The long term effects of intra uterine growth restriction (IUGR) on calcium homeostasis and bone health

A thesis submitted to the University of Manchester, for the Degree of Master of Philosophy in the Faculty of Medical and Human Sciences

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Abstract

Background

Epidemiological studies have shown that poor growth in-utero and during infancy might increase an individual's risk of developing osteoporosis in later life. Mineral accretion by the fetus is an important element of such growth. Animal studies have shown that maternofetal transport of calcium across the placenta is reduced in intra uterine growth restriction (IUGR), it is therefore plausible that a deficit in skeletal mineralisation persists during childhood. Furthermore it has been proposed that renal tubular reabsorption of calcium and magnesium might be altered in utero in response to impaired placental transport of these ions.

Aims

We hypothesised that children aged between 5 and 10 years born IUGR would have reduced volumetric bone mineral density (mg/cm$^3$) at the distal and mid-radius compared to age matched controls. Furthermore we hypothesised that urinary calcium (Ca) and magnesium (Mg) excretion would be reduced.

Methods

Eighty-three children were invited to participate, of these 12 (11%) were successfully recruited. Anthropometric measurements were taken and bone parameters measured using peripheral quantitative computed tomography (pQCT) on 8 white-Caucasian children mean age 8.55 years (7.0-10.3). Data was converted into age and height standard deviation scores (SDS) using local reference data. Urine was analysed to measure Ca and Mg/ creatinine ratios.

Results

Anthropometric measurements of children who had IUGR was not different compared to UK normative growth standards. In the distal and mid radius mineral content was not different but important differences in the geometry of the bone
were found. Their radii were slender with thicker cortices, which resulted in reduced pQCT derived bone strength parameters. Muscle area and grip force were also reduced. Results are summarised below:

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<thead>
<tr>
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<th>Mean(SD)</th>
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<tr>
<td>Distal Radius</td>
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<tr>
<td>Total area</td>
<td>-0.80 (0.55)</td>
<td>0.004</td>
</tr>
<tr>
<td>Mid radius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>-0.84 (0.70)</td>
<td>0.012</td>
</tr>
<tr>
<td>SSI</td>
<td>-0.58 (0.67)</td>
<td>0.046</td>
</tr>
<tr>
<td>AMI</td>
<td>-0.70 (0.73)</td>
<td>0.029</td>
</tr>
<tr>
<td>CSMA</td>
<td>-1.00 (0.87)</td>
<td>0.014</td>
</tr>
<tr>
<td>Grip force</td>
<td>-0.78 (0.77)</td>
<td>0.024</td>
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**Conclusion**

Results of this small study suggest that 5 to 10 year old children who were born IUGR had adequately mineralised slender bones with thicker cortices. Their pQCT measured bone strength parameters were reduced, which might put them at increased risk of forearm fractures. An adequately powered longitudinal study is required to confirm these findings, and explore the possible contribution of muscle size and function in programming of bone strength parameters in children who suffer IUGR.
Declaration

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Abbreviations

1,25(OH)$_2$D  \(1,25\text{-dihydroxyvitamin D}\)

25(OH)D  \(25\text{-hydroxyvitamin D}\)

aBMD  \(\text{Areal bone mineral density}\)

AGA  \(\text{appropriate for gestational age}\)

AMI  \(\text{axial moment of inertia}\)

BMAD  \(\text{Bone mineral apparent density}\)

BMC  \(\text{Bone mineral content, g}\)

BMD  \(\text{Bone mineral density, g/cm}^2\)

BMU  \(\text{basic multicellular unit}\)

BWt SDS  \(\text{birth weight standard deviation score}\)

Ca  \(\text{calcium}\)

CSMA  \(\text{cross sectional muscle area}\)

DXA  \(\text{dual energy x-ray absorptiometry}\)

IUGR  \(\text{intra uterine growth restriction}\)

Mg  \(\text{magnesium}\)

pQCT  \(\text{peripheral quantitative computed tomography}\)

PTH  \(\text{parathyroid hormone}\)

PTHrP  \(\text{parathyroid hormone related protein}\)

SD  \(\text{standard deviation}\)

SDS  \(\text{standard deviation scores}\)

SGA  \(\text{small for gestational age}\)

SSI  \(\text{stress strain index}\)
Chapter 1

Introduction

Osteoporosis is defined by the World Health Organisation as a skeletal disorder, characterised by low bone mass and micro architectural deterioration of bone tissue. A painful and debilitating disease that leads to an increased risk of fracture, osteoporosis is an important cause of morbidity and mortality particularly among older populations. Hip fractures alone are reported to have an incidence in excess of 70,000 per year within the United Kingdom. Hip fractures in women are said to occupy a similar number of inpatient bed days to that of cardiovascular disease, breast cancer and chronic obstructive pulmonary disease (Kanis et al., 1997). The cost of this to the NHS is estimated to be in excess of £2.3 billion per year (Torgerson et al., 1997) thus making osteoporosis an important public health issue, particularly within the current financial climate in the National Health Service.

There is increasing evidence that the risk of osteoporotic fractures in later life might be influenced by environmental factors that affect growth in prenatal and early postnatal life (Petersen et al., 1989, Pohlandt and Mathers, 1989, Minton et al., 1983, Beltrand et al., 2008). Furthermore, epidemiological studies have shown that bone mineral density and bone mineral content in older life, surrogate markers for bone strength, are lowest amongst those who had low birth weights (Cooper et al., 1995, Cooper et al., 1997, Gale et al., 2001, Oliver et al., 2007). Thus, poor growth in-utero and during infancy might increase an individual's risk of developing osteoporosis in later life. Mineral accretion by the fetus is an important element of skeletal development and growth and it has previously been shown that the maternofetal transport of calcium (Ca) is reduced in intra uterine growth restricted (IUGR) rats (Mughal et al., 1989). Offspring of diabetic rats and
humans, who also have reduced maternofetal transfer of Ca, have been shown to have reduced urinary Ca and magnesium (Mg) excretion compared to offspring of controls, and these differences persisted up to young adulthood (Mughal et al., 2005, Bond et al., 2005). These results suggest that renal tubular reabsorption of Ca and Mg might be altered in utero, probably in response to impaired placental transport of these ions. It is therefore plausible that urinary Ca and Mg excretion will also be lower in children who were born IUGR. The idea that permanent changes in structure and function can be caused by environmental stimuli at critical periods of early development is known as ‘programming’.

The goal of work undertaken in fulfilment of my Master of Philosophy thesis was to investigate the effects that IUGR might have on bone development and mineralisation in children aged 5 to 10 years. Within this thesis, I have discussed the definition, diagnosis and aetiology of IUGR. Following this, evidence from current animal and human studies relating to the ‘programming’ of skeletal mineralisation and calcium homeostasis is discussed, particularly in relation to growth restriction. Skeletal mineralisation and development of bone is described and the techniques that can be used to measure the skeletal is evaluated. The hypothesis and methods of the study designed to investigate the long term effects of intra-uterine growth restriction on calcium homeostasis and bone health are detailed. The results are then presented followed by critical discussion and evaluation of the study and its findings, shortcomings and implications for the future.
Chapter 2

Intra Uterine Growth Restriction (IUGR)

Fetal growth and development is dependent on genetic, maternal and environmental factors (Peleg et al., 1998). Fetal insulin and insulin-like growth factors (IGF’s), the primary hormones that regulate fetal growth, are released in response to nutrient delivery to the fetus via the placenta. A balance of the requirements of the fetus, the mother and the placenta is necessary for normal growth and development to be achieved (Léger et al., 2004). Under optimal conditions the fetus will achieve an inherent growth potential that results in a baby of appropriate size for gestation. Some babies may be small at birth due to genetic factors but they have actually achieved their maximum growth potential. However, if conditions are not optimal, the fetus may not achieve their growth potential and growth will be limited. Known as IUGR, this is a significant cause of perinatal mortality and morbidity. Infants weighing less than 2.5kg at birth have a perinatal mortality rate 5-30 times higher than babies with birth weights on the 50\textsuperscript{th} centile (Peleg et al., 1998). The incidence of IUGR is approximately 5\% of all newborns (Mullis and Tonella, 2008) and Peleg et al (1998) suggested that IUGR is present in 30\% of all infants with birth weights below the 10\textsuperscript{th} centile for gestational age (Peleg et al., 1998). Small for gestational age (SGA) is a term also commonly used in the literature to describe babies that are small at birth. SGA is defined as birth weight and/or birth length standard deviation score (SDS) of less than -2 standard deviations (SD) according to the population reference data for gestational age (Lee et al., 2003, Mullis and Tonella, 2008), or birth weight between the 3\textsuperscript{rd} and 10\textsuperscript{th} centile for gestational age (Lee et al., 2003, Mandruzato et al., 2008), (Léger et al., 2004). IUGR may also be defined in this way and these terms are often used interchangeably (Lee et al., 2003, Mullis and Tonella, 2008).
It is however important to be able to distinguish between the two as they are not the same, this is particularly important when interpreting data and comparing studies. SGA refers to birth weight and includes those that are small but have achieved their appropriate growth potential, whereas IUGR refers to a pathological condition where a fetus has not achieved its genetically determined growth potential due to a slowing down of the growth trajectory in utero. Only a proportion of all SGA babies will be small due to IUGR. On reading the literature the definitions, diagnosis and management of IUGR and SGA infants is very much under debate.

2.1 Clinical indicators of IUGR

Due to the multi factorial nature of IUGR, ante natal diagnosis can be difficult and no single method can be completely accurate. Screening is however essential to detect those at risk. Assessment is made using a combination of the following methods.

1. Firstly, establishment of an accurate gestational age is essential for detection, monitoring and assessment of the IUGR fetus. This can be calculated from the certain date of last menstrual cycle in women with regular cycles or ultrasound measurement. Early ultrasound measurement for determining gestation is most accurate, ideally performed between 8 and 13 weeks gestation (Peleg et al., 1998).

2. Fetal growth can be assessed in utero clinically by palpation of the uterus and fundal height measurement. Where there is a lag in fundal height of 3-4cm behind gestational age, IUGR is suspected (Peleg et al., 1998).

3. IUGR can be diagnosed when a reduction in growth velocity is detected by serial ultrasound measurements (Lee et al., 2003). The measurements most commonly used are bi-parietal diameter, head circumference, abdominal
circumference and femur length (Mandruzzato et al., 2008). Abdominal circumference reflects liver size relating to glycogen storage and therefore nutritional status. Low abdominal circumference percentile has the highest sensitivity for diagnosing IUGR (Baschat and Weiner, 2000).

4. Amniotic fluid volume can also be estimated by ultrasound measurement. A reduction in amniotic fluid volume is recognised as another indicator of IUGR (Banks and Miller, 1999).

5. Umbilical artery doppler measurements are essential for monitoring placental insufficiency in the IUGR fetus and should guide clinical management. A reduction in umbilical artery diastolic flow is a strong indicator of uteroplacental dysfunction (Mandruzzato et al., 2008).

6. Post-natal examination of the newborn is also crucial to confirm diagnosis of IUGR.

2.2 Classification of IUGR

IUGR can be classified as symmetrical or asymmetrical. Babies who are proportionally small are symmetrically growth restricted and thought to have been exposed to a sub-optimal environment in the first two trimesters of development. Whereas those babies, who are disproportionately small, are asymmetrically growth restricted and are thought to have suffered growth restriction during the third trimester. This group generally have poorer outcomes (Mandruzzato et al., 2008). Asymmetric IUGR resulting from uteroplacental insufficiency is characterised by a small abdominal size (reflective of liver volume and subcutaneous fat), scrawny limbs (decreased muscle mass) and thinned skin (decreased fat) but normal head circumference (Peleg et al., 1998, Sheridan, 2005). This reflects the ability of the fetus to adapt to the sub-optimal
environment and redistribute the blood flow to the vital organs of brain, heart and placenta thereby preserving head circumference.

It has however been suggested that there remains insufficient evidence to relate different degrees or proportionality of growth restriction to the cause (Léger et al., 2004).

2.3 Aetiology of IUGR

The aetiology of IUGR is multifactorial and therefore it is not always possible to distinguish cause. Table 2.1 shows some of the known risk factors.

**Table 2.1 Maternal, fetal and placental risk factors for IUGR.**

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<tr>
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<td>Increased parity</td>
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<td>Previous IUGR pregnancy</td>
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<td>Maternal disease</td>
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<td></td>
<td>• Chronic hypertension</td>
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<td>• Pre-gestational diabetes</td>
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<td>• Cardiovascular disease</td>
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<td>• Pre-eclampsia</td>
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<td>• Infection</td>
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<td></td>
<td>Insufficient uteroplacental perfusion</td>
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<td>Sub-optimal placental implantation site</td>
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Chapter 3

Programming

Fetal development is dependent on the interaction of genetic, endocrine and environmental factors. As well as the intrinsic genetic influence on the development of the fetus and resulting genotype, there is a period of time when the fetus is said to be ‘plastic’. This is a period of time during which the fetus has the ability to adapt to the environment it is in by making permanent structural and functional changes, thereby altering the phenotype and increasing chances of survival. During fetal development, there are critical time periods when the different systems and organs in the body undergo rapid cell division and are most sensitive to their environment. These time periods are when adaptations can occur. For most systems this will be in utero, however some adaptations may still occur during the post natal period of growth. The adaptation that the fetus makes to its environment, known as ‘programming’, changes the pathways of development resulting in a permanent alteration in structure, physiology and/or metabolism.

Programming can be a response to enable survival in the immediate environment or what is know as a predictive adaptive response (PAR) to enable long term survival (Gluckman et al., 2005). In many instances this allows the baby to be better matched to its environment, for example if the mother is poorly nourished such as during times of famine, it signals to the baby that it is about to enter an environment with little food. The baby responds to this signal and adapts by reducing birth size and altering its metabolism to enable it to survive food shortage (Barker, 2004). This adaptation allows immediate survival, trading off immediate advantage for possible later consequences if the environment changes and is known as the ‘thrifty phenotype’. The ‘thrifty phenotype’ results in a
permanent alteration in the setting of hormones and metabolism to ensure increased insulin resistance. This allows better handling of food to maintain blood glucose level for the benefit of brain development but at the expense of glucose transport to muscles and growth (Hales and Barker, 1992). The reset growth trajectory is therefore suited to a life of deprivation. Another example of adaptation is that infants are born with similar numbers of sweat glands at birth however they do not become functional until around three years of age. The hotter the environment that the child is exposed to during this time, the greater the number of sweat glands that become functional thus enabling children who are born into a hot country to cope better with the heat of their environment.

In recent years, however, there has been a rapidly growing body of evidence that the adaptations that a fetus makes in utero may result in an increase in disease risk in later life. Known as the ‘fetal origins of adult disease’, this theory was first described by Barker (Barker, 2004, Barker, 1997).

This discovery came about as the result of studies examining men and women in mid to late life who were born between 1911-1930 in Hertfordshire for whom there was comprehensive anthropometric documentation made by midwives and health visitors from birth into early childhood (Cooper et al., 1997). From these data the authors concluded that low birth weight, indicating poor fetal growth, was associated with an increased incidence of coronary heart disease, stroke and hypertension and type 2 diabetes in later life. Results from a study in Sheffield supported this concept; infants who were born small because they were growth restricted rather than pre-term were at increased risk of disease in adult life (Gale et al., 2001). Furthermore, it has been shown that children who are most at risk are those who had impaired fetal growth and low birth weight in infancy followed
by rapid catch up in growth and adiposity in early childhood (Eriksson et al., 2001), (Eriksson et al., 2003), (Barker, 2006).

Figure 3.1 Diagram illustrating programming effects of the intra uterine environment on hormonal and metabolic development leading to risk of adult disease. Taken from www.som.soton.ac.uk/.../dohad/groups/endocrine/

There are many factors to incorporate when considering the fetal origins of adult disease. In animal studies, there is evidence that maternal nutritional status at conception could be linked with adult disease (Kwong et al., 2004, Bloomfield et al., 2003). The timing of the environmental insult during development is also crucial. As mentioned previously, there are different critical time periods during fetal development when the different systems and organs in the body undergo rapid cell division and are most sensitive to their environment. For example in the rat kidney, nephrogenesis begins mid gestation and is completed one week after birth. Under nutrition that occurs mid to late gestation in the rat has been show to cause a significant reduction in the number of nephrons developed in the affected kidney (Langley-Evans et al., 2003). Skeletal mineralisation occurs in the last trimester of pregnancy when calcium accrual is greatest so perhaps this is the
critical time period when an environmental insult causes an alteration in the mineralisation of the skeleton. It is therefore crucial that we consider the fetal growth pattern during development not just the resultant birth weight. For example there may be different implications for two infants with the same birth weight, one of whom developed normally in utero for the first two trimesters and then became malnourished and the pattern of growth slowed, compared with the infant whose growth was restricted in the first trimester but then grew steadily in the last two trimesters.

Another theory hypothesising the fetal origins of adult disease has been described by Gluckman as the mis-match theory (Gluckman et al., 2005). This suggests that the adult disease risk is dependant on the mis-match between the environment predicted during plastic developmental phase whereby the adaptive responses are made to anticipate a future adverse environment. This can have maladaptive consequences if the environment is mismatched to that predicted. The greater the mismatch, the greater the disturbance in physiology and therefore the greater the risk of disease (Figure 3.2). Gluckman et al (2005) suggests that this may explain why lifestyle diseases such as obesity, coronary heart disease, hypertension and type 2 diabetes are higher in populations undergoing socioeconomic transition. This is illustrated below in Figure 3.2.
Figure 3.2 Diagram illustrating the relationship between the developmental and adult environment. The horizontal lines represent limits of environment the individual is exposed to. The shaded area represents appropriate predictive adaptive responses associated with reduced risk of adult disease. Individual A exposed to normal intra uterine environment and able to tolerate greater variation in environment without consequences than individual B. (Silveira et al., 2007) adapted from Gluckman and Hanson.

The concept that the fetal origins of disease not only has an effect on individuals but can also effect future generations was first recognised through studying the grandchildren of a group of individuals who were conceived during the Dutch famine of 1944 (Lumey et al., 2007). It has been shown that birth size of grandchildren of those who experienced the famine was still reduced even though they were conceived after the famine was over (Lumey, 1992). This demonstrated that adaptations made by a fetus in response to environmental stimuli can have an epigenetic effect, whereby a change can occur in the phenotype of an individual that can last for multiple generations. This occurs without a change in underlying
DNA but can cause a lasting modification in gene expression. The mechanism by which this occurs in humans is not yet clear however recent studies have suggested this may be related to DNA methylation (Heijmans et al., 2008, Waterland, 2009).

Thus, further study into the developmental origins of adult disease is vital to lead us to the knowledge that will help formulate strategies to relieve the burden of disease not only on this, but future generations. Such is the growing interest in this field there is now an international society dedicated to promoting and disseminating research into the fetal and developmental origins of disease (www.DOHaD.co.uk) and January 2010 has seen the launch of a Journal of Developmental Origins of Disease to be published six times a year by Cambridge Journals (www.journals.cambridge.org). A further indication of just how rapidly this field of research is growing is that in 2006, a pub med search for ‘fetal programming’ yielded 50 references (Fernandez-Twinn and Ozanne, 2006) however that same search descriptor used in March 2010 yielded 1188 references (Figure 3.3).

Figure 3.3 Results from PubMed search for ‘fetal programming’ 2006/2010
3.1 Programming of skeletal development

Many studies worldwide have shown that fetal growth, low birth weight and growth in infancy are associated with susceptibility of developing not only the metabolic diseases such as Type 2 diabetes (McCance et al., 1994) but other diseases such as osteoporosis (Cooper et al., 1995).

The next sections (3.1.1, 3.1.2) will discuss previous animal and human studies that provide evidence that an adverse intra uterine and early developmental environment can have a lasting detrimental effect on bone health leading to an increased risk of osteoporosis in later life.

3.1.1 Animal studies

There are many examples of animal studies that provide evidence for the adverse programming of bone due to environmental stimulus in utero. It has been shown in rats that maternal diet during pregnancy can have lasting effects on bone and that these effects alter both the structure and density of bone and that these effects may be site specific. (Lanham et al., 2008a), (Lanham et al., 2008b). Mehta et al (2002), also provides evidence to suggest that maternal malnutrition might program skeletal growth by modifying how the growth plate responds to environmental influence during intrauterine and early post natal life. In this study, the adult offspring of rats who were malnourished during pregnancy had lower bone mineral content (BMC) and bone area (BA) of the tibia even after adjusting for weight and gender. Structural changes were also seen with the rats having widened epiphyseal growth plates (Mehta et al., 2002).

It has been shown that small size at birth is related to reduced BMC. The most common cause of small size at full term birth is due to intra uterine growth restriction (IUGR) which is commonly caused by uteroplacental insufficiency. This was discussed in greater detail in the previous chapter. Uteroplacental
insufficiency can be induced experimentally in rats using bilateral ligation of uterine arteries (Wigglesworth, 1964). Using this method, it has been shown that the maternofetal transfer of calcium across the IUGR rat placenta is reduced. The mechanism by which this occurs is not known, however it is suggested that a reduction in blood flow across the placenta may cause a reduction in the energy available for the active process of calcium transfer across the placenta (Mughal et al., 1989), or perhaps an alteration in hormone regulation of calcium transport (Robinson et al., 1989). Other factors that may have an effect on the amount of calcium transfer across the placenta include maternal and fetal blood flow through the placenta, maternal and fetal calcium concentrations, placental transport mechanisms and the hormones that regulate them such as 1,25(OH)₂ vitamin D₃, parathyroid hormone (PTH), PTH related peptide and calcitonin (Javaid and Cooper, 2002, Husain and Mughal, 1992). Oyhenart et al (2002) show that reduced blood flow across the placenta alters skeletal growth in rat pups. This study also suggests some site specificity with axial growth being most affected and the humerus unaffected. There is also some evidence of catch up growth in the femur (Oyhenart et al., 2002). Engelbregt et al (2004), also found that total body bone mineral content (TBBMC) in male and female IUGR rats was reduced at six months and at puberty but that this was appropriate for size at puberty, suggesting that if reduced bone mass is present at birth in IUGR it has disappeared i.e. caught up by puberty (Engelbregt et al., 2004). Two other animal studies suggest possible mechanisms for alteration in the BMC in IUGR offspring. Mollard et al (2004) found that at 21 days of age, small for gestational age (SGA) piglets had significantly lower TBBMC measured by dual x-ray absorptiometry (DXA) than appropriate for gestational (AGA) piglets even after adjustment for weight and length. Interestingly the SGA piglets showed catch up growth and had
the same body size as that of the AGA piglets. The SGA piglets also showed an increased calcium absorption rate in the proximal intestine. This suggests that there may be permanent up-regulation of intestinal calcium absorption in the SGA piglet and that this may be sufficient to facilitate catch up growth but not mineralisation of the bone. It does not explain however if this is a permanent under mineralisation or a lag that will catch up (Mollard et al., 2004). Bond et al (2005) have also suggested the possibility of programming of calcium homeostasis which will have an effect on bone health in rats. In this group, the offspring were from streptozotocin induced diabetic rats who like IUGR rats have been shown to have reduced maternofetal transfer of calcium and also magnesium across the placenta. The offspring of the diabetic rats showed reduced urinary calcium and mineral excretion that persisted into adulthood. This suggests intrauterine programming of calcium and magnesium handling in the renal tubule with evidence that the mechanism by which this occurs is through up-regulation of the transport proteins responsible for re-absorption of filtered calcium from the distal renal tubule (Bond et al., 2005).

Romano et al (2009) measured bone parameters in the adult IUGR rat femur using both dual x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). This study showed a reduction in DXA measured BMC which was not significant after size adjustment. However the pQCT measured parameters showed smaller size bones with a reduced stress strain index (SSI), a pQCT derived measurement of the bones strength, in the femur. This was 14% lower in males with less of an effect in females indicating that there may also be some sex linked differences in the effect IUGR has on bone outcomes. The study also suggests some evidence of the ability to reduce the effects of adverse bone programming in female rat pups by providing improved lactation in first 6
months. The study confirms that it is crucial to define the period of poor growth in relation to bone outcomes as a period of poor growth following a normal birth weight later followed by accelerated growth after weaning was also shown to have an adverse effect on bone geometry density and strength (Romano et al., 2009).

3.1.2 Human Studies

A number of human studies provide evidence that the adult risk of osteoporosis might be linked to environmental influences during early development and growth. Several studies have investigated the effect of IUGR on bone by measuring the amount of mineral in the bone by various methods, whilst others have looked at biochemical markers of bone homeostasis to investigate mechanisms by which bone health is altered. Perhaps the best way of presenting this is to group the studies into the following, those studies where;

3.1.2.1 Bone parameters were measured in infancy and childhood

3.1.2.2 Bone parameters were measured in adult cohorts for whom birth and childhood health records were available.

3.1.2.3 Physiological studies where biochemical markers of bone homeostasis were measured and related to bone health outcomes.

3.1.2.1. Bone parameters measured in infancy and childhood

Minton et al (1983), used single-beam photon absorptiometry adapted for use in neonates to measure BMC at the distal third of the radius. Although his numbers were small, with only five term small for gestation (SGA) infants, he found that at birth they had significantly lower BMC than appropriate size for gestation (AGA) infants. Although post natal mineralisation was delayed with the difference in BMC still present at 8 weeks of age, his results did show evidence of a catch up in mineralisation with BMC the same between both groups at 12 weeks of age. This difference was not however seen in preterm SGA and AGA leading to speculation
that the reduced mineralisation could occur in last trimester (Minton et al., 1983). Using the same method but with larger numbers and measuring BMC at the right mid humerus, Pohlandt and Mathers (1989), used preterm and term light for gestational age (LGA) and AGA infants to show that BMC was related to weight and not gestation. He found that LGA defined as $<3^{rd}$ centile infants had lower BMC than AGA infants of the same gestation but the same BMC as AGA infants of the same weight (Pohlandt and Mathers, 1989). Using dual-photon absorptiometry Petersen et al (1989) measured total body bone mineral content of SGA and AGA newborn infants. Even when the results were adjusted for weight or length there was a significant reduction in TBBMC in term SGA infants (Petersen et al., 1989)

More recently, DXA is recognised as a more precise and efficient method of assessing total body composition and mineralisation of the skeleton. Lapillone et al (1997) compared the BMC measured by DXA of term symmetric SGA and AGA infants. BMC was significantly reduced between the 2 groups of the same gestation, however like Pohlandt and Mather (1989) there was no difference when BMC was compared between SGA infants and AGA of the same weight. Lapillone et al (1997) suggested that BMC should be investigated in those with asymmetric IUGR. This study also showed that accumulation of calcium and therefore increase in BMC dramatically increases from 32-41 weeks gestation (Lapillonne et al., 1997).

Walther looked at the skeletal maturity of children aged 3 who were malnourished in utero. He found significant skeletal retardation at birth. This difference was no longer significant at 3 years suggesting a catch up in skeletal maturity in all those except those with a length below 10$^{th}$ centile for age suggesting a link between skeletal maturity and height (Walther et al., 1981).
There are some differences in the results reported above and the reason for this has been suggested by Namgung et al (1993). The author suggests that the degree of IUGR moves from mild to moderate depending on the timing and the degree of adverse environment to which the fetus is exposed. Following on from this Namgung et al (1993) suggests that weight is affected first then length then head circumference and that the effect this has on bone mineralisation is that first bone is affected less than weight then weight in proportion to bone and finally in more severe cases BMC more than weight (Namgung et al., 1993). It is also important to use same definition for IUGR/ SGA when comparing results from studies (Karlberg et al., 1996). Most studies use less than 10th centile as a definition but Pohlandt used those less than 3rd centile (Pohlandt and Mathers, 1989). More recently, DXA measured BMC was found to be reduced in a cohort of newborn infants for whom serial fetal growth assessments were made from 22 to 36 weeks gestation. This showed that the fetal growth pattern and birth weight to be independent predictors of BMC. In this cohort the SGA group displayed reduced BMC but also, AGA infants who had displayed some growth restriction between 22 to 36 weeks displayed reduced BMC (Beltrand et al., 2008). The fetal growth pattern should therefore always be considered where the data is available when assessing bone parameters in children.

3.1.2.2 Bone parameters measured in adult cohorts where birth and childhood health records are available.

Epidemiological evidence of an association between birth weight, weight at 1 year and BMC at the lumbar spine and femoral neck was reported by Cooper et al (1997). This came from a cohort of women from Bath born in 1968-1969 who were studied age 21 years and a group of men and women from Hertfordshire studied between 60 and 75 years of age (Cooper et al., 1995, Cooper et al., 1997).
Cooper had previously linked childhood growth with later hip fracture risk in a cohort born 1924-1933 in Helsinki (Cooper et al., 2001). Gale et al (2001) used DXA to measure BMC and body composition in a cohort of 143 men and women from Sheffield born between 1922 and 1926 who still resided in Sheffield and for whom there were complete records on neonatal anthropometric data. Birth weight was found to be a significant predictor of BMC at the lumbar spine, proximal femur and whole body after adjustment for age, sex and adult height. They also looked at asymmetric IUGR but found that ratio of head to abdominal circumference, a measure of brain sparing when the fetus is exposed to late adversity, was not predictive of bone size or BMD. These were lasting effects as they were found 7 decades after birth and included adjustment for known adult lifestyle determinants of bone loss such as inactivity, low calcium intake and smoking (Gale et al., 2001). A study using monozygotic and dizygotic twins to examine the association of birth weight with adult phenotypes found adult bone mass, particularly BMC was associated with birth weight (Antoniades et al., 2003). In a follow up study of the Hertfordshire cohort using pQCT, birth weight and weight at 1 year were found to have independent associations with measures of bone strength at the tibia and radius. Birth weight was associated with cortical bone area in men and tibial periosteal circumference was associated with birth weight and weight at 1 year in both sexes (Oliver et al., 2007).

3.1.2.3. Physiological studies where different endocrine measurements are taken and related to health records.

Calcium absorption in post menopausal women was investigated by Arden et al (2002), who found that as with pigs, humans with low birth weights had increased intestinal calcium absorption again suggesting that a poor intra uterine environment leads to permanent up regulation of adult intestinal calcium.
absorption. Arden et al (2002) suggests that the mechanism by which this occurs could be involving 1,25 \((\text{OH})_2\) vitamin D, as levels of this active metabolite of vitamin D were also increased in the low birth weight group (Arden et al., 2002). Other studies have investigated the link between birth weight and adult hormone levels involved in skeletal homeostasis. Birth weight and growth in infancy have also been linked with cortisol and growth hormone levels in later life. An adaptation in these levels as a result of early environmental stressor could result in reduced peak skeletal size, mineralisation and predisposition to an increased rate of bone loss in later life. (Dennison et al., 1997, Dennison et al., 1999, Dennison et al., 2003). Harrast and Kalkwarf (1998) investigated whether the low BMC seen in IUGR infants was as a result of lower bone formation or increased bone resorption by measuring biochemical markers of bone turnover in amniotic fluid (Harrast and Kalkwarf, 1998). The authors found evidence of decreased fetal bone formation which is in accordance with Namgung et al (1993). In Namgung et al’s study a 22\% reduction in BMC and 40\% reduction in cord serum osteocalcin, a marker of bone formation, was found in SGA infants compared to controls (Namgung et al., 1993). Namgung et al (2006) also measured significantly low serum 1,25-(OH)\(_2\)D concentrations in SGA infants; it has been suggested that 1,25-(OH)\(_2\)D has a role in the transport of calcium across the placenta (Robinson et al., 1989), which could result in the low BMC and osteocalcin seen in SGA infants. Bone specific alkaline phosphatase (B-ALP) is another marker of bone formation. Alkaline phosphatase (ALP) is a cell surface enzyme of osteoblasts and has a significant role in skeletal mineralisation; calcification is blocked when ALP activity is inhibited. B-ALP reflects early osteoblastic activity and osteocalcin (OC) reflects late osteoblastic activity. B-ALP was higher in SGA again suggesting adaptive mechanisms in fetal osteoblastic activity due to reduced
Placental calcium transport across the placenta. These studies lead Namgung et al (2006) to suggest that reduced mineral supply across the placenta directly affects bone formation (Namgung et al., 1996). However, more recently Briana et al (2008) showed no significant differences in the markers of bone formation, BALP, ALP, OC, parathyroid hormone (PTH), urine cross-linked N-telopeptide of type 1 collagen (NTx), calcium or phosphorus between IUGR cases and AGA controls (Briana et al., 2008). This was in maternal samples and samples from cord blood reflecting fetal measurements and neonates on days 1 and 4 postpartum to reflect stabilization to extra uterine life in a cohort of asymmetric growth restricted infants. Furthermore, Briana also indicated that the low bone mass seen in IUGR infants does not seem to be attributed to high bone resorption by finding no differences in osteoprotegerin (OPG), and RANK ligand markers of bone resorption (Briana et al., 2009).

In summary, there is evidence of low BMC in IUGR infants at birth, with some studies suggest that this is a lasting effect, however, the mechanism by which this occurs is not known. It is known that calcium is crucial for the mineralisation of bone and that large amounts of calcium are transported across the placenta to provide for the mineralisation of the growing fetus. As with the IUGR placenta, it has been shown that there is a reduction in the transport of calcium across the placenta of the diabetic rat (Husain et al., 1994). We have previously shown that in humans this results in an alteration of calcium and magnesium homeostasis by reduced excretion of urinary calcium and magnesium which persisted to young adulthood (Mughal et al., 2005). So although no conclusive mechanism for the alteration in bone mineralisation has been reported the above literature suggests low mineral supply is related.
Chapter 4

Bone

4.1 Structure of bone

Bone is a highly specialised composite material comprised of mineralised connective tissue and cartilage. One third of bone is organic, mostly collagen and the remaining two thirds consists of inorganic mineral, carbonate crystals called hydroxyapatite. Hydroxyapatite contains calcium and phosphate and gives bone its characteristic hardness. Macroscopically there are two types of bone, cortical and trabecular bone. Trabecular, also known as cancellous or spongy bone consists of an intricate lattice surrounded by bone marrow. It is lighter, has a larger surface area and a higher metabolic turnover than cortical bone. Trabecular bone is found within the vertebrae of the spine and at the ends of the long bones. Cortical or compact bone is dense more solid bone. It consists of building blocks called osteons which are concentric layers of matrix tissue called lamellae with a central ‘Haversian’ canal containing blood vessels and nerves. Cortical bone accounts for 80% of adult bone mass and is mainly found in diaphysis of the long bones and as the surrounding outer layer of other bones. Long bones, for example the radius, consist of a) the diaphysis, a tubular shaft of cortical bone that surrounds the medullary cavity and b) the epiphyses, expanded ends of the bone that consists of trabecular spongy bone surrounded by hard cortical bone on the outside. The diaphysis and epiphyses are separated by a growing zone of cartilage known as the epiphyseal growth plate.

4.2 Skeletal development

The human skeleton has a multi-functional role, providing a mechanical framework and support for the body to facilitate movement, protection for vital internal organs and the storage of essential minerals for the body such as calcium
and phosphate. It also houses bone marrow essential for the process of haematopoiesis.

The skeleton consists of a) the axial skeleton including the skull, vertebral column (spine), sternum and ribs which are mostly flat bones with a large surface area to facilitate the attachment of muscles and b) the appendicular skeleton which includes the long bones of the limbs and the hip and shoulder. Embryonic development of the skeleton begins at eight weeks when a cartilage template of the skeleton has been formed and continues post natally. Bone is formed by two different processes briefly described below in section 4.2.1 and 4.2.2;

4.2.1 Intra membranous ossification

Intra membranous ossification is the process by which bone is formed directly from embryonic connective tissue called mesenchyme. Mesenchymal cells become capillaries and osteoprogenitor cells differentiate into osteoblasts (bone forming cells). Osteoblasts secrete bone matrix (osteoid) which is subsequently mineralised. Mature osteoblasts ultimately become surrounded with matrix to form osteocytes which are mature bone cells. The matrix then calcifies and ultimately forms trabecular bone. Smooth compact peristeum forms on the bone surface. This is the process by which the flat bones within the axial skeleton are formed.

4.2.2 Endochondral ossification

Endochondral ossification is the process by which bone growth continues postnatally. The long bones are formed by endochondral ossification. This is a complex multi-step process where a cartilage model of bone is first formed from mesenchymal cells. Chondrocytes then divide and differentiate to form matrix and
primary ossification sites in the centre of the bone. Osteoblasts deposit bone matrix over the calcified cartilage and trabecular bone is formed. Secondary ossification centres form at the ends of the bone following birth. When the child reaches skeletal maturity (18 to 25 years of age), all of the cartilage is replaced by bone, fusing the diaphysis and both epiphyses together (epiphyseal closure). Bone remodeling shapes, forms and repairs the bone. Bone modeling results in net growth.

Figure 4.1 Diagram showing endochondral ossification and structure of long bone. Adapted from teaching package on bone and skeletal tissue available from www.itech.pjc.edu/fduncan/bsc1093/ap1c6pp.

4.3 Maternofetal transfer of calcium

To facilitate the mineralisation of the fetal skeleton large amounts of calcium are transferred from the mother to the fetus across the placenta. Two thirds of total body calcium is accrued by the fetus during the last trimester of pregnancy (Husain and Mughal, 1992). As well as providing for mineralisation of the skeleton extra cellular calcium is required for cell activity. Calcium is actively transported across the placenta using enzymes and transport proteins calbindin-
$D_28k$ and $Ca^{2+}$-ATPase. Maternal hormones such as PTH, PTHrP and 1,25-dihydroxyvitamin D are present in the fetal circulation and are thought to be involved in the process of calcium homeostasis in the fetus (Kovacs and Kronenberg, 1997). It has been shown that maternofetal calcium transport is maintained in times of maternal hypocalcaemia and hormone deficiency. As discussed earlier this is thought to be facilitated by an up-regulation of transport proteins and maintained by a calcium sensing receptor (Bond et al., 2008, Kovacs and Kronenberg, 1997).

### 4.4 Calcium homeostasis

To maintain sufficient calcium levels for the mineralisation of bone post natally, calcium is absorbed through the small intestine. Following birth this is at first a passive process, but later vitamin D receptors develop in the intestinal cells and 1,25(OH)$_2$D becomes necessary for the active transport of calcium within the intestine. When serum ionized calcium falls due to insufficient dietary intake, PTH increases which stimulates the renal conversion of 25(OHD) to 1,25(OH)$_2$D. 1,25(OH)$_2$D and PTH then induce osteoblasts to secrete RANK ligand. This in turn stimulates the production of osteoclasts to resorb bone thereby releasing calcium into the extracellular space. To maintain calcium homeostasis calcium is also reabsorbed in the renal tubule via a negative feedback system.

### 4.5 Post natal bone growth

Throughout life bone is constantly being broken down and re-built for the purpose of growth, calcium homeostasis and to replace worn out or damaged bone. The processes that determine this are modelling and re-modelling and enables bone to grow and maintain its mechanical properties of shape and strength.
4.5.1 Bone modelling

Bone modelling is the process whereby osteoclasts (bone resorbing cells) lining the inside of the cortex of the bone (known as the endosteum) resorb bone therefore increasing the medullary cavity of the bone. At the same time osteoblasts (bone forming cells) derived from mesenchymal stem cells, line the outside of the cortex of the bone, known as the periosteum, and deposit bone matrix known as osteoid that becomes mineralised and therefore results in an increase in the bone circumference and an alteration in micro-architecture. This process is regulated by a number of factors including the secretion of parathyroid hormone and vitamin D.

4.5.2 Bone re-modelling

Bone re-modelling is when the process of bone resorption by osteoclast activity is followed by bone formation by osteoblast activity on the same surface, replacing old bone with new bone. This process enables the bone to repair micro fractures that occur in the bone due to daily activity and enables the minerals in the bone to be utilised by the body for other processes. Osteoclasts and osteoblasts work together in the bone re-modelling process and are known as a basic multicellular unit (BMU). The resorption and formation of bone should occur at the same rate to maintain the quantity and quality of bone. If the rate of resorption exceeds formation, the quality of the bone is compromised, for example in post menopausal osteoporosis.

4.5.3 Mechanostat Model

First described by Wolff, bone modelling and re-modelling can be initiated by a response to mechanical loads (Wolff, 1892). Further to this, first proposed by Frost in 1987 and later refined and updated, Frost described the model of bone growth and loss as a mechanostat, whereby bone responds to a local mechanical
elastic deformation caused by forces exerted by muscles (Frost, 2003). Thus exercise, resulting in the contraction of muscle thereby exerting a force on bone is important in the development of strong bones. There are many examples of how physically active children have greater BMD or BMC or adaptations in bone size or geometry than sedentary children (Ward et al., 2005, Slemenda et al., 1991, Bailey et al., 1999).

4.6 Summary of determinants of bone strength
The strength and mechanical function of bone is determined by a combination of factors as follows;

1. the volume of bone
2. the amount of mineral within the bone
3. the organisation of bone – trabecular/cortical
4. the architectural arrangement of the bone
5. the loading conditions to which it is subjected

Alterations in any of these parameters will compromise bone strength.

4.7 Techniques for studying the skeleton
To be able to evaluate the bone health of children who were growth restricted in utero, an in vivo measurement of bone is required. As stated above bone strength is determined by a combination of size, shape, mineral content and architecture of the bone. A combination of bone densitometry techniques is required to give us all the information needed to fully evaluate bone strength. For the purpose of looking at bone outcomes in this study; bone density measurements and architecture parameters, the following two commonly used non-invasive techniques have been used (DXA section 4.7.1 and pQCT section 4.7.2).
4.7.1 Dual energy x-ray absorptiometry (DXA)

DXA has been available since the 1980’s and is the most commonly used technique for measuring bone in children in the UK and throughout the world. It allows both whole body and selected regions of interest to be measured using low levels of ionising radiation, it is a quick, precise and minimally invasive technique (Fewtrell, 2003). The minimal amounts of radiation exposure (Table 4.1) and the quick scanning time make it ideal for use with children (Figure 4.2). In my experience children as young as 5 years are able to remain still for the time it takes to scan the whole body, particularly when they are rewarded with a picture of their skeleton to take home (see figure 4.3).

![Figure 4.2 Child having a DXA scan](image1)

| Table 4.1 Ionising radiation doses for DXA and comparisons:* (NOS, 2004) |
|-----------------|-----------------|
| **DXA**         | Effective dose µSv |
| Lumbar Spine    | 1-3.4            |
| Whole body      | 3-5              |
| Total Hip       | 3-5              |
| Other           |                  |
| Chest x-ray     | 20               |
| Manchester      |                  |
| background radiation | 7 per day    |
| Transatlantic return flight | 80        |

![Figure 4.3 Whole body DXA scan](image2)
Bone density measurements are calculated using DXA when high and low energy x-ray beams are projected through the body from an x-ray source. By measuring the differential attenuation of the x-rays that are transmitted, the density of the body tissue through which the beams pass can be calculated. This enables measurements of the area and composition of mineral in the bone and the amount composition of fat and lean mass in the adjacent soft tissue to be derived. Using imaging techniques this data can be converted into a grey scale to produce an image. Using edge detection techniques to define the bone of interest, measurements of areal bone mineral density (BMDa) and bone area (BA) are made from which bone mineral content (BMC) can be calculated (Crabtree and Ward, 2009).

DXA does have some limitations. DXA is unable to distinguish between trabecular and cortical compartments of bone. There can also be inaccuracies in the measurements made due to changes in body composition and the measurement of BMDa is size dependent (Crabtree and Ward, 2009). BMDa is not a true volumetric measurement of the mineral in the bone but a ratio of the total amount of bone tissue within the projected area of bone (g/cm$^2$). DXA uses a 2 dimensional projection technique to measure bone, a 3 dimensional object and cannot therefore take into account the depth of the bone. This makes the measurement dependent on bone size and will under-estimate BMD in a small child with small bones and overestimate BMD in a tall child with bigger bones even though the volumetric density within the bone may be the same (Crabtree and Ward, 2009). See figure 4.4.
Figure 4.4 Schematic representation of the size dependence of DXA measured areal BMD. The cubes represent 2 different size vertebrae. They have the same volumetric BMD but different areal BMD.

To facilitate accurate interpretation of DXA data, size adjustment must be made using one of the following techniques.

1. The simplest size adjustment method is to report BMC for height.

2. Most commonly used for hip and spine data is the calculation of bone mineral apparent density (BMAD g/cm$^3$) using the methods of Carter, and Lu (Carter et al., 1992, Lu et al., 1996).

3. The Mølgaard model assesses bones to see if they are short for height, narrow or light (Molgaard et al., 1997).

4. The Crabtree/ Hogler method including lean mass (Crabtree et al., 2004, Hogler et al., 2003)

5. The Prentice model, linear regression, calculating a size adjusted bone mineral content (Prentice et al., 1994)

The World Health Organisation uses bone mineral density measurements derived from DXA in the criteria for diagnosing Osteoporosis in adults (1994). The International Society of Clinical Densitometry recommends that bone density
results in children are size adjusted, and that they are reported as low for chronological age if the SDS score $\leq 2$ (Lewiecki et al., 2008). When evaluating DXA measurements in children it is also essential to know the reference population and type of machine used to ensure correct interpretation of results (Fewtrell, 2003). If inappropriate reference data is used, incorrect conclusions can be drawn from research and furthermore incorrect clinical diagnosis and management of children with bone disease may take place.

### 4.7.2 Peripheral Quantitative Computed Tomography (pQCT)

Peripheral quantitative computed tomography is another technique that has been used with increasing frequency in research studies for assessing volumetric BMD and particularly geometric and architectural parameters of the appendicular skeleton and muscle (see Figure 4.5). It has the ability of providing a 3 dimensional cross-sectional assessment of both the structure and the geometric properties of the bone yet with a low radiation exposure (Fewtrell, 2003). The single slice technique is the most commonly used and the ionising radiation dose per single slice is $<1 \, \mu$Sv (Crabtree and Ward, 2009). pQCT has the ability to assess BMC, bone volume and vBMD, without influence from body (skeletal) size, which is a major advantage when used in children. pQCT is also able to separately assess both the trabecular and cortical compartment of bone (Crabtree and Ward, 2009). This enables researchers and clinicians to evaluate which compartment of bone is affected by different diseases and conditions. pQCT is also able to measure bone and muscle geometry which enables assessment of the muscle bone unit (Schoenau et al., 2002). Furthermore, measurements of the biomechanical properties of the bone can be estimated from pQCT derived parameters. These relate to the strength of the bone; Stress Strain Index (SSI)
being a measurement of torsional strength and Axial Moment of Inertia (AMI) relating to the bones bending ability.

Unlike DXA, pQCT provides a true volumetric measurement (mg/cm³) making size adjustment unnecessary for most values. Measurements are less influenced by size so age matched controls are sufficient (Crabtree and Ward, 2009) however height adjustment is still recommended for geometric and strength parameters (Ashby et al., 2009). The ISCD recommends that for clinical assessment measurements of trabecular and total BMC and BMD are made at the distal radius, (diaphyseal 4% site) and measurements of cortical BMC, cortical thickness, total bone area and cross sectional muscle area at the mid radius (metaphyseal, 50% site) (Zemel et al., 2008), see Figure 4.6. As with all data it is important for the accuracy of interpretation of results that appropriate reference data is used. For this study the Manchester paediatric reference data for the Stratec XCT- 2000 will be used (Ashby et al., 2009).

Figure 4.5 Child having a pQCT scan of radius

Figure 4.6 Sites of measurements pQCT radius
Chapter 5

Methodology

5.1 Hypothesis

To the best of my knowledge, no previous studies have assessed the accrual of BMC and BMD using DXA and pQCT in children aged 5-10 years old who were born IUGR. The following specific hypotheses will be tested in this study:

(a) DXA measured BMC (g) and areal BMD (g/cm²) at the lumbar spine and total hip will be lower in children aged 5-10 years old who were born IUGR compared to age matched controls, even after allowing for differences between height and weight in the two groups.

(b) The distal radial (total and trabecular) and mid-radial (cortical) volumetric BMD (vBMD mg/cm³) measured by pQCT will be lower in children aged 5-10 years old who were born IUGR compared to age matched controls.

(c) Urinary Ca and Mg excretion will be lower in children aged 5-10 years old who were born IUGR compared to age matched controls.

5.2 Study design

For this cross-sectional pilot study, children aged between 5-10 years who were born at Saint Mary’s Hospital, Manchester, UK, full term (38-41 weeks gestation), with evidence of IUGR were recruited. BMC and BMD of the lumbar spine and total hip was measured using DXA. Volumetric BMD of the distal & mid radius was measured by pQCT. Bone age was assessed from non-dominant hand x-ray. Urinary calcium/creatinine ratio and magnesium/creatinine ratio was measured in first void urine samples taken on the same day as the bone densitometry measurements. The DXA and pQCT measured bone outcomes in the IUGR group were analysed and compared to a locally derived reference
population in whom reference data curves for age or height are available. Appropriate SDS scores were calculated. Only white Caucasian children were recruited as currently there is no control data available for other races within the published DXA and pQCT data from the Manchester population (Ward et al., 2007, Ashby et al., 2009)

5.3 Ethics and funding

The study was funded following successful application by the author to the Central Manchester and Manchester Children’s University Hospitals NHS Trust research grant scheme. A sum of £14,410 was awarded to the author for the study (Appendix 1).

The study was granted Ethical approval by the Central Manchester Research Ethics Committee on 30th March 2007; REC reference 07/Q1407/17(Appendix 2). Further amendments were approved by the committee on 1st December 2008 and 21st May 2009.

Approval for the project to be undertaken was made by the Director of Central Manchester and Manchester Children’s University Hospitals NHS Trust Research and Development office on 22nd October 2007 (Appendix 3).

5.4 Inclusion criteria

Pre-pubertal white Caucasian children aged between 5 and 10 years who were born at Saint Mary’s Hospital with IUGR defined as birth weight<10th centile.

5.5 Exclusion criteria

1. Children whose IUGR was caused by chromosomal problems, known intrauterine infection or maternal use of illicit drugs.
2. Children with a close family member who suffers from a bone disorder.
3. Children taking or who have taken regular systemic steroids.

5.6 Recruitment of Participants

The original aim of the study was to recruit 50 children aged 5-8 years who were born between 38-42 weeks gestation (full term) and were IUGR defined as birth weight <10th centile. The age of recruitment was later extended to 10 years following ethical approval due to difficulty in recruitment. The Saint Mary’s Hospital perinatal database was used to identify potential suitable participants. Following this, the mother’s maternity notes and the child’s delivery and hospital medical records were screened to ensure they fulfilled inclusion criteria and none of the exclusion criteria applied. Following this, the General Practitioner (GP) of potential participants was sent a screening questionnaire (Appendix 5) to confirm that the family were indeed suitable to be invited to participate in the study. This was necessary as the child may not have been assessed at the hospital since birth and therefore their circumstances may have changed making the child/family unsuitable to be contacted for either social or medical reasons. Following satisfactory response from the GP, the child and parents were sent an invitation pack including an invitation letter describing the study, parent and child information leaflets and a reply slip to return to advise us if they wished to participate in the study (Appendix 6 and 7). Those that replied indicating their willingness to participate in the study were offered a date to attend for participation via post or telephone call and a participant pack that included consent (Appendix 8) and assent forms, lifestyle questionnaire, food diary and urine specimen pot were sent via post.
5.7 Outcome measures

5.7.1 Bone mineral content & density measurements

The Hologic QDR Discovery (Hologic Inc, Bedford, MA, U.S.A.) DXA fan beam scanner (software version 12.6, fast array mode) was used to measure lumbar spine (LS: L1-L4) bone mineral content (LSBMC; g), bone area (LSBA: cm²) and areal bone mineral density (LSaBMD; g/cm²) and BMC and BA of the left femoral neck (FN). The auto low-density algorithms were used for analysis to improve the edge detection of the bones in children. The bone mineral apparent density of lumbar spine (LSBMAD; g/cm³) and femoral neck (FNBMA; g/cm³) was calculated using the methods of Carter and Lu respectively (Carter et al., 1992), (Lu et al., 1996). The scans were performed with subjects lying supine and wearing light clothes. The in-vivo coefficient of variation (CV) for adult measurements of the lumbar spine and proximal femur are 1.1% and 1.3% respectively.

Control data for the DXA measured variables was taken from a previously published study where four hundred and forty two (239 males) healthy white Caucasian children were recruited for a study of bone mass acquisition during childhood. Detailed recruitment and relevant inclusion and exclusion criteria for the population have been previously described (Ward et al., 2007). Briefly, subjects who participated in this study did not suffer from primary or secondary disorders associated with diminished bone strength, were not taking medication and had not sustained a fracture within the past 12 months.

pQCT measurements were made using a Stratec XCT-2000 scanner, software version 5.5d (Stratec, Pforzheim, Germany). All measurements were taken at the non-dominant arm in accordance with the manufacturer’s recommendations. Detailed scan protocols have been previously published (Ashby et al., 2009).
Briefly, forearm length, defined as the distance from the ulna styloid process to the olecranon, was measured using a flexible tape measure (Sunlight Medical, Israel, Tel Aviv). Tomographic ‘slices’ of the radius were performed at the distal metaphysis (proximal to the growth plate) and the midshaft diaphysis, corresponding with 4% and 50% of the forearm length respectively. Each 1.2 mm thick slice was sampled using the scan speed of 25mm/sec and voxel size of 0.4mm. The resulting cross-sectional images and data were analysed using manufacturer’s software version 5.5d. The volumetric BMD (mg/cm³) were determined at the distal radius (4% site) for the total cross-section (cortical & trabecular) and for the trabecular compartment (defined as 45% of the total radius area). At the 50% mid-radial shaft, the cross-sectional muscle area (CSMA, mm²), cortical bone area (mm²), cortical thickness (mm), and the volumetric cortical bone mineral density (mg/cm³) were determined as previously described (Ashby et al., 2009). Surrogate bone strength markers were also derived from the pQCT data. SSI (mm⁴) relating to the torsional strength of the bone and the axial moment of inertia (AMI) (mm⁴), which is a measure of the distribution of cortical bone mass about the centre of the cross section of a tubular bone and is related to bending strength of bone, was also measured (Brennan et al., 2005). The in-vivo CV for total and trabecular volumetric BMD at the distal radius in adults are 0.78%, cortical 0.86%.

The reference data for the pQCT derived variables was taken from a previously published study of 625 white Caucasian children and young adults using the Stratec XCT-2000 pQCT scanner (Ashby et al., 2009).

5.7.2 Urine data

Urinary analysis for calcium/creatinine (UCa/Cr and magnesium/creatinine (UMg/Cr) ratio was undertaken at the Biochemistry laboratory of Manchester
Royal Infirmary. In children, accurate 24 hour urine collections can be difficult to obtain, and therefore molar ratio’s of UCa/Cr and UMg/Cr of first void urines were used as these variables correlate well with 24 hour urinary excretion of these minerals (Ghazali and Barratt, 1974). After collection, urine samples were frozen at -40º C before analysis. A 2ml sample of thawed urine was acidified with 30µl 5 M HCL, and mixed and pH adjusted to a value of 1-2. Samples were centrifuged, and the supernatant analysed for Ca, Mg and Cr using the Hitachi 917 auto analyser (Hitachi,Tokyo,Japan).

5.7.3 Anthropometric data
Gestational age at birth was estimated from the first day of the last menstrual period (LMP) or from the dating scan if LMP was not available or unreliable as documented in the mothers’ maternity notes. Birth weight (grams) and birth head circumference (cm) were retrieved from birth notes recorded at time of delivery and converted to standard deviation score (SDS). Standing height was measured to the nearest millimetre using a portable stadiometer (Leicester Height Metre, Child Growth Foundation, UK). Participants were weighed fully clothed, except for shoes and coat, to the nearest 0.1kg using Seca digital scales (Auto weight Scales, UK). Body mass index (BMI; kg/m²) was calculated as weight (kg) divided by height² (m).

5.7.4 Lifestyle information
An estimation of the number of hours per week of weight bearing physical activity and sedentary activity such as watching television and using the computer was calculated using a questionnaire adapted from Slemenda (Slemenda et al., 1991). A 3-day food diary was completed by parents and children to estimate daily intake of minerals and vitamin D. However these data were not analysed due to small numbers recruited and data being inaccurate and incomplete. Infant
weight measurements recorded at Child Health Clinics by family practitioners and health visitor were obtained from parent held records and converted to SDS scores using 1990 Growth Reference data (Cole et al., 1995).

5.8 Statistical Analysis

Anthropometric data

Height weight and BMI were converted to SDS scores using the 1990 Growth Reference data (Freeman et al., 1995, Cole et al., 1995). Birth weight and head circumference at birth were converted to SDS using the regional St Mary’s neonatal unit database (British growth foundation reference data, September 1996). The SDS scores were tested using a one sample t-test to see if they differed from zero.

Bone data

Height and gender and age and gender matched SDS scores were calculated for bone outcomes using the published LMS curves derived from a normal reference database for DXA and pQCT bone outcomes (Ward et al., 2007, Ashby et al., 2009). The SDS scores were tested using a one sample t-test to see if they differed from zero.

Urine data

The mean and standard deviations of Ca/Cr and Mg/Cr were compared with those unpublished data from the previously discussed reference population (Ward et al., 2007). The data were analysed and used as control data for a previous study (Mughal et al., 2005). Analysis was performed by Dr Steve Roberts, statistician, at Manchester University who kindly provided the urine references values for this study.
Chapter 6

Results

6.1 Study population

A total of 291 maternal and child health records of babies born at St Mary’s Hospital between 1999 and 2004 with birth weight <10\textsuperscript{th} centile were screened. Of these 149 (51\%) appeared to fulfil the study inclusion criteria and screening questionnaires were sent to their GP’s. Ninety six GP’s (65\%) replied from whom 83 children were suitable to invite to participate in the study. Twelve families replied and were willing to participate in the study however only 9 children completed the study. Three children did not attend initial or repeat appointments that were made for scans, or return telephone calls. This represents 11\% successful recruitment from those that were invited to participate. A summary of recruitment is given in Figure 6.1 followed by a CONSORT flow diagram in Figure 6.2.

![Flow of recruitment for IUGR study](image)

**Figure 6.1 Bar chart showing flow of recruitment**

Nine children (6 male) successfully completed the study. Of these, data from 8 children (5 male) were analysed; one child was subsequently found to be of mixed race for which there was no reference data.
Birth records assessed for eligibility n=291

Fulfil initial screening-letter sent to GP n=149
Did not fulfil inclusion criteria n=91

GP replies received n=96
No GP reply received n=53

Children who fulfil inclusion criteria, invited to participate n=83

Children who did not fulfil inclusion criteria n=13

Willing to participate n=12
No response from invite letter n=71

Moved out of area no contact details available n=8

Study protocol completed n=9
Did not attend appointments n=3

Behavioural problems/non-compliance n=2

Excluded from analysis n=1

Data analysed n=8

Recurrent non attenders n=2

Child protection issues n=1

Figure 6.2 CONSORT diagram to show participant flow
Relevant medical history noted from the participants; 1 child suffered from mild asthma and 1 child had suffered a traumatic fracture of the tibia aged 10 years.

### 6.2 Maternal/Pregnancy data

The mean maternal age at delivery was 31.75 years (25-40) SD 4.89. Method of delivery for the 8 pregnancies were as follows: 2 normal spontaneous deliveries, 4 induced, 1 elective caesarean section due to breech, 1 emergency caesarean section due to placental abruption. Following delivery 4 placentas were noted to be ‘ragged’ and one placenta was noted to be small. Asymmetrical IUGR was confirmed by ultra sound scan in 5 of the 8 babies. Figure 6.3 gives additional maternal/pregnancy data.

![Figure 6.3](chart.png)

**Figure 6.3 Bar chart to show descriptive maternal/pregnancy data**

### 6.3 Birth data

All 8 of the infants were breast fed at time of discharge with 5 (62%) infants being discharged at 1 day of age despite their low birth weight; mean(SD) 2.88 (2.7) days. The mean (SD) birth weight (kg) was 2.5 (0.14) and mean (SD) head circumference at birth (cm) 33.2 (0.98). The birth weight SDS was lower than the birth head circumference SDS for all of the infants. Birth length was not measured.
at St Mary’s Hospital therefore this data was not available. As shown in table 6.1 below the study participants had a significantly lower birth weight and head circumference at birth than a gestation and gender matched reference data.

Table 6.1 Mean(SD), 95% CI and p values of birth weight and head circumference SDS

<table>
<thead>
<tr>
<th></th>
<th>Mean(SD)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight SDS</td>
<td>-2.05(0.31)</td>
<td>-2.32 to -1.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Head circumference</td>
<td>-1.18(0.60)</td>
<td>-1.68 to -0.68</td>
<td>0.001</td>
</tr>
<tr>
<td>at birth SDS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.4 Anthropometric data

The mean age at scan for the children was 8.55 (7.0-10.3) (years). As shown in table 6.2 below, the study participants showed a trend to being slightly smaller in stature as a group (p=0.06) but were not lighter or lower in BMI than the general population.

Table 6.2 Mean(SD), 95% CI and p values of height, weight and BMI SDS at time of study

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight SDS</td>
<td>-0.35(0.54)</td>
<td>-0.80 to 0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Height SDS</td>
<td>-0.50(0.63)</td>
<td>-1.04 to 0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>-0.11(0.68)</td>
<td>-0.68 to 0.46</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The range of the number of hours of exercise the children participated in per week was too large to be meaningful for such a small dataset (2-35 hours) and was not analysed further.
6.5 Bone data

6.5.1 Axial skeleton

Unfortunately when it came to analysing these data, it was found that the DXA software had been upgraded to a different version from when the reference data was collected. The reference data could not be updated in time for submission of my thesis. Hence there are no DXA results at this time as data should not be compared with data analysed using different analysis software (Kocks et al., 2010, Fewtrell, 2003). It is however anticipated that the reference data will be updated therefore the study data will be analysed when this becomes available.

6.5.2 Appendicular skeleton

As seen in table 6.3 below, when compared to height and age reference data separately both the distal radius and the mid radius were significantly smaller in size in the study group.

Cortical content is increased and cortical thickness is significantly increased in the study group compared to height matched reference data.

Some differences are seen in the architecture and geometry of the radius with AMI and SSI, surrogate measures of the bending and torsional strength of the bones respectively, being significantly reduced compared to an age matched reference population. In comparison to height matched reference population the differences were reduced.

Muscle area, as a proxy for muscle strength of the radius is significantly lower than both the age and height matched population. Confirmatory of this is the reduction of grip force for age.

Mean bone age was lower than chronological age (n=6). Bone age Mean (SD) 7.25 years (2.48); Chronological age Mean (SD) 8.28 years (1.06).
Table 6.3 Bone and muscle outcomes derived from pQCT measurements of the distal and mid radius. Measurements are compared to gender and height, and/or gender and age matched reference data as stated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean(SD)</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Distal radius 4% site</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total area for age SDS score</td>
<td>-0.80 (0.55)</td>
<td>-1.26 to -0.35</td>
<td>0.004</td>
</tr>
<tr>
<td>Total area for ht SDS score</td>
<td>-0.55 (0.50)</td>
<td>-0.97 to -0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Total vBMD for age SDS score</td>
<td>0.41 (0.82)</td>
<td>-0.28 to 1.1</td>
<td>0.20</td>
</tr>
<tr>
<td>Trabecular vBMD for age SDS score</td>
<td>-0.11 (1.2)</td>
<td>-1.1 to 0.90</td>
<td>0.80</td>
</tr>
<tr>
<td><strong>Mid radius 50% site</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total area for age SDS score</td>
<td>-0.84 (0.70)</td>
<td>-1.43 to -0.25</td>
<td>0.012</td>
</tr>
<tr>
<td>Cortical content for age SDS score</td>
<td>-0.14 (0.80)</td>
<td>-0.80 to 0.52</td>
<td>0.631</td>
</tr>
<tr>
<td>Cortical thickness for age SDS score</td>
<td>0.34 (0.77)</td>
<td>-0.30 to 0.98</td>
<td>0.257</td>
</tr>
<tr>
<td>SSI for age SDS score</td>
<td>-0.58 (0.67)</td>
<td>-1.15 to -0.01</td>
<td>0.046</td>
</tr>
<tr>
<td>AMI for age SDS score</td>
<td>-0.70 (0.73)</td>
<td>-1.32 to -0.09</td>
<td>0.029</td>
</tr>
<tr>
<td>CSMA for age SDS score</td>
<td>-1.00 (0.87)</td>
<td>-1.23 to 0.27</td>
<td>0.014</td>
</tr>
<tr>
<td>Total area for ht SDS score</td>
<td>-0.55 (0.58)</td>
<td>-1.04 to -0.06</td>
<td>0.031</td>
</tr>
<tr>
<td>Cortical content ht SDS score</td>
<td>0.39 (0.50)</td>
<td>-0.02 to 0.81</td>
<td>0.060</td>
</tr>
<tr>
<td>Cortical thickness for ht SDS score</td>
<td>0.73 (0.70)</td>
<td>0.15 to 1.3</td>
<td>0.021</td>
</tr>
<tr>
<td>SSI for ht SDS score</td>
<td>-0.22 (0.49)</td>
<td>-0.63 to 0.19</td>
<td>0.251</td>
</tr>
<tr>
<td>AMI for ht SDS score</td>
<td>-0.39 (0.55)</td>
<td>-0.85 to 0.07</td>
<td>0.081</td>
</tr>
<tr>
<td>CSMA for ht SDS score</td>
<td>-0.66 (0.75)</td>
<td>-1.29 to -0.04</td>
<td>0.040</td>
</tr>
<tr>
<td>Grip force SDS score</td>
<td>-0.78 (0.77)</td>
<td>-1.41 to -0.13</td>
<td>0.024</td>
</tr>
</tbody>
</table>
6.6 Urine results

Mean value for Ca/Cr was 0.34 mmol/mmol, the normal reference range for Ca/Cr is 0.01-0.52 mmol/mmol. Therefore calcium excretion is not altered in this group.

Table 6.4 Values of urinary calcium to creatinine ratio (mmol/mmol) and magnesium to creatinine ratio (mmol/mmol) for study group and reference data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (CI) study data</th>
<th>Mean (CI) reference data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Ca/Cr</td>
<td>0.34 (0.07 to 0.93)</td>
<td>0.39 (0.34 to 0.43)</td>
</tr>
<tr>
<td>Urinary Mg/Cr</td>
<td>0.74 (0.34 to 1.27)</td>
<td>0.60 (0.55 to 0.65)</td>
</tr>
</tbody>
</table>

6.7 Dietary Data

As stated previously, the food diary’s containing the dietary information were poorly completed and therefore were not analysed.
Chapter 7

Discussion

7.1 Main findings

To the best of my knowledge this is the first study to investigate bone size, mineral content, geometry and architecture in pre-pubertal children, whose growth was constrained in utero. Urinary calcium excretion was also studied to investigate alterations in calcium homeostasis that could be impacting bone outcomes.

Although the group were not significantly smaller than the reference population they had smaller bone cross sectional area at the radius. The radius was however appropriately mineralised. Their pQCT measured bone strength parameters were lower. They also had less muscle area and reduced muscle function in the radius. However, their urinary calcium excretion was normal.

7.2 Discussion of findings

As expected, birth weight and head circumference at birth was significantly lower in the study group than the gestation and gender matched reference population. Length of newborns is not measured at birth at St Mary’s therefore this data was not available but it would have been informative to compare linear size at birth to height at time of scan. Asymmetric IUGR was confirmed in 5 out of the 8 cases by ultrasound measurements extracted from the maternal notes. Abdominal circumference was reduced more than head circumference from scans performed in the last trimester. Scans were not performed in the last trimester for the remaining 3 participants, however the birth weight SDS was lower than the birth head circumference SDS suggesting asymmetric IUGR in all 8 children. As discussed in Chapter 2 asymmetric IUGR indicates that the fetus has adapted to the suboptimal environment by prioritising nutrition for the vital organs therefore
preserving brain growth (Sheridan, 2005, Peleg et al., 1998). Asymmetric IUGR is also thought to occur in the third trimester of pregnancy, a crucial time for the mineralisation of bone when two thirds of the bodies calcium is accrued by the fetus (Mughal et al., 1989).

Measurements taken at the time of study suggest a trend for the group to be smaller in height ($p=0.06$), however the children were not significantly different in weight or BMI than the reference population suggesting catch up of weight from low to normal had been virtually complete in this age group. It was part of the study protocol to investigate the rate of catch up growth in this group, however, despite these children being so small at birth it was surprising to find that growth parameters of weight and height in the first two years had not been documented in all of the hand held child health records. The data was therefore incomplete and thus analysis investigating the growth trajectory could not be performed. More recently, accurate and regular measurement of growth in the first two years is of particular importance as since 2001 growth hormone has become licensed for "long-term treatment of growth failure in children who were born small for gestational age (SGA) who fail to manifest catch-up growth by age 2.” It is therefore hoped that this data would be available for future studies.

When looking at future health outcomes, body composition particularly distribution of fat is an important indicator. As previously discussed, despite being small at birth, the children in this study have demonstrated a catch up in weight by a mean age of 8.5 years. We have previously shown in a different cohort of children of diabetic mothers that they had a 32% higher fat mass than controls by a median age of 10 years (Mughal et al., 2010). It is therefore unfortunate that body composition parameters cannot be further explored in this group as at the time of analysis for this thesis there was no reference data for body composition
available; it is hoped that the body composition outcomes derived from the DXA data for this study can be compared to a reference population in the future.

The pQCT data has yielded novel data which provide important insights into the effect of IUGR on the cross sectional muscle area and bone geometry of the radius. In essence, this group of children who were IUGR have more slender bones in the radius with slightly thicker cortices and an appropriate amount of mineral in the periosteal envelope (Figure 7). As the children are slightly smaller in stature it is important to adjust the total area measured for height, following this the bones remain significantly smaller than controls. Due to the analysis of the DXA data not being available at this time, it is unclear if the axial skeleton is also affected. In addition, the study has found that size independent bone strength parameters derived from the pQCT measurements are reduced. SSI a measure of the bones ability to resist torsional resistance and AMI representing the bending ability of the bone are reduced respectively ($p= 0.04, 0.02$) when compared with an age and gender matched reference population. Bone strength is influenced by the distance that the bone mass is distributed away from the midline and it has been shown in other groups that the laying down of bone on the periosteal surface may compensate for alterations in bone strength (Eelloo et al., 2008, Brennan et al., 2005), however this has not occurred in this group as the bones remain small. It is therefore plausible that children who were IUGR might be at increased risk of long bone fractures arising from undue application of forces from bending or torsion. This is supported by Oliver et al (2007) who demonstrated a positive association between birth weight and weight at one year to bone strength parameters in later life.
As discussed in chapter 4, bone has been described as a mechanostat, whereby bone has the ability to adapt its mechanical properties, size, shape, mass and therefore strength by responding to forces exerted by muscles (Frost, 2003). Muscle therefore has a crucial role in skeletal development and bone health. This study found that CSMA was significantly lower than both height ($p=0.004$) and age ($p=0.014$), gender matched controls. Furthermore, grip force, a functional measure of muscle strength was significantly lower than age matched controls ($p=0.024$). These findings suggest that children who were IUGR may have altered muscle mass and function which in turn has had a detrimental affect on bone. Further study is required to elucidate the mechanisms by which these differences occur. It could however be hypothesised that the IUGR children have a smaller muscle area which results in reduced muscle force being exerted on the bone resulting in lower bone modelling and therefore growth. It is unfortunate that the body composition data was not available at this time to explore the possibility of an alteration in whole body lean muscle mass which would further support this theory. It would be pertinent in future studies to also explore these data in the tibia, a weight bearing long bone.
It could be further hypothesised that as well as there being less muscle, that the muscle function may be reduced. This could be explored in a functional study using jumping mechanography to measure muscle force in the legs or perhaps an intervention study using exercise or vibrating plates to detect muscle and bone response. A third hypothesis to be explored is that IUGR has led to alteration in bone modelling or re-modelling. Bone turnover marker studies may give an insight into this.

The background to the hypotheses of this study was that the IUGR fetus has a reduced calcium transfer from across the placenta (Mughal et al., 1989). In a previous study, calcium homeostasis was found to be altered and urinary calcium excretion reduced suggesting an adaptation had occurred in response to having reduced calcium transfer across the placenta (Mughal et al., 2005). There is no evidence to suggest that this has occurred in the IUGR group as urinary calcium excretion does not appear to be altered.

7.3 Shortcomings of the study

There are a number of shortcomings to this study. Principally, the small number of participants recruited to the study. The design of the study was a pilot to gain initial data from which to base a larger study. St Mary’s hospital is a tertiary referral centre for maternity and neonatal care and with approximately 4000 birth per year, therefore it was realistic to aim to recruit 50 children who were <10th centile for birth weight over a three year period. However, upon screening the maternity notes of those babies who were born <10th centile, a higher number than expected had to be excluded as they did not fulfil inclusion criteria. Perhaps, this could have been anticipated due to the nature of tertiary referral centres being referred difficult and unusual cases that would preclude inclusion. In addition, St Mary’s hospital is a large inner city hospital. Thus, many of the babies were from
ethnic origins that could not be included due to lack of robust reference data, and a significant number of mothers were alcohol and drug abusers who also had to be excluded. Despite this, there were still 291 children who could proceed to the next stage of recruitment. Surprisingly, at the next stage of recruitment it was noted that for the majority of the infants there was little increase in monitoring during the pregnancy and the infants returned to the maternity wards soon after delivery with no special measures and were discharged home soon after delivery with no follow up and no special advice despite the low birth weight. Following this, it was necessary to alter the original wording on the participant information leaflets so as not to alarm parents who may never have been told that their baby was IUGR; IUGR was replaced by ‘babies who were small at birth’. This may have been a significant factor for the low response from families invited to participate as they would not perceive their child to be at any increased risk than other AGA infants. Another stage of the recruitment where the numbers of suitable participants was significantly reduced was in retrieving the screening information from GP’s. Despite trying to make this process simple and time efficient for the GP’s by sending ‘tick sheets’ with the screening questions on and providing self addressed envelopes the response rate was relatively poor (65%). This response included repeat letters being sent to those that had not replied after the first contact. Although it is entirely necessary to ensure it is suitable to contact families, for both medical and social reasons, perhaps this could be more effectively done in the future via the telephone and perhaps with another suitable practitioner such as a practice nurse. It would also help to have access to the NHS database to enable the checking of current addresses and GP details before contacting them as several families had moved. As successful recruitment represented only 11% of the families contacted, it would be useful to get some
feedback from those families that were approached and decided not to participate before a larger study was to be undertaken. It could be deduced anecdotally that there is no incentive for the families to participate as they do not perceive that their child is at any more risk than other children as they were small at birth but have caught up with their peers now in growth and have no other health issues. It is a significant limitation that all of the data collected from the participants could not be analysed. Lessons to be learnt from this are that it is imperative to check availability and suitability of reference data before designing the study. Other shortcomings include that of the nature of all cross sectional studies in that there is very little accurate information on lifestyle that may have affected bone and muscle outcomes between birth and time of study. This can only be achieved by longitudinal studies. Another weakness is the multiplicity of the study. Due to the low number of participants recruited, there are more outcomes measured than participants studied, however many of the outcomes are interrelated.

7.4 Future direction

The findings of this pilot study provide evidence to warrant further investigation of bone and muscle parameters in the IUGR population. Detail of additional outcomes has been suggested within the discussion namely whole body lean muscle mass, axial skeleton bone parameters and tibial bone and muscle parameters. Long bone fractures in children are relatively common however it would be informative to interrogate GP databases to stratify number of fractures by the birth weight of the children to see if there is any increase in those of lower birth weights. In the design of a study to replicate these outcomes in a larger cohort it would be necessary to follow the children longitudinally throughout puberty to see if the effects seen are lasting.
7.5 Summary

In spite of the small numbers recruited to this study, it has yielded a unique insight into the bone geometry of the appendicular skeleton of the IUGR child. It has revealed that this group, have narrow bones at the radius with slightly thicker cortices and normal mineral density. This has resulted in reduced bone strength parameters. It is therefore plausible that these children may be at increased susceptibility to fractures of the long bones arising from torsional or bending forces. Furthermore it is likely that an alteration in muscle size and force has contributed to this. Confirmation and further investigation of these findings needs to be studied in a larger prospective longitudinal study through childhood and adolescence.
Reference List


MEHTA, G., ROACH, H. I., LANGLEY-EVANS, S., TAYLOR, P., READING, I., OREFFO, R. O., AIHIE-SAYER, A., CLARKE, N. M. & COOPER, C.


## APPLICATION FOR A TRUST RESEARCH GRANT SCHEME 2005/06

<table>
<thead>
<tr>
<th>Surname of Applicant (Principal Investigator first)</th>
<th>Forenames</th>
<th>Title</th>
<th>Qualifications</th>
<th>Age</th>
<th>Employer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eelloo</td>
<td>Judith</td>
<td>Mrs</td>
<td>RGN, RSCN</td>
<td></td>
<td>Central Manchester and Manchester University</td>
</tr>
<tr>
<td>Mughal</td>
<td>Zulf</td>
<td>Dr</td>
<td>MBChB,FRCP,</td>
<td></td>
<td>Children’s Hospitals NHS Trust (CMMUCH NHS Trust)</td>
</tr>
<tr>
<td>Roberts</td>
<td>Steve</td>
<td>Dr</td>
<td>FRCpCH, DCH</td>
<td></td>
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<tr>
<td>Ward</td>
<td>Kate</td>
<td>Dr</td>
<td>PhD</td>
<td></td>
<td>CMMUCH NHS Trust</td>
</tr>
<tr>
<td>Adams</td>
<td>Judith</td>
<td>Professo r</td>
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<tr>
<td>Emmerson</td>
<td>Anthony</td>
<td>Dr</td>
<td>PhD</td>
<td></td>
<td>CMMUCH NHS Trust</td>
</tr>
</tbody>
</table>

1. Name and address for correspondence (please include telephone and email)

   Judith Eelloo, Paediatric Research Nurse, 1st Floor Old Gynaec Block, Saint Mary’s Hospital, Hathersage Road, Manchester, M13 0JH. Tel: 0161 276 4159 E-mail: judith.eelloo@cmmc.nhs.uk

2. Place (inc. address) of research if different from above

   (1) Department of Chemical Pathology, Central Manchester & Manchester Children’s University Hospitals Trust, Manchester Royal Infirmary

   (2) Department of Clinical Radiology, Stopford Building, University of Manchester, Oxford Road, Manchester

Title of proposed research (25 words max)
The long term effects of intra uterine growth restriction (IUGR) on calcium homeostasis and bone health

3. Keywords describing research
IUGR, urinary mineral excretion, bone mineral density, pre-pubertal children

6. Is this a continuation of a Trust funded project?
NO

7. Is this a re-submission of an application for a Trust Project Grant?
NO

8. Will animals be used?
NO

9. Proposed starting date
July 2006

10. Proposed duration (months)
24

11. Has ethics approval for this project been obtained?
NO

If ‘Yes’ please attach a copy of the approval letter to this application.
If ‘No’, could you please indicate when you will be submitting an ethics application or justify why ethics approval is not needed.
Ethics approval for this pilot study will be sought if successful. We have previously had Ethical approval granted for a study with a protocol of similar methodology and measurements (CEN/01/217). We currently have approval from the Central Manchester LREC (CEN/98/036) and the North-West MREC (MREC 04/8/006) for bone mineral density measurement in healthy children & adolescents.

12 Current awards held by applicants (give title, sum awarded, funding body and end date).
Explain any apparent overlap with this application and current awards (append separate sheet if necessary)

Project details

14. Abstract

**Background & Hypothesis:** (1) Epidemiological studies have shown that poor growth *in-utero* might increase an individual's risk of developing osteoporosis in later life. Mineral accretion by the fetus is an important element of such growth. We have previously shown that the maternofetal transport of calcium across *in situ* perfused rat placentae of intra uterine growth retarded (IUGR) rats was significantly lower than in control animals. Calcium is the most important mineral constituent of the bone mineral content (BMC) and bone mineral density (BMD). (2) We have also shown that renal tubular reabsorption of calcium (Ca) and magnesium (Mg) is programmed *in-utero*, in response to impaired placental transport of these ions.

We hypothesise that in children aged 5-8 years old who were born IUGR: (1) the whole body and regional bone BMC & BMD will be lower compared to age matched controls, even after allowing for differences between height & weight. (2) Urinary Ca and Mg excretion be lower compared to age matched controls.

**Methods:** Fifty 5-8 year old children who were born IUGR will be recruited. BMC and BMD of the lumbar spine, total hip and whole body will be measured using dual energy x-ray absorptiometry (DXA) and volumetric BMD of the distal & mid radius will be measured by peripheral quantitative tomography (pQCT). Urinary calcium/creatinine ratio and magnesium/creatinine ratios will be measured in first void samples. The DXA and pQCT measured bone outcome measures in the IUGR and control groups will be compared after controlling for height, weight, calcium intake and physical activity.

**Future plans:** Results of this pilot study will be used to design a larger and adequately powered study and seek external funding from the Wellcome Trust or the National Osteoporosis Society.
15. Lay Summary

It has been shown that babies who grow poorly in the womb have an increased risk of developing osteoporosis, a brittle bone disease of late adulthood. Osteoporosis costs the NHS in excess of £940 million per year and hip fractures (>70,000 per year) are an important cause of morbidity and mortality among older populations. Results of our previous research have shown that there is reduced transfer of calcium across the placenta (after-birth) in experimental models of fetal growth restriction in the womb. Calcium is necessary for building healthy bones. The main aim of our proposed pilot research study is to find out if bone mineral content (BMC) and bone mineral density (BMD), measured non-invasively using state of the art scanners, are lower in 5 to 8 year old children who had impaired growth in the womb, compared with age matched children who grew normally in the womb. BMC and BMD are surrogate markers of bone strength. We will also find out if the amounts of calcium and magnesium minerals lost in the urine are different in the two groups of children. We believe that results of such a study might help to identify potentially modifiable factors during pregnancy and early childhood that may have long lasting beneficial effects on bone health.

16a. Please describe how “users” (i.e. patients & their carers) have been involved in this project e.g. involved in formulating the research idea, or are involved in conducting the research. If users have not been involved please justify and explain this.

This research group has strong links with the National Osteoporosis Society (N.O.S) both at local and national levels. Professor Judy Adams is currently chair of Bone Densitometry Forum and on the Board of Trustees of the council. One of the priorities of the N.O.S is to identify perinatal and childhood factors that may contribute to development of osteoporosis during adulthood. The applicants have extensive experience in research within children and constantly re-evaluate any feedback they may have from users who have been involved in their research such as information leaflet format, questionnaire design and response to outcome measures.

16b. Please describe the anticipated outcomes and benefits of the research on the Trust services.

We believe that the results of this pilot study will enable us to seek substantial external funding to conduct a larger and adequately powered study. Results of such a study might help to identify potentially modifiable factors that occur ante-natally and during childhood, which may have long lasting beneficial effects on bone health.

17. Description of the project (under the suggested headings: background, hypothesis, aims, plan of investigation, outcomes and references (maximum 2 sides)

(a) BACKGROUND

Possible effects of intrauterine growth retardation on bone mass acquisition children & adolescents.

Epidemiological studies have shown that cardiovascular disease and type 2-diabetes are associated with small size at birth (1). The fetus is thought to be ‘programmed’ as an adaptation to undernourishment, permanently changing its structure, physiology and metabolism. Placental transport of minerals and their accretion in to the fetal skeleton might affect an individual’s bone health through the process of perinatal programming of bone and mineral metabolism. In a retrospective study of adults aged 61-73 in Herefordshire, investigators from the MRC Environmental Epidemiology Unit in Southampton showed that bone mineral density (BMD) was lower among those with the lowest birth weights (2). Thus, poor growth in-utero might increase an individual's risk of developing osteoporosis in later life. Mineral accretion by the fetus is an important element of such growth. We have previously shown that the maternofetal transport of calcium across in situ perfused rat placentae of intra uterine growth retarded (IUGR) rats was significantly lower than in control animals (3). Calcium is the most important mineral constituent of the bone mineral content and BMD. In animals and humans, the BMD measured shortly after birth and up to 12 weeks of age is reduced in IUGR infants, compared to appropriate for gestation infants (4,5,6). It is plausible that the deficit in skeletal mineralisation in ex-IUGR infants might persist during childhood.
Possible effects of intrauterine growth retardation on urinary mineral excretion during children & adolescents.

We have shown that offspring of diabetic rats & humans have reduced urinary calcium (Ca) and magnesium (Mg) excretion compared to offspring of controls, and these differences persisted up to young adulthood (7,8). These results suggest that renal tubular reabsorption of Ca and Mg might be programmed in utero, probably in response to impaired placental transport of these ions. Uptregulation of calbindin-D_{28K} and the plasma-membrane-Ca^{2+}-ATPase in the fetal nephron, which are responsible for re-absorption of filtered Ca, has been confirmed in offspring of diabetic rats (9). As placental calcium transport is impaired in IUGR fetuses (3), it is plausible that the urinary Ca and Mg excretion be lower in children who were born IUGR.

(b) AIMS AND HYPOTHESIS

To our knowledge, no studies have assessed the accrual of bone mineral content (BMC) and BMD assessed using the dual-energy x-ray absorptiometry (DXA) and peripheral quantitative tomography (pQCT) in children aged 5-8 years old who were born IUGR. The following specific hypothesis will be tested:

1. (a) DXA measured BMC (g) and areal BMD (g/cm^2) at the lumbar spine, total hip and whole body will be lower in children aged 5-8 years old who were born IUGR compared to age matched controls, even after allowing for differences between height & weight in the two groups.

   (b) The distal radial (cortical & trabecular) and mid-radial (cortical) volumetric BMD (vBMD mg/cm³; is less dependent on bone size ) measured by pQCT will be lower in children aged 5-8 years old who were born IUGR compared to age matched controls.

   (c) Within the IUGR group, the above mentioned DXA and pQCT measured variables will be inversely related to severity of IUGR.

2. Urinary Ca and Mg excretion be lower in children aged 5-8 years old who were born IUGR compared to age matched controls.

(c) PLAN OF INVESTIGATION

For this pilot study we will recruit 50 children aged 5-8 years old who were IUGR, defined as birthweight < 10th centile, at the time of their birth. The existing comprehensive perinatal database at St Mary’s hospital will be used to identify the potential subjects for the study. Subjects whose IUGR was caused by chromosomal problems, intrauterine infections and maternal use of illicit drugs will be excluded. Other exclusion criteria include those children who suffer from a bone disorder, such as osteogenesis imperfecta and chronic childhood illnesses, such as cystic fibrosis will also be excluded. Only white Caucasian subjects will be recruited as currently there are no control data for other races that are gathered using DXA & pQCT densitometers in the University Department of Radiology. Bone & urine mineral (Ca & Mg) excretion data gathered on age matched white Caucasian children, who were recruited for a study of bone mass acquisition during childhood, will serve as controls.

(d) STUDY TIMESCALE & OUTCOME MEASURES

We are confident that the pilot study will be complete in 2 years, allowing the first 6 months for obtaining ethical approval and recruitment, 12 months for the gathering outcome data, and 6 months for data analysis and write up.

Bone mineral content & density measurements. Whole body, lumbar spine and total hip bone area, BMC and BMD will be measured using the Hologic QDR Discovery DXA fan beam scanner (software version 12.1, fast array mode). The in-vivo coefficient of variation (CV) for these variables in adults ranges from 1% to 1.3%. The distal radial (cortical & trabecular) and mid-radial (cortical) vBMD will be measured using the Stratec-Norland XCT 2000 pQCT scanner. The pQCT has the ability to assess BMC, bone volume and vBMD, without influence from body (skeletal) size, which is a major advantage when used in children. The in-vivo the CV is <1.0%.

Urine calcium and magnesium excretion. A first void urine sample will be collected at analysis of urinary calcium/creatinine ratio and magnesium/creatinine ratio will be undertaken at the Biochemistry laboratory, Manchester Royal Infirmary (8).
Other variables. Standing height will be measured using a wall mounted stadiometer and weight will be measured using digital scales. A 3-day food diary will be completed by parents and children and daily intake of minerals and vitamin D will be estimated using the Comp-Eat Nutritional Software. In each subject, a validated questionnaire will be used to assess subjects’ weight-bearing physical activity (10).

(e) DATA ANALYSIS
The DXA and pQCT bone outcome measures in the IUGR and control groups will be compared using appropriate analysis of covariance as in previous studies (8,11). The bone parameters will be appropriately transformed as determined previously. Appropriate covariate adjustment will be dependent on the parameters but will always include careful adjustment for age using cubic spline functions. Urine data will be similarly analysed as in previous studies (8). Within the IUGR group, we will also explore the relationship between bone size, BMD and vBMD variables and the severity of IUGR as reflected by gestation -specific birth weight standard deviation scores.

(f) REFERENCES

There is currently no data to determine if children who were born following IUGR have altered bone size, mass or structure that may put them increased risk of fracture or osteoporosis as an adult. If the results of this study suggest that this group of children are indeed at risk of poor bone health we will seek external funding from the National Osteoporosis Society or the Wellcome Trust to conduct a larger study.

Details of support requested
Basic salary (including increments) must be shown separately from National Insurance and Superannuation. A provision for nationally agreed pay awards during the term of the grant must be included.

(Please continue on separate sheet if necessary)
## STAFF

<table>
<thead>
<tr>
<th>Name</th>
<th>Basic Salary:</th>
<th>NI &amp; Superan:</th>
<th>Prov. for pay award:</th>
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<tr>
<td>0.1 WTE Band 6 Paediatric Research Nurse (remainder of 0.5 WTE will be supplemented as nurse already employed by NNMMU)</td>
<td>£2515</td>
<td>£352+£322</td>
<td>£210 (increment)</td>
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</table>

Name: ........................................... ...........................................

Name: ...........................................

Total salaries: £3399 £3511 £6910

## CONSUMABLES AND INVESTIGATIONS
Please include a full description of all consumables and investigations and their associated costs—continue on a separate sheet if necessary

1. Bone density scans for 50 participants @ £125 each
   - 1st Year: £3125
   - 2nd Year: £3125
   - Total: £6250

2. Urine specimen analysis including provision of specimen pots for 50 participants @ £10 each
   - 1st Year: £250
   - 2nd Year: £250
   - Total: £500

3. Travel cost reimbursement for 50 participants @ £10
   - 1st Year: £250
   - 2nd Year: £250
   - Total: £500

4. Stationary and postage
   - 1st Year: £250
   - 2nd Year: £250
   - Total: £500

Total consumables: £3875 £3625

## TOTAL SUPPORT REQUESTED
- 1st Year: £5112
- 2nd Year: £1370
- Total: £428

TOTAL SUPPORT REQUESTED: £14410

---

I have read the grant guidance notes and regulations and agree to abide by the rules should the application be successful. I note that no alterations can be made without prior approval from the Trust Research Grants Committee.

Applicant’s Name (Caps): ...........................................................

Signature: ...........................................................................

Date: ___________________________
The application form must be signed by the Clinical Director and accountant of the Trust department in which the research will be taking place.

I confirm that I have read and support this application, and if successful, have the necessary accommodation and facilities in my department. I also confirm that the salary of the applicant/principal investigator, unless applied for here, is guaranteed during the term of the grant.

Clinical/ Divisional Director’s Name (Caps): Signature:

Date:
(Trust –based)

I confirm that I have read and accept the conditions under which grants are awarded and that the salary details given are correct and include a provision for nationally agreed pay awards.

Directorate/Divisional Accountants Name (Caps): Signature:

Date:
(Trust-based)
Appendix 2 Grant Award Letter

Central Manchester and Manchester Children's University Hospitals
NHS Trust

10 May 2006

Mrs Judith Eelloo
Paediatric Research Nurse
1st Floor Old Gynaec Block
St Mary's Hospital
Hathersage Rd
Manchester M15 6FH

Dear Mrs Eelloo

Re: Trust Research Grant Scheme 2005/06

R&D Ref: 10021
Project Title: The Long term effects of intra uterine growth restriction (IUGR) on calcium homeostasis and bone health

The Trust Research Grants Committee met on the 26th April 2006 and I am pleased to inform you that your application was successful. The amount you have been awarded is £14410.

We received many excellent applications and wish to encourage as many researchers as possible. We have, therefore, reduced the amount awarded to some of the grants from that which was originally requested.

To arrange payment of these funds, please contact Gisela Taylor, Finance Department, on 0161 276 8974.

We would expect all grants to be active by August 2006 and continue for 24 months (as specified in your application). If you envisage difficulties in commencing your study by then please let us know. Please also keep me informed of any changes which may occur during the life of the project.

The Committee would be pleased to see how the Scheme impacts on the Trust's research portfolio and as such has requested that awardees write a short report at the closure of the study. This could detail any successes relating to new discoveries, improvements in service and/or successful grant applications which may have been secured as a result of receiving the pump-priming award.
Please ensure that a copy of your ethics submission and ethics favourable opinion are sent to the R&D Office when they are available. This will enable R&D to provide approval for you to commence the study.

I would like to take this opportunity of congratulating you on your success and also to wish you all the best with this research. If you have any questions regarding the award please contact Alison Robinson on 276 4902.

Yours sincerely

[Signature]

Professor David Henson
Chair
Research Grants Committee

cc: Gisela Taylor, Finance Department, Cobbet House
    Alison Robinson, Research & Development
Appendix 3 Ethics approval letter

National Research Ethics Service

Central Manchester Research Ethics Committee
Room 181
Gateway House
Piccadilly South
Manchester
M60 7LP

Telephone: 0161 237 2163
Fax: 0161 237 2383

1 July 2008

Dr Z Mughal
Consultant Paediatrician and Honorary Senior Lecturer in Child Health
Division of Paediatric Medicine
St Mary's Hospital
Hatherleigh Road
Whalley Range
Manchester
M13 9JH

Dear Dr Mughal,

Study title: The Long Term Effects of Intra Uterine Growth Restriction (IUGR) on Calcium Homeostasis and Bone Health

REC reference: 07/Q14007/17

This study was given a favourable ethical opinion by the Committee on 30th of March 2007.

It is a condition of approval by the Research Ethics Committee that the Chief Investigator should submit a progress report for the study 12 months after the date on which the favourable opinion was given, and then annually thereafter. To date, the Committee has not yet received the annual progress report for the study, which was due on 30th of March 2008. It would be appreciated if you could complete and submit the report by no later than 1st of August 2009.

Guidance on progress reports and a copy of the standard NRES progress report form is available at: http://www.hra.nhs.uk/applicants/overview/progressreport.htm

There is also guidance on declaring the end of the study at: http://www.mrepp.nhs.uk/applicants/overview/declareendofproject.htm

[Failure to submit progress reports may lead to a suspension of the favourable ethical opinion for the study.]

The Research Ethics Committee is an advisory committee to North West Strategic Health Authority.

The National Research Ethics Service (NRES) represents the NHS Ethical review bodies of the National Research Ethics Service, the National Research Ethics Committee, and Research Ethics Committees in England.
Appendix 4 CMFT/ Manchester University approval letter

Central Manchester and Manchester Children’s University Hospitals
NHS
Trust
Research & Development
1st Floor Post Graduate Centre
Manchester Royal Infirmary
Oxford Road
Manchester M13 9WL
Tel: 0161-276-4902
Fax: 0161-276-5766
Alton.robinson@cmtrc.nhs.uk

22 October 2007

Ref: 10021- Ltr-2- Mughal

Dr Zulf Mughal
Consultant Paediatrician and
Honorary Senior Lecturer in Child Health
St Mary’s Hospital
Hathersage Road
Manchester M13 0JH

Dear Dr Mughal,

Research Study: “The long-term effects of intrauterine growth restriction (IUGR) on calcium homeostasis and bone health.”

PIN: 10021 (please use this reference number in any future correspondence)

Thank you for submitting a Pan Manchester Research Notification for the above study. I am pleased to be able to confirm that the R&D Office now has all the required information concerning this research and that the Trust’s Director of Research and Development has given approval for the project to be undertaken.

We acknowledge that the Central Manchester and Manchester Children’s University Hospitals has accepted the role of Research Sponsor for this study (ref. Research Governance Framework, as issued by the Department of Health).

Details of the project have been recorded on the Trust R&D Management Information System and the project has been given a unique identification number (PIN), as shown above. (A copy of the signed notification form is enclosed for your records).

Please note, it is a requirement of the approval given by the Trust that the research project is being conducted in line with the guidance given within the Research Governance Framework. Further guidance is available on the R&D web pages (see above), or request a CD from the R&D office.

Please draw your attention to the need to comply with both the Health and Safety at Work Act and the Data Protection Act. If you require further information or advice in any of these latter areas please contact the Trust’s Health & Safety Advisor, Mr Ken Wood, on 276 4262 or the Trust’s Data Protection Officer, Ms Cara Lally on 276 4878.

In line with this framework I would be grateful if you would inform me of the actual start date of this particular project and any changes that might be made to it during its course. Your help and support would be gratefully received.

I would like to take this opportunity to wish you well with your research

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http://www.doh.gov.uk/research/ridohsrsp/strategyandresearch/governance/govhome.htm

82
Ref: 10023. Lt 2. Mughal

Yours sincerely,

Alison Robinson
Research Operations Manager

Enc (signed PMNF)

Cc

Mrs Judith Edlooe
Paediatric Research Nurse
1st Floor Old Gynae Block
St Mary's Hospital
Hatherage Road
Manchester M13 0JH
Appendix 5 GP screening questionnaire

To: Judith Eelloo
Paediatric Research Nurse
The New Manchester Children’s Hospital
Oxford Road
Manchester
M13 9WL
Judith Eelloo-Paediatric Research Nurse Direct Line :0161 276 4159
Fax No : 0161-276 6907

Name:               D.O.B
Address:
□ Please tick here if the family has moved out of the area and give forwarding address if known.
□ Please tick here if the above details are correct and child is still alive.
Please state childs ethnic group:
Does he/she suffer from any of the following conditions?

YES  NO

➢ Cerebral Palsy
➢ Any problem with mobility
➢ Epilepsy
➢ Registered blind /Deaf-(hearing aids)
➢ Autistic spectrum disorder
➢ Chronic illness

If yes please, state the diagnosis e.g cystic fibrosis

YES  NO

➢ Has he/she required regular oral steroids
➢ Is there a family history of osteoporosis

➢ Is this family suitable to send a letter of invitation for the study

Any other Comments:

Many thanks for your co-operation
Parent/Guardian Information Leaflet

You and your child are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what taking part will involve for your child.
- Part 2 gives you more detailed information about the conduct of the study.

Please ask us if there is anything that is not clear or if you would like more information. Take time to decide with your child whether or not you wish to take part.

What is the background to and purpose of the study?

We at Saint Mary’s Hospital and the University of Manchester are doing some research to try and find out what makes children’s bone strong. We already know from previous research that birth weight and bone size affects bone health. In this study we want to find out if being born at full term but with a lower birth weight than average has any lasting effect on the size and strength of children’s bones. Babies are sometimes born small as a result of growing more slowly in the womb, this may be referred to as intra uterine growth restriction, IUGR. Previous studies have shown that in babies who grow more slowly in the womb, less calcium may pass from the mother to the baby in the womb. Calcium and magnesium are necessary for the building of healthy bones. It is normal for us all to pass some calcium and magnesium out of our bodies in our urine. Based on results of our previous research studies, we believe that babies who grow more slowly in the womb may adapt to having less calcium by passing less minerals (calcium and magnesium) in the urine.

The goals of our proposed research are to measure the size and the amount of minerals in the bone (a marker of the bones strength) by taking special x-ray pictures of the children’s bones called bone scans. We would also like to measure the amount of calcium and magnesium that is passed in the urine of children who were small when they were born. We will then compare these measurements with a group of children who were not small at birth. The findings from the study are of great importance to ensure that we continue to give the best possible care and advice to mothers when their babies are found to be growing more slowly both during pregnancy and early childhood in the future.

Why has my child been chosen?

There is no reason to worry about your child’s bones. We are contacting you because our records show that your child was born at full term here at St.Mary’s and was either smaller than average at birth or showed some signs of slower growth in the womb. You may have had extra scans antenatally to check the
babies' growth. We would like to look at the bones of 50 children and compare them to babies who were not small at birth and see if there has been any lasting difference in the size and strength of their bones or the amount of minerals that they excrete in their urine.

**Does my child have to take part?**

No. This is a voluntary research study; it is up to you and your child to decide whether or not you would like to take part. Even if you initially agree to take part you can withdraw your child from the study at any time without giving a reason. If your child is not happy about having the scans when you arrive there will be no pressure to continue. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives.

If you and your child are happy to take part and are happy with the information and explanations you have received, you will be asked to sign a consent form. If your child wants to, and is able, they will be asked to write their name on an assent form with you to show that they are happy to take part. You will be given a copy of the information leaflets, consent and assent forms to keep.

**What will happen to my child if we agree to take part?**

Your child will attend the x-ray department at Manchester Royal Infirmary for approximately one hour to have bone density scans and a plain hand x-ray to determine exact bone age. Your child will also be weighed and measured and relevant medical information will be extracted from the maternity notes and your child’s birth notes and medical records. We would also like to look at how your child has grown by looking at the weights and measurements recorded by your health visitor in your parent held child health records (red book).

You will also be sent the following to complete at home with your child prior to attending for the scans:

- A general health questionnaire e.g. family history, medication or exercise to help detect factors that may affect your child’s bone health
- A 3-day food diary to see how much calcium and magnesium your child currently eats in their diet.
- If your child is 9 or above, we will ask you to fill in a puberty self-assessment chart to indicate your child’s stage of development. It is important for us to know your child’s stage of development as many changes occur in bones during puberty.
- A pot to collect a small urine sample from your child at home and bring to the appointment with you so that we can measure the amount of calcium and magnesium that are excreted in the urine.

**Bone Density scans**

The size, thickness and density of the bones in your child’s hip, spine, forearm

This machine on the right is called Dual Energy X-Ray Absorptiometry (DXA for short). It will be used to measure the size and strength of the bones in your child’s hip, spine and whole body. This scan will take about 10 minutes. Your child can have a picture of their skeleton and bones to keep from this scan! You will be able to stay with your child during the scan.
What will having the scans be like for my child?
You can be with your child for all of the scans unless you are pregnant. Your child may wear their own clothes as long as there is no metal e.g. Zips or hooks, jogging pants or a skirt and t-shirt are ideal. Your child will need to remain very still for the scans; music, story tapes and television will be used where possible to aid this. To avoid disruption to schooling, we will make every effort to schedule visits for after school or during school holidays. Drinks and biscuits will be available! Travel expenses will be reimbursed.

Are the X-ray scans dangerous?
The scans are safe, do not hurt and are easy to perform. There is a small risk from the ionising radiation that is used to carry out the bone scans and hand X-ray. The bone scans are taken using special X-ray equipment that uses only a small amount of ionising radiation. We are exposed to ionising radiation in our natural environment from rocks and buildings (background radiation) and during air flights. The amount of extra ionising radiation that your child will be exposed to by taking part in the study is 0.012mSv, which is equivalent to 2 days UK background radiation.

What are the possible benefits to my child of taking part?
We cannot promise the study will help your child but the information we get may potentially alter the advice we give to mothers whose babies are small or grew more slowly in the womb both during pregnancy and early childhood and this may have a beneficial effect on the bone health of these children in the future.

What happens when the research study stops?
Your child will need no further follow up. In the unexpected event that a problem is found with your child’s scans your child’s GP will be informed and you will be referred to see Dr Mughal in his clinic.

What if there is a problem?
If you have any complaint about the way that you or your child have been dealt with during the study or any possible harm you feel you might have suffered it will be addressed in the same way as NHS patients. The detailed information on this is given in Part 2.

Will taking part in the study be kept confidential?
Yes. All the information about your child’s participation in this study will be kept confidential. The details are included in Part 2.

**Whom should I contact if I have questions or would like more information about the study?**
Judith Eelloo, paediatric research nurse on 0161 276 4159 or Dr Mughal, Consultant Paediatrician and Principal Investigator- 0161 276 6501:
St Mary’s Hospital
Hathersage Road
Manchester
M13 0JH

This completes Part 1 of the information sheet. If you are considering participation, please continue to read the additional information in Part 2 before making any decision.

**Part 2**

What if relevant new information becomes available?
A letter from Dr Mughal will advise you of this

**What will happen if my child or I don’t want to carry on with the study?**
This is a voluntary research study, you or your child do not have to take part. Even if you initially agree to take part you can withdraw your child from the study at any time without giving a reason. If your child is not happy about having the scans when you arrive there will be no pressure to continue.

What if there is a problem?
All complaints will be handled in accordance with the NHS complaints procedure. If you have a concern about any aspect of this study, you should ask to speak to one of the researchers who will do their best to answer any concerns or questions: Dr Mughal 0161 276 6501 or Judith Eelloo 0161 276 4159. If you remain unhappy or would like to speak to somebody not directly involved in the study please contact the Patient Advisory and Liaison Service on 0161 276 8686 or the trust research and development department on 0161 276 3565.
All NHS and University of Manchester employees who are involved in this study are indemnified for their activities in the event that anything untoward happen during the course of the study.

**Will my child’s taking part in this study be kept confidential?**
Each child who takes part in the study will be given a unique study number and all identifying data such as name and date of birth will be removed. The information will be stored anonymously in accordance with the Data Protection Act 1998. Publications and reports on the results of the study will not include the name of your child. Data will be retained for 21 years and then disposed off securely.

**What will happen to the results of the research study?**
The results will be processed as quickly as possible at the end of the study and should be published about 12 -18 months later. A copy of the published articles can be sent to you.

Who has reviewed and approved this research study?
A Local Research Ethics Committees has approved the study. The Research and Development department at the Central Manchester and Manchester Children’s University Hospitals NHS Trust have also approved and funded the study.

What should I do now?
If you or your child have any questions or would like some more information please call Judith Eelloo, paediatric research nurse on 0161 276 4159 or Dr Mughal on 0161 276 6501. If you have decided with your child that they would be willing to take part in this important study we are most grateful. Please complete the enclosed slip and return it in the stamped addressed envelope provided. You will be contacted within the next two weeks to discuss the study in more detail.

Thank you for taking the time to read this information even if you have decided not to take part in the study.
A study to look at the size and strength of the bones of children who were born small

Children's Information Leaflet
(Please read this with your mum or dad)

Hello. My name is Judith, I am a children's nurse and I work at Saint Mary's hospital in Manchester where you were born. Here at Saint Mary's we are trying to find out what makes children's bones strong. We would like to see if the bones of children who were small babies like you are different to babies who were bigger than
you when they were born. This is nothing to worry about and does not mean there is anything wrong with your bones.

Calcium and minerals that we get from eating foods like milk, cheese & broccoli help to build strong and healthy bones but we also pass some calcium and minerals that our bones don’t need out in our urine (wee).

We would therefore like to take some special pictures of your bones called ‘bone scans’ and test a sample of your wee.

This leaflet tells you all about the research study. Ask your mum or dad about it and then you can decide if you would like to come and help.

If you would like to come and take part in the study, you will come to the Manchester Royal Infirmary with your mum or dad to have some pictures taken of your bones. To take the pictures we use special machines called scanners. We will try to do this after school or during your school holidays so that you do not have to miss time off school.

**How will the pictures be taken?**

Here is a picture of one of the scanners. This will take a picture of all the bones in your body. You will have to lie still on the bed and the scanner will move over you and take the pictures. Your mum or dad can
Does it hurt when the pictures are taken?

No. The scans are safe and do not hurt. You will not feel the pictures being taken. To take the pictures the scanners use a small amount of something called ionising radiation. Ionising radiation is present in the air around us in small amounts in rocks and buildings and when we go on aeroplanes. If we have too much ionising radiation there is a small risk that it can harm our body so the doctors have made sure that they will not take too many pictures.

What else will I have to do if I take part?

Calcium is a mineral that helps make our bones strong and healthy. We all eat it everyday in our food, for example it is in milk. We would like to see how much calcium you eat, so we would like you to keep a food diary for 3 days. This means that you need to write down everything that you eat or drink. Your mum or dad will help you.

Doing exercise also helps make your bones strong so we would like you to write down all the exercise that you do like P.E at school, or football, swimming and bike riding at home.

We will also send you a pot to collect a small amount of your wee in at home before you come for the scans. Mum or dad can help you.
If you are 9 years or older, we will also send you a chart to fill in at home with your parents that will tell us what stage of development your body is in.

When you come to Manchester to have your bone pictures taken I will measure you to see how tall you are and weigh you to see how heavy you are and we will take an x-ray picture of your hand.

**What If I Change My Mind?**
You can change your mind about coming at any time. Even if you come to have your bone pictures taken and then change your mind and want to go home that’s all right. If you are not happy about anything in the study please tell your mum or dad. You can ask me any questions at any time you feel you want to.

**What shall I do now?**
Talk to your mum or dad. If you, your mum or dad have any questions about the study you can call me, Judith (research nurse) on 0161 276 4159. If you and your mum or dad decides that you would like to come and take part in the study your mum or dad can fill in the form that I have sent and send it back to me. I will then ring your mum or dad to arrange a suitable time when you will be able to come and have the pictures taken.

It does not matter if you have decided that you do not want to take part in this study.

Thank-you for reading this leaflet.
Appendix 8 Parent/Guardian Research Consent Form

Participant Identification Number:

Title of Study: The long-term effects of intra uterine growth restriction (IUGR) on calcium homeostasis and bone health

Name of Researchers:
Dr M Z Mughal - Consultant Paediatrician & Honorary Senior Lecturer in Child Health
Professor J Adams - Professor of Diagnostic Radiology,
Judith Eelloo – Paediatric Research Nurse

Please initial box
1. I confirm that I have read and understand the information sheet dated 08/05/09 (Version 5) for the above study. My child and I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my child’s participation is voluntary and that he/she is free to withdraw, at any time, without giving any reason, without their medical care or legal rights being affected.

3. I understand that relevant sections of maternity notes and any of my child’s medical notes and data collected during the study, may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my child taking part in this research. I give permission for these individuals to have access to my child’s records.

4. I agree to my child’s GP being informed of my participation in the study.

5. I agree to my child taking part in the above study.

______________________ ________________ ____________________
Name (Print) Date Signature

Relationship to child

______________________ Date ____________________
Researcher Date Signature

Name of person taking consent if not researcher

______________________ Date ____________________
Name of person taking consent if not researcher Date Signature

1 copy to participant, 1 copy for researcher file, 1 copy (original) to be kept in medical notes
Appendix 9 Author publications/ presentations whilst studying for MPhil

Manuscripts
1. Body Composition and Bone Status of Children Born to Mothers with Type 1 Diabetes Mellitus
Archives of Disease in Childhood (95): 281-285 2010.

2. Bone status of children aged 5-8 years, treated with dexamethasone for chronic lung disease of prematurity.

Published Abstracts


3. Forearm muscle and bone parameters in pre-pubertal children with neurofibromatosis type 1(NF1) KA Ward, JE Adams, J Eelloo, SM Huson, DGR Evans, MZ Mughal Bone, Volume 45, Supplement 2, July 2009, Pages S61


Oral/ Poster Presentations
2. Bone mineral density (BMD) in 5-8 year olds who were treated with Dexamethasone (Dx) for chronic lung disease of prematurity

**Eelloo JA, Roberts SA, Emmerson AJB, Ward KA, Adams JE, Mughal MZ**

Oral presentation at Manchester Paediatric Club Annual General Meeting Manchester 2006

Oral Presentation at Royal College of Paediatrics and Child Health Spring Meeting York 2007

Poster presentation at 4th International Childrens Bone health Conference Montreal Canada 2007

3. Intrauterine programming of urinary calcium and magnesium excretion in children born to mothers with insulin dependent diabetes mellitus


Poster Presentation American Society of Bone and Mineral Research Annual Meeting 2003 Minnesota USA Poster won plenary prize