. MW would like to know exactly what research and evidence was out	More information is needed, possibly in the pregnancy book, leaflet but if it becomes a bigger issue there may be a need for a leaflet all of it's own	When MW see's the patient it can be too late especially if she is consuming a lot of coffee. MW would like to know how effective the advice is. If there is a risk MW feels that more	A health based website that is government run but aimed at women		Campaigns because they can't be ignored	Information on worries should be highlighted and provided in a very clever way. Campaigns in the media are good but only work for about 4-6 months.	MW feels more information is needed as the only information given to women is in the Tommy's leaflet-maybe a short concise leaflet	Any information given should be backed up with a conversation. Give information out preconception. Opportunity for both women and MWs to address any concerns they have in the future appointments.	Lack of research makes it difficult for MWs, need gold standard research instead of traditional methods. Need more information to back up what MWs are saying-women will then take notice

	there.		informaiton should be out there, possibly on the side of coffee jars.						Posters-visual information, visual leaflet in many languages

Information needs to go to the professionals first and then be disseminated down. Any format-newsletters, emails etc	Credit card size information cards	Ask a question during the booking appointment	A better information leaflet that gives the reason why a woman should reduce her intake. Media, it should be advertised	Women and MWs aren't given enough information and they can't make informed decisions. No information on new research, MW feels she should know about	Credit card size information cards or an internet website		Something other than leaflets, posters around the hospital	Campaigns in surgeries, TV's in waiting rooms. Need better information. Need more research	Drinking coke needs to be addressed. Further training for MWs or a session telling what should be said. If people are drinking	Leaflets need to be consistent. Information in glossy magazines. More information on coke and energy drinks

				what she's advising especially since women listen to MWs. Timing if information needs to be considered.				and there is concern then more research is needed	

Appendix 7: Placental and Myometrial Biopsy Consent Form

MANCHESTER 1824

The University of Manchester

Central Manchester University Hospitals NHS
Foundation Trust

NHS

Consent Form (Version 5: 14/02/2010)

Giving Placental Tissue, Maternal Tissue Samples and Blood for Research into Pregnancy Complications

To give us your consent, please can you confirm 'yes' to the questions below by placing your initials in the box provided. Please then sign your name in the box and have this witnessed at the same time as you sign it.

Please initial box

1. I confirm that I have read and understand the information sheet dated14/02/2010 (version 5) and have had the opportunity to ask questions.

2. I understand that giving samples for research is voluntary and that I am free to withdraw my consent at any time, providing my samples are

still being stored by the Maternal & Fetal Health Research Group. I do not have to give a reason and my medical treatment or legal rights will not be affected by withdrawal of my consent.

3. I give permission for my samples to be stored by, and distributed to researchers within, The Maternal & Fetal Health Research Group.

4. I understand that sections of my medical notes may be looked at by responsible individuals from the Maternal & Fetal Health Research Group where it is relevant to my taking part in research. I give permission for these individuals to access my records.

5. I understand that stored information will be kept confidential at all times; data will only pass to researchers in an anonymous way that protects my identity.

6. I understand that I will not personally benefit, financially or otherwise, from my gift of samples. This includes where my samples are involved in research resulting in the development of a new treatment or medical test.

7. I give permission for my samples, stored by The Maternal & Fetal Health Research Group, to be distributed to researchers in the USA and Canada provided that appropriate ethical approval is in place and that the research focuses on pregnancy complications.

8. I give permission for DNA analyses to be performed on blood samples collected from me and my baby to permit the investigation of genes that may be linked to pregnancy complications.

9. I give permission for my samples to be utilized by researchers within The Maternal & Fetal Health Research Group in a study supported by Ark Therapeutics Ltd., London, UK.

Consent Form (Version 5: 14/02/2010)

Giving Placental Tissue, Maternal Tissue Samples and Blood for Research into Pregnancy Complications

To give us your consent, please can you confirm 'yes' to the questions below by placing your initials in the box provided. Please then sign your name in the box and have this witnessed at the same time as you sign it.

Please initial box

10.   I give permission for a biopsy of my womb (a myometrial tissue biopsy) to be taken at the time of my Caesarean Section.

11.   I give permission for a biopsy of my omentum (an omental tissue biopsy) to be taken at the time of my Caesarean Section.

Name of Patient	**Date**	**Signature**

Name of Witness	**["Role"]**	**Date**	**Signature**

This witness can be a healthcare worker, relative, other patient, other adult, etc as available.

Appendix 8: Media Headlines

BBC News:

Caffeine limits for pregnant women (10/10/2001)

Coffee pregnancy warning (21/02/2003)

Pregnancy caffeine reassurance (26/01/2007)

Cut caffeine, pregnant women told (02/11/2008)

How much coffee is safe? (01/12/2011)

Coffee and cake lovers IVF success (03/07/2012)

Coffee, Wine, Cheese- How much can pregnant women have? (23/08/2013)

The Guardian:

Miscarriage and health risk warning over excessive coffee consumption (01/12/2011)

Pregnant women urged to give up coffee (18/02/2013)

Is it dangerous to drink coffee during pregnancy? (24/02/2013)

Coffee and wine: How much can pregnant women have? (26/08/2013)

Daily Mail:

Pregnancy dangers of coffee

Coffee may raise child cancer risk: New evidence that caffeine could damage babies DNA (26/01/2009)

A coffee a day for mums-to-be won't hurt baby (23/07/2010)

Drinking coffee during pregnancy won't turn your child hyperactive (12/07/2012)

Pregnant women CAN drink alcohol and coffee (18/08/2013)

Two cups of coffee a day while pregnant 'raise baby's leukaemia risk' (18/08/2014)

Coffee safety warning: Drink no more than four coffees a day - or two if you're pregnant, say experts (27/05/2015)

The Independent:

Want to conceive? Then shop drinking coffee, says study (26/05/3011)

Coffee, wine and sushi! New pregnancy book says OK (28/08/2013)

Hearld Sun:

Pregnant women can safely drink two glasses of wine and four cups of coffee a day, says author (16/08/2013)

Daily Star:

Coffee in pregnancy may be risky (27/11/2005)

Too much coffee in pregnancy tied to smaller, later new-borns (23/02/2013)

Appendix 9: Quantities of Caffeine in Different Beverages

Table 1 | **The caffeine content of various beverages, foods and drugs**

Beverages, foods and drugs	*Caffeine content (mg)
Sprite or Fanta (12 oz/360 ml)	0
Decaffeinated coffee (8 oz/240 ml)	1–5
Milk chocolate (1 oz/28 g)	6
Green tea (8 oz/240 ml)	15–20
Dark chocolate (1 oz/28 g)	20
Pepsi Cola (12 oz/360 ml)	38
Dr Pepper (12 oz/360 ml)	40
Coca-Cola (12 oz/360 ml)	46
Black tea (8 oz/240 ml)	40–60
Espresso (2 oz/60 ml)	50–120
Red Bull (8.2 oz/246 ml)	80
Instant coffee (8 oz/240 ml)	65–100
Brewed coffee (8 oz/240ml)	80–135
Drip coffee (8 oz/240ml)	115–175
Typical caffeine pill	200

*Values supplied by the US Food and Drug Administration.

Appendix 9a: Quantities of caffeine according to Nature paper (Foster & Wulff 2005).

Food or Drink	Portion Size	Caffeine Content (mg)
Coffee, brewed	1 cup	111mg (range 102-200mg)
Coffee, instant	1 cup	78mg (range 27-173mg)
Coffee, decaffeinated	1 cup	4mg (range 3-12mg)
Espresso	1 shot	40mg (range 30-90mg)
Tea, brewed	1 cup	44mg (range 40-120mg)
Snapple (fruit ad diet versions)	1 bottle (480ml)	42mg
Pepsi	1 bottle (500ml)	32mg
Pepsi Max	1 bottle (500ml)	30mg
Diet Coke	1 bottle (500ml)	64mg
Coke	1 bottle (500ml)	48mg
Caffeine-free Coke	1 bottle (500ml)	0mg
7-up (diet and regular)	1 bottle (500ml)	0mg
Red Bull	1 can (250ml)	80mg
Hot cocoa	1 cup	8mg
Milk chocolate	1 bar	11mg
Dark chocolate	1 bar	31mg
Coffee flavoured ice-cream	1 scoop	16mg (range 15-17mg)

Appendix 9b: Quantities of caffeine according to the Food Standards Agency Ireland (could not identify a UK equivalent).

Appendix 10: Systematic Review Table

Papers	Characteristics Of Study
Caffeine intake during pregnancy, late miscarriage and stillbirth (Greenwood et al. 2010)	• Cohort of 2643 pregnant women (18-45 years) • Validated tool to assess caffeine intake; questionnaire at booking and subsequent appointments. Recall bias. • Smoking confounder adjusted for. • Strong association between caffeine intake and 1st trimester and later miscarriage and stillbirth. • Pregnancies resulting in miscarriage/ stillbirth; women indicated higher caffeine intake • Mean= 145mg/day v's <103mg/day • Study states that is strengthens the observational evidence. • Small number of miscarriage and stillbirth detected-low power thus limiting ability to detect small associations and thus wide confidence intervals.
Caffeine intake during pregnancy and adverse birth outcomes: a systematic review and dose-response meta-analysis. (Greenwood et al. 2014)	• Systematic review • Meta-analysis of dose response curves for associations between caffeine and SA, low birth weight, SGA. • 60 publications from 53 cohort and case control studies. • Increase of 100mg associated with 14% increae in SA, 19% increase in still birth, 2% increase in preterm delivery, 7% increase in incidence in low birth weight and 10% increase in SGA. • Substantial heterogeneity in all models • Some of studies corrected for confounders such as smoking and obstetric history. • Limitation of small study effects. • No identifiable threshold below which associations were identified • Insufficient evidence to support further reductions in the maximum recommended intake. • Suggest maintaining current recommendations as a precaution. • None of the studies corrected for nausea / pregnancy signal.
Serum caffeine and paraxanthine as markers for reported caffeine intake in pregnancy. (Klebanoff et al. 1998)	• Uses serum paraxanthine levels rather than self-reporting. • Recall diary also utilised. • HPLC; serum concentrations. • Smokers v's non-smokers; levels were lower in smokers (correlates with literature). • Suggest serum paraxanthine and caffeine are useful to distinguish between women with varying levels of caffeine intake. • Wide inter-individual metabolism rates.

	- No consideration of nausea. - No necessarily a paper for poor outcome but discusses serum levels during pregnancy and the necessity of controlling for smoking.
Maternal Serum Paraxanthine, a caffeine metabolite and the risk of spontaneous abortion (Klebanoff et al. 1999)	- Nested case control study. - 591 women with SA 140 days into gestation. - Control: 2558 women with live births at 28 weeks gestation or later. - Years 1959 to 1966, measured over 30 years. - Serum paraxanthine levels were increased in those with SA. - Only extremely high serum paraxanthine concentrations were associated with SA. - Moderate caffeine consumption unlikely to have an effect. - Considered vomiting but not nausea. - Acknowledges that serum paraxanthine only a marker of short term caffeine intake. - Serum was stored for over 30 years; does long term storage have an effect?
Association of cytochrome P450 1B1 polymorphism with first-trimester miscarriage. (Karypidis et al. 2006)	- Population based case control study of early SA. - Interviews and questionnaire. - Maternal age, smoking habits, alcohol intake, caffeine intake, fetal karyotype, nausea and vomiting into consideration. - Carriers of CYP1B1 polymorphism was associated with first trimester SA and thus modifies the risk amongst coffee drinkers. - Polymorphism is rather common and therefore a potential indicator for clinicians.
Caffeine metabolism and the risk of spontaneous abortion of normal karyotype fetuses. (Signorello et al. 2001)	- Rate of caffeine metabolism influencing SA. - Case controlled study. - 101 women with normal karyotype SA and 953 pregnant women- 6 – 12 weeks gestation. - Adjusted for maternal age, gestational age, smoking and nausea score. - High CYP1A2 activity may increase the risk of SA, independently or by modifying the effect of caffeine. - Some missing urine results. - Caffeine intake as low as 100 - 300 mg/ day was associated with doubled the rish of SA amongst certain populations. - Potential for recall bias, exaggerated reporting amongst those who experience SA. - Limited sample size, statistically imprecise estimates. - Further studies are needed.
Caffeine intake, CYP1A2 polymorphism and the risk of	- Case control study. - 58 cases with two or more recurrent pregnancy losses

recurrent pregnancy loss. (Sata et al. 2005)	v's fertile 147 controls. • Low response rate to the questionnaires. • Overestimation of caffeine consumption amongst those who experienced SA. • Multifactorial polygenetic disease therefore more molecular epidemiological studies are further needed. • Cases with specific homozygous allele along with an increased caffeine intake deteriorates the fecundity among susceptible women. • Pregnancy signal/ nausea was not considered.

Caffeine intake and the risk of first-trimester spontaneous abortion.
 	
(Cnattingius et al. 2000)
	• Is ingestion of caffeine in early pregnancy a reflection, rather than having an effect, on fetal viability? Investigate whether caffeine is associated with an increased risk of early SA.
	• Over 2 years 562 women who suffered a SA between 6 to 12 weeks were recruited.
	• 101 fetuses were chromosomally normal, 157 were chromosomally abnormal.
	• Control: selected to match the women who had SA in duration of gestation (in weeks) and area of residence. To limit bias women with induced abortions were included into the control group.
	• Midwives conducted interviews using a structured questionnaire with women who had suffered SA, doctors conducted interviews with women who had undergone induced abortions. It was necessary to conduct some interviews over the telephone (50 women, 5 control).
	• Considered many different sources of caffeine, offered from four different cup sizes and conversion factors included (mean values for coffee's, tea and soft drinks).
	• Multivariate analysis included caffeine intake, smoking status, age, number of previous pregnancies, history of SA, alcohol consumption, presence or absence of nausea, vomiting, fatigue and the adjusted OR for SA in women who consumed at least 100mg of caffeine a day in comparison with women who consumed less than 100mg a day;
	    o OR: 100mg-299mg per day =1.3
	    o 300-499mg per day = 1.5
	    o 500mg + per day = 1.4
	• There was significant interaction between caffeine and smoking with regard to risk of SA. Among smokers, the ingestion of caffeine was not associated with an excess risk of SA whereas with non-smokers high intake was associated with a doubling in risk.
	• States that there is a possibility of confounding by other constituents in coffee.

	• The general message from the paper was that ingestion of caffeine during early stages of pregnancy is associated with an increased risk of first-trimester SA of a fetus of normal karyotype and that the increase in risk associated with caffeine is present among non-smokers.
Risk factors for first trimester miscarriage-results from a UK-population-based case-control study. (Maconochie et al. 2007)	• Population based case control study. • Two-stage postal survey on reproductive histories of women. • 603 women experiencing SA in first trimester and 6116 women who pregnancy progressed beyond 12 weeks. • Nausea was considered. • Caffeine was associated with SA however when nausea was considered this trend was non-significant. • Other risk factors were given higher priority within study.
Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. (Rasch 2003)	• Case control study; 330 women with SA vs 1168 pregnant women • Variables of maternal age, gestational age, cigarette, alcohol and caffeine consumption • Consumption of 375mg or more caffeine was associated with an increased risk of SA, regardless of tobacco consumption. • Questionnaire utilised? Not necessarily verified. • Did however consider multiple sources of caffeine; tea, chocolate, carbonated drinks. • No consideration of nausea however it was mentioned.
Cigarette, Alcohol and Coffee Consumption and Spontaneous Abortion. (Armstrong et al. 1991)	• Over a 2 year period 56,000 women were interviewed. These included women who had a normal delivery or spontaneous abortion. 47,146 were valid for analysis however this was whittled down to 35,848 (21.6% SA rate) once coffee consumption was analysed. • Cups of coffee per day and drinks of each type (alcohol) per week were recorded. • Confounders such as maternal age, education level, ethnicity and employment during pregnancy were considered. Previous live births, spontaneous abortions and specific risk factors were considered. • OR: risk of spontaneous abortion increased on average by a factor of 1.017 (1.004-1.030) for each cup per day. State that this risk may be as a result of residual confounding, however their survey data suggest that total caffeine and coffee consumption to be highly correlated. It suggested that coffee consumption accounted for 2% of SA, 16% in women who drank 10 cups of coffee a day. • General measurement for 'cup of coffee' but not

	explicitly stated.
Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. (Zusterzeel et al. 2000)	• 187 women with unexplained recurrent SA. • Serum collection • Particular polymorphisms, in combination with coffee consumption, may increase the risk of recurrent SA. • Nausea not considered as a confounder.
Dietary factors and risk of spontaneous abortion. (Di Cintio et al. 2001)	• Explored the dietary habits and risk of spontaneous abortion using data from a case-controlled study. • 912 women with the median age being 31 years (14 - 46 years) that were admitted for SA within 0-12 weeks of pregnancy. Control group included women recruited who gave birth to healthy infants at term. • Cases and controls were interviewed by trained professionals using a standard questionnaire. • Socioeconomic status, personal habits such as alcohol and smoking habits were noted as well as gynaecological and obstetric history. • Those who drank coffee before pregnancy and suffered a SA had an OR of 1.4. • Limitations included the fact that data was collected at different times between cases and controls and may have resulted in misrepresentation. Would have been better to design a prospective study comparing women suffering miscarriage/SA to those with a pregnancy proceeding beyond 12 weeks. • Questionnaire was not validated before the study and dietary questions were only a small part of the questionnaire and less attention may have been paid to this section. Information was only collected on a small number of food items also and portion/size was not precise. • As with all studies on SA in a hospital setting (hospital-based case control) it only includes women who suffered a SA requiring hospital admission with the consequent exclusion of women with subclinical abortions or very early pregnancy losses.
Risks of repeated miscarriage. (George et al. 2006)	• Matched case control study. • In person interviews combined with serum sampling. • Among non-smoking women with high caffeine intake, there was an increased risk of repeated miscarriage. • 108 women who experienced SA vs 953 control pregnancies. • Caffeine was associated with an increased risk of recurrent SA. • Nausea was not considered as a confounder although an extensive list was considered.
Coffee consumption and risk	• Case control study; 782 women with SA vs 1543

of hospitalized miscarriage before 12 weeks of gestation. (Parazzini et al. 1998)	controls. - Interviews were conducted post SA - Coffee consumption was associated with SA - Only some of the cases controlled for pregnancy signal / nausea. - Many confounders considered, including paternal coffee consumption.
Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion?. (Tolstrup et al. 2003)	- Nested cased control study 303 women with SA vs 1381 controls - Self-reporting caffeine consumption; 2 interviews and follow up. - High risk of SA associated with preconception caffeine consumption. - Acknowledge nausea but do not consider as a confounder.
Caffeine and miscarriage risk. (Savitz et al. 2008)	258 women with SA amongst 2407 women. - Interviews conducted. - Coffee consumption in all 3 trimesters was not associated with increased risk of SA. - Pregnancy signal / nausea was considered as a confounder.
Caffeine Consumption and Miscarriage: A prospective Cohort Study (Pollack et al. 2010)	- Prospective cohort study with longitudinal measurement of caffeine consumption. - No evidence that caffeine consumption increases SA risk. 2637 women were contacted. - Initial interview and further caffeine diary. - Did not consider the pregnancy signal / nausea
The associations of maternal caffeine consumption and nausea with spontaneous abortion. (Wen et al. 2001)	- Population based prospective study - Interview and questionnaire 1152 women - SA is unrelated to maternal caffeine consumption before conception. - Nausea was considered as a confounding factor. - Maternal caffeine consumption after nausea starts in 1st trimester is associated with an increased risk of SA.
Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. (Weng et al. 2008)	- Population based prospective cohort study. - In person interviews. 172 women experienced SA - High doses of caffeine during pregnancy increase risk of SA independent of pregnancy related symptoms. - Nausea and pregnancy signal was considered.
Moderate caffeine use and the risk of spontaneous abortion and intrauterine growth retardation.	- Cohort of 431 women - Fetal growth assessed with ultrasonography. - No evidence that moderate caffeine use increased the risk of SA IUGR or microcephaly

(Mills et al. 1993)	
Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. (Khoury et al. 2004)	• Women interviewed monthly using standardized questionnaires. • Women with T1D • Caffeine consumption during early pregnancy increases risk of SA. • Smoking with caffeine consumption is associated with a reduced risk of pre-eclampsia. • Nausea not considered as a confounder.
A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-Fallopian transfer (Klonoff-Cohen et al. 2002)	• Prospective study of 221 couples. • 2 questionnaires. • Caffeine should be minimized prior to IVF. • Women recruited already had reduced their caffeine intake. • Nausea was not considered as a confounding factor.
Teratogen update: evaluation of the reproductive and developmental risks of caffeine (Christian & Brent 2001)	• Review summarizing animal and human research. • More of an association between coffee consumption and tobacco/alcohol consumption that teratogenic effects. • Nausea was considered as a confounder. • Very extensive review.
Evaluation of the reproductive and developmental risks of caffeine (Brent et al. 2011)	• Review article • Scientific epidemiology literature concerning the reproductive and development toxicology risks of caffeine. • Dietary exposures of caffeine are not teratogenic or are directly responsible for an increased risk in SA or FGR. • Nausea was considered as a confounding factor.
Reproductive effects of caffeine: a review and theoretical analysis (Dlugosz & Bracken 1992)	• Review article • Insufficient evidence to support the reduction of caffeine during pregnancy. • Further studies are urgently needed. • Nausea as a confounder was considered.
Maternal Caffeine Consumption and Spontaneous Abortion : A Prospective Cohort Study (Dlugosz et al. 1996)	• Data was collected from an investigation of the possible effects of several environmental risk factors on pregnancy, over a 4 year period. Overall 2967 women completed interviews. Data was collected on health, pregnancy history, demographic, lifestyle characteristic and habits. • There were 2714 singleton, live births and 135 (4.5%) miscarriages. • Confounding factors taken into consideration were mothers' age, ethnicity, education, gravidity, parity, smoking, alcohol intake, prior induced and SA history, pregnancy weight, age at menarche and

	gestational stage at time of interview.
	• The mean daily caffeine consumption during the first month of pregnancy was 89.0mg per day for the women who miscarried and 72.4mg per day for the women who delivered liveborn infants.
• Not a terribly reliable paper as they investigated the relationship between caffeine consumption and several known and suspected risk factors for SA.
• Do provide information on risk estimates of SA for cigarette smoking, caffeine consumption and alcohol consumption and these are adjusted for maternal age, gestational age and each of the other two exposures.
• Consumption of more than 300mg per day caffeine almost doubled the odds of SA occurring however the linear dose-response was weak and adjustments for covariates lowered the risk estimate. SA is substantially increased when daily consumption reaches three or more cups of coffee or tea. They noted very little association between increased carbonated drink intake and SA.
Moderate to Heavy Caffeine Consumption During Pregnancy and Relationship to Spontaneous Abortion and Abnormal Fetal Growth: A Meta-analysis

(Fernandes et al. 1998) | • Meta-analysis.
• Computerized literature search and manual search.
• Not possible to completely consider confounders.
• Nausea as a confounder was not considered.
• Small, but statistically significant increase in the risk of SA and low birth weight amongst women consuming more than 150mg caffeine per day. |
| Reproduction and caffeine consumption- a literature review

(Golding 1995) | • Review article
• Animal and human epidemiological studies.
• Large scale studies are required.
• Few studies within this review consider the pregnancy signal
• Good example of older studies on caffeine. |
| Caffeine and its Effects on Pregnancy and the Neonate

(McKim 1991) | • Review article
• Advise on informing women on caffeine and the inability of the neonate to metabolize the compound. |
| A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: a 2000-2009 update

(David et al. 2010) | • Review articles; 2000-2009
• Review of human studies.
• Acknowledge inability to draw conclusive results due to confounders.
• Only some studies considered the pregnancy signal / nausea.
• Weight of the evidence did not support a positive relationship between caffeine consumption and adverse reproductive or perinatal outcomes.
• On the whole, observations indicated that there were no harmful effects associated with caffeine |

	consumption during the gestational period.
	• Extensive review
Maternal Caffeine Consumption and Spontaneous Abortion A Review of the Epidemiologic Evidence. (Signorello & McLaughlin 2004)	• Review article • 15 Epidmiologic studies. • Majority of studies reported positive associations between caffeine intake during pregnancy and risk of SA. • Discuss the many limitations; extensive review. • Nausea as a confounder was considered in some, but not all studies.
Caffeinated Beverages, Decaffeinated Coffee and Spontaneous Abortion. 1997 (Fenster et al. 1997)	• 5144 pregnant women • Interview in the first trimester. • No increased risk for SA associated with caffeine consumption. • Increased risk for SA associated with heavy consumption of decaffeinated coffee during 1st trimester. • Nausea was considered as a confounding factor.
A review of the literature relating caffeine consumption by women to their risk of reproductive hazards (Leviton & Cowan 2002)	• Review article • Concluded that no reproductive adversity had been consistently associated with caffeine consumption. • Nausea and pregnancy signal had been considered as a confounding factor in some, but not all studies.
The effect of caffeine consumption and nausea on the risk of miscarriage (Giannelli et al. 2003)	• Case control study restricted to nulliparous women. • 474 women identified. • Structured questionnaire administered by trained interviewer • High caffeine consumption during pregnancy (greater than 300 mg / day) was an independent risk factor for increased risk of SA. • Nausea is a protective factor. • Recall bias
Miscarriage, caffeine, and the epiphenomena of pregnancy: the casusal model (Stein & Susser 1991)	• Review article • Some articles consider nausea and pregnancy signal as confounding factor. • Inconclusive results as heterogeneity of studies and inability to consider all confounding factors.
Caffeine consumption during pregnancy and fetal growth (Fenster, Eskenazi, Windham & S. H. H. Swan 1991)	• Case controlled study of SA, over a period of a year. SA was identified from pathology reports and 68% of SA's occurred before 13 weeks (mean . • Detailed questions were asked about consumption 1 month pre-conception and about any changes during the first half of pregnancy. • Two controls per case were selected with frequency matching to case pregnancies by last menstrual period and hospital. 607 cases/ 1284 controls completed interviews and were suitable for analysis.

	- Tap water consumption was examined as a potential confounder because a report found an increased risk of SA associated with reported consumption of tap water. Confounding variables were entered in the models if they were related to SA and/or caffeine consumption. - Study indicated a slightly increased risk for SA associated with heavy caffeine consumption. There was double the risk for heavy consumers who reported nausea while this was not observed for those who did not report nausea. This could be because the non-nauseated women may be at higher risk of SA to begin with making it difficult to detect any added risk associated with the exposure to caffeine. Those who reduced their caffeine intake early in pregnancy had a risk of SA equal to non-consumers. - Crude measurement of caffeine as there is substantial variation in caffeine content according to the method of beverage preparation. Recall and selection bias all potential study confounders.
The association between low birth weight and caffeine consumption during pregnancy (Martin & Bracken 1987)	- Prospective study of 3891 women - Interview with standardized schedule. - Significant dose response relation between caffeine consumption during the gestational period and low fetal weight. - No identified effect on preterm delivery. - Nausea was not considered as a confounder.
Association of maternal caffeine consumption with decrements in fetal growth (Bracken et al. 2003)	- Cohort study; 2291 women identified. - Initial interview, regular checks throughout the pregnancy to evaluate changes in health indicators and validate exposure. - Moderate caffeine consumption during pregnancy does not significantly influence fetal growth *in utero*. - Nausea and pregnancy signal was not considered as a confounding factor.
Coffee Consumption, Birthweight and Reproductive Failures (Olsen et al. 1991)	- Questionnaire on smoking habits and dietary habits. - 11858 women were identified. - Coffee and tea consumption may interfere with fetal growth; may be more pronounced in smokers. - Consider the strength of the coffee. - Associations between coffee consumption and preterm births or congenital malformations were very weak. - Nausea and pregnancy signal was not considered as a confounding factor.
Relation of Caffeine intake during pregnancy to intrauterine growth	- Population based study - 7025 women identified. - Trained interviewer, standardized interview using

retardation and preterm birth (Fortier et al. 1993)	telephone. • Results indicated that caffeine consumption was associated with an increased risk of IUGR. • Nausea and pregnancy signal was not considered as a confounder.
Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study (Konje 2008)	• Prospective longitudinal observational study. • 2635 women identified • Caffeine assessment using standardized tool; questionnaire. • Salivary samples • Maternal caffeine intake is associated with an increased risk of FGR. • Suggest sensible advice for women. • Nausea as a confounder was considered.
The factors affecting pregnancy outcomes in the second trimester pregnant women (Bang & Lee 2009)	• A study investigating the factors which potentially affect birth weight and gestational age and thus provide basic data to promote a more favourable pregnancy outcome. • 403 in second trimester (20-36 weeks) were recruited over a 2 year period but only 234 pregnant women were included for analysis. • Maternal age, occupation status, number of family members, pregnancy experiences and morning sickness were recorded. Pre-pregnancy BMI was calculated however this was based on self-reported pre-pregnancy weight and height, and thus unreliable. • 24 hour recall method for 1 day was used to calculate alcohol and coffee consumption by trained field workers with the help of food models to estimate portion sizes. • Very brief paragraph stating that 10% of the subjects consumed more than 5 cups of coffee per day, however it was stated that there was no significance between coffee consumption and poor outcome (low birth weight). • Respondents were not randomly selected and were taken from a limited number of geographical areas. Dietary intake was only considered for one day and could not possibly reflect the ordinary diet of the subjects.
Caffeine metabolites in umbilical cord blood, cytochrome P-450 1A2 activity, and intrauterine growth restriction (Grosso et al. 2006)	• 2478 women recruited. • Interview on caffeine consumption prior to delivery and postpartum.. • Umbilical cord blood collection. • No associations with coffee consumption and preterm delivery. • Association of CYP1A2, rather than caffeine, and IUGR. • Nausea and pregnancy signal was not considered as a

	confounder.
Coffee and fetal death: a cohort study with prospective data (Bech et al. 2005)	• Cohort Prospective study. • 101032 women agreed to participate. • Conducted telephone interviews. • Coffee and caffeine were found to have no association on fetal death however researchers recommend to limit coffee per day during pregnancy. • No data on nausea or coffee aversion; not considered as a confounder.
Smoking and caffeine and alcohol intake during pregnancy in a northern population: effect on fetal growth (Godel & Pabst 1992)	• Questionnaire survey and collection of maternal and new-born measurements. • 162 women recruited. • There was no significant correlation between the total caffeine intake and infant measurements. • Nausea was not considered as a confounding factor.
High caffeine consumption in the third trimester of pregnancy : gender-specific effects on fetal growth (Vik et al. 2003)	• Population based case control study. • 111 mothers with SGA infants vs 747 controls. • 3 days of dietary records prospectively recorded. • High caffeine intake of caffeine in 3rd trimester was associated with increased risk of SGA in male fetuses. • Nausea as a potential confounding factor was not considered.
Effects on birthweight of alcohol and caffeine consumption in smoking women (Peacock et al. 1991)	• Prospective population study. • 1309 women. • 3 interviews incl. interview prior to booking. • Additional risk of SGA infants in smoking mothers that consume alcohol and high amounts of caffeine. • Difficult study as women investigated were smokers and tobacco consumption is a known risk factor. • Nausea was controlled for as a known confounding factor.
Effects of cigarette smoking, alcohol, coffee and tea consumption on preterm delivery. (Berkowitz et al. 1982)	• Case control study. • 175 mothers experiencing preterm delivery vs 313 term deliveries. • Structured standardized questionnaires • Interviews. • Lack of any significant associations between coffee consumption and preterm delivery; acknowledge that these are preliminary results. • Nausea was not considered a confounder and thus not include.
Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial	• Randomised double blind controlled trial. • 1207 pregnant women • Caffeinated coffee: 568 women • Decaffeinated coffee: 629 women. • Interview • Women replaced their usual coffee with those bought

(Bech et al. 2007)	by researchers. • Moderate reduction in caffeine intake in the second half of pregnancy has no effect on birth weight or length of gestation. • Nausea was not considered as a confounding factor.
Coffee drinking and risk of preterm birth (Chiaffarino et al. 2006)	• Case control study. • 502 women who delivered preterm vs 1966 controls. • Interview • Compared with no consumption, a low consumption of coffee during pregnancy may not have significant effects on preterm birth. • More marked inverse association for coffee consumption in the 3rd trimester of pregnancy in SGA cases compared with NGA. • Nausea was not considered as a confounding factor.
Smoking during pregnancy and pre term birth (Wisborg et al. 1996)	• Follow up study. • 2 questionnaires. • The association between smoking and preterm birth was only present among women with a high caffeine intake. • Nausea was not considered as a confounding factor.
Caffeine consumption during pregnancy and risk of preterm birth (Maslova et al. 2010)	• Meta-analysis. • No observed association between caffeine intake during pregnancy and the risk of preterm birth for cohort and case control studies.

Appendix 11: Example CASP tool for Cohort Study

CRITICAL APPRAISAL SKILLS PROGRAMME (CASP): Making Sense of Evidence

12 Questions to Help You Make Sense of a Cohort Study

General Comments
- Three broad issues need to be considered when appraising a cohort study.
  - Are the results of the study valid?
  - What are the results?
  - Will the results help locally?
- The 12 questions on the following pages are designed to help you think about these issues systematically.
- The first two questions are screening questions and can be answered quickly. If the answer to those two is "yes", it is worth proceeding with the remaining questions.
- There is a fair degree of overlap between several of the questions.
- You are asked to record a "yes", "no" or "can't tell" to most of the questions.
- A number of hints are given after each question. These are designed to remind you why the question is important. There may not be time in the small groups to answer them all in detail!

A. Are the results of the study valid?

Screening Questions

1. Did the study address a clearly focused issue?

   ☐ Yes   ☐ Can't Tell   ☐ No

   HINT: A question can be focused in terms of:
   - the population studied
   - the risk factors studied
   - the outcomes considered
   - is it clear whether the study tried to detect a beneficial or harmful effect?

2. Did the authors use an appropriate method to answer their question?

   ☐ Yes   ☐ Can't Tell   ☐ No

   HINT: Consider
   - Is a cohort study a good way of answering the question under the circumstances?
   - Did it address the study question?

Is it worth continuing?

Detailed Questions

3. Was the cohort recruited in an acceptable way?

   ☐ Yes   ☐ Can't Tell   ☐ No

   HINT: We are looking for selection bias which might compromise the generalisability of the findings:
   - Was the cohort representative of a defined population?
   - Was there something special about the cohort?
   - Was everybody included who should have been included?

4. Was the exposure accurately measured to minimize bias?

   ☐ Yes   ☐ Can't Tell   ☐ No

   HINT: We are looking for measurement or classification bias:
   - Did they use subjective or objective measurements?
   - Do the measures truly reflect what you want them to (have they been validated)?
   - Were all the subjects classified into exposure groups using the same procedure?

5. Was the outcome accurately measured to minimize bias?

   ☐ Yes   ☐ Can't Tell   ☐ No

   HINT: We are looking for measurement or classification bias:
   - Did they use subjective or objective measurements?
   - Do the measures truly reflect what you want them to (have they been validated)?
   - Has a reliable system been established for detecting all the cases (for measuring disease occurrence)?
   - Were the measurement methods similar in the different groups?
   - Were the subjects and/or the outcome assessor blinded to exposure (does this matter)?

6. A. Have the authors identified all important confounding factors?

   ☐ Yes   ☐ Can't Tell   ☐ No

   List the ones you think might be important, that the authors missed.

   _____

   _____

   _____

6. B. Have they taken account of the confounding factors in the design and/or analysis?

   ☐ Yes   ☐ Can't Tell   ☐ No

   HINT:
   Look for restriction in design, and techniques eg modelling, stratified-, regression-, or sensitivity analysis to correct, control or adjust for confounding factors

7. A. Was the follow up of subjects complete enough?

☐ Yes   ☐ Can't Tell   ☐ No

7. B. Was the follow up of subjects long enough?

☐ Yes   ☐ Can't Tell   ☐ No

HINT:
- o   The good or bad effects should have had long enough to reveal themselves
- o   The persons that are lost to follow-up may have different outcomes than those available for assessment
- o   In an open or dynamic cohort, was there anything special about the outcome of the people leaving, or the exposure of the people entering the cohort?

B. What are the results?

8. What are the results of this study?

_____

_____

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HINT:
- o   What are the bottom line results?
- o   Have they reported the rate or the proportion between the exposed/unexposed, the ratio/the rate difference?
- o   How strong is the association between exposure and outcome (RR)?
- o   What is the absolute risk reduction (ARR)?

9. How precise are the results? How precise is the estimate of the risk?

_____

_____

_____

_____

_____

_____

HINT: Size of the confidence intervals

10. Do you believe the results?

☐ Yes   ☐ Can't Tell   ☐ No

HINT:
- o   Big effect is hard to ignore!
- o   Can it be due to bias, chance or confounding?
- o   Are the design and methods of this study sufficiently flawed to make the results unreliable?
- o   Consider Bradford Hills criteria (eg time sequence, dose-response gradient, biological plausibility, consistency).

Is it worth continuing?

C. Will the results help me locally?

11. Can the results be applied to the local population?

☐ Yes   ☐ Can't Tell   ☐ No

HINT: Consider whether
- o   The subjects covered in the study could be sufficiently different from your population to cause concern.
- o   Your local setting is likely to differ much from that of the study
- o   Can you quantify the local benefits and harms?

12. Do the results of this study fit with other available evidence?

☐ Yes   ☐ Can't Tell   ☐ No

One observational study rarely provides sufficiently robust evidence to recommend changes to clinical practice or within health policy decision making.

However, for certain questions observational studies provide the only evidence.

Recommendations from observational studies are always stronger when supported by other evidence.

Bibliography

Aalkjær, C. & Mulvany, M., 1981. Functional and morphological properties of human omental resistance vessels. *Journal of Vascular Research*. Available at: http://www.karger.com/Article/Abstract/158357 [Accessed November 11, 2014].

Aaronson, L.S., 1989. Perceived and received support: Effects on health behavior during pregnancy. *Nursing Research*. Available at: http://journals.lww.com/nursingresearchonline/Abstract/1989/01000/Perceived_and_Received_Support__Effects_on_Health.2.aspx [Accessed April 7, 2014].

Aaronson, L.S., Mural, C.M. & Pfoutz, S.K., 1988. Seeking Information: Where Do Pregnant Women Go? *Health Education and Behaviour*, 15(3), pp.335–345. Available at: http://heb.sagepub.com/cgi/doi/10.1177/109019818801500307 [Accessed February 6, 2014].

Adeney, K.L. et al., 2007. Coffee consumption and the risk of gestational diabetes mellitus. *Acta Obstetricia Et Gynecologica Scandinavica*, 86(2), pp.161–166. Available at: <Go to ISI>://000244922700006.

Agardh, E.E. et al., 2004. Coffee consumption, type 2 diabetes and impaired glucose tolerance in Swedish men and women. *Journal of internal medicine*, 255(6), pp.645–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15147528.

Aguilera, G. & Rabadan, Diehl, C., 2000. Regulation of vasopressin V1b receptors in the anterior pituitary gland of the rat. *Experimental physiology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-445X.2000.tb00004.x/abstract [Accessed November 15, 2014].

Ahmed, A., Li, X. & Dunk, C., 1995. Colocalisation of vascular endothelial growth factor and its Flt-1 receptor in human placenta. *Growth Factors*. Available at: http://informahealthcare.com/doi/abs/10.3109/08977199509036883 [Accessed October 21, 2014].

Albrecht, W., 2010. Words of wisdom. Re: maternal smoking, alcohol, and coffee use during pregnancy and son's risk of testicular cancer. *European urology*, 57(2), pp.357–358. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20116771.

Alderete, E., Eskenazi, B. & Sholtz, R., 1995. Effect of Cigarette Smoking and Coffee Drinking on Time to Conception. *Epidemiology*, 6(4), pp.403–408.

Aldridge, A., Aranda, J. V & Neims, A., 1979. Caffeine metabolism in the newborn. *Clinical pharmacology and therapeutics*, 25(4), p.447. Available at: http://ukpmc.ac.uk/abstract/MED/428190 [Accessed December 14, 2011].

Alessandro, D.M.D. et al., 2004. A Randomized Controlled Trial of an Information Precription for Pediatric Patient Education on the Internet. *American Medical Association*, 158, pp.857–862.

al-Kanhal, M. & Bani, I., 1994. Food habits during pregnancy among Saudi women. *International journal for vitamin and nutrition ...*. Available at: http://europepmc.org/abstract/MED/8830001 [Accessed May 4, 2014].

Allen, D., Benner, P. & Diekelmann, N., 1986. Three paradigms for nursing research: Methodological implications. *Nursing Research*. Available at: http://scholar.google.co.uk/scholar?q=allen+paradigm+nursing+research&btnG =&hl=en&as_sdt=0,5#0 [Accessed May 9, 2014].

Almeida, M. De & Graca, P., 1997. Sources used and trusted by nationally-representative adults in the European Union for information on healthy eating. *European Journal of Clinical Nutrition*. Available at: http://europepmc.org/abstract/MED/9222719 [Accessed August 26, 2014].

Altura, B.M. et al., 1972. Effects of vasoactive agents on isolated human umbilical arteries and veins. *The American journal of physiology*, 222(2), pp.345–355. Available at: http://www.researchgate.net/profile/Burton_Altura2/publication/18764489_Effe cts_of_vasoactive_agents_on_isolated_human_umbilical_arteries_and_veins/lin ks/00b4952a4e9cff3d0d000000.pdf [Accessed March 5, 2015].

Anderson, A.S., 2007. Pregnancy as a time for dietary change? *Proceedings of the Nutrition Society*, 60(04), pp.497–504. Available at: http://www.journals.cambridge.org/abstract_S0029665101000581 [Accessed October 27, 2013].

Anderson, A.S., Campbell, D. & Shepherdt, R., 1993. Nutrition knowledge , attitude to healthier eating and dietary intake in pregnant compared to non-pregnant women. , pp.335–353.

Anderson, A.S., Campbell, D.M. & Shepherd, R., 1995. The influence of dietary advice on nutrient intake during pregnancy. *The British journal of nutrition*, 73(2), pp.163–77. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7718538.

Anderson, G.D., 2005. Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clinical pharmacokinetics*, 44(10), pp.989–1008. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16176115 [Accessed May 14, 2015].

Anderson, T., 2000. Feeling safe enough to let go: the relationship between a woman and her midwife during the second stage of labour. *The Midwife-Mother Relationship.(Kirkham M ed.) ...*. Available at: http://scholar.google.co.uk/scholar?q=Anderson,+T.,+2000.+Feeling+safe+enou gh+to+let+go:+the+relation-+ship+between+a+woman+and+her+midwife+during+the+second+stage+of+la bour.&btnG=&hl=en&as_sdt=0,5#0 [Accessed November 12, 2013].

Anon, 1981. Coffee: Should we stop drinking it? *Lancet*, p.256.

Anon, 2013. SIGMA-ALDRICH Safety Sheet. , (1907), pp.1–7.

Appiah, I. et al., 2009. Hydrogen peroxide affects contractile activity and anti-oxidant enzymes in rat uterus. *British journal of pharmacology*, 158(8), pp.1932–41. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2807655&tool=pmcentrez&rendertype=abstract [Accessed March 7, 2015].

Arion, W.J. et al., 1997. Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose 6-phosphatase. *Archives of biochemistry and biophysics*, 339(2), pp.315–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9056264.

Armitage, A., 2007. Mutual research designs: Redefining mixed methods research design. *European conference on research*. Available at: http://books.google.co.uk/books?hl=en&lr=&id=VtYcSTUV0nQC&oi=fnd&pg=PA29&dq=redefining+mixed+methods+research+design+&ots=bC3BJNqiPd&sig=WWbylphtvB2Sz6s1MZm01oqDAGU [Accessed June 27, 2012].

Armstrong, B.G., McDonald, A.D. & Sloan, M., 1991. Cigarette, Alcohol and Coffee Consumption and Spontaneous Abortion. , pp.1991–1993.

Armstrong, D.S., 2002. Emotional Distress and Prenatal Attachment in Pregnancy After Perinatal Loss. *Journal of Nursing Scholarship*.

Arthur, V.A.M., 1995. Written patient information: a review of the literature. *Journal of Advanced Nursing*, 21(6), pp.1081–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7665771.

Ashworth, J. & Warren, A., 1997. Loss of endothelium dependent relaxation in myometrial resistance arteries in pre- eclampsia. *British journal of Obstetrics and Gynaecology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1471-0528.1997.tb10939.x/full [Accessed November 11, 2014].

Athearn, P.N. et al., 2004. Awareness and acceptance of current food safety recommendations during pregnancy. *Maternal and child health journal*, 8(3), pp.149–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15499871.

Atkinson, R. & Flint, J., 2004. Snowball sampling. , 10(2), pp.141–163. Available at: http://eprints.gla.ac.uk/38647/.

Azuma, K., Ippoushi, K. & Nakayama, M., 2000. Absorption of chlorogenic acid and caffeic acid in rats after oral administration. *Journal of agricultural and Food Chemistry*. Available at: http://pubs.acs.org/doi/abs/10.1021/jf000483q [Accessed September 11, 2014].

Babbie, E., 2010. *The Practice of Social Research*,

Bandura, A., 2004. Health promotion by social cognitive means. *Health Education and Behaviour*, 31(2), pp.143–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15090118 [Accessed January 21, 2014].

Bang, S.W. & Lee, S.S., 2009. The factors affecting pregnancy outcomes in the second trimester pregnant women. *Nutrition research and practice*, 3(2), pp.134–40. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2788170&tool=pmc entrez&rendertype=abstract [Accessed August 22, 2012].

Barclay, L., Cm, R.N. & Med, B.A., 1997. Becoming a mother — an analysis of women's experience of early motherhood. *Journal of Advanced Nursing*, 25, pp.719–728.

Barger, M.K., 2010. Maternal nutrition and perinatal outcomes. *Journal of midwifery & women's health*, 55(6), pp.502–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20974412 [Accessed October 30, 2013].

Bech, B.H. et al., 2005. Coffee and fetal death: a cohort study with prospective data. *American journal of epidemiology*, 162(10), pp.983–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16207803 [Accessed August 8, 2013].

Bech, B.H. et al., 2007. Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. *Epidemiology*, 334(January), pp.409–412B. Available at: <Go to ISI>://000244654400033.

Becker, C.G. & Hajjar, D.P., 1985. Tobacco Constituents Are Mitogenic for Arterial Smooth-Muscle Cells. *Cancer Causes Control*, pp.1–5.

Beckman, J. & Koppenol, W., 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *American Journal of Physiology*. Available at: http://ajpcell.physiology.org/content/ajpcell/271/5/C1424.full.pdf [Accessed January 14, 2015].

Bekker, H. et al., 1999. Informed decision making: an annotated bibliography and systematic review. *Health Technology*, 3(1). Available at: http://discovery.ucl.ac.uk/15902/1/15902.pdf [Accessed May 8, 2014].

Benassayag, C., Mignot, T. & Haourigui, M., 1997. High polyunsaturated fatty acid, thromboxane A2, and alpha-fetoprotein concentrations at the human feto-maternal interface. *Journal of lipid research*. Available at: http://www.jlr.org/content/38/2/276.short [Accessed November 12, 2014].

Benirschke, K. & Kaufmann, P., 2000. *Pathology of the Human Placenta*, Springer Science & Business Media. Available at: http://books.google.com/books?id=NR1Pcv4Dxs4C&pgis=1 [Accessed March 6, 2015].

Benirschke, K., Kaufmann, P. & Baergen, R., 1995. The human placenta. Available at: http://eknygos.lsmuni.lt/springer/343/Contents and Front Matter.pdf [Accessed March 7, 2015].

Benoit, W. & Strathman, A., 2004. Source credibility and the elaboration likelihood model. *Association for Consumer Research*. Available at: http://scholar.google.co.uk/scholar?q=+2004+Source+Credibility+and+the+elaboration&btnG=&hl=en&as_sdt=0,5#6 [Accessed August 26, 2014].

Benson, P., 1980. Neonatal neuronal necrosis: its relationship to the distribution and maturation of oxidative enzymes of newborn cerebral and cerebellar cortex. *Medicine*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8749.1980.tb04392.x/abstract [Accessed August 7, 2015].

Berg, M., Lundgren, I. & Hermansson, E., 1996. Women ' s experience of the encounter with the midwife during childbirth. *Midwifery*, (December 1995).

Berkowitz, G.S., Holford, T.R. & Berkowitz, R.L., 1982. Effects of cigarette smoking, alcohol, coffee and tea consumption on preterm delivery. *Early human development*, 7(3), pp.239–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7160334.

Bernhardt, J. & Felter, E.E.M., 2004. Online pediatric information seeking among mothers of young children: results from a qualitative study using focus groups. *Journal of medical Internet research*, 6(1), p.e7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1550581&tool=pmcentrez&rendertype=abstract [Accessed October 29, 2013].

Bessell, T. & McDonald, S., 2002. Do Internet interventions for consumers cause more harm than good? A systematic review. *Health Psychology*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1369-6513.2002.00156.x/full [Accessed April 25, 2014].

Binder, P. & Borné, Y., 2012. Shared language is essential: communication in a multiethnic obstetric care setting. *Health Psychology*. Available at: http://www.tandfonline.com/doi/abs/10.1080/10810730.2012.665421 [Accessed October 6, 2014].

Black, N., 1994. Why we need qualitative research. *Journal of Epidemiology and Community Health*, (July), pp.425–426.

Blatter, L. & Wier, W., 1994. Nitric oxide decreases i in vascular smooth muscle by inhibition of the calcium current. *Cell Calcium*. Available at: http://www.sciencedirect.com/science/article/pii/0143416094900515 [Accessed March 10, 2015].

Bliss, L.A. et al., 2003. Taking the Next Step : Mixed Methods Research in Organizational Systems. *Information Technology, Learning and Performance*, 21(1), pp.19–29.

Boekschoten, M. V, Schouten, E.G. & Katan, M.B., 2004. Coffee bean extracts rich and poor in kahweol both give rise to elevation of liver enzymes in healthy volunteers. *Nutrition journal*, 3, p.7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=481079&tool=pmce ntrez&rendertype=abstract [Accessed November 22, 2011].

Bondarianzadeh, D., 2008. *Food safety in pregnancy : an exploration of lay and professional perspectives*.

Bonita, J.S. et al., 2007. Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies. *Pharmacological research : the official journal of the Italian Pharmacological Society*, 55(3), pp.187–98. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17368041 [Accessed October 25, 2011].

Borland, S.E. et al., 2009. Women's Compliance with nutrition and lifestyle recommendations before pregnancy: general population cohort study. *British Medical Journal*, pp.6–11.

Bouayed, J., Rammal, H. & Dicko, A., 2007. Chlorogenic acid , a polyphenol from Prunus domestica (Mirabelle), with coupled anxiolytic and antioxidant effects. *Animals*, 262, pp.77 – 84.

Bracken, M.B. et al., 2003. Association of maternal caffeine consumption with decrements in fetal growth. *American Journal of Epidemiology*, 157(5), pp.456–466. Available at: <Go to ISI>://000181410900009.

Bracken, M.B. et al., 2002. Heterogeneity in Assessing Self-Reports of Caffeine Exposure: Implications for Studies of Health Effects. *Epidemiology*, 13, pp.165–171.

Bradley, E.H., Curry, L. a LA & Devers, K.K.J., 2007. Qualitative data analysis for health services research: developing taxonomy, themes, and theory. *Health services research*, 42(4), pp.1758–72. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1955280&tool=pmc entrez&rendertype=abstract [Accessed October 5, 2012].

Brannen, J., 2005. Mixing Methods: The Entry of Qualitative and Quantitative Approaches into the Research Process. *International Journal of Social Research Methodology*, 8(3), pp.173–184. Available at: http://www.tandfonline.com/doi/abs/10.1080/13645570500154642 [Accessed April 28, 2014].

Braun, V. & Clarke, V., 2006. Using Thematic Analysis in Psychology. *Qualitative Research in Psychology*, 3, pp.77–101.

Brent, R.L., Christian, M.S. & Diener, R.M., 2011. Evaluation of the reproductive and developmental risks of caffeine. *Birth defects research. Part B, Developmental and reproductive toxicology*, 92(2), pp.152–87. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3121964&tool=pmc entrez&rendertype=abstract [Accessed August 22, 2012].

Brett, K. et al., 2014. Maternal–Fetal Nutrient Transport in Pregnancy Pathologies: The Role of the Placenta. *International Journal of Molecular Sciences*, 15(9), pp.16153–16185. Available at: http://www.mdpi.com/1422-0067/15/9/16153/.

Brewer, J. & Hunter, A., 1989. *Multimethod research: A synthesis of styles.*, Available at: http://psycnet.apa.org/psycinfo/1989-98711-000 [Accessed July 28, 2013].

Brewer, N. & Weinstein, N., 2004. Risk perceptions and their relation to risk behavior. *Annals of behavioral medicine*. Available at: http://link.springer.com/article/10.1207/s15324796abm2702_7 [Accessed April 30, 2014].

Brier, N., 2004. Anxiety after miscarriage: a review of the empirical literature and implications for clinical practice. *Birth*, 31(2), pp.138–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15153134.

Broeder, L. Den, Wagemakers, A. & Woerkum, C. van, 1999. Towards a communication policy of the Royal Dutch Organisation of Midwives. *Wageningen University, Wageningen*. Available at: http://scholar.google.co.uk/scholar?hl=en&q=den+Broeder,+L.,+Wagemakers,+A.,+van+Woerkum,+C.,+1999.+Towards+a+communication+policy+of+the+Royal+Dutch+Organisa-+tion+of+Midwives.&btnG=&as_sdt=1,5&as_sdtp=#0 [Accessed April 28, 2014].

Brosens, I., Robertson, W. & Dixon, H., 1967. The physiological response of the vessels of the placental bed to normal pregnancy. *The Journal of Pathology*. Available at: http://onlinelibrary.wiley.com/doi/10.1002/path.1700930218/abstract [Accessed October 21, 2014].

Brown, C.R. et al., 1988. Changes in rate and pattern of caffeine metabolism after cigarette abstinence. *Clinical pharmacology and therapeutics*, 43, pp.488–491.

Brownbill, P. et al., 2008. Vasoactivity to and endogenous release of vascular endothelial growth factor in the in vitro perfused human placental lobule from pregnancies complicated by preeclampsia. *Placenta*, 29(11), pp.950–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18845336 [Accessed August 22, 2012].

Browne, M.L., 2006. Maternal exposure to caffeine and risk of congenital anomalies: a systematic review. *Epidemiology*, 17(3), pp.324–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16570025 [Accessed November 22, 2011].

Browner, C. & Preloran, H.M., 2003. Genetic counseling gone awry: miscommunication between prenatal genetic service providers and Mexican-origin clients. *Social science & medicine*. Available at: http://www.sciencedirect.com/science/article/pii/S0277953602002149 [Accessed October 6, 2014].

Brueggemann, L. & Martin, B., 2005. Low voltage-activated calcium channels in vascular smooth muscle: T-type channels and AVP-stimulated calcium spiking. *American Journal of Physiology-Heart and Circulatory Physiology*. Available at: http://ajpheart.physiology.org/content/288/2/H923.short [Accessed November 15, 2014].

Bryce, R.L. & Enkin, M.W., 1984. Lifestyle in Pregnancy. *Canadian Family Physician*, pp.2127–2130.

Bryman, A., 2006. Integrating quantitative and qualitative research: how is it done? *Qualitative Research*, 6(1), pp.97–113. Available at: http://qrj.sagepub.com/cgi/doi/10.1177/1468794106058877 [Accessed November 1, 2012].

Bryman, A., 1988. Quantity and quality in social research. Available at: http://books.google.co.uk/books?hl=en&lr=&id=7pyt0yqQAcQC&oi=fnd&pg=PP1&dq=bryman+2004+quality+and+quantity&ots=4aRADyZMxJ&sig=pt9-7UK_gQVWczDRY8e1QzPDWqo [Accessed June 27, 2012].

Buijink, A., Visser, B. & Marshall, L., 2012. Medical apps for smartphones: lack of evidence undermines quality and safety. *Evidence-Based Medicine*. Available at: http://ebm.bmj.com/content/early/2012/08/24/eb-2012-100885.short?rss=1&ssource=mfr [Accessed August 26, 2014].

Bunting, L. & McAuley, C., 2004. Research Review: Teenage pregnancy and motherhood: the contribution of support. *Child and Family Social Work*, 9(2), pp.207–215. Available at: http://doi.wiley.com/10.1111/j.1365-2206.2004.00328.x.

Burgess, R., 1989. *The Ethics of Educational Research*,

Burns, R., 1997. *Introduction to research methods*, Available at: http://scholar.google.co.uk/scholar?q=burns+1997+introduction+to+research+methods&btnG=&hl=en&as_sdt=0,5#2 [Accessed November 12, 2012].

Burton, G., 2009. Oxygen, the Janus gas; its effects on human placental development and function. *Journal of anatomy*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-7580.2008.00978.x/full [Accessed June 29, 2015].

Burton, G., Jauniaux, E. & Watson, A., 1999. Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy: the Boyd collection revisited. *American Journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/S0002937899705181 [Accessed November 5, 2014].

Buscariollo, D.L. et al., 2011. Caffeine acts via A1 adenosine receptors to disrupt embryonic cardiac function. *PLoS Medicine*, 6(12), p.e28296. Available at:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3229565&tool=pmc entrez&rendertype=abstract [Accessed August 22, 2012].

Butt, M.S. & Sultan, M.T., 2011. Coffee and its consumption: benefits and risks. *Critical reviews in food science and nutrition*, 51(4), pp.363–73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21432699 [Accessed July 22, 2011].

Calkins, K. & Devaskar, S.U., 2011. Fetal Origins of Adult Disease. *Current Problems in Pediatric and Adolescent Health Care*, 41(6), pp.158–176. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21684471 [Accessed September 30, 2015].

Cameron, R., 2011. Mixed Methods Research : The Five P's of Framework. *Journal of Business Research Methods*, 9(2), pp.96–108.

Cano-Marquina, A., Tarín, J.J. & Cano, A., 2013. The Impact of Coffee on Health. *Maturitas*, 75(1), pp.7–21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23465359 [Accessed November 11, 2013].

Carbillon, L., Uzan, M. & Uzan, S., 2000. Hemodynamics : A Crucial Adaptation. , 55(9), pp.574–581.

Cardwell, M., 2013. Stress: pregnancy considerations. *Obstetrical & gynecological survey*. Available at: http://journals.lww.com/obgynsurvey/Abstract/2013/02000/Stress___Pregnancy _Considerations.21.aspx [Accessed March 3, 2015].

Carolan, M., 2007. Health literacy and the information needs and dilemmas of first-time mothers over 35 years. *Journal of Clinical Nursing*.

Carrillo, J.A. & Benitez, J., 2000. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. *Clinical pharmacokinetics*, 39(2), pp.127–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10976659 [Accessed July 29, 2013].

Carroll, D. & Benoit, C., 2004. Aboriginal midwifery in Canada: Merging traditional practices and modern science. *Reconceiving midwifery*. Available at: http://books.google.com/books?hl=en&lr=&id=VKad-RUFgHwC&oi=fnd&pg=PA263&dq=traditional+practices+modern+midwifery &ots=ZFb23XV9gx&sig=JXCKGRx8gnmJ_D4_Prv2kBHugEA [Accessed January 2, 2015].

Carter, A., 1997. When is the maternal placental circulation established in man? *Placenta*. Available at: http://www.sciencedirect.com/science/article/pii/S0143400497900758 [Accessed March 7, 2015].

CASP, 2006. 10 questions to help you make sense of qualitative research How to use this appraisal tool. *Public Health*.

Castellucci, M. et al., 1990. Anatomy and Embryology The development of the human placental villous tree. , pp.117–128.

Cavin, C. et al., 2002. Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity. *Food and Chemical Toxicology*, 40(8), pp.1155–1163. Available at: <Go to ISI>://000177393700016 [Accessed December 15, 2011].

Cavin, C. et al., 2001. Protective effects of coffee diterpenes against aflatoxin B1-induced genotoxicity: mechanisms in rat and human cells. *Food and Chemical toxicology*, 39(6), pp.549–556. Available at: http://www.sciencedirect.com/science/article/pii/S027869150000168X [Accessed December 15, 2011].

Cazeneuve, C. et al., 1994. Biotransformation of caffeine in human liver microsomes from foetuses, neonates, infants and adults. *British journal of clinical pharmacology*, 37(5), pp.405–12. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1364894&tool=pmcentrez&rendertype=abstract.

Chaddha, V. et al., 2004. Developmental biology of the placenta and the origins of placental insufficiency. *Seminars in fetal & neonatal medicine*, 9(5), pp.357–69. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15691771 [Accessed November 4, 2014].

Chaiken, S., 1992. Defensive processing of personally relevant health messages. *Personality and Social Psychology Bulletin*. Available at: http://psp.sagepub.com/content/18/6/669.short [Accessed May 1, 2014].

Chan, D. et al., 2004. Recent developments in meconium and hair testing methods for the confirmation of gestational exposures to alcohol and tobacco smoke. *Clinical biochemistry*, 37(6), pp.429–38. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15183290 [Accessed January 1, 2015].

Chan, W., Wen, P. & Chiang, H., 1994. Structure-activity relationship of caffeic acid analogues on xanthine oxidase inhibition. *Anticancer research*. Available at: http://europepmc.org/abstract/med/7645946 [Accessed November 9, 2014].

Chang, W. et al., 2010. Chlorogenic acid attenuates adhesion molecules upregulation in IL-1b-treated endothelial cells. *European Journal of Nutrition*, pp.267–275.

Chang, W. & Hsu, F., 1992. Inhibition of Platelet Activation and Endothelial Cell Injury by Polyphenolic Compounds Isolated from Lo & era japonica Thunb. *Pharmacology*, (June 1991), pp.307–312.

Charnock-Jones, D., 1993. Identification and localization of alternately spliced mRNAs for vascular endothelial growth factor in human uterus and estrogen regulation in endometrial carcinoma. *Biology of reproduction*. Available at: http://www.biolreprod.org/content/48/5/1120.short [Accessed October 21, 2014].

Chen, J.H. & Ho, C.-T., 1997. Antioxidant Activities of Caffeic Acid and Its Related Hydroxycinnamic Acid Compounds. *Journal of Agricultural and Food Chemistry*, 45(7), pp.2374–2378. Available at: http://pubs.acs.org/doi/abs/10.1021/jf970055t.

Chen, Z. et al., 2009. Anti-hypertensive nutraceuticals and functional foods. *Agricultural and Food Chemistry*, pp.4485–4499. Available at: http://pubs.acs.org/doi/abs/10.1021/jf900803r [Accessed January 9, 2012].

Cheung, R.J., Gupta, E.K. & Ito, M.K., 2005. Acute coffee ingestion does not affect LDL cholesterol level. *The Annals of pharmacotherapy*, 39(7-8), pp.1209–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15928258 [Accessed February 21, 2012].

Chiaffarino, F. et al., 2006. Coffee drinking and risk of preterm birth. *European journal of clinical nutrition*, 60(5), pp.610–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16391589 [Accessed August 8, 2014].

Chief Nursing Officers of England, Northern Ireland, S. and W., 2011. Midwifery 2020. Delivering expectations. *Practising Midwife*, 14, pp.42–43.

Choi, M.J. et al., 2011. Cafestol, a coffee-specific diterpene, induces apoptosis in renal carcinoma Caki cells through down-regulation of anti-apoptotic proteins and Akt phosphorylation. *Chemico-biological interactions*, 190(2-3), pp.102–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21334318 [Accessed November 22, 2011].

Choudhry, U., 1997. Traditional practices of women from India: pregnancy, childbirth, and newborn care. *Journal of Obstetric, Gynaecologic & Neonatal Nursing*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1552-6909.1997.tb02156.x/full [Accessed August 26, 2014].

Christensen, K. & Mulvany, M.J., 2001. Location of resistance arteries. *Journal of vascular research*. Available at: http://scholar.google.co.uk/scholar?hl=en&as_sdt=0,5&q=christensen+mulvaney+1994+resistance#0 [Accessed November 5, 2014].

Christian, M.S. & Brent, R.L., 2001. Teratogen update: evaluation of the reproductive and developmental risks of caffeine. *Teratology*, 64(1), pp.51–78. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11410911.

Christman, B. et al., 1992. An Imbalance between the Excretion of Thromboxane and Prostacyclin Metabolites in Pulmonary Hypertension. *The New England Journal Of Medicine*, pp.70–5.

Chung, T.-W. et al., 2004. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 18(14),

pp.1670–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15522912 [Accessed October 24, 2014].

Ciliska, D., Thomas, H. & Buffett, C., 2008. An Introduction to Evidence-Informed Public Health and A Compendium of Critical Appraisal Tools for Public Health Practice A Compendium of Critical Appraisal Tools for Public Health Practice. *National Collaborating Centre for Methods and Tools*, (February).

Di Cintio, E. et al., 2001. Dietary factors and risk of spontaneous abortion. *European journal of obstetrics, gynecology, and reproductive biology*, 95(1), pp.132–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11267735.

CIOMS & WHO, 2002. *International ethical guidelines for biomedical research involving human subjects.*, Available at: http://www.ncbi.nlm.nih.gov/pubmed/14983848.

Cipolla, M., Binder, N. & Osol, G., 1997. Myoendometrial versus placental uterine arteries: structural, mechanical, and functional differences in late-pregnant rabbits. *American Journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/S0002937897704642 [Accessed November 5, 2014].

Cipolla, M. & Osol, G., 1994. Hypertrophic and hyperplastic effects of pregnancy on the rat uterine arterial wall. *American Journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/0002937894901023 [Accessed November 5, 2014].

Claes, S.J., 2004. Corticotropin-releasing hormone (CRH) in psychiatry: from stress to psychopathology. *Annals of medicine*, 36(1), pp.50–61. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15000347 [Accessed June 29, 2015].

Clark, A.M., 1998. The qualitative-quantitative debate: moving from positivism and confrontation to post-positivism and reconciliation. *Journal of Advanced Nursing*, 27(6), pp.1242–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9663876.

Clark, L., Mugford, M. & Paterson, C., 1991. How does the mode of delivery affect the cost of maternity care? *British journal of obstetrics and gynaecology*, 98(6), pp.519–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1873239.

Clavel, J. et al., 2005. Childhood leukaemia, polymorphisms of metabolism enzyme genes, and interactions with maternal tobacco, coffee and alcohol consumption during pregnancy. *European Journal of Cancer Prevention*, 14(6), pp.531–540. Available at: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00008469-200512000-00007.

Clifford, K., Rai, R. & Regan, L., 1997. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Human Reproduction*, 12(2), pp.387–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9070732.

Clifford, M.N., 2000. Chlorogenic acids and other cinnamates – nature , occurrence , dietary burden , absorption and metabolism. *Journal of the Science of Food and Agriculture*, 1043(October 1999), pp.1033–1043.

Clifton, V. & Challis, J., 1997. Placental corticotropin releasing hormone function during human pregnancy. *The Endocrinologist*. Available at: http://journals.lww.com/theendocrinologist/Abstract/1997/07060/Placental_Corticotropin_Releasing_Hormone_Function.7.aspx [Accessed March 10, 2015].

Cnattingius, S. et al., 2000. Caffeine intake and the risk of first-trimester spontaneous abortion. *New England Journal of Medicine*, pp.1839–1845.

Cockell, A.P. et al., 1997. Human placental syncytiotrophoblast microvillous membranes impair maternal vascular endothelial function. , 104(February), pp.235–240.

Collins, J.A. & Fauser, B.C.J.M., 2005. Balancing the strengths of systematic and narrative reviews. *Human Reproduction*, 11(2), pp.103–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15618290 [Accessed July 28, 2011].

Connor, J., 1967. Selective dissemination of information: a review of the literature and the issues. *The Library Quarterly*. Available at: http://www.jstor.org/stable/4305823 [Accessed September 30, 2014].

Cook, T. & Campbell, D.T., 1979. *Design and Analysis Issues for Field Settings*,

Cooper, E.J. et al., 2005. Effects of Oxygen Tension and Normalization Pressure on Endothelin Induced Constriction of Human Placental Chorionic Plate Arteries. *Journal of the Society for Gynecologic Investigations*, 12(7), pp.488–494. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16202925.

Cremin, P., Kasim-Karakas, S. & Waterhouse, a L., 2001. LC/ES-MS detection of hydroxycinnamates in human plasma and urine. *Journal of agricultural and food chemistry*, 49(4), pp.1747–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11308321.

Crescimanno, C., Marzioni, D. & Persico, M., 1995. Expression of bFGF, PlGF and their receptors in the human placenta. *Placenta*. Available at: http://scholar.google.co.uk/scholar?q=crescimanno+1995+bFGF&btnG=&hl=en&as_sdt=0,5#0 [Accessed October 21, 2014].

Creswell, J. et al., 2006. How interpretive qualitative research extends mixed methods research. *Research in the Schools*. Available at:

http://www.msera.org/Rits_131/Creswell_131.pdf [Accessed November 11, 2012].

Creswell, J., 2003. *Research Design: Qualitative, Quantitative and Mixed Methods Approaches*, Available at: http://scholar.google.co.uk/scholar?q=creswell+2003+research+design:+qualitative&btnG=&hl=en&as_sdt=0,5#2 [Accessed June 27, 2012].

Creswell, J. & Clarke, V., 2007. *Designing and conducting mixed methods research*, Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1753-6405.2007.00097.x/full [Accessed November 29, 2012].

Creswell, J. & Hanson, W., 2007. Qualitative Research Designs Selection and Implementation. *The Counseling Psychologist*, 35, pp.236–264. Available at: http://tcp.sagepub.com/content/35/2/236.short [Accessed November 29, 2012].

Crozier, T.W.M. et al., 2012. Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. *The Royal Society of Chemistry*, 3(1), pp.30–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22130653 [Accessed April 26, 2012].

Cruchten, S.T.J. Van et al., 2010. Absorption , Distribution , and Biliary Excretion of Cafestol , a Potent Cholesterol-Elevating Compound in Unfiltered Coffees , in Mice. *Pharmacology*, 38(4), pp.635–640.

Cruchten, S.T.J. Van et al., 2010. The role of epoxidation and electrophile-responsive element-regulated gene transcription in the potentially beneficial and harmful effects of the coffee components cafestol and kahweol..pdf. *Journal of Nutritional Biochemistry*, 21(8), pp.757–763. Available at: http://dx.doi.org/10.1016/j.jnutbio.2009.05.001.

Cummiskey, M., 2011. There's an App for That Smartphone Use in Health and Physical Education. *Journal of Physical Education, Recreation & ….* Available at: http://www.tandfonline.com/doi/pdf/10.1080/07303084.2011.10598672 [Accessed August 26, 2014].

Curtis, B.P., Ball, L. & Kirkham, M., 2002. Why do midwives leave ? (not) being the kind of midwife you want to be. *British Journal of Midwifery*, (Table 3), pp.27–30.

Daly, J., McDonald, I. & Willis, E., 2007. *Researching Health Care: Designs, Dilemmas, Diciplines*,

Daly, L.E. & Bourke, G.J., 2000. *Interpretation and Uses of Medical Statistics* L. E. Daly & G. J. Bourke, eds., Oxford, UK: Blackwell Science Ltd. Available at: http://doi.wiley.com/10.1002/9780470696750.

Van Dam, R.M. & Hu, F.B., 2005. Coffee consumption and risk of type 2 diabetes - A systematic review. *Journal of American Medical Association*, 294(1), pp.97–104. Available at: <Go to ISI>://000230210200030.

Damsky, C., Fitzgerald, M. & Fisher, S., 1992. Distribution patterns of extracellular matrix components and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the. *Journal of Clinical Investigation*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC442839/ [Accessed October 28, 2014].

Darvill, R., Skirton, H. & Farrand, P., 2010. Psychological factors that impact on women's experiences of first-time motherhood: a qualitative study of the transition. *Midwifery*, 26(3), pp.357–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18783860 [Accessed February 6, 2014].

Darwish, O., Amine, E. & Abdalla, S., 1982. Food habits during pregnancy and lactation in Iraq. *Food and Nutrition Bulletin*. Available at: http://agris.fao.org/agris-search/search/display.do?f=2012/OV/OV2012060900609.xml;XF19830897515 [Accessed May 4, 2014].

Dautzenberg, M. & Just, A., 2013. Temporal characteristics of nitric oxide-, prostaglandin-, and EDHF-mediated components of endothelium-dependent vasodilation in the kidney. *American Journal of Physiology*. Available at: http://ajpregu.physiology.org/content/305/9/R987.abstract [Accessed November 11, 2014].

David, J. et al., 2010. A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: a 2000-2009 update. *Food and Chemical Toxicology*, 48(10), pp.2549–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20558227 [Accessed October 5, 2012].

Davidoff, F. & Haynes, B., 1995. Evidence based medicine; A New Journal to Help Doctors Identify the Information they need. *British Medical Journal*. Available at: http://www.bmj.com/content/310/6987/1085.short [Accessed June 13, 2012].

Davis, M., 2004. Nausea and vomiting of pregnancy: an evidence-based review. *The Journal of perinatal & neonatal nursing*. Available at: http://journals.lww.com/jpnnjournal/Abstract/2004/10000/Nausea_and_Vomiting_of_Pregnancy__An.2.aspx [Accessed May 7, 2014].

Davis, M., Ferrer, P. & Gore, R., 1986. Vascular anatomy and hydrostatic pressure profile in the hamster cheek pouch. *American Journal of Physiology*. Available at: http://ajpheart.physiology.org/content/ajpheart/250/2/H291.full.pdf [Accessed November 5, 2014].

Deave, T., Johnson, D. & Ingram, J., 2008. Transition to parenthood: the needs of parents in pregnancy and early parenthood. *BMC Pregnancy and Childbirth*. Available at: http://www.biomedcentral.com/1471-2393/8/30/ [Accessed December 5, 2013].

DeJoy, S.B., 2010. "Midwives are nice, but . . .": perceptions of midwifery and childbirth in an undergraduate class. *Journal of midwifery & women's health*,

55(2), pp.117–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20189130 [Accessed October 30, 2013].

DeLano, F. & Schmid-Schönbein, G., 1991. Penetration of the systemic blood pressure into the microvasculature of rat skeletal muscle. *Microvascular.* Available at: http://www.sciencedirect.com/science/article/pii/002628629190011Y [Accessed November 5, 2014].

Demir, R., 2009. Expression of VEGF receptors VEFGR-1 and VEGFR-2, angiopoietin receptors Tie-1 and Tie-2 in chorionic villi tree during early pregnancy. *Folia Histochemica et Cytobiologica.* Available at: http://czasopisma.viamedica.pl/fhc/article/view/4326 [Accessed November 4, 2014].

Demir, R., Kaufmann, P. & Castellucci, M., 1989. Fetal vasculogenesis and angiogenesis in human placental villi. *Acta Anatomica.* Available at: http://www.karger.com/Article/Abstract/146886 [Accessed March 7, 2015].

Demir, R. & Kosanke, G., 1997. Classification of human placental stem villi: review of structural and functional aspects. *Microscopy research and technique.* Available at: http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0029(19970701/15)38:1/2%3C29::AID-JEMT5%3E3.0.CO;2-P/abstract [Accessed November 4, 2014].

Denicola, A., Souza, J. & Radi, R., 1998. Diffusion of peroxynitrite across erythrocyte membranes. *PNAS.* Available at: http://www.pnas.org/content/95/7/3566.short [Accessed January 14, 2015].

Denscombe, M., 2008. Communities of Practice: A Research Paradigm for the Mixed Methods Approach. *Journal of Mixed Methods Research*, 2(3), pp.270–283. Available at: http://mmr.sagepub.com/cgi/doi/10.1177/1558689808316807 [Accessed October 30, 2012].

Denzin, N. & Lincoln, Y., 2005. *The Sage handbook of qualitative research*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=X85J8ipMpZEC&oi=fnd&pg=PR9&dq=ethics+and+politics+in+qualitative+research+2000&ots=D1PdI9R4dt&sig=2jqlt76vJ7sR7q21WGYt_qLo16c [Accessed November 11, 2012].

Desai, I. & Tavares, M., 1980. Food habits and nutritional status of agricultural migrant workers in Southern Brazil. *The American journal of Clinical Nutrition.* Available at: http://ajcn.nutrition.org/content/33/3/702.short [Accessed May 4, 2014].

Deutsch, F. & Ruble, D., 1988. Information-seeking and maternal self-definition during the transition to motherhood. *Journal of personality and social psychology.* Available at: http://psycnet.apa.org/journals/psp/55/3/420/ [Accessed December 5, 2013].

Dews, P.B., O'Brien, C.P. & Bergman, J., 2002. Caffeine: behavioral effects of withdrawal and related issues. *Food and Chemical Toxicology*, 40(9), pp.1257–61. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12204389.

Dhillon, A.S. et al., 2003. Internet use and perceptions of information reliability by parents in a neonatal intensive care unit. *Journal of perinatology : official journal of the California Perinatal Association*, 23(5), pp.420–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12847540 [Accessed April 23, 2014].

Diaz, J., Griffith, R. & Ng, J., 2002. Patients' use of the Internet for medical information. *Journal of General Internal Medicine*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1525-1497.2002.10603.x/full [Accessed April 24, 2014].

DiClemente, C., 2000. The process of pregnancy smoking cessation: implications for interventions. *Tobacco …*. Available at: http://tobaccocontrol.bmj.com/content/9/suppl_3/iii16.short [Accessed September 9, 2013].

Dillen, S. Van & Hiddink, G., 2003. Understanding nutrition communication between health professionals and consumers: development of a model for nutrition awareness based on qualitative consumer. *Journal of Clinical Nutrition*. Available at: http://ajcn.nutrition.org/content/77/4/1065S.short [Accessed August 26, 2014].

Dindyal, S., 2002. How Personal Factors, Including Culture And Ethnicity, Affect The Choices And Selection Of Food We Make. *Internet Journal of Third World Medicine*. Available at: http://scholar.google.com/scholar?hl=en&as_sdt=0,5&q=food+pregnancy+religion+culture+allergy#0 [Accessed August 26, 2014].

Dipietro, J. a et al., 2003. Psychosocial influences on weight gain attitudes and behaviors during pregnancy. *Journal of the American Dietetic Association*, 103(10), pp.1314–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14520249.

Ditto, P. & Lopez, D., 1992. Motivated skepticism: Use of differential decision criteria for preferred and nonpreferred conclusions. *Journal of Personality and Social Psychology*. Available at: http://psycnet.apa.org/journals/psp/63/4/568/ [Accessed May 1, 2014].

Dizdaroglu, M. & Jaruga, P., 2002. Free radical-induced damage to DNA: mechanisms and measurement< sup> 1, 2</sup>. *Free Radical Biology and Medicine*. Available at: http://www.sciencedirect.com/science/article/pii/S0891584902008262 [Accessed January 14, 2015].

Dlugosz, L. et al., 1996. Maternal Caffeine Consumption and Spontaneous Abortion : A Prospective Cohort Study. *Epidemiology*, 7(3), pp.250–255.

Dlugosz, L. & Bracken, M.B., 1992. Reproductive effects of caffeine: a review and theoretical analysis. *Epidemiologic reviews*, 14, pp.83–100. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1289118.

Dodd, J.M., Crowther, C. a & Robinson, J.S., 2008. Dietary and lifestyle interventions to limit weight gain during pregnancy for obese or overweight women: a systematic review. *Acta obstetricia et gynecologica Scandinavica*, 87(7), pp.702–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18607830 [Accessed November 12, 2013].

Donoghuea, J. et al., 2000. Fetal placental vascular responses to corticotropin-releasing hormone in vitro. Effects of variation in oxygen tension. *Placenta*. Available at: http://www.sciencedirect.com/science/article/pii/S0143400400905484 [Accessed March 10, 2015].

Dórea, J.G. & da Costa, T.H.M., 2007. Is coffee a functional food? *British Journal of Nutrition*, 93(06), p.773. Available at: http://www.journals.cambridge.org/abstract_S0007114505001200 [Accessed August 25, 2011].

Dosman, D., Adamowicz, W. & Hrudey, S., 2001. Socioeconomic determinants of health- and food safety- related risk perceptions. *Risk Analysis*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/0272-4332.212113/full [Accessed April 30, 2014].

Doyle, L., Brady, A.-M. & Byrne, G., 2009. An overview of mixed methods research. *Journal of Research in Nursing*, 14(2), pp.175–185. Available at: http://jrn.sagepub.com/cgi/doi/10.1177/1744987108093962 [Accessed April 28, 2014].

Drentea, P. & Moren-Cross, J.L., 2005. Social capital and social support on the web: the case of an internet mother site. *Sociology of Health and Illness*, 27(7), pp.920–943. Available at: http://doi.wiley.com/10.1111/j.1467-9566.2005.00464.x [Accessed February 6, 2014].

Dubois, S. et al., 1997. Ability of the Higgins Nutrition Intervention Program to Improve Adolescent Pregnancy Outcome. *Journal of American Dietetic Association*.

Dubois, S. & Dougherty, C., 1991. Twin pregnancy: the impact of the Higgins Nutrition Intervention Program on maternal and neonatal outcomes. *Journal of Clinical Nutrition*. Available at: http://ajcn.nutrition.org/content/53/6/1397.short [Accessed March 7, 2015].

Duff, P. & Séror, J., 2005. Computers and qualitative data analysis: Paper, pens, and highlighters vs. screen, mouse, and keyboard. *Tesol Quarterly*. Available at: http://onlinelibrary.wiley.com/doi/10.2307/3588315/abstract [Accessed October 9, 2012].

Dumont, E. & Lamontagne, D., 1995. No role of ATP-sensitive potassium channels in the vasoconstriction produced by vasopressin. *Journal of vascular research*. Available at: http://www.karger.com/Article/Abstract/159088 [Accessed November 15, 2014].

Dykes, F. & Williams, C., 1999. Falling by the wayside: a phenomenological exploration of perceived breast-milk inadequacy in lactating women. *Midwifery*. Available at: http://www.sciencedirect.com/science/article/pii/S0266613899901857 [Accessed November 11, 2012].

Echeverri, D. et al., 2010. Caffeine's Vascular Mechanisms of Action. *International journal of vascular medicine*, p.834060. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3003984&tool=pmc entrez&rendertype=abstract [Accessed June 25, 2013].

Edwards, A., Elwyn, G. & Mulley, A., 2002. Explaining risks: turning numerical data into meaningful pictures. *BMJ: British Medical Journal*. Available at: http://www.bmj.com/content/324/7341/827.1?variant=full-text [Accessed May 1, 2014].

Eijsden, M. Van, 2006. Folic acid knowledge and use in a multi- ethnic pregnancy cohort: the role of language proficiency. *Birtish Journal of Gynaecology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1471-0528.2006.01096.x/full [Accessed August 9, 2015].

Ekbom, A., 1998. Growing evidence that several human cancers may originate< i> in utero</i> *Seminars in cancer biology*. Available at: http://www.sciencedirect.com/science/article/pii/S1044579X98900736 [Accessed May 16, 2012].

El-Khairy, L. et al., 2003. Plasma total cysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. *The American journal of clinical nutrition*, 77(2), pp.467–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12540409.

Ellis, A. & Triggle, C., 2003. Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. *Canadian journal of physiology and ….* Available at: http://www.nrcresearchpress.com/doi/abs/10.1139/y03-106 [Accessed June 24, 2015].

Elmenhorst, D. et al., 2011. Adenosine A(1) receptors in human brain and transfected CHO cells: Inhibition of [(3)H]CPFPX binding by adenosine and caffeine. *Neuroscience letters*, 487(3), pp.415–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21056087 [Accessed December 13, 2011].

Elrad, H. & Gleicher, N., 1985. Physiologic changes in normal pregnancy. *Principles of Medical Therapy in Pregnancy*. Available at:

http://link.springer.com/chapter/10.1007/978-1-4613-2415-7_4 [Accessed May 4, 2014].

Enders, A.C. & Welsh, A.O., 1993. Structural interactions of trophoblast and uterus during hemochorial placenta formation. *The Journal of Experimental Zoology*, 266(6), pp.578–87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8371099.

English, P., Kharrazi, M. & Guendelman, S., 1996. Pregnancy outcomes and risk factors in Mexican Americans: the effect of language use and mother's birthplace. *Ethnicity & Disease*. Available at: http://europepmc.org/abstract/med/9467706 [Accessed August 10, 2015].

Eskes, T.K., 2001. Clotting disorders and placental abruption: homocysteine--a new risk factor. *European journal of obstetrics, gynecology, and reproductive biology*, 95(2), pp.206–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11301173.

Eteng, M.U. et al., 1997. Recent advances in caffeine and theobromine toxicities: a review. *Plant foods for human nutrition (Dordrecht, Netherlands)*, 51(3), pp.231–43. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9629863.

Evans, B.C., Coon, D.W. & Ume, E., 2012. Use of Theoretical Frameworks as a Pragmatic Guide for Mixed. *Journal of Mixed Methods Research*, 5(4), pp.276–292.

Evans, S. & Griffiths, R., 1992. Caffeine tolerance and choice in humans. *Psychopharmacology*. Available at: http://link.springer.com/article/10.1007/BF02245285 [Accessed August 10, 2015].

Fairburn, C., Stein, A. & Jones, R., 1992. Eating habits and eating disorders during pregnancy. *Psychosomatic Medicine*. Available at: http://www.psychosomaticmedicine.org/content/54/6/665.short [Accessed May 4, 2014].

Falloon, B. & Stephens, N., 1995. Comparison of small artery sensitivity and morphology in pressurized and wire-mounted preparations. *American Journal of Physiology*. Available at: http://ajpheart.physiology.org/content/ajpheart/268/2/H670.full.pdf [Accessed November 11, 2014].

Farah, A. et al., 2008. Chlorogenic acids from green coffee extract are highly bioavailable in humans. *The Journal of nutrition*, 138(12), pp.2309–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19022950.

Fattman, C., Schaefer, L. & Oury, T., 2003. Extracellular superoxide dismutase in biology and medicine. *Free Radical Biology and Medicine*. Available at: http://www.sciencedirect.com/science/article/pii/S0891584903002752 [Accessed January 14, 2015].

Feldman, J., 1966. The dissemination of health information. A case study in adult learning. *The dissemination of health information. A case study ....* Available at: http://www.cabdirect.org/abstracts/19672705086.html [Accessed September 30, 2014].

Fenster, L. et al., 1997. Caffeinated Beverages , Decaffeinated Coffee , and Spontaneous Abortion. *Epidemiology*, 8(5), pp.515–523.

Fenster, L., Eskenazi, B., Windham, G.C. & Swan, S.H.H., 1991. Caffeine consumption during pregnancy and fetal growth. *American journal of public health*, 81(4), pp.458–61. Available at: http://www.jstor.org/stable/10.2307/25759877 [Accessed January 16, 2012].

Fenster, L., Eskenazi, B., Windham, G.C. & Swan, S.H., 1991. Caffeine consumption during pregnancy and spontaneous abortion. *Epidemiology*, pp.168–174. Available at: http://www.jstor.org/stable/10.2307/25759877 [Accessed January 16, 2012].

Fereday, J. & Muir-Cochrane, E., 2006. Demonstrating Rigor Using Thematic Analysis : A Hybrid Approach of Inductive and Deductive Coding and Theme Development. *International Journal of Qualitative Methods*, 5(March), pp.1–11.

Ferguson, T., 1997. Health online and the empowered medical consumer. *The Joint Commission journal on quality improvement*. Available at: http://europepmc.org/abstract/MED/9179717 [Accessed April 25, 2014].

Ferlie, E.B. & Shortell, S.M., 2001. Improving the quality of health care in the United Kingdom and the United States: a framework for change. *The Milbank quarterly*, 79(2), pp.281–315. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2751188&tool=pmc entrez&rendertype=abstract.

Fernandes, O. et al., 1998. Moderate to Heavy Caffeine Consumption During Pregnancy and Relationship to Spontaneous Abortion and Abnormal Fetal Growth: A Meta-analysis. *Journal of Reproductive Toxicology*, 12(4), pp.435–444.

Fernandez, M., Saenz, M. & Garcia, D., 1998. Natural Products: Anti-inflammatory Activity in Rats and Mice of Phenolic Acids Isolated from Scrophularia frutescens. *Journal of Pharmacy and Pharmacology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.2042-7158.1998.tb03332.x/abstract [Accessed November 9, 2014].

Fesen, M., Kohn, K. & Pommier, Y., 1993. Inhibitors of human immunodeficiency virus integrase. *PNAS*. Available at: http://www.pnas.org/content/90/6/2399.short [Accessed November 9, 2014].

Fink, A., 1998. Conducting Research Literature Reviews: From Paper to the Intern. Available at: http://pdf1.caodbooks.com/conducting-research-literature-reviews-from-paper-to-the-intern-PDF-697347.pdf [Accessed January 10, 2014].

Fishbein, M., Y-Rit, J. & Lando, U., 1980. The relationship of vascular injury and myocardial hemorrhage to necrosis after reperfusion. *Circulation*. Available at: http://circ.ahajournals.org/content/62/6/1274.short [Accessed June 29, 2015].

Fisher, S. & Damsky, C., 1993. Human cytotrophoblast invasion. *Cell Biology*, 4.

Fishman, D., 1991. The experimental versus the pragmatic paradigm: Summary and conclusions. *Evaluation and Program Planning*. Available at: http://www.sciencedirect.com/science/article/pii/014971899190025C [Accessed November 12, 2012].

Fitzgerald, D.J. et al., 1990. Thromboxane A2 Synthesis in Pregnancy-induced Hypertension. *Lancet*, 335, pp.751–754.

Fitzpatrick, R. & Boulton, M., 1996. Qualitative research in health care: I. The scope and validity of methods. *Journal of evaluation in clinical practice*, 2(2), pp.123–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9238582.

Fleming, V.E.M., 1998. Women and midwives in partnership: a problematic relationship? *Journal of Advanced Nursing*, 27(1), pp.8–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9515602.

Fluehr-Lobban, C., 1998. *Handbook of Methods in Cultural Anthrapology*,

Ford-Gilboe, M., 1995. Stories and numbers: Coexistence without compromise. *Advances in Nursing ...*. Available at: http://journals.lww.com/advancesinnursingscience/Abstract/1995/09000/Stories_and_numbers__Coexistence_without.3.aspx [Accessed November 29, 2012].

Fortier, I., Marcoux, S. & Beaulac-Baillargeon, L., 1993. Relation of Caffeine intake during pregnancy to intrauterine growth retardation and preterm birth. *Epidemiology*, 137(9), pp.931–940.

Foster, R.G. & Wulff, K., 2005. The rhythm of rest and excess. *Nature reviews. Neuroscience*, 6(5), pp.407–14. Available at: http://dx.doi.org/10.1038/nrn1670 [Accessed October 12, 2015].

Fouda, L.M., Ahmed, M.H. & Shehab, N.S., 2012. Nutritional Awareness of Women during Pregnancy. *Journal of American Science*, 8(7), pp.494–502.

Fox, S., 2012. Mobile Health 2012.

Frank, J. et al., 2003. The Dietary Hydroxycinnamate Caffeic Acid and Its Conjugate Chlorogenic Acid Increase Vitamin E and Cholesterol Concentrations in Sprague − Dawley Rats. *Journal of Agricultural and Food Chemistry*, (22), pp.2526–2531.

Frary, C.D., Johnson, R.K. & Wang, M.Q., 2005. Food sources and intakes of caffeine in the diets of persons in the United States. *Journal of the American Dietetic Association*, 105(1), pp.110–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15635355 [Accessed March 28, 2012].

Fredholm, B.B., 1995. Adenosine , Adenosine Receptors and the Actions of Caffeine *. , pp.93–101.

Freeman, L.M., D, L.M.F.P. & Lecturer, R.S., 2006. Continuity of carer and partnership. A review of the literature. *Women and birth : journal of the Australian College of Midwives*, 19(2), pp.39–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16890901 [Accessed November 12, 2013].

Freeman, L.M. & Griew, K., 2007. Enhancing the midwife-woman relationship through shared decision making and clinical guidelines. *Women and birth : journal of the Australian College of Midwives*, 20(1), pp.11–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17127115 [Accessed November 12, 2013].

Frenkel, K. et al., 1993. Inhibition of tumor promoter-mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. *Cancer Research*. Available at: http://cancerres.aacrjournals.org/content/53/6/1255.short [Accessed November 9, 2014].

Freshwater, D., 2005. Writing, rigour and reflexivity in nursing research. *Journal of Research in Nursing*, 10(3), pp.311–315. Available at: http://jrn.sagepub.com/cgi/doi/10.1177/174498710501000307 [Accessed September 3, 2013].

Fuhr, U., Klittich, K. & Staib, A., 1993. Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *British journal of clinical Pharmacology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2125.1993.tb04162.x/abstract [Accessed March 10, 2015].

Furber, C., 2010. Framework analysis : a method for analysing qualitative data. *African Journal of Midwifery and Women's Health*, 4(2), pp.97–100.

Furchgott, R. & Zawadzki, J., 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. Available at: http://dialogues-cvm.org/pdf/10/DCVM10_07.pdf [Accessed July 3, 2012].

Gadsby, R., Barnie-Adshead, A. & Jagger, C., 1997. Pregnancy nausea related to women's obstetric and personal histories. *Gynecology and Obstetrics*. Available at: http://www.karger.com/Article/Abstract/291833 [Accessed April 4, 2014].

Gage, N., 1989. The Paradigm Wars and Their Aftermath A "Historical" Sketch of Research on Teaching Since 1989. *Educational Researcher*, 18(7), pp.4–10.

Available at: http://edr.sagepub.com/cgi/doi/10.3102/0013189X018007004 [Accessed November 11, 2012].

Gaillard, R. & Jaddoe, V.W.V., 2014. Assessment of Fetal Growth by Customized Growth Charts. *Annals of Nutrition and Metabolism*, 65(2-3), pp.149–155. Available at: http://www.karger.com?doi=10.1159/000361055.

Gardosi, J. & Mongelli, M., 1995. An adjustable fetal weight standard. *Ultrasound in Obstetrics ...*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1469-0705.1995.06030168.x/full [Accessed September 27, 2014].

Garg, U. & Hassid, A., 1991. Nitric oxide decreases cytosolic free calcium in Balb/c 3T3 fibroblasts by a cyclic GMP-independent mechanism. *Journal of Biological Chemistry*. Available at: http://www.jbc.org/content/266/1/9.short [Accessed March 10, 2015].

Gaytan, S. & Saadani-Makki, F., 2006. Effect of postnatal exposure to caffeine on the pattern of adenosine A1 receptor distribution in respiration-related nuclei of the rat brainstem. *Autonomic ...*. Available at: http://www.sciencedirect.com/science/article/pii/S1566070206000828 [Accessed September 17, 2013].

Genazzani, A. et al., 1975. Immunoreactive ACTH and cortisol plasma levels during pregnancy. Detection and partial purification of corticotrophin-like placental hormone: the human chorionic corticotrophin (HCC). *Clinical Endocrinology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2265.1975.tb03299.x/abstract [Accessed October 29, 2014].

Genbacev, O., 1997. Regulation of Human Placental Development by Oxygen Tension. *Science*, 277(5332), pp.1669–1672. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.277.5332.1669 [Accessed October 10, 2014].

Gentzkow, G., 1985. Recurrent miscarriage I : definition and epidemiology. *Postgraduate Medical Journal*, pp.673–675.

George, L. et al., 2006. Risks of repeated miscarriage. *Paediatric and perinatal epidemiology*, 20(2), pp.119–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16466430.

George, S.E., Ramalakshmi, K. & Mohan Rao, L.J., 2008. A perception on health benefits of coffee. *Critical reviews in food science and nutrition*, 48(5), pp.464–86. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18464035 [Accessed September 14, 2011].

Gerber, B.S. & Eiser, A.R., 2001. The Patient –Physician Relationship in the Internet Age: Future Prospects and the Research Agenda. *Journal of Medical Internet Research*.

Gerrard, M. et al., 1996. A longitudinal study of the reciprocal nature of risk behaviors and cognitions in adolescents: what you do shapes what you think, and vice versa. *Health Psychology*, 15(5), pp.344–354. Available at: http://psycnet.apa.org/journals/hea/15/5/344/ [Accessed May 1, 2014].

Giannelli, M. et al., 2003. The effect of caffeine consumption and nausea on the risk of miscarriage. *Paediatric and perinatal epidemiology*, 17(4), pp.316–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14629312.

Gilbert, S. et al., 1988. Adverse pregnancy outcome in the monkey (Macaca fascicularis) after chronic caffeine exposure. *Journal of Pharmacology and ....* Available at: http://jpet.aspetjournals.org/content/245/3/1048.short [Accessed July 29, 2013].

Giles, W., 1985. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. *Birtish Journal of Gynaecology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1471-0528.1985.tb01045.x/full [Accessed October 21, 2014].

Gill, J., 2002. Reported levels of alcohol consumption and binge drinking within the UK undergraduate student population over the last 25 years. *Alcohol and Alcoholism*. Available at: http://alcalc.oxfordjournals.org/content/37/2/109.short [Accessed November 5, 2014].

Glazener, M.A. & Statistician, M., 1997. Satisfaction and the three C's: continuity, choice and control. Women's views from a randomised controlled trial of midwife-led care. , 104(November), pp.1273–1280.

Gleich, J., 1954. Effect of smoking and of drinking of coffee and alcohol in pregnancy on the fetus. *Cas Lek Cesk*, 93(36-37), pp.1007–1012. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=13209609.

Godel, J. & Pabst, H., 1992. Smoking and caffeine and alcohol intake during pregnancy in a northern population: effect on fetal growth. *CMAJ*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1336160/ [Accessed August 6, 2013].

Gold, K.J. et al., 2012. Internet message boards for pregnancy loss: who's on-line and why? *Women's health issues : official publication of the Jacobs Institute of Women's Health*, 22(1), pp.e67–72. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3237941&tool=pmcentrez&rendertype=abstract [Accessed October 30, 2013].

Golding, J., 1994. Caffeine, health and commercial interests. *Addiction (Abingdon, England)*, 90(7), pp.988–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7663323.

Golding, J., 1995. Reproduction and caffeine consumption- a literature review. *Early human development*, 43(1), pp.1–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8575347.

Golias, C. et al., 2007. The kinin system-bradykinin: Biological effects and clinical implications. Multiple role of the kinin system-bradykinin. *Hippokratia*, 11(3), pp.124–128.

Gonthier, M.-P.M. et al., 2003. Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *The Journal of nutrition*, 133(6), pp.1853–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12771329 [Accessed September 11, 2014].

Goodwin, T., 2002. Nausea and vomiting of pregnancy: an obstetric syndrome. *American Journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/S0002937802800163 [Accessed May 7, 2014].

Gordon, M.C., 2012. Maternal Physiology. In *Physiology*. pp. 55–84.

Gostin, L.O., 1991. Ethical Principles for the Conduct of Human Subject Research : Population-Based Research and Ethics. *Medical and Health Care*, 201.

Gothill, M. & Armstrong, D., 1999. Dr . No-body : the construction of the doctor as an embodied subject in British general practice Matthew Gothill and David Armstrong. *Sociology of Health and Illness*, 21(1), pp.1–12.

Graf, R., Langer, J. & Schönfelder, G., 1994. The extravascular contractile system in the human placenta. *Anatomy and …*. Available at: http://link.springer.com/article/10.1007/BF00190104 [Accessed November 4, 2014].

Graff, C.J., 2014. Mixed Methods Research. *Evidence-based practice: An integrative approach to research, administration, and practice*, pp.45–64.

Graham, W.J., 1997. Midwife-led care. *British journal of obstetrics and gynaecology*, azc(1997 Apr;104(4):410-8), pp.396–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9141573.

Granado-Serrano, A. et al., 2007. Molecular mechanisms of (-)-epicatechin and chlorogenic acid on the regulation of the apoptotic and survival/proliferation pathways in a human hepatoma cell line. *Journal of Agricultural and Food Chemistry*. Available at: http://pubs.acs.org/doi/abs/10.1021/jf062556x [Accessed January 9, 2012].

Green, B.N., Johnson, C.D. & Adams, A., 2006. Writing narrative literature reviews for peer-reviewed journals: secrets of the trade. *Journal of chiropractic medicine*, 5(3), pp.101–17. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2647067&tool=pmcentrez&rendertype=abstract.

Greene, J., 2006. Toward a methodology of mixed methods social inquiry. *Research in the Schools*. Available at: http://www.msera.org/Rits_131/Greene_131.pdf [Accessed November 11, 2012].

Greene, J. & Caracelli, V.J., 1997. Defining and describing the paradigm issue in mixed-method evaluation. *New directions for evaluation*. Available at: http://onlinelibrary.wiley.com/doi/10.1002/ev.1068/abstract [Accessed November 11, 2012].

Greenwood, D.C. et al., 2014. Caffeine intake during pregnancy and adverse birth outcomes: a systematic review and dose-response meta-analysis. *European journal of epidemiology*, 29(10), pp.725–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25179792 [Accessed November 30, 2014].

Greenwood, D.C. et al., 2010. Caffeine intake during pregnancy, late miscarriage and stillbirth. *European Journal of Epidemiology*, pp.275–280.

Grewal, J. et al., 2008. Maternal periconceptional smoking and alcohol consumption and risk for select congenital anomalies. *Birth Defects Research*. Available at: http://onlinelibrary.wiley.com/doi/10.1002/bdra.20461/full [Accessed January 16, 2012].

Griendling, K. & FitzGerald, G., 2003. Oxidative stress and cardiovascular injury part I: basic mechanisms and in vivo monitoring of ROS. *Circulation*. Available at: http://circ.ahajournals.org/content/108/16/1912.short [Accessed June 29, 2015].

Griffiths, R., Evans, S. & Heishman, S., 1990a. Low-dose caffeine discrimination in humans. *Journal of Pharmacology and Experimental Therapeutics*. Available at: http://jpet.aspetjournals.org/content/252/3/970.short [Accessed October 28, 2013].

Griffiths, R., Evans, S. & Heishman, S., 1990b. Low-dose caffeine physical dependence in humans. *The of Pharmacology and experimental therapeutics*. Available at: http://jpet.aspetjournals.org/content/255/3/1123.short [Accessed October 28, 2013].

Griffiths, R.R. & Woodson, P.P., 1988. Caffeine physical dependence : a review of human and laboratory animal studies. *Psychopharmacology*, 94, pp.437–451.

Groisser, D.S., Rosso, P. & Winick, M., 1982. Coffee consumption during pregnancy: subsequent behavioral abnormalities of the offspring. *Journal of Nutrition*, 112(4), pp.829–832. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7069517.

Gross, G., Jaccaud, E. & Huggett, a C., 1997. Analysis of the content of the diterpenes cafestol and kahweol in coffee brews. *Food and Chemical*

Toxicology, 35(6), pp.547–54. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/9225012.

Grosso, L.M. et al., 2006. Caffeine metabolites in umbilical cord blood, cytochrome
P-450 1A2 activity, and intrauterine growth restriction. *American journal of
epidemiology*, 163(11), pp.1035–41. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/16641310 [Accessed September 17,
2013].

Grosso, L.M. et al., 2001. Maternal caffeine intake and intrauterine growth
retardation. *Epidemiology*, 12(4), pp.447–55. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/11428387.

Grosso, L.M. & Bracken, M.B., 2005. Caffeine metabolism, genetics, and perinatal
outcomes: a review of exposure assessment considerations during pregnancy.
Annals of epidemiology, 15(6), pp.460–6. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/15967394 [Accessed January 15, 2012].

Grover, A. et al., 1999. Effects of peroxide on contractility of coronary artery rings
of different sizes. *Molecular and Cellular Biology*, 194(1-2), pp.159–164.
Available at: http://link.springer.com/article/10.1023/A:1006902603056
[Accessed March 8, 2015].

Guba, E. & Lincon, Y., 2000. Competing Paradigms in Qualitative Research.pdf. In
Major Paradigms and Perspectives. pp. 105–117.

Guilbert, J.J., 2003. The world health report 2002 - reducing risks, promoting healthy
life. *Education for health (Abingdon, England)*, 16(2), p.230. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/14741909 [Accessed January 26, 2014].

Guillemin, M. & Gillam, L., 2004. Ethics, reflexivity, and "ethically important
moments" in research. *Qualitative inquiry*. Available at:
http://qix.sagepub.com/content/10/2/261.short [Accessed May 9, 2013].

Gülçin, I. & Gulcin, I., 2006. Antioxidant activity of caffeic acid (3 , 4-
dihydroxycinnamic acid). *Toxicology*, 217(2-3), pp.213–220. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/16243424 [Accessed August 9, 2014].

Gupton, A., 2001. Complicated and uncomplicated pregnancies: women's perception
of risk. *Journal of Obstetric, Gynaecologic & Neonatal Nursing*. Available at:
http://onlinelibrary.wiley.com/doi/10.1111/j.1552-6909.2001.tb01535.x/full
[Accessed October 28, 2013].

Gyun, H. et al., 2006. The coffee diterpene kahweol inhibits tumor necrosis factor-α-
induced expression of cell adhesion molecules in human endothelial cells.
Toxicology and Applied Pharmacology, 217, pp.332 – 341.

Hakkola, J. et al., 1998. Xenobiotic-metabolizing cytochrome P450 enzymes in the
human feto-placental unit: role in intrauterine toxicity. *Critical reviews in*

toxicology, 28(1), pp.35–72. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/9493761 [Accessed June 30, 2015].

Halldórsdóttir, S. & Karlsdóttir, S., 1996. Empowerment or discouragement:
women's experience of caring and uncaring encounters during childbirth.
Health care for women international. Available at:
http://www.tandfonline.com/doi/abs/10.1080/07399339609516251 [Accessed
November 12, 2013].

Halpern, W. & Kelley, M., 1991. In vitro methodology for resistance arteries.
Journal of Vascular Research. Available at:
http://www.karger.com/Article/Abstract/158869 [Accessed November 5, 2014].

Hamilton, W., Boyd, J. & Mossman, H., 1945. Human embryology (prenatal
development of form and function). *Human embryology*. Available at:
http://www.cabdirect.org/abstracts/19461402460.html [Accessed March 7,
2015].

Hammer, E. & Cipolla, M., 2005. Arterial wall hyperplasia is increased in placental
compared with myoendometrial radial uterine arteries from late-pregnant rats.
American Journal of Obstetrics and Gynaecology. Available at:
http://www.sciencedirect.com/science/article/pii/S0002937804007173
[Accessed November 5, 2014].

Hammersley, M., 2008. *Questioning qualitative inquiry: Critical essays*, Available
at:
http://books.google.co.uk/books?hl=en&lr=&id=PvfFJkfAQHUC&oi=fnd&pg=
PP6&dq=hammersley+2008&ots=2gY4jiIc4c&sig=JhVyQbxwuKmiYFEJo2Y
_I8qzK04 [Accessed November 29, 2012].

Hampl, V. & Jakoubek, V., 2009. Regulation of fetoplacental vascular bed by
hypoxia. *Physiological Research*, 58, pp.87–94.

Hanson, B., 2008. Wither Qualitative/Quantitative?: Grounds for Methodological
Convergence. *Quality & Quantity*, 42(1), pp.97–111. Available at:
http://www.springerlink.com/index/10.1007/s11135-006-9041-7 [Accessed
November 29, 2012].

Hardey, M., 2001. "E-health": the internet and the transformation of patients into
consumers and producers of health knowledge. *Information, Communication &
Society*, 4(3), pp.388–405. Available at:
http://www.tandfonline.com/doi/abs/10.1080/713768551 [Accessed April 18,
2014].

Hardy, M. & Bryman, A., 2004. *Handbook of Data Analysis*,

Hart, A., Henwood, F. & Wyatt, S., 2004. The role of the Internet in patient-
practitioner relationships: findings from a qualitative research study. *Journal of
medical Internet research*, 6(3), p.e36. Available at:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1550614&tool=pmc entrez&rendertype=abstract [Accessed October 30, 2013].

Hart, C., 1998. *Doing a literature review: Releasing the social science research imagination*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=tc8LS6qa_KIC&oi=fnd&pg=P R6&dq=Hart,+C.+(1998).+Doing+a+Literature+Review:+Releasing+the+Socia l+Science+Research+Imagination,&ots=1y- fg11E66&sig=uIMhzmDJ5QufInK13Yn_FZc1kcU [Accessed January 10, 2014].

Hatem, M. et al., 2009. Midwife-led versus other models of care for childbearing women (Review). , (4).

Hatzoglou, A. & Margioris, A., 1996. Identification, characterization and localization of corticotropin-releasing hormone receptors in human placenta. *Life sciences*. Available at: http://www.sciencedirect.com/science/article/pii/S0024320596005346 [Accessed March 10, 2015].

Hawker, S. et al., 2002. Appraising the Evidence: Reviewing Disparate Data Systematically. *Qualitative Health Research*, 12(9), pp.1284–1299. Available at: http://qhr.sagepub.com/cgi/doi/10.1177/1049732302238251 [Accessed October 5, 2012].

Hedberg, A., Mento, P. & Liu, E., 1989. Evidence for functional thromboxane A2-prostaglandin H2 receptors in human placenta. *American Journal of Physiology*. Available at: http://ajpendo.physiology.org/content/ajpendo/256/2/E256.full.pdf [Accessed November 12, 2014].

Heidemann, B. & McClure, J.H., 2003. Changes in Maternal Physiology. *Anaesthesia*, 3(Figue 1), pp.21–24.

Heifetz, S., 1996. The umbilical cord: obstetrically important lesions. *Clinical obstetrics and gynecology*. Available at: http://journals.lww.com/clinicalobgyn/Abstract/1996/09000/The_Umbilical_Co rd__Obstetrically_Important.7.aspx [Accessed March 7, 2015].

Heijden, O. van der, 2005. Uterine artery remodeling in pseudopregnancy is comparable to that in early pregnancy. *Biology of reproduction*. Available at: http://www.biolreprod.org/content/73/6/1289.short [Accessed November 5, 2014].

Hemingway, P., 2009. What is a systematic review ? *Evidence-Based Medicine*, (April), pp.1–8.

Hempstock, J., Bao, Y. & Bar-Issac, M., 2003. Intralobular differences in antioxidant enzyme expression and activity reflect the pattern of maternal arterial bloodflow within the human placenta. *Placenta*. Available at:

http://www.sciencedirect.com/science/article/pii/S0143400402909550 [Accessed January 14, 2015].

Henwood, F. et al., 2003. "Ignorance is bliss sometimes": constraints on the emergence of the "informed patient" in the changing landscapes of health information. *Sociology of health & illness*, 25(6), pp.589–607. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12919447.

Higdon, J. V & Frei, B., 2006. Coffee and health: a review of recent human research. *Critical reviews in food science and nutrition*, 46(2), pp.101–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16507475 [Accessed June 14, 2011].

Higgins, A. et al., 1989. Impact of the Higgins Nutrition Intervention Program on birth weight: a within-mother analysis. *Journal of the American Dietetic Association*. Available at: http://europepmc.org/abstract/med/2760370 [Accessed March 7, 2015].

Higgins, A., 1976. Nutritional status and the outcome of pregnancy. *J Can Diet Assoc*. Available at: http://www.ddm-mdd.org/files/pdf/eng/1976 Higgins abstract.pdf [Accessed March 7, 2015].

Higgins, J.P.T. et al., 2003. Measuring inconsistency in meta-analyses. *BMJ: British Medical Journal*, 327(7414), pp.557–60. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=192859&tool=pmcentrez&rendertype=abstract.

Higgins, J.P.T. & Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. *Statistics in medicine*, 21(11), pp.1539–58. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12111919 [Accessed January 23, 2014].

Hildingsson, I. et al., 2002. Women ' s expectations on antenatal care as assessed in early pregnancy : number of visits , continuity of caregiver and general content. *Acta obstetricia et gynecologica Scandinavica*, (6), pp.118–125.

Hofberg, K. & Ward, M.R., 2003. Fear of pregnancy and childbirth. *Postgraduate medical journal*, 79(935), pp.505–10, quiz 508–10. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1742837&tool=pmcentrez&rendertype=abstract.

Hoffman, S. & Hatch, M.C., 1996. Stress, social support and pregnancy outcome: a reassessment based on recent research. *Paediatric and perinatal epidemiology*, 10(4), pp.380–405. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8931053.

Hofman, M.K. et al., 2004. Nutrient-Gene Interactions CYP7A1 A-278C Polymorphism Affects the Response of Plasma Lipids after Dietary Cholesterol or Cafestol Interventions in Humans 1 , 2. *Atherosclerosis*, (April), pp.2200–2204.

Holland, D.T. et al., 1998. Simple high-performance liquid chromatography method for the simultaneous determination of serum caffeine and paraxanthine following rapid sample preparation. *Journal of Chromatography*, 707(1-2), pp.105–110. Available at: <Go to ISI>://000073238700014.

Holloway, I., 2001. Revisiting Qualitative Inquiry- Interviewing in nursing and midwifery research.

Hollyer, T. et al., 2002. The use of CAM by women suffering from nausea and vomiting during pregnancy. *BMC complementary and alternative medicine*, 2, p.5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=113747&tool=pmce ntrez&rendertype=abstract.

Holmes, C., Landry, D. & Granton, J., 2004. Science Review: Vasopressin and the cardiovascular system part 2-clinical physiology. *Critical Care*. Available at: http://www.biomedcentral.com/content/pdf/cc2338.pdf [Accessed November 15, 2014].

Holmes, C. & Patel, B., 2001. Physiology of vasopressin relevant to management of septic shock. *CHEST*. Available at: http://journal.publications.chestnet.org/article.aspx?articleid=1079959 [Accessed November 15, 2014].

Holtzman, S. & Finn, I., 1988. Tolerance to behavioral effects of caffeine in rats. *Pharmacology Biochemistry and Behavior*. Available at: http://www.sciencedirect.com/science/article/pii/0091305788901797 [Accessed August 10, 2015].

Hope, T., Savulescu, J. & Hendrick, J., 2003. *Medical Ethics and Law: The Core Curriculum*, Available at: https://www.eu.elsevierhealth.com/product.jsp?isbn=9780443103377 [Accessed September 2, 2012].

Hornig, B., Kohler, C. & Drexler, H., 1997. Role of Bradykinin in Mediating Vascular Effects of Angiotensin-Converting Enzyme Inhibitors in Humans. *Circulation*, 95(5), pp.1115–1118. Available at: http://circ.ahajournals.org/content/95/5/1115.full [Accessed June 29, 2015].

Hoshmand, L., 2003. Can lessons of history and logical analysis ensure progress in psychological science? *Theory & Psychology*. Available at: http://tap.sagepub.com/content/13/1/39.short [Accessed November 11, 2012].

Howarth, A.M., Swain, N.R. & Treharne, G.J., 2012. First-time mothers' perspectives on relationships with and between midwives and doctors: insights from a qualitative study of giving birth in New Zealand. *Midwifery*, 28(4), pp.489–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21903307 [Accessed October 30, 2013].

Huang, X., Lin, J. & Demner-Fushman, D., 2006. Evaluation of PICO as a knowledge representation for clinical questions. *AMIA ... Annual Symposium proceedings / AMIA Symposium. AMIA Symposium*, pp.359–63. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1839740&tool=pmcentrez&rendertype=abstract [Accessed June 2, 2015].

Huber, W.W. et al., 2008. Effects of coffee and its chemopreventive components kahweol and cafestol on cytochrome P450 and sulfotransferase in rat liver. *Food and Chemical Toxicology*, 46(4), pp.1230–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17983700 [Accessed November 22, 2011].

Huber, W.W. et al., 2004. Potential chemoprotective effects of the coffee components kahweol and cafestol palmitates via modification of hepatic N-acetyltransferase and glutathione S-transferase activities. *Environmental and molecular mutagenesis*, 44(4), pp.265–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15468054 [Accessed November 22, 2011].

Huber, W.W. et al., 2002. The coffee components kahweol and cafestol induce?-glutamylcysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. *Archives of toxicology*, 75(11), pp.685–694. Available at: http://www.springerlink.com/index/HKLH4N8YGWPAYVED.pdf [Accessed December 15, 2011].

Hughes, K. et al., 2008. Alcohol, nightlife and violence: the relative contributions of drinking before and during nights out to negative health and criminal justice outcomes. *Addiction*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1360-0443.2007.02030.x/full [Accessed November 5, 2014].

Hughes, R.N. & Beveridge, I.J., 1991. Behavioral effects of exposure to caffeine during gestation, lactation or both. *Neurotoxicology and teratology*, 13(6), pp.641–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1779952.

Huie, R. & Padmaja, S., 1993. The reaction of NO with superoxide. *Free radical research*. Available at: http://informahealthcare.com/doi/abs/10.3109/10715769309145868 [Accessed January 14, 2015].

Hundley, V., Cruickshank, F. & Lang, G., 1994. Midwife managed delivery unit: a randomised controlled comparison with consultant led care. *BMJ: British Medical Journal*. Available at: http://www.bmj.com/content/309/6966/1400.abstract [Accessed August 26, 2014].

Hunt, D.-J. et al., 2002. Effects Nutrition Education programs on anthropometric measurements and pregnancy outcomes of adolescents. *Research and Professional Briefs*.

Hunter, B. et al., 2008. Relationships: The hidden threads in the tapestry of maternity care. *Midwifery*, 24(2), pp.132–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18378051 [Accessed November 12, 2013].

Hunter, B., 2006. The importance of reciprocity in relationships between community-based midwives and mothers. *Midwifery*, 22(4), pp.308–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16616398 [Accessed November 12, 2013].

Hurley, K.M. et al., 2005. Psychosocial influences in dietary patterns during pregnancy. *Journal of the American Dietetic Association*, 105(6), pp.963–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15942549 [Accessed September 28, 2014].

Hustin, J. & Schaaps, J., 1987. Echocardiograhic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy. *American Journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/S000293788780371X [Accessed November 5, 2014].

Hutchinson, F., 1957. The distance that a radical formed by ionizing radiation can diffuse in a yeast cell. *Radiation Research*. Available at: http://www.rrjournal.org/doi/abs/10.2307/3570395 [Accessed January 14, 2015].

Hwa, J. et al., 2009. The coffee diterpene kahweol induces apoptosis in human leukemia U937 cells through down-regulation of Akt phosphorylation and activation of JNK. *Apoptosis*, pp.1378–1386.

Hwan, S. et al., 2010. Cafestol, a coffee-specific diterpene, is a novel extracellular signal-regulated kinase inhibitor with AP-1-targeted inhibition of prostaglandin E2 production in lipopolysaccharide-activated macrophages. *Biological & pharmaceutical bulletin*, 33(1), pp.128–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20045950.

IFIC, 2007. *Caffeine and Health: Clarifying the controversies*,

Iglesias, I. et al., 2006. Chronic intake of caffeine during gestation down regulates metabotropic glutamate receptors in maternal and fetal rat heart. *Amino acids*. Available at: http://link.springer.com/article/10.1007/s00726-005-0293-7 [Accessed September 17, 2013].

Imai, M., 1980. Effects of vasopressin on water and NaCl transport across the in vitro perfused medullary thick ascending limb of Henle's loop of mouse, rat, and rabbit kidneys. *Pflügers Archiv*. Available at: http://link.springer.com/article/10.1007/BF00587521 [Accessed November 15, 2014].

Impicciatore, P. et al., 1997. Reliability of health information for the public on the world wide web: systematic survey of advice on managing fever in children at home. *British Medical Journal*, 314(June).

Inagami, T., 1999. Molecular biology and signaling of angiotensin receptors: an overview. *Journal of the American Society of Nephrology: JASN*. Available at: http://europepmc.org/abstract/med/9892133 [Accessed November 16, 2014].

Infante-Rivard, C., Fernández, A. & Gauthier, R., 1993. Fetal loss associated with caffeine intake before and during pregnancy. *Journal of American Medical Association*. Available at: http://jama.jamanetwork.com/article.aspx?articleid=409969 [Accessed January 3, 2015].

Jacelon, C.S. & O'Dell, K.K., 2005. *Analyzing qualitative data.*, Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2474845&tool=pmc entrez&rendertype=abstract.

Jackson, W.F., 2005. Potassium Channels in the Peripheral Microcirculation. *Microcirculation*, 12(1), pp.113–127.

Jacques, P. et al., 1999. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *New England Journal of Medicine*. Available at: http://www.nejm.org/doi/full/10.1056/NEJM199905133401901 [Accessed July 2, 2012].

Jagger, J.. et al., 1998. Ca2+ channels, ryanodine receptors and Ca2+-activated K+ channels: a functional unit for regulating arterial tone. *Acta physiologica Scandinavica*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1365-201X.1998.00462.x/abstract [Accessed November 15, 2014].

Jahanfar, S. & Jaafar, S., 2013. Effects of restricted caffeine intake by mother on fetal , neonatal and pregnancy outcome (Review). *The Cochrane Collaboration*, (2).

James, J.E., 2004. Critical review of dietary caffeine and blood pressure: A relationship that should be taken more seriously. *Psychosomatic Medicine*, 66(1), pp.63–71. Available at: http://www.psychosomaticmedicine.org/cgi/doi/10.1097/10.PSY.0000107884.7 8247.F9 [Accessed November 6, 2011].

Janatpour, M.J. et al., 2000. Id-2 regulates critical aspects of human cytotrophoblast differentiation, invasion and migration. *Development*. Available at: http://dev.biologists.org/content/127/3/549.short [Accessed November 4, 2014].

Jarvis, W.T., 1983. Food faddism, cultism, and quackery. *Annual review of nutrition*, 3, pp.35–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6315036.

Jauniaux, E., Watson, A. & Hempstock, J., 2000. Onset of maternal arterial blood flow and placental oxidative stress: a possible factor in human early pregnancy

failure. *The American Journal Of Pathology*. Available at: http://www.sciencedirect.com/science/article/pii/S0002944010648493 [Accessed November 5, 2014].

Jensen, T. et al., 1998. Caffeine Intake and Fecundability: A Follow-Up Study Among 430 Danish Couples Planning Their First Pregnancy. *Reproductive Toxicology*, 12(3), pp.289–295.

Jepson, R.G. et al., 2010. The effectiveness of interventions to change six health behaviours: a review of reviews. *BMC public health*, 10(2), p.538. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18339056.

Jewell, D. & Young, G., 2009. Interventions for nausea and vomiting in early pregnancy (Review). , (4).

Johnson, A., 1999. Do parents value and use written health information. *Neonatal, Paediatric and Child Health Nursing*. Available at: http://scholar.google.co.uk/scholar?q=Johnson,+A.,+1999.+Do+parents+value+and+use+written+health+information?&btnG=&hl=en&as_sdt=0,5#2 [Accessed April 28, 2014].

Johnson, A. & Sandford, J., 2005. Written and verbal information versus verbal information only for patients being discharged from acute hospital settings to home: systematic review. *Health education research*, 20(4), pp.423–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15572437.

Johnson, B. & Turner, L., 2003. Data collection strategies in mixed methods research. *Handbook of mixed methods in social …*. Available at: http://books.google.co.uk/books?hl=en&lr=&id=F8BFOM8DCKoC&oi=fnd&pg=PA297&dq=questionnaire+qualitative+data+collection&ots=gTeLBCstLb&sig=m2r9ZPiPyuGhCdz-xMClF6KgTHs [Accessed June 28, 2012].

Johnson, J. et al., 2003. The prevalence of domestic violence in pregnant women. *Birtish Journal of Gynaecology*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1471-0528.2003.02216.x/full [Accessed November 5, 2014].

Johnson, R.B.B. & Onwuegbuzie, A.J.J., 2004. Mixed Methods Research: A Research Paradigm Whose Time Has Come. *Educational Researcher*, 33(7), pp.14–26. Available at: http://edr.sagepub.com/cgi/doi/10.3102/0013189X033007014 [Accessed November 23, 2011].

Jones, S.C., Crookes, P. a & Johnson, K.M., 2011. Teaching critical appraisal skills for nursing research. *Nurse education in practice*, 11(5), pp.327–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21474381 [Accessed February 5, 2014].

De Jong, C.L.E. et al., 1999. Fetal growth rate and adverse perinatal events. *Ultrasound in Obstetrics and Gynaecology*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1469-0705.1999.13020086.x/abstract [Accessed September 27, 2014].

Jootun, D., McGhee, G. & Marland, G.R., 2009. Reflexivity: promoting rigour in qualitative research. *Nursing standard*, 23(23), pp.42–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19263905.

Juliano, L. & Griffiths, R., 2004. A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. *Psychopharmacology*. Available at: http://link.springer.com/article/10.1007/s00213-004-2000-x [Accessed October 28, 2013].

Jumonville, J., Smith, E. & Pope, M., 2013. Breast Milk Ethanol Screening System. *Upspring Ltd.* Available at: http://www.freepatentsonline.com/y2013/0040289.html [Accessed November 5, 2013].

Jung, H. et al., 2010. Chemico-Biological Interactions The coffee diterpene kahweol sensitizes TRAIL-induced apoptosis in renal carcinoma Caki cells through down-regulation of Bcl-2 and c-FLIP. *Chemico-Biological Interactions*, 186(1), pp.36–42. Available at: http://dx.doi.org/10.1016/j.cbi.2010.04.013.

Källén, B., Lundberg, G. & Aberg, A., 2003. Relationship between vitamin use, smoking, and nausea and vomiting of pregnancy. *Acta obstetricia et gynecologica Scandinavica*, 82(10), pp.916–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12956841.

Kam, C.D., Wilking, J.R. & Zechmeister, E.J., 2007. Beyond the "Narrow Data Base": Another Convenience Sample for Experimental Research. *Political Behavior*, 29(4), pp.415–440. Available at: http://link.springer.com/10.1007/s11109-007-9037-6 [Accessed July 10, 2015].

Karthikesan, K., Pari, L. & Menon, V.P., 2010. Chemico-Biological Interactions Antihyperlipidemic effect of chlorogenic acid and tetrahydrocurcumin in rats subjected to diabetogenic agents. *Chemico-Biological Interactions*, 188(3), pp.643–650. Available at: http://dx.doi.org/10.1016/j.cbi.2010.07.026.

Karypidis, A.-H. et al., 2006. Association of cytochrome P450 1B1 polymorphism with first-trimester miscarriage. *Fertility and sterility*, 86(5), pp.1498–503. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16978616 [Accessed October 2, 2012].

Kasai, H., Fukada, S. & Yamaizumi, Z., 2000. Action of chlorogenic acid in vegetables and fruits as an inhibitor of 8-hydroxydeoxyguanosine formation< i> in vitro</i> and in a rat carcinogenesis model. *Food and Chemical Toxicology*. Available at:

http://www.sciencedirect.com/science/article/pii/S0278691500000144 [Accessed July 29, 2013].

Kashiwada, Y. & Nishizawa, M., 1995. Anti-AIDS agents, 18. Sodium and potassium salts of caffeic acid tetramers from Arnebia euchroma as anti-HIV agents. *Journal of natural …*. Available at: http://pubs.acs.org/doi/abs/10.1021/np50117a007 [Accessed November 9, 2014].

Keidel, A. et al., 2010. Discrimination of Green Arabica and Robusta Coffee Beans by Raman Spectroscopy. *Journal of Agricultural and Food Chemistry*, pp.11187–11192.

Kelle, U., 2008. Combining qualitative and quantitative methods in research practice : purposes and advantages. *Qualitative Research in Psychology*, 3(November 2012), pp.293–311.

Kelley, K., Bond, R. & Abraham, C., 2001. Effective approaches to persuading pregnant women to quit smoking: a meta-analysis of intervention evaluation studies. *British journal of health psychology*, 6(Pt 3), pp.207–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14596723.

Kendler, K. & Prescott, C., 1999. Caffeine intake, tolerance, and withdrawal in women: a population-based twin study. *American Journal of Psychiatry*. Available at: http://ajp.psychiatryonline.org/article.aspx?articleid=173266&RelatedWidgetAr ticles=true [Accessed October 28, 2013].

Kennedy, H.P. & Lowe, N.K., 2001. Science and Midwifery: Paradigms and Paradoxes. *Journal of Midwifery and Womens Health*, 46(2), pp.91–97.

Kenny, L. & Baker, P.N., 2002. The role of gap junctions in mediating endothelium-dependent responses to bradykinin in myometrial small arteries isolated from pregnant women. *British Journal of Pharmacology*. Available at: http://onlinelibrary.wiley.com/doi/10.1038/sj.bjp.0704817/full [Accessed November 11, 2014].

Kenny, L., Baker, P.N. & Kendall, D., 2002. Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and pre-eclampsia. *Clinical Science*. Available at: http://www.clinsci.org/cs/103/cs1030067.htm [Accessed November 11, 2014].

Khan, A. et al., 1998. Effects of 3-mercaptopicolinic acid and a derivative of chlorogenic acid (S-3483) on hepatic and islet glucose-6-phosphatase activity. *European Journal of Pharmacology*, 349(2-3), pp.325–331. Available at: http://dx.doi.org/10.1016/S0014-2999(98)00188-5 [Accessed May 15, 2012].

Khong, T.Y., 2004. Placental vascular development and neonatal outcome. *Seminars in neonatology : SN*, 9(4), pp.255–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15251142 [Accessed June 28, 2013].

Khoury, J.C. et al., 2004. Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, 15(1), pp.44–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15101611 [Accessed October 2, 2012].

Khun, T., 1962. Paradigm shift. Available at: http://scholar.google.co.uk/scholar?hl=en&q=khun+1962&btnG=&as_sdt=1,5&as_sdtp=#0 [Accessed November 29, 2012].

Kilby, M. et al., 1996. Localisation of hepatocyte growth factor and its receptor (c-met) protein and mRNA in human term placenta. *Growth Factors*. Available at: http://informahealthcare.com/doi/abs/10.3109/08977199609034573 [Accessed October 21, 2014].

Kim, H.G., Hwang, Y.P. & Jeong, H.G., 2009. Kahweol blocks STAT3 phosphorylation and induces apoptosis in human lung adenocarcinoma A549 cells. *Cell Biology*, 187, pp.28–34.

Kim, J.Y., Jung, K.S. & Jeong, H.G., 2004. Suppressive effects of the kahweol and cafestol on cyclooxygenase-2 expression in macrophages. *Febs Letters*, 569(1-3), pp.321–326. Available at: <Go to ISI>://000222548500057.

Kingdom, J. et al., 2000. Development of the placental villous tree and its consequences for fetal growth. *European Journal of Obstetrics and Gynaecology*, 92(1), pp.35–43. Available at: http://www.sciencedirect.com/science/article/pii/S0301211500004231 [Accessed March 7, 2015].

Kirkham, M., 2000. The midwife-mother relationship. Available at: http://scholar.google.co.uk/scholar?q=2000+The+Midwife–Mother+Relationship&btnG=&hl=en&as_sdt=0,5#0 [Accessed November 12, 2013].

Kirkinen, P., Jouppila, P. & Koivula, A., 1983. The effect of caffeine on placental and fetal blood flow in human pregnancy. *American journal of Obstetrics and Gynaecology*. Available at: http://europepmc.org/abstract/MED/6650631 [Accessed August 6, 2013].

Kjær, A., 1993. Vasopressin as a neuroendocrine regulator of anterior pituitary hormone secretion. *Acta endocrinologica*. Available at: http://www.eje-online.org/content/129/6/489.short [Accessed November 15, 2014].

Klebanoff, M. et al., 1998. Serum caffeine and paraxanthine as markers for reported caffeine intake in pregnancy. *Annals of epidemiology*, 8(2), pp.107–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9491935.

Klebanoff, M.A. et al., 1999. Maternal Serum Paraxanthine, a caffeine metabolite and the risk of spontaneous abortion. *The New England Journal Of Medicine*, 341(22), pp.1639–1644. Available at: <Go to ISI>://000083847500002.

Kliman, H., 2000. Uteroplacental blood flow: the story of decidualization, menstruation, and trophoblast invasion. *The American journal of pathology*. Available at: http://scholar.google.com/scholar?q=kliman+2000+oxygen+tension&btnG=&hl=en&as_sdt=0%2C5#0 [Accessed January 14, 2015].

Kline, J. et al., 1991. Caffeine and Spontaneous Abortion of Known Karyotype. *Epidemiology*, 2(6), pp.409–417.

Klonoff-Cohen, H., Bleha, J. & Lam-Kruglick, P., 2002. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-Fallopian transfer. *Human Reproduction*, 17(7), pp.1746–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12093834.

Knutti, R., Rothweiler, H. & Schlatter, C., 1981. Effect of Pregnancy on the Pharmacokinetics of Caffeine. , 1, pp.1–6.

Koblinsky, M., Filippi, V., et al., 2006. Maternal health in poor countries: the broader context and a call for action. *Lancet*. Available at: http://www.sciencedirect.com/science/article/pii/S0140673606693847 [Accessed August 26, 2014].

Koblinsky, M., Matthews, Z. & Hussein, J., 2006. Going to scale with professional skilled care. *Lancet*. Available at: http://www.sciencedirect.com/science/article/pii/S0140673606693823 [Accessed August 26, 2014].

Kohnen, G. & Kertschanska, S., 1996. Placental villous stroma as a model system for myofibroblast differentiation. *Histochemistry and cell biology*. Available at: http://link.springer.com/article/10.1007/BF01457655 [Accessed November 4, 2014].

Kolasa, K.M., 2009. Handbook of Nutrition and Pregnancy. *Journal of Nutrition Education and Behavior*, 41(4), p.305.e3. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1499404608008737 [Accessed October 30, 2013].

Kommunehospital, A. & Western, T., 1986. Oxytocin on various smooth muscle tissues within the human uteroplacental unit. *Acta Physiologica Scandinavica*, (Lundgren 1957), pp.23–31.

Konje, J., 2008. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. *British Medical Journal*, 337(nov03 2), pp.a2332–a2332. Available at: http://www.bmj.com/cgi/doi/10.1136/bmj.a2332 [Accessed July 24, 2012].

Kono, Y. et al., 1997. Antioxidant activity of polyphenolics in diets. Rate constants of reactions of chlorogenic acid and caffeic acid with reactive species of oxygen and nitrogen. *Biochimica et biophysica acta*, 1335(3), pp.335–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9202196.

Koren, G., 2000. Caffeine during pregnancy? In moderation. , 46, pp.801–803.

Koshihara, Y., 1984. Caffeic acid is a selective inhibitor for leukotriene biosynthesis. , 792, pp.92–97.

Kramer, M.S., 2003. Overview The Epidemiology of Adverse Pregnancy Outcomes : An Overview 1. , pp.1592–1596.

Krebs, C. et al., 1996. Intrauterine growth restriction with absent end-diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree. *American Journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/S0002937896701035 [Accessed October 21, 2014].

Krenz, M., Oldenburg, O. & Wimpee, H., 2002. Opening of ATP-sensitive potassium channels causes generation of free radicals in vascular smooth muscle cells. *Basic research in …*. Available at: http://link.springer.com/article/10.1007/s003950200045 [Accessed March 8, 2015].

Kroke, A. et al., 2004. History of the concept of "levels of evidence" and their current status in relation to primary prevention through lifestyle interventions. *Public health nutrition*, 7(2), pp.279–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15003135 [Accessed May 16, 2012].

Kroon, P. & Williamson, G., 1999. Hydroxycinnamates in plants and food: current and future perspectives. *Journal of the Science of Food and Agriculture*. Available at: http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0010(19990301)79:3%3C355::AID-JSFA255%3E3.0.CO;2-G/abstract [Accessed November 9, 2014].

Krsnjavi, H. & Mimica, M., 1987. Coffee and alcohol consumption and smoking during pregnancy. *Arh Hig Rada Toksikol*, 38(2), pp.141–147. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3442498.

Kublickiene, K.R. et al., 1997. Role of nitric oxide in the regulation of vascular tone in pressurized and perfused resistance myometrial arteries from term pregnant women. *American Journal of Obstetrics and Gynaecology*, 177(5), pp.1263–1269. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0002937897700486.

Kuczkowski, K.M., 2009. Caffeine in pregnancy. *Archives of gynecology and obstetrics*, 280(5), pp.695–8. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/19238414 [Accessed December 13, 2011].

Kuhn, T., 2012. *The structure of scientific revolutions*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=3eP5Y_OOuzwC&oi=fnd&pg=PR5&dq=Kuhn+T.S.+(1970)+The+Structure+of+Scientific+Revolutions.+University+of&ots=xT4SDcoJuM&sig=L_bUeXxkdpFpP3zff_UHCLIbasc [Accessed May 8, 2014].

Kunst, H. et al., 2002. Accuracy of information on apparently credible websites : survey of five common health topics Follow up of quality of public oriented health information on the world wide web : systematic re-evaluation. *British Medical Journal*, pp.581–582.

Kurman, R. et al., 1984. Immunocytochemical localization of placental lactogen and chorionic gonadotropin in the normal placenta and trophoblastic tumors, with emphasis on intermediate. *International Journal of Gynaecology Pathology*. Available at: http://journals.lww.com/intjgynpathology/Abstract/1984/03010/Immunocytochemical_Localization_of_Placental.9.aspx [Accessed October 28, 2014].

Kwek, K. et al., 2001. Vasoactive effects of 8-epi-prostaglandin F(2alpha)in isolated human placental conduit and resistance blood vessels in vitro. *Placenta*, 22(6), pp.526–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11440540.

Kweon, M., Hwang, H. & Sung, H., 2001. Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (Phyllostachys edulis). *Journal of Agricultural and Food Chemistry*. Available at: http://pubs.acs.org/doi/abs/10.1021/jf010514x [Accessed July 29, 2013].

Kwok, J.C.K. et al., 2014. Human placenta as an ex vivo vascular model for neurointerventional research. *Journal of neurointerventional surgery*, 6(5), pp.394–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4033111&tool=pmcentrez&rendertype=abstract [Accessed March 7, 2015].

Lacey, A. & Luff, D., 2009. Qualitative Data Analysis. *National Institute for Health Research*.

Lacro, R., Jones, K. & Benirschke, K., 1987. The umbilical cord twist: Origin direction, and relevance. *American Journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/S0002937887800674 [Accessed March 5, 2015].

Lacroix, R., Eason, E. & Melzack, R., 2000. Nausea and vomiting during pregnancy: A prospective study of its frequency, intensity, and patterns of change. *American Journal of Obstetrics and Gynaecology*, pp.931–937. Available at: http://www.sciencedirect.com/science/article/pii/S0002937800703498 [Accessed May 7, 2014].

Lafay, S. et al., 2006. Chlorogenic acid is absorbed in its intact form in the stomach of rats. *The Journal of Nutrition*. Available at: http://jn.nutrition.org/content/136/5/1192.short [Accessed September 11, 2014].

Lagan, B.M., Sinclair, M. & Kernohan, W.G., 2011a. A Web-based survey of midwives' perceptions of women using the Internet in pregnancy: a global phenomenon. *Midwifery*, 27(2), pp.273–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19700228 [Accessed October 30, 2013].

Lagan, B.M., Sinclair, M. & Kernohan, W.G., 2010. Internet Use in Pregnancy Informs Women ' s Decision Making : A Web-Based Survey. *Birth*, 37(June), pp.106–115.

Lagan, B.M., Sinclair, M. & Kernohan, W.G., 2011b. What is the impact of the Internet on decision-making in pregnancy? A global study. *Birth*, 38(4), pp.336–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22112334.

Lankford, A.R. et al., 2006. Effect of modulating cardiac A1 adenosine receptor expression on protection with ischemic preconditioning. *American Journal of Physiology-Heart and Circulatory Physiology*, 290(4), pp.H1469–73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16299262 [Accessed January 11, 2012].

Larsson, M., 2007. A descriptive study of the use of the Internet by women seeking pregnancy-related information. *Midwifery*, 25(1), pp.14–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17408822 [Accessed October 30, 2013].

Laudan, L., 1970. From theories to research traditions. In *From Theories to Research Traditions*.

Lavender, T. et al., 2001. Midwives' views on redefining midwifery. *Professional Issues*, 9(11), pp.666–670.

Lavender, T., Edwards, G. & Alfirevic, Z., 2004. *Demystifying Qualitative Research in Pregnancy and Childbirth*,

Lederman, R., 1990. Anxiety and stress in pregnancy: significance and nursing assessment. *NAACOGS*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2206746 [Accessed October 28, 2013].

Lee, K.J., Choi, J.H. & Jeong, H.G., 2007. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. *Food and Chemical Toxicology*, 45(11), pp.2118–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17590492 [Accessed November 22, 2011].

Lepping, P., 2007. Philosophy, Ethics, and Humanities in Medicine. *Philosophy, Ethics, and Humanities in Medicine*. Available at: http://www.biomedcentral.com/content/pdf/1747-5341-2-5.pdf [Accessed July 3, 2012].

Leviton, A. & Cowan, L., 2002. A review of the literature relating caffeine consumption by women to their risk of reproductive hazards. *Food and Chemical Toxicology*, 40(9), pp.1271–310. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12204391.

Li, P., Dietz, R. & Harsdorf, R. von, 1997. Differential effect of hydrogen peroxide and superoxide anion on apoptosis and proliferation of vascular smooth muscle cells. *Circulation*. Available at: http://circ.ahajournals.org/content/96/10/3602.short [Accessed January 14, 2015].

Librach, C., Werb, Z. & Fitzgerald, M., 1991. 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. *Journal of Cell Biology*. Available at: http://jcb.rupress.org/content/113/2/437.abstract [Accessed October 28, 2014].

Liguori, A., Hughes, J.R. & Grass, J. a, 1997. Absorption and subjective effects of caffeine from coffee, cola and capsules. *Pharmacology, biochemistry, and behavior*, 58(3), pp.721–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9329065.

Linn, S. et al., 1982. No association between coffee consumption and adverse outcomes of pregnancy. *New England Journal of Medicine*, 306(3), pp.141–145. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7054656.

Little, B.B., 1997. Pharmacokinetics During Pregnancy: Evidence Based Maternal Dose Formulation. *Obstetrics and gynecology*, 7844(98), pp.858–868.

Little, P. et al., 2001. Observational Study Of Effect Of Patient Centredness And Positive Approach On Outcomes Of General Practice Consultations All use subject to JSTOR Terms and Conditions Primary care Observational positive consultations study of effect of patient centredness. *British Medical Journal*.

Liu, Y., Fiskum, G. & Schubert, D., 2002. Generation of reactive oxygen species by the mitochondrial electron transport chain. *Journal of neurochemistry*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.0022-3042.2002.00744.x/full [Accessed January 14, 2015].

Loke, W.M. et al., 2010. Specific Dietary Polyphenols Attenuate Atherosclerosis in Apolipoprotein E – Knockout Mice by Alleviating Inflammation and Endothelial Dysfunction. *Pathology*, pp.749–757.

Long, V., Martin, T. & Janson-Sand, C., 2002. The Great Beginnings Program: Imapct of a nutrition curriculum on nutrition knowledge, diet quality, and birth outcomes in pregnant and parenting teens. *Research and Professional Briefs*.

Longo, L., 1983. Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. *American Journal of Physiology*.

Available at: http://ajpregu.physiology.org/content/245/5/R720.short [Accessed May 4, 2014].

Lu, C. & Shulman, S., 2008. Rigor and flexibility in computer-based qualitative research: Introducing the Coding Analysis Toolkit. *International Journal of Multiple Research Approaches*. Available at: http://pubs.e-contentmanagement.com/doi/pdf/10.5172/mra.455.2.1.105 [Accessed October 9, 2012].

Lu, X.-Z. et al., 2015. Activation of M3 cholinoceptors attenuates vascular injury after ischaemia/reperfusion by inhibiting the Ca(2+) /calmodulin-dependent protein kinase II pathway. *British journal of pharmacology*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25953628 [Accessed June 29, 2015].

Lucas, A., Fewtrell, M. & Cole, T., 1999. Fetal origins of adult disease—the hypothesis revisited. *BMJ: British Medical Journal*. Available at: http://www.bmj.com/content/319/7204/245?rss=1&ssource=mfc [Accessed August 26, 2014].

Lucas, M. et al., 2011. Coffee, caffeine, and risk of depression among women. *Archives of internal medicine*, 171(17), pp.1571–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21949167.

De Lucia, M. et al., 2009. Differential reactivity of purified bioactive coffee furans, cafestol and kahweol, with acidic nitrite: product characterization and factors controlling nitrosation versus ring-opening pathways. *Chemical research in toxicology*, 22(12), pp.1922–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19894707.

Ludwig, I. a et al., 2014. Variations in caffeine and chlorogenic acid contents of coffees: what are we drinking? *Food & function*, 5(8), pp.1718–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25014672 [Accessed September 30, 2014].

Lundgren, I. & Berg, M., 2007. Central concepts in the midwife – woman relationship. *Scandinavian Journal of Caring Science*, 21(2), pp.220–228. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17559441.

Luyben, A.G. & Fleming, V.E.M., 2005. Women's needs from antenatal care in three European countries. *Midwifery*, 21(3), pp.212–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15967548 [Accessed April 24, 2014].

Lyall, F., 2005. Priming and remodelling of human placental bed spiral arteries during pregnancy - A Review. *Placenta*, 26(SUPPL.).

Lytle, L.A. et al., 2004. School-based approaches to affect adolescents' diets: results from the TEENS study. *Health Education and Behaviour*, 31(April 2004), pp.270–287.

MacCornack, F., 1977. The Effects of Coffee Drinking on the Cardiovsculr System. *Preventive Medicine*, 119(6), pp.104–119.

Mackenzie, N. & Knipe, S., 2006. Research dilemmas: Paradigms, methods and methodology. *Issues in educational research*. Available at: http://www.iier.org.au/iier16/mackenzie.html [Accessed November 11, 2012].

Macklin, K. & Maus, A., 1998. Human vascular endothelial cells express functional nicotinic acetylcholine receptors. *Journal of Pharmacology and Experimental Therapeutics*. Available at: http://jpet.aspetjournals.org/content/287/1/435.short [Accessed June 29, 2015].

Maconochie, N. et al., 2007. Risk factors for first trimester miscarriage-results from a UK-population-based case-control study. *Birtish Journal of Gynaecology*, 114(2), pp.170–186. Available at: http://doi.wiley.com/10.1111/j.1471-0528.2006.01193.x [Accessed September 25, 2012].

Maigaard, S., 1986. Relaxant and contractile effects of some amines and prostanoids in myometrial and vascular smooth muscle within the human uteroplacental unit. *Acta physiologica ...*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1748-1716.1986.tb07946.x/abstract [Accessed November 11, 2014].

Majer, B.J. et al., 2005. Coffee diterpenes prevent the genotoxic effects of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and N-nitrosodimethylamine in a human derived liver cell line (HepG2). *Food and Chemical Toxicology*, 43(3), pp.433–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15680679 [Accessed November 9, 2011].

Mak, K. & Gude, N., 1984. Effects of vasoactive autacoids on the human umbilical-fetal placental vasculature. *Birtish Journal of Gynaecology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1471-0528.1984.tb05890.x/abstract [Accessed November 11, 2014].

Manach, C. et al., 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American journal of clinical nutrition*, 81(1 Suppl), p.230S–242S. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15640486.

Mandala, M. & Osol, G., 2012. Physiological remodelling of the maternal uterine circulation during pregnancy. *Basic & clinical pharmacology & ...*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1742-7843.2011.00793.x/full [Accessed November 5, 2014].

Mangabeira, W., 2004. Computers and Qualitative Research Adoption, Use, and Representation. *Social science computer ...*. Available at: http://ssc.sagepub.com/content/22/2/167.short [Accessed October 9, 2012].

Many, A. & Westerhausen-Larson, A., 1996. Xanthine oxidase/dehydrogenase is present in human placenta. *Placenta*. Available at:

http://www.sciencedirect.com/science/article/pii/S0143400496900612 [Accessed January 14, 2015].

Margetts, B. et al., 2001. Developing an evidence-based approach to Public Health Nutrition: translating evidence into policy. *Public Health Nutrition*, 4(6a), pp.1393–1397. Available at: http://www.journals.cambridge.org/abstract_S1368980001000428 [Accessed April 16, 2012].

Maron, B.A. & Loscalzo, J., 2009. The treatment of hyperhomocysteinemia. *Annual review of medicine*, 60, pp.39–54. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2716415&tool=pmc entrez&rendertype=abstract [Accessed June 30, 2015].

Martin, T.R. & Bracken, M.B., 1987. The association between low birth weight and caffeine consumption during pregnancy. *American journal of epidemiology*, 126(5), pp.813–21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3661529.

Martins, R.K. & McNeil, D.W., 2009. Review of Motivational Interviewing in promoting health behaviors. *Clinical Psychology Review*, 29(4), pp.283–93. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19328605 [Accessed October 29, 2013].

Maslova, E. et al., 2010. Caffeine consumption during pregnancy and risk of preterm birth. *American Journal of Clinical Nutrition*, pp.1120–1132.

Matsuoka, S. et al., 2006. Caffeine induces apoptosis of human umbilical vein endothelial cells through the caspase-9 pathway. *Gynaecological Endocrinology*, 22, pp.48–53. Available at: http://informahealthcare.com/doi/abs/10.1080/09513590500476198 [Accessed March 7, 2015].

Maurice, P., 1986. *Politics and Ethics in Qualitative Research*,

Maxcy, S., 2003. Pragmatic threads in mixed methods research in the social sciences: The search for multiple modes of inquiry and the end of the philosophy of formalism. *Handbook of mixed methods in social and behavioral …*. Available at: http://books.google.co.uk/books?hl=en&lr=&id=F8BFOM8DCKoC&oi=fnd&p g=PA51&dq=maxcy+2003&ots=gTfNzBrwJg&sig=4VaOxfRHWsPtbuQy4jGu UcMa1Uo [Accessed November 11, 2012].

McCalden, T.A., Nath, R.G. & Thiele, K., 1984. The role of prostacyclin in the hypercapnic and hypoxic cerebrovascular dilations. *Life sciences*, 34(19), pp.1801–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6376989 [Accessed July 6, 2015].

McCarthy, A. & Woolfson, R., 1994. Functional characteristics of small placental arteries. *American journal of Obstetrics and Gynaecology*. Available at:

http://www.sciencedirect.com/science/article/pii/S0002937894703116 [Accessed November 11, 2014].

McCartney, P., 2000. Information technology in maternal/child nursing: past, present, and future. *The American Journal of Maternal/Child Nursing*. Available at: http://journals.lww.com/mcnjournal/Abstract/2000/11000/Information_Technol ogy_in_Maternal_Child_Nursing_.12.aspx [Accessed April 23, 2014].

McGowan, J., 1988. Neonatal withdrawal symptoms after chronic maternal ingestion of caffeine. *Southern Medical Journal*. Available at: http://scholar.google.com/scholar?hl=en&q=caffeine+neonatal+serum+levels& btnG=&as_sdt=1%2C5&as_sdtp=#4 [Accessed January 4, 2015].

McKenzie, P.J., 2009. Informing choice: The organization of institutional interaction in clinical midwifery care. *Library & Information Science Research*, 31(3), pp.163–173. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0740818809000577 [Accessed October 30, 2013].

McKim, E.M., 1991. Caffeine and its Effects on Pregnancy and the Neonate. *Journal of Nursing and Midwifery*, 36(4).

McLennan, C., 1942. Antecubital and femoral venous pressure in normal and toxemic pregnancy. Available at: http://scholar.google.co.uk/scholar?q=Antecubital+and+femoral+venous+pressu re+pregnancy&btnG=&hl=en&as_sdt=0,5#0 [Accessed May 4, 2014].

McMaster, M. et al., 1995. Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *Journal of Immunology*. Available at: http://www.jimmunol.org/content/154/8/3771.short [Accessed October 28, 2014].

McMullan, M., 2006. Patients using the Internet to obtain health information: how this affects the patient-health professional relationship. *Patient education and counseling*, 63(1-2), pp.24–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16406474 [Accessed October 30, 2013].

McNanley, T. & Woods, J., 2004. Placental Physiology. In *Gynecology and Obstetrics*. Available at: https://www.glowm.com/resources/glowm/cd/pages/v3/v3c059.html [Accessed June 28, 2015].

McNulty, M., 1987. Pregnancy Police: The Health Policy and Legal Implications of Punishing Pregnant Women for Harm to their Fetuses. In *Review of Law and Social Change*.

McQuail, D., 2010. McQuail's mass communication theory. Available at: http://scholar.google.com/scholar?q=McQuail,+D.,+2000.+McQuail's+Mass+C

ommunication+Theory,+fourth+edn.+Sage+Publications,+London.&btnG=&hl=en&as_sdt=0,5#0 [Accessed August 26, 2014].

McQueen, J. & Jardine, A., 1990. Vascular angiotensin II and atrial natriuretic peptide receptors in normal and growth-retarded human placentae. *Journal of Endocrinology*. Available at: http://joe.endocrinology-journals.org/content/126/2/341.short [Accessed November 16, 2014].

Medawar, P., 1953. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol*. Available at: http://scholar.google.co.uk/scholar?hl=en&q=Medawar%2C+P.B.+%281953%29+Some+immunological+and+endocrinologi-+cal+problems+raised+by+the+evolution+of+viviparity+in+verte-+brates.&btnG=&as_sdt=1%2C5&as_sdtp=#0 [Accessed October 27, 2014].

Meekins, J. & Pijnenborg, R., 1994. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Journal of Obstetrics and Gynaecology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1471-0528.1994.tb13182.x/full [Accessed November 4, 2014].

Melmon, K. & Cline, M., 1968. Kinins: possible mediators of neonatal circulatory changes in man. *Journal of Clinical Investigation*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC297285/ [Accessed November 23, 2014].

Melnyk, B. & Fineout-Overholt, E., 2011. *Evidence-based practice in nursing and healthcare*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=hHn7ESF1DJoC&oi=fnd&pg=PT15&dq=traditional+evidence+based+practices&ots=HkNtggkY36&sig=_SLsPg_7oy6oblyf-Sz-EiD_HbA [Accessed October 29, 2013].

Menegaux, F. et al., 2005. Maternal coffee and alcohol consumption during pregnancy, parental smoking and risk of childhood acute leukaemia. *Cancer Detect Prev*, 29(6), pp.487–493. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16289502.

Mertens, D., 2005. *Research and evaluation in education and psychology: integrating diversity within quantitative, qualitative and mixed methods*, Available at: http://scholar.google.co.uk/scholar?q=mertens+2005+research+methods+in+education&btnG=&hl=en&as_sdt=0,5#1 [Accessed November 12, 2012].

Michaluart, P. et al., 1999. Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. *Cancer Research*. Available at: http://cancerres.aacrjournals.org/content/59/10/2347.short [Accessed November 9, 2014].

Michell, J., 2003. Pragmatism, positivism and the quantitative imperative. *Theory & Psychology*. Available at: http://tap.sagepub.com/content/13/1/45.short [Accessed November 11, 2012].

Miller, J. & Williamson, E., 1980. Fetal Loss After Implantation. *Journal of Human Genetics*, pp.554–556.

Miller, S., 2006. Mixed methods as methodological innovations: Problems and prospects. *Methodological Innovations Online*. Available at: http://scholar.google.co.uk/scholar?hl=en&as_sdt=0,5&q=miller+2006+mixed+methods+and+methodological+innovations#3 [Accessed July 28, 2013].

Mills, J. et al., 1993. Moderate caffeine use and the risk of spontaneous abortion and intrauterine growth retardation. *Journal of American Medical Association*, pp.328–328. Available at: http://jama.jamanetwork.com/article.aspx?articleid=403189 [Accessed August 6, 2014].

Mills, T.A. et al., 2009. Acute and chronic modulation of placental chorionic plate artery reactivity by reactive oxygen species. *Free radical research*, 47(2), pp.159–166. Available at: http://www.sciencedirect.com/science/article/pii/S0891584909002342 [Accessed July 3, 2012].

Mills, T.A. et al., 2005. Chorionic plate artery function and Doppler indices in normal pregnancy and intrauterine growth restriction. *European Journal of Clinical Intervention*, 35(12), pp.758–764. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16313252.

Mills, T.A. et al., 2006. Differential effects of hydrogen peroxide on human fetoplacental and non-pregnant uteroplacental arterial tone. *Proceedings of The Physiological Society*. Available at: http://www.physoc.org/3rd-innovations-in-drug-formulation-and-delivery/proceedings/abstract/Proc Physiol Soc 3C113 [Accessed March 7, 2015].

Mills, T.A., Baker, P.N. & Wareing, M., 2007. The effect of mode of delivery on placental chorionic plate vascular reactivity. *Hypertens Pregnancy*, 26(2), pp.201–210. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17469010.

Milne, E. et al., 2011. Maternal consumption of coffee and tea during pregnancy and risk of childhood ALL: results from an Australian case-control study. *Cancer Causes Control*, 22(2), pp.207–218. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21113653.

Milošević, M., Shrove, M. & Jovanov, E., 2011. Applications of smartphones for ubiquitous health monitoring and wellbeing management. *Journal of*

Information Technology and Applications. Available at: http://www.doisrpska.nub.rs/index.php/jita/article/view/357 [Accessed August 26, 2014].

Milunsky, A., Jick, H. & Jick, S., 1989. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *Journal of the American Medical Association*. Available at: http://jama.ama-assn.org/content/262/20/2847.short [Accessed July 2, 2012].

Minamisawa, M., Yoshida, S. & Takai, N., 2004. Determination of biologically active substances in roasted coffees using a diode-array HPLC system. *Analytical sciences : the international journal of the Japan Society for Analytical Chemistry*, 20(2), pp.325–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15055960.

Mirzoeva, O. et al., 1996. Inhibition of ICE-family cysteine proteases rescues murine lymphocytes from lipoxygenase inhibitor-induced apoptosis. *FEBS letters*. Available at: http://www.sciencedirect.com/science/article/pii/0014579396011118 [Accessed November 9, 2014].

Mitchell, M. & Bibby, J., 1978. Thromboxane B2 and human parturition: concentrations in the plasma and production in vitro. *Journal of Endocrinology*. Available at: http://joe.endocrinology-journals.org/content/78/3/435.short [Accessed November 12, 2014].

Mohazzab-H, K., 1994. NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. *American Journal of Physiology*. Available at: http://ajpheart.physiology.org/content/ajpheart/266/6/H2568.full.pdf [Accessed January 14, 2015].

Mohazzab-H, K. & Wolin, M., 1994. Properties of a superoxide anion-generating microsomal NADH oxidoreductase, a potential pulmonary artery PO2 sensor. *American Journal of Physiology*. Available at: http://ajplung.physiology.org/content/ajplung/267/6/L823.full.pdf [Accessed January 14, 2015].

Moll, W., Künzel, W. & Herberger, J., 1975. Hemodynamic implications of hemochorial placentation. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. Available at: http://scholar.google.co.uk/scholar?q=Hemodynamic+implications+of+hemochorial+placentation+&btnG=&hl=en&as_sdt=0%2C5#0 [Accessed November 4, 2014].

Momoi, N. et al., 2008. Modest maternal caffeine exposure affects developing embryonic cardiovascular function and growth. *American Journal of Physiology-Heart and Circulatory Physiology*, 294(5), pp.H2248–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18359892 [Accessed February 25, 2013].

Mongraw-Chaffin, M.L. et al., 2009. Maternal smoking, alcohol, and coffee use during pregnancy and son's risk of testicular cancer. *Alcohol*, 43(3), pp.241–245. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19303242.

Montgomery, J., 2007. Café culture and the city : The role of pavement cafés in urban public social life Café Culture and the City : The Role of Pavement Cafés in Urban Public Social Life. *Management*, (March 2012), pp.37–41.

Morel, J.L. et al., 2003. Crucial role of type 2 inositol 1,4,5-trisphosphate receptors for acetylcholine-induced Ca2+ oscillations in vascular myocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 23(9), pp.1567–1575.

Morgan, D.L., 2007. Paradigms Lost and Pragmatism Regained: Methodological Implications of Combining Qualitative and Quantitative Methods. *Journal of Mixed Methods Research*, 1(1), pp.48–76. Available at: http://mmr.sagepub.com/cgi/doi/10.1177/2345678906292462 [Accessed October 30, 2012].

Morgan, S. & Winship, C., 2007. *Counterfactuals and causal inference: Methods and principles for social research*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=lFTP49c5wJoC&oi=fnd&pg=PR9&dq=morgan+2007+social&ots=QlYL58vNaP&sig=oTiYe-n5vx99cqveAqYZIlQU7qM [Accessed November 29, 2012].

Moridani, M.Y. et al., 2001. CAFFEIC ACID , CHLOROGENIC ACID , AND DIHYDROCAFFEIC ACID METABOLISM : GLUTATHIONE CONJUGATE FORMATION ABSTRACT : , 29(11), pp.1432–1439.

Morris, M. & Weinstein, L., 1981. Caffeine and the fetus: is trouble brewing? *American Journal of Obstetrics and Gynaecology*. Available at: http://europepmc.org/abstract/MED/7258232 [Accessed August 6, 2013].

Mossman, H., 1937. Comparative morphogenesis of the fetal membranes and accessory uterine structures. Available at: http://scholar.google.co.uk/scholar?q=mossman+1937+comparative+morphogenesis&btnG=&hl=en&as_sdt=0,5#0 [Accessed October 21, 2014].

Motulsky, A., 1996. Nutritional ecogenetics: homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid. *American journal of human genetics*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1914958/ [Accessed July 2, 2012].

Mounce, H., 1997. *The two pragmatisms: from Peirce to Rorty*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=f5dQRUqk3QUC&oi=fnd&pg=PP1&dq=mounce+1997&ots=bKD02roRsQ&sig=HIU_qRQz6y1wPzZnE5QgW9psZHE [Accessed November 29, 2012].

Moyes, A.J., Gray, G. a & Denison, F.C., 2014. Bradykinin B1 receptor-mediated vasodilation is impaired in myometrial arteries from women with pre-eclampsia. *Hypertension in pregnancy : official journal of the International Society for the Study of Hypertension in Pregnancy*, 33(2), pp.177–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24304135 [Accessed June 26, 2014].

Mulder, E. & Medina, P. De, 2002. Prenatal maternal stress: effects on pregnancy and the (unborn) child. *Early human ….* Available at: http://www.sciencedirect.com/science/article/pii/S0378378202000750 [Accessed March 3, 2015].

Mulliner, C.M., Spiby, H. & Fraser, R.B., 1995. A study exploring midwives' education in, knowledge of and attitudes to nutrition in pregnancy. *Midwifery*, 11(1), pp.37–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7731375.

Mulrow, C.D., 1994. Rationale for systematic reviews. *British Medical Journal*, 309(September), pp.597–599.

Mulvany, M.J. & Aalkjær, C., 1990. Physiological Reviews of Small Arteries. *The American Physiological Society*.

Mulvany, M.J. & Halpern, W., 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circulation Research*. Available at: http://circres.ahajournals.org/content/41/1/19.full.pdf [Accessed July 3, 2012].

Mulvany, M.J. & Halpern, W., 1976. Mechanical Properties of Vascular Smooth Muscle Cells In Situ. *Nature*.

Muriel, P. & Arauz, J., 2010. Coffee and liver diseases. *Fitoterapia*, 81(5), pp.297–305. Available at: http://dx.doi.org/10.1016/j.fitote.2009.10.003 [Accessed September 4, 2011].

Myatt, L. et al., 1997. Endothelial nitric oxide synthase in placental villous tissue from normal, pre-eclamptic and intrauterine growth restricted pregnancies. *Human Reproduction*. Available at: http://humrep.oxfordjournals.org/content/12/1/167.short [Accessed March 10, 2015].

Myatt, L. & Brewer, A., 1992. Attenuation of the vasoconstrictor effects of thromboxane and endothelin by nitric oxide in the human fetal-placental circulation. *American journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/0002937892918636 [Accessed July 10, 2012].

Myatt, L. & Cui, X., 2004. Oxidative stress in the placenta. *Histochemistry and cell biology*, 122(4), pp.369–82. Available at: http://link.springer.com/article/10.1007/s00418-004-0677-x [Accessed February 19, 2014].

Myers, J.E. et al., 2005. Altered endothelial function in isolated human myometrial vessels induced by plasma from women with pre-eclampsia is not reproducible in isolated mouse vessels. *Clinical Science*, 462, pp.457–462.

Myers, J.E. et al., 2006. The effect of maternal characteristics on endothelial-dependent relaxation of myometrial arteries. *European journal of obstetrics, gynecology, and reproductive biology*, 124(2), pp.158–163. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16139415.

Myren, M. et al., 2007. The human placenta – An alternative for studying foetal exposure. , 21, pp.1332–1340.

Naidoo, N. et al., 2011. Cholesterol-raising diterpenes in types of coffee commonly consumed in Singapore, Indonesia and India and associations with blood lipids: a survey and cross sectional study. *Nutrition journal*, 10(1), p.48. Available at: http://www.nutritionj.com/content/10/1/48.

National Collaborating Centre for Methods and Tools, 2006. Critical appraisal tools to make sense of evidence. , (2006), pp.1–3. Available at: http://www.nccmt.ca/registry/view/eng/87.html [Accessed February 5, 2014].

National Institute for Health and Care Excellence, 2008. Antenatal care: Woman-centred-care, Guidance and guidelines. Available at: https://www.nice.org.uk/guidance/cg62/chapter/woman-centred-care [Accessed October 13, 2015].

Nawrot, P. et al., 2003. Effects of caffeine on human health. *Food additives and contaminants*, 20(1), pp.1–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12519715 [Accessed January 11, 2012].

Nehlig, A., 1999. Are we dependent upon coffee and caffeine? A review on human and animal data. *Neuroscience and biobehavioral reviews*, 23(4), pp.563–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10073894.

Nehlig, A., Debry, G. & Rard, G.I., 1994. Potential teratogenic and neurodevelopmental consequences of coffee and caffeine exposure: a review on human and animal data. *Neurotoxicology and teratology*, 16(6), pp.531–543. Available at: http://www.sciencedirect.com/science/article/pii/0892036294900329 [Accessed July 8, 2012].

Nielsen, A.H., Schauser, K.H. & Poulsen, K., 2000. The Uteroplacental Renin – Angiotensin System. *Placenta*, pp.468–477.

Nielsen, J.J.N. et al., 2006. Interventions to improve diet and weight gain among pregnant adolescents and recommendations for future research. *American Dietetic Association*, 106, pp.1825–1840. Available at: http://www.sciencedirect.com/science/article/pii/S0002822306018311 [Accessed March 7, 2015].

Nilsen, P., 2009. Brief alcohol intervention to prevent drinking during pregnancy: an overview of research findings. *Current opinion in obstetrics & gynecology*, 21(6), pp.496–500. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19797951 [Accessed September 19, 2014].

Niven, C., 1994. Coping with labour pain: the midwife's role. *Midwives, research and childbirth*. Available at: http://scholar.google.co.uk/scholar?q=niven+1994+midwife&btnG=&hl=en&as_sdt=0,5#5 [Accessed November 12, 2013].

Nonoguchi, H. & Owada, A., 1995. Immunohistochemical localization of V2 vasopressin receptor along the nephron and functional role of luminal V2 receptor in terminal inner medullary collecting ducts. *Journal of Clinical Investigation*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/pmc185813/ [Accessed November 15, 2014].

Nurminen, M.L. et al., 1999. Coffee, caffeine and blood pressure: a critical review. *European journal of clinical nutrition*, 53(11), pp.831–839. Available at: http://ukpmc.ac.uk/abstract/MED/10556993.

Nyborg, N. et al., 1987. Active, passive and myogenic characteristics of isolated rat intramural coronary resistance arteries. *Pflügers Archiv*. Available at: http://link.springer.com/article/10.1007/BF00581329 [Accessed November 11, 2014].

Nygård, O. et al., 1997. Coffee consumption and plasma total homocysteine: The Hordaland Homocysteine Study. *The American journal of clinical nutrition*, 65(1), pp.136–43. Available at: <Go to ISI>://A1997WA65800020.

O'Brien, B. & Naber, S., 1992. Nausea and vomiting during pregnancy: effects on the quality of women's lives. *Birth*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1523-536X.1992.tb00671.x/full [Accessed May 7, 2014].

O'Brien, B. & Zhou, Q., 1995. Variables related to nausea and vomiting during pregnancy. *Birth*, 22(2), pp.93–100. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7779229.

O'Brien, T., Ray, J. & Chan, W., 2003. Maternal body mass index and the risk of preeclampsia: a systematic overview. *Epidemiology*. Available at: http://journals.lww.com/epidem/Abstract/2003/05000/Maternal_Body_Mass_Index_and_the_Risk_of.20.aspx [Accessed August 26, 2014].

O'Brien, W. et al., 1988. Serum prostacyclin binding and half-life in the umbilical circulation. *Prostaglandins*. Available at: http://www.sciencedirect.com/science/article/pii/009069808890086X [Accessed March 10, 2015].

O'Cathain, A. et al., 2002. Use of evidence based leaflets to promote informed choice in maternity care: randomised controlled trial in everyday practice. *BMJ: British Medical Journal*, 324(7338), p.643. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=84396&tool=pmcentrez&rendertype=abstract.

O'Connor, M. & Whaley, S., 2007. Brief intervention for alcohol use by pregnant women. *American Journal of Public Health*. Available at: http://ajph.aphapublications.org/doi/abs/10.2105/AJPH.2005.077222 [Accessed September 9, 2013].

O'Leary, Z., 2004. *The essential guide to doing research*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=ItKeqNfgNW0C&oi=fnd&pg=PA1&dq=o+leary+2004&ots=pJeldC9r7J&sig=A8YVU7E78HkbpQxFlpitpPOoE_s [Accessed November 12, 2012].

Ochiai, R. et al., 2004. Green Coffee Bean Extract Improves Human Vasoreactivity. *Hypertension Research*, pp.731–737.

Ockene, J. et al., 2002. Spontaneous cessation of smoking and alcohol use among low-income pregnant women. *American journal of preventive medicine*, 23(3), pp.150–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12350446.

Office for National Statistics & Britain, I.G., 2013. *Internet Access - Households and Individuals , 2013*,

Ohman, S.G., Grunewald, C. & Waldenström, U., 2003. Women's worries during pregnancy: testing the Cambridge Worry Scale on 200 Swedish women. *Scandinavian Journal of Caring Science*, 17(2), pp.148–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12753515.

Okatani, Y., Watanabe, K. & Sagara, Y., 1997. Effect of nitric oxide, prostacyclin, and thromboxane on the vasospastic action of hydrogen peroxide on human umbilical artery. *Acta obstetricia et gynecologica Scandinavica*, 76, pp.515–520.

Okuda, T. et al., 1983. Studies on the activities of tannins and related compounds from medicinal plants and drugs. I. Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. *Chemical & pharmaceutical bulletin*, 31(5), p.1625. Available at: http://ukpmc.ac.uk/abstract/MED/6616711 [Accessed January 9, 2012].

Olsen, J. et al., 1989. Changing Smoking, Drinking, and Eating Behaviour among Pregnant Women in Denmark Evaluation of a health campaign in a local region. *Scandinavian*. Available at: http://sjp.sagepub.com/content/17/4/277.short [Accessed May 4, 2014].

Olsen, J., 1991. Cigarette smoking, tea and coffee drinking, and subfecundity. *American journal of epidemiology*, 133(7), pp.734–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2018028 [Accessed December 9, 2011].

Olsen, J., Overvad, K. & Frische, G., 1991. Coffee Consumption, Birthweight and Reproductive Failures. *Epidemiology*, 2(5), pp.370–374.

Olthof, M.R., Hollman, P.C.H., Zock, P.L., et al., 2001. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *American Journal of Clinical Nutrition*, 73(3), pp.532–538. Available at: <Go to ISI>://000167111900008.

Olthof, M.R. et al., 2003. Human Nutrition and Metabolism Chlorogenic Acid , Quercetin-3-Rutinoside and Black Tea Phenols Are Extensively Metabolized in Humans 1. *Human Nutrition and Metabolism*, (October 2002), pp.1806–1815.

Olthof, M.R., Hollman, P.C.H. & Katan, M.B., 2001. Human Nutrition and Metabolism Chlorogenic Acid and Caffeic Acid Are Absorbed in Humans. *Nutrition*, 131(September 2000), pp.66–71. Available at: <Go to ISI>://000166501100011.

Ong, L.M. et al., 1995. Doctor-patient communication: a review of the literature. *Social science & medicine (1982)*, 40(7), pp.903–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18666041.

Oppen, A. van, Stigter, R. & Bruinse, H., 1996. Cardiac output in normal pregnancy: a critical review. *Obstetrics and gynecology*. Available at: http://www.sciencedirect.com/science/article/pii/0029784495003487 [Accessed May 4, 2014].

Osol, G. & Mandala, M., 2009. Maternal uterine vascular remodeling during pregnancy. *Physiology*, 24, pp.58–71. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2760472&tool=pmcentrez&rendertype=abstract [Accessed May 21, 2013].

Pagano, P., 1993. Superoxide anion production by rabbit thoracic aorta: effect of endothelium-derived nitric oxide. *American Journal of Physiology*. Available at: http://ajpheart.physiology.org/content/ajpheart/265/2/H707.full.pdf [Accessed January 14, 2015].

Pagano, P. & Ito, Y., 1995. An NADPH oxidase superoxide-generating system in the rabbit aorta. *American Journal of Physiology*. Available at: http://ajpheart.physiology.org/content/ajpheart/268/6/H2274.full.pdf [Accessed January 14, 2015].

Page, N.M. et al., 2000. Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. *Nature*, 405(6788), pp.797–800. Available at: http://europepmc.org/abstract/med/10866201 [Accessed June 29, 2015].

Painter, R.C., Roseboom, T.J. & Bleker, O.P., 2005. Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reproductive Toxicology*, 20(3), pp.345–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15893910 [Accessed August 8, 2014].

Pandey, A. et al., 2013. Smartphone apps as a source of cancer information: changing trends in health information-seeking behavior. *Journal of Cancer Education*. Available at: http://link.springer.com/article/10.1007/s13187-012-0446-9 [Accessed August 26, 2014].

Parazzini, F. et al., 1996. Coffee and alcohol intake, smoking and risk of multiple pregnancy. *Human Reproduction*, 11(10), pp.2306–2309. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8943546.

Parazzini, F. et al., 1998. Coffee consumption and risk of hospitalized miscarriage before 12 weeks of gestation. *Human Reproduction*, 13(8), pp.2286–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9756312.

Parazzini, F. et al., 2005. Maternal coffee drinking in pregnancy and risk of small for gestational age birth. *European Journal of Clinical Nutrition*, 59(2), pp.299–301. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15454971.

Parsons, W. & Neims, A., 1978. Effect of smoking on caffeine clearance. *Clinical pharmacology and therapeutics*, 24(1), p.40. Available at: http://ukpmc.ac.uk/abstract/MED/657717 [Accessed January 15, 2012].

Party, M., 2001. Modernising maternity care. *A Commissioning Toolkit for Primary Care Trusts in ...*. Available at: http://scholar.google.co.uk/scholar?q=Maternity+Care:+a+commissioning+toolkit+for+Primary+Care&btnG=&hl=en&as_sdt=0,5#1 [Accessed September 3, 2013].

Patel, B. et al., 2002. Beneficial effects of short-term vasopressin infusion during severe septic shock. *Anesthesiology*. Available at: http://journals.lww.com/anesthesiology/Abstract/2002/03000/Beneficial_Effects_of_Short_term_Vasopressin.11.aspx [Accessed November 15, 2014].

Patel, M.X., 2003. Challenges in recruitment of research participants. *Advances in Psychiatric Treatment*, 9, pp.229–238.

Patton, M., 2001. *Qualitative research & evaluation methods*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=FjBw2oi8El4C&oi=fnd&pg=PP23&dq=m+patton+2002+qualitative+research&ots=buq-hGLJyI&sig=Qzgendsk646JYvQSdvWLBuDyiqw [Accessed November 12, 2012].

Paulesu, L., Romagnoli, R. & Bigliardi, E., 2005. Materno-fetal immunotolerance: is Interleukin-1 a fundamental mediator in placental viviparity? *Developmental and comparative immunology*, 29(5), pp.409–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15707662 [Accessed October 18, 2014].

Peacock, J.L., Bland, J.M. & Anderson, H.R., 1991. Effects on birthweight of alcohol and caffeine consumption in smoking women. *Journal of Epidemiology and Community Health*, pp.159–163.

Peadon, E. et al., 2011. Attitudes and behaviour predict women's intention to drink alcohol during pregnancy: the challenge for health professionals. *BMC public health*, 11(1), p.584. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3155919&tool=pmc entrez&rendertype=abstract [Accessed November 24, 2011].

Pearce, J., Knox, B. & Kremer, J., 1991. FOOD PREFERENCE DURING HUMAN PREGNANCY : A REVIEW. *Food Quality and Preference*, 2(1990), pp.131–154.

Pereira, R.D. et al., 2015. Angiogenesis in the Placenta : The Role of Reactive Oxygen Species Signaling. , 2015.

Petraglia, F. & Florio, P., 1996. Human placenta and fetal membranes express human urocortin mRNA and peptide. *The Journal of Clinical Endocrinology and Metabolism*. Available at: http://press.endocrine.org/doi/abs/10.1210/jcem.81.10.8855842 [Accessed March 10, 2015].

Petticrew, M., 2003. Why certain systematic reviews reach uncertain conclusions. *BMJ: British Medical Journal*, 326(7392), pp.756–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1125658&tool=pmc entrez&rendertype=abstract.

Phillips-Bute, B.G. & Lane, J.D., 1997. Caffeine withdrawal symptoms following brief caffeine deprivation. *Physiology & behavior*, 63(1), pp.35–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9402612.

Picard, N. et al., 2008. Maternal caffeine ingestion during gestation and lactation influences respiratory adaptation to acute alveolar hypoxia in newborn rats and adenosine A2A and GABA A receptor mRNA transcription. *Neuroscience*, 156(3), pp.630–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18706486 [Accessed November 11, 2013].

Pierce, G. & Midgley, A., 1963. The origin and function of human syncytiotrophoblastic giant cells. *The American Journal Of Pathology*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1949753/ [Accessed October 29, 2014].

Pijnenborg, R. et al., 1981. The Pattern of Interstitial Trophoblastic Invasion of the Myometrium in Early Human Pregnancy. *Placenta*, pp.303–315.

Pijnenborg, R. et al., 1983. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. *Placenta*. Available at: http://www.sciencedirect.com/science/article/pii/S0143400483800435 [Accessed November 4, 2014].

Pijnenborg, R., Vercruysse, L. & Hanssens, M., 2006. The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta*. Available at: http://www.sciencedirect.com/science/article/pii/S0143400405003206 [Accessed November 5, 2014].

Pipkin, F. & Symonds, E., 1977. Factors affecting angiotensin II concentrations in the human infant at birth. *Clin Sci Mol Med*. Available at: http://www-05.all-portland.net/cs/052/0449/0520449.pdf [Accessed November 16, 2014].

Pittman, R.N., 2011. Regulation of Tissue Oxygenation. , pp.1–100.

Plichart, M. et al., 2008. Parental smoking, maternal alcohol, coffee and tea consumption during pregnancy and childhood malignant central nervous system tumours: the ESCALE study (SFCE). *European Journal of Cancer Prevention*, 17(4), pp.376–383. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18562965.

Ploemen, J.H.T.. et al., 1993. In vitro and in vivo reversible and irreversible inhibition of rat glutathione S-transferase isoenzymes by caffeic acid and its 2-S-glutathionyl conjugate. *Food and Chemical Toxicology*, 31(7), pp.475–482. Available at: http://www.sciencedirect.com/science/article/pii/0278691593901069 [Accessed November 9, 2014].

Polit, D. & Beck, C., 2004. *Nursing research: Principles and methods*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=5g6VttYWnjUC&oi=fnd&pg=PA3&dq=polit+beck+2004&ots=__hRrJmqDA&sig=6BORaDUHBgE88hfvqVzgnCFlIPg [Accessed November 18, 2014].

Pollack, A. et al., 2010. Caffeine Consumption and Miscarriage: A prospective Cohort Study. *National Institute for Health Research*, 93(1), pp.304–306.

Pollard, K.C., 2011. How midwives' discursive practices contribute to the maintenance of the status quo in English maternity care. *Midwifery*, 27(5), pp.612–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20692743 [Accessed October 30, 2013].

Pollock, K. & Grime, J., 2002. Primary care practice consultations for depression : qualitative study. , 325(September).

Pope, C. & Mays, N., 1995. Reaching the parts other methods cannot reach: an introduction to qualitative methods in health and health services research. *BMJ: British Medical Journal*, 311(6998), pp.182–4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2550229&tool=pmcentrez&rendertype=abstract [Accessed October 9, 2012].

Pope, C., Mays, N. & Pope, C., 1995. Qualitative Research Observational methods in health care settings. *British Medical Journal*, 311(July), pp.182–184. Available at:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2550229&tool=pmc
entrez&rendertype=abstract [Accessed October 9, 2012].

Pope, C., Ziebland, S. & Mays, N., 2000. Analysing qualitative data. *Analysis*,
320(January), pp.5–7.

Posmontier, B., 2002. Antepartum care in the twenty-first century. *Nursing Clinics of
North America*. Available at:
http://scholar.google.co.uk/scholar?q=Posmontier+(2002)&btnG=&hl=en&as_s
dt=0,5#3 [Accessed April 24, 2014].

Poston, L. et al., 2006. Vitamin C and vitamin E in pregnant women at risk for pre-
eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet*. Available at:
http://www.sciencedirect.com/science/article/pii/S014067360668433X
[Accessed July 7, 2015].

Poston, L., McCarthy, a. L. & Ritter, J.M., 1995. Control of vascular resistance in the
maternal and feto-placental arterial beds. *Pharmacology & Therapeutics*, 65(2),
pp.215–239. Available at:
http://linkinghub.elsevier.com/retrieve/pii/016372589400064A.

Proctor, S., 1998. What determines quality in maternity care? Comparing the
perceptions of childbearing women and midwives. *Birth*, 25(2), pp.85–93.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/9668742.

Pugalendhi, K.V. & Ramakrishnan, S., 1990. Roasting of coffee-quality changes and
flavour development. *Journal of Coffee Research …*. Available at:
http://orton.catie.ac.cr/cgi-
bin/wxis.exe/?IsisScript=CAFE.xis&method=post&formato=2&
cantidad=1&expresion=mfn=002068 [Accessed July 9, 2012].

Quesada, A.R. & Medina, M.A., 2011. Anti-Angiogenic and Anti-Inflammatory
Properties of Kahweol , a Coffee Diterpene. , 6(8).

Quiñones, M. & Guerrero, L., 2014. Involvement of nitric oxide and prostacyclin in
the antihypertensive effect of low-molecular-weight procyanidin rich grape seed
extract in male spontaneously. *Journal of Functional Foods*. Available at:
http://www.sciencedirect.com/science/article/pii/S1756464613002624
[Accessed November 11, 2014].

Rabiee, F., 2007. Focus-group interview and data analysis. *Proceedings of the
Nutrition Society*, 63(04), pp.655–660. Available at:
http://www.journals.cambridge.org/abstract_S0029665104000874 [Accessed
October 5, 2012].

Rai, R. et al., 1997. Randomised controlled trial of aspirin and aspirin plus heparin in
pregnant women with recurrent miscarriage associated with phospholipid
antibodies (or antiphospholipid antibodies). *BMJ: British Medical Journal*,
314(7076), pp.253–7. Available at:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2125731&tool=pmc entrez&rendertype=abstract.

Ramalakshmi, K. & Raghavan, B., 2010. Caffeine in Coffee : Its Removal . Why and How ? Caffeine in Coffee : Its Removal . Why and How ? , (April 2012), pp.37–41.

Ramlau-Hansen, C.H. et al., 2008. Semen quality according to prenatal coffee and present caffeine exposure: two decades of follow-up of a pregnancy cohort. *Human Reproduction*, 23(12), pp.2799–2805. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18757446.

Rasch, V., 2003. Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. *Acta obstetricia et gynecologica Scandinavica*, 82(2), pp.182–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12648183.

RCOG, 2004. *Couples with recurrent miscarriage : What the RCOG guideline means for you*,

Read, M., Leitch, I. & Giles, W., 1999. U46619-mediated vasoconstriction of the fetal placental vasculature in vitro in normal and hypertensive pregnancies. *Journal of Hypertension*. Available at: http://journals.lww.com/jhypertension/Abstract/1999/17030/U46619_mediated_vasoconstriction_of_the_fetal.12.aspx [Accessed July 10, 2012].

Regan, L., Braude, P.R. & Trembath, P.L., 1989. Influence of past reproductive performance on risk of spontaneous abortion. *BMJ: British Medical Journal*, 299(6698), pp.541–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1837397&tool=pmc entrez&rendertype=abstract.

Regnault, T.R.H. et al., 2002. Placental development in normal and compromised pregnancies-- a review. *Placenta*, 23 Suppl A, pp.S119–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11978069 [Accessed June 28, 2013].

Reissig, C.J., Strain, E.C. & Griffiths, R.R., 2009. Caffeinated energy drinks--a growing problem. *Drug and alcohol dependence*, 99(1-3), pp.1–10. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2735818&tool=pmc entrez&rendertype=abstract [Accessed October 21, 2013].

Renfree, M., 2013. The origin and evolution of genomic imprinting and viviparity in mammals. *Philosophical Transactions of the Royal Society*. Available at: http://rstb.royalsocietypublishing.org/content/368/1609/20120151.short [Accessed October 18, 2014].

Renkert, S. & Nutbeam, D., 2001. Opportunities to improve maternal health literacy through antenatal education: an exploratory study. *Health promotion*

international, 16(4), pp.381–8. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/11733456.

Resch, B. & Papp, J., 1983. Effects of caffeine on the fetal heart. *American Journal of Obstetrics and Gynaecology*. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/6846450 [Accessed September 17, 2013].

Reynolds, L.P. et al., 2010. Uteroplacental vascular development and placental function: an update. *The International journal of developmental biology*, 54(2-3), pp.355–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19924632 [Accessed June 25, 2013].

Reynolds, L.P. & Redmer, D. a, 1995. Utero-placental vascular development and placental function. *Journal of animal science*, 73(6), pp.1839–51. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/7545661.

Ribeiro, J. a. & Sebastio, A.M., 2010. Caffeine and adenosine. *Journal of Alzheimer's Disease*, 20(SUPPL.1).

Richardson, W. et al., 1995. The well-built clinical question: a key to evidence-based decisions. *ACP J Club*. Available at:
http://www.lusiada.br/cursos/posgraduacao/mestrado-clinica-medica/images/artigos/key-evidence-based-decisions.pdf [Accessed October 29, 2013].

Ricketts, M. et al., 2007. The Cholesterol-Raising Factor from Coffee Beans , Cafestol , as an Agonist Ligand for the Farnesoid and Pregnane X Receptors. *Molecular Endocrinology*, 21(7), pp.1603–1616. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/17456796 [Accessed August 30, 2011].

Rising, S., 1998. Centering pregnancy: An interdisciplinary model of empowerment. *Journal of Nurse-Midwifery*. Available at:
http://onlinelibrary.wiley.com/doi/10.1016/S0091-2182(97)00117-1/full [Accessed December 5, 2013].

Ritchie, J. & Spencer, L., 1994. Qualitative Data Analysis for Applied Policy Research. In *Analyzing Qualitative Data*. pp. 173–194.

Ritter, J.M. et al., 1982. Prostanoid Synthesis by Human Umbilical Artery. *Prostaglandins*, 24(6), pp.881–886.

Robaut, C. et al., 1991. Regional distribution and pharmacological characterization of endothelin-1 binding sites in human fetal placental vessels. *Placenta*. Available at:
http://www.sciencedirect.com/science/article/pii/014340049190510M [Accessed November 16, 2014].

Robson, A. et al., 2012. Uterine natural killer cells initiate spiral artery remodeling in human pregnancy. *FASEB Journal*, 26(12), pp.4876–4885.

Robson, S. & Hunter, S., 1989. Serial study of factors influencing changes in cardiac output during human pregnancy. *American Journal of Physiology*. Available at: http://ajpheart.physiology.org/content/256/4/H1060.short [Accessed May 4, 2014].

Rodeck, C.H. & Whittle, M.J., 2009. *Fetal Medicine: Basic Science and Clinical Practice*, Elsevier Health Sciences. Available at: https://books.google.com/books?id=xzMeQbHJX0AC&pgis=1 [Accessed October 12, 2015].

Rodesch, F. & Simon, P., 1992. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstetrics and gynecology*. Available at: http://journals.lww.com/greenjournal/Abstract/1992/08000/Oxygen_Measurements_in_Endometrial_and.25.aspx [Accessed November 5, 2014].

Rogan, F. et al., 1997. "Becoming a mother'--developing a new theory of early motherhood. *Journal of advanced nursing*, 25(5), pp.877–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9147193.

De Roos, B. et al., 1998. Absorption and urinary excretion of the coffee diterpenes cafestol and kahweol in healthy ileostomy volunteers. *Journal of internal medicine*, 244(6), pp.451–460. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2796.1998.00386.x/full [Accessed January 12, 2012].

Roseboom, T., de Rooij, S. & Painter, R.C., 2006. The Dutch famine and its long-term consequences for adult health. *Early human development*, 82(8), pp.485–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16876341 [Accessed August 10, 2014].

Rosenberg, W. & Donald, A., 1995. Evidence based medicine: an approach to clinical problem-solving. *BMJ: British Medical Journal*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2549505/ [Accessed October 29, 2013].

Rothman, a J. & Kiviniemi, M.T., 1999. Treating people with information: an analysis and review of approaches to communicating health risk information. *Journal of the National Cancer Institute*, 55455(25), pp.44–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10854457.

Rubayiza, A. & Meurens, M., 2005. Chemical Discrimination of Arabica and Robusta Coffees by Fourier Transform Raman Spectroscopy. *Journal of Agricultural and Food Chemistry*, pp.4654–4659.

Rudolph, A. & Heymann, M., 1967. The circulation of the fetus in utero methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circulation research*. Available at: http://circres.ahajournals.org/content/21/2/163.short [Accessed October 29, 2014].

Rumbold, A. & Crowther, C., 2006. Vitamins C and E and the risks of preeclampsia and perinatal complications. *New England Journal of Medicine*. Available at: http://www.nejm.org/doi/full/10.1056/NEJMoa054186 [Accessed July 7, 2015].

Russell, J. et al., 2008. Vasopressin versus norepinephrine infusion in patients with septic shock. *New England Journal of Medicine*. Available at: http://www.nejm.org/doi/full/10.1056/NEJMoa067373 [Accessed November 15, 2014].

Rutherford, R., 1995. Nitric oxide synthase in human placenta and umbilical cord from normal, intrauterine growth- retarded and pre- eclamptic pregnancies. *British journal of Pharmacology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1476-5381.1995.tb15111.x/abstract [Accessed March 10, 2015].

Ryan, A.B., 2006. Post-Positivist Approaches to Research. In *Researching and Writing your thesis: a guide for postgraduate students*.

Ryan-Nicholls, K.D. & Will, C.I., 2009. Rigour in qualitative research: mechanisms for control. *Nurse researcher*, 16(3), pp.70–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19425402.

Sabry, S. & Mondon, F., 1995. In vitro contractile and relaxant responses of human resistance placental stem villi arteries of healthy parturients: role of endothelium. *Fundamental & clinical pharmacology*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1472-8206.1995.tb00264.x/abstract [Accessed November 11, 2014].

Sachse, C. & Bhambra, U., 2003. The cytochrome P450 CYP1A2 gene (CYP1A2) in colorectal cancer patients and controls: allele frequencies, linkage disequilibrium and influence on caffeine. *British journal of Clinical Medicine*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2125.2003.01733.x/full [Accessed March 10, 2015].

Sadler, T., 2011. *Langman's medical embryology*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=DKrovd6TKMwC&oi=fnd&pg=PP2&dq=langman's+embryology&ots=iYtPDCsUMP&sig=szO1qYyO4Par1HlmT4jr3gbRzvc [Accessed October 21, 2014].

Sagrestano, L.M. et al., 1999. Ethnicity and Social Support During Pregnancy 1. , 27(6), pp.869–898.

Salín-Pascual, R.J. et al., 2006. Caffeine challenge in insomniac patients after total sleep deprivation. *Sleep medicine*, 7(2), pp.141–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16459138 [Accessed October 17, 2013].

Salmeron, J. & Manson, J., 1997. Dietary fiber, glycemic load, and risk of non—insulin-dependent diabetes mellitus in women. *Journal of the American Medical Association*. Available at: http://jama.ama-assn.org/content/277/6/472.short [Accessed May 15, 2012].

Salo, D., Perez, C. & Lavery, R., 2004. Patient education and the Internet: do patients want us to provide them with medical web sites to learn more about their medical problems? *Journal of Emergency Medicine*. Available at: http://www.sciencedirect.com/science/article/pii/S0736467903003755 [Accessed April 23, 2014].

Samangaya, R.A. et al., 2011. Phosphodiesterase inhibitor effect on small artery function in preeclampsia. *Hypertens Pregnancy*, 30(2), pp.144–152. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20818967.

Sandelowski, M., 2008. Reading, Writing and Systematic Review. *Journal of advanced nursing*, 64(1), pp.104–110.

Sandelowski, M., 1986. The problem of rigor in qualitative research. *ANS. Advances in nursing science*, 8, pp.27–37.

Sanderson, S., Tatt, I.D. & Higgins, J.P.T., 2007. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. *International journal of epidemiology*, 36(3), pp.666–76. Available at: http://www.ije.oxfordjournals.org/cgi/doi/10.1093/ije/dym018 [Accessed November 13, 2013].

Sandoo, A. et al., 2010. The endothelium and its role in regulating vascular tone. *The open cardiovascular medicine journal*, 4, pp.302–12. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3040999&tool=pmc entrez&rendertype=abstract.

Sankar, P., 2004. Communication and miscommunication in informed consent to research. *Medical anthropology quarterly*, 18(4), pp.429–46. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20154295.

De Santis, M. et al., 2010. Use of the Internet by women seeking information about potentially teratogenic agents. *European journal of obstetrics, gynecology, and reproductive biology*, 151(2), pp.154–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20478650 [Accessed October 30, 2013].

Santos, I.S., Matijasevich, A. & Domingues, M.R., 2012. Maternal caffeine consumption and infant nighttime waking: prospective cohort study. *Pediatrics*, 129(5), pp.860–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3566755&tool=pmc entrez&rendertype=abstract [Accessed November 11, 2013].

Sarkadi, a & Bremberg, S., 2005. Socially unbiased parenting support on the Internet: a cross-sectional study of users of a large Swedish parenting website. *Child: care, health and development*, 31(1), pp.43–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15658965.

Sastry, B., Barnwell, S. & Moore, R., 1984. Factors affecting the uptake of α-amino acids by human placental villus: Acetylcholine, phospholipid methylation, Ca++ and cytoskeletal organization. *Fetal Nutrition, Metabolism, and Immunology*. Available at: http://link.springer.com/chapter/10.1007/978-1-4684-1191-1_8 [Accessed November 23, 2014].

Sata, F. et al., 2005. Caffeine intake, CYP1A2 polymorphism and the risk of recurrent pregnancy loss. *Molecular human reproduction*, 11(5), pp.357–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15849225 [Accessed October 2, 2012].

Savaş, S. & Evcik, D., 2000. Do undereducated patients read and understand written education materials? A pilot study in Isparta, Turkey. *Scandinavian journal of rheumatology*. Available at: http://europepmc.org/abstract/MED/11324797 [Accessed August 26, 2014].

Savitz, D.A. et al., 2008. Caffeine and miscarriage risk. *Epidemiology*, 19(1), pp.55–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18091004 [Accessed February 9, 2014].

Savitz, D.A., 2008. Invited commentary: disaggregating preterm birth to determine etiology. *American journal of epidemiology*, 168(9), pp.990–2; discussion 993–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18756017 [Accessed October 2, 2012].

Schardt, C. et al., 2007. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC medical informatics and decision making*, 7(1), p.16. Available at: http://www.biomedcentral.com/1472-6947/7/16 [Accessed July 30, 2015].

Schilling, B., 1986. Cross-Cultural Counseling. A Guide for Nutrition and Health Counselors. Available at: http://eric.ed.gov/?id=ED291849 [Accessed August 26, 2014].

Schneider, Z., 2002. An Australian study of women's experiences of their first pregnancy. *Midwifery*, 18(3), pp.238–249. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0266613802903098 [Accessed March 24, 2014].

Schulte, G. et al., 2004. Adenosine A receptors are necessary for protection of the murine heart by remote, delayed adaptation to ischaemia. *Acta physiologica Scandinavica*, 182(2), pp.133–43. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15450109.

Schwarting, R., 1993. Little missed markers and Ki-67. *Laboratory investigation; a journal of technical ....* Available at: http://europepmc.org/abstract/MED/8099985 [Accessed October 28, 2014].

Scialli, A., Lione, A. & Padgett, G., 1995. Reproductive effects of chemical, physical, and biologic agents: Reprotox. Available at: http://www.getcited.org/pub/103324659 [Accessed August 7, 2013].

Scott, J.C., 1990. *Domination and the Arts of Resistance: Hidden Transcripts*,

Sebire, N.J. & Talbert, D., 2002. The role of intraplacental vascular smooth muscle in the dynamic placenta: a conceptual framework for understanding uteroplacental disease. *Medical hypotheses*, 58(4), pp.347–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12027531 [Accessed October 28, 2014].

Sebire, N.J. & Talbert, D.G., 2001. The dynamic placenta: a closer look at the pathophysiology of placental hemodynamics in uteroplacental compromise. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*, 18(6), pp.557–61. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11844188.

Seefat-van Teeffelen, A., Nieuwenhuijze, M. & Korstjens, I., 2011. Women want proactive psychosocial support from midwives during transition to motherhood: a qualitative study. *Midwifery*, 27(1), pp.e122–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19931954 [Accessed October 30, 2013].

Selhub, J., 2008. Public health significance of elevated homocysteine. *Food and nutrition bulletin*, 29(2 Suppl), pp.S116–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18709886.

Sesti, F., Liu, S. & Cai, S., 2010. Oxidation of potassium channels by ROS: a general mechanism of aging and neurodegeneration? *Trends in cell biology*. Available at: http://www.sciencedirect.com/science/article/pii/S096289240900227X [Accessed March 8, 2015].

Shakespeare, D. & Issues, P., 2004. Exploring midwives ' attitude to teenage pregnancy. *Professional Issues*, 12(5).

Shams, M. & Ahmed, A., 1994. Localization of mRNA for basic fibroblast growth factor in human placenta. *Growth Factors*. Available at: http://informahealthcare.com/doi/abs/10.3109/08977199409001052 [Accessed October 21, 2014].

Sheard, C. & Garrud, P., 2006. Evaluation of generic patient information: effects on health outcomes, knowledge and satisfaction. *Patient education and counseling*, 61(1), pp.43–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16533676 [Accessed September 3, 2013].

Shi, J. & Benowitz, N., 1993. Pharmacokinetic-pharmacodynamic modeling of caffeine: Tolerance to pressor effects. *Clinical Pharmacology …*. Available at: http://onlinelibrary.wiley.com/doi/10.1038/clpt.1993.3/abstract [Accessed August 10, 2015].

Shi, W. et al., 2007. Arginine vasopressin inhibits Kir6.1/SUR2B channel and constricts the mesenteric artery via V1a receptor and protein kinase C. *American Journal of Physiology-Heart and Circulatory Physiology*, 293(1), pp.R191–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17428891 [Accessed November 15, 2014].

Shore, V. et al., 1997. Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblast. *Placenta*. Available at: http://www.sciencedirect.com/science/article/pii/S0143400497900072 [Accessed October 21, 2014].

Shweiki, D. et al., 1992. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*. Available at: http://www.med.upenn.edu/timm/documents/DunaiefTIMMShweikietal-1.pdf [Accessed October 22, 2014].

Shweiki, D. & Itin, A., 1993. Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. *Journal of Clinical Investigation*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC288226/ [Accessed October 22, 2014].

Signorello, L.B. et al., 2001. Caffeine metabolism and the risk of spontaneous abortion of normal karyotype fetuses. *Obstetrics and gynecology*, 98(6), pp.1059–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11755554.

Signorello, L.B. & McLaughlin, J.K., 2004. Maternal Caffeine Consumption and Spontaneous Abortion A Review of the Epidemiologic Evidence. *Epidemiology*, 15(2), pp.229–239.

Di Simone, N. et al., 2004. Effect of folic acid on homocysteine-induced trophoblast apoptosis. *Molecular human reproduction*, 10(9), pp.665–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15286211 [Accessed December 8, 2011].

Di Simone, N. et al., 2003. Homocysteine induces trophoblast cell death with apoptotic features. *Biology of reproduction*, 69(4), pp.1129–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12773435 [Accessed December 7, 2011].

Sinclair, M., 2007. A guide to understanding theoretical and conceptual frameworks. *Evidence Based Midwifery*, 5, p.2007.

Singh, J., 2013. Critical appraisal skills programme. *Journal of Pharmacology and Pharmacotherapeutics*, 4(1), p.76. Available at: http://www.jpharmacol.com/text.asp?2013/4/1/76/107697 [Accessed February 5, 2014].

Sjöström, H., Langius-Eklöf, A. & Hjertberg, R., 2004. Well-being and sense of coherence during pregnancy. *Acta obstetricia et gynecologica Scandinavica*, 83(12), pp.1112–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15548141.

Slattery, M.M. & Morrison, J.J., 2002. Preterm delivery. *Lancet*, 360(9344), pp.1489–97. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18598119.

Smith, D.K. et al., 1994. Lack of knowledge in health professionals: a barrier to providing information to patients? *Quality in health care : QHC*, 3(2), pp.75–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1055200&tool=pmc entrez&rendertype=abstract.

Smith, J. & Firth, J., 2011. Qualitative data analysis: the framework approach. *Nurse researcher*, 18(2), pp.52–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21319484.

Smith, T., 1999. Ethics in medical research: a handbook of good practice. Available at: http://books.google.co.uk/books?hl=en&lr=&id=vFjd2aKG_5QC&a mp;oi=fnd&pg=PR13&dq=need+ethics+research+medical&ots= 28Xt3tEYQ1&sig=KoqfJ4xcFCXwn5esYXlyrPxE9T0 [Accessed June 27, 2012].

Soares, M.J. & Hunt, J.S., 2006. Methods and Protocols: Overview I. In *Placenta and Trophoblast*. pp. 3–6.

Soltani, B.H. & Dickinson, F.M., 2005. Exploring women's views on information provided during pregnancy. *British Journal of Midwifery*, 13(10), pp.633–636.

Spratt, C., Walker, R. & Robinson, B., 2004. *Mixed research methods*,

Srivastava, A. & Thomson, S.B., 2009. Framework Analysis: A Qualitative Methodology for Applied Policy Research. *Journal of Administration and Governance*, 4(2), pp.72–79.

Stanton, C.K. & Gray, R.H., 1995. Effects of caffeine consumption on delayed conception. *American journal of epidemiology*, 142(12), pp.1322–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7503053.

Stapleton, H., Kirkham, M. & Thomas, G., 2002. Qualitative study of evidence based leaflets in maternity care. *BMJ: British Medical Journal*, 324(7338), p.639. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=84395&tool=pmcen trez&rendertype=abstract.

Statham, H., Solomou, W. & Chitty, L., 2000. Prenatal diagnosis of fetal abnormality: psychological effects on women in low-risk pregnancies. *Baillière's best practice & research. Clinical obstetrics & gynaecology*, 14(4), pp.731–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10985942 [Accessed November 5, 2014].

Stavri, G. et al., 1995. Hypoxia and platelet-derived growth factor-BB synergistically upregulate the expression of vascular endothelial growth factor in vascular

smooth muscle cells. *Febs Letters*. Available at: http://www.sciencedirect.com/science/article/pii/001457939401458D [Accessed October 22, 2014].

Stein, Z. & Susser, M., 1991. Miscarriage, caffeine, and the epiphenomena of pregnancy: the casusal model. *Epidemiology*.

Stirpe, F. & Corte, E. Della, 1969. The regulation of rat liver xanthine oxidase conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O). *Journal of Biological Chemistry*. Available at: http://www.jbc.org/content/244/14/3855.short [Accessed January 14, 2015].

Stone, J. & Yang, S., 2006. Hydrogen Peroxide: A Signaling Messenger. *Antioxidants and Redox Signaling*, 8, pp.243–270. Available at: http://www.liebertonline.com/doi/abs/10.1089/ars.2006.8.1391.

Stoner, B., 1986. Understanding medical systems: traditional, modern, and syncretic health care alternatives in medically pluralistic societies. *Medical Anthropology Quarterly*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1937-6219.1986.tb01021.x/abstract [Accessed January 2, 2015].

Street, R.L., 1991. Information-giving in medical consultations: the influence of patients' communicative styles and personal characteristics. *Social science & medicine (1982)*, 32(5), pp.541–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2017721.

Suh, K.S., 1999. Impact of communication medium on task performance and satisfaction: an examination of media-richness theory. *Information & Management*, 35(5), pp.295–312. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0378720698000974.

Susser, M. & Stein, Z., 1994. Timing in prenatal nutrition: a reprise of the Dutch Famine Study. *Nutrition reviews*, 52(3), pp.84–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8015751.

Sutcliffe, R. et al., 1982. The protein composition of the fibrinoid material at the human uteroplacental interface. *Placenta*. Available at: http://www.sciencedirect.com/science/article/pii/S0143400482800064 [Accessed March 7, 2015].

Suvorava, T. & Kojda, G., 2009. Reactive oxygen species as cardiovascular mediators: Lessons from endothelial-specific protein overexpression mouse models. *Biochimica et biophysica acta*, 1787(7), pp.802–810. Available at: http://dx.doi.org/10.1016/j.bbabio.2009.04.005.

Suzuki, A. et al., 2006. Improvement of hypertension and vascular dysfunction by hydroxyhydroquinone-free coffee in a genetic model of hypertension. *FEBS Letters*, 580, pp.2317–2322.

Swan, G., Carmelli, D. & Cardon, L., 1997. Heavy consumption of cigarettes, alcohol and coffee in male twins. *Journal of Studies on Alcohol.* Available at: http://www.jsad.com/jsad/authdownload/Heavy_Consumption_of_Cigarettes_Alcohol_and_Coffee_in_Male_Twins/404.html [Accessed October 28, 2013].

Swanson, J. a., Lee, J.W. & Hopp, J.W., 1994. Caffeine and nicotine: A review of their joint use and possible interactive effects in tobacco withdrawal. *Addictive Behaviors*, 19(3), pp.229–256.

Sweeney, M. et al., 2008. Characterisation of tone oscillations in placental and myometrial arteries from normal pregnancies and those complicated by pre-eclampsia and growth restriction. *Placenta*, 29(4), pp.356–365. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18336903.

Sweeney, M. et al., 2006. Ultrastructural Features of Smooth Muscle and Endothelial Cells of Isolated Isobaric Human Placental and Maternal Arteries. *Placenta*, 27, pp.635–647.

Symon, A. & Wrieden, W., 2003. A qualitative study of pregnant teenagers' perceptions of the acceptability of a nutritional education intervention. *Midwifery*. Available at: http://www.sciencedirect.com/science/article/pii/S0266613803000044 [Accessed March 7, 2015].

Szeremeta, M. et al., 2001. Snapshots of five clinical ethics committees in the UK. *Journal of medical ethics*. Available at: http://jme.bmj.com/content/27/suppl_1/i9.short [Accessed July 10, 2012].

Szwajcer, E.M. et al., 2007. Nutrition awareness and pregnancy: implications for the life course perspective. *European journal of obstetrics, gynecology, and reproductive biology*, 135(1), pp.58–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17178186 [Accessed October 17, 2013].

Szwajcer, E.M. et al., 2005. Nutrition-related information-seeking behaviours before and throughout the course of pregnancy: consequences for nutrition communication. *European journal of clinical nutrition*, 59 Suppl 1, pp.S57–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16052197 [Accessed August 8, 2014].

Szwajcer, E.M. et al., 2009. Written nutrition communication in midwifery practice: what purpose does it serve? *Midwifery*, 25(5), pp.509–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18243449 [Accessed October 30, 2013].

Taggart, N., 1961. Food habits in pregnancy. *The Proceedings of the Nutrition Society*, 20, pp.35–40. Available at: http://journals.cambridge.org/abstract_S0029665161000123 [Accessed May 4, 2014].

Tahanian, E. & Lord-dufour, S., 2010. Inhibition of Tubulogenesis and of Carcinogen- mediated Signaling in Brain Endothelial Cells Highlight the Antiangiogenic Properties of a Mumbaistatin Analog. *Chemical Biology And Drug Design*, (9), pp.481–488.

Tanaka, T., Kojima, T. & Kawamori, T., 1993. Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. …. Available at: http://carcin.oxfordjournals.org/content/14/7/1321.short [Accessed November 9, 2014].

Tanner, C., Medin, D. & Iliev, R., 2008. Influence of deontological versus consequentialist orientations on act choices and framing effects: When principles are more important than consequences. *European Journal of Social Psychology*. Available at: http://onlinelibrary.wiley.com/doi/10.1002/ejsp.493/abstract [Accessed September 2, 2012].

Tanuma, a, 2003. Caffeine Enhances the Expression of the Angiotensin II Type 2 Receptor mRNA in BeWo Cell Culture and in the Rat Placenta. *Placenta*, 24(6), pp.638–647. Available at: http://linkinghub.elsevier.com/retrieve/pii/S014340040300033X [Accessed November 22, 2011].

Tashakkori, A. & Teddlie, C., 2002. *Handbook of mixed methods in social & behavioral research*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=F8BFOM8DCKoC&oi=fnd&pg=PR9&dq=tashakkori+paradigm&ots=gTfNzAqxPe&sig=9mOZxezNDOqWoPZBFiJ8CIeBlGg [Accessed November 11, 2012].

Tate, R.M. et al., 1984. Oxygen Metabolites Stimulate Thromboxane Production and Vasoconstriction in Isolated Saline- perfused Rabbit Lungs interactions are unknown . We hypothesized that reactive in lungs and that vasoactive products of arachidonate ,. , 74(August), pp.608–613.

Tausig, J. & Freeman, E., 1988. The next best thing to being there: Conducting the clinical research interview by telephone. *American Journal of Orthopsychiatry*. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1939-0025.1988.tb01602.x/abstract [Accessed June 27, 2012].

Taylor, S. & Brown, J., 1988. Illusion and well-being: a social psychological perspective on mental health. *Psychological bulletin*. Available at: http://doi.apa.org/psycinfo/1988-16903-001 [Accessed May 1, 2014].

Van Teijlingen, E. et al., 1998. *Effectiveness of interventions to promote healthy eating in pregnant women and women of childbearing age- a review.pdf*,

Van Teijlingen, E.R. et al., 2003. Maternity satisfaction studies and their limitations: "What is, must still be best". *Birth*, 30(2), pp.75–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12752163.

Temple, J.L., 2009. Caffeine Use in Children: What we know, what we have left to learn and why we should worry. , 33(6), pp.793–806.

Tencé, M. & Petit, A., 1989. Characterization of angiotensin II binding sites in the human term placenta. *Molecular and cellular endocrinology*. Available at: http://www.sciencedirect.com/science/article/pii/0303720789900877 [Accessed November 16, 2014].

Thaler, I. et al., 1990. Changes in uterine blood flow during human pregnancy. *American journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/000293789090834T [Accessed November 4, 2014].

Theunissen, I. & Parer, J., 1994. Fluid and electrolytes in pregnancy. *Clinical obstetrics and Gynecology*. Available at: http://journals.lww.com/clinicalobgyn/Abstract/1994/03000/Fluid_and_Electrol ytes_in_Pregnancy.5.aspx [Accessed May 4, 2014].

Thompson, K. a et al., 2004. Women's perceptions of support from partners, family members and close friends for smoking cessation during pregnancy--combining quantitative and qualitative findings. *Health education research*, 19(1), pp.29–39. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15020543 [Accessed October 29, 2013].

Tolstrup, J.S. et al., 2003. Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion? *Human Reproduction*, 18(12), pp.2704–2710. Available at: http://www.humrep.oupjournals.org/cgi/doi/10.1093/humrep/deg480 [Accessed October 2, 2012].

Tosun, M., Paul, R. & Rapoport, R., 1998. Role of Extracellular Ca++ Influx viaL-Type and Non-L-Type Ca++ Channels in Thromboxane A2 Receptor-Mediated Contraction in Rat Aorta. *Journal of Pharmacology and Experimental Therapeutics*. Available at: http://jpet.aspetjournals.org/content/284/3/921.short [Accessed November 14, 2014].

Touitou, Y. et al., 2004. Ethical Principles and Standards for the Conduct of Human and Animal Biological Rhythm Research. *Chronobiology International*, 21(1), pp.161–170. Available at: http://informahealthcare.com/doi/abs/10.1081/CBI-120030045 [Accessed July 13, 2012].

Touyz, R.M., 2004. Reactive oxygen species and angiotensin II signaling in vascular cells: implications in cardiovascular disease. *Brazilian Journal of Medical and Biological Research*, 37(8), pp.1263–1273.

Toyofuku, K. & Nishimura, J., 1995. Effects of U46619 on intracellular Ca++ concentration and tension in human umbilical artery. *American journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/0002937895904719 [Accessed November 14, 2014].

Tranfield, D., Denyer, D. & Smart, P., 2003. Towards a Methodology for Developing Evidence-Informed Management Knowledge by Means of Systematic Review. *British Journal of Management*, 14(3), pp.207–222. Available at: http://doi.wiley.com/10.1111/1467-8551.00375.

Trevathan, W., 2011. *Human birth: An evolutionary perspective*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=Potlqpl-jxgC&oi=fnd&pg=PR7&dq=viviparity+mammals+humans&ots=OJqXi5j09U&sig=ZyWKluvS063KvSHD5-hRCOtwcn0 [Accessed October 18, 2014].

Triandis, H., 1977. Interpersonal behavior. Available at: http://library.wur.nl/WebQuery/clc/305594 [Accessed August 25, 2014].

Trist, E., 1951. SOME SOCIAL AND PSYCHO. *Human relations*. Available at: https://noppa.aalto.fi/noppa/kurssi/tu-53.1410/luennot/TU-53_1410_luettava_artikkeli.pdf [Accessed September 30, 2014].

Troyer, R.J.R.J.R.J.R.J.R.J.R.J. et al., 1984. Coffee Drinking : An Emerging Social Problem? *Society for the Study of Social Problems*, 31(4), pp.403–416. Available at: http://www.jstor.org/stable/10.2307/800387 [Accessed December 9, 2011].

Tsubouchi, H. et al., 2006. Effect of coffee intake on blood flow and maternal stress during the third trimester of pregnancy. *Int J Gynaecol Obstet*, 92(1), pp.19–22. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16242134.

Tsukahara, H., Sekine, K. & Miura, M., 2002. Vasoactive and natriuretic mediators in umbilical cord blood: a report of our observation and review of the literature. *Early human ...*. Available at: http://www.sciencedirect.com/science/article/pii/S0378378202000385 [Accessed November 16, 2014].

Tsutsumi, K. & Kotegawa, T., 2001. The effect of pregnancy on cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activities in humans&ast. *Clinical Pharmacology ...*. Available at: http://www.nature.com/clpt/journal/v70/n2/abs/clpt200173a.html [Accessed January 15, 2012].

Tuffrey, C. & Finlay, F., 2002. Use of the internet by parents of paediatric outpatients. *Archives of disease in childhood*, 87(6), pp.534–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1755829&tool=pmcentrez&rendertype=abstract.

Tulenko, T., 1979. Regional sensitivity to vasoactive polypeptides in the human umbilicoplacental vasculature. *American Journal of Obstetrics and Gynaecology*. Available at: http://europepmc.org/abstract/MED/507115 [Accessed November 5, 2014].

Tulppala, M. & Marttunen, M., 1997. Low-dose aspirin in prevention of miscarriage in women with unexplained or autoimmune related recurrent miscarriage: effect on prostacyclin and thromboxane A2. *Human embryology*. Available at: http://humrep.oxfordjournals.org/content/12/7/1567.short [Accessed November 12, 2014].

Umemura, T. et al., 1998. Direct injection determination of theophylline and caffeine in blood serum by high-performance liquid chromatography using an ODS column coated with a zwitterionic bile acid derivative. *Blood*, 123(August), pp.1767–1770.

Valero De Bernabé, J. et al., 2004. Risk factors for low birth weight: a review. *European journal of obstetrics, gynecology, and reproductive biology*, 116(1), pp.3–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15294360 [Accessed August 6, 2014].

Verbeke, W. & De Bourdeaudhuij, I., 2007. Dietary behaviour of pregnant versus non-pregnant women. *Appetite*, 48(1), pp.78–86. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17005297 [Accessed October 17, 2013].

Verhoef, M.J. & Casebeer, a L., 1997. Broadening horizons: Integrating quantitative and qualitative research. *The Canadian journal of infectious diseases*, 8(2), pp.65–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3327344&tool=pmcentrez&rendertype=abstract.

Verhoef, P. et al., 2002. Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. *The American journal of clinical nutrition*, 76(6), pp.1244–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12450889.

Verzelloni, E. et al., 2011. Antiglycative and antioxidative properties of coffee fractions. *Food Chemistry*, 124(4), pp.1430–1435. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0308814610009714 [Accessed December 13, 2011].

Vieira, O. et al., 1998. Cholesteryl ester hydroperoxide formation in myoglobin-catalyzed low density lipoprotein oxidation: concerted antioxidant activity of caffeic and p-coumaric acids with. *Biochemical pharmacology*. Available at: http://www.sciencedirect.com/science/article/pii/S000629529700470X [Accessed November 9, 2014].

Vik, T. et al., 2003. High caffeine consumption in the third trimester of pregnancy : gender-specific effects on fetal growth. *Paediatric and perinatal epidemiology*, pp.324–331.

Voorde, J., 1997. The influence of pregnancy on endothelium- derived nitric oxide mediated relaxations in isolated human resistance vessels. *Fundamental & clinical pharmacology*. Available at:

http://onlinelibrary.wiley.com/doi/10.1111/j.1472-8206.1997.tb00851.x/abstract [Accessed November 23, 2014].

Vries, R. De et al., 2001. *Birth by design: Pregnancy, maternity care and midwifery in North America and Europe*, Available at: http://books.google.co.uk/books?hl=en&lr=&id=_LsgY1UezT8C&oi=fnd&pg=PP1&dq=midwifery+north+america&ots=Y_qoafQY0M&sig=qGC2PwiNbVfD6xOVC0gZKsaw8Is [Accessed September 28, 2014].

Vuorela, P. & Hatva, E., 1997. Expression of vascular endothelial growth factor and placenta growth factor in human placenta. *Biology of reproduction*. Available at: http://www.biolreprod.org/content/56/2/489.short [Accessed October 21, 2014].

Wakatsuki, T., Nakaya, Y. & Inoue, I., 1992. Vasopressin modulates K ()-channel activities of cultured smooth muscle cells from porcine coronary artery. *American Journal of Physiology*. Available at: http://ajpheart.physiology.org/content/ajpheart/263/2/H491.full.pdf [Accessed November 15, 2014].

Waldenström, U. & Turnbull, D., 1998. A systematic review comparing continuity of midwifery care with standard maternity services. *British journal of obstetrics and gynaecology*, 105(11), pp.1160–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9853764.

Walker, N.J. & Bucher, J.R., 2009. A 21st century paradigm for evaluating the health hazards of nanoscale materials? *Toxicological sciences : an official journal of the Society of Toxicology*, 110(2), pp.251–4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2708598&tool=pmcentrez&rendertype=abstract [Accessed May 8, 2014].

Walker, R. et al., 2005. The development and process evaluation of an information-based intervention for pregnant women aimed at addressing rates of caesarean section. *Birtish Journal of Gynaecology*, 112(12), pp.1605–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16305562.

De Wals, P. et al., 2007. Reduction in neural-tube defects after folic acid fortification in Canada. *New England Journal of Medicine*. Available at: http://www.nejm.org/doi/full/10.1056/NEJMoa067103 [Accessed July 2, 2012].

Walsh, S. & Wang, Y., 1995. Trophoblast and placental villous core production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. *The Journal of Clinical Endocrinology & Metabolism*. Available at: http://press.endocrine.org/doi/abs/10.1210/jcem.80.6.7775637 [Accessed November 12, 2014].

Walters, W. & Boura, A., 1991. Regulation of fetal vascular tone in the human placenta. *Reproduction, Fertility and Development*. Available at: http://www.publish.csiro.au/?paper=RD9910475 [Accessed November 16, 2014].

Wang, J. & Mazza, G., 2002. Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN/gamma-activated RAW macrophages. *Journal of agricultural and food chemistry*, 50(4), pp.850–857. Available at: http://pubs.acs.org/doi/abs/10.1021/jf010976a [Accessed January 9, 2012].

Wang, Y., 2010. *Vascular Biology of the Placenta*,

Wareing, M. et al., 2002. Characterization of Small Arteries Isolated From the Human Placental Chorionic Plate. *Placenta*, 23(5), pp.400–409. Available at: http://www.sciencedirect.com/science/article/pii/S0143400402908258.

Wareing, M., Bai, X., Seghier, F., et al., 2006. Expression and function of potassium channels in the human placental vasculature. *American Journal of Physiology*, 291(2), pp.R437–46. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16914430.

Wareing, M., Myers, J.E., O'Hara, M., et al., 2006. Phosphodiesterase-5 inhibitors and omental and placental small artery function in normal pregnancy and pre-eclampsia. *European journal of obstetrics, gynecology, and reproductive biology*, 127(1), pp.41–49. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16815471.

Wareing, M., Greenwood, S.L., et al., 2006. Reactivity of human placental chorionic plate vessels from pregnancies complicated by intrauterine growth restriction (IUGR). *Biol Reprod*, 75(4), pp.518–523. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16707770.

Wareing, M., Myers, J.E., et al., 2005. Sildenafil citrate (Viagra) enhances vasodilatation in fetal growth restriction. *Journal of Clinical Endocrinology & Metabolism*, 90(5), pp.2550–2555. Available at: <Go to ISI>://000228914200011.

Wareing, M., O'Hara, M., et al., 2005. The involvement of Rho-associated kinases in agonist-dependent contractions of human maternal and placental arteries at term gestation. *American Journal of Obstetrics and Gynaecology*, 193(3 Pt 1), pp.815–824. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16150280.

Wareing, M., 2003. Vasoactive effects of neurokinin B on human blood vessels. *American Journal of Obstetrics and Gynaecology*, 188(1), pp.196–202. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0002937802714440 [Accessed November 22, 2011].

Wareing, M. et al., 2003. Vasoactive responses of veins isolated from the human placental chorionic plate. *Placenta*, 24(7), pp.790–796. Available at:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&d
opt=Citation&list_uids=12852871.

Wareing, M. & Baker, P.N., 2004. Vasoconstriction of small arteries isolated from
the human placental chorionic plate in normal and compromised pregnancy.
Hypertens Pregnancy, 23(3), pp.237–246. Available at:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&d
opt=Citation&list_uids=15617623.

Wareing, M., Greenwood, S.L. & Baker, P.N., 2006. Reactivity of human placental
chorionic plate vessels is modified by level of oxygenation: Differences
between arteries and veins. *Placenta*, 27, pp.42–48.

Watson, A. et al., 1997. Variations in expression of copper/zinc superoxide
dismutase in villous trophoblast of the human placenta with gestational age.
Placenta. Available at:
http://www.sciencedirect.com/science/article/pii/S0143400497800641
[Accessed January 14, 2015].

Watson, J., 1981. Nursing's Scientific Quest. *Nursing Outlook*. Available at:
http://www.eric.ed.gov/ERICWebPortal/recordDetail?accno=EJ248174
[Accessed November 29, 2012].

Weathersbee, P. & Lodge, J., 1977. Caffeine: its direct and indirect influence on
reproduction. *The Journal of reproductive medicine*, 19(2), p.55. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/197235 [Accessed December 14, 2011].

Weaver, K. & Olson, J.K., 2006. Understanding paradigms used for nursing research.
Journal of advanced nursing, 53(4), pp.459–69. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/16448489.

Weigel, R.M. & Weigel, M.M., 1989. Nausea and vomiting of early pregnancy and
pregnancy outcome. A meta-analytical review. *British journal of obstetrics and
gynaecology*, 96(11), pp.1312–8. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/2611170.

Weinstein, N., 1998. Accuracy of smokers' risk perceptions. *Annals of behavioral
medicine*. Available at: http://link.springer.com/article/10.1007/BF02884459
[Accessed April 30, 2014].

Weiss, E. & Moore, K., 2003. An assessment of the quality of information available
on the internet about the IUD and the potential impact on contraceptive choices.
Contraception, 68(5), pp.359–364. Available at:
http://linkinghub.elsevier.com/retrieve/pii/S0010782403001847 [Accessed
January 23, 2014].

Wen, L.M. et al., 2010. Dietary behaviours during pregnancy: findings from first-
time mothers in southwest Sydney, Australia. *The international journal of
behavioral nutrition and physical activity*, 7, p.13. Available at:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2830165&tool=pmc entrez&rendertype=abstract.

Wen, W. et al., 2001. The associations of maternal caffeine consumption and nausea with spontaneous abortion. *Epidemiology*. Available at: http://journals.lww.com/epidem/Abstract/2001/01000/The_Associations_of_Ma ternal_Caffeine_Consumption.8.aspx [Accessed August 7, 2013].

Wendler, C.C. et al., 2009. Embryonic caffeine exposure induces adverse effects in adulthood. *Faseb Journal*, 23(4), pp.1272–1278. Available at: <Go to ISI>://000266651600031 [Accessed August 22, 2012].

Weng, X., Odouli, R. & Li, D.-K., 2008. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. *American Journal of Obstetrics and Gynaecology*, 198(3), pp.279.e1–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18221932 [Accessed April 6, 2012].

Whorton, A.R. et al., 1982. Mechanism of bradykinin-stimulated prostacyclin synthesis in porcine aortic endothelial cells. *Biochimica et biophysica acta*, 712(1), pp.79–87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6810948 [Accessed July 6, 2015].

Wigglesworth, J.S., 1969. Vascular Anatomy of the Human Placenta and Its Significance for Placental Pathology. *Birtish Journal of Gynaecology*, 76(11), pp.979–989. Available at: http://doi.wiley.com/10.1111/j.1471-0528.1969.tb09463.x.

Wilcox, C.S., 2002. Reactive oxygen species: roles in blood pressure and kidney function. *Current hypertension reports*, 4(2), pp.160–166.

Wilcox, M., Gardosi, J. & Mongelli, M., 1993. Birth weight from pregnancies dated by ultrasonography in a multicultural British population. *BMJ: British Medical Journal*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1678907/ [Accessed September 27, 2014].

Wilkes, B., Mento, P. & Hollander, A., 1990. Endothelin receptors in human placenta: relationship to vascular resistance and thromboxane release. *American Journal of Physiology*. Available at: http://ajpendo.physiology.org/content/ajpendo/258/5/E864.full.pdf [Accessed November 16, 2014].

Wills, G. & Forster, D., 2008. Nausea and vomiting in pregnancy: what advice do midwives give? *Midwifery*, 24(4), pp.390–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17850938 [Accessed October 27, 2013].

Winklbaur, B. et al., 2008. Treating pregnant women dependent on opioids is not the same as treating pregnancy and opioid dependence: a knowledge synthesis for better treatment for women. *….* Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1360-0443.2008.02283.x/full [Accessed November 5, 2014].

Wisborg, K. et al., 2003. Maternal consumption of coffee during pregnancy and stillbirth and infant death in first year of life: prospective study. *BMJ: British Medical Journal*, 326(7386), p.420. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12595379.

Wisborg, K. et al., 1996. Smoking during pregnancy and pre term birth. , 103(August), pp.800–805.

Wisdom, S. et al., 1991. Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. *American journal of Obstetrics and Gynaecology*. Available at: http://www.sciencedirect.com/science/article/pii/000293789190018M [Accessed January 14, 2015].

WMA, 2013. *Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects*, Available at: http://www.wma.net/en/30publications/10policies/b3/ [Accessed August 14, 2015].

Wolf, F. De, 1973. Ultrastructure of the spiral arteries in the human placental bed at the end of normal pregnancy. *American journal of Obstetrics and Gynaecology*. Available at: http://europepmc.org/abstract/MED/4745182 [Accessed November 4, 2014].

Wolf, M., Kettyle, E. & Sandler, L., 2001. Obesity and preeclampsia: the potential role of inflammation. *Obstetrics and gynecology*. Available at: http://journals.lww.com/greenjournal/Abstract/2001/11000/Obesity_and_Preeclampsia__The_Potential_Role_of.9.aspx [Accessed August 26, 2014].

Wolin, M., Burke-Wolin, T. & Mohazzab-H, K., 1999. Roles for NAD (P) H oxidases and reactive oxygen species in vascular oxygen sensing mechanisms. *Respiration physiology*. Available at: http://www.sciencedirect.com/science/article/pii/S0034568799000237 [Accessed January 14, 2015].

Wollmann, H.A., 1998. Intrauterine Growth Restriction : Definition and Etiology. *Hormone Research*, 49(suppl 2), pp.1–6.

Woods, N., 1985. New models of women's health care. *Health Care for Women International*. Available at: http://www.tandfonline.com/doi/abs/10.1080/07399338509515696 [Accessed October 29, 2013].

World Health Organisation, 1965. *Nutrition in pregnancy and lactation: report of a WHO Expert Committee*, Available at: https://extranet.who.int/iris/restricted/handle/10665/38431 [Accessed May 4, 2014].

Worp, H.B. Van Der et al., 2010. Can Animal Models of Disease Reliably Inform Human Studies ? *PLoS Medicine*, 7(3).

Wrieden, W. & Symon, A., 2003. The development and pilot evaluation of a nutrition education intervention programme for pregnant teenage women (food for life). *Journal of Human Nutrition and dietetics*. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1365-277X.2003.00426.x/full [Accessed March 7, 2015].

Wright, A. & Walker, J., 2007. Management of women who use drugs during pregnancy. *Seminars in fetal and neonatal medicine*. Available at: http://www.sciencedirect.com/science/article/pii/S1744165X07000066 [Accessed November 5, 2014].

Yanagisawa, M., Kurihara, H. & Kimura, S., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*. Available at: http://web.mit.edu/hst527/www/readings/Lecture 11/Nature.pdf [Accessed November 16, 2014].

Yang, Y. et al., 2008. PKA-dependent activation of the vascular smooth muscle isoform of K ATP channels by vasoactive intestinal polypeptide and its effect on relaxation of the mesenteric resistance artery. *Biochimica et Biophysica Acta*. Available at: http://www.sciencedirect.com/science/article/pii/S0005273607003392 [Accessed November 15, 2014].

Young, J. et al., 2004. The coffee diterpene kahweol suppress the inducible nitric oxide synthase expression in macrophages. *Cancer Letters*, 213, pp.147–154.

Yukawa, G.S. et al., 2004. Effects of Coffee Consumption on Oxidative Susceptibility of Low Density Lipoproteins and Serum Lipid Levels in Humans. *Biochemistry Moscow*, 69(1), pp.70–74.

Yung, L.M. et al., 2006. Reactive oxygen species in vascular wall. *Cardiovascular & hematological disorders drug targets*, 6(1), pp.1–19.

Yvonne Feilzer, M. & Feilzer, M., 2009. Doing Mixed Methods Research Pragmatically: Implications for the Rediscovery of Pragmatism as a Research Paradigm. *Journal of Mixed Methods Research*, 4(1), pp.6–16. Available at: http://mmr.sagepub.com/cgi/doi/10.1177/1558689809349691 [Accessed November 9, 2012].

Zeh, D. & Zeh, J., 2000. Reproductive mode and speciation: the viviparity-driven conflict hypothesis. *Bioessays*. Available at: http://wolfweb.unr.edu/homepage/jaz/eecb752/lecture07/Zeh&Zeh2000.pdf [Accessed October 18, 2014].

Zhang, Z. et al., 2001. Characterization of antioxidants present in hawthorn fruits. , 12, pp.144 –152.

Zhao, Y. et al., 2012. Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertension Research*, 35(4), pp.370–374. Available at: http://dx.doi.org/10.1038/hr.2011.195.

Zhou, Y. et al., 1997. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? *The Journal of Clinial Investigation*. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC508044/ [Accessed November 4, 2014].

Zhu, B.T. et al., 2009. Inhibition of human catechol-O-methyltransferase (COMT)-mediated O-methylation of catechol estrogens by major polyphenolic components present in coffee. *The Journal of steroid biochemistry and molecular biology*, 113(1-2), pp.65–74. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2674329&tool=pmcentrez&rendertype=abstract [Accessed December 8, 2011].

Ziebland, S. et al., 2004. How the internet affects patients' experience of cancer: a qualitative study. *British Medical Journal*, 328(March).

Zusterzeel, P.L. et al., 2000. Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. *Molecular human reproduction*, 6(5), pp.474–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10775653.

Zygmunt, M. et al., 2003. Angiogenesis and vasculogenesis in pregnancy. *European Journal of Obstetrics and Gynaecology*, 110, pp.S10–S18. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0301211503001684 [Accessed October 27, 2014].

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