THE EFFECT OF MATERNAL MALARIA DURING PREGNANCY ON BIRTH SIZE, EARLY CHILDHOOD GROWTH AND BLOOD PRESSURE IN NIGERIAN CHILDREN

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LIST OF ABBREVIATIONS

ACE	-	Angiotensin Converting Enzyme
AMH	-	Adeoyo Maternity Hospital
BF for MP	-	Blood Film for Malaria Parasites
BP	-	Blood Pressure
CHD	-	Coronary Heart Disease
CV	-	Coefficient of Variation
CVD	-	Cardiovascular Disease
CVS	-	Cardiovascular System
DBP	-	Diastolic Blood Pressure
FBC	-	Full Blood Count
GH	-	Growth Hormone
HDL-C	-	High Density Lipoprotein-Cholesterol
HIV	-	Human Immunodefficiency Virus
IGF-I	-	Insulin-like Growth Factor I
IGF-II	-	Insulin-like Growth Factor II
IGFBP-I	-	Insulin-like Growth Factor Binding Protein I
IGFBP-3	-	Insulin-like Growth Factor Binding Protein 3
IPT	-	Intermittent Preventive Therapy
IUGR	-	Intrauterine Growth Retardation
LBW	-	Low Birth Weight
LDL-C	-	Low Density Lipoprotein-Cholesterol
MUAC	-	Mid-Upper Arm Circumference
NCHS	-	National Centre for Health Statistics
NHANES	-	National Health and Nutritional Examination Survey
OFC	-	Occipito-Frontal Circumference
PAR	-	Predictive Adaptive Responses
PCV	-	Packed Cell Volume
RTM	-	Regression to the Mean
SBP	-	Systolic Blood Pressure
SDS	-	Standard Deviation Score
SGA	-	Small for Gestational Age
SOP	-	Standard Operating Procedure
SP	-	Sulphadoxine – Pyrimethamine
TC	-	Total Cholesterol
TLC	-	Total Leucocyte Count
TG	-	Triglycerides
TNF	-	Tumour Necrosis Factor
UCH	-	University College Hospital
WHO	-	World Health Organization

ABSTRACT

Background: In Nigeria, there is an escalating incidence of hypertension, its complications and other cardiovascular risks, likely to have their origins in early life. Malaria is still hyperendemic, with pregnant women at increased risk, with associated consequences of maternal anemia and high rates of delivering low birth-weight babies.

Aims and Hypothesis: In this study, we have tested the hypothesis that malaria in pregnancy will not only enhance the risk of small birth size and poor infant growth, but will also generate higher blood pressures in infancy and beyond. We also tested the hypothesis that metabolic markers in pregnant mothers affected by malaria would relate to infant birth size. Thus the aims of this project were: 1) to define relationships between the type of malaria exposure and birth size, 2) to characterize the association between maternal and cord metabolic biomarkers and birth size on the background of prenatal malaria exposure and 3) to examine the effect prenatal malaria exposure on first year growth and whether higher blood pressure (BP) is generated.

Methods: Healthy pregnant women were recruited and followed at Adeoyo Maternity Hospital, Ibadan. Anthropometric, BP, and biomarkers (lipids, glucose, insulin and TNFα) measurements were obtained in the mothers at booking. Birth size and growth at 3 and 12 months along with biomarkers (as above) and IGF-I measures in cord blood were assessed in the infants. Blood films for malaria parasites were taken throughout pregnancy including delivery and in all babies. Women were grouped to distinguish between the timing of malaria parasitaemia (either during pregnancy only or during pregnancy and at delivery) and the severity of malaria infection (low vs high parasite load). At birth, 436 mother-baby pairs were measured. 467 maternal samples were obtained for metabolic profile and 187 cord blood samples. 318 babies were all followed from birth to 3 and 12 months.

Results: Malaria parasitaemia was found in 48% of the women, associated with younger maternal age, being primigravid and a lower haematocrit. Babies of mothers with high parasitaemia through pregnancy had the smallest birth growth parameters compared with those without malaria (weight, length, and head circumference were smaller by 300g, 1.1cm and 0.7cm respectively, all $p \leq 0.005$) but their systolic BP (SBP) and diastolic BP (DBP) adjusted for weight were higher than those with low parasitaemia by 1.7 and 1.4 mmHg/kg respectively. SBPs were lowest in babies of mothers with malaria at delivery implying an acute effect on the babies' circulation.

Mothers with malaria had significantly lower lipids (except triglycerides) but higher TNFα, effect not seen in cord blood. Cord IGF-I was significantly lower in babies whose mothers had malaria. Significant determinants of birth size were maternal total cholesterol, LDL-cholesterol, insulin, malarial status and cord insulin and IGF-I.

Babies exposed to maternal malaria remained smaller at 1 year, most marked in boys, whose SBP adjusted for weight at 3 and 12 months was higher than those not exposed. Change in SBP over the first year was greater in boys than girls while the change in girls was greater in those exposed to maternal malaria than those not exposed (18.7 vs 12.7 mmHg, 95% CI 1-11, p=0.02). 11% of boys (>twice expected) had BP $\geq 95^{th}$ percentile (hypertensive, US criteria) of whom 68% had maternal malaria exposure. Gender, maternal malaria exposure and weight change were all independently associated with increased change in BP to 1 year.

Conclusion: Intrauterine exposure to malaria appears not only to have an important impact on birth size but also gender-dependent effects on growth and changes in infant BP. These findings have potential implications for cardiovascular health in sub-Saharan Africa and may contribute to the global burden of hypertension.

DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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CONTRIBUTION

This is to confirm that Omolola Ayoola played a key role in the conception of the study detailed in this thesis and in writing the papers which have been submitted for publication. She wrote the grant proposal with Professor Clayton and Professor Cruickshank and obtained funding for the project.

At the commencement of the study, Omolola prepared and submitted the ethics application, developed the standard operating procedures for the study and set up the study database. She undertook tasks that were required for setting up and smooth running of the study which was carried out at the Adeoyo maternity hospital and surrounding community in Ibadan, Nigeria.

With Dr Whatmore, she arranged for the ordering and procurement of equipment and supplies for the study which were shipped to Ibadan.

Omolola went to Ibadan to establish the study and undertook the following tasks:

- Advocacy visits to the study site with meetings with the senior management of the hospital.
- Recruitment of research staff such as: nurses, laboratory technicians, data manager, administrative support staff and field support staff.
- Preparation of inventories for all equipment and supplies and procurement of laboratory supplies, printer, scanner, stationeries, stabilizers, computer consumables and other things required for the study in Ibadan.
- Preparation of data collection forms for the mothers and their babies, protocols and materials for the research staff training,
- Training of research staff in anthropometric measurement, blood, laboratory techniques and data management.
- With the research team, consenting, recruitment and follow-up of study subjects (mothers and babies), their measurements, blood sample collection and some sample analysis in Ibadan and data entry.
- Shipment of blood samples on dry ice in batches to the University of Manchester for laboratory analysis.

Dr Whatmore assisted with sample storage and provided other logistics support for the project. She carried out some biochemical laboratory analyses of samples obtained at the University of Manchester laboratory with Dr Whatmore.

She received statistical support for data analysis from Dr Gemmell at the University of Manchester and wrote the three papers with Professors Clayton and Cruickshank.

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DEDICATION

This work is dedicated to the Lord my God, the King eternal, immortal and the only wise God, my strength, wisdom, Glory and the Lifter up of my head

THE AUTHOR

Omolola Ayoola graduated from the College of Medicine, University of Ibadan in 1991 with the Bachelor of Medicine, Bachelor of Surgery (MB,BS) honours. She completed postgraduate training in general paediatrics in 2000 and obtained the Fellowship of the West African College of Physicians (Paediatrics), FWACP. In the same year, she was appointed Lecturer in Paediatrics and Paediatric Endocrinology at the University of Ibadan with the remit to develop the endocrinology unit of the Department of Paediatrics, with emphasis on both teaching and research. At the same time, she was also appointed an honorary consultant paediatrician to the University College Hospital.

In 2003, she won a NIH / Fogarty scholarship award for International Training in Medical Informatics and Epidemiology. She completed 5 months study (funding limited) towards MSc in Epidemiology & Medical Informatics, working with Professor Robert Woolson at the Medical University of South Carolina, USA from August to December 2003. She returned to the University of Ibadan and completed the Masters programme in 2004.

She applied for and was awarded the 2004 Heinz Fellowship of the Royal College of Paediatrics and Child Health. She was a clinical research fellow for 3 months under Professor Clayton at Royal Manchester Children's Hospital, Pendlebury.

In 2004, Omolola became a Senior Lecturer. In order to further develop expertise in endocrine science research, she also applied for and was successfully awarded one of 2 prestigious Lancet International Fellowship. She returned to the UK to work with Professor Clayton and Cruickshank in the Cardiovascular / Endocrine Science Research groups at the University of Manchester. She gained significant experience in managing an ongoing cohort research study – 'the Manchester Children's Heart and Growth study', (a model for this project work in Nigeria).

In 2006, she was awarded the Wellcome Trust Research Training Fellowship and began her PhD under the supervision of Professor Peter Clayton and Professor Kennedy Cruickshank.

PRESENTATIONS AND PUBLICATIONS RELATED TO THIS THESIS

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2. **Ayoola OO**, Whatmore A, Cruickshank JK, Clayton PE. Association of malaria in pregnancy with maternal metabolic biomarkers, cord blood IGF-I and birth size in Nigerian infants: 'The Ibadan growth and vascular health study'. Horm Res 2010; 74(S3): 101.

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CHAPTER 1

INTRODUCTION

1.1. Context of the Research

In developing countries like Nigeria, non-communicable diseases are replacing the traditional burden of infections as leading causes of disability and premature death in adults. Sudden cardiac death associated with hypertension is not an uncommon occurrence in Nigerians especially in those who are apparently healthy. There is limited information on cardiovascular disease (CVD) patterns and an epidemic is inevitable if current trends go unchecked. In Nigeria, about 25 million women become pregnant annually and are at increased risk of infection with *Plasmodium falciparum*, leading to adverse consequences during pregnancy such as anaemia and low birth weight (LBW) babies, particularly in their first two pregnancies. It is recognised in many studies worldwide that there is an inverse relationship between birth weight and blood pressure (BP). In this study, our aim was to unravel potential links between maternal malarial timing and load, birth size, early growth and BP patterns in their children's early life. This project was directed at identifying the potential early-life risk factors underlying high BP.

Therefore, we established a birth cohort in Nigeria to evaluate the early impact of maternal malaria in pregnancy on growth pattern and BP over the first year of life.

1.2. Rationale for presenting Thesis in Alternative Format

In order to explore these relationships, a prospective longitudinal cohort study design was employed. The alternative format was used to present the findings of this thesis so that the results could be presented in a format suitable for publication in peer-reviewed journals. The three papers presented have been submitted for publication. These results show continuous observations from intrauterine life to the age of one year in this cohort. Using the alternative format provided an opportunity to present these findings in a sequential and logical order with the format outlined below

1.3. Thesis Format

The outline of the proposed thesis structure is as follows:

1. Context of the research
    - Rationale for Alternative Format
    - Study Aims and Hypothesis
2. Review of previous work
    - Cardiovascular diseases burden, risks and relation to ethnicity and age
    - Birth size and early growth in Nigeria children – the role of malaria in pregnancy
    - Developmental origins of adult disease
3. Subjects and Methods
4. Results in format suitable for publication
    - Maternal malaria, birth size and blood pressure in Nigerian infants: Insights into the developmental origins of hypertension from Ibadan Growth Cohort. Ayoola OO, Gemmell I, Omotade OO, Adeyanju OA, Cruickshank JK, Clayton PE
    - Maternal malaria status and metabolic profiles in pregnancy and in cord blood: relationships with birth size in Nigerian infants Ayoola OO, Whatmore A, Cruickshank JK, Clayton PE
    - The impact of maternal malaria in pregnancy on changes in blood pressure in children over the first year of life. Ayoola OO, Gemmell I, Clayton PE, Cruickshank JK
5. Discussions
6. Appendices

1.2. Aims and Hypothesis

1.2.1. Study Aims

We have established a cohort of healthy pregnant mothers followed through pregnancy to delivery and their infants born in Nigeria. Anthropometric and BP measures were carried out on mothers and babies, all followed from birth to 3 and 12 months. Assays for lipids, glucose, insulin and TNFα were obtained in pregnancy and these analytes and Insulin-like Growth Factor-I (IGF-I) were obtained from cord blood. Blood films for malaria parasites were obtained through pregnancy and at delivery and women were grouped into three categories to distinguish between the timing of malaria through pregnancy and at delivery as 'No Malaria', 'Malaria during pregnancy only' or 'Malaria at delivery ± during pregnancy'. This study aims to explore the relationships between malaria in pregnancy, its effect on birth size and early growth and BP patterns at one year of age in Nigerian infants. We also investigated the relationship between birth size and maternal and cord metabolic biomarkers such as lipids, glucose, insulin and TNF-α, and cord IGF-I.

1.2.2. Key Questions

1. Does maternal malaria in pregnancy affect birth size, growth and BP of Nigerian infants at birth?
2. What are the effects of malaria in pregnancy on maternal and cord blood metabolic biomarkers and how do these impact on size at birth?
3. Does maternal malaria in pregnancy affect growth and BP of Nigerian infants over the first year of life?

1.2.3. Hypotheses

We propose that:

i) Size at birth and at one year will be related inversely to malaria load in pregnancy.

ii) Malaria in pregnancy will induce changes in maternal and cord blood metabolic markers, which will be related to birth size.

iii) Malaria load in pregnancy will be related positively to BP at birth and one year.

CHAPTER 2

REVIEW OF PREVIOUS WORK

2.1. Cardiovascular diseases burden, risks and relation to ethinicity and age

2.1.1. Introduction

This section provides an overview of the epidemiology and burden of cardiovascular diseases (CVDs) worldwide, in Africa and particularly Nigeria and the relation of cardiovascular (CVS) risks to ethnicity and age.

2.1.2. Epidemiology of cardiovascular diseases worldwide

Globally, non-communicable diseases are leading causes of deaths in men and women accounting for about 60% mortality (1). CVDs are a major cause of chronic non-communicable diseases and commonest cause of death worldwide being responsible for 29% of mortality in 1996 and 32% of mortality in women and 27% in men in 2004 (1;2). CVD entities include hypertension, stroke, coronary heart disease (CHD), valvular, muscular and congenital heart disease.

Hypertension is the commonest cause of CVD and premature death among the adult population worldwide (3). It was estimated in 2000, that the prevalence of hypertension and CVD risk factors worldwide was about 25%. This figure is expected to rise to 29% by 2025. (4). Figure 2.1 shows the rates of hypertension in all regions of the world. In 2000, the rates for men and women in sub-Saharan Africa were 26·9% and 28·3% respectively (4). Hypertension rates have increased in developing countries, while in developed countries the rates have remained stable or decreased in the past decade (5). In Africa, there are reports that the rates are relatively lower especially in some rural parts but can be as high as 33% in some urban communities (6;7).

The components of the metabolic syndrome 'X' which includes insulin resistance, hypertension, dyslipidaemia and impaired glucose tolerance are CVS risk factors and as elsewhere have also been reported to cluster in elderly African persons who are apparently healthy (8). The rise in prevalence of CVS risk factors in many developing

countries is related to changes in diet and other lifestyle factors. Forrester et al reported that up to 70% of the variance in the prevalence of hypertension in the tropics can be accounted for by salt intake and body mass index (9;10). The development of obesity and hypertension has been linked to dietary habits and obesity appears to amplify or interact with the effects of other CVS disease risk factors such as insulin resistance, hyperinsulinemia and dyslipidemia (11). Other known risk factors for the development of hypertension and CHD are increased total cholesterol (TC) and triglycerides (TG) and reduced high-density lipoprotein-cholesterol (HDL-C) (12).

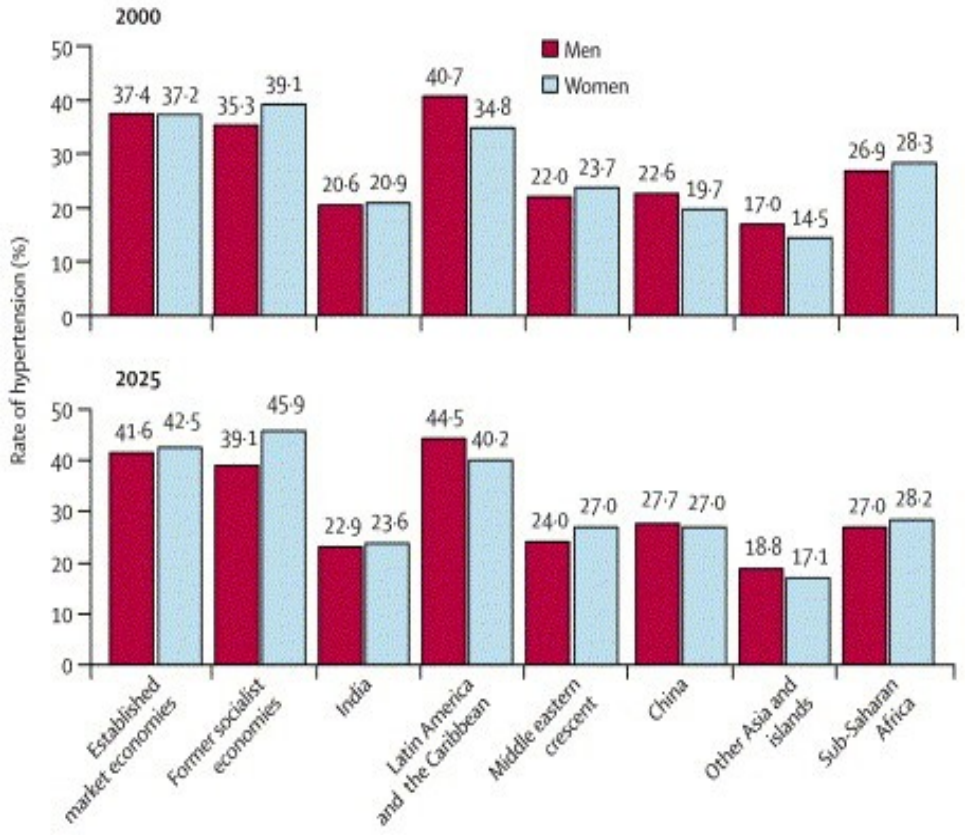

Figure 2.1 Hypertension rates in people aged 20 years and older by world region and sex in 2000 (upper) and 2025 (lower)

Source: Kearney PM et al (4)

2.1.3. Cardiovascular risk factors, Ethnicity and Age

Hypertension is a well recognized common risk factor for stroke, renal failure, CHD, heart failure and peripheral vascular disease (13). In population-based studies conducted in the United States, there were reports of a significant increase in hypertension prevalence from 1988 to 2004 compared to prior findings from the National Health and Nutritional Survey (NHANES) from 1960 to 1991 which suggested a decline in hypertension rates (14). There were also significant differences in BP between African-Americans and white-Americans. They demonstrated higher mean BP in adult African-Americans, and overall, hypertension was more common in blacks (32%) than in whites (23%) with some studies reporting higher prevalence of up to two-fold (15-17).

In the United Kingdom, there are also ethnic disparities in CVD risks, prevalence, and mortality. White-Europeans had higher mean systolic BP (SBP) but lower diastolic BP (DBP) compared to the South-Asians (18), however, the South-Asian men had higher mean BP levels (19;20).

There are conflicting reports over whether hypertension is more common among the South-Asians (21). Cruickshank et al reported no significant difference in either SBP or DBP among White-Europeans, Asians and Black West Indians (22). Others found lower SBP and higher DBP in South-Asians except South Asian women. Agyemang et al describes this as a complex picture, which could be explained by variations in body size, body fat, study methods, and heterogeneity in the South Asian population. There are many differences in the CVD risks among South Asians with lower BP in Bangladeshis, slightly higher BP in Pakistanis and much higher BP in Indians (18). There is also a higher incidence and mortality from CHD among South Asians compared to other ethnicities associated with high prevalence of diabetes and metabolic syndrome, as a result of insulin

resistance, central obesity due to sedentary lifestyle, high serum TG and low HDL-C (23;24).

In Afro-Caribbeans and Africans living in the United Kingdom, there are higher mean BP levels and a high prevalence of hypertension. There is also evidence that hypertension is usually present at an earlier age in the Afro-Caribbeans than in Africans. The risk of death from stroke and end-stage renal failure are high in both groups but the risk of death from CHD is low (24;25). This risk pattern is associated with a high prevalence of hypertension, low serum TG, diabetes and obesity especially in the women (24).

Thus while higher rates of diabetes and CVD are well established in South Asians and excess prevalence of high BP in people of African descent, whether and how these are related to early life factors and child growth remains unclear.

In a 10 year longitudinal study carried out by Dekker et al in Black-American and White-American subjects aged 5 to 27.5 years, there were ethnic differences in both SBP and DBP in male and female subjects with Black children showing significantly higher BP than Whites. Black girls had higher SBP levels than White girls from childhood to early adulthood while Black boys had significantly higher SBP than White boys from early adolescence onward, thus the ethnic difference is observed earlier in girls than boys. There was higher DBP in Black boys and girls than Whites from childhood through adulthood and the difference was stable over time. These ethnic differences in BP trajectories persisted in both males and females after controlling for the effects of socio-economic status, adiposity and/or height velocity (26).

Cruickshank et al suggested that prenatal effects account for 'black-white' differences in adolescent BP while postnatal growth and BP itself also affect later BP (27). Data from the population-based Bogalusa heart study also showed that BP measured initially from 4 years and older was the most powerful determinant of later BP levels (28). There are other reports that increased prevalence of childhood CVS risk factors is a determinant of CVS

events confirming that BP during childhood is an established predictor of adult BP (29;30). However, the role of sex and ethnicity in early development of BP from childhood to adulthood is poorly understood and there is a paucity of data especially in African children (26).

2.1.4. Cardiovascular risk factors in Sub-saharan Africa particularly Nigeria

Sub-Saharan Africa is a diverse region comprising 47 countries and a total population of approximately 480 million people. Many countries of sub-Saharan Africa, have been undergoing epidemiological transition with non-communicable diseases replacing the traditional burden of infections as a leading cause of adult morbidity and mortality (31;32). Mortality from cardiovascular causes in developing countries was 63% of global deaths in 1990 and is expected to rise to 70% of deaths in adults by the year 2020 (10;33). This can be attributed to a range of factors – improving economy, individual wealth, lifestyle modification and the changing age structure as seen in Nigeria where the proportion of the population aged $\geq$ 65 years was less than 3% but is now rising to 4-7% (34).

There is a high prevalence of hypertension, a powerful independent risk factor for mortality from stroke and cardiac disease, especially in suburban and urban areas (35). However, it is usually under-diagnosed and associated with severe complications such that case fatality and age-standardised deaths from stroke in sub-Saharan Africa are now similar to or even higher than that of developed countries (36). Therefore, hypertension is now a public health problem of significant economic importance in sub-Saharan Africa. (37).

In Nigeria, hypertension prevalence rates are as high as 30-32% in middle-income urban and some rural areas although the overall prevalence is 10-15% (38-40). Hypertensive heart disease is the most common cause of cardiac death with many of the hypertensive cases previously undiagnosed (41). As shown in figure 2.2, the age-standardized mortality rates from stroke in adults aged 30–69 years was higher in Nigeria than rates in some

other developing countries like India and Tanzania in 2005 and many-fold higher than those of developed countries like Canada and the United Kingdom (42).

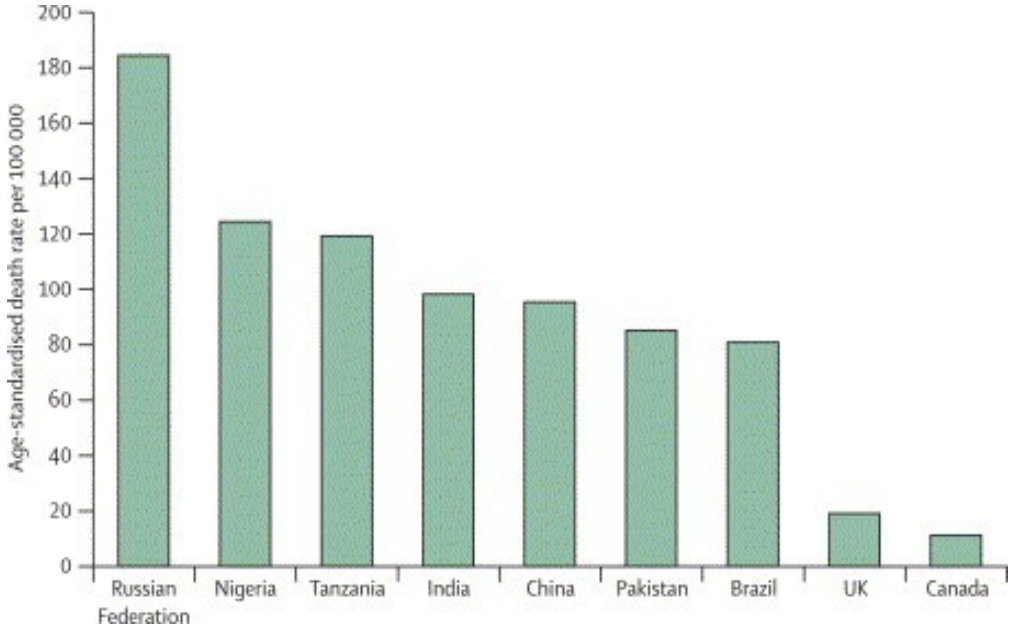

Figure 2.2 Age-standardised mortality from stroke per 100 000 for ages 30–69 years in Nigeria and other selected countries, projections for 2005.

Source: Strong K et al (42)

Furthermore, CVD and renal diseases are now major public health burdens with a relatively high prevalence of multiple CVD risk factors such as insulin resistance, diabetes, hyperlipidaemia and elevated serum homocysteine levels (43-45). Sudden cardiac death is increasingly common especially in those who are apparently healthy and its incidence may not be as rare among Nigerians as previously thought (8;39;46). The emergence of Type 2 diabetes (47) may also be contributing to cases of CHD and/or covert myocardial infarction underlying these sudden cardiac deaths. It has also been reported that prevalence of CVD risk factors was higher in elderly non-diabetic Nigerian women (8) contrary to male sex preponderance in the White European population (48). Other studies reported a higher

prevalence of hypertension, obesity and hypercholesterolaemia in female patients who were already at increased risk for CVD (49).

However, the genesis of this excess CVS risk in Nigeria is unclear and whether there is a relationship between this excess CVS risk, prenatal effects, birth size and early childhood growth. Data on hypertension and CVD risks in childhood in Nigeria are limited despite the increasing rates of CVD risk profile in adulthood.

According to Barker and others, LBW is inversely related to risk of chronic diseases, such as hypertension and diabetes in adult life (50-52). Furthermore, LBW rates are high in Nigeria as a result of high prevalence of maternal malaria in pregnancy (53;54).

2.1.5. Summary

There has been a steady rise in the prevalence of hypertension, in Nigeria, as in other parts of sub-Saharan Africa. There is also a relatively high prevalence of multiple coronary heart disease (CHD) risk factors such as diabetes, hyperlipidaemia and elevated serum homocysteine levels. Low birth weight has been associated with increased risk of adult hypertension,

There is limited understanding of the genesis of this excess cardiovascular risk in Nigeria and whether it may be related to prenatal effects, birth size and postnatal growth.

2.2. Birth size and early growth in Nigerian children – the role of malaria in pregnancy and metabolic biomarkers

2.2.1. Introduction

This section reviews birth size and natural history of early growth in Nigerian babies.The burden and consequences of malaria in pregnancy, its effect on maternal and cord biochemical markers and birth size are also disussed.

2.2.2. Birth Size and Early Growth in Nigerian Children

Birth weight is the single most important determinant of neonatal and infant survival and health. In Nigeria, LBW (defined as birth weight less than 2.5kg) rates vary from 9% to 24% with higher rates being reported in studies carried out in rural communities (55-58). These babies are small for gestational age (SGA) i.e their birth weight and or birth length is 2 standard deviations (SDS) or less below the mean for their gestational age. This is usually as a result of IUGR when their birth length is 2 SDS or less below the mean for their gestational age (56;59) These babies tend to be proportionately small inferring adaptation throughout pregnancy, rather than disproportionate growth restriction typical of deprivation in late gestation (as seen frequently in developed countries (60). It is a common consequence of maternal malaria in pregnancy. In addition to female sex, maternal factors are associated with LBW such as young age, primiparity, lack of antenatal care, low socio-economic status, short-stature, multiple pregnancies, obstetric disorders like pre-eclampsia and antepartum hemorrhage (55;61;62) .

Furthermore, maternal infections, especially malaria, have been associated with an increased incidence of LBW with reports of higher positive rates of malaria parasite and/or pigment in the placentae of LBW babies (62-64) .

Few studies have attempted to examine the growth pattern of Nigerian babies in early life. Most were cross-sectional and generally limited to babies exclusively breast-fed for the first 6 months of life (57;65). The growth curves of exclusively breast-fed infants, recruited within 14 days of life and weighing 2.5kg and above, showed increasing weight from birth to six months as expected, however boys gained weight faster than the girls and were heavier at six months (57;66;67). Additionally, the 50th centile curves of these infants (both genders) for the first six months were higher than the 50th centile curves of the World Health Organisation and National Centre for Health Statistics (WHO/NCHS) reference for the same age and sex (57;66;67). These babies represented the middle to high socioeconomic group with normal weight at birth while those who were SGA were not included. However, studies carried out in the rural parts of Nigeria demonstrate that the length, weight and head circumference standard deviation scores (SDS) of infants from birth to 2 years were below World Health Organisation (WHO) standards and boys were more malnourished than girls (65;68).

Kalanda et al reported that maternal malaria at delivery was associated with reduced weight for age and weight for length at 6 and 12 months (69) but there is paucity of data on height and other anthropometry in these children and the effect of maternal malaria on their growth pattern.

2.2.3. Malaria in Pregnancy

Falciparum malaria remains a major cause of morbidity and mortality worldwide, estimated at 300-500 million cases and 1-2 million deaths annually, 90% of which occur in sub-Saharan Africa (70). In Nigeria, as in other countries in sub-Saharan Africa, malaria is hyperendemic and the incidence remains unacceptably high in pregnant women with the prevalence ranging from 20% to 44% (71;72).

Malaria infection in pregnancy can be defined either on the basis of parasitaemia during pregnancy or placental infection at the time of delivery, with pregnant women being more likely to have higher density parasitaemia (73). Although the majority of these infections are low-grade and frequently sub-clinical (63), therefore undetected and untreated, because of immunity acquired during previous exposures which protects against clinical malaria (71;74), the parasites can sequester in the placenta and alter its structure (75;76). This has been attributed to the transient depression of cell-mediated immunity which allows fetal allograft retention but this also causes a compromise in body resistance to various infectious diseases (77;78).

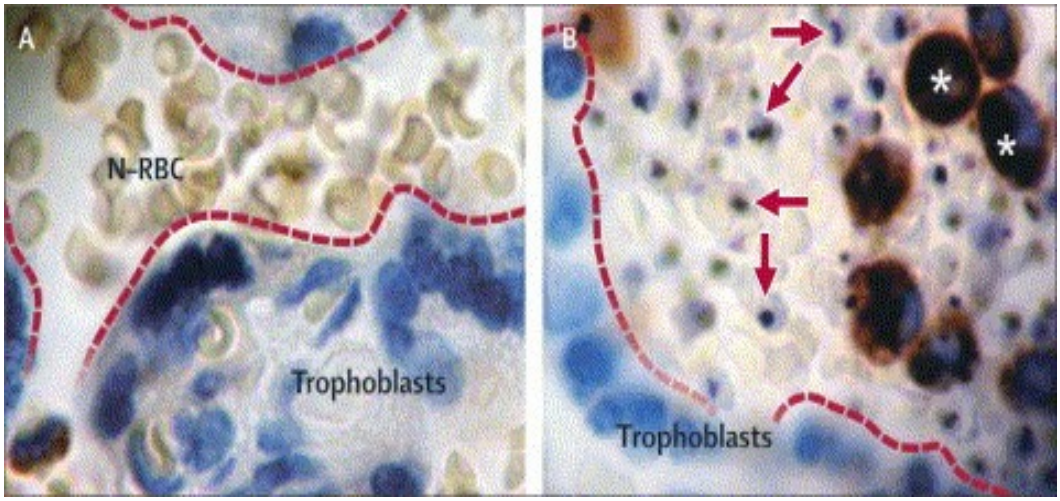

Figure 2.3: Histological appearances of normal and malaria-infected placentae

Placental tissues from (A) normal and (B) malaria-infected women showing parasites and monocyte-macrophage infiltrates

"Sections were stained with monoclonal antibody to CD68, specific to monocytes and macrophages, and developed with diaminobenzidine (brown colour).
Asterisks indicate CD68 staining of monocytes/macrophages in the intervillous space. Arrows indicate parasitised erythrocytes.
The lines indicate the outline of the trophoblast layer.
Original magnification (oil immersion) ×1000,
N-RBC=non-parasitised erythrocytes"

Quoted from: Rogerson SJ et al (78)

The placenta is a site that is preferred for replication by Plasmodium falciparum leading *to* placental sequestration and the reason for this has been attributed to reduced immunity to malaria infection (79). The pathogenesis of malaria in pregnancy resulting in LBW is not fully understood but it is linked mainly to placental sequestration as shown in Figure 2.3. Placental sequestration leads to cytoadherence which likely impairs placental function in addition to other factors which may be hormonal or nutritional. The placentae of LBW babies have higher positive rates of malaria parasite and/or pigment (64). which interferes with nutrient transfer to the fetus resulting in LBW babies (80). It has been proposed that the parasites may not be directly responsible for the placental structural changes and impairment of fetal nutrient transfer but this may be linked to production of non-chemotactic cytokines by leucocytes. This results in the thickening of the trophoblastic basement membrane and might cause mechanical blockage of placental oxygen and nutrient transport to the fetus (79). In pregnant women with malaria, it has been observed that there is accumulation of parasite-infected red blood cells and fibrin deposits in the maternal vascular area of the placenta (the intervillous space) to higher densities than in the peripheral blood. There is also increased numbers of maternal phagocytic cells, especially monocytes, in the intervillous space and deposition of malaria pigment i.e haemozoin (a byproduct of parasite haemoglobin digestion) in phagocytic white blood cells. The trophozoite and schizont stages of malaria parasites that are normally absent from the circulation are also found in the placentae (78;81;82)

2.2.4. Consequences of Malaria in Pregnancy

The impact of malaria during pregnancy varies greatly according to the intensity of transmission. In Nigeria, with stable endemicity, where many women have asymptomatic infections, these are associated with significant consequences to maternal and infant health such as maternal anemia, prematurity and LBW due to IUGR (83).

Anemia in pregnancy is common in Nigerian women and the commonest cause is malaria resulting in 11% of maternal deaths (71;72). In southern Nigeria, the prevalence of anaemia in pregnancy secondary to malaria was 56% (84) and at booking in pregnant women in the University College Hospital (UCH), Ibadan, it was 15% (85). Anaemic mothers tend to deliver premature babies with LBW, with a higher mortality rate compared with those of non-anaemic mothers; these babies have higher BP. This observation has also been demonstrated in animal studies where maternal iron restriction in the rat reduced birth weight and led to elevated BP at 40 days of age (86). Other data show that mothers with lower haemoglobin had thinner skinfold thicknesses, and the child's S BP increased by 2.6 mmHg for each 1 g/dl decline in the mother's haemoglobin (87). These findings support the theory that maternal anemia is linked to the genesis of raised BP in children (86;87).

Malaria in pregnancy also results in IUGR leading to LBW (63;73;80). It is responsible for 5–12% of all LBW or 35% of preventable LBW (88) and contributes to 75,000–200,000 infant deaths each year (89). The intermittent preventive therapy (IPT) regimen with sulphadoxine pyrimethamine (SP) recommended for prevention of malaria in pregnancy in Nigeria is not very effective because there is poor compliance by pregnant women due to costs. Despite prophylactic regimens, the incidence of LBW among neonates born to women in neighbouring Mali in West Africa was 33.2% for IPT with chloroquine and 23.3% for IPT with SP (90).

Infants of mothers with placental malaria infection are more susceptible to malaria in the first 2 years of life (91). Infections, including malaria, during infancy are likely to contribute further to the adverse long-term health profile of these children (92). There is a high prevalence of infection in Nigerian children - 46% of postneonatal febrile infants had malaria, while 38% had a bacteraemia (93). Acute attacks of malaria also cause a

significant reduction in weight gain in childhood due to the cachectic effects of TNFα production (94).

Malaria rates remain high in sub-Saharan Africa and transitional changes in lifestyles for many women may alter their offspring's growth performance on the background of high malarial load in utero.

2.2.5. Maternal metabolic markers in pregnancy and cord metabolic profile

Studies in various populations including Nigerian pregnant women show an elevation in serum lipids, except high density lipoprotein- cholesterol (HDL-C), in normal pregnancy. The elevation is higher in the third trimester (95-97). Some have reported an elevation in HDL-C while others reported no significant changes during pregnancy (98-100). The causes of the lipid changes in pregnancy are uncertain, but elevation of total cholesterol (TC) might be due to increased synthesis and / or decreased catabolism. As previously documented, this adverse lipid profile of increased TC and low-density lipoprotein-cholesterol (LDL-C) and low to normal HDL-C indicate that Nigerian women are more prone to hyperlipidemia during pregnancy though not atherogenic (95;96).

Furthermore, there is evidence that maternal lipid levels in pregnancy influence cord lipid levels (101). Data from studies in many countries including a study at Ibadan about 30 years ago suggest that cord lipids were much lower than those in the maternal blood. Cord blood lipid profile is unique and different from adult values (102-106). Cord HDL-C and TG are about one-half that of adults while TC and LDL-C are about one-third (107;108). However there are inconsistencies in the associations between maternal and cord lipid profile probably as a result of variations in the study methods (102;104;109-111).

Cord lipid profile has been linked to childhood lipid profile and its changes which are predictive of adult profile (112-114).

2.2.6. Relation of maternal and cord metabolic markers with malaria in pregnancy and birth weight

Metabolic parameters such as lipids, glucose and insulin have been studied in relation to malaria in adults and children, however, there is a paucity of data on their relationship with malaria in pregnancy.

In non-pregnant adults with acute malaria, higher TG, lower TC and HDL-C have been reported (115), as well as no change in TC (116). The mechanisms for these changes in lipid profile associated with malaria are not yet clear. Some in vitro experimental findings indicate that the HDL-C fraction appears to be a major lipid source for parasite growth because there is a selective uptake of HDL-C particles by P. falciparum (117) and increased intraparasitic cholesterol and phospholipids (118).

Hypoglycemia is less common in adults with malaria than children but it has been reported in pregnant women who have severe malaria. This is often in relation to quinine and quinidine therapy. Hypoglycaemia is associated with increased glucose turnover attributed to enhanced pancreatic β-cell function and quinine-induced hyperinsulinaemia, which causes increased peripheral uptake of glucose (119-121).

Furthermore, in peripheral and placental blood of pregnant women in Malawi and Kenya, higher TNFα has been associated with malaria as well as other cytokines such as interleukin 8 (IL-8), gamma interferon IL-6, and IL-10 (122;123). There is paucity of data on cord metabolic profile in relation to malaria in pregnancy. .

Maternal and cord blood levels of lipids, glucose and insulin have been investigated as possible determinants of birth weight (124-126). In non-diabetic pregnant women with hyperglycaemia, fasting TG was positively independently associated with birth weight and with fetal growth and birth size in women with gestational diabetes (125;127;128). Maternal fasting plasma insulin was inversely associated with birth weight while plasma glucose was positively related (124;129;130).

Cord blood glucose and insulin levels are positively related to birth weight in normal and LBW babies (131). The mechanism of this relationship is not yet well understood but it has been proposed that it may result from reduced fetal glucose transport from the mother and increased fetal anaerobic glucolysis. The fetal hypoglycaemia has important role for fetal adaptation and survival when there is limited availability of maternal glucose. It maintains the materno-fetal glucose concentration gradient,hence increasing placental glucose transfer to the fetus limits insulin secretion(131). The relationship between cord blood lipids and birth weight is inconsistent (132;133), however, LBW has been associated with high TG,low LDL and HDL (134;135). It is suggested that intrauterine growth retardation which leads to LBW is associated with lipid disorders with change in fetal metabolism and use of other sources of fuel instead of lipids. These associations have not been explored in relation to fetal intrauterine exposure to malaria.

2.2.7. Summary

Malaria in pregnancy has significant impact on maternal and infant wellbeing being responsible for high prevalence of maternal anemia in Nigeria women which is linked to the genesis of raised BP in children (86;87).

It is also responsible for high LBW rates. The mediators of the relationships between prenatal malaria, birth size and maternal and cord metabolic profile have not been clearly defined.

2.3. Developmental origins of adult disease

2.3.1. Introduction

The evidence for the association between birth size and chronic diseases in adult life and proposed mechanisms and hypotheses for the developmental origins of adult disease are reviewed in this section.

2.3.2. Long term consequences of birth size

Low birth weight, an indicator of IUGR for a given gestational age, has been associated with some chronic diseases in adult life. About two decades ago, David Barker et al reported an inverse relation between adult BP and birth weight. They documented a mean (SD) decrease of 0.80 (0.39, 1.22) mm Hg in SBP for every kilogram increase in birth weight (136). Subsequently, in numerous studies around the world, LBW has been associated with increased risk of adult hypertension (137;138), reduced arterial compliance (139), atherogenic lipid profiles (140;141) and increased left ventricular mass (142) resulting in an increased incidence of CHD and stroke (143-145). Most studies have been in adults in Europe and the United States reporting consistently that those who had lower birth weights had higher BP (146-148). There are detractors from this interpretation. Huxley et al examined studies that had reported regression coefficients of SBP on birth weight and those that reported only the direction of this association. They suggested that there may have been an over-estimation of the strength of the association between birth weight and BP due to bias in the reporting of results. They also reported that there was inappropriate and inadequate adjustment of the relationship for potential confounding factors some of which are current weight, sex, height, parental socioeconomic status and gestational age (149). However, there were contrary findings and views to this interpretation from longitudinal studies that report inverse association between birth weight

and BP with or without adjustment for current weight (150;151) The association is robust and amplifies with age and the weaker associations in large studies reported by Huxley et al are due to error from 'recalled' rather than recorded birth weight and inaccurate BP measurements for routine medical examination as opposed to research purpose (152;153).

The association has been reported in children but there were some inconsistencies in studies in adolescents like Menezes et al who reported no inverse association between birth weight and BP but positive association between birth length and BP in Brazil (154-158). The inverse relationship between birth weight and BP is strengthened by adjustment for later size (159), therefore there are arguments that postnatal "catch-up" growth rather than prenatal development is more critical (160;161).

2.3.3. Mechanisms for the relationship between birth size, early growth and later health outcome

Large-scale studies and experimental animal data provide convincing evidence about the inverse relationship between birth size and chronic diseases in adult life (162;163). When low birth weight is followed by accelerated postnatal growth, this is associated with increased risk of insulin resistance and later type2 diabetes mellitus but when there is no catch-up growth, there is higher risk of hypertension (164;165).

The knowledge of the mechanisms involved in the association between fetal growth impairment and risk of chronic disease in adult life is key in helping to develop strategies for disease prevention and control, but their pathophysiology has not been fully elucidated. Various models of the mechanisms have been proposed.

Figure 2.4 shows some of the proposed mechanisms to explain the association between fetal growth impairment and the risk of chronic disease mainly insulin resistance in adult life. During fetal life, growth retardation occurs as a result of fetal malnutrition and partly

genetic predisposition, and this results in reprogramming of the metabolic and endocrine systems ultimately leading to insulin resistance. In postnatal life, rapid catch-up growth with obesity if present, may cause reduced peripheral insulin sensitivity leading to early tissue stem cell exhaustion (166).

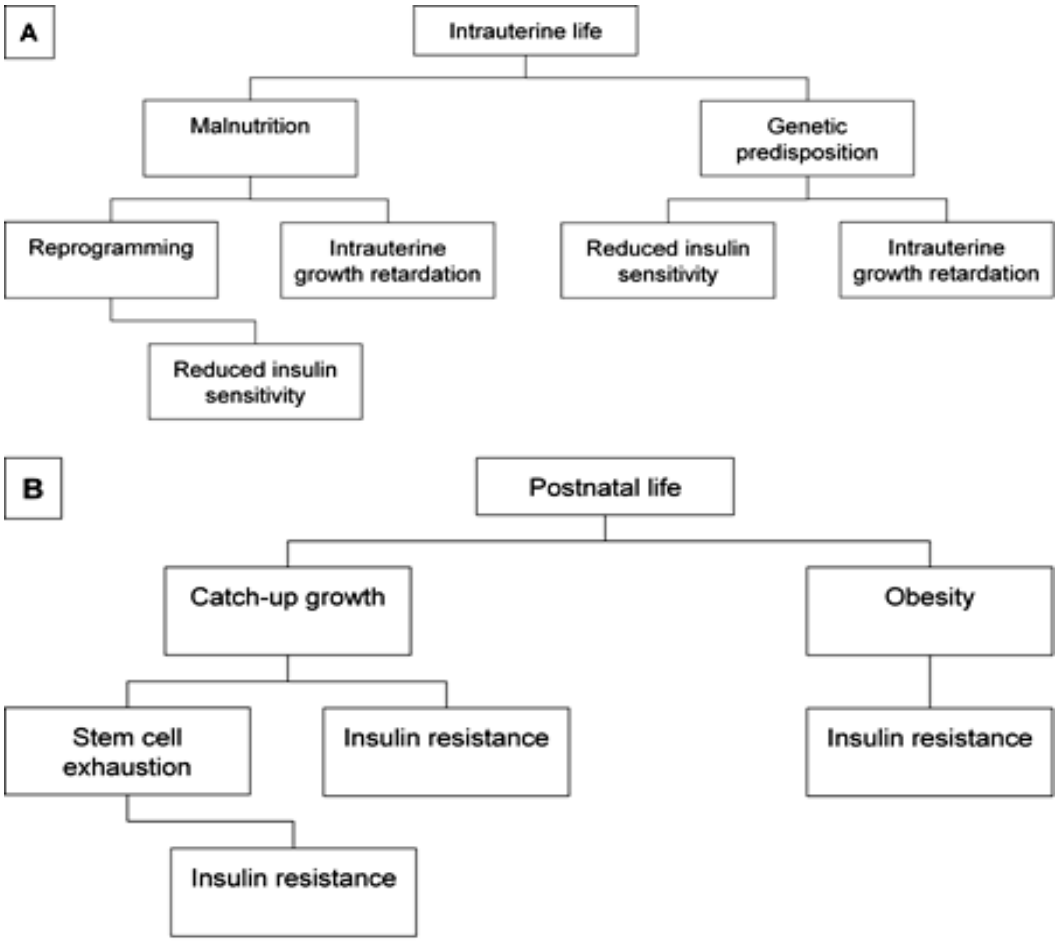

Figure 2.4: A summary of proposed mechanisms explaining the association between fetal growth impairment and risk of chronic disease in adult life

Source: Geremia C et al 2004 (166)

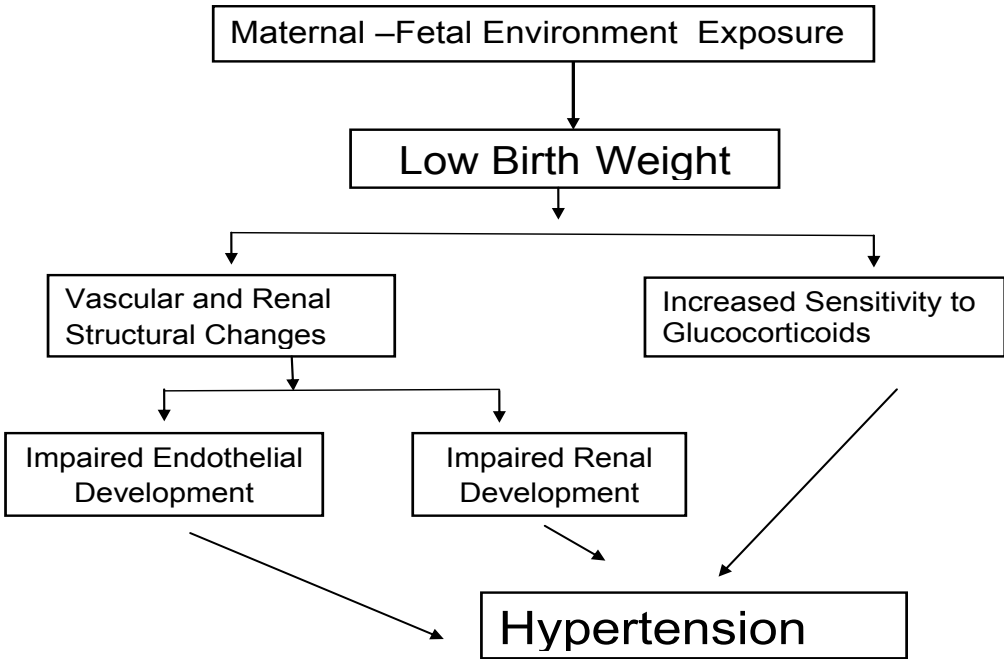

Figure 2.5: Proposed mechanisms for developmental origin of hypertension

Source: Lackland et al (167)

Figure 2.5 shows some of the proposed mechanisms for the birth weight/hypertension relationship as reported by Lackland et al (167). During intrauterine life, adverse environment leads to growth retardation and low birth weight. This leads to structural changes to blood vessels and the kidney. Arterial compliance is directly related to size and decreased compliance is associated with high BP probably due to reduced elastin in vessel wall of LBW babies (139;168;169). This leads to impaired development of the endothelium and kidneys related to reduced number of nephrons (170). These subsequently result in hypertension. In addition, increased glucocorticoid exposure due to stress in utero may enhance angiotensin-converting enzyme activity and increase angiotensin 2 also resulting in hypertension (171;172).

Geremia et al suggests that each hypothesis might represent a different piece of the same puzzle (166).

2.3.4. Hypotheses related to birth weight- adult chronic disease association

Epidemiological and clinical studies led to the proposal of the “thrifty phenotype hypothesis” and experimental studies led to the development of many other hypotheses to explain the mechanisms for the association between birth size and adult chronic diseases as discussed below.

As far back as 70 years ago, the early fetal environment was associated with subsequent health and survival (173). The Dutch famine study reported the association of birth weight with higher rates of obesity (174). Many other studies demonstrated an association between LBW and higher SBP, TG levels and development of impaired glucose tolerance and CVD (174-176). In Pima Indians where there is a very high rate of type 2 diabetes, there were two peaks in the risk of developing diabetes related to both extremes of birth weight (177). For those with LBW, there was a fourfold higher risk of developing diabetes. However, some studies reported that there was no relationship between catch up growth in the first 6 months of life and BP (150;178).

In addition to observational studies, longitudinal studies on children who were born small also provide evidence for the relationship between LBW and chronic disease in adult life (179;180). Controlling for confounders such as lifestyle factors and socioeconomic status strengthened the association between LBW and increased CVS risk (181). The risk of disease is reported to be higher in those born smaller who later become relatively obese as adolescents or adults (182-184). Consequently, the thrifty phenotype hypothesis was proposed to explain this epidemiological association.

2.3.5. The Thrifty Phenotype Hypothesis

The “thrifty phenotype hypothesis” was proposed by Hales and Barker in 1992 in order to explain the association between LBW and adult type 2 diabetes (185). This hypothesis supposes that the fetus responds to an adverse environment by reducing its somatic

growth but not cranial growth and making irreversible changes in its developmental trajectory termed ‘programming’(186). Programming was a term first used to explain the longer-term consequences of different forms of infant feeding (187;188). These changes lead to adaptation of the physiology of the mature organism for a nutritionally deprived environment. These adaptations include changes in insulin sensitivity, a reduced vascular bed in several organs such as pancreas, liver and muscle and reduced nephron number(189). Thus the biological risk factors for CVD including levels of BP are ‘programmed’ in-utero through influences which alter fetal growth. Fetal adaptations to lack of nutrients are thought to be a major influence in programming because the main determinant of fetal growth is the supply of nutrients (190). In healthy pregnancies some environmental factors that limit nutrient supply to the fetus, such as multiple pregnancy, parity, maternal size and age can influence birth size (62;191;192) In addition to nutrition, factors such as infections, socio-economic status and smoking have significant effects on birth size (193;194). Furthermore, it has been shown that adverse fetal environment can affect disease risk even in babies with normal birth size.

In this thrifty phenotype model, the relative roles of environmental and genetic factors were evaluated such that birth size was noted to be a reflection of altered rates of fetal growth and have only a small genetic component (162). The change in maternal and placental function leads to an inadequate supportive environment for fetal growth resulting in altered fetal growth rate due to intrauterine constraints. Thus, the relationship between birth size and disease risk does not imply a causal role of being born small but reflects the sensitivity of fetal growth to adverse intrauterine influences (162). Therefore, the causal trigger is the effect of environmental influences acting during early fetal development. Consequently, in a suboptimal intrauterine environment, the adaptations may provide a survival advantage on the fetus but if there is exposure to an energy-rich environment, it leads to a disadvantage resulting in the risk of chronic disease in adult life (162).

2.3.6. Evidence from Experimental studies

Various changes in the periconceptual, embryonic, fetal and neonatal environment significantly alter the metabolic and CVS function of the offspring. This phenomenon has been demonstrated in many animal models of early growth restriction. These experiments were carried out not only to unravel the basis of the association between early growth restriction and postnatal metabolic and CVS risk but also to provide an understanding of the underlying molecular mechanisms. Many models were produced by dietary manipulations such as maternal total undernutrition, low-protein diet, high-fat diet and others were glucocorticoid administration and induction of uteroplacental insufficiency in pregnant animals (195-197).

When low-protein diet was given to pregnant rats throughout pregnancy and lactation, their offspring were significantly growth retarded throughout life, had altered glucose homeostasis and in adulthood developed hypertension. Some offspring developed an abnormal increase in β-cell secretion of insulin (198), some developed insulin resistance (199) while some had altered expression of the hypothalamic-pituitary-adrenal axis (196). In old age, some offspring had defects in insulin signaling in liver, muscle and fat cells leading to hyperglycemia (200;201). Some offspring had reduced numbers of nephrons and increased renal plasma flow leading to hypertension in later life (163). This finding was corroborated by autopsy studies in man that revealed that people with hypertension dying in accidents had half the number of nephrons of those without known hypertension (202).

In models where maternal glucocorticoid was given, the consequences were similar to those produced by low-protein diet (200). The fetus was exposed to excess maternal steroid and the offspring developed high BP. This results from the suppression of placental 11-ß-hydroxysteroid dehydrogenase type 2 enzyme which inactivates cortisol (196).

A uteroplacental insufficiency model is produced by ligation of the uterine artery. This leads to IUGR. In the fetus, there is progressive dysfunction in insulin secretion and action

leading to type 2 diabetes and obesity in adult offspring (203). The association of LBW, postnatal insulin resistance and high BP have also been reported in normal non-manipulated guinea pigs and pigs (199;204).

2.3.7. The Role of Hormones

The mediators of the relationships between birth weight and later CVS morbidity have not been clearly identified, but 'programming' of hormonal axes in the SGA baby has been defined in animal models and humans (205;206). Hormonal 'programming', in which the hormonal axis is permanently reset as a result of in-utero events in terms of secretion and/or tissue sensitivity, has been well-characterised for insulin, growth hormone (GH) and the insulin-like growth factor (IGF) axis. The association of LBW with subsequent catch-up has also been linked to changes in IGF-I and leptin (207).

The IGF system, comprising IGF-I and -II, their binding proteins and receptors, is widely expressed throughout the body and is the principle mediator of cell growth and differentiation. The regulation of fetal growth by GH and IGF-I is complex. Between the 8^{th} and 12^{th} weeks of gestation, placental GH can be detected in maternal serum and increases throughout pregnancy replacing pituitary GH and resulting in increased maternal serum IGF-I (207). IGF-I levels also rise in the fetal circulation from the 18^{th} to the 40^{th} week of gestation. It is believed that both fetal and maternal IGF-I levels regulate fetal growth (207).

IGF action is modified by its binding proteins. The most abundant binding protein in serum is insulin-like growth factor binding protein 3 (IGFBP-3), which transports IGF-I and -II in the circulation in a ternary complex with the acid-labile subunit. IGF-I and II can then be transferred to the smaller insulin-like growth factor binding protein I (IGFBP-I) for tissue access. Levels of IGFBP-I in the blood are dynamically regulated by insulin and glucose (207). Heald et al reported that IGFBP-I is inversely related to insulin, and low levels are

likely to allow IGF-I to be more bioavailable for cellular actions (208). Figure 2.6 shows hormonal regulation of fetal growth.

Cord blood IGF-I is positively related to birth weight with lower levels reported in IUGR twins compared to their normal twin (209). However, small babies who then catch-up in weight have higher IGF-I levels at age 5 years than those born with a normal weight (210). There are consistent reports of higher concentrations of IGF-I than expected for height and weight in LBW babies especially those that catch up in weight postnatally. Therefore, it is proposed that a high IGF-I concentration is a mechanism linking IUGR with high BP in adult life (205;211).

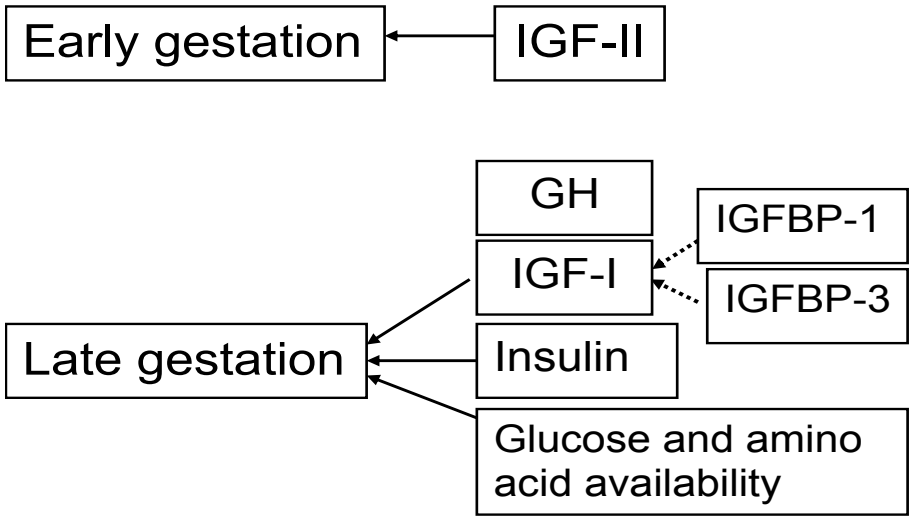

Figure 2.6: The hormonal regulation of fetal growth

The IGF system has been linked to the development of CVS pathology with animal data indicating that IGF-I is a mediator of the hypertrophic and hyperplastic effects of hypertension, the development of atheromatous plaques, cardiomegaly and cardiac failure (212-215). Thus IGF system changes in the infant are likely to have a key role in CVS development and perhaps its abnormalities, a scenario not yet explored in the West African setting.

Studies in adults have revealed differences in the IGF system in relation to disease profile in different ethnic groups. For instance, Pakistanis, who have the highest rates of CHD, had lower IGF-I levels than white Europeans, while IGF-I was highest in African-Caribbeans, who also had the lowest levels of IGFBP-1 and the highest incidence of hypertension and stroke (216). These data indicate that there is a complex but identifiable association of the IGF system with CVD risk factors that differ by ethnic background. In the Nigerian population, where hypertension is the predominant CVS risk, it is likely that IGF-I will be high and IGFBP-1 relatively low. Our study will identify the role of IGF-I in relation to BP in infancy.

Furthermore, experimental models have strengthened the role of the endocrine system in the developmental origin of adult disease. In experimental models where maternal glucocorticoids were administered, the offspring had an imprint on the hypothalamo-pituitary-adrenal axis leading to permanent modification of the neuroendocrine response to stress throughout life (217;218). In monkeys, prenatal exposure to dexamethasone led to a reduction in hippocampal volume and an increase in plasma cortisol levels postnatally (219). In addition, Phillips *et al*. have reported that in LBW babies, an increased risk of diabetes might be a consequence of elevated cortisol concentrations which might lead to insulin resistance (220).

Some hypotheses have been proposed to explain the role of the endocrine system in the mechanism of the developmental origin of adult disease.

2.3.8. The"fetal salvage" hypothesis

The basis of the “fetal salvage” model is that growth retarded prepubertal children have evidence of impaired insulin sensitivity but normal glucose transport (221). The model proposes that the growth retarded fetus develops peripheral insulin resistance, leading to redistribution of nutrients, such as glucose, in favour of essential organs like the brain, thus contributing to the ‘brain sparing’ phenomenon. The number or function of skeletal muscle glucose transporters is also permanently reduced and stimulation of β cells to produce high insulin levels to achieve normoglycaemia because of the reduced peripheral insulin sensitivity eventually results in β cell exhaustion (222-224).

2.3.9. The "catch-up growth" hypothesis

In growth retarded babies, there is an effect on the endocrine system such that at birth, there are high levels of GH, IGFBP-1 and IGFBP-2 while those of insulin, IGF-I and IGFBP-3 are low. These parameters usually become normal within the first three months of life (225;226). This rapid adaptation to extra-uterine life is the basis of the "catch-up growth" hypothesis. It is proposed that tissues chronically depleted of nutrients and, consequently, insulin and IGFs during fetal life, when exposed suddenly to high levels of insulin and IGFs in early postnatal life as a result of normal nutrient supply, may develop insulin resistance to counteract the additional insulin-like actions (224). According to this model, those infants who were small at birth and show early and complete recovery from IUGR would be at higher risk for metabolic disturbances. This theory is supported by various studies including animal experiments (226-230)

2.3.10. Gene versus environment

There is a close relationship between genes and the environment. There have been previous reports that genetic factors account for 30-60% of BP varaiations (231).

Angiotensin converting enzyme (ACE) genotype has been associated with hypertension and Hindmarsh PC et al reported differences in ACE genotype with birth weight and body size at one year of age as possible explanation for the epidemiological findings (232;233). The maternal intrauterine environment can affect fetal gene expression such that there are variations in gene expression from the same gene structure which develops into different phenotypes. In addition, the maternal gene expression can also alter the fetal environment (234). There is much debate on the roles played by genes and the environment in programming and its molecular mechanisms.

The "fetal insulin" hypothesis: Hattersley and Tooke proposed the hypothesis which states that the association between LBW and adult insulin resistance is principally genetically mediated (235). They suggested that genetically determined insulin resistance produces low insulin-mediated fetal growth in utero as well as insulin resistance in childhood and adulthood. Thus, fetal genetic factors regulate either fetal insulin secretion or the sensitivity of fetal tissues to the effects of insulin and this affects insulin-mediated fetal growth (235). Therefore, genetic factors that increase insulin resistance in fetal and subsequently adult life are polygenic and would produce two phenotypes: a) a small, thin baby and b) an adult with increased risk of CVD particularly associated with obesity (235). In support of this hypothesis, Dunger et al reported an association between birth weight and common allelic variation (class I or II) at the variable number of tandem repeat locus in the promoter region of the insulin gene (236). In fetal life, insulin is one of the major growth factors, thus, monogenic disorders that affect fetal insulin secretion and resistance also affect fetal growth (237;238). Furthermore, LBW and maturity onset diabetes of the young occur with mutations in the gene that encodes glucokinase (237;238) but this is rare. Furthermore, Vaessen N et al reported that genetic variations result in low circulating IGF-I concentrations, reduced height in adulthood, diminished insulin-secreting capacity, and a high risk of type 2 diabetes and myocardial infarction (239). They also reported an

association between a polymorphism in the promoter region of IGF-I gene and birth weight (240). Consequently, the association between LBW, diabetes, and CVS disease could be a result of genetic variations in the IGF-I gene, affecting both fetal growth and susceptibility to late-onset disease. However, there are indications that the increased risk of chronic disease associated with small birth size is a result of a combination of genetic factors and adverse intrauterine environment. It was proposed that this interaction occurs through the processes of developmental plasticity (241).

2.3.11. Developmental Plasticity and Mismatch

The ability of organisms to change their structure and function in response to environmental cues is termed “developmental plasticity" (162). The hypothesis is that the organism usually responds during critical time windows and the changes induced become irreversible and a single genotype develops into many phenotypes in response to environmental cues through continuous postnatal adaptation (162). These adaptive responses are believed to be made in order to prepare the organism for its future environment but have no immediate value to the organism. This concept termed “Predictive adaptive responses” (PARs) can be classified as appropriate or inappropriate (187).

In appropriate PARs, there is low risk of disease because the physiological responses of the organism match the environmental exposure. However, inappropriate PARs occur when there is mismatch between the adaptive response and the environment leading to high risk of disease in later life (187). According to Gluckman et al, the shape of the relationship between the settings established during the developmental plastic phase and the settings required in the mature phase is nonlinear as a result of adverse intrauterine environment, shown in Figure 2.7 (162). The critical windows of potential induction differ in various organs in the body. Some are only within the intrauterine life while some extend

postnatally. The fetus relies usually on cues received from the placenta and the mother to assess its current and predict its future environment (242). Adaptive responses may not be dichotomous (i.e. as either immediately adaptive or predictively adaptive), but may have features that include both. For example, insulin resistance may be advantageous prenatally and postnatally in an undernourished environment. However, the degree of match or mismatch to the postnatal environment determines the risk of disease (242). There is now an increasing focus on the role of epigenetic mechanisms on the biological basis of developmental plasticity.

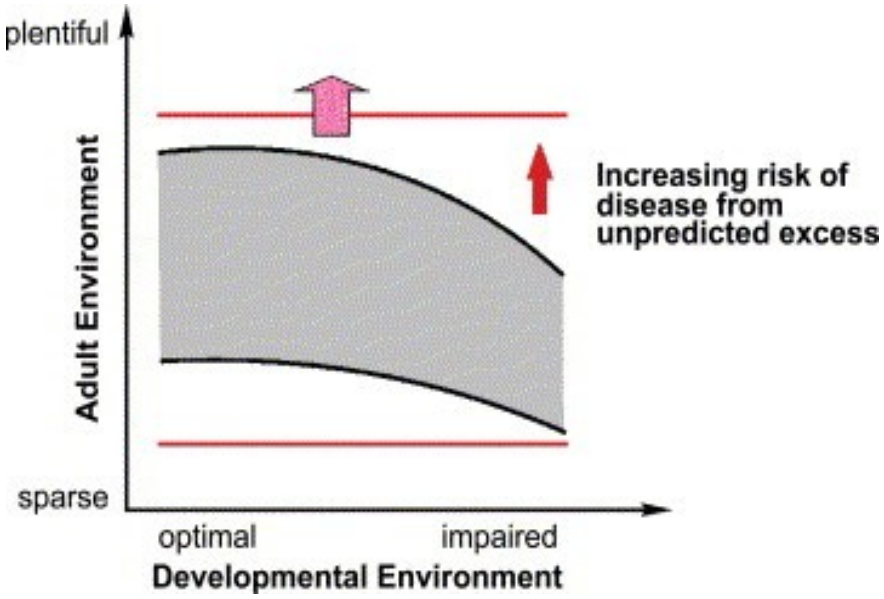

Figure 2.7: The shape of the relationship between the settings established during the developmental plastic phase and mature phase

The red lines are the upper and lower boundary of the variation in the environment that the mature organism can be exposed to.
The PARs model proposes that the predicted environment (shaded area) of the developing organism growing optimally (left side) matches with its adaptive response.
If there is impaired environment, (right side), then there is mismatch between the environment for which the organism is adapted by PARs and the mature environment. This leads to mismatch (space between the shade area and upper red line) - high risk of disease.

Source: Gluckman et al ; 2004 (162)

2.3.12. Epigenetic Processes - Definitions and Mechanisms

According to Waddington, 'epigenetic' processes are the ways in which the developmental environment can influence the mature phenotype (243). Godfrey et al observed that alternative phenotypes can be induced from a genotype during development as a result of environmental cues (244). During the plastic phase of development, the environment factors interact with the genome of the organism to produce heritable effects (244). These factors can act in one generation while their effects are revealed in subsequent generations.

The term "epigenetic" is now used to refer to structural changes to genes that do not alter the nucleotide sequence (244). Epigenetic inheritance is defined as biologic processes that regulate mitotically or meiotically heritable changes in gene expression without altering the DNA sequence (244).

There is considerable evidence that epigenetic mechanisms are implicated in dietary and endocrine exposures and some disease conditions (245-247). Thus, some heritable elements of disease susceptibility could be transmitted by nongenomic means. Furthermore, the environmental cues in early stages of development determine the risk of disease in later life through epigenetic modifications in non-imprinted genes whereby there is modification in gene expression without alteration in DNA sequences. These changes lead to life-long alterations in gene expression (244).

Figure 2.8 is a summary of the mechanisms by which the environmental cues influence the developmental program. Before implantation, the environmental effects alter gene expression through epigenetic modification of the DNA (162). In addition, non-imprinted genes can also undergo epigenetic modification (162). According to Gluckman et al epigenetic modification is the primary basis of the programming phenomenon and environmentally induced changes in gene expression produce the structural and regulatory

effects on many organ systems (242). The broader implication is that the understanding of epigenetic processes may form the basis for the development of intervention strategies.

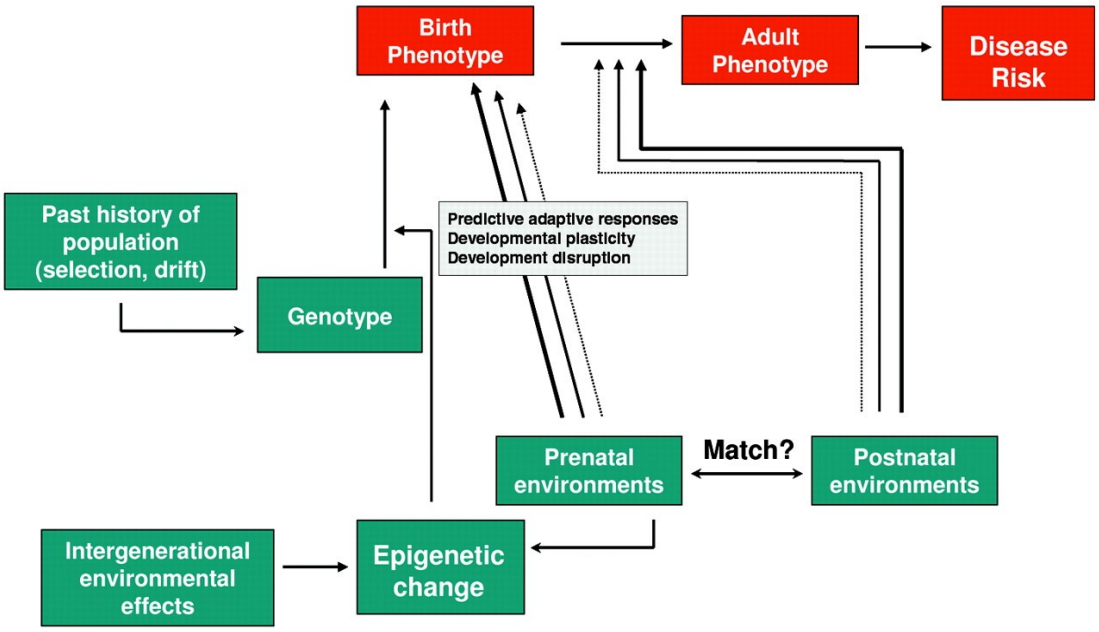

Figure 2.8: A general model of interaction between intergenerational, genetic and environmental factors

If the prenatal and postnatal environments match, the physiological settings achieved through the processes of developmental plasticity will leave the organism well prepared for the postnatal environment. Conversely, a mismatch between the prenatal and postnatal environment may be pathogenic.

Source: Gluckman et al 2004 (162)

Table 2.1: Summary of Hypotheses and Supporting Literature

Hypothesis	***Literature First Author (Reference)***
Thrifty Phenotype	Hales CN et al (185)
Fetal Salvage	Hofman PL et al (221)
Catch-up Growth	Cianfarani S et al (224)
Fetal-Insulin	Hattersley AT et al (235)
Developmental Plasticity	Gluckman PD et al (162)
Epigenetic Mechanism	Godfrey KM et al (244)

2.3.13. Summary

Several hypotheses have been proposed over the past 10 years to explain the inverse association between birth size and later development of chronic diseases. Table 2.1 is a summary of these hypotheses and their supporting literature. The focus of each hypothesis is either the effect of an adverse environment or genetic susceptibility or gene-environment interaction. The gene-environment interaction hypothesis is now the most attractive.

These epidemiological, clinical and animal studies provide evidence that the intrauterine environment influences the growth and development of the fetus as well as the risk of subsequent development of chronic disease in adult life. Despite various mechanisms that have been proposed to demonstrate this concept, the pathophysiological link is still unclear. Furthermore, there is paucity of data demonstrating these concepts in African children where there is a high rate of LBW babies resulting from IUGR often due to maternal malaria in pregnancy.

Summary of Literature

This section has provided an overview of key areas of work as they relate to cardiovascular outcomes in the context of Sub-Saharan Africa, under 3 headings: (1) the rise in hypertension and cardiovascular disease (CVD) risk factors; (2) high prevalence of maternal malaria and its consequences for birth size, infant growth and risk of CVD; (3) the mechanisms of developmental origins of adult disease.

It is quite clear that there is an increased rate of CVS risk factors in Nigeria, with an escalating frequency of hypertension proportionally greater than other risks. Mortality from CVS causes is also on the increase. There are modifications in lifestyle and age structure associated with economic transition that are related to these changes.

Furthermore, malaria, which is still hyperendemic and common in pregnant women, causes maternal anemia and low birth-weight, which, as in many experimental models, may predispose to increased vascular disease in the offspring. It is recognised in many studies worldwide that there is an inverse relationship between birth weight and BP. This observation has been supported by epidemiological and experimental studies and various hypotheses have been examined to explain the mechanisms underlying this important observation.

The key focus of this thesis was to examine this phenomenon in Nigerian infants in the context of exposure to malaria in utero and early growth with reference to overall impact on the BP in the first year of life. This project sought to determine when and if the relationship between LBW due to malaria in pregnancy results in raised BP in the first year of life thereby setting the scene for the risk of chronic diseases in adult life as earlier proposed by the ‘developmental origins of adult disease’ hypothesis.

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CHAPTER 3

SUBJECTS AND METHODS

3.1. Introduction

This chapter gives an overview of the study design, study population, data collection procedures and statistical analysis.

3.2. Study Design

This is an observational prospective cohort design. This analytical study design is appropriate to assess the effect of intrauterine exposure to malaria on birth size, growth and BP at one year of age in this birth cohort. The subjects are free from outcome at baseline and they are followed to document incidence of outcome variables. The exposure to malaria in pregnancy will be assessed; hence causal influences could be drawn. However, cohort studies are subject to influence of many factors over which the investigator may not have full control. Therefore, it may be difficult to ascertain the causality of the association between the exposure and the outcome variable (1). Another disadvantage of cohort studies is that they may require many years of follow-up of the outcome after the exposure and hence there may be loss to follow-up. However, a major strength of cohort studies is that the design does not require random assignment of subjects, which in many cases may be unethical or improbable. It is usually less expensive than experiments or surveys because direct observation of the group is made over time (1).

This study is novel because previously, a prospective cohort has not been followed up to examine the effects of intrauterine exposure to malaria on growth, metabolic profile and BP in Nigerian children. Furthermore, the role of maternal and cord blood metabolic markers as possible mediators of these relationships has not been investigated.

3.3. Study Site

A semi-urban community, Yemetu-Adeoyo, Ibadan in relatively affluent Southwest Nigeria, was studied. Families come from a range of socioeconomic backgrounds, including those with improving lifestyles. Transmission of malaria is perennial. Recruitment of participants was carried out at the local hospital in the community, Adeoyo Maternity Hospital (AMH). It is the oldest maternity hospital in Nigeria dating from 1927, and provides primary and secondary medical care with over 4000 deliveries each year. Ethical approval for the study was obtained from the joint University of Ibadan / UCH Ethics committee and the University of Manchester Ethics committee.

3.4. Study Administration and Set Up

The following activities were carried out before the recruitment of subjects.

- The participant information sheet and consent forms were prepared and translated to the local language, Yoruba (Appendix 1 and 2).
- The standard operating procedures (SOPs) for the study were prepared (Appendix 3).
- The case record forms and follow-up forms for data collection from the mothers and their babies were prepared (Appendix 4 and 5).
- Research staff comprising nurses, laboratory officers, data administrator and field officers were recruited
- Protocols and materials were prepared for the training of research staff.
- Training of research staff was carried out in the following areas:
    - General information about the study, study aims and milestones
    - General Procedures – High standard, Ethics and Principles of Good Practice
    - Health and Safety
    - Anthropometry measurement
    - Blood Pressure measurement
    - Blood and Laboratory procedures

When the research staff training was completed, advocacy visits were made to the study site, AMH Ibadan, with meetings with the senior management of the hospital. The ante-natal clinic was also visited to talk to the women about the study, create awareness and

answer their questions. The study information sheets (Appendix 1) were given to the women who were interested in participating in the study.

3.5. Subject Selection Criteria

Healthy women aged 18-45 years presenting at AMH for antenatal care at or before 36 weeks gestation and residing within the catchment area of the study centre for at least 2 years were recruited. Those babies born ≥37 weeks gestation at AMH were included.

A cohort of 500 pregnant women in good general health was planned to be recruited at AMH. The sample of 500 women was to ensure that a minimum of 400 infants were not only enrolled but followed to the age of one year. Table 3.1 shows the details of study design.

Pregnant women were enrolled over one year to cover both dry and wet seasons. In the year, 3496 women booked for ante-natal care at the hospital, of which 659 women were eligible because the others were not planning their delivery at AMH. Of 659 eligible women, 624 were recruited but 161 still did not deliver at AMH. Therefore, the final cohort included 463 mother-baby pairs. Of these, 27 were excluded due to 4 (0.9%) maternal deaths, 11 (2.4%) stillbirths, 5 (1.1%) miscarriages and 7 (1.5%) neonatal deaths leaving 436 mother-baby pairs measured at birth. At the second antenatal visit, 467 maternal samples were obtained for metabolic profile and 187 cord blood samples at birth. At 3 months, 384 babies were measured. There had by then been one infant and one maternal death. At 12 months of age, 380 babies were measured. There had been 9 infant deaths mainly from febrile illnesses. Others had relocated from the study catchment area or were no longer interested. Our follow-up response rate was 87%.

Only infants who were measured at all three time points (birth, 3 and 12 months, n=318, 173 boys, 145 girls) were included in the analysis at one year of age.

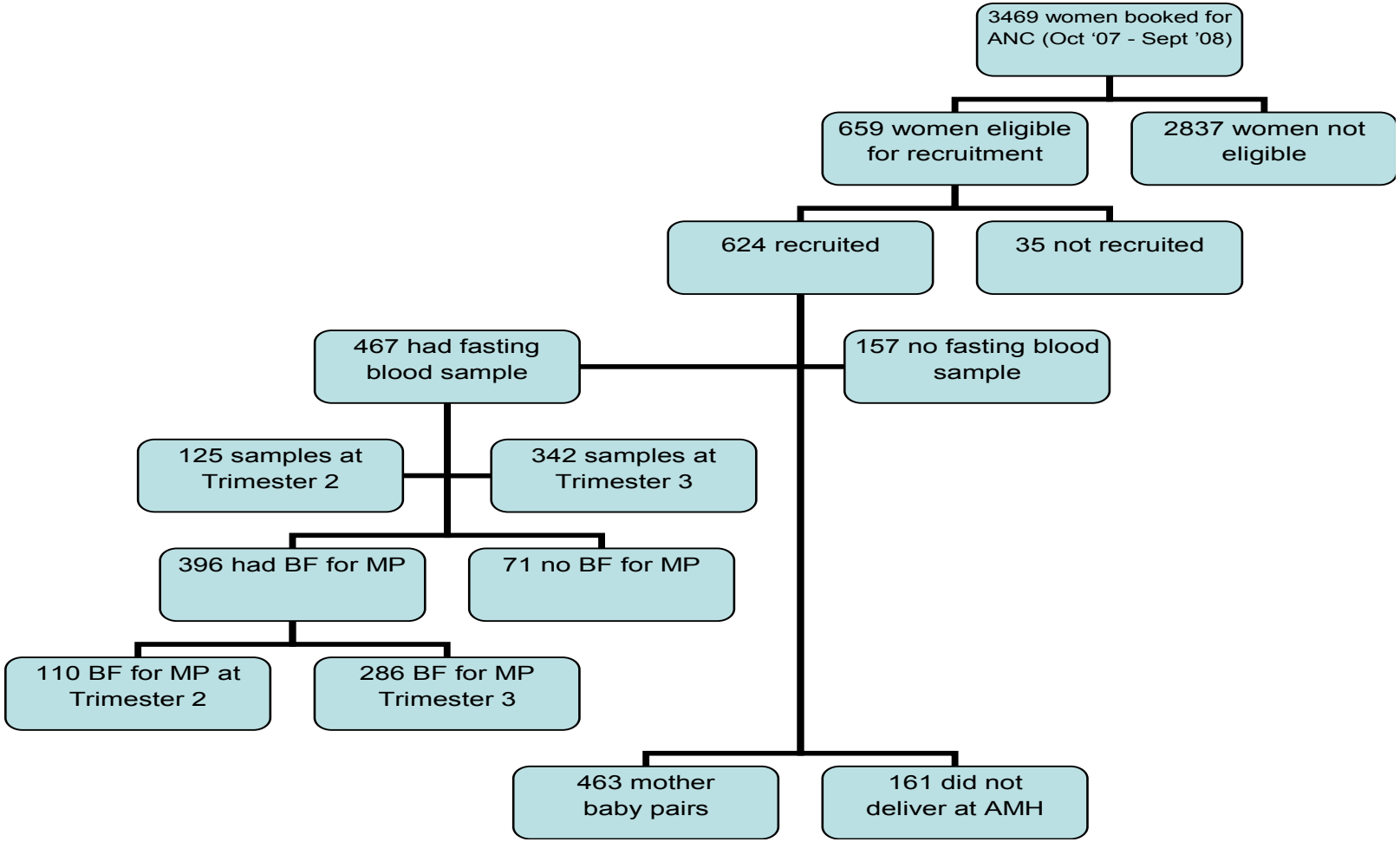

Figure 3.1: Flow chart showing details of mothers recruited in pregnancy until delivery

AMH – Adeoyo Maternity Hospital: BF for MP – Blood film for Malaria Parasites

3.5.1. Exclusion Criteria

Women with multiple pregnancies or with chronic diseases such as hypertension and diabetes, or those positive for human immunodeficiency virus (HIV) or sexually transmitted infections at booking and those with pregnancy-induced hypertension were excluded. Those with preterm deliveries (26 babies), babies with known syndromes, metabolic defects, major congenital abnormalities or severe birth trauma were also excluded.

3.6. Recruitment Procedures, Time points and measurements – Table 3.1

3.6.1. Consent

The participant information sheet and consent forms were given to the subjects after explaining to them what the study entails carefully in the appropriate language, most commonly Yoruba or English. The study protocol and the rationale for the study were also explained. The women were given the opportunity to ask questions, and then an informed consent was obtained.

3.6.2. Mothers - At Booking

After obtaining informed consent, socio-demographic, obstetric, family, and health history, particularly related to malaria were obtained including history of fever in this pregnancy and use of antimalarial drugs. Women were recruited at second (277) and at third (347) trimesters. All participants are issued prescriptions of SP for IPT according to standard practice in the hospital.

3.6.3. Maternal Anthropometry and Blood Pressure

Standardized measures of anthropometry were carried out on the women at every visit till delivery. Mother's height was measured on a purpose built stadiometer without shoes,

according to SOP and training video (Appendix 3). The maternal weight was measured with light clothing, without shoes to the nearest 0.1 kg on scale (SECA model) according to SOP and training video instructions (Appendix 3).

The maternal BP was taken at every visit during pregnancy and after delivery according to SOPs, with validated Datascope BP monitor using appropriate sized cuffs (i.e the bladder length of the cuff used was ≥80% and width was > 40% of the arm circumference). Before the BP reading, the woman was comfortably seated and relaxed with the back and arm supported, the legs uncrossed, for at least 5 minutes and not moving or speaking. Her upper arm was supported at the level of the heart with no tight clothing constricting the arm and measurement was taken on the left arm. Three readings were taken at least one minute apart with means of the last two analysed (Appendix 3).

3.6.4. Blood Measurements

During the first visit, a sample of blood was obtained for the following investigations which were carried out at the laboratory in Ibadan.

(i) 2ml of blood in EDTA tube for haemoglobin genotype and full blood count (FBC).

(ii) Thick and thin blood films were prepared, stained with 3% Giemsa at pH 7.2 and examined for malaria parasites under light microscopy. Thick smears were recorded as negative only after 200 high-power microscope fields had been scanned. In those with malaria, absolute parasite counts were determined as previously reported (2;3) by counting the number of parasites (np) among 200 leucocytes on the thick film using the following equation:

Absolute parasite counts (i.e. parasites per microlitre of blood) = (np / 200) x TLC where TLC= subject's total leucocyte count).

For quality control, 40% of positive and negative samples were re-examined by two different trained microscopists. (Appendix 3).

3.7. Follow-up of pregnant women

3.7.1. Anthropometry and Blood Pressure

These women are seen regularly until delivery of their babies. The frequency of antenatal visits followed routine hospital practice and was determined by the gestational age of the pregnancy, which is 4 weekly from the first trimester till 28 weeks gestation, two weekly till 36 weeks gestation and weekly till delivery. During the visits, health data were obtained and their anthropometry and BP by standard procedures.

Before their expected date of delivery, information about the baby's follow up procedures was given to them again and an informed consent was obtained.

3.7.2. Blood Measurements

During the second visit, i.e second and third trimesters, 10mls of blood was taken under fasting conditions of which 6 mls was put in a lithium heparin tube and put immediately on ice for TNFα, insulin, and lipids i.e TC, LDL-C, HDL-C and TG determinations. 2mls of blood was put in fluoride oxalate tube for glucose assay. Plasma was separated by centrifugation at 3000rpm and 4^0C for 10 minutes and aliquoted into microtubes and stored immediately in a -80^0C freezer (Appendix 3).

During this second and every subsequent visit until delivery, thick blood films for malaria parasites were taken, stained with Giemsa, examined for malaria parasites by microscopy and absolute parasite counts were determined (Appendix 3).

Table 3.1: SUBJECTS, METHODS AND TIMEPOINTS

Mother - Task	Booking	28 to 36 wks
HIV Status	✓	
History of previous pregnancies	✓	
Health / medication history i.e antimalarials etc	✓	✓
Family & Social history	✓	
Anthropometry (height, weight)	✓	✓
Physical examination	✓	✓
Blood Pressure	✓	✓
Haematology samples – Malaria films	✓	✓
Full blood count		✓
HB genotype	✓	
Metabolism & Inflammatory markers		
Glucose, Insulin, Lipids		✓
TNF		✓

Child - Task	Birth	3m	9m	1yr
Birth history	✓			
Weaning diet, Health / Medication history		✓	✓	✓
Anthropometry (height, weight, MUAC)	✓	✓	✓	✓
OFC, skinfold measurements	✓	✓		✓
Blood pressure	✓	✓		✓
Haematology samples – Malaria films (cord blood)	✓	✓		✓
	✓			
Placental smears for malaria parasites				
Full blood count	✓	✓		✓
Metabolism & Inflammatory markers				
Glucose, Insulin, Lipids (cord blood)	✓			✓
TNF (cord blood)	✓			✓
Growth factor - IGF-I (cord blood)	✓			✓

3.8. Delivery and Recruitment of Babies

At the delivery of each baby, 10mls of cord blood was collected from the umbilical vein on the fetal surface of the placenta. 2mls of the sample was put in EDTA bottle for FBC, 2mls in fluoride oxalate tube for plasma glucose. The remaining sample was put in the lithium heparin tube which was put immediately on ice for lipids, IGF-I, TNFα and insulin determinations.

The placenta was weighed and turned to the maternal surface. The cotyledons were exposed and 1ml of blood obtained from the intervillous space which was used to prepare a blood smear on a slide for malaria parasite examination. A blood film for malaria parasite was also prepared from cord blood sample.

The delivery and birth data forms were completed to obtain full information about the baby's delivery (appendix 4).

3.9. Anthropometry of Babies

Babies were measured within 72 hours of life. They were weighed without a nappy to the nearest 0.1kg. Length was measured from crown to heel on a stadiometer. Head circumference was measured around the widest circumference of the head using a non-stretchable tape measure. Skinfolds (triceps, biceps, sub-scapular, and suprailiac) were measured using Holtain calipers on the left side of the body. All anthropometry measures were done according to SOP and training video instructions (appendix 3).

All measurements were carried out at every visit in duplicate or triplicate if disagreeing by >15%.

3.10.Infant BP

Before taking the BP reading, the baby was comfortably lying on the mother's lap for at least 5 minutes, and many times they were asleep. The measurement was carried out

using appropriate newborn cuffs selected according to baby's upper arm length, extending completely around the arm with the bladder width covering at least two-thirds of the upper arm. Measurement was obtained according to SOP on the left arm with the Datascope BP monitor, specifically validated for infants (appendix 3) and repeated three times and the mean of the last two readings analysed.

3.11. Follow-up of babies (*birth, 3, 9, 12 months)* - **Table 3.1**

3.11.1. Anthropometry and Health Checks

Breast-feeding, weaning and other histories were taken including full physical examination. Anthropometry and BP were carried out by standard procedures. Every mother was asked to keep a diary record of any febrile illness, checked at each visit. Any infant febrile at the time of assessment had a full blood count and malaria smear and any other clinically relevant investigations. Diagnosis and treatment followed normal clinical practice.

3.11.2. Blood measurements

In addition to cord blood obtained at birth, blood was obtained at 12 months for FBC and thick blood smear for malaria parasites, and plasma for lipids, glucose, insulin, TNFα, and IGF-I. The samples were stored at -80^{0}C prior to being sent on dry ice to Manchester for analysis. Thick blood smears were taken, examined for malaria parasites by microscopy and absolute parasite counts were determined (appendix 3).

Figure 3.2 is a summary of the details of the cohort follow-up.

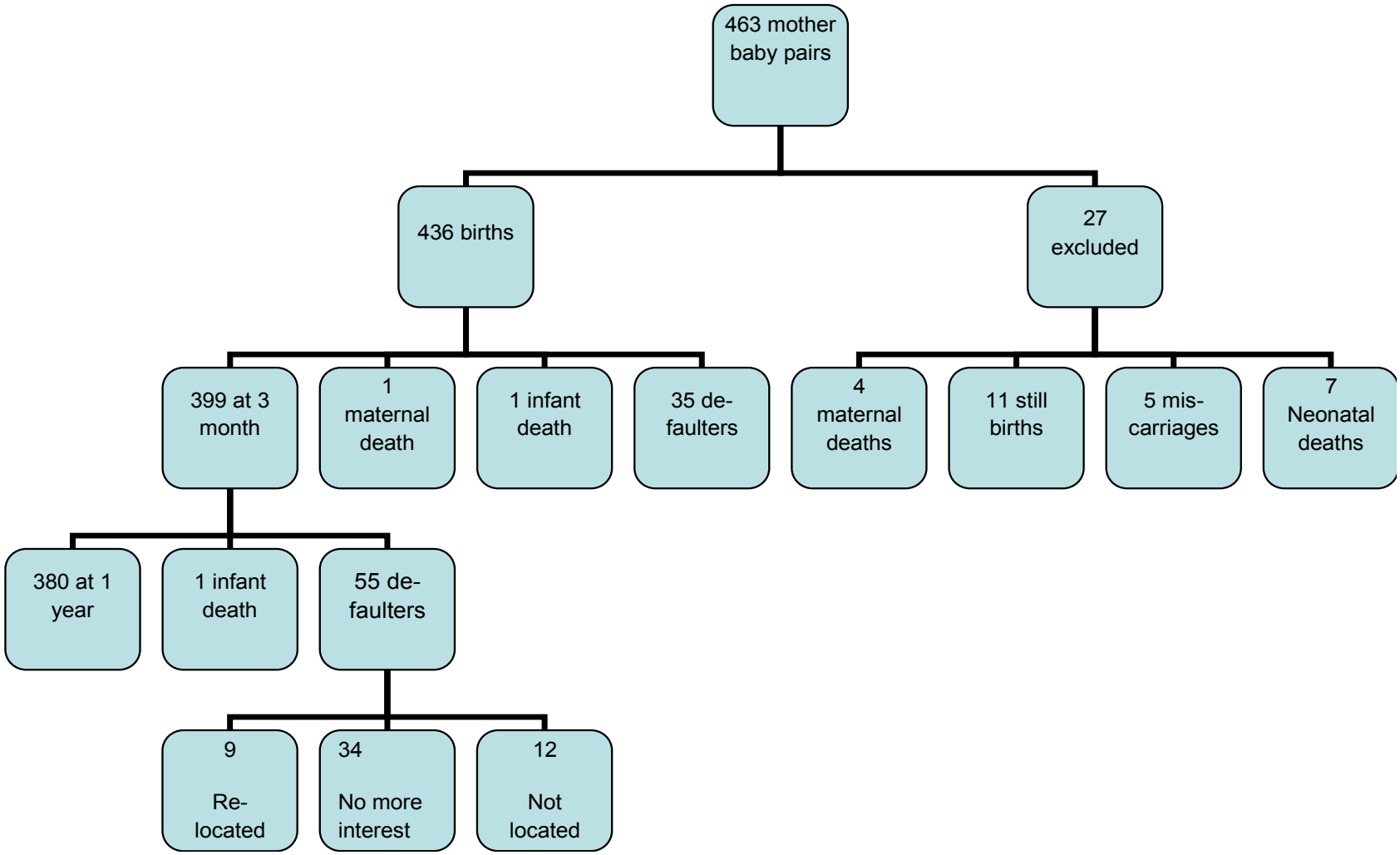

Figure 3.2: Flow chart showing details of Infant recruitment and follow-up from birth till one year of age

The demographics and clinical characteristics of the mothers and infants followed up are shown in Tables 3.2 and 3.3.

Table 3.2 shows the clinical and demographic characteristics of all women at recruitment in relation to presence of malaria in the women. All women recruited had at least 2 antenatal clinic attendances, 94% attended 3 times, 80% four times and 63% five times before delivery. The median (range) durations of the pregnancy at booking and at delivery were 28 (12 – 36) and 39 (37 – 42) weeks respectively with 28% of women being primigravida.

Table 3.3 shows the infant demographic and clinical characteristics at birth. The placenta-fetal weight ratios for infants whose mothers have malaria were significantly lower than those whose mothers did not have malaria. The placenta-fetal weight ratio is a way placental efficiency can be assessed. These lower numbers in babies of mothers with malaria suggests that their placentas have impaired function.

Table 3.2: Maternal clinical characteristics at recruitment by malarial status

Parameter	Total n= 436		Malaria Absent n=225 (52%)		Malaria present n=211 (48%)			
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**t test**	**P value**
Age (years)	28.6	5.0	29.4	4.8	27.7	5.1	3.50	**0.001**
Gestational age (weeks)	27.1	5.1	27.3	4.9	26.7	5.4	0.89	0.374
Body temperature (^{0}C)	37.0	0.3	37.1	0.6	37.2	0.6	0.01	0.992
Weight (kg)	64.2	11.2	64.5	11.5	63.9	10.8	0.53	0.596
Height (cm)	160.2	5.7	160.4	5.9	160.1	5.3	0.52	0.600
SBP (mmHg)	104.1	8.6	104.0	9.4	104.2	7.7	-0.20	0.840
DBP (mmHg)	60.9	6.9	60.9	7.2	61.0	6.7	-0.10	0.921
Packed cell volume (%)	31.8	3.2	32.2	2.9	31.5	3.5	2.21	**0.028**
Gravidity	1.7	1.5	1.9	1.6	1.5	1.3	2.80	**0.005**

SBP- Systolic blood pressure; DBP- Diastolic blood pressure

Table 3.3: Infant clinical characteristics at birth by maternal malarial status

Parameter	Total N = 436		Malaria Absent n = 225		Malaria Present n = 211			
	Mean	SD	Mean	SD	Mean	SD	t test	p value
Placental Weight (g)	565.15	115.4	557.19	111.5	573.65	119.2	-1.33	0.186
Placental-fetal weight ratio	5.39	1.4	5.56	1.5	5.20	1.2	2.35	**0.019**
Weight (kg)	2.91	0.4	2.97	0.4	2.85	0.4	2.72	**0.007**$^{+}$
Length (cm)	48.67	2.3	48.97	2.4	48.35	2.1	2.82	**0.005**$^{+}$
OFC (cm)	34.32	1.3	34.50	1.4	34.2	1.2	2.21	**0.028**$^{+}$
MUAC (cm)	9.86	0.9	9.95	0.8	9.77	0.9	2.11	**0.036**$^{+}$
Subscapular (cm)	4.21	0.9	4.28	0.9	4.14	0.8	1.67	0.097
Suprailiac (cm)	4.35	0.9	4.42	0.9	4.27	0.9	1.60	0.111
Triceps (cm)	4.14	0.9	4.19	0.8	4.08	0.9	1.28	0.200
Biceps (cm)	3.63	0.7	3.70	0.8	3.55	0.6	2.15	**0.032**$^{+}$
Subscap/Triceps Ratio	1.03	0.1	1.03	0.1	1.02	0.1	0.13	0.899
SBP (mmHg)	71.00	12.9	72.40	13.4	69.50	12.2	2.34	**0.020**$^{+}$
DBP (mmHg)	36.11	9.0	37.00	9.5	35.20	8.4	1.97	**0.049**$^{+}$
Weight SDS	0.74	0.0	0.08	0.0	0.07	0.0	2.89	0.004
Length SDS	1.24	0.1	1.25	0.1	0.23	0.1	3.03	0.003
OFC SDS	0.87	0.0	0.88	0.0	0.89	0.0	2.46	0.014
MUAC SDS	0.25	0.0	0.25	0.0	0.25	0.0	2.28	0.023
Subscapular SDS	0.11	0.0	0.11	0.0	0.11	0.0	1.60	0.110
Triceps SDS	0.11	0.0	0.11	0.0	0.10	0.0	1.33	0.186
Biceps SDS	0.09	0.0	0.10	0.0	0.10	0.0	2.17	0.031

OFC – Occipito-frontal circumference; MUAC – Mid-upper arm circumference
SBP- Systolic blood pressure; DBP- Diastolic blood pressure

3.12. Metabolic Assays

Total cholesterol, HDL-C and TGs concentrations were determined by using standard enzymatic procedures on an automatic analyser (COBAS MIRA/ HITACHI 704 - Roche Diagnostics, Germany). The inter- and intra-assay coefficient of variation (CV)s for all parameters was < 5%. LDL-cholesterol was calculated using the Friedewald formula (4).

Glucose was measured by the glucose oxidase method using a commercial kit (Randox, Crumlin, UK) on a YSI 2300 stat plus analyser (YSI, Farnborough, Hants, UK). The intra-assay CV was 1.5% at 4.1mmol/l, and inter-assay CVs were 2.8% and 1.7% at 4.1 and 14.1 mmol/l respectively.

Insulin was measured by ELISA using a commercial kit (Mercodia, Uppsala, Sweden). Assay sensitivity was 1 mU/l. Intra-assay CVs were 3.4% and 3.2% at 11 and 154mU/l, and equivalent inter-assay CVs were 3.6% and 2.9%.

IGF-I and TNF-α were measured using Immulite 2000 assays (DPC, Lumigen Inc, Southfield, UK). Respective assay sensitivities were 25µg/l and <0.09 µg/l. Inter-assay CV values at 48.9 and 158.5µg/l were 7.6 and 9.2% for IGF-1. For TNF-α, intra-assay CVs were 6.7% and 5.3% at 6.3 and 19pg/ml, and the inter-assay CV values at 6.1 and 18.6 pg/ml were 8.2 and 9.7% respectively.

3.13. Validity of Anthropometric Measurements

Three nurses, already trained in paediatric venepuncture, were trained in anthropometry methods and they carried out all measurements throughout the study. These methods are based on the WHO manual (1995). Inter-observer and within-observer error were minimized through 3 monthly refresher training sessions where we revisited the SOPs and training videos. This was to ensure standardization of the technique of measurement throughout the study. All equipment used in measuring the women and the babies were

calibrated regularly and the same equipment was used consistently throughout the study in order to minimize measurement errors related to different equipment.

3.14. Cohort maintenance

Multiple contact addresses and mobile telephone numbers for about 95% of the women were obtained. All participants received a telephone call to confirm their appointment and attendance the day before the visit.

In addition, most participants were visited at home between main visits, and small gifts as a token of thanks and as a proxy for 'out of pocket' expenses were provided. After the blood sampling at the second visit, all women received a tin of evaporated milk. After the delivery of the baby, all mothers were given a pack of baby nappies when they are discharged from hospital. In addition, all women also had the project contact telephone number in case they had any concerns.

3.15. Definitions

Anaemia in the mothers was defined as packed cell volume (PCV) < 30%.

Malaria parasitaemia was considered positive if the asexual blood stages of Plasmodium falciparum were identified in peripheral blood during pregnancy or at delivery, in the placenta or in cord blood.

For the purposes of this study, all women were grouped into 2 categories:

a) 'No Malaria' - no parasites detected throughout pregnancy or at delivery (n=225).
b) 'Malaria present' - parasites present at least once during pregnancy and/or at delivery (n=211).

All women were stratified into two categories to distinguish between the *timing* of malaria through pregnancy and at delivery:

a) 'Malaria during pregnancy only' - presence of malaria parasites at least once during pregnancy but not at delivery (n=138).

b) 'Malaria at delivery' - mothers with parasites present in the placenta and/or their peripheral blood sample at delivery and/or in the cord blood (n=73). Of these, parasites were present during pregnancy in (n=15) and not present in (n=58).

All visits during pregnancy and at delivery were taken into account.

In order to examine the effects of parasite load during pregnancy and at delivery, parasite density was initially classified into low (<1000 parasites/μl), high (1000-10000/μl) and very high (>10000/μl). Due to small numbers, the latter two categories were merged.

3.16. Catch up Growth

Data from repeated measures over time can be subject to regression to the mean (5). Actual change in standard deviation scores (SDS) and change in SDS predicted by regression to mean (RTM) were calculated. If the observed change in SDS is greater than that predicted by RTM, then true catch up growth has occurred (6). If the observed change in SDS is less than RTM, then that child's growth is failing.

3.17. Power Calculations

Hypothesis i: Assuming 50% malarial infection during pregnancy (7), henceaffecting 200 babies of the 400 pregnancies recruited, the study will have 80% (90%) power at $p<0.05$ to detect differences at one year of 1 (1.2) cm in length, 600 (700) grams in weight, and 3.1 (3.6) mmHg in SBP between babies *whose mothers had malaria* and those without. At birth we could detect birth weight and birth length differences of 170g and 1.2cm assuming respective birthweights of 2840 (SD 533) gms in babies whose mothers had malaria and 2960 (450) gms in those without as in a previous study (8).

Hypothesis iii: Assuming a median birth weight of 2800gms (based on previous study on Malawian babies), and having a birth weight less than the median being relatively small, if 50% of mothers of all infants had malaria, we will have 80 (90) % power to detect differences at age one year of 4.3 (5.0) mmHg in SBP between *babies* of mothers with malaria and those without. Differences of this magnitude are well within the range found at these ages.

3.18. Statistical Analysis

Data were analysed using SPSS version 14 (SPSS Inc, Chicago, IL).

Results were expressed as mean (SD) or median (IQ range). The main predictors were maternal malarial status, lipids, and glucose levels. Other predictors such as insulin, IGF-I and TNF-α were positively skewed. Most outcomes (length, weight, BP at birth, 3 and 12 months and change in BP over the first year) were normally distributed. Analyses were with parametric and non-parametric tests

The initial analysis comparing mean levels of each outcome between groups defined by the predictors was by t-test or analysis of variance.

Levels of insulin, IGF-I and TNF-α were skewed and therefore tested non-parametrically, while lipids and glucose levels were normally distributed and tested parametrically. Correlations were by Spearman's test. Associations between categorical variables and malaria were assessed by means of Chi-square test, simple linear and stepwise multiple regressions were used to examine the relationship between an outcome and several predictors simultaneously to adjust for potential confounders to assess the determinants of infant size and BP. Interaction terms were added to these regression models to test for effect modification between predictors. Two-sided P values < 0.05 were considered significant.

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CHAPTER 4

Chapters 4 to 6 present three papers in format suitable for submission for publication in a peer-reviewed journal. The three papers have been submitted for publication.

Publication Number – 1

This chapter will present the first paper titled: **'Maternal malaria, birth size and BP in Nigerian infants: Insights into developmental origins of hypertension from Ibadan Growth Cohort'.** In this paper, we examined the impact of maternal malaria in pregnancy on size and BP at birth.

Authors: Ayoola OO, Gemmell I, Omotade OO, Adeyanju OA, Cruickshank JK, Clayton PE

Authors' contributions

OA participated in overall study conception and design, data collection, analysis interpretation and manuscript preparation
IG was involved in study design and data analysis.
OO supervised and assisted in data collection and manuscript preparation.
OA assisted in data collection and manuscript preparation.
KC and PC are my Supervisors and both participated in overall study conception and design, data interpretation and manuscript preparation.

Maternal malaria, birth size and blood pressure in Nigerian infants: Insights into the developmental origins of hypertension from Ibadan Growth Cohort

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Short title: maternal malaria, birth size and infant BP

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Abstract

Background: Hypertension is an increasing health issue in sub-Saharan Africa where malaria remains common in pregnancy. We established a birth cohort in Nigeria to evaluate the early impact of maternal malaria on blood pressure (BP).

Methods: Anthropometric measurements, BP, blood films for malaria parasites and haematocrit were obtained in 436 mother-baby pairs. Women were grouped to distinguish between the *timing* of malaria parasitaemia as 'No Malaria', 'Malaria during pregnancy only' or 'Malaria at delivery', and parasite density as low (<1000 parasites/µl of blood) and high (≥1000/µl).

Results: Prevalence of maternal malaria parasitaemia was 48% and was associated with younger maternal age ($p<0.001$), being primigravid ($p=0.022$), lower haematocrit ($p=0.028$). High parasite density through pregnancy had the largest effect on mean birth indices so that weight, length, head and mid-upper arm circumferences were smaller by 300g, 1.1cm, 0.7cm and 0.4cm respectively compared with 'No malaria' (all $p\leq0.005$).

In pregnancies having 'Malaria at delivery', systolic (BP) SBPs adjusted for other confounders were lower respectively by 4.3 and 5.7mmHg/kg compared with 'malaria during pregnancy only' or 'none'. In contrast the mean SBP and diastolic BPs (DBP) adjusted for weight were higher by 1.7 and 1.4mmHg/kg respectively in babies whose mothers had high compared with low parasitaemia.

Conclusions: As expected, prenatal malarial exposure had a significant impact on infant birth weight. Malaria at delivery was associated with the lowest BPs while malaria through pregnancy, which may attenuate growth of the vascular network, generated higher BPs adjusted for size. These neonatal findings have potential implications for cardiovascular health in sub-Saharan Africa.

Key words: malaria in pregnancy; infants; birth size; blood pressure; Sub-saharan Africa

Introduction

Hypertension is now a public health and economic problem in Sub-Saharan Africa with a prevalence up to 33% in urban areas in Nigeria.(1;2) Its well-known complications and mortality occur at a younger age than in developed countries (3;4). In sub-Saharan Africa, malaria is hyperendemic, particularly in pregnancy with prevalence rates from 20 to 44% in Nigerian women (5). Most cases of malaria in pregnancy are asymptomatic because of immunity acquired during previous exposures (5;6). However, asymptomatic infection still has significant consequences for maternal and infant health resulting in maternal anaemia and intrauterine growth retardation (IUGR), which causes 43% of preventable low birth weight babies (LBW, birth weight <2500g), contributing to 75,000–200,000 infant deaths each year (7-10). In Nigeria about 12-24% of newborns are LBW as a result of IUGR (11;12).

In numerous global studies, adult risk of hypertension and other chronic disease is associated with LBW (13;14). In an emerging economy, LBW babies who show catch-up growth may be at particular risk of developing such disease in midlife (15). These observations subsequently led to the 'developmental origins' hypothesis.

Blood pressure (BP) correlates with birth weight in newborns (16-18). There may be differences in the relationship between birth weight and BP in preterm babies small (SGA) and appropriate for gestational age (AGA) in the first week of life (18). AGA babies showed the expected positive correlation between birth weight and BP while SGA babies did not (19). There are limited data on neonatal BP in African children, in particular exploring the relationships between BP, birth size and exposure to malaria in utero.

Therefore, we tested the hypothesis that BP at birth would be higher and birth size would be smaller in babies whose mothers had malaria in pregnancy, defined by its timing during pregnancy and/or at delivery and the magnitude of parasite density. This in turn may affect the rate of BP rise through childhood and set the scene for hypertension in later life.

Subjects and Methods

Ethics Statement

Ethical approval was obtained from the joint University of Ibadan / University College Hospital Ethics committee and the University of Manchester Ethics committee. The study protocol and the rationale for the study were explained carefully in appropriate language, most commonly Yoruba or English, with questions answered as needed and written informed consent was obtained from all participants. After the delivery of their babies, another written informed consent was also obtained for the participation of their babies in the study.

Study Site

A semi-urban community, Yemetu-Adeoyo, Ibadan in Southwest Nigeria, where malaria transmission is perennial, was the site for the study. Families come from a range of socioeconomic backgrounds. The local community hospital, Adeoyo Maternity Hospital (AMH), the oldest maternity hospital in Nigeria dating from 1927, provides primary and secondary medical care with over 4000 deliveries each year.

Participants

Healthy women aged 18-45 years presenting at AMH before 36 weeks gestation and residing within the catchment area of the study centre for at least 2 years were recruited. Singleton babies born at ≥37 weeks gestation were included.

Women with chronic diseases such as hypertension and diabetes, or those positive for HIV or sexually transmitted infections at booking, were excluded. Preterm deliveries, babies with known syndromes, metabolic defects, congenital abnormalities or severe birth trauma were excluded from the study.

Women were enrolled over one year to cover both wet and dry seasons.

In the year, 3496 women booked for ante-natal care, but many were not planning their delivery at AMH so 659 women were eligible, of which 624 were recruited but 161 still did

not deliver at AMH; thus the final cohort included 463 mother-baby pairs. Of these, 27 were excluded due to 4 (0.9%) maternal deaths, 11 (2.4%) stillbirths, 5 (1.1%) miscarriages and 7 (1.5%) neonatal deaths, leaving 436 pairs. There was no significant difference in the socio-demographic and clinical data of excluded women.

Procedures: Time points and measurements

Standard operating procedures (SOPs) were developed. Informed consent, using forms translated into Yoruba, was taken at booking, then demographic, obstetric, family and health details, malarial history and use of antimalarial drugs was recorded. All women were issued with prescriptions for sulphadoxine-pyrimethamine (SP) for Intermittent Preventive Therapy (IPT) for malaria according to standard hospital practice.

Maternal Anthropometry

Standardized measures of anthropometry and BP were taken at every antenatal visit until delivery, weight to the nearest 0.1 kg (SECA scale), height on a stadiometer to the nearest 0.1 cm, both without shoes, according to the SOP and training video.

Blood Samples

At booking, 2ml of blood obtained in EDTA tubes and blood films were prepared, stained with 3% Giemsa at pH 7.2 and examined for malaria parasites (MP) under light microscopy. Thick smears were recorded as negative only after 200 high-power microscope fields had been scanned. In those with malaria, absolute parasite counts were determined as previously reported (20) by counting the number of parasites (np) among 200 leucocytes on the thick film as follows: Absolute parasite counts (per microlitre of blood) = (np / 200) x TLC where TLC= subject's total leucocyte count).

For quality control, 40% of negative and positive samples were re-examined by two different trained microscopists.

Follow-up, Delivery and Recruitment of Babies

Women were followed up until delivery based on routine ante-natal practice, determined by gestational age. Repeat MP blood films were obtained every visit and at delivery when a film was also prepared from cord blood. The placenta was weighed, turned to the maternal surface, cotyledons exposed and 1ml of blood obtained from the intervillous space for a placental malarial blood smear. Detailed delivery information was recorded.

Infant Anthropometry and Skinfold Measures

Babies were weighed naked to the nearest 0.1kg, length was measured from crown to heel on an infant stadiometer to the nearest 0.1cm and occipito-frontal circumference (OFC) around the widest circumference of the head using a non-stretchable tape to the nearest 0.1cm. Skinfold thicknesses (triceps, biceps, sub-scapular, and suprailiac) were measured using Holtain calipers on the left side to the nearest 0.1mm. Measurements were obtained in duplicate or triplicate if disagreeing by >15%. All babies were examined within 72 hours of birth.

Maternal and Infant BP

Maternal and Infant BPs were taken according to a standard protocol and SOP. Before the BP reading, the woman was comfortably seated and relaxed with the back and arm supported, the legs uncrossed, for at least 5 minutes and not moving or speaking. Her upper arm was supported at the level of the heart with no tight clothing constricting the arm. The measurement was carried out on the left arm with validated Datascope BP monitor using appropriate-sized cuffs (i.e the bladder length and width of the cuff supplied were $\geq$80% and 40%, respectively, of the arm circumference).

Before performing the BP reading, the baby was comfortably lying on the mother's lap for at least 5 minutes, and many times they were asleep. The measurement was again done with the Datascope BP monitor, specifically validated for infants, using appropriate newborn cuffs on the left arm.

In both mother and child, measurement was repeated three times and the mean of the last two readings analysed.

Validity of Anthropometric and BP Measurements

Three nurses trained in anthropometry and BP methods, based on the WHO manual (1995) and SOPs, carried out all measurements throughout the study on the same equipment. Inter-observer and within-observer error were minimized through 3-monthly refresher training sessions.

Definitions

Anaemia was defined as packed cell volume (PCV) < 30%.

Malaria was defined as asexual blood stages of Plasmodium falciparum in peripheral blood or placenta of the pregnant women or cord blood at delivery. All visits during pregnancy and at delivery were taken into account.

For this study, women were first grouped into 2 categories:

a) 'No Malaria' - no parasites detected throughout pregnancy or at delivery (n=225).

b) 'Malaria present' - parasites present at least once during pregnancy and/or at delivery (n=211).

Women with malaria were then stratified to distinguish between the *timing* of malaria through all visits in pregnancy and at delivery:

a) 'Malaria during pregnancy only' - presence of malaria parasites at least once during pregnancy but not at delivery (n=138).

b) 'Malaria at delivery' - mothers with parasites present in the placenta and/or their peripheral blood sample at delivery and/or in the cord blood (n=73).

To examine effects of parasite load during pregnancy and at delivery, parasite density was classified into low (<1000 parasites/µl), or high (≥1000/µl).

Statistical Analysis

Data were analysed using SPSS version 14 (SPSS Inc, Chicago, IL). Socioeconomic index scores were based on occupations and educational attainment of both parents on scales I to V, as previously (21). Means of the four scores to the nearest whole number were assigned. Analyses included Chi squared tests, t-tests, analysis of variance, simple linear and multiple regressions. Two-sided P values < 0.05 were considered significant.

Results

Clinical characteristics of mothers and malaria status

Parasitaemia was present at least once in pregnancy and/or delivery in 211 of the 436 recruited mothers (total 48%, with 30% having low parasitaemia and 18% high parasitaemia). Classified by *timing*, 138 (31%) had malaria parasitaemia at some time during pregnancy only and 73 (17%) at delivery.

56% of those with parasitaemia were primigravid, so malaria and first pregnancy were significantly associated (X^2 = 5.276, p=0.022). About half of the women reported the use of preventive measure such as chemoprophylaxis / insecticide spray or coil / bed nets / netted windows but these were not associated with protection from malaria (p>0.05). Social class and maternal malaria parasitaemia were also not associated (X^2 = 1.557, p=0.212).

Most women were asymptomatic. A complaint of fever in the week preceding recruitment and at every visit until delivery was reported in 9 women and fever recorded in only 6 women. Women with malaria were younger (27.7 vs 29.4 years, p= 0.001) more likely to be anaemic than those without parasitaemia. Malaria parasitaemia and anaemia was found in 19% of all women, but anaemia was present in 32%. Mean (SD) PCV was 32.2 (2.9)% in women without malaria, 31.9 (3.4)% in those with low parasitaemia and 30.7(3.6)% in those with high parasitaemia (p=0.003).

There were no differences in other clinical characteristics such as pregnancy duration at booking, weight, height, body temperature, SBP and DBP in women with and those without malaria.

Clinical characteristics of babies and malarial status: parasite density compared with timing

Growth variables

At birth, anthropometric measures were similar in boys and girls except for OFC which was greater by 0.3cm (0.08-0.58, p=0.011) in boys. The boys had lower unadjusted systolic (S)BP (-2.3mmHg, p=0.067) and diastolic (D)BP (-1.6mmHg, p=0.067). The differences in BP were not statistically significant (data not shown).

The placental weights of infants born to women with and without malaria parasitaemia were not significantly different but the placenta-fetal weight ratios for infants whose mothers have malaria were significantly lower than those whose mothers did not have malaria (p=0.019) - Table 4.1.

Anthropometric variables and skinfolds of infants born to women with malaria parasitaemia were globally smaller than those of women without (Table 4.1 and 4.2a).

Based on *parasite density*, birth weight, length, OFC, and MUAC of infants born to women with high parasitaemia were smaller by 300 (95% CI 100-400)gm, 1.1 (0.5-1.6)cm, 0.7 (0.3-1)cm and 0.4 (0.2-0.6)cm respectively compared with those without parasitaemia (Table 4.2a). These babies were also thinner, shorter, had smaller OFC and MUAC than those whose mothers had low parasitaemia; skinfold thicknesses (biceps, triceps and subscapular) were also lower (Table 4.2a).

Analysis by *malaria timing* showed that parasitaemia at delivery had no additional impact on anthropometric variables compared to parasitaemia during pregnancy only (Table 4.2b).

Regression analyses testing effects on growth parameters showed that birth length, gestational age at birth and maternal weight were each independently associated with birth weight, while parasite density was inversely related (Table 4.3a). Only birth weight and

maternal height were independently related to birth length with no effect of malarial status (Table 4.3a). There were no significant gender effects on birth weight and length.

Impact of maternal malaria parasitaemia on Infant BP

Babies whose mothers had no parasitaemia had higher mean SBP (p=0.02) and DBP (p=0.049) than those with parasitaemia (Table 4.1). This effect can be attributed to malaria timing: babies whose mothers had parasitaemia at delivery had SBP lower by 4.3 (0.6-8.0)mmHg/kg than those of women with parasitaemia in pregnancy only, and 5.7 (0.8-8.9)mmHg/kg lower than those without parasitaemia (Table 4.2b).

In contrast when evaluating the effect of parasite density through pregnancy (Table 4.2a), mean SBP and DBP were not different. However neonatal BP is size dependent and when adjusted for weight, SBP and DBP were higher by 1.7 (0.2, 3.3)mmHg/kg, p=0.024 and 1.4 (0.4, 2.3)mmHg/kg, p=0.006 respectively in babies whose mothers had high parasitaemia compared to those with low (Table 4.4).

In regression analyses, infant SBP was independently associated with birth weight, gender (girls) maternal age, and maternal SBP and inversely associated with birth length and maternal height. Malaria parasitaemia timing, specifically at delivery was also a determinant of infant SBP (Table 4.3b). Maternal weight and DBP were not. Infant DBP at birth was positively associated with gender (girls), birth weight, gestational age and inversely with maternal height and also with parasitaemia at delivery *(Table* 4.3b).

Discussion

General features of malaria

This study illustrates the continuing impact of malaria in otherwise healthy pregnant Nigerian women, almost half of whom in the study (48%) were affected. The rates confirm recent reports in pregnant Nigerian women (5;22) and are also in agreement with findings

from Malawi, Gabon and Ghana, but lower than in Western Kenya (23-26). The latter areas have similar endemic rates to that in Nigeria. Most of our study cohort was asymptomatic, in line with previous findings that malaria in pregnancy in Africa rarely results in fever or any symptoms and therefore remains mostly undetected and untreated (8).

For those women reporting the use of preventive measures against malaria, including the 53% who said they used chemoprophylactic drugs, there was no difference in malaria parasitaemia frequency. Previous findings have shown that netted windows, insecticide sprays, mosquito repellent and insecticide-treated nets were effective in protecting against malaria (27;28). In Nigeria, there is low use of SP for IPT as recommended for prevention of malaria in pregnancy; this is due to cost, as was the case in this study. When used appropriately, IPT with SP is effective in preventing malaria in pregnancy (29). In Mali, there was a reduction in the incidence of LBW among neonates born to women who used IPT with SP compared to other anti-malarial drugs (30).

In the suburban women in this study, malaria parasitaemia was not associated with social class, similar to a Kenyan report (25) but contrasting with those from Burkina Faso and India, with a higher incidence in low-income groups in rural areas (31;32). Primigravid women were more affected by malaria, as described (33), likely related to protective anti-adhesion antibodies against chondroitin sulphate A-binding parasites developing only over successive pregnancies (34).

In this study, diagnosis of malaria was based on microscopy of peripheral, placental and cord blood samples. Malaria in pregnancy still presents diagnostic challenges and has relied mainly on microscopy in studies in sub-Saharan Africa. Microscopy, though valuable, requires well-trained and skilled staff (35). It can be used for speciation and quantification of parasites as done in this study, as well as assessing response to treatment. Histological examination, although reported to be more sensitive than

microscopy on placental blood samples (36), was not available for this study. Placental blood, in addition to peripheral blood smears, were used to ensure that placental malarial infections were detected when peripheral parasitaemia may be negative (37). Therefore we included all these sites in our definition of malaria in pregnancy and delivery. The finding of lower placenta-fetal weight ratio in infants born to women with malaria parasitaemia confirms previous reports of lower placental weight in infants of mothers with malaria parasitaemia (38). The placenta-fetal weight ratio is a way placental efficiency can be assessed. These lower numbers in babies of mothers with malaria suggests that their placentas have impaired function.

Birth outcomes

As previously reported in Nigeria, birth weight for boys and girls were similar in this study. All other anthropometry were not previously reported in boys and girls (39).

Babies of mothers with parasitaemia were globally smaller, most marked among babies of mothers with high parasite density during pregnancy. Similarly, mean birth weights in Burkina Faso, Tanzania, Mali and Pakistan for babies born to mothers with malaria in pregnancy were lower by 105gm (40), 371gm (41), 382gm (33) and 461gm (42) than those without malaria. Other anthropometry was not reported in these studies.

Birth weight is the single most important determinant of neonatal and infant survival and health, and malaria reduced all growth parameters, (43) probably related to chronic placental infection and insufficiency (43;44).

Effects on infant BP

Some of the mechanisms linking small birth size and adult hypertension are poor maternal nutrition and maternal iron deficiency anaemia, which reduces vascular elasticity (45;46). In mothers with reduced skinfold thicknesses and lower haemoglobin concentrations, their

children's SBP at the age of 10-12 years increased by 2.6 mmHg for each 1g/dl decline in the mother's haemoglobin (45). This confirms findings in experimental studies, where maternal iron restriction in the rat reduced birth weight and led to elevated BP at 40 days of age (47). In this study anaemia at 32% is lower than previously reported in more rural Southern Nigeria but higher than rates from the nearby tertiary University College Hospital, Ibadan, with better obstetric facilities and managing patients in higher social classes (48;49). As elsewhere, anaemia is significantly associated with parasitaemia in primigravid women (5;50). These findings support the theory that maternal anaemia, an important consequence of malaria in pregnancy, could be linked to the genesis of raised BP in children. Our finding also confirms those of other settings (51), that maternal age was associated with newborn BP, which is generally related to birth weight (52;53).

We also found a significant negative association between birth weight and blood pressure in girls as previously reported (54).

In this cohort we found that babies whose mothers had malaria parasitaemia at delivery had lower SBP and DBP (Table 4.3b). This observation could be accounted for in part by findings of lower mean BP in LBW babies and higher mean BP in those with higher birth weight (55), but this may also be related to the acute haemodynamic effects occurring in placental parasitaemia which may lead to lower newborn BP.

Overall, malarial load through pregnancy had the greatest impact on birth size (Table 4.3a). Thus those babies who were exposed to high parasite loads through pregnancy were the smallest, and rather than having lower BP, both SBP and DBP corrected for weight were higher than in those exposed to a low parasite load (Table 4.4). This is in keeping with a developmental origins hypothesis linking placental insufficiency, IUGR and later hypertension. On-going follow-up will reveal whether small babies, having been exposed to intra-uterine malaria, who then show early catch-up growth, will have higher blood pressure. As far as we are aware, this is the first study to follow such a cohort, and

the recognition of raised BP in early childhood could have important implications to health in Africa.

This leads to the question of how these two contrasting observations on BP related to timing and parasite density are mediated. Our hypothesis is that in these LBW babies, placental parasitaemia is associated with significant inflammation in the placental and infant arterial tree leading to more acute placental changes and significant infant vascular dilatation as an initial protective mechanism and hence lower BP at birth in those with parasitaemia at delivery. In contrast, parasitaemia *during* pregnancy leads to general growth restriction and smaller birth size and relatively higher BP for size. The more limited vascular tree of lighter infants may not be able to meet end-organ oxygen and nutritional demand without reactive peripheral vasoconstriction and higher BPs over time. Marginally but progressive higher BPs in infancy and early childhood may result, leading to a higher risk of hypertension in later life. Recurrent post-natal malaria will intermittently alter peripheral blood flow, but again at the expense of optimal supply to particular organs, hence restricting growth. The balance between the overall size of the fetus' vascular tree, how well particular organs grow during pregnancy, notably the renal glomeruli with their afferent and efferent arterioles, and continuing environmental hazards (e.g. infections, limited food supply and food quality) or opportunities (e.g. plentiful physical activity) will determine vascular performance, now measurable by pulse wave velocity (56).

Conclusions

There is a high incidence of malaria and anaemia in this apparently healthy cohort of pregnant women, particularly the younger mothers and primigravids. Malaria in pregnancy adversely affects birth size, with high parasite density during pregnancy having the greatest impact on all growth parameters and being associated with higher BP corrected for weight. Follow-up studies to extend these observations into early childhood and to

provide a better understanding of the influence of maternal malaria on BP in this cohort are underway.

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Table 4.1: Associations between maternal malarial status, infant growth characteristics and blood pressure at birth

Parameter	Malaria Absent n = 225		Malaria Present n = 211			
	Mean	**SD**	**Mean**	**SD**	**t test**	**p value**
Placental Weight (g)	557.19	111.5	573.65	119.2	-1.33	0.186
Placental-fetal weight ratio	5.56	1.5	5.20	1.2	2.35	**0.019**
Weight (kg)	2.97	0.4	2.85	0.4	2.72	**0.007**
Length (cm)	48.97	2.4	48.35	2.1	2.82	**0.005**
OFC (cm)	34.5	1.4	34.2	1.2	2.21	**0.028**
MUAC (cm)	9.95	0.8	9.77	0.9	2.11	**0.036**
Subscapular (cm)	4.28	0.9	4.14	0.8	1.67	0.097
Suprailiac (cm)	4.42	0.9	4.27	0.9	1.60	0.111
Triceps (cm)	4.19	0.8	4.08	0.9	1.28	0.200
Biceps (cm)	3.70	0.8	3.55	0.6	2.15	**0.032**
Subscap/Triceps Ratio	1.03	0.1	1.02	0.1	0.13	0.899
SBP (mmHg)	72.4	13.4	69.5	12.2	2.34	**0.020**
DBP (mmHg)	37.0	9.5	35.2	8.4	1.97	**0.049**

OFC – Occipito-frontal circumference; MUAC – Mid-upper arm circumference; Subscap – Subscapular skinfold thickness; SBP- Systolic blood pressure;
DBP- Diastolic blood pressure

Table 4.2: Associations between growth characteristics and blood pressure at birth defined by Parasite density and Timing of Malaria

	a. Parasite density #					b. TIMING of maternal malaria *				
Variable	**Malaria**	**mean**	**Differ-ence**	**95% CI of Difference**	**p-value[+]**	**Malaria**	**mean**	**Differ-ence**	**95% CI of Difference**	**p-value[+]**
Weight kg	None **n = 225**	3.0	0.3	0.1,0.4	**0.005**	No malaria **n = 225**	3.0	0.1	0.0, 0.2	**0.006**
	Low **n = 131**	3.0	0.3	0.1,0.4	**0.005**	In pregnancy **n = 138**	2.8	*		
	High **n = 80**	2.7	**#**			At delivery **n = 73**	2.9	0.1	-0.1, 0.2	0.425
Length cm	None	49.0	1.1	0.5,1.6	**0.001**	No malaria	49.0	0.7	0.2, 1.2	**0.006**
	Low	48.6	0.7	0.04,1.3	**0.037**	In pregnancy	48.3	*		
	High	47.9	**#**			At delivery	48.5	0.2	-0.5, 0.9	0.565
OFC cm	None	34.5	0.7	0.3,1.0	**<0.001**	No malaria	34.5	0.3	-0.0, 0.6	0.065
	Low	34.4	0.6	0.3,1.0	**0.001**	In pregnancy	34.2	*		
	High	33.8	**#**			At delivery	34.1	-0.1	-0.4, 0.3	0.792
MUAC mm	None	9.9	0.4	0.2,0.6	**0.001**	No malaria	10.0	0.3	0.0, 0.4	**0.024**
	Low	9.9	0.4	0.1,0.6	**0.006**	In pregnancy	9.7	*		
	High	9.5	**#**			At delivery	9.8	0.1	-0.1, 0.4	0.388
Sub-scapular mm	None	4.4	0.4	0.0,0.7	**0.026**	No malaria	4.3	0.2	0.0, 0.4	0.088
	Low	4.2	0.2	-0.1,0.6	0.199	In pregnancy	4.1	*		
	High	4.0	**#**			At delivery	4.2	0.1	-0.2, 0.4	0.467
Triceps mm	None	4.2	0.3	0.0,0.5	**0.031**	No malaria	4.2	0.1	0.0, 0.3	0.139
	Low	4.2	0.3	-0.2,0.5	0.074	In pregnancy	4.1	*		
	High	3.9	**#**			At delivery	4.1	0.0	-0.2, 0.3	0.453
Biceps mm	None	3.7	0.2	0.0,0.4	**0.029**	No malaria	3.7	0.2	0.0, 0.3	**0.024**
	Low	3.6	0.1	-0.1,0.3	0.365	In pregnancy	3.5	*		
	High	3.5	**#**			At delivery	3.6	0.1	-0.1, 0.3	0.446

Suprailiac mm	None	4.4	0.2	-0.0, 0.5	0.05	No malaria	4.4	-0.1	-0.0, 0.4	0.135
	Low	4.3	0.1	-0.1, 0.4	0.26	In pregnancy	4.3	*		
	High	4.2	**#**			At delivery	4.3	0.0	-0.3, 0.3	0.865
Subs/Triceps Ratio	None	1.0	0.0	-0.0, 0.0	0.717	No malaria	1.03	0.0	-0.0, 0.0	0.975
	Low	1.0	0.0	-0.0, 0.0	0.693	In pregnancy	1.03	*		
	High	1.0	**#**			At delivery	1.02	-0.01	-0.0, 0.0	0.857
SBP mmHg	None	72.4	3.0	-0.4,6.4	0.082	No malaria	72.4	1.4	-1.3, 4.3	0.292
	Low	69.5	0.1	-3.6,3.8	0.956	In pregnancy	71.0	*		
	High	69.4	**#**			At delivery	66.7	-4.3	-8.0, -0.6	**0.001**
DBP mmHg	None	37.0	1.0	-1.4,3.3	0.442	No malaria	37.0	1.1	-0.9, 3.0	0.294
	Low	34.7	-1.3	-3.9,1.3	0.329	In pregnancy	35.9	*		
	High	36.0	**#**			At delivery	33.9	-2.0	-4.7, 0.6	0.125

+ Analysis of Variance
 # Reference group = High parasitaemia. * **Reference group = Malaria in pregnancy**
There were no differences between Low parasitaemia and None.

OFC – Occipito-frontal circumference; MUAC – Mid-upper arm circumference; Subs – Subscapular skinfold thickness; SBP- Systolic blood pressure; DBP- Diastolic blood pressure

Table 4.3a: Multiple regression analyses for determinants of infant birth size including maternal malaria timing and density

Variable	Regression for Birth Weight				Regression for Birth Length		
	β	95% CI	P value		β	95% CI	P value
Sex	0.000	-0.07 to 0.07	0.988		-0.207	-0.60 to 0.19	0.307
Gestational age at birth (weeks)	0.057	0.03 to 0.08	**<0.001**		0.108	-0.05 to 0.26	0.169
Length (cm)	0.089	0.07 to 0.11	**<0.001**	Weight (kg)	2.842	2.30 to 3.38	**<0.001**
Placental weight (g)	0.000	0.00 to 0.00	0.337		0.003	0.00 to 0.00	**0.004**
Parasite density+	-0.084	-0.17 to 0.000	**0.05**		-0.051	-0.53 to 0.43	0.835
Malarial timing*	0.050	-0.03 to 0.13	0.245		0.010	-0.47 to 0.49	0.966
Gravidity	0.001	-0.04 to 0.04	0.959		0.123	-0.09 to 0.34	0.262
Maternal age (year)	0.003	-0.01 to 0.01	0.611		0.033	-0.02 to 0.09	0.239
Maternal weight (kg)	0.006	0.00 to 0.01	**0.003**		0.003	0.00 to 0.01	0.782
Maternal height (cm)	0.000	-0.01 to 0.01	0.972		0.024	-0.02 to 0.06	0.244
Maternal SBP (mmHg)	0.002	-0.00 to 0.01	0.504		-0.016	-0.05 to 0.02	0.382
Maternal DBP (mmHg)	-0.004	-0.01 to 0.00	0.376		-0.021	-0.07 to 0.02	0.361

SBP- Systolic blood pressure; DBP- Diastolic blood pressure
*Malarial timing: Coding 0 = no malaria, 1= malaria in pregnancy, 2= malaria at delivery ± pregnancy
+Parasite density: Coding 0= no malaria, 1= low parasite density, 2= high parasite density

Table 4.3b: Multiple regression analyses for determinants of infant SBP and DBP at birth including maternal malaria timing and density

Variable	Regression for SBP			Regression for DBP		
	β	95% CI	P value	β	95% CI	P value
Sex	2.764	0.04 to 5.49	**0.047**	2.142	0.29 to 4.00	**0.024**
Gestational age at birth (weeks)	0.941	-0.15 to 2.03	0.09	0.813	0.07 to 1.55	**0.031**
Weight (kg)	8.386	4.11 to 12.66	**<0.001**	3.378	0.47 to 6.29	**0.023**
Length (cm)	-1.311	-2.08 to -0.55	**0.001**	-0.261	-0.78 to 0.26	0.323
Placental weight (g)	-0.002	-0.01 to 0.01	0.767	-0.003	-0.01 to 0.01	0.523
Parasite density+	1.523	-1.75 to 4.79	0.36	1.445	-0.78 to 3.67	0.202
Malarial timing *	-3.355	-6.60 to -0.11	**0.043**	-2.294	-4.50 to -0.09	**0.042**
Gravidity	-1.074	-2.55 to 0.41	0.154	-0.545	-1.55 to 0.46	0.288
Maternal age (year)	0.390	0.02 to 0.77	**0.041**	0.037	-0.22 to 0.29	0.776
Maternal weight (kg)	0.004	-0.15 to 0.16	0.956	0.036	-0.07 to 0.14	0.5
Maternal height (cm)	-0.363	-0.64 to -0.09	**0.01**	-0.273	-0.46 to -0.09	**0.004**
Maternal SBP (mmHg)	0.163	-0.08 to 0.41	0.193	-0.021	-0.19 to 0.15	0.807
Maternal DBP (mmHg)	-0.044	-0.35 to 0.26	0.778	0.165	-0.04 to 0.37	0.119

SBP- Systolic blood pressure; DBP- Diastolic blood pressure
*Malarial timing: Coding 0 = no malaria, 1= malaria in pregnancy, 2= malaria at delivery ± pregnancy
+Parasite density: Coding 0= no malaria, 1= low parasite density, 2= high parasite density

Table 4.4: Association between maternal malaria parasite load and infant SBP and DBP adjusted for birth weight and length

Variable	Malaria Parasite density	mean	Difference	95% CI of difference	p-value+
SBP/weight	None	24.8	-0.9	-0.5, 2.3	0.185
	Low	24.0	-1.7	0.2, 3.3	**0.024**
	High	25.7			
SBP/length	None	1.48	0.03	-0.11, 0.04	0.417
	Low	1.43	-0.02	-0.06, 0.10	0.663
	High	1.45			
DBP/weight	None	12.6	-0.7	-0.2,1.7	0.105
	Low	12.0	-1.4	0.4, 2.3	**0.006**
	High	13.4			
DBP/length	None	0.75	0.0	-0.05, 0.05	0.958
	Low	0.72	-0.3	-0.02, 0.09	0.164
	High	0.75			

SBP- Systolic blood pressure; DBP- Diastolic blood pressure
+ Analysis of Variance

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CHAPTER 5

Publication Number – 2

This chapter will present the second paper titled: **'Maternal malaria status and metabolic profiles in pregnancy and in cord blood: relationships with birth size in Nigerian infants**

In this paper, we examined the effects of maternal malaria in pregnancy on maternal biochemical markers and cord blood biochemical markers and IGF-I. The relationships of these biochemical markers on birth size were also documented.

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Authors' contributions

OA participated in overall study conception and design, data collection, analysis interpretation and manuscript preparation

AW was involved in sample assay, data analysis, interpretation and manuscript preparation

KC and PC are my Supervisors and both participated in overall study conception and design, data interpretation and manuscript preparation.

Maternal malaria status and metabolic profiles in pregnancy and in cord blood: relationships with birth size in Nigerian infants

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Abstract

Malaria is more common in pregnant than non-pregnant Nigerian women, and is associated with small birth size and the consequent short- and long-term health risks. The evaluation of maternal metabolic status in pregnancy in relation to malaria, in order to identify potential biomarkers associated with birth size, has not been studied.

Objective: To define relationships between maternal and cord serum metabolic markers, maternal malaria status and birth size.

Methods: During pregnancy, anthropometric measurements, blood films for malaria parasites and assays for lipids, glucose, insulin and tumour necrosis factor alpha (TNFα) were obtained from 467 mothers at the second and third trimesters and these analytes and insulin-like growth factor-I (IGF-I) were obtained from cord blood of 187 babies.

Results: Overall prevalence of maternal malaria was 52%, and this was associated with younger age, anaemia and smaller infant birth size. Mothers with malaria had significantly lower cholesterol and higher TNFα concentrations with no difference in triglyceride levels. In contrast, there was no effect of maternal malaria on cord blood lipids, but the median (range) cord IGF-I was significantly lower in babies whose mothers had malaria: 60.4 (24 - 145)µg/L versus none: 76.5 (24 - 150)µg/L, p=0.03. On regression analysis, significant determinants of birth size included maternal total cholesterol, LDL-C, insulin and malarial status while associated fetal factors included cord insulin and IGF-I.

Conclusions: Malaria in pregnancy was common and associated with reduced birth size, lower maternal lipids and higher TNFα. In the setting of endemic malaria maternal total cholesterol during pregnancy and cord blood insulin and IGF-I levels are potential biomarkers of fetal growth.

Introduction

Low birth weight with and without early catch-up growth is associated with an increased risk of mid-life chronic diseases, such as hypertension and diabetes (1;2). In Nigeria, increasing mortality from stroke and end-stage renal failure is associated with a high prevalence of hypertension, obesity, diabetes and high serum triglycerides especially in women (3). Malaria remains endemic in Nigeria and is more common among pregnant women with significant consequences to maternal and infant health, such as maternal anemia and low birth weight (LBW) (4). In keeping with the developmental origins hypothesis, malaria may contribute to later life morbidities. However mediators of the relationships between LBW, malaria and later cardiovascular and metabolic morbidity have not been clearly identified.

Maternal and cord blood levels of lipids, glucose and insulin and cord insulin-like growth factor-I (IGF-I) levels have been investigated as possible determinants of birth weight (5-7). Maternal fasting triglyceride (TG) was independently associated with birth weight in non-diabetic women with maternal hyperglycaemia and with fetal growth and birth size in women with gestational diabetes (6;8;9). Maternal plasma glucose was positively associated with birth weight while fasting plasma insulin was inversely related (5;10;11). The relationship between birth weight and cord blood lipids is inconsistent (12;13) but positive correlations between birth weight and cord blood glucose and insulin levels in both normal and low birth weight babies are reported (14). Fetal IGF-I may also be involved in the control of fetal size during late gestation(15;16).

Increased placental expression of cytokines such as TNFα, interleukin (IL) 8, γ-interferon, IL-6 and IL-10 occurs in pregnancies affected by malaria but only TNFα has been linked to LBW (17;18).

We hypothesised that malaria parasitaemia in pregnancy would induce changes in maternal metabolic markers leading to significant consequence on infant birth size. We

have used our established cohort of mothers and infants born in Nigeria to examine the relationships between maternal and cord blood metabolic profiles and birth size in the setting of endemic malaria, and to identify potential pregnancy biomarkers of LBW.

Subjects and Methods

Study Site

The study was carried out in Yemetu-Adeoyo, a semi-urban community in Ibadan in Southwest Nigeria where transmission of malaria is perennial. The hospital in this community, Adeoyo Maternity Hospital (AMH), is the oldest maternity hospital in Nigeria, dating from 1927. There are over 4000 deliveries annually. Ethical approval for the study was obtained from the joint University of Ibadan / University College Hospital ethical committee and the University of Manchester Ethics committee.

Study Procedures, Follow-up, Delivery and Recruitment of Babies

Healthy pregnant women aged 18-45 years presenting at AMH before 36 weeks gestation and all babies born ≥37 weeks gestation were eligible. Women who were HIV positive or had sexually transmitted infections at booking, those with preterm deliveries, as well as those with multiple pregnancies or with chronic diseases such as hypertension and diabetes were excluded. Babies with known syndromes, metabolic defects, major congenital abnormalities or severe birth trauma were also excluded.

The study protocol and the rationale for the study were explained carefully in appropriate language, most commonly Yoruba or English, with questions answered as needed and written informed consent was obtained from all participants. After the delivery of their babies, another written informed consent was also obtained for the participation of their babies in the study.

Information on socio-demographic, obstetric, family, and health history including malarial frequency and use of antimalarial drugs, was collected. All participants were issued prescriptions of sulphadoxine-pyrimethamine for Intermittent Preventive Therapy (IPT) for malaria according to standard hospital practice.

Antenatal visits followed routine practice with frequency of attendance determined by gestational age. Standardised measures of anthropometry were carried out on all women at every visit until delivery. Maternal weight was measured to the nearest 0.1 kg on a SECA scale, and height on a stadiometer, both without shoes, according to our standard operating procedure (SOP) and training video.

Of 624 healthy pregnant women enrolled over one year to cover wet and dry seasons, 161 did not deliver at AMH. At the second antenatal visit, we obtained blood samples from 467 women. Of these, 27 were excluded due to 4 (0.9%) maternal deaths, 11 (2.4%) stillbirths, 5 (1.1%) miscarriages and 7 (1.5%) neonatal deaths, leaving 436 mother-baby pairs. Based on adequacy of maternal and cord blood samples, 467 samples from the mothers and 187 from the cord blood were available.

Blood Measurements

In brief, 2mls of blood was collected at booking into an EDTA tube for full blood count and blood films were prepared, stained with 3% Giemsa at pH 7.2 and examined for malaria parasites (MP) under light microscopy with parasite densities determined as absolute parasite number per µL of blood. Repeat thick blood films for MP were obtained at every subsequent visit and at delivery. The placenta was weighed, turned to the maternal surface, cotyledons exposed and 1ml of blood obtained from the intervillous space for a placental malarial blood smear.

Fasting blood was obtained from the women at the second antenatal visit with 125 samples obtained during second trimester while 342 samples were obtained from women

in their third trimester of pregnancy. At delivery cord blood was collected from the umbilical vein on the fetal surface of the placenta. Plasma was separated by centrifugation at 3000rpm and 4^0C for 10 minutes and aliquoted into microtubes and stored at -80^0C prior to lipid, glucose, insulin, TNFα and IGF-I (cord blood only) assays. Cord glucose could not be measured immediately and so was not included in the assay.

Biochemical Assays

Total cholesterol (TC), HDL-cholesterol and triglyceride concentrations were determined by using standard enzymatic procedures on an automatic analyser (COBAS MIRA/ HITACHI 704 - Roche Diagnostics, Germany). The inter- and intra-assay coefficients of variation (CVs) for all parameters were < 5%. LDL-cholesterol and VLDL-cholesterol were calculated using the Friedewald formula (19). The normal values of lipids (mmol/L) in adult women are TC 3-5, HDL-C 1.2-2.2, LDL-C 2-3 and TG 0.6-1.68.

Maternal glucose was measured by the glucose oxidase method using a commercial kit (Randox, Crumlin, UK) on a YSI 2300 stat plus analyser (YSI, Farnborough, Hants, UK). The intra-assay CV was 1.5% at 4.1mmol/L, and inter-assay CVs were 2.8% and 1.7% at 4.1 and 14.1mmol/L respectively. Normal fasting glucose values are 3.9-6mmol/L

Insulin was measured by ELISA using a commercial kit (Mercodia, Uppsala, Sweden). Assay sensitivity was 1mU/L. Intra-assay CVs were 3.4% and 3.2% at 11 and 154mU/L, and equivalent inter-assay CVs were 3.6% and 2.9%.

IGF-I and TNF-α were measured using Immulite 2000 assays (DPC, Lumigen Inc, Southfield, UK). Respective assay sensitivities were 25μg/L and <0.09 ng/L. Inter-assay CV values for IGF-1 at 48.9 and 158.5μg/L were 7.6 and 9.2%. For TNF-α, intra-assay CVs were 6.7% and 5.3% at 6.3 and 19ng/L, and the inter-assay CVs at 6.1 and 18.6ng/L were 8.2 and 9.7% respectively.

Infant Anthropometry

Babies were measured within 72 hours of birth. They were weighed naked to the nearest 0.1kg and length measured on an infant stadiometer from crown to heel to the nearest 0.1cm. Occipito-frontal circumference (OFC) was taken around the widest circumference of the head using a non-stretchable tape.

Validity of Anthropometric Measurements

Based on the WHO manual (1995), three nurses, already proficient in paediatric venepuncture, were trained in anthropometry methods. They carried out all measurements on the same equipment throughout the study. They also had 3-monthly protocol-refresher training sessions and used training videos to minimize inter-observer and within-observer errors.

Definitions

Malaria was defined as the presence of asexual blood stages of Plasmodium falciparum in peripheral blood. There were two definitions of malaria: a) 'Malaria at recruitment' = Malaria at second antenatal visit. This was only used in analyses of effects on maternal biochemical markers at the same time-point. Women were grouped into 2 categories:

i. 'No Malaria' - no parasites detected throughout pregnancy or at delivery (n=314).
ii. 'Malaria present' - parasites present at least once during pregnancy and/or at delivery (n=72).

b) 'Maternal Malaria' = Malaria parasitaemia in peripheral blood at least once during pregnancy and/or at delivery and/or in the placenta. This was used in analyses of relationships with cord parameters and effects on birth indices. Women were grouped into 2 categories:

i. ‘No Malaria’ - no parasites detected throughout pregnancy or at delivery (n=225).

ii. ‘Malaria present’ - parasites present at least once during pregnancy and/or at delivery (n=211).

To examine effects of parasite load during pregnancy and at delivery, parasite density was classified into low (<1000 parasites/μl), or high (≥1000/μl).

Anaemia was defined as packed cell volume (PCV) < 30%.

Statistical Analysis

Data were analysed using SPSS version 14 (SPSS Inc, Chicago, IL). Results were expressed as mean (SD) or median (IQ range), using Student t and Chi square tests for associations between maternal and infant clinical characteristics and malaria. Levels of insulin, IGF-I and TNF-α were skewed and therefore tested non-parametrically, while lipids and glucose levels were normally distributed and tested parametrically. Correlations were by Spearman’s test and simple linear and stepwise multiple regressions used to assess the determinants of infant size. All tests were 2-sided and P values < 0.05 were considered ‘significant’.

Results

Maternal clinical characteristics and biochemical markers at recruitment (Table 5.1) show that the age and BMI of women were similar irrespective of trimester at recruitment. Plasma levels of all lipids except HDL-C were significantly elevated in the third compared to the second trimester but fasting plasma glucose was reduced. Insulin and TNF-α levels were similar.

Effect of maternal malaria at recruitment on maternal characteristics and biochemical markers

The prevalence of malaria parasitaemia at recruitment was 18%. Most women were asymptomatic and only 7 had fever. Malaria was associated with younger maternal age (29.4 versus 27.7 years, p=0.001) and lower packed cell volume [31.7(4.7)% versus 32.8(3.3)%, p=0.006].

Malaria had an effect on all lipid parameters except TG. Mothers with malaria had significantly lower TC, lower HDL-C, lower LDL-C, no change in TG but higher TNFα in both second and third trimesters (Table 5.2), but presence of malaria parasitaemia had no effect on glucose and insulin levels.

Analysis based on parasite density, showed that all lipid parameters except TG were lowest in women with high parasitaemia than those with low parasitaemia and none. In addition, TNFα levels were highest in women with high parasitaemia than those with low parasitaemia and none.

Effect of maternal malaria on newborn birth indices and cord blood biochemical markers

There was no gender difference in cord blood lipid profile, TNFα, glucose, insulin and IGF. Prevalence of maternal malaria defined as malaria parasitaemia in pregnancy and/or at

delivery overall was 52%. Mean birth weight, length, OFC and mid-upper arm circumference (MUAC) of infants born to women with malaria were globally smaller than those of women without malaria (Table 5.3a), with no gender differences. Of the four skinfold thicknesses (biceps, triceps, suprailiac and subscapular) only biceps skinfolds were significantly lower in those with malaria.

Cord blood IGF-I levels were significantly lower in babies of mothers with malaria, with no differences in cord blood lipids, insulin and TNFα (Table 5.3b). Analysis based on parasite density showed similar results with lowest IGF-I levels in infants of women with high parasitaemia.

Correlations between maternal and cord blood biochemical markers

Maternal TC and cord TC (r=0.17, p=0.02) and HDL-C (r=0.26, p=0.001) were significant correlated, as were maternal HDL-C and cord HDL-C (r=0.22, p=0.004), maternal LDL-C and cord TC (r=0.16, p=0.037) and HDL-C (r=0.17, p=0.03), and maternal TG and cord IGF-I (r=-0.18, p=0.02). Maternal insulin correlated positively with cord blood insulin (r=0.23, p=0.002). However, there were no significant correlations between maternal and cord LDL-C nor maternal and cord TNF-α.

Correlations between maternal and cord blood biochemical markers and birth indices

All maternal biochemical markers were significantly correlated (Table 5.4) with birth weight at $p \leq 0.003$ with triglyceride (p=0.018) and glucose (p=0.036) less strongly. Maternal TC and LDL correlated positively with all skinfold thicknesses (all $p<0.01$), and TG (p=0.018) and insulin (p=0.03) also correlated with subscapular, while maternal glucose (p=0.02) and insulin (p=0.001) correlated positively with suprailiac skinfolds.

Birth weight was positively associated with cord blood insulin (p=0.017) and IGF-I (p=0.025) which was correlated with MUAC (p=0.01) but neither cord biochemical marker was associated with birth length or OFC (Table 5.4).

Cord insulin correlated positively (all p<0.012) and cord TNFα negatively (all p<0.03) with all skinfold thicknesses. Cord IGF-I was positively associated with suprailiac skinfold (p=0.03).

Determinants of infant size

To determine independent effects, three multiple stepwise regression models were derived (Table 5.5).

(1) Influence of malaria at recruitment and maternal biochemical markers: In the first model, we evaluated the influence of malaria at recruitment and all maternal biochemical markers on birth indices. Maternal TC was a powerful, independent determinant of birth weight (p=0.001), such that for every 0.15mmol/L increase in TC, there was a 100g increase in birth weight.

Maternal TC was also a significant independent determinant of MUAC (p<0.001) and subscapular skinfolds (p=0.005), while maternal LDL and insulin were determinants of triceps skinfolds.

(2) Influence of maternal malaria and cord biochemical markers: In this model, we examined the influence of maternal malaria through pregnancy and at delivery and all cord biochemical markers on birth indices. Maternal malaria was the only significant determinant of birth weight (p=0.007), cord IGF-I was the only independent determinant of MUAC (p<0.036) and cord insulin for subscapular skinfolds.

(3) Influence of maternal malaria and maternal and cord biochemical markers: In this final model, we included maternal malaria, maternal and cord biochemical markers that had shown significant correlations to birth indices.

Maternal malaria was the only significant determinant of birth weight, with cord blood insulin and IGF-I associated with MUAC. Maternal glucose was the only significant determinat of OFC. Cord blood insulin was related to subscapular and triceps skinfolds.

Discussion

Effect of maternal malaria on maternal and cord blood biochemical markers

In these pregnant women, there is a marked cholesterol-lowering impact of malaria, including HDL and LDL; but triglyceride levels were unaffected. Our findings corroborate older reports of lower TC and HDL associated with acute malaria but in that setting higher TG (20). A study of lipid profiles associated with acute malaria in non-pregnant people showed higher TG but no change in TC (21). The mechanisms involved in changes in lipid profile associated with malaria are still unclear. In vitro experiments have shown a selective uptake of HDL-C by P. falciparum indicating that the HDL-C fraction appears to be a major lipid source for parasite growth (22) and increased intraparasitic cholesterol and phospholipid levels (23).

Maternal plasma glucose and insulin were unaltered by malaria which may in part be due to our study participants being asymptomatic. The result contrasts with a previous study in symptomatic pregnant women with acute malaria, who had hypoglycaemia associated with increased glucose turnover, attributed to enhanced pancreatic β-cell function (24). We found a positive association between maternal and cord insulin as found in a previous study not in West Africa (9).

Maternal TNFα concentrations in the second trimester were 9 fold higher in those with malaria, but only doubled if malaria occurred in the third trimester. This higher TNFα, associated with malaria was found previously in Malawian and Kenyan pregnant women in peripheral and placental blood (17;25). However, we found no associations between TNF-α and any biochemical parameter or birth size.

In agreement with other studies in various populations including Nigerian pregnant women, our data show an elevation in serum lipids, except HDL-C, during pregnancy. The elevation was highest in the third trimester (26-28), Jimenez et al reported no significant changes in HDL-C during pregnancy while others report an elevation (29;30). These

increases in TC and LDL-C and low to normal HDL-C are believed not to be atherogenic (26;28) and are likely related to energy transfer to the fetus.

In contrast to previous reports of higher cholesterol levels in girls than in boys (13;31),,we found no gender difference in cord lipid profile and other metabolic parameters. The cord lipid profiles are similar to those in our Manchester children from the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study (32) and to those in Ibadan about 30 years ago but slightly lower than that reported in Eastern Nigeria previously (13;33;34). Cord HDL-C and TG levels are about half that of adults while TC and LDL-C are about one-third, hence HDL is the major lipid moiety (31;35). Our data also show significant correlations between maternal and cord lipids except LDL, in contrast to a previous Nigerian report (31).

Our data also suggest that malaria had no effect on the cord lipid profile, insulin and TNF α levels, findings not previously described in an endemic malaria area. As previously reported (36;37), we found that malaria in pregnancy was associated with younger age and maternal anaemia, as well as smaller, shorter, thinner babies with smaller head circumferences , although the effect on birth length was not independent of other factors

Maternal and cord blood biochemical markers and birth size

There was a significant association between maternal lipids during the second and third trimesters of pregnancy and birth weight, length, MUAC and all four skinfold thicknesses. This is in contrast to findings in Nigerian newborns about 15 years ago when maternal lipid samples were obtained at delivery not during pregnancy. (31;33). Variations in maternal lipid metabolism may affect fetal growth during pregnancy leading to effects on birth size. In women with altered glucose tolerance, serum TG concentrations have predicted birth weight (6;8).

Published data on relationships between skinfold thicknesses and maternal lipids in pregnancy are limited and examined mothers with diabetes. Our data corroborate the findings in a group of women with tightly controlled gestational diabetes, where maternal TG correlated with birth indices including neonatal fat mass (6).

There were positive relationships between suprailiac skinfolds, birth weight and maternal glucose, insulin and cord blood insulin which confirms findings in the HAPO study that mid-pregnancy maternal fasting or postprandial plasma glucose level is an independent predictor of birth weight (11). In addition, maternal insulin is one of the major growth factors in fetal life as shown by monogenic disorders that affect fetal insulin secretion (38).

An important finding in this study is that babies whose mothers had malaria had lower cord blood IGF-I levels but no effect on any other cord blood biochemical markers. Birth weight and MUAC had significant correlations with cord blood IGF-I, corroborating previous reports and suggesting that IGF-I is involved in the control of fetal size during the third trimester(16;39).

Determinants of birth size

The regression models highlight the most significant maternal factors that determine birth size, which were cholesterol, LDL, glucose, insulin and maternal malaria, albeit weakly, while associated fetal factors included cord insulin and IGF-I.

Birth weight remains the single most important determinant of neonatal and infant survival and health; similar to our findings, there has been previous evidence that cord blood insulin and IGF-I correlates with birth weight (16;39;40) but we are not aware of any study examining the impact of maternal malaria on cord IGF-I. Overall, maternal TC, LDL and cord IGF-I, all affected by malaria, are the most significant determinants of birth weight, and may mediate the effect of malaria on fetal growth.

Our findings are strongly supported by animal experiments which demonstrate that Plasmodium falciparum is unable to synthesise lipids required during its erythrocytic cycle. Hence there is lipid transport through membrane flux into the parasite. The main lipid transported is HDL that is an essential requirement for parasite growth and which may contribute to reduced cholesterol in the pregnant women. Therefore, malaria in pregnancy results in demands for cholesterol from three sources including the parasite for its growth and probably its attachment properties, the placenta for production of progesterone to maintain the pregnancy and the fetus itself for growth (22;23). This combination may lead to low cholesterol levels resulting in prematurity and low birth weight babies.

Conclusions

We have identified that maternal, but not cord lipids, and cord IGF-I are reduced in the presence of maternal malaria. These factors also associate with birth size, and in this setting of endemic malaria can therefore be considered as potential biomarkers of fetal growth.

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Conflict of interest statement

None declared.

Table 5.1: Maternal clinical characteristics and biochemical markers at recruitment

Parameter (n=467) Mean (SD) or Median (IQ Range)	**Trimester 2 n=125**	**Trimester 3 n=342**	**P value[a,b]**
Age (year)	29.0 (4.9)	28.5 (5.3)	0.280
Gestational age (weeks)	22.8 (3.2)	31.9 (3.3)	**<0.001**
BMI (kg/m^2)	25.2 (3.9)	24.9 (3.9)	0.501
Total cholesterol (mmol/L)	4.44 (3.93, 5.45)	4.98 ((4.28, 5.69)	**0.002**
HDL-C (mmol/L)	1.54 (1.27, 1.83)	1.55 (1.27, 1.81)	0.922
LDL-C (mmol/L)	2.49 (1.97, 3.13)	2.74 (2.17, 3.25)	**0.021**
Triglyceride (mmol/L)	1.31 (1.05, 1.51)	1.47 (1.23, 1.79)	**<0.001**
Glucose (mmol/L)	4.33 (4.04, 4.55)	4.13 (3.87, 4.41)	**<0.001**
Insulin (mU/L)	4.32 (2.76, 6.90)	3.92 (2.21, 6.09)	0.204
TNF-α (ng/L)	0.17 (0.09, 0.83)	0.35 (0.09, 0.83)	0.102

Note: [a] Student t test done for variables presented as mean(SD); [b]Mann Whitney U test for variables reported as median (IQ range)
BMI, Body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TNF-α, Tumour necrosis factor-alpha

Table 5.2: Effect of malaria parasitaemia at recruitment on maternal biochemical markers

Maternal Biochemical Markers	Trimester 2 n=110		P value	Trimester 3 n=286		P value+
Total n=396 Median (IQ range)	**Malaria Absent n=83**	**Malaria Present n=17**		**Malaria Absent n=231**	**Malaria Present n=55**	
TC (mmol/L)	4.73(4.26,5.71)	3.73(2.80,4.28)	**<0.001**	5.17(4.50,5.81)	4.43(3.67,5.41)	**<0.001**
HDL-C (mmol/L)	1.58(1.37,2.02)	0.86(0.70,1.38)	**<0.001**	1.64(1.39,1.84)	1.27(0.97,1.63)	**<0.001**
LDL-C (mmol/L)	2.66(2.03,3.28)	2.10(1.51,2.53)	**0.010**	2.77(2.31,3.43)	2.44(1.70,3.08)	**0.006**
TG (mmol/L)	1.29(1.02,1.51)	1.35(1.19,1.62)	0.112	1.47(1.24,1.77)	1.51(1.28,1.99)	0.125
Glucose (mmol/L)	4.31(4.06,4.58)	4.40(3.89,4.48)	0.795	4.10(3.85,4.37)	4.23(3.97,4.44)	0.060
Insulin (mU/L)	4.58(3.06,7.56)	2.95(1.87,5.10)	0.056	3.86(2.16,6.11)	4.34(2.66,6.06)	0.316
TNF α (ng/L)	0.09(0.09,0.55)	0.79(0.45,1.91)	**<0.001**	0.29(0.09,0.78)	0.55(0.09,1.76)	**0.003**

Note: TC, Total Cholesterol; TG, Triglyceride; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TNFα, Tumour necrosis factor-alpha

+ Analysis of covariance

Table 5.3a: Effect of maternal malaria on birth indices

Mean (SD)	Malaria Absent		Malaria Present		P value[+]
N= 436	**n= 225**		**n= 211**		
Birth weight (kg)	3.03	0.4	2.87	0.4	**0.007**
Birth length (cm)	48.97	2.4	48.35	2.0	**0.005**
OFC (cm)	34.42	1.3	34.07	1.1	**0.028**
MUAC (cm)	9.98	0.8	9.80	0.9	**0.036**
Biceps (cm)	3.70	0.8	3.55	0.6	**0.032**

Note: OFC, Occipito-frontal Circumference; MUAC, Mid-upper arm circumference
+- Student t test

Table 5.3b: Effect of maternal malaria on cord blood biochemical markers

Median (IQ Range)	Malaria Absent n= 85		Malaria Present n= 97		P value[+]
Total cholesterol (mmol/L)	1.65	0.82, 5.53	1.66	0.71, 6.78	0.136
HDL-C (mmol/L)	0.66	0.32, 2.16	0.63	0.25, 1.91	0.10
LDL-C (mmol/L)	0.70	0.03, 3.19	0.68	0.01, 3.64	0.246
Triglyceride (mmol/L)	0.79	0.20, 2.77	0.62	0.26, 3.52	0.30
Insulin (mU/L)	2.82	0.1, 59.21	2.61	0.26, 50.95	0.677
TNFα (ng/L)	2.96	0.01, 97.01	2.23	0.03, 61.58	0.769
IGF-I (µg/l)	74.2	24.0, 150.0	61.3	24.0, 145.0	**0.031**

Note: HDL-C, High-density Lipoprotein cholesterol; LDL-C,

Low-density lipoprotein cholesterol; TNFα, Tumour necrosis factor-alpha

+ - Mann Whitney U test

Table 5.4: Correlations (r values) between maternal and cord biochemical markers and birth indices

Biochemical Markers	Birth Weight (kg)	Birth Length (cm)	OFC (cm)	MUAC (cm)
Maternal Biochemical Markers (n=467)				
Total cholesterol (mmol/L)	0.194*	0.108*	0.097	0.204*
HDL-C (mmol/L)	0.062	0.024	0.015	0.141*
LDL-C (mmol/L)	0.179*	0.095	0.080	0.174*
Triglyceride (mmol/L)	0.119*	0.079	0.096	0.076
Glucose (mmol/L)	0.107*	0.098	0.159*	0.090
Insulin (mU/L)	0.150*	0.062	0.023	0.052
Cord Biochemical Markers (n=187)				
Cord Insulin (mU/L)	0.181*	0.003	0.085	0.041
Cord IGFI (µg/L)	0.169*	0.093	0.054	0.193*

* $p<0.05$ Note: OFC, Occipito-frontal Circumference; MUAC, Mid-upper arm circumference; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TNFα, Tumour necrosis factor-alpha

Table 5.5: Determinants of birth size on multiple stepwise regressions

Birth Indices	Model 1			Model 2			Model 3		
		ß	P-value		ß	P-value		ß	P-value
Birth weight	TC (mmol/L)	0.172	**0.001**	Maternal malaria (No/Yes)	-0.221	**0.007**	Maternal malaria (No/Yes)	-0.155	**0.05**
								0.157	**0.045**
							Cord IGF-I (µg/L)	0.158	**0.042**
							Cord Insulin (mU/L)		
OFC	None			None			Maternal Glucose (mmol/L)	0.163	**0.039**
MUAC	TC (mmol/L)	0.149	**<0.001**	Cord IGF-I (µg/l)	0.173	**0.036**	Cord IGF-I (µg/L)	0.179	**0.024**
Subscapular	TC (mmol/L)	0.111	**0.005**	Insulin (mU/L)	0.019	**0.013**	Cord Insulin (mU/L)	0.022	**0.005**
	Insulin (mU/L)	0.021	**0.002**						
Triceps	LDL (mmol/L)	0.122	**0.006**				LDL (mmol/L)	0.184	**0.007**
	Insulin (mU/L)	0.023	**<0.001**				Cord Insulin (mU/L)	0.015	**0.034**

Model 1 - Maternal Markers and Malaria at recruitment

Model 2 - Cord Markers and Malaria during pregnancy and delivery

Model 3 - Maternal and Cord Markers and Malaria during pregnancy and delivery

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CHAPTER 6

Publication Number – 3

This chapter will present the third paper titled: **'The impact of maternal malaria in pregnancy on changes in blood pressure in children over the first year of life'** In this paper, we examined the impact of maternal malaria in pregnancy on growth and BP over the first year of life.

Authors: Ayoola OO, Gemmell I, Cruickshank JK, Clayton PE

Authors' contributions

OA participated in overall study conception and design, data collection, analysis interpretation and manuscript preparation

IG was involved in study design and data analysis.

KC and PC are my Supervisors and both participated in overall study conception and design, data interpretation and manuscript preparation.

THE IMPACT OF MATERNAL MALARIA IN PREGNANCY ON CHANGES IN BLOOD PRESSURE IN CHILDREN OVER THE FIRST YEAR OF LIFE

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Abstract

As in most sub-Saharan Africa, hypertension and its complications are increasingly common in Nigeria, where malaria is hyper-endemic. We established a birth cohort to assess the impact of maternal malaria on growth and blood pressure (BP) in Nigerian infants over one year.

Healthy pregnant women were recruited and followed at Adeoyo Maternity Hospital, Ibadan with blood films for malaria parasites throughout pregnancy including delivery. Anthropometric and BP measures were carried out on 318 babies, all followed from birth to 3 and 12 months. Analysis used multiple regression techniques for longitudinal data, including estimating 'regression to the mean'.

Babies exposed to maternal malaria were shorter, smaller and thinner at birth and remained smaller at 1 year, most marked in boys. Their systolic BP (SBP) adjusted for weight at 3 and 12 months was higher than those unexposed (at 3 months 0.63 mmHg/kg, 95% CI 0-1.2, p=0.04). Change in SBP over the first year was greater in boys than girls (20.9 vs 15.7mmHg p=0.002) but greater in girls exposed to maternal malaria (18.7 vs 12.7 mmHg, 95% CI 1-11, p=0.02). 11% of boys (> twice expected) had BP $\geq 95^{th}$ percentile (hypertensive, US criteria) of whom 68% had maternal malaria exposure.

On regression analysis, gender, maternal malaria exposure and weight change all independently increased change in BP to 1 year.

Maternal malaria exposure had greater adverse effect on growth in boys than girls. Malaria exposed boys had a higher than expected incidence of hypertension at one year, but the girls had a greater increase in BP. Thus intrauterine exposure to malaria appears to have important gender-dependent effects on growth and changes in infant BP and may contribute to the global burden of hypertension.

Key words: maternal malaria in pregnancy, infants, early growth, blood pressure, Nigeria

Introduction

In developing countries, cardiovascular mortality continues to rise with economic transition and changing age structures; hypertension is now thought to be the commonest global risk factor (1). In Nigeria, as for much of sub-Saharan Africa, non-communicable diseases are rapidly replacing but co-exist with traditional infections (2;3). The steady rise in hypertension prevalence at younger ages than in developed countries (4) leads to earlier complications and premature death from hypertensive heart disease, stroke and end-stage renal failure (5;6).

Malarial infection is hyperendemic in Nigeria, particularly in pregnancy leading to maternal anaemia, intrauterine growth retardation (IUGR) and low birth weight (LBW,birth weight <2500g) (7-9). About 12-24% of newborns have been LBW, usually small for gestational age from IUGR (10). Malaria in pregnancy may cause 5–12% of all LBW, 35% of preventable LBW (11) and contributes to 75,000–200,000 infant deaths each year in Africa (12).

Globally, LBW is associated with increased risks of hypertension and diabetes in later life (13-15). Based in part on the 'developmental origins' hypothesis, intrauterine and early experience appears to program anatomic, physiological and endocrine pathways resulting in excess adult disease. Accelerated weight gain in early childhood, particularly by smaller babies exhibiting 'catch-up' growth, is also linked with excess risk in midlife (16). There are arguments that postnatal "catch-up" growth rather than prenatal development is more critical because the association between birth weight and blood pressure (BP) is strengthened by adjustment for later size (16;17). Weight gain in the first 3 months is a driver of BP at 1 year (18;19). In sub-Saharan Africa, there are to our knowledge no studies on the role that maternal malarial infection may play on the relationships between infant growth and development of BP. In Nigerian infants, we tested the hypothesis that

malaria in pregnancy, with its effects on size at birth and early growth, would be related to the pattern of BP change by 12 months of age.

Methods

Study Site and Participants: Families came from a range of socioeconomic backgrounds in the semi-urban community of Yemetu-Adeoyo, Ibadan in Southwest Nigeria, where malaria transmission is perennial. Over 4000 deliveries occur annually at Adeoyo Maternity Hospital (AMH). Ethical approval was obtained from University of Ibadan/ University College Hospital and University of Manchester's Ethics committees.

Healthy women aged 18-45 years presenting before 36 weeks gestation, subsequently delivered at ≥37 weeks gestation at AMH, were eligible. Exclusions included HIV infection, sexually transmitted infections, or multiple pregnancies. Chronic disease (hypertension, diabetes etc) and babies with known syndromes, metabolic defects, major congenital abnormalities or severe birth trauma were also exclusions.

Over one year to cover wet and dry seasons, 624 women were recruited, of whom 161 did not deliver at AMH. Of 463 mother-baby pairs, 27 were excluded due to 4 (0.9%) maternal deaths, 11 (2.4%) stillbirths, 5 (1.1%) miscarriages and 7 (1.5%) neonatal deaths, leaving 436 pairs at birth. After a further maternal and 10 infant deaths from febrile illnesses, relocation or refusal (87% response rate overall), infants measured at all three time points (birth, 3 and 12 months, n=318, 173 boys, 145 girls) were analysed here.

Study Procedures included informed consent at booking with socio-demographic, obstetric, family, and health history including malaria and use of antimalarial drugs. All women were issued prescriptions of sulphadoxine-pyrimethamine for Intermittent Preventive Therapy for malaria according to standard hospital practice.

Blood Measurements*:* 2mls of blood were collected at booking into EDTA tubes for full blood count. Thick and thin blood films were stained with 3% Giemsa at pH 7.2 and examined for malaria parasites (MP) under light microscopy with repeat MP films at subsequent visits, delivery and from cord blood. Thick smears were recorded as negative only after scanning 200 high-powered microscope fields. In those with malaria, absolute parasite counts were determined (20;21) by counting the number of parasites (np) among 200 leucocytes on thick film using the equation: Absolute parasite counts (i.e parasites per µl of blood) = (np/200) x TLC where TLC= subject's total leucocyte count. For quality control, 40% of negative and positive samples were re-examined by two different trained microscopists.

Malaria was defined as asexual blood stages of Plasmodium falciparum during any pregnancy visit or at delivery, in the placenta or cord blood. Women were grouped into 2 categories: a)'No Malaria' - no parasites detected throughout pregnancy or delivery; b) 'Malaria present' - parasites present at least once during pregnancy and/or at delivery. To examine effects of parasite load during pregnancy and at delivery, parasite density was classified into low (<1000 parasites/µl), or high (≥1000/µl).

Anthropometric and BP Measurements: Three nurses, proficient in paediatric venepuncture, were trained in anthropometry and BP measures from WHO's manual (1995) and standard operating procedures (SOPs), using the same equipment throughout. They had 3-monthly refresher training sessions with SOPs and training videos to minimize inter- and within-observer errors.

Within 72 hours of birth, babies were weighed naked to the nearest 0.1kg and crown-heel length measured on an infant stadiometer. Other measures included occipito-frontal circumference (OFC, widest circumference of the head using a non-stretchable tape);. mid

upper arm circumference (MUAC) halfway between the scapula's acromium process and the olecranon with the infant's arm bent; left-sided skinfold thicknesses (triceps, biceps, sub-scapular, and suprailiac) using Holtain calipers. All were obtained in duplicate, or triplicate if disagreeing by >15%. Before performing the BP reading, the baby was comfortably lying on the mother's lap for at least 5 minutes, and many times they were asleep. The measurement was done with the Datascope BP monitor, specifically validated for infants using appropriate newborn cuffs, on the left arm, repeated three times and the mean of the last two readings analysed. The cuff was selected according to baby's upper arm length, extending completely around the arm with the bladder width covering at least two-thirds of the upper arm.

Statistical Analysis

Anthropometric data were transformed into standard deviation scores (SDS) using WHO child growth standards (WHO Anthro version 3.1). T-tests were used to test for differences in anthropometry and blood pressure associated with maternal malaria status. Actual change in SDS and change in SDS predicted by regression to mean (RTM) were calculated and the effect of malaria on growth adjusted for RTM was assessed (22;23). BP percentiles at one year were calculated using BP tables developed by the National High BP Education Program Working Group, using the mean sex-specific heights at 1year of the infants (24). Multiple regression models were used to examine the relationship of BP to maternal malaria status, gender, and anthropometry at birth, 3 and 12 months. Data were analysed using SPSS version 14 (SPSS Inc, Chicago, IL).

Results

Malaria parasitaemia occurred at least once in pregnancy and/or delivery in 160 (50%) of 318 mothers, but its prevalence in babies at 3 and 12 months was only 2.6% and 5.3% respectively.

Anthropometry, growth and BP measures in boys compared to girls

All infants were breastfed, about 97% for >12 months, and had average weight and length for age SD scores <0. Weight SDS < -1 occurred in 42% and 53%, and length SDS <-1 in 36% and 37% at birth and 12 months respectively.

Anthropometric measures differed between boys and girls. At birth, boys were longer by 0.6cm (95%CI 0.07-1.06, p=0.04), had greater OFC by 0.3cm (0.06-0.60, p=0.017) but lower unadjusted systolic (S)BP (-3.3mmHg, p=0.023) and diastolic (D)BP (-2.2mmHg, p=0.034), and when adjusted for weight.

At 3 months, weight, length, OFC, MUAC, biceps and triceps skinfolds were greater in boys than girls (all p<0.01). Unadjusted BPs were not different but BPs adjusted for weight were still lower (both p<0.01) in boys. At 12 months, boys were heavier and taller than girls with larger OFCs and MUACs *(*all p<0.01); skinfolds were similar but BPs adjusted for weight were still significantly lower in boys.

Effect of maternal malaria on infant anthropometry and growth patterns in boys compared to girls

Mean birth anthropometry and SD scores of all infants, genders combined, of mothers with malaria were significantly smaller than those of women without malaria (data not shown). In boys and girls separately, all measures and their SD scores were smaller in those whose mothers had malaria if not statistically significantly so (Table 6.1).

At 3 months, babies with maternal malaria remained shorter by 0.6cm (0.01-1.11, p=0.04), lighter by 0.2kg (0.04 -0.40, p=0.019), and had smaller OFC by 0.3cm (0.01-0.65, p=0.04)

and MUAC by 0.3cm (0.04-0.56, p=0.02) than those without malaria - as a result of effects on boys only (Table 6.1). At 12 months, these effects persisted in boys.

These changes in weight / length SDS in boys with maternal malaria exposure (-0.30, -0.2) were significantly greater than the changes in girls (-0.08, -0.1) after adjusting for the effect of RTM (for weight β=0.352, p=0.043; for length β=0.331, p=0.036, Table 6.2).

Effect of maternal malaria on infant BP and its early changes in boys versus girls

These anthropometric effects of malaria in pregnancy had important influences on infant BP. At birth, mean SBP, unadjusted for size differences, of babies with maternal malaria were lower than those without (69.4 vs. 73.1 mmHg, p=0.01, significant in girls, Table 6.1). At 3 and 12 months, unadjusted mean SBP and DBP were similar but SBP adjusted for weight was now significantly *higher* in boys of mothers with malaria (Table 6.1).

Changes in BP were overall greater in boys than girls. The unadjusted mean changes in SBP from birth to 3 and to 12 months were greater in both sexes who had had maternal malaria. These changes were most prominent in girls: with changes in SBP from birth to 3 (19.4 vs 13.9 mmHg, difference, 5.5, 0.3 -10.7mmHg, p=0.04,) and 12 months (18.7 vs 12.7 mmHg, difference, 6.0, 1.1 -10.9mmHg, p=0.02) with and without maternal malaria respectively. Their mean SBP changes adjusted for length from birth to 3 and 12 months were also greater.

Comparison with US BP percentiles at age 1 year (Table 6.3)

At 1 year, 33 (19%) boys had SBP >90th percentile ('pre-hypertension'), about double that expected, of whom near three-fifths (58%) had had maternal malaria; 11% were $\geq$95th percentile ('hypertensive') of whom near three-quarters (68%) had maternal malaria(X^2 = 5.53, p= 0.02). Of girls, 27(18%) had SBP >90th percentile of whom less than half (44%) had maternal malaria; 8% were hypertensive with about half having had maternal malaria (X^2 =1.79, p= 0.2).

Determinants of changes in blood pressure over the first year of life

In multiple regression analyses (Table 6.4), significant independent determinants of positive changes in SBP from birth to 12 months were being male, presence of maternal malaria. For each cm increase in length from birth to 3 months, SBP was lower by 2.1mmHg and higher by 2.2mmHg for every kg increase in weight from birth to 12 months. Analysis by malaria parasite density showed similar results with the relationship between maternal malaria and change in SBP being stronger with p = 0.04 (data not shown). For change in DBP from birth to 12 months, change in weight from birth to 3 months was the only significant determinant. Analysis by malaria parasite density showed similar results as maternal malaria was not a determinant of change in DBP.

Discussion

These Nigerian babies fell below WHO standards for weight and length at birth, features which persisted until one year old, when arm and skinfold measures were also still well below standards. These results corroborate many previous reports from over 20 years ago, recently re-documented in rural Nigeria (25-27). To our knowledge, this is the first report on the effect of maternal malaria on BP during infancy. We did not adjust for any further effects of malaria in their first year because, despite systematic searching, it was detected in fewer than 5%. At birth infants with maternal malaria had lower BPs but when adjusted for any birth dimensions, BPs did not differ from those of unaffected infants, as reported for BP adjusted for height and weight previously (28-30). In contrast, SBP adjusted for weight was *higher* at 3 months in these infants, significantly so at both 3 and 12 months in boys. DBP adjusted for weight was also greater in malaria-affected infants but not significantly. Affected babies had a greater change in unadjusted SBP from birth to

3 and 12 months, notably in girls in whom change in SBP adjusted for length was also higher.

Few studies have examined longitudinal growth of West African infants in early life. Most were cross-sectional(27;31-34), of babies exclusively breastfed for 4 to 6 months of life as our cohort was and subsequently up to a year. Maternal malaria continued to exaggerate reduced size and affected boys fared worse than girls (Table 6.3). The impact of malaria on growth here, as elsewhere (35-38), seems related to chronic placental infection and insufficiency (39;40) leading to proportionate fetal growth retardation (41). Apart from length SDS from birth to 3 months, the babies did not show significant catch up growth beyond that expected due to regression to the mean. Similar results occurred in Malawian infants in whom maternal malaria at delivery was associated with reduced weight for age and thinness at age 12 months (42). In our cohort, the deficit in catch up growth was more marked among boys, but not for girls from birth to 12 months (Table 6.2). Similar findings of low weight gain in the first year of life have reported in boys who are then at risk of high SBP, especially if they end up with short adult stature (43). Lack of early catch up is sometimes followed by increased weight gain in childhood which has been linked to the risk of developing metabolic and cardiovascular disease later in life particularly stroke which is thought to result from hypertension (43-45).

Compared with US BP centiles, nearly a fifth of these generally small babies had BP above the 90th centile (ie: some 60-70% more than expected), despite differing but controlled measurement settings and generally much warmer conditions than for the US readings. Further, 8-11% were 'hypertensive' on these standardised readings but taken on just the one occasion as the mean of the 2 last of 3 values; more than half had had maternal malaria. US BP centiles are unavailable at birth or 3 months but our 1 year results indicate that these Nigerian BPs were relatively high, despite or because of their

smaller size. Follow-up is continuing so the contribution of these factors to their later BP profiles will become clearer.

The US BP tables are based on auscultation for BP measurement. Some oscillometric devices have been developed with tailored algorithms for young children to improve accuracy (46;47) and are validated at this age group, as we used here. When so validated or calibrated, oscillometric devices reduce observer error, are convenient and are preferred for BP measurement in newborns and young infants, in whom auscultation is difficult (46;47).

Limitations of this work include our use of what some observers may regard as an unusual adjustment to the BPs dividing by weight at the time of measurement. That manoeuvre is not generally done, in part perhaps because BP in infancy has only recently been considered much and its influence by systematic small size at birth and subsequent 'catch-up' or not is infrequently measured. We consider it quite logical in the context here of such systematic birth restriction that malaria induces, and the effect of malaria remains, even if 'borderline' when using weight or length SDS simultaneously, as in the regression analysis. High malaria parasitaemia amplified the relationship between intrauterine exposure to malaria and change in SBP over the first year of life. This finding may be related to the impact of malaria on birth dimensions which leads to lighter, shorter babies as previously reported (8;35;48).

Both fetal and early postnatal growth have been related to BP in adolescents and adults (49;50). Children who are thin at birth and rapidly increase in size, particularly in adiposity, in the first 6 months of life develop higher early childhood SBP at three years of age (51). Here we find similar results by one year, which may set the scene for higher BP later. In Gambian children, there was no relation between BP and birth weight in children aged 1-8 years but there was an inverse relation in those older than 8y.(52). In children in Jamaica

aged 6–16y, in Zimbabwe aged 6y and South African children aged 5y, there were inverse relations between birth weight and BP, after adjustment for current weight (53-55).

Longitudinal data on tracking of BP strongly suggests that childhood BP is a predictor of adult BP in most population settings so far examined, mainly in westernised countries (56). Two sets of data from the Bogalusa Heart Study, comparing African-American & European-origin children in the same setting, suggested important early life effects. In one cohort, birth weight inversely, then strong early growth as well as achieved body mass directly influenced BP in adolescence, and accounted for the 'black-white' BP difference emerging at that age (50). In a separate cohort, BP measured initially from 4 years and older was the most powerful determinant of later BP levels (57).

We are unaware of previous work on intra-uterine exposure to malaria and its impact on infant BP in African children;. LBW results from maternal malaria because placental sequestration and insufficiency lead to significant inflammation in the placental and infant arterial tree but the mechanisms underlying a putative link suggested here between malaria in pregnancy and later infant BP are not yet elucidated. We suggest the hypothesis that due to maternal malaria, there is resultant smaller birth size, general growth restriction and consequently, a smaller vascular tree. As the child grows, the more limited vascular tree of the smaller infants would not meet end-organ oxygen and nutritional demands without higher BPs and reactive peripheral vasoconstriction over time, manifested as progressively higher BPs leading to a higher risk of hypertension in later life. (58;59).

Perspective

Nigerian babies were smaller, shorter and thinner than WHO standards at birth and failed to catch up over their first year. The findings were more pronounced in babies with maternal malaria particularly boys. Changes in BP were overall greater in boys than girls. Mean change in SBP during infancy were higher in children with maternal malaria

particularly girls whose length adjusted SBP were also higher. At one year of age, 8-11% had BPs already in the hypertensive range and more than half had been exposed to maternal malaria, suggesting a potentially important role for intrauterine exposure to malaria in influencing early BP changes. These BP patterns may not be unrelated to Nigeria and sub-Saharan Africa's high risk of hypertension in later life.

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Table 6.1: Anthropometry and blood pressure at birth, 3 and 12 months in boys compared to girls by maternal malarial status

	BIRTH n = 318				3 MONTHS n = 318				12 MONTHS n = 318			
	BOYS n = 173		GIRLS n = 145		BOYS n = 173		GIRLS n = 145		BOYS n = 173		GIRLS n = 145	
Mean	**MP NO** n = 86	**MP YES** n = 87	**MP NO** n = 72	**MP YES** n = 73	**MP NO** n = 86	**MP YES** n = 87	**MP NO** n = 72	**MP YES** n = 73	**MP NO** n = 86	**MP YES** n = 87	**MP NO** n = 72	**MP YES** n = 73
Weight (kg)	2.99	2.87	2.88	2.80	**6.14**	**5.84***	5.52	5.40	**8.79**	**8.40***	7.91	7.94
Length (cm)	49.14	48.52	48.47	48.06	61.50	61.04	60.41	59.73	74.22	73.56	72.7	72.5
BMI (kg/m^2)	12.34	12.19	12.23	12.12	**16.16**	**15.65***	15.12	15.10	15.88	15.47	14.94	15.08
OFC (cm)	34.60	34.23	34.13	34.03	**41.13**	**40.73***	40.11	39.86	**46.04**	**45.48***	44.60	44.80
MUAC (cm)	10.01	9.79	9.83	9.74	**13.52**	**13.02***	12.89	12.82	**14.70**	**14.17***	13.88	13.84
Subs (cm)	4.07	4.05	4.34	4.12	7.34	7.14	7.23	7.01	**5.65**	**5.30***	5.54	5.35
Triceps (cm)	4.08	4.00	4.15	4.02	7.69	7.54	7.43	7.03	**6.67**	**6.25***	6.35	6.31
SBP (mmHg)	70.9	68.6	**75.8**	**70.4***	90.1	90.2	89.5	89.6	90.1	89.9	88.8	89.0
SBP/W	24.1	24.4	26.7	25.6	**15.0**	**15.7***	16.5	17.0	**10.4**	**10.9***	11.4	11.4
SBP/L	1.45	1.42	1.56	1.47	1.46	1.48	1.49	1.50	1.21	1.22	1.22	1.23
DBP (mmHg)	36.2	34.0	38.2	36.3	49.8	48.7	48.3	49.4	50.2	49.4	51.3	49.7
DBP/W	12.2	12.1	13.5	13.2	8.26	8.5	8.9	9.4	5.8	6.00	6.6	6.4
DBP/L	0.73	0.74	0.79	0.76	0.81	0.80	0.80	0.83	0.68	0.67	0.71	0.69
Weight SDS	-0.78	-1.07	-0.83	-1.02	**-0.51**	**-0.93***	-0.58	-0.78	**-0.95**	**-1.37***	-1.12	-1.08
Length SDS	-0.43	-0.78	-0.44	-0.63	-0.12	-0.31*	0.19	-0.13	-0.67	-0.98	-0.56	-0.63
BMI SDS	-0.94	-1.11	-0.97	-1.10	**-0.62**	**-0.98***	-0.93	-0.87	**-0.79**	**-1.14***	-1.11	-1.00
OFC SDS	0.05	-0.22	0.15	0.08	**-0.37**	**-0.03***	0.27	0.20	**-0.02**	**-0.47***	-0.23	-0.09
MUAC SDS					**0.14**	**-0.56***	-0.21	-0.29	**-0.01**	**-0.50***	-0.32	-0.36
Subs SDS					-0.26	-0.53	-0.44	-0.70	**-0.92**	**-1.31***	-1.05	-1.22
Triceps SDS					-1.39	-1.61	-1.46	-1.75	**-1.11**	**-1.45***	-1.20	-1.29

BMI – Body Mass index; OFC – Occipito-frontal circumference; MUAC – Mid-upper arm circumference; Subs – Subscapular skinfold thickness; Tric – Triceps skinfold thickness; SBP- Systolic blood pressure; DBP- Diastolic blood pressure; W – Weight; L – Length

* - $p < 0.05$

Table 6.2: Change in SDS adjusted for regression to the mean from 0 to 3 and 0 to 12 months by maternal malarial status and by gender

		0-3 months						0-12 months					
		Weight			Length			Weight			Length		
		MP No	MP Yes	p-value	MP No	MP Yes	p-value	MP No	MP Yes	p-value	MP No	MP Yes	p-value
Boys	ΔSDS	0.28	0.14		0.31	0.47		-0.16	-0.30		-0.25	-0.20	
(n=173)	RTM	0.44	0.55	*0.063*	0.14	0.56	0.746	0.54	0.69	*0.066*	0.21	0.48	0.239
Girls	ΔSDS	0.25	0.24		0.63	0.25		-0.23	-0.08		-0.10	-0.01	
(n=145)	RTM	0.45	0.48	0.710	0.50	0.33	0.365	0.56	0.63	0.248	0.29	0.40	0.881
p-value		0.681	0.363		0.145	0.347		0.322	**0.043**		0.616	**0.036**	

ΔSDS – Observed change in SDS, RTM – Expected Change due to Regression to the Mean

p-values are from the analysis of the difference in ΔSDS adjusted for RTM by maternal malaria status (columns) and gender (row) (22).

Table 6.3: Comparison of Infant BP by Maternal malaria with US BP percentiles at age one year

12 MONTHS n = 318								
	BOYS n = 173				GIRLS n = 145			
BP	**MP NO**		**MP YES**		**MP NO**		**MP YES**	
Percentile	n = 86	**%**	n = 87	%	n = 72	**%**	n = 73	%
<90th	70	81.4	70	80.5	60	83.3	58	79.5
90th -94th	10	11.6	4	4.6	7	9.7	8	11
≥95th	6	7.0	13	14.9	5	7	7	9.5

Table 6.4: Regression analyses for determinants of change in infant blood pressure from birth to one year

Variable	Δ SBP				ΔDBP			
	ß	95% CI	P-value	R^2	ß	95% CI	P-value	R^2
0 - 12 months								
Sex (boy/girl)	-4.794	-8.11 to -1.48	**0.005**		-1.688	-4.08 to 0.70	0.16	
Malaria status	3.233	-0.07 to 6.53	0.05		0.66	-1.717 to 3.031	0.59	
Length SDS 0-3	-2.129	-3.65 to -0.61	**0.006**		-.463	-1.586 to 0.66	0.42	
Weight SDS 0-3					1.405	.153 to 2.66	**0.028**	
Weight SDS 0-12	2.273	0.85 to 3.70	**0.002**	**0.10**				**0.06**

SBP- Systolic blood pressure; DBP- Diastolic blood pressure
Malarial status: Coding 0 = 'No malaria', 1= 'Malaria present'

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CHAPTER 7

DISCUSSION

7.1. Introduction

This chapter presents a summary of the major findings from this work, the limitations of the study and future directions of research.

7.2. Paper 1

The main thrust of this paper was to evaluate the impact of intrauterine exposure to malaria on size and BP at birth. In order to adequately explore this, we classified malaria in pregnancy based on a) timing of infection, i.e 'During pregnancy' and 'At delivery' and b) parasite density i.e 'Low' (<1000 parasites/μl) and 'High' (≥ 1000 parasites/μl).

In this cohort, malaria in pregnancy rates were high (48%) and as in other studies in Africa, these women were asymptomatic (1-4). This asymptomatic infection was however associated with maternal anaemia which has been linked to the genesis of raised BP in children (5-7).

Furthermore, there was significant consequence on birth anthropometry particularly in those with high parasitaemia such that mean weight, length and head circumference were lower in the babies by 300g, 1.1 cm and 0.7 cm respectively. Similar findings in birth weight have been reported in other studies in Africa but there is lack of data on other anthropometric measures (8;9).

It is noteworthy that in babies of mothers with malaria at delivery, SBP were lower compared with those with malaria during pregnancy and no malaria. Overall, babies exposed to high malarial load through pregnancy had the smallest birth size but rather than having significantly lower BP as expected, both SBP and DBP corrected for weight were higher than in those exposed to a low parasite load.

These observations corroborate developmental origins hypothesis linking placental insufficiency, IUGR and later hypertension. This could have important implications for BP levels through childhood and subsequently the development of hypertension in midlife in Nigeria.

7.3. Paper 2

In Paper 2, we examined maternal and cord blood metabolic markers in relation to maternal malaria in pregnancy and their relationships to birth size with the aim of defining the mediators of the relationships between prenatal malaria and birth size which is linked to later cardiovascular and metabolic morbidity.

All plasma lipids levels except HDL were higher in the third trimester than the second trimester of pregnancy This is believed not to be atherogenic and confirms previous studies in Nigeria and other populations (10-14). Contrary to a previous Nigerian report, there were significant correlations between maternal and cord lipids except LDL (15).

Presence of malaria in pregnancy was associated with higher maternal TNFα, lower lipids except TG, and lower cord blood IGF-I. This is in line with previous reports of lower TC and HDL-C in acute malaria and higher TNFα in peripheral and placental blood of Malawian and Kenyan pregnant women (16-18). However, there was no effect on the cord lipid profile, insulin and TNF α levels.

Significant determinants of birth size were intrauterine exposure to malaria, maternal cholesterol, LDL-C, insulin and cord IGF-I and insulin. Maternal malaria was associated with TC, LDC-C and cord IGF-I which are strong determinants of birth size. These factors are likely linked to mechanisms of the effect of intrauterine malaria on fetal growth. The mechanisms for malaria-induced fetal growth restriction have not been fully elucidated. There are proposals that it is a multifactorial process involving placental insufficiency. It is believed to be immune related (19;20) and linked with its effect on lipid metabolism as

confirmed in this study where malaria was associated with lower lipid levels except TG. It is believed that cholesterol is necessary for normal progesterone synthesis which is essential for maintaining the pregnancy (21) The growing fetus also requires adequate amount of cholesterol. This demand is aggravated in the presence of malaria in pregnancy because Plasmodium falciparum cannot synthesize cholesterol but it is an essential requirement for its growth (21). This leads to low cholesterol levels associated with malaria in pregnancy and the consequences to maternal and fetal health.

7.4. Paper 3

Paper 3 was aimed at exploring the impact of intrauterine exposure to malaria on birth size, growth and BP over the first year of life. Data from 318 babies that were measured at birth, 3 and 12 months of life provided strictly longitudinal assessment of growth and BP development. There are limited longitudinal data of this type particularly in those with previous intrauterine exposure to malaria in Sub-saharan Africa.

Anthropometry of babies of mothers with malaria were smaller in all dimensions at birth and remained so at one year of life, most marked in boys.

In babies of mothers with high parasitaemia particularly boys, SBP and DBP adjusted for weight were higher at birth and remained so at 3 and 12 months. Furthermore, girls whose mothers had malaria in pregnancy, had a greater change in BP from birth to 3 and 12 months. 11% of boys (> twice expected) had BP $\geq 95^{th}$ percentile (hypertensive, US criteria) of whom 68% had maternal malaria exposure.

On regression analysis, gender, maternal malaria exposure and weight change all independently increased change in BP to 1 year.

These observations are of great importance particularly because little is known about the association between birth weight, early growth and BP in Nigeria where there are high

rates of LBW as a consequence of high prevalence of malaria in pregnancy. Possible mechanisms for this observation are still unclear but there has been proposal that it involves decreased arterial compliance in these LBW babies which is associated with high BP. This is probably due to decreased amount of elastin present in the vessel wall resulting from in utero effects on vascular elastogenesis (22-24) which could be amplified by the vascular effects of placental malaria in this environment.

In addition, longitudinal data on tracking of BP show strongly that childhood BP is a strong predictor of adult BP (25-27). These findings therefore suggest that there is a significant role for intrauterine exposure to malaria in influencing early BP and this set the scene for development of high BP in midlife. This has important implications in relation to the burden of hypertension in Sub-saharan Africa where malaria is hyperendemic and very common in pregnancy.

7.5. Study Limitations

Our original plan was to recruit a cohort of 500 pregnant women to ensure a minimum sample size of 400 infants not only enrolled but followed to one year old. This was to enable us to investigate longitudinal growth and BP data in at least 400 babies from birth to 2 years. We actually recruited 624 pregnant women to ensure that we met our target. However, 161 women still did not deliver their baby at AMH; thus the final cohort included 463 mother-baby pairs. Of these, 27 were excluded due to 4 (0.9%) maternal deaths, 11 (2.4%) stillbirths, 5 (1.1%) miscarriages and 7 (1.5%) neonatal deaths, leaving 436 pairs at birth. At 3 months, 384 babies of the 436 babies were measured, but 15 more were measured at 6 months. At 12 months, 380 were measured. There had been 9 infant deaths mainly from febrile illnesses. Others had relocated from the study catchment area or were no longer interested. Our follow-up response rate was 87%. However, our

analyses at 12 months were on 318 infants who were measured at all the three time points (birth, 3 and 12 months).

The study was originally powered for 400 infants so our follow-up numbers were lower and we also performed some analysis on subgroups of high and low parasitaemia and timing of malaria parasitaemia. These could have an impact on the ability to detect the effects of exposure to intrauterine malaria. It is possible that the effects are more than we have reported and that impact of intrauterine exposure to malaria on the offspring could be underestimated. This further emphasizes the fact that intrauterine malaria plays an important role in the association of lower birth size with high BP in this environment.

In this study, diagnosis of malaria was based on microscopy of peripheral, placental and cord blood samples. Malaria in pregnancy still presents diagnostic challenges and laboratory diagnosis which has relied mainly on microscopy is the commonest method of diagnosis in sub-Saharan Africa. According to the WHO, microscopy is a valuable technique when performed correctly but it requires well-trained and skilled staff (28)]. It has the advantages that it can be used for speciation and quantification of parasites, as done in this study, as well as assessing response to antimalarial treatment. Histological examination, although reported to be more sensitive than microscopy on placental blood samples for detection of malaria (29)], was not available for this study. Placental blood samples, in addition to peripheral blood smears, were used to ensure that placental malarial infections were detected when peripheral parasitaemia may be negative (30)]. Therefore we included all these sites in our definition of malaria in pregnancy and delivery.

At the second antenatal visit, we obtained fasting blood samples from 467 women. However, 187 samples from the cord blood were available due to difficulty with collection and transfer of sample for storage. This was because most babies were born after midnight and the freezer was not at AMH because of problems of power cut amongst

others. Cord blood glucose could not be measured immediately and so was not included in the assay.

Another limitation of this study is the use of an unusual adjustment to the BPs dividing by weight at the time of measurement. That manoeuvre is not generally done, in part perhaps because BP in infancy has only recently been considered much and its influence by systematic small size at birth and subsequent 'catch-up' or not is infrequently measured. It is quite logical in the context here of such systematic birth restriction that malaria induces, and the effect of malaria remains, even if 'borderline' when using weight or length SDS simultaneously, as in the regression analysis. Both fetal and early postnatal growth have been related to BP in adolescents and adults (25;31).

7.6. Conclusions and Future Research Direction

In this apparently healthy cohort of pregnant women in Nigeria, there is a high incidence of malaria and anaemia, particularly associated with younger age and being primigravid. The consequence of malaria in pregnancy included adverse effect on birth size such as shorter, smaller and thinner babies with high parasite density during pregnancy having the greatest impact on all growth parameters and being associated with higher BP corrected for weight.

Malaria in pregnancy is associated with lower that maternal lipids (except TGs) and cord blood IGF-I. These factors are strong determinants of birth size. Therefore, the effect of intrauterine malaria exposure on birth size may be mediated by maternal total cholesterol, LDC-C and in part by fetal IGF-I and TNF.

Maternal malaria exposure had greater adverse effect on growth in boys than girls. Malaria exposed boys had a higher than expected incidence of hypertension at one year, but the girls had a greater increase in BP.

Therefore, intrauterine exposure to malaria seems to be an important factor influencing early rise in blood pressure. These observations support the 'developmental origin hypothesis' confirming the significant influence of prenatal environment on birth size and later BP. This could have important implications in the genesis of high rates of hypertension in Nigeria and hence adult cardiovascular health.

Recent discoveries about the mechanisms of pathogenesis of IUGR leading to LBW associated with malaria in pregnancy have revealed that such babies are predisposed to hypertension and vascular dysfunction (20). It is proposed that placental malaria induces accumulation of monocytes in the intervillous space with increased cytokine and complement release which leads to impairment of placental nutrient transporters, can alter fetal growth hormone levels and impair trophoblast invasion thereby hindering vascular development and angiogenesis. This leads to changes in placental blood flow. Furthermore, maternal anemia which results from malaria can lead to IUGR (19;20;32;33). These processes lead to placental insufficiency and small birth size.

Furthermore, in a cohort of babies exposed to placental malaria but who were not LBW at birth, there was reduced weight gain over the first year of life as reported in this study. These infants were 2.4 times more likely to be wasted at the age of three months and 3.1 times more likely to be underweight at the age of 12 months than infants born to mothers without placental malaria (24).

The possible explanations for this observation are disturbed innate immunity in early life which could certainly enhance susceptibility to other pathogens and antigens and therefore result in poorer growth development. Furthermore, during pregnancy, the placenta acts as an additional source of leptin but placental malaria causes reduced leptin and disrupts its relationship with birth weight and an early lack of leptin responsiveness could contribute to poor growth in these infants (34;35). In our follow up study, we are exploring the role of leptin in this cohort.

This study confirms previous observations that LBW is associated with increased risk of high BP which is a major risk factor for stroke in Nigeria. This association is independent of the length of gestation and therefore reflects slow fetal growth. In our cohort, there was lack of catch up growth which has also been reported to be associated with later development of hypertension, particularly slow growth between birth and two years of age. This observation was made in children exposed to malaria in utero. (24;36).

The findings from this study are of importance in the elucidation of the mechanisms for the birth-weight–hypertension relationship in the West African setting where malaria in pregnancy contributes significantly to LBW. This result suggests that antenatal factors, particularly prenatal exposure to malaria, reflected in altered rates of growth in this apparently healthy cohort of pregnant women, play a role in generating higher blood pressure. This has implications for targeting early intervention and preventative programs for malaria in pregnant women in Nigeria.

Follow-up studies to extend these observations into early childhood and to provide a better understanding of the influence of maternal malaria on BP in this cohort are underway. The children are now two to three years of age and we are going to explore their growth, BP measures and metabolic parameters at the age of one. An area of great interest that would also be explored is the role of the IGF- axis and leptin and adiponectin in the mechanism of these observations.

Comparisons of the data from Nigeria with those of similar data from the Manchester cohort of White European and South Asian babies to analyse the impact of ethnicity on cardiovascular risks will also be done.

In this study, malaria in pregnancy was assessed without making any changes to routine clinical management in order to document and substantiate the above findings. Future research would include intervention studies in which malaria in pregnancy would be controlled and the babies would be followed up.

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CHAPTER 8

APPENDICES

APPENDIX ONE

Title of Research Project**: The effect of maternal health, particularly malaria, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children**

Names of Researchers: DR OO AYOOLA, PROF P CLAYTON, PROF K CRUICKSHANK, PROF O OMOTADE

Participant Information Sheet

Invitation to take part

We would like to invite you, your husband and baby to join this study. Below we have outlined what this study is all about and the information, and samples we would like to collect. We would be grateful if you could spend some time reading through this information. We would be very happy to answer any questions you may have.

Introduction

In recent years, we have learnt that the size of a baby at birth and the speed that they grow and gain weight in the first months of life can be one of the many factors that lead to some diseases that develop later in life. These include conditions like high blood pressure, diabetes and heart attacks.

We do not yet fully understand how this link happens. In our study we want to look at a number of possible factors, like presence of malaria in mother's blood during pregnancy, how your baby grow and gains weight over his/her first 2 years and the amount of hormones related to growth and other metabolic substances & proteins that your baby produces. We would like to see if there is any early evidence in your baby's blood of increased risk of heart and blood vessel problems and diabetes in later life.

We also want to see how your baby's heart grows, how his/her blood pressure and blood flow through the arteries of the neck and tummy (abdomen) develops over the first two years. The heart size is measured by placing an ultrasound probe on your baby's chest while the blood flow is recorded using a pencil-like probe on the skin. Neither of these tests hurt at all.

We also know that people from different ethnic groups actually have different rates of disease like diabetes, high blood pressure and heart attacks. For example, those originating from Nigeria in Africa have higher rates of high blood pressure and stroke than White Europeans. Clearly there is something about a person's background that contributes.

To improve prevention and treatments for all these problems, we believe it is really important to know what these links are between the baby and its health later in life. In the future, that will allow us to advise people, perhaps including your family, how best to reduce the chance of having health problems later.

What will I have to do if I take part?

You will be seen in pregnancy and we will ask questions about your health, feeding, and lifestyle. We will also undertake measurements of your height, weight and blood pressure.

We will take a sample of blood to check for presence of malaria in your blood in addition to your blood sugar, insulin, fat and other factors that help to assess how your heart works.

Your baby will be examined at birth to check on his/her size and health. We will take blood sample from the placenta to check for presence of malaria and blood test for malaria, sugar and insulin will be taken from the umbilical cord.

We would like to invite you and your baby to see us five times over his/her first 2 years. The visits will be at 3, 6 or 9, 12, 18 and 24 months. We will ask you to come to the Immunization Clinic, Adeoyo Hospital, Ibadan. We will ask you to come in the morning to stay for an hour or so while we ask you about your baby, his/her health and diet, and your diet, and measure the following:

1. your baby's length, weight, and head size, skin & fat thickness in the arms and over the back
2. your baby's blood pressure in the arm, and blood flow via a light probe on the neck and tummy (abdomen) and heart size, again using a simple ultrasound. None of these should trouble your baby at all.
3. take a small blood sample from your baby's heel or arm to detect presence of malaria and other infections, measure hormones that control growth, other metabolic and protein factors and blood fats like cholesterol and markers of inflammation in the blood. This sample only takes a minute or so to do. We also ask permission to store part of the sample to do newer tests later which may become important and relevant to your baby or child's general as well as blood vessel and heart health – but which we do not know of as yet. To reduce the chance that this will hurt your baby, we can put anesthetic cream on the skin well before the test.
4. to ask you yourself to have these measures – that is, rechecking your blood flow and heart size at the same visit and your blood for the same types of substances as for your baby and as for them, to store part of the sample for future tests. All these simple measurements, for both you and your baby, will take less than an hour.

You and your husband will also be asked whether you could provide a sample of blood for DNA studies and whether a sample of blood from your baby's umbilical cord for DNA could be taken, or if not from the cord blood, then when we see your baby at a later time in the project. DNA is the genetic material that can be used to examine genes. We are interested in examining genes that control insulin and hence sugar levels and also genes related to babies' growth. Small natural changes in the genetic code in any of these genes might be associated with particular levels of blood sugar and insulin, particular patterns of growth in the baby or in their heart blood vessels or in their blood pressure and related conditions. We want to test whether these small changes in a gene have these effects.

We will only use your DNA samples to look at these genes, and the researchers involved in this study will have no other information available to them than that collected in this study. DNA samples are very valuable for research. In the future, we may wish to study other genes that might influence growth, growth, hormones or heart and blood pressure development of a baby. Our plan is therefore to store these DNA samples securely for possible future study.
For any other study, we would return to our local ethics Committee to ask for their permission to do it. If you do **not want** DNA from yourself or baby to be stored for this purpose, then please let us know and the samples **will be destroyed.**

We will be able to cover expenses for coming and provide you and your baby with some feeding utensils while you are visiting us.

What are the possible risks to taking part?
We do not think there are any risks to taking part.

Are there any possible benefits?
We hope to learn a great deal about the links between malaria in pregnancy, malaria episodes in childhood, these markers of metabolism (chemistry) or inflammation in your baby's blood, their growth and related blood growth factors & hormones, their genes and his/her heart, blood pressure & blood flow development. We should also understand how your own 'heart health' progresses, be able to advise you appropriately and see how this links into that of your baby.

We hope that this will help us to understand how malaria load, a baby's size and growth can influence diseases like high blood pressure, diabetes and heart trouble later in life. It is unlikely that any of the pieces of information we collect will directly affect your baby. We will however be able to show you how your baby is growing, and talk to you about his/her health. If you or your baby has any health problems, we can also put you in touch with the right person to help.

Do I have to take part?
No, taking part is **entirely** voluntary. We would also add that you can take part in only one or two of the tests if you wish.

If you would prefer not to take part you do not have to give a reason. We will not be upset and your treatment through pregnancy and after will not be affected. If you take part but later change your mind you can withdraw at any time from the study without disadvantage to your future treatment.

What do I do now?
The researcher and the team organizing the study can answer any questions and you can let them know if you are interested in taking part.

Thank you very much for considering taking part in our research. Please discuss this information with your family, friends or personal physician if you wish.
If you wish to obtain independent advice about this research you may contact:

Professor O Omotade, Institute of Child Health, College of Medicine, University of Ibadan, Ibadan, Nigeria. Tel: + 234 2751 0698

OR

Dr Omolola Ayoola, Department of Paediatrics, College of Medicine,University of Ibadan, Ibadan, Nigeria. Tel: + 234 2751 0698

APPENDIX TWO

CONSENT FORM (Mothers)

Title of Project: The effect of maternal health, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children

Names of Researchers: DR OO AYOOLA, PROF P CLAYTON, PROF K CRUICKSHANK, PROF O OMOTADE

Please initial box

1. I understand that I am providing consent for **myself** ☐
2. I confirm that I have read and understand the information sheet dated …… for the above study and / or the study has been well explained to me and I have had the opportunity to ask questions. ☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected . ☐
4. I understand that sections of any of my medical notes may be looked at by responsible individuals involved in the study where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
5. I understand that part of the (separated) blood samples will be stored for future study of metabolic & protein substances relevant to the child's blood vessel and general health. Please initial this box if you are happy for your sample to be stored. If this box is <u>not initialed,</u> that sample will be destroyed. ☐
6. I understand that DNA samples will be stored for future study. Please initial this box if you are happy for your sample to be stored. If this box is <u>not initialed,</u> the DNA sample will be destroyed at the end of the study ☐
7. I understand that information and records held by the hospital may be used to keep in touch with me and follow up my health status. ☐
8. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Researcher	Date	Signature

CONSENT FORM (Children)

Title of Project**: The effect of maternal health, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children**

Names of Researchers: DR OO AYOOLA, PROF P CLAYTON, PROF K CRUICKSHANK, PROF O OMOTADE

Please initial box

1. I understand that I am providing consent on behalf of **my child** ☐
2. I confirm that I have read and understand the information sheet dated ……. for the above study and / or the study has been well explained to me and I have had the opportunity to ask questions. ☐
3. I understand that my child's participation is voluntary and that I am free to withdraw him/ her at any time, without giving any reason. His /her medical care or legal rights will not be affected . ☐
4. I understand that sections of any of my child's medical notes may be looked at by responsible individuals involved in the study where it is relevant to his/her taking part in research. .I give permission for these individuals to have access to my child's records. ☐
5. I understand that part of the (separated) blood samples will be stored for future study of metabolic & protein substances relevant to the child's blood vessel and general health. Please initial this box if you are happy for your sample to be stored. If this box is <u>not initialed,</u> that sample will be destroyed. ☐
6. I understand that DNA samples will be stored for future study. Please initial this box if you are happy for your child's sample to be stored. If this box is <u>not initialed,</u> the DNA sample will be destroyed. ☐
7. I understand that information and records held by the hospital may be used to keep in touch with me and follow up my child's health status. ☐
8. I agree that my child can take part in the above study. ☐

_____	_____	_____
Name of Parent	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Researcher	Date	Signature

APPENDIX THREE

STANDARD OPERATING PROCEDURE 1

Title – Anthropometry and Skin folds Measurements

Author: Dr Ayoola

Implementation 08/10/07

Review Date: 08/10/08

Version Number 02

Purpose:
The purpose of this Standard Operating Procedure (SOP) is to ensure that measurements are carried out on mothers and children safely and accurately with minimal discomfort to them.

Scope
This SOP covers any research staff who is undertaking these measurements on children and mothers.

Applicability:
This SOP must be used in conjunction with the study protocol.

Associated Documentation:
Manufacturers' instructions.

Methods: ***Weight and Height***

Mothers

1. The maternal weight should be measured without shoes to the nearest 0.1 kg on scale (SECA model) according to training video instructions.
2. Height should be measured on a purpose built stadiometer according to manufacturer's instructions and training video.

Infants

1. Undress infant with help from parent/carer or ask parent/carer to undress infant if this is more acceptable to them.
2. Infants should be weighed without their nappy to the nearest 0.1 kg on infant pan scales (SECA model) according to training video instructions.

3. Length should be measured from the crown to heel on a purpose built infantometer. The infants head will be put against the head board of the infantometer and their legs gently flattened and extended. Length will be the furthest point that their feet reach the foot board. Take measurement in centimeters.

Methods: ***Head Circumference***

1. Measurement must be in accordance with training provided by a competent trainer.
2. Explain to the parent/career what you are doing and seek verbal assent for the procedure.
3. Assemble all necessary equipment ensuring it is clean and in proper working order.
4. Head circumference must be measured at the widest point on the skull using the seca 212 head circumference tape measure and should be recorded in centimetres

Methods: ***Blood Pressure***

Mothers

1. The maternal blood pressure will be taken at every visit during pregnancy and after delivery
2. Before performing a blood pressure reading, the subject will be comfortably seated and relaxed with the back and arm supported, the legs uncrossed, for at least 5minutes and not moving or speaking.
3. The upper arm will be supported at the level of the heart with no tight clothing constricting the arm.
4. Proper cuff size selection is critical to accurate measurement. The bladder length and width of the cuff will be 80% and 40%, respectively, of the arm circumference.
5. The cuff will be placed neatly, with the indicator mark over the brachial artery.
6. Blood pressure measurement errors are generally worse in cuffs that are too small compared to those that are too big.
7. The measurement will be repeated three times and recorded as displayed on the monitor.

Infants

1. The child's blood pressure will be taken at every visit.
2. Before performing the blood pressure reading, the child will be comfortably seated on mother's lap or lying down and relaxed for at least 5minutes.
3. The upper arm will be supported at the level of the heart with no tight clothing constricting the arm.
4. Proper cuff size selection is critical to accurate measurement. The bladder length and width of the cuff will be 80% and 40%, respectively, of the arm circumference.
5. The cuff will be placed neatly, with the indicator mark over the brachial artery.
6. Blood pressure measurement errors are generally worse in cuffs that are too small compared to those that are too big.
7. The measurement will be repeated three times and recorded as displayed on the monitor.

Cuff Sizes

Indication	Width (cm)*	Length (cm)*	BHS Guidelines Bladder width & Length (cm)*	Arm cir. (cm)*
Small Adult/Child	10 - 12	18.24	12 x 18	<23
Standard Adult	12 – 13	23 – 35	12 x 26	<33
Large Adult	12 – 16	35 – 40	12 x 40	<50
Adult Thigh Cuff**	20	42		<53

Methods: ***Skinfold Thickness***

1. Skinfolds (biceps, triceps, sub-scapular, flank) will be measured using Holtain callipers
2. Equipment must be calibrated regularly in accordance with the manufacturer's instructions. This is in order to ensure constancy of measurement and elimination of error.

3. Measurements are to be made on the left arm.
4. Mark the mid point of upper arm with indelible marker. Mid point is halfway between the tip of the acromium process on the scapula and the tip of the acromium process on the scapula and the olecranon process on the ulna with the infants arm bent.
5. Take a measurement of the mid upper arm circumference in centimetres
6. Triceps skin fold is measured at the mid point level on the back of the arm over the triceps muscle.
7. Pick up the skin fold between the thumb and forefinger of the left hand. Place the callipers on the skin fold just below the fingers.
8. Remove the fingers and take a reading once the dial has settled 2 – 3 seconds later.
9. Biceps is measured in the same way on the biceps muscle.
10. Turn the infants on his/her front
11. Subscapula skin fold is measured 1cm below the tip of the scapula bone. There is natural potential crease at an angle of 45 degrees towards the spine.
12. Suprailiac skin fold is measured in the natural fold just above the iliac crest in the mid axillary line.
13. All measurements must be taken in duplicate and compared against national and international standards

Hygiene: Infants will be measured on the same equipment to exclude error due to inter-equipment variability. To minimize the risk of cross – infection all rings and bracelets and watches should be removed and hands thoroughly washed. Measuring instruments and work surfaces should be cleaned with alcohol wipes, before infants are touched. The tape measure and Holtain calipers should also be wiped before and after every use.

STANDARD OPERATING PROCEDURE 2

Title – Blood Protocol

Author: Dr Ayoola

Implementation 22/10/07

Review Date: 22/10/08

Version Number 02

Purpose:
The purpose of this Standard Operating Procedure is to ensure that all staff carry out all blood sampling procedures consistently and maintain high standard.

Scope
This SOP covers all research staff who are working in the laboratory.

Applicability:
This SOP must be used in conjunction with the study protocol.

Methods

MOTHERS

Booking / Visit 1

Tests Required

- 2ml of blood in EDTA (purple capped) tube will be collected for both Haemoglobin electrophoresis and Full Blood Count.
- Blood film for MP will be taken.

Visit 2

- Collect 6ml of blood into lithium heparin (green capped) tube for Insulin, Lipids, TNF determination.
- Collect 2ml of blood into fluoride oxalate (grey capped) tube for fasting glucose analysis

Visit 3

- PCV and Blood film for MP

Visit 4

- PCV and Blood film for MP

Visit 5 / Delivery of baby

- PCV and Blood film for MP

CHILDREN

At Delivery

- Put 6ml of cord blood into lithium heparim (green-capped) tube and put on ice immediately for Insulin, Lipids, IGFI, IGFBPI, CRP and TNF determination.
- 2ml of cord blood in EDTA (purple-capped) tube will be collected for Full Blood Count and Blood Film for Malaria Parasite
- 2ml of cord blood in EDTA (purple-capped) will be collected for DNA analysis and put on ice immediately
- Intervillious placental blood smear for malaria parasite will be taken.
- Finger prick of mother for PCV and Blood film for MP

3 / 6 month

- PCV and Blood film for MP

9 month

- 2ml of blood in EDTA (purple-capped) tube will be collected for Haemoglobin electrophoresis
- 2ml of blood in EDTA (purple-capped) tube will be collected for DNA analysis.

1 year

- 2ml of blood in EDTA (purple- capped) tube will be collected for Full Blood Count and Blood film for MP
- 6ml of blood will be collected into lithium heparin (green- capped) tube for Insulin, Lipids, IGF-I and TNF determination.
- Collect 2ml of blood into fluoride oxalate (grey capped) tube for fasting glucose analysis

STANDARD OPERATING PROCEDURE 3

Title – Laboratory Protocol

Author: Dr Ayoola

Implementation 22/10/07

Review Date: 22/10/08

Version Number 02

Purpose:
The purpose of this Standard Operating Procedure is to ensure that all staff working in the laboratory carry out all laboratory procedures consistently and maintain high standard.

Scope
This SOP covers all research staff working in the laboratory.

Applicability:
This SOP must be used in conjunction with the study protocol.

Processing Of Samples

Hb electrophoresis sample

- store in the fridge.

TNF, Lipids, Insulin sample

- Centrifuge at 3,000 rpm at 4^{o}C
- Separate the plasma into micro tubes and store at - 80^{o}C

DNA sample

- Store at -80^{o}C

Packed cell volume (PCV)

- Mix the anti coagulated blood carefully by repeated inversion
- Fill the capillary tube with blood up to three quarter length
- Seal the dry end with flame or sealant
- Centrifuge for 5 minutes at RCF-12,000g /5000 rpm using a Microhaematocrit centrifuge
- Read result with the aid of heamatocrit reader

Malaria Parasite Staining Techniques

Density of Parasite And Differential WBC Count

1. Make a good smear of blood on a clear slide (thick and thin)
2. Allow to air dry naturally
3. Fix the thin film with methanol and allow to air dry. Make sure the methanol does not touch the thick film
4. Apply the Giemsa stain and allow to stain for 20min
5. Rinse the stained slide with clean water
6. Place the stained slide in a slide rack in an inverted position and allow to air dry
7. Put the slide under the microscope and observe first with x40 objective to see the distribution of the cells and then with oil immersion objective
8. Count the number of malaria parasite seen with a counter against the total number of WBC seen at different field until at least a total of WBC is 200.

9a. **Calculation of malaria density**

Absolute parasite counts i.e Density (i.e. parasites per microlitre of blood) $= \frac{\text{No of parasites seen}}{200} \times \text{TLC}$

where TLC= subject's total leucocyte count).

9b. **Differential Counting**

- Place a drop of immersion oil on the lower third of the blood film
- Examine under microscope using 100x objective.
- Systematically examine the blood film and count the different, white cells seen in each field (count a total of 100 cells)
- Report the presence of white cells abnormality
- Report the appearance of Red cells
- Comment on platelet number and appearance

APPENDIX FOUR

The effect of maternal health, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children

CASE RECORD FORM (MOTHERS)

Study No _____

SECTION A: DEMOGRAPHIC DATA

1. Hospital/Clinic No. _____ 2. Date ___/___/____

3. Family Name _____ 4. First Name: _____

5. Contact Address _____

_____

_____

6. Telephone Nos: _____

7. Age _____ 8. Date of birth ___/___/____

9. Religion _____

10. Marital Status 1. Single 2. Married 3. Separated/Divorced 4. Other

11.Ethnic group 1. Yoruba 2. Ibo 3. Hausa 4. Other

SECTION B: OBSTETRIC HISTORY

12. LMP _____/____/_____ 13. Expected date of delivery ____/_____/_____

14 Gestational age _____ (weeks)

15. Ultrasound Expected date of delivery _____

16a. Do you know your weight before you became pregnant 1. Yes 2. No

16b. If Yes, what was it? _____

17a. Do you know your own birth weight? 1. Yes 2. No

17b. If yes, what was it? _____

18 No of previous pregnancies _____

19. No of living children _____

20. History of any illnesses in previous pregnancies
a. Hypertension 1. Yes 2. No
b. Diabetes Mellitus 1. Yes 2. No
c. Others 1. Yes 2. No

d. If Yes, mention them _____

21. History of complications in previous pregnancy 1. Yes 2. No If Yes
a. Long labour > 12 hours
b. Excessive bleeding
c. Convulsion not caused by fever
d. Premature birth
e. Low birth weight baby
f. Others specify _____

22 Are you on any regular medication? 1. Yes 2. No

If Yes, list them _____

SECTION C: FAMILY & SOCIAL HISTORY

23a. Type of family? 1. Monogamous 2. Polygamous

If polygamous, how many wives? _____

23b.What position are you? _____

24. How many individuals including yourself live in your house or apartment?

25. How many of those individuals are children?
a. Under the age of 18? _____
b. Under the age of 5? _____

26. Does the father of your baby live in the same household as you?
1. Yes 2. No

27a. Do you know your husband's birth weight? 1. Yes 2. No

27b. If yes, what is it? _____

28a. Do you know your husband's height? 1. Yes 2. No

28b. If yes, what is it? _____

29. Educational status

	Woman	Husband's
1. No formal education		
2. ≤ 6 years of education / Primary school		
3. ≤ 12 years of education / School certificate		
4. ≥ 12 years of education / OND / Grade II Teacher certificate		
5. University graduate / HND		

30. Occupation

	Woman	Husband's
1.Unemployed / Housewife / Students		
2.Petty trader / Labourer / Messenger / Subsistence farmer		
3.Primary school teacher / Driver / Artisan eg carpenter, plumber		
4.Secondary school teacher / Intermediate grade public servant		
5.Senior civil servant / Professional / Manager / Lecturer / Business men		

31a Any family history of medical illnesses? 1. Yes 2. No

31b.If Yes, what are they? 1. Diabetes Mellitus 2. Hypertension 3. Asthma 4. Epilepsy 5. Others- specify _____

31c. Who has the illness in the family? 1. Father 2. Mother 3. Siblings 4. Grandparents 5. Uncle/Aunt 6. Great-grandparents 7.Other-specify

32a. Do you take alcohol? 1. Yes 2. No

32b. If yes, how many drinks of alcohol have you taken in a typical day on average during this pregnancy? 1. None 2.Less than one drink per day
3. 1-2 drinks per day 4. More than 2 drinks per day 5. Don't know

33a. Do you smoke cigarettes? 1. Yes 2. No

33b.If yes, how many sticks of cigarettes have you smoked in a typical day on average during this pregnancy?
1. None 2. 1-10 3. >10 4. Don't know

SECTION D: PREGNANCY HISTORY

34. Was this pregnancy planned? 1. Yes 2. No
35. How would you rate your general health prior to this pregnancy on a scale from 1-10. 1. poor and 10. excellent _____

36a. Have you been taking any medication since you got pregnant?
1. Yes 2. No

36b. If yes, what medication? (Please circle all that apply)

1. Seizure Medication 2. Ferrous tablet 3. Folic acid
4..Multivitamin tablets 5. Antibiotics 6. Other (specify) _____

37. Do you do any of the following at home to prevent malaria in this pregnancy?

	YES	NO
1. Use an insecticide		
2. Sleep under a mosquito net		
3. Use mosquito coil		
4. Sleep in a room with netted windows		
5. Take Sulphadoxine-Pyrimethamine		
6. Take Daraprim ("Sunday-Sunday")		
7. Use any other anti-mosquito measure		

38a. Do you have any medical complaints today? 1. Yes 2. No

38b. If Yes,

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appepite			
d. Vomitting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			
i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

39. Drugs used prior to presentation for the medical complaint

- Duration includes how long ago the drug use and for how long it was used.

*Key to source of drug: 1. Hospital 2. Health centre 3. Chemist 4. Left over at home
5. Obtained from neighbour 6. Traditional healer 7. Other source (specify)

	YES	NO	IF YES, duration (days)	SOURCE
a. Paracetamol				
b. Chloroquine/Nivaquine				
c. Camoquine				
d. Fansidar				
e. Quinine				

f. Halofantrine				
g. Artemether (Paluther)				
h. Coartem				
i. Septrin				
j. Ampicillin/Ampiclox				
k. Native medication				
l. Any other drug (specify)				

40. Which of the following places have you been to before coming here?

	YES	NO
a. Private hospital		
b. Health centre		
c. Chemist		
d. Traditional healer		
e. Church/Mosque		
f. Other (specify)		

1

41. During this pregnancy, did you take any drugs to prevent you from getting malaria? 1. Yes 2. No

42. If Yes which drug?
1. Paracetamol 2. Chloroquine . 3. Fansidar 4. Daraprim
5. Camoquine 6. Coartem 7. Dart 8. Septrin 9. Ampicillin /Ampiclox 10 Native medication 11. Any other drug (specify) _____

43. No of malarial episodes in last pregnancy _____

44. No of malarial episodes so far in this pregnancy _____

46. No of malarial episodes in other pregnancies _____

SECTION E: EXAMINATION

Temperature - _____^{o}C
Pallor 1. Yes 2. No Jaundice 1. Yes 2. No

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				

Abdominal Examination: Fundal Height _____ Lie _____

Position _____ Foetal Heart rate _____

_____ _____ _____
Name of Interviewer Date Checker

SECTION F: INVESTIGATIONS

	Adeoyo	Visit 0	Visit 1	Visit 2	Visit 3
Date					
Parasite count					
PCV					
Hb genotype					
Blood group					
WBC					
Neutrophil					
Lymphocyte					
Monocyte					
Platelet count					
Urine protein					
Urine sugar					

	Visit 4	Visit 5	Visit 6	Visit 7	Delivery
Date					
Parasite Count					
PCV					

Adeoyo: Hb Genotype _____ Blood Group _____

Project: Hb Genotype_____

The effect of maternal health, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children

FOLLOW - UP FORM (MOTHERS)

Study No _____

SECTION A: DEMOGRAPHIC DATA

1. Hospital/Clinic No. _____ 2. Date ___/___/____

3. Family Name _____ *4. First Name:* _____

5. Telephone Nos: _____

6. Age _____ 7. Date of birth ___/___/____

8. LMP _____/____/_____ 9. Expected date of delivery ____/_____/_____

10 Gestational age _____ (weeks)

11a. Do you have any medical complaints today? 1. Yes 2. No

12b. If Yes,

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appepite			
d. Vomitting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			
i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

13. Drugs used prior to presentation for the medical complaint

- Duration includes how long ago the drug use and for how long it was used.

*Key to source of drug: 1. Hospital 2. Health centre 3. Chemist 4. Left over at home 5. Obtained from neighbour 6. Traditional healer 7. Other source (specify)

	YES	NO	IF YES, duration (days)	SOURCE
a. Paracetamol				
b. Chloroquine/Nivaquine				

c. Camoquine				
d. Fansidar				
e. Quinine				
f. Halofantrine				
g. Artemether (Paluther)				
h. Coartem				
i. Septrin				
j. Ampicillin/Ampiclox				
k. Native medication				
l. Any other drug (specify)				

14. Which of the following places have you been to before coming here?

	YES	NO
a. Private hospital		
b. Health centre		
c. Chemist		
d. Traditional healer		
e. Church/Mosque		
f. Other (specify)		

15. No of malarial episodes so far in this pregnancy _____

SECTION E: EXAMINATION

Temperature - _____^{o}C
Pallor 1. Yes 2. No
Jaundice 1. Yes 2. No

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				

Abdominal Examination: Fundal Height _____ Lie _____

Position _____ Foetal Heart rate _____

SECTION F: INVESTIGATIONS

PCV _____ Blood Film for MP _____

_____ _____ _____
Name of Interviewer Date Checker

LABOR AND DELIVERY DATA

Study No _____

1. Hospital/Clinic No. _____ 2. Date ___/___/____

4. Family Name _____ *4. First Name:* _____

6. Age _____ 7. Date of birth ___/___/____

8. LMP _____/____/_____ 9. Ultrasound EDD ____/_____/_____

10 Gestational age _____ (weeks) PLACENTAL WEIGHT _____

11. Date of delivery _____/ _____/ _____

12. Any maternal complications during this pregnancy? 1. Uncomplicated
2. Minor complications not requiring hospitalization
3. Moderate complications with < 1 week hospitalization
4. Severe complications with > 1 week hospitalization

13a. Was lytic cocktail therapy given? 1. Yes 2. No 3. Unknown

13b. If given, indication for lytic therapy 1. Tocolysis 2. Preeclampsia 3. Pregnancy-induced hypertension 4. Chronic hypertension 5.Tocolysis and preeclampsia 6. Unknown

14. If yes, did mother receive lytic cocktail treatment on the day of delivery? 1. Yes 2. No 3. Unknown
15a. Other maternal medications prior to delivery: 1. Yes 2. No
15b. If Yes, what are they? 1. Indomethacin 2. Dexamethasone
3. Antibiotics 4. Other 5. Unknown

16. Was labour induced with pitocin? 1.Yes 2.No 3.Unknown

17. Mode of delivery 1. Spontaneous vaginal 2. Assisted vaginal (forceps, vacuum) 3.Caesarean section after onset of labour 4. Caesarean section without labour

18. Complications during labour. 0. None 1. Failure to Progress 2. Cephalopelvic Disproportion 3. Fetal Distress 4. Infection 5. Placental abruption 6. Other
7. Unknown

_____ _____

Name of Nurse Name of Lab Officer

APPENDIX FIVE

CASE RECORD FORM (CHILDREN)

Mother's Study No _____ Baby's Study No _____

SECTION A: DEMOGRAPHIC DATA/ BIRTH HISTORY

1. Hospital/Clinic No. _____ 2. Date ___/___/____

3. Family Name _____ 4. First Name: _____

5. Date of birth ___/___/____ 6. Time of birth _____ (24 hours)

7. Sex 1. Male 2. Female 10. Birth weight (kg) _____

8. Apgar Score at 1min _____ 9. Apgar Score at 5 min _____

11. Gestational Age by USS (weeks)_____

12. Type of Feeding at time of discharge 1. Exclusive Breastfeeding
2. Formula feeding 3. Combination

13. Neonatal Diagnoses _____

SECTION B: EXAMINATION

Temperature _____^{o}C

Pallor 1. Yes 2. No Jaundice 1. Yes 2. No

OFC (cm) _____ MUAC (cm) _____

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				
Subscapular SF (cm)				
Triceps SF (cm)				
Biceps SF (cm)				
Suprailiac SF (cm)				

The effect of maternal health, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children

3 MONTH FOLLOW UP

SECTION A: DEMOGRAPHIC DATA

1. Mother's Study No: _____ 2. Baby Study No: _____
3. Date First Seen ___/___/____ 4. Date ___/___/____
5. Interval since last visit_____
6. Family Name _____ 7. First Name _____
8. Mother's Name _____
9. Address _____
10. Telephone Nos _____
11. Age of Child (months) _____ 12. Date of Birth ___/___/____
13. Sex 1. Male 2. Female

14a. Was Child seen when visit due 1. Yes 2. No

14b. If No Why? a. Travelled b. Did not come for immunization c. No longer interested in the study d. Other reasons (specify) _____

14c. Has your child received any immunisations? 1. Yes 2. No

14d. If Yes, a. BCG b. OPV0 c. DPT1 d. OPV1

SECTION B: FAMILY AND SOCIAL HISTORY

15a.Type of Family 1. Monogamous 2. Polygamous

15b. If Polygamous, how many wives? _____

15c. What position is the mother? _____

15d. How many children are in the family? _____

15e. What position is this child? _____

SECTION C: FEBRILE ILLNESS & OTHER MEDICAL HISTORY

16a. Has your child been on any regular drugs since delivery? 1. Yes 2. No

16b. If Yes, what are they? 1. Pharmadec/Abidec 2. Vitamin C

3. Others (specify) _____

17. Do you do any of the following at home (for the child) to prevent malaria

	YES	NO
1. Use an insecticide		
2. Sleep under a mosquito net		
3. Use mosquito coil		
4. Sleep in a room with netted windows		
5. Take Sulphadoxine-Pyrimethamine		
6. Take Daraprim (“Sunday-Sunday”)		
7. Use any other anti-mosquito measure		

18a.Did the child have any episode of fever since last visit? 1. Yes 2. No

18b.If Yes, How many episodes? _____

18c.How long ago were these? _____

19a.Did the child have any other symptoms with or without fever?1. Yes 2. No

19b.If Yes, what are they?

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appetite			
d. Vomiting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			
i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

20a. Has the child been treated? 1. Yes 2. No

20b. If Yes, what

1. Antimalarial –(which type) _____
2. Antipyretics – (which type) _____
3. Antibiotics – (which type) _____
4. Others – (specify)

21a.Was BF for MP done 1. Yes 2. No

21b.MP results _____ 15c.PCV _____

22a.Any other investigation done? 1. Yes 2. No

22b.If Yes specify test and results _____

23. Final Diagnosis (1) Malaria (2) Others specify _____

24a.Is your child well today? 1. Yes 2. No

24b.If No, what are the symptoms?

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appepite			
d. Vomitting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			
i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

25a. Have you given any treatment at home? 1. Yes 2. No

25b. If Yes, Drugs used prior to presentation for the medical complaint

- Duration includes how long ago the drug use and for how long it was used.

*Key to source of drug: 1. Hospital 2. Health centre 3. Chemist 4. Left over at home 5. Obtained from neighbour 6. Traditional healer 7. Other source (specify)

	YES	NO	IF YES, duration (days)	SOURCE
a. Paracetamol				
b. Chloroquine/Nivaquine				
c. Camoquine				
d. Fansidar/Amalar/Maloxim				
e. Quinine				
f. Halofantrine				
g. Artesunnate				
h. Coartem				
i. Septrin				
j. Ampicillin/Ampiclox				
k. Native medication				
l. Any other drug (specify)				

26. Did you take child to any of the following places?

	YES	NO
a. Private hospital		
b. Health centre		
c. Chemist		
d. Traditional healer		

e. Church/Mosque		
f. Other (specify)		

SECTION D: EXAMINATION

27. Pallor 1. Yes 2. No 28. Jaundice 1. Yes 2. No

29. OFC (cm) _____ 30. MUAC (cm) _____

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				
Subscapular SF (cm)				
Triceps SF (cm)				
Biceps SF (cm)				
Suprailiac SF (cm)				

31. **Mothers Name:**

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				

SECTION E: INVESTIGATIONS

34. PCV _____

35. BF for MP result _____

The effect of maternal health, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children

9 MONTH FOLLOW UP

SECTION A: DEMOGRAPHIC DATA

2. Mother's Study No: _____ 2. Baby Study No: _____

3. Date First Seen ___/___/____ 4. Date ___/___/____

5. Interval since last visit_____

6. Family Name _____ 7. First Name _____

8. Mother's Name _____

9. Address _____

10. Telephone Nos _____

11. Age of Child (months) _____ 12. Date of Birth ___/___/____

13. Sex 1. Male 2. Female

14a. Was Child seen when visit due 1. Yes 2. No

14b. If No Why? a. Travelled b. Did not come for immunization c. No longer interested in the study d. Other reasons (specify) _____

14c. Has your child received any immunisations? 1. Yes 2. No

14d. If Yes, a. BCG & OPV0 b. DPT1 & OPV1 c. DPT2 & OPV2 d. DPT3 & OPV3 e. Measles f. Hepatititis 1,2,3

g. Yellow fever h. Others _____

SECTION B: FAMILY AND SOCIAL HISTORY

15a.Type of Family 1. Monogamous 2. Polygamous

15b. If Polygamous, how many wives? _____

15c. What position is the mother? _____

15d. How many children are in the family? _____

15e. What position is this child? _____

Educational status

	Mother	**Father**
1. No formal education		
2. ≤ 6 years of education / Primary school		
3. ≤ 12 years of education / School certificate		
4. ≥ 12 years of education / OND / Grade II Teacher certificate		

5. University graduate / HND		

30. Occupation

	Mother	**Father**
1.Unemployed / Housewife / Students		
2.Petty trader / Labourer / Messenger / Subsistence farmer		
3.Primary school teacher / Driver / Artisan eg carpenter, plumber		
4.Secondary school teacher / Intermediate grade public servant		
5.Senior civil servant / Professional / Manager / Lecturer / Business men		

SECTION C: FEBRILE ILLNESS & OTHER MEDICAL HISTORY

16a. Has your child been on any regular drugs since delivery? 1. Yes 2. No

16b. If Yes, what are they? 1. Pharmadec/Abidec 2. Vitamin C

3. Others (specify) _____

17. Do you do any of the following at home (for the child) to prevent malaria

	YES	NO
1. Use an insecticide		
2. Sleep under a mosquito net		
3. Use mosquito coil		
4. Sleep in a room with netted windows		
5. Take Sulphadoxine-Pyrimethamine		
6. Take Daraprim (“Sunday-Sunday”)		
7. Use any other anti-mosquito measure		

18a.Did the child have any episode of fever since last visit? 1. Yes 2. No

18b.If Yes, How many episodes? _____

18c.How long ago were these? _____

19a.Did the child have any other symptoms with or without fever?1. Yes 2. No

19b.If Yes, what are they?

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appetite			
d. Vomiting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			

i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

20a. Has the child been treated? 1. Yes 2. No

20b. If Yes, what

5. Antimalarial –(which type) _____
6. Antipyretics – (which type) _____
7. Antibiotics – (which type) _____
8. Others – (specify) _____

21a. Was BF for MP done ? 1. Yes 2. No

21b.MP results _____ 15c.PCV _____

22a. Any other investigation done? 1. Yes 2. No

22b.If Yes, specify test and results _____

23. Final Diagnosis (1) Malaria (2) Others specify _____

24a.Is your child well today? 1. Yes 2. No

24b.If No, what are the symptoms?

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appepite			
d. Vomitting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			
i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

25a. Have you given any treatment at home? 1. Yes 2. No

25b. If Yes, Drugs used prior to presentation for the medical complaint

- Duration includes how long ago the drug use and for how long it was used.

*Key to source of drug: 1. Hospital 2. Health centre 3. Chemist 4. Left over at home 5. Obtained from neighbour 6. Traditional healer 7. Other source (specify)

	YES	NO	IF YES, duration (days)	SOURCE
a. Paracetamol				
b. Chloroquine/Nivaquine				
c. Camoquine				
d. Fansidar/Amalar/Maloxim				
e. Quinine				
f. Halofantrine				
g. Artesunnate				
h. Coartem				
i. Septrin				
j. Ampicillin/Ampiclox				
k. Native medication				
l. Any other drug (specify)				

26. Did you take child to any of the following places?

	YES	NO
a. Private hospital		
b. Health centre		
c. Chemist		
d. Traditional healer		
e. Church/Mosque		
f. Other (specify)		

SECTION D: NUTRITIONAL/ WEANING HISTORY

27. What was the main way that you first fed your baby?

a. breastfeeding b. cup & spoon c. both/combination

28. When did you decide to use these feeding methods?

a. before the baby was born b. after the baby was born

29. Were you given any information on feeding methods before delivery of your baby?

1. Yes 2. No

29b. If Yes, who was the main person who gave you this information?

a. nurse b. doctor c. friends d. mother/mother-in-law

e. other (specify)_____

30. Which feeding methods did they encourage?

a. breastfeeding b. bottlefeeding c. combination of breast & bottle

d. cup & spoon e. other (please specify) _____

31. How old was your baby when you began breastfeeding?

a. immediately after delivery b. within a few hours of delivery

c. other (please specify) _____

32. How long have you breastfed for? _____ (months)

33. Have you stopped breastfeeding? 1. Yes 2. No

33b. If Yes, please state reasons for stopping breastfeeding

a. Inconvenience e.g. working mother b. difficulties in breastfeeding i.e. not enough milk c. artificial milk is as good as or better than breast milk

d. less fashionable to breast feed e. Embarrassing-lack of privacy

f. other (please specify) _____

34. Have you introduced weaning diet? 1. Yes 2. No

34b. If Yes, how old was your baby then? _____ months

35. Please state reasons why you started weaning diet

a. Resumed at work (working mother) b. breastfeeding no longer adequate

c. child is 6 months or older d. other (please specify) _____

36. Does your child currently have: a. a bottle b. dinky feeder

c. feeding cup d. combination (please state) _____

e. other (specify) _____

37. Do you give pap regularly to your baby? 1. Yes 2. No

37b. If Yes, what do you add to the pap? a. nothing b. soya milk

c. formula milk d. weaning milk eg peak 1,2,3 e. full cream milk (peak, nunu etc) f. crayfish g. sugar h. other (specify) _____

38. Did anyone influence the types of food you give your baby? 1. Yes 2. No

38b. If Yes, who influenced you? a. Mother/mother-in-law b. Nurse

c. Doctor d. Friend e. other (please specify) _____

39. At what age did you introduce your child to solids food _____ months

40. What was the first food given? a. baby rice b. cerelac (maize-based)

c. golden morn d. white or red pap e. family food e.g. amala

f. other (please specify) _____

41. What was the first food given? a. manufactured baby foods

b. homemade baby foods c. Both

42. If you use manufactured baby foods currently, are they: a. sweet

b. savoury c. all of the above d. other (specify) _____

43. Did you add any of the following when preparing solid food for your baby?

a. sugar b. salt c. spices d. all of the above e. none of the above f. other (specify) _____

44. Do you give finger foods (e.g. banana, carrot, etc)? 1. Yes 2. No

44b. If Yes, (please specify) _____

45. Do you give your baby sweets or chocolates 1. Yes 2. No

46. Do you give vitamin drops to your baby? 1. Yes 2. No

47. At what age did you begin to give vitamin drops? _____ weeks

48. What type of vitamin drops do you give? (brand name) _____

49. At what age did the baby cut his/her teeth? _____months

50. Do you clean your baby's teeth with toothpaste? 1. Yes 2. No

51. Did your baby have PWV measured at birth? 1. Yes 2.No

52. Did your baby have PWV measured at 3 month / 6 month visit? 1. Yes 2. No

SECTION E: EXAMINATION

53. Pallor 1. Yes 2. No 54. Jaundice 1. Yes 2. No

54. OFC (cm) _____ 56. MUAC (cm) _____

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				
Subscapular SF (cm)				
Triceps SF (cm)				
Biceps SF (cm)				
Suprailiac SF (cm)				

57. Mothers Name:

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				

SECTION E: INVESTIGATIONS

58. PCV _____

59. BF for MP result _____

60. DNA Sample collected _____

The effect of maternal health, fetal size and early childhood growth on cardiovascular (CVS) development in Nigerian children

12 MONTHS FOLLOW UP

SECTION A: DEMOGRAPHIC DATA

3. Mother's Study No: _____ 2. Baby Study No: _____

3. Date First Seen ___/___/____ 4. Date ___/___/____

5. Interval since last visit_____

6. Family Name _____ 7. First Name _____

8. Mother's Name _____

9. Address _____

10. Telephone Nos _____

11. Age of Child (months) _____ 12. Date of Birth ___/___/____

13. Sex 1. Male 2. Female

14a. Was Child seen when visit due 1. Yes 2. No

14b. If No Why? a. Travelled b. Did not come for immunization c. No longer interested in the study d. Other reasons (specify) _____

14c. Has your child received any immunisations? 1. Yes 2. No

14d. If Yes, a. BCG & OPV0 b. DPT1 & OPV1 c. DPT2 & OPV2 d. DPT3 & OPV3 e. Measles f. Hepatititis 1,2,3

g. Yellow fever h. Others _____

SECTION B: FAMILY AND SOCIAL HISTORY

15a.Type of Family 1. Monogamous 2. Polygamous

15b. If Polygamous, how many wives? _____

15c. What position is the mother? _____

15d. How many children are in the family? _____

15e. What position is this child? _____

Educational status

	Mother	**Father**
1. No formal education		
2. ≤ 6 years of education / Primary school		
3. ≤ 12 years of education / School certificate		
4. ≥ 12 years of education / OND / Grade II Teacher certificate		

5. University graduate / HND		

30. Occupation

	Mother	**Father**
1.Unemployed / Housewife / Students		
2.Petty trader / Labourer / Messenger / Subsistence farmer		
3.Primary school teacher / Driver / Artisan eg carpenter, plumber		
4.Secondary school teacher / Intermediate grade public servant		
5.Senior civil servant / Professional / Manager / Lecturer / Business men		

SECTION C: FEBRILE ILLNESS & OTHER MEDICAL HISTORY

16a. Has your child been on any regular drugs since delivery? 1. Yes 2. No

16b. If Yes, what are they? 1. Pharmadec/Abidec 2. Vitamin C

3. Others (specify) _____

17. Do you do any of the following at home (for the child) to prevent malaria

	YES	NO
1. Use an insecticide		
2. Sleep under a mosquito net		
3. Use mosquito coil		
4. Sleep in a room with netted windows		
5. Take Sulphadoxine-Pyrimethamine		
6. Take Daraprim (“Sunday-Sunday”)		
7. Use any other anti-mosquito measure		

18a.Did the child have any episode of fever since last visit? 1. Yes 2. No

18b.If Yes, How many episodes? _____

18c.How long ago were these? _____

19a.Did the child have any other symptoms with or without fever?1. Yes 2. No

19b.If Yes, what are they?

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appetite			
d. Vomiting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			

i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

20a. Has the child been treated? 1. Yes 2. No

20b. If Yes, what

9. Antimalarial –(which type) _____
10. Antipyretics – (which type) _____
11. Antibiotics – (which type) _____
12. Others – (specify) _____

21a.Was BF for MP done 1. Yes 2. No

21b.MP results _____ 15c.PCV _____

22a.Any other investigation done? 1. Yes 2. No

22b.If Yes specify test and results _____

23. Final Diagnosis (1) Malaria (2) Others specify_____

24a.Is your child well today? 1. Yes 2. No

24b.If No, what are the symptoms?

	YES	NO	IF YES, duration in days
a. Fever			
b. Chills/Rigors			
c. Loss of appepite			
d. Vomitting			
e. Diarrhoea			
f. Jaundice			
g. Pallor			
h. Cough			
i. Catarrh/Nasal discharge			
j. Difficult/Fast breathing			
k. Weakness/Lethargy			
i. Body pains/aches			
m. Convulsion			
n. Irritability			
o. Clouded consciousness			
p. Others (specify)			

25a. Have you given any treatment at home? 1. Yes 2. No

25b. If Yes, Drugs used prior to presentation for the medical complaint

- Duration includes how long ago the drug use and for how long it was used.

*Key to source of drug: 1. Hospital 2. Health centre 3. Chemist 4. Left over at home 5. Obtained from neighbour 6. Traditional healer 7. Other source (specify)

	YES	NO	IF YES, duration (days)	SOURCE

a. Paracetamol				
b. Chloroquine/Nivaquine				
c. Camoquine				
d. Fansidar/Amalar/Maloxim				
e. Quinine				
f. Halofantrine				
g. Artesunnate				
h. Coartem				
i. Septrin				
j. Ampicillin/Ampiclox				
k. Native medication				
l. Any other drug (specify)				

26. Did you take child to any of the following places?

	YES	NO
a. Private hospital		
b. Health centre		
c. Chemist		
d. Traditional healer		
e. Church/Mosque		
f. Other (specify)		

SECTION D: NUTRITIONAL/ WEANING HISTORY

43. How long have you breastfed for? _____ (months)

44. Have you stopped breastfeeding? 1. Yes 2. No

28b. If Yes, please state reasons for stopping breastfeeding

a. Inconvenience e.g. working mother b. difficulties in breastfeeding i.e. not enough milk

c. artificial milk is as good as or better than breast milk

d. less fashionable to breast feed e. Embarrassing-lack of privacy

f. other (please specify) _____

45. Have you introduced weaning diet? 1. Yes 2. No

29b. If Yes, how old was your baby then? _____ months

46. Please state reasons why you started weaning diet

a. Resumed at work (working mother) b. breastfeeding no longer adequate

c. child is 6 months or older d. other (please specify) _____

47. Does your child currently have: a. a bottle b. dinky feeder

c. feeding cup d. combination (please state) _____

e. other (specify) _____

48. Do you give pap regularly to your baby? 1. Yes 2. No

32b. If Yes, what do you add to the pap? a. nothing b. soya milk

c. formula milk d. weaning milk eg peak 1,2,3 e. full cream milk (peak, nunu etc) f. crayfish g. sugar h. other (specify) _____

49. Did anyone influence the types of food you give your baby? 1. Yes 2. No

33b. If Yes, who influenced you? a. Mother/mother-in-law b. Nurse

c. Doctor d. Friend e. other (please specify) _____

50. At what age did you introduce your child to solids food _____ months

51. What was the first food given? a. baby rice b. cerelac (maize-based)

c. golden morn d. white or red pap e. family food e.g. amala

f. other (please specify) _____

52. What was the first food given? a. manufactured baby foods

b. homemade baby foods c. Both

53. If you use manufactured baby foods currently, are they: a. sweet

b. savoury c. all of the above d. other (specify) _____

54. Did you add any of the following when preparing solid food for your baby?

a. sugar b. salt c. spices d. all of the above e. none of the above f. other (specify) _____

55. Do you give finger foods (e.g. banana, carrot, etc)? 1. Yes 2. No

39b. If Yes, (please specify) _____

56. Do you give your baby sweets or chocolates 1. Yes 2. No

57. At what age did baby attain neck control? _____

58. At what age did baby sit without support? _____

59. At what age did baby crawl _____

60. At what age did baby stand without support? _____

61. At what age did baby start walking? _____

62. At what age did baby say the first word? _____

63. At what age did the baby cut his/her teeth? _____months

64. Do you clean your baby's teeth with toothpaste? 1. Yes 2. No

65. Did your baby have PWV measured at birth? 1. Yes 2.No

66. Did your baby have PWV measured at 3 month / 6 month visit? 1. Yes 2. No

SECTION E: EXAMINATION

67. Pallor 1. Yes 2. No 54. Jaundice 1. Yes 2. No

68. OFC (cm) _____ 56. MUAC (cm) _____

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				
Subscapular SF (cm)				
Triceps SF (cm)				
Biceps SF (cm)				
Suprailiac SF (cm)				

61. Mothers Name:

Measurement	1st	2nd	3rd	Initials
Weight (kg)				
Height (cm)				
Blood pressure (mmHg)				

SECTION E: INVESTIGATIONS

60. PCV _____

61. BF for MP result _____

62. DNA Sample collected _____

APPENDIX SIX

STUDY SITE – ADEOYO MATERNITY HOSPITAL, IBADAN, NIGERIA