A LONGITUDINAL ULTRASOUND STUDY OF FETAL GROWTH AND INTRAUTERINE GROWTH RESTRICTION IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

by
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ABSTRACT
SARAH HENRY LANDIS: A longitudinal ultrasound study of fetal growth and intrauterine growth restriction in Kinshasa, Democratic Republic of Congo
(Under the direction of Steven Meshnick)

Each year, 24% of births in resource poor countries are small-for-gestational age (SGA). Most SGA infants suffer from intrauterine growth restriction (IUGR); a pathologic process characterized by insufficient transfer of nutrients and oxygen to the fetus and impaired fetal growth. In resource poor countries, IUGR is frequently due to malaria or maternal under-nutrition. This dissertation addresses clinically important questions concerning the pathogenesis of malaria infection *in utero* and the identification of IUGR in resource poor settings.

The data source is a prospective, longitudinal ultrasound study of 182 pregnant women conducted in Kinshasa, Democratic Republic of Congo between May 2005 and May 2006. Women participated in monthly follow-up visits during which malaria, maternal anthropometrics, and ultrasound estimated fetal weight (EFW) were measured.

We estimated the effect of malaria on the risk of IUGR, and assessed whether maternal under-nutrition modified this relationship. Data from 178 women and 758 ultrasounds were included. IUGR was defined as EFW below the 10^{th} percentile of a standardized fetal weight nomogram. Log-binomial models using generalized estimating equations were fitted separately for malaria and maternal anthropometric exposures and including a product interaction term between them. A single incident malaria infection was not significantly associated with IUGR (Risk ratio (RR)=1.2, 95% confidence interval (CI): 0.7, 2.2); women with ≥3 episodes were at increased risk (RR=2.3, 95% CI: 0.8, 6.3). The effect of malaria was significantly stronger among under-nourished women. Prompt treatment of antenatal

malaria infections may prevent IUGR, especially in under-nourished women.

We developed a fetal size nomogram for Congo using data from 144 women with certain gestational dates and 755 ultrasound scans. A linear mixed effect model was fitted for EFW as a function of gestational age that incorporated random effects for the intercept and slope. Reference intervals were derived from this model and compared with intervals derived from industrialized countries. The 50^{th} centile EFW for Congo fetuses was consistently lower than fetuses born in industrialized populations. Comparison of the outer centiles showed inconsistent patterns, owing primarily to differing statistical techniques. This fetal size nomogram should improve diagnosis of IUGR in resource poor settings with endemic malaria.

To Melvin Fox Landis, Jr.,

who would have been so proud

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LIST OF ABBREVIATIONS

AC	Abdominal circumference
BPD	Biparital diameter
BMI	Body mass index
CI	Confidence interval
CSA	Chondroitin sulphate A
EFW	Estimated fetal weight
FL	Femur length
HC	Head circumference
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
IRBC	Infected red blood cell
IUGR	Intrauterine growth restriction
LBW	Low birth weight
LMP	Last menstrual period
MUAC	Mid upper arm circumference
PTD	Preterm delivery
RR	Risk ratio
SES	Socioeconomic status
SGA	Small for gestational age
SD	Standard deviation
SP	Sulfadoxine Pyrimethamine
VSA	Variant surface antigen

CHAPTER 1.
BACKGROUND AND SIGNIFICANCE

Each year, 24% of births in resource poor countries are small-for-gestational age (SGA).[1] Most SGA infants suffer from intrauterine growth restriction (IUGR); a pathologic process characterized by insufficient transfer of nutrients and oxygen to the fetus and impaired fetal growth. In resource poor countries, IUGR is frequently due to malaria or maternal under-nutrition.[2] The specific aims of this dissertation address two clinically important questions concerning the pathogenesis of malaria infection *in utero* and the identification of IUGR in resource poor settings.

> **Specific Aim 1: Describe the association between maternal malaria infection and intrauterine growth restriction in Kinshasa, Democratic Republic of Congo and determine if maternal nutritional status modifies this relationship.**
>
> **Specific Aim 2: Utilize prospectively collected ultrasound data to develop a fetal size for gestational age nomogram for a resource poor population with high malaria prevalence.**

Overview of intrauterine growth restriction

Overview: Normal fetal growth and development can be divided into three physiologic stages: i) cell replication and proliferation, also know as the hyperplastic phase; ii) cell migration and aggregation to form tissue and rudimentary organs; and iii) increase in cell size and formation of functional organ structures, also known as the hypertrophic phase.[3] Thus in early pregnancy, very high mitotic activity (DNA replication) is paired with very little change in mass, while in late pregnancy; mitosis slows with a coincident rapid gain in weight. As a result, genetic factors most influence fetal growth during the first half of pregnancy, and hormonal or environmental factors dominate later in pregnancy.[4]

In a healthy pregnancy, fetuses normally gain about 5 grams per day from 14-15 weeks,

10 grams per day by 20 weeks, and 30-35 grams per day from 32-36 weeks. During the last month of pregnancy, the growth rate decreases and then levels off around the 40th week of gestation.[5]

The placenta plays a key role in fetal growth. During normal placental formation, trophoblasts from the fetus invade the uterine endometrial lining and obliterate the muscular walls of the uterine spiral arteries. The spiral arteries convert to maximally dilated uteroplacental arteries allowing development of a high volume, low resistance circulation.[6] During the second and third trimesters, there is also a proliferation in the number of villi and small vessels in the placental vascular bed. As the number of vessels increases, placental vascular resistance decreases and blood flow volume through the umbilical artery increases.[7] This increase in blood flow volume through the uterine and umbilical arteries is necessary to meet the increasing nutritional demands of the rapidly-growing fetus in late pregnancy.[6] Pathophysiological processes that inhibit trophoblastic invasion, obstruct blood flow, or decrease the number of umbilical tertiary villious arteries and arteriols may result in decreased delivery of oxygen and nutrients to and from the fetus.[8] This, in turn, will likely impair fetal growth.[9,10,11,12,13,14]

Impaired fetal growth manifests as decreased fetal body mass and infant low birth weight (LBW, defined as birth weight less than 2500 grams).[15] A diagnosis of LBW indicates three possible fetal conditions, one of which is normal and two that are pathologic in nature. The normal condition refers to an infant that is constitutionally (or genetically) small and otherwise healthy. The pathologic conditions are preterm delivery (PTD) or delivery before 37 completed weeks of gestation, and small for gestational age (SGA) or low attained birth weight for a given gestational age at delivery. Most SGA infants suffer from intrauterine growth restriction (IUGR) which occurs when there is sub-optimal transfer of nutrients and oxygen to the fetus *in utero* resulting in impaired growth of fetal organs and tissues.

Risk factors for IUGR: Risk factors associated with IUGR can be categorized into three

broad groups: fetal factors, maternal factors, and uteroplacental factors.

***Fetal factors*:** Fetal causes of IUGR include chromosomal disorders such as trisomy 13, 18 and 21, viral infections such as rubella, toxoplasmosis or cytomegalovirus, multiple pregnancy, fetal gender and birth order. Chromosomal abnormalities and congenital malformations are estimated to be responsible for approximately 20% of IUGR while infections are estimated to account for 5-10% of all IUGR cases.[16]

***Maternal factors**:* Maternal factors often lead to IUGR through pathways that result in decreased oxygen-carrying capacity secondary to maternal vascular disease (i.e., diabetes, hypertension, or renal disease) or placental damage resulting from maternal disease or environmental exposure (i.e., smoking/drugs, thrombophilia, various autoimmune diseases or malaria).[17] Maternal vascular disease is estimated to account for 25-30% of all IUGR.[16] Maternal under-nutrition and micronutrient deficiency may also lead to IUGR if the availability of substrates for fetal growth is deprived.[15]

Uteroplacental factors: Uteroplacental factors may manifest as abnormal placental size, morphology or function. When IUGR is caused by placental abnormalities, the growth aberration is usually the consequence of decreased delivery of nutritional substrates or oxygen to the fetus. Small placental size, placental previa, and pathologic features such as placental abruption, circumvallate placenta, chorioangioma and vaginal bleeding of unknown etiology have been associated with IUGR.[16,18,19]

Types of IUGR: IUGR is often categorized as being symmetrical or asymmetrical, although growth restriction can fall anywhere on a spectrum between these two extremes.[20] Symmetrical growth restriction accounts for about 10% of all IUGR and is characterized by smaller growth than expected of all fetal biometry, showing equally poor growth of the head, abdomen and short and long bones. Alternatively, asymmetrical growth describes infants whose body weight is low, with relative preservation of the head, which is close to or being normally sized. This is thought to be due to the brain-sparing effect that results when the

fetus is challenged with decreased nutritional reserves, and redistributes blood flow to the brain, heart, adrenals, and placenta, resulting in diminished relative flow to the bone marrow, muscles, soft tissue and liver.

The pattern of growth inhibition often depends on the timing of the insult.[16] Insults that occur early in pregnancy or that result in an overall reduction of cellular hyperplasia such as a genetic abnormality, teratogen exposure (i.e., tobacco, alcohol, drugs) or early viral infection, are more associated with symmetrical IUGR. Conversely, factors that arise later in pregnancy (i.e., maternal vascular disease or gestational diabetes) result in uteroplacental insufficiency and affect fat deposition and the size and protein content of the cells. This results in brain sparing and an asymmetrical growth pattern.

Perinatal and neonatal complications of IUGR: IUGR fetuses have higher rates of perinatal morbidity and mortality and are at increased risk of sudden infant death syndrome and stillbirth. In the early neonatal period, IUGR infants are at increased risk of hypoglycemia, hypocalcemia, thrombocytopenia, temperature instability, and renal failure[2,21] and during childhood, they are more likely to have poor cognitive development and neurological impairment.[22]

Identification of intrauterine growth restriction: In the early 1960's Lubchenco and colleagues demonstrated an increased risk of perinatal mortality among infants that were less than the 10th percentile of birth weight for their gestational age.[23] Further, the risk of both perinatal mortality and morbidity increases rapidly as birth weight for age falls from the 10th to the 1st percentile. These findings served as a foundation for the techniques used today to characterize fetuses as IUGR. The most commonly used method is to compare either a single fetal biometric indicator, or estimated fetal weight (EFW), against a standardized fetal weight-for-gestational age nomogram.

Individual biometric measurements are less commonly used and the sensitivity of a single measurement to diagnosis IUGR is generally lower than using EFW. Of the single biometric

measurements, abdominal circumference (AC) has the best correlation with IUGR.[24] AC reflects hepatic size and disposition of subcutaneous fat, both of which are diminished in the growth restricted fetus. Sensitivity estimates for a cutoff of AC below the 25th centile are 83-86%, specificity ranges from 79-80%.[25] The sensitivity of this measure can be improved to over 95% if a 2.5% centile is used.[26] Utilization of biparietal diameter (BPD) alone is not recommended as these measurements correlate poorly with IUGR; sensitivity estimates for BPD range from 43.8% to 100% with most values between 50% and 60%.[25] BPD can be challenging to measure due to factors including fetal lie and variation in fetal head shape. Head circumference (HC) gives slightly better positive predictive value than BPD alone as it is not influenced as much fetal head shape. Femur length (FL) alone is also not recommended because femur growth is affected early in symmetrical growth restriction but late in asymmetrical IUGR.

Algorithms that combine HC, BPD, AC and FL to calculate EFW in grams are the most accurate predictors of IUGR and birth weight.[27] IUGR assessment using estimated fetal weight has been found to be accurate to within 10% of fetal weight in about 90% of pregnancies.[24] A cutoff of estimated fetal weight <10th percentile for a standardized nomogram has been shown to have a sensitivity in the range of 87%-90%, with specificities of 80%-87%.[25]

Specific Aim 1: Describe the association between maternal malaria infection and intrauterine growth restriction in Kinshasa, Democratic Republic of Congo and determine if maternal nutritional status modifies this relationship

Rationale: In areas of stable malaria transmission, non-pregnant adults have developed a sufficient level of acquired immunity such that *P. falciparum* infection does not often result in fever or other clinical symptoms. However, pregnant women are more susceptible to malaria infection and its disease consequences, especially in their first and second pregnancies. A review by Steketee of 34 studies of malaria infection, adverse pregnancy outcomes and

pregnancy associated conditions (such as anemia) showed that *P. falciparum* malaria in pregnancy consistently contributed to LBW through both PTD and IUGR.[28] Nine studies reported risk ratios for LBW ranging from 1.4 to 1.8, with population attributable risks of 8 to 14%. Five studies reported results separately for preterm-LBW and IUGR-LBW. In these studies, maternal malaria infection accounted for approximately 8 to 36% of preterm-LBW and 13-70% for IUGR-LBW. Malaria is also a major cause of anemia in sub-Saharan Africa which is independently a risk factor for LBW.[29,30]

In the developing world, it has long been recognized that childhood malnutrition and its sequelae influences susceptibility to malaria infection and can influence the severity of malaria associated morbidity and mortality.[31-33] Repercussions of childhood malnutrition and malaria infection, such as stunting and low young adult BMI place reproductive age women at increased risk of poor birth outcome. In women who have become pregnant, the joint effects of these conditions may act on similar physiologic pathways to reduce uteroplacental blood flow. Further, these conditions both contribute to maternal anemia, which independently contributes to LBW through decreased maternal-fetal oxygen transfer.[34,35] Thus, in pregnant women, it is likely that maternal nutritional status will modify the relationship between malaria and fetal growth.

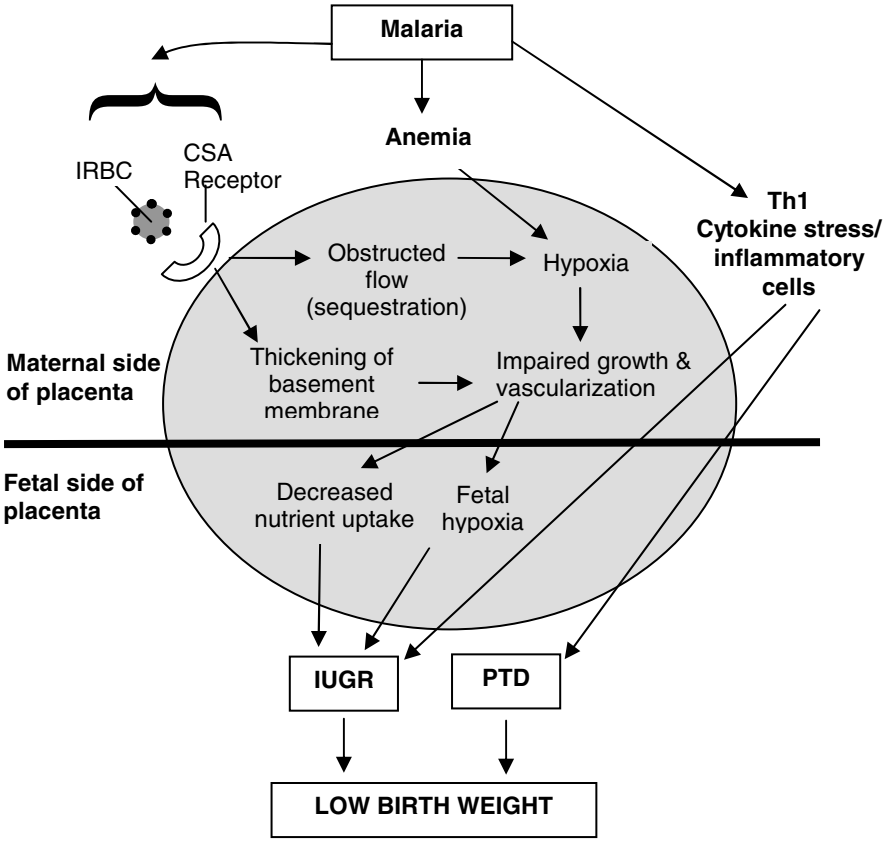
Biologic mechanisms linking malaria to poor birth outcome: The unique relationship between malaria infection and pregnancy results from the inability of pregnant women to mount an adequate immune response against *P. falciparum* parasites. The malaria parasite life cycle has multiple stages occurring in both the human host and mosquito vector.[36] Malaria illness occurs during the erythrocytic stage, when the merozoite form of the parasite invades a red blood cell, matures, and ruptures the red cell, releasing multiple daughter merozoites who then repeat this cycle. During *P. falciparum* parasite maturation, variant surface antigens (VSAs) are expressed on the surface of infected red blood cells (IRBC). These VSAs bind to endothelial cell receptors and allow the IRBC to sequester in the

vascular bed of various tissues. Sequestration allows the parasite to avoid the immune system's surveillance, thus facilitating parasite replication and progression to symptoms.[37] In non-pregnant adults, IRBCs express erythrocyte membrane protein 1 (PfEMP1) antigens that binds to the placental glycoprotein CD-36 receptor. However, in pregnant women, a new subpopulation of parasites arise that express different PfEMP1 antigens with distinct binding phenotypes, uniformly binding chondroitin sulphate A (CSA) on the surface of the placenta (and not CD-36)[37,38] (Figure 1.1).

During a first pregnancy, women posses antibody titers against common PfEMP1. However there is an absence of antibodies to the subpopulation that binds to CSA.[39,37] Since the primigravid immune system has never "seen" this antigen before, there is no pre-existing immunity and thus primigravida have higher density malarial infections. Conversely, antibodies that block parasite adhesion to CSA are found in sera from multigravida. Thus, multigravid women have acquired antibodies that can limit parasite sequestration in the placenta and provide some protection from placental infections. This pattern suggests that antibodies to the CSA binding PfEMP1 antigen develop only over successive pregnancies, accounting for the increased susceptibility of primigravida and secundigravida to infection. This also explains in part why pregnant women have more frequent and higher density infections than non-pregnant women.

After IRBC cytoadherence to the placental surface has taken place, uteroplacental function and fetal growth can be affected through a variety of mechanisms (Figure 1.1). First, malaria infections (and the immune response to them) early in pregnancy (<20 weeks) might affect uterine artery blood flow by impairing the process of trophoblast invasion and uteroplacental vascular and arterial development, as occurs in preeclampsia.[40] This would result in decreased placental vascularization and uteroplacental circulation, rendering the placenta unable to meet the fetal metabolic demands of late pregnancy.[17,41]

Figure 1.1. Biologic mechanisms linking malaria infection to low birth weight



The next two mechanism center around the pregnant host's impaired immune response to placental malaria infection. Because pregnant women do not posses antibody titers against the subpopulation of VSAs that binds to CSA, parasites are able to sequester in the intervillious spaces of the placenta which may alter the dynamics of the maternal-fetal exchange.[42,43] As large numbers of parasites and macrophages accumulate in the intervillious space, a nearly solid mass of reticuloendothelial cells is formed. As well, *P. falciparum*-infected placentas show a thickening of the basement membrane of placental trophoblast cells.[42] Both the large mass of reticuloendothelial cells and the thickening of the basement membrane can inhibit growth and vascularization of the placenta, leading to decreased transplacental nutrient and gas transport and/or fetal hypoxia.

The second mechanism involves a Type 1 and Type 2 immune system imbalance. The

Type 1 arm of the human immune system (also called the cellular or pro-inflammatory response) is the major host response against intracellular pathogens like malaria. The Type 2 response (also called the humoral or anti-inflammatory response) is the major host response against extracellular pathogens such as bacteria and helminthes. Cytokines that stimulate Type 1 tend to inhibit Type 2, and vice versa and thus, the immune response is often biased either toward Type 1 or Type 2. Wegman and colleagues proposed that a normal healthy pregnancy requires the inhibition of the Type 1 response to prevent a woman from mounting an immune response against fetal tissue.[44] Malaria infections during pregnancy induce proinflammatory Type 1 cytokines, such as TNF-α, IFN-γ and other cytokines in the IFN-γ pathway like IL-12.[41,45,46] This increase in Type 1 cytokines may accompany inflammatory cell infiltration into the placenta. Monocytes also produce TNF[45] and chemokines that attract further monocytes into the intervillious space.[47] These data suggest that it may be the inflammation and not that actual infection that is a proximal cause of poor pregnancy outcome. Placental levels of cytokines have been repeatedly associated with lower birth weight.[45,48,49]

The final mechanism relates to malaria's effect on maternal anemia. Malaria causes maternal anemia through a combination of dyserythropoesis (inability to make new red blood cells) and destruction of infected and uninfected erythrocytes.[34] Anemia might contribute to placental hypoxia, and to impaired placental growth and vascularization. Moderate or severe anemia is a recognized independent cause of LBW.[35]

HIV and malaria co-infection in pregnancy: Human immunodeficiency virus (HIV) has emerged as a major public health problem in areas of sub-Saharan Africa with endemic *P. falciparum* malaria. Due to the high prevalence of both diseases in sub-Saharan Africa, co-infections are common. The ability of HIV to reduce the host immune response to other infectious agents exacerbates the adverse maternal health effects of malaria during pregnancy.

HIV-positive women appear to be particularly vulnerable to malaria infection,[50] and co-infected women have a higher risk of malaria associated SGA.[51,52,53,54] HIV may increase susceptibility to malaria by exerting effects on humoral [55] and cellular immunity,[41,56] thus impairing development of antibodies to malaria at all gravidities. The greatest impairment is in recognition of parasites that express placenta specific VSAs.[55] Women who are most immunosuppressed (lowest CD4 cell counts) have the least antibodies to these VSAs. Due to this impaired immunity, HIV-infected women have a higher prevalence and density of both peripheral *and* placental parasitemia compared to HIV negative women.[57,58,59,60] This increase is seen among both multigravida and primigravida, suggesting a shift in the burden of high parasitemic malaria from primarily primi- and secundigravida to all pregnant women and altering the well established gravidity specific pattern of malaria susceptibility.

HIV may also worsen malaria effects through anemia; HIV and malaria co-infected women are at greater risk of anemia, possibly due to a larger parasite burden and longer duration of malaria infection in HIV positive women.[50]

Biologic mechanisms linking maternal nutritional status to IUGR: During pregnancy, unique maternal metabolic and physiologic processes occur to meet increasing energy demands related to fetal development.[61] An additional energy demand of 200 to 300 kcal per day above non-pregnant energy needs is required to support growth of the fetus, placenta and maternal tissues.[62] Women with nutritional deficiencies are often unable to meet this increased demand. Within the context of pregnancy, nutritional deficiencies often take on one of two distinct forms: (i) under-nourished at the time a woman becomes pregnant; and (ii) inability to meet or sustain adequate protein or caloric intake during the antenatal period. In both the developed and developing world, maternal nutritional status before and during pregnancy is a well-recognized determinant of infant birth size.[63]

Chronic under-nutrition and acute episodes of low food intake render the mother incapable of meeting the increasing metabolic demands of pregnancy through a variety of

physiologic pathways. In a healthy pregnancy, plasma volume begins to increase near the end of the first trimester and increases by greater than 50% by 34 weeks gestation.[64] This expansion is necessary to sustain the elevated cardiac output required to supply blood to maternal tissues and organs. Inadequate energy or protein intake prevents adequate plasma volume expansion limiting maternal cardiac output and blood perfusion to the placenta and uterus.[65,66,67] Inadequate plasma volume expansion is associated with poor obstetrical outcomes including spontaneous abortion, stillbirth and SGA.[64,66]

In a second pathway, acute episodes of starvation during gestation result in a reduction in plasma glucose levels which can induce changes in the normal transfer of nutrients from mother to fetus.[67] Coincident with decreased plasma glucose, the mother will reserve amino acids to be utilized by her own liver for gluconeogenesis, thus decreasing the transfer of several essential amino acids to the fetus. Further, fetal insulin levels will decrease to compensate for reduced availability of glucose, which influences fetal fat deposition and protein synthesis.

A final pathway involves maternal body fat composition and fat deposition. In a healthy pregnancy, woman lay down subcutaneous fat stores as a maternal energy reserve and to support the rapid growth of the fetus during the latter part of pregnancy.[68] Inadequate energy intake can affect both a woman's baseline body composition and her ability to accumulate adequate fat stores to sustain fetal demand.[8,69]

Anthropometric indicators of maternal nutritional status: Maternal nutritional status is often measured using anthropometric indicators collected before and longitudinally during pregnancy.[70] Commonly used indicators include: 1) pre-pregnancy weight and body mass index (BMI), 2) maternal height, 3) pregnancy weight gain, and 4) mid upper arm circumference (MUAC). Maternal anthropometric indicators have been demonstrated to be better predictors of pregnancy outcome than measures of dietary intake.[71]

The sections below provide detailed information about each indicator including a

discussion of the physiologic basis for the indicator, reported epidemiologic associations between the indicator and poor birth outcomes including SGA and LBW, and proposed cutoff values for monitoring nutritional status.

***Maternal height**:* In the developing world, reduced maternal stature is often a consequence of chronic under-nutrition in early childhood.[33,67] Height has been used to predict neonatal outcomes and to identify women at greatest risk of obstetrical complications including prolonged or obstructed labor, cephalopelvic disproportion, or cesarean section.[72] The use of height as a predictor of poor fetal growth needs to be interpreted with caution however, as height is often highly correlated with other maternal anthropometric indicators. For example, taller women are generally heavier but have lower BMI measures than shorter women; so any effect of height may be secondary to, or confounded by, maternal weight or muscle and fat reserves.[70]

In general, taller women appear have higher birth weight infants than smaller women.[73,74,75,76,77,78] Studies in resource poor settings have found an effect of maternal height in the range of 10-22 grams per centimeter increase in height.[70] Women who are shorter also appear to be a significantly increased risk of delivering a LBW[79,80] or SGA[81] infant. However, two studies in resource poor settings (Senegal and Peru) showed no effect of height on birth weight after controlling for maternal muscle or fat reserves[82,83] and studies from the US and Sweden showed that a height effect was attenuated after adjustment for maternal pre-pregnancy weight.[76,84] Naeye and Tafari found that height affected birth length, but not birth weight, in a study of pregnant Ethiopian women.[85]

Height is a highly desirable anthropometric indicator to utilize in pregnancy studies in resource poor settings as it is fairly easy to measure and can be recorded at any time during the pregnancy. Several potential cut-offs values ranging from 140 to 150 cm have been explored as indicators of LBW in resource poor settings.[70,86]

***Maternal pre-pregnancy weight/body mass index (BMI)**:* Body fat is often estimated

using a formula that incorporates weight and height, most commonly the body mass index (BMI=weight in cm/height in m^2), under the assumption that most of the variation in weight is due to fat and not lean body mass. BMI is strongly correlated with fat mass measured by Dual-energy-x-ray absorptionometry.[87] Low maternal pre-pregnancy weight and BMI are considered markers for minimal nutrient reserves. Further, as plasma volume is highly correlated with body weight, low pre-pregnancy weight or BMI may play a role in insufficient plasma volume expansion early in pregnancy.[65] In general, women in resource poor countries have lower pre-pregnancy weight and BMI than women in industrialized countries,[88] and low pre-pregnancy weight is often associated with poor weight gain during pregnancy.[70]

In both industrialized and resource poor countries, women with low pre-pregnancy weight or BMI have consistently delivered lower birth weight babies.[76,89] In a study of indigent women from the southern United States, Neggers and colleagues demonstrated a nearly 300 gram increase in absolute birth weight comparing women in the 90th percentile of pre-pregnancy weight to women in the 10th percentile and concluded that pre-pregnancy weight was the best predictor of infant birth weight.[74]

Maternal pre-pregnancy weight and BMI status have substantial effects on the risk of LBW and SGA. A study of over 20,000 infants in the United States showed a decrease in the percent of infants born LBW with increasing pre-pregnancy weight.[90] An investigation of risk factors for LBW showed that nearly 60% of infants born <2,500 grams were born to women weighing less than 50.8kg; short women with low pre-pregnancy weight had the highest rate of LBW.[80] In a large prospective study of predominantly black indigent women in the United States, there was a 3-fold increase in SGA risk among women with low pre-pregnancy weight after adjustments were made for other confounders.[81] A study in India found a similar magnitude of effect for women weighing less than 40kg prior to pregnancy and showed that pre-pregnancy weight was the strongest predictor of SGA in bivariate and multivariate

analyses.[91]

Using data from 46 national surveys of mothers aged 15–49 from 36 resource poor countries, Nestel and Rutstein defined de facto reference BMI cutoffs for pregnant women.[92] Four reproductive outcomes were compared according to BMI categories: neonatal and infant mortality, size at birth, birth weight, and miscarriage or stillbirth. Women with low BMI had babies that were smaller and of lower birth weight than women with normal or high BMI; neonatal and infant mortality rates were also highest in the lowest BMI group. Studies from New Guinea and Jamaica confirm these findings.[77,78]

In resource poor settings where women often initiate prenatal care in the second trimester, a major challenge to implementing this indicator is the lack of an estimate of "true" pre-pregnancy weight. However, because women in resource poor settings often gain little weight early in pregnancy, weight at booking can often be used as a proxy measure.[70] The Institute of Medicine recommends the following categories for use in categorizing women into pre-pregnancy weight categories: BMI of <19.8 kg/m^2 (underweight), 19.8-26.0 (normal weight), 26.0-29.0 (overweight) and >29.0 (obese). A slightly lower cutpoint of BMI <18.5 kg/m^2 has also been used in developing countries as suggestive of chronic energy deficiency.[70]

Pregnancy weight gain: Women typically need to gain between 11-15 kg in weight to meet the metabolic needs of pregnancy. This weight gain can be characterized into fetal components including the conceptus (3.2-3.6 kg), the placenta and amniotic fluid (1.4-1.8 kg), and the enlarged uterus (0.9-1.8 kg), as well as maternal components of tissue fluid (2.3-2.7 kg), plasma volume (1.4-1.8 kg) and body mass or fat stores (2.3-2.6 kg).[64] Weight gain is minimal early in gestation and mostly reflects uterine growth and blood volume expansion; in the latter half of pregnancy the growing fetus and placenta make up most of the maternal weight gain.[64] In resource poor countries, average total gestational weight gains range from 4.8-9.0 kgs; much less than the 10.5-13.5 kgs reported from industrialized countries.[70]

Among women living in resource poor settings, fetal weight makes up a larger proportion of overall weight gain than in industrialized areas, indicating that the maternal components of weight gain, including plasma volume expansion and fat store deposition, may be compromised.

A strong body of evidence supports the finding of a positive association between total pregnancy weight gain and LBW or SGA.[15,73,75,89,93,94] A weekly weight gain of <0.24 kg or 0.24-0.57 kg (compared to 0.58-0.74 kg/week) was associated with a 3-fold and 2-fold increased risk of SGA, respectively, in a study of rural indigent women in the southern United States.[81]

Previous research suggests that pregnancy weight gain has varying affects on infant outcome depending on the timing of weight gain. With the exception of a study by Brown *et al,*[95] first trimester weight gain has largely found to be unrelated to infant birth weight.[96-98] In contrast, gains in the second, or second and third, trimester have been associated with newborn weight in a variety of settings. In Guatemala, Villar *et al* showed that late 2nd trimester and early 3rd trimester weight gain was associated with higher mean birth weight.[99] In another Guatemalan population, mid-pregnancy weight gain was shown to be more important than late pregnancy gain in predicting birth weight.[100] In Tanzania, Nyaruhucha and colleagues showed that weight gain in the third trimester was significantly associated with birth weight in multivariate analysis.[101] Studies from industrialized countries have demonstrated similar strong effects of second trimester or mid-pregnancy gains.[93,96,98] These trimester specific weight gain findings may suggest that maternal physiologic changes occurring in the earlier half of pregnancy, including plasma volume expansion and fat deposition, play a large part in maternal weight gain and an important role in fetal outcome.

Many studies of pregnancy weight gain have also demonstrated a variable effect based on pre-pregnancy weight or BMI. In general, the importance of maternal weight gain appears to diminish as pre-pregnancy weight increases. Among women with low weight gain, Strauss *et*

al showed a decreasing trend in the risk of SGA with increasing baseline BMI.[97] Niswander demonstrated a similar trend using percent LBW as an outcome.[76] Abrams and Laros saw a statistically significant effect of maternal weight gain on birth weight in women who were underweight, ideal weight and moderately overweight, but not among women in the obese pre-pregnancy category.[102] Similarly, Simpson noted an increase in mean birth weight with increasing pregnancy weight gain in women with low and normal pre-pregnancy weight, but not among women in the highest group (>160 pounds).[90] Findings such as these lead to the 1990 Institute of Medicine recommendation that pregnancy weight gain norms be stratified by maternal pre-pregnancy BMI status.[103]

Pregnancy weight gain can be assessed using measurements taken at least one month apart and it is not absolutely necessary that an accurate estimate of gestational age be known in order for the indicator to be useful. Cut offs for weight gain of <1.0 kg and <1.5 kg per month throughout the second and third trimester have been suggested to identify women with inadequate dietary intake and at risk of adverse pregnancy outcome.[70] Lack of weight gain or weight loss is indicative of a serious problem that requires immediate nutritional intervention.

Mid-upper arm circumference (MUAC): Maternal body fat represent the largest component of tissue gain during pregnancy.[99] MUAC and skinfold thickness measures reflect both fat and lean tissues stores. Early in pregnancy, maternal fat cells hypertrophy, fat synthesis is increased, and lipolysis is inhibited to expand maternal deep and subcutaneous fat stores and build an energy reserve.[61,69,104] The deposition of fat in the early stages of pregnancy indicate a positive energy balance.[105] Later in pregnancy, there is a shift in metabolism that favors lipolysis, and the fat mass diminishes in order to meet the increased nutritional demands of the fetus.[73,104] MAUC and skinfold thicknesses are highly correlated with both maternal weight gain and BMI.[70,104]

Studies from resource poor populations have had conflicting results concerning this

indicator. For example, in Guatemalan women, Li and colleagues[100] showed no association between MUAC change while Lechtig found that MUAC was as good as third trimester weight gain for predicting birth weight.[106] In Zimbabwe, Friis *et al* demonstrated a nearly 2-fold increase in the risk of LBW among women with low arm fat area.[79]

Previous studies of MUAC and skinfold thickness in industrialized and resource poor settings have identified certain trimester patterns of fat accrual associated with LBW and SGA. For example, studies concentrating on first trimester have shown no relationship between fat mass and poor birth outcome.[94,107] However, failure to accrue fat during the second trimester of pregnancy has been associated with lower birth weight.[73,99,105] In contrast, MAUC that continues to increase into the third trimester are associated with lower attained birth weight[73,85,82] which may indicate that the fetus was not able adequately mobilize maternal fat stores late in pregnancy.[103]

For one time screening, MUAC cutoffs between <20.8 cm and < 23.5 cm have been suggested to identify women with inadequate nutritional status.[70] MUAC in this range have been associated with predicting LBW, fetal, and infant deaths with an adequate level of sensitivity and specificity. Change in monthly or trimester specific MUAC or arm fat area measurements have also been used to describe fat accretion in several studies. However, in resource poor settings, where women may gain very little, or even lose MUAC, the usefulness of change in MUAC as a monitoring tool may be limited.[70]

Specific Aim 2: Utilize prospectively collected ultrasound data to develop a fetal size for gestational age nomogram for a resource poor population with high malaria prevalence

Rationale: IUGR is often defined as an estimated fetal weight of less than the 10th percentile of a standardized fetal weight-for-gestational age nomogram.[16] The choice of standardized nomogram can influence the predictive properties of this definition. In fact, depending on the standard population, cut-offs to define IUGR can vary by up to 500 grams.[108] Most fetal weight nomograms were derived from European or American Caucasian

populations and represent a narrow range of genetic diversity or environmental conditions.[109] Environmental factors that are prevalent in resource poor populations, such as maternal infections and chronic under-nutrition and micronutrient deficiency, can significantly affect fetal growth.[2,15] Therefore, It is likely that fetal weight nomograms created from industrialized countries are not appropriate standards for diagnosing IUGR in resource poor populations.

Previous ultrasound studies of African populations have largely found lower mean values for fetal biometry and estimated fetal weight for gestational age compared to industrialized standards. Okonofau and colleagues conducted two ultrasound studies among Nigerian women during the late 1980s. They found that the BPD[110] and AC[111] of Nigerian fetuses were consistently lower at all gestational ages between 20 to 40 weeks when compared to a European standard. They also calculated the BPD to AC ratio as a possible means to assess symmetrical vs. asymmetrical IUGR patterns.[111] A study conducted in Zimbabwe showed nearly identical smoothed centile results for the modeled BPD measurements as demonstrated by Okonofau and colleagues.[112] Another study from Nigeria, however, found no significant differences between the BPD of Nigerian fetuses and European fetuses until late in pregnancy when the Nigerian fetuses had slightly smaller measurements.[113]

Research conducted in other resource poor populations display a similar trend as the African data. In Bangladesh, Spencer and colleagues found that the AC and EFW of Bangladeshi fetuses were smaller than fetuses of white women at 28, 32 and 36 weeks gestation.[114] With advancing gestational age, the 50th percentile HC, AC and FL for Peruvian fetuses fell progressively below the 50th percentile of reference populations from the United States and Britian.[115] Two studies of Indian women found lower mean fetal AC [116,117] and BPD[117] after 24 weeks, compared to whites.

In sum, these studies provide a body of evidence that fetal size nomograms developed for industrialized populations are likely to overestimate fetal weight centiles for resource poor populations, thus leading to diagnosis of an inappropriately high proportion of fetus as IUGR.

Statistical development of fetal size nomograms: There are three main uses of fetal nomograms in obstetric practice: 1) to assess the size of a fetus of known gestational age against a reference standard at a certain point in time (size nomogram), 2) to assess the growth rate of a fetus between two time points against reference data (growth nomogram), and 3) to estimate the gestational age of a fetus from its fetal size.[118] The second and third uses are beyond the scope of this dissertation; however, it is important to make a distinction between fetal size nomograms and fetal growth nomograms. Fetal size nomograms are often erroneously referred to in the literature as "growth nomograms" or "growth curves" but they should not be used to make assumptions about, or to monitor, fetal growth progress over time.

Because growth nomograms inherently describe how a fetus is growing *conditional upon* its size a few weeks or months prior, it is essential that they be derived from longitudinal (or serial) measurements from each fetus. Many past researchers have also attempted to develop fetal size nomograms from longitudinal ultrasound data, but they utilized inappropriate statistical techniques for this application. For example, many investigators treated the longitudinal data as if it were cross-sectional data (i.e., each women scanned only once).[119,120,121] By assuming statistical independence, this approach ignores the high correlation amongst biometric parameters over time (gestational age) and presumes that the fetal growth velocity, and the estimated model residual errors, are constant over time. These assumptions are not appropriate for fetal growth data, which generally demonstrates a pattern of increased variability in EFW with increasing gestational age. Several authors attempted to improve the statistical methods used to analyze longitudinal data for creating a fetal size nomogram by fitting a separate regression curve to each fetus and using the average variation (i.e., average of the individual regression coefficients) among these curves to derive the size centiles.[122,123] This method, however, is also flawed and leads to outer centiles that are too narrow because it only accounts for between-fetus variation.[124] In a

series of papers published in the mid-1990's, Altman and Chitty pointed out the errors in these techniques and recommended that fetal size nomograms only be derived from cross-sectional data.[118,124] Further, they proposed a set of techniques to appropriately analyze cross-sectional data which modeled the mean and standard deviation (SD) of the data separately and then produced the centiles from the relationship mean ± z(SD) where z is the standard normal deviate.

The introduction of mixed effects modeling (also known as hierarchical or multilevel modeling), provided a new statistical technique that addresses some of the limitations discussed above by considering both the between- and within-fetus variation in the calculation of fetal size nomogram reference centiles from longitudinal data. For this specific aim, we utilized a mixed effect modeling approach suggested by Royston,[125] to develop a fetal size nomogram for this Congolese population.

CHAPTER 2:
RESEACH DESIGN AND METHODS

The specific aims of this dissertation are explored with data collected from a longitudinal cohort study of 182 pregnant women identified during routine antenatal care at Binza Maternity in Kinshasa, Democratic Republic of Congo between May 2005 and May 2006. Information regarding study design and data collection methodology that is relevant to both specific aims is provided first. Specific details about the study population (inclusion and exclusion criteria), variable definitions, and statistical methods for each specific aim analysis are provided separately in subsequent sections.

Study design and data collection

Setting: Binza maternity is Kinshasa's second busiest maternity with approximately 7,000 deliveries per year. The maternity has been operating in urban Kinshasa for over 30 years. Italian nuns are responsible for the overall functioning/administration of the maternity; Congolese nationals conduct all medical and nursing aspects. The maternity includes simple but effective facilities for caring for premature infants, and an outpatient unit for seeing infants in post-partum follow-up. Women with significant blood loss during delivery or who require surgical intervention are transferred to a nearby obstetrical referral hospital. The mean age of women delivering at Binza Maternity is 27 years and the mean gestational age at first antenatal presentation is 26 weeks. Nearly one-third of women are parasitemic at first antenatal presentation. Over 80% of the women who receive antenatal care at Binza return to the maternity to deliver.

Recruitment: Pregnant women were recruited from the population of new antenatal care

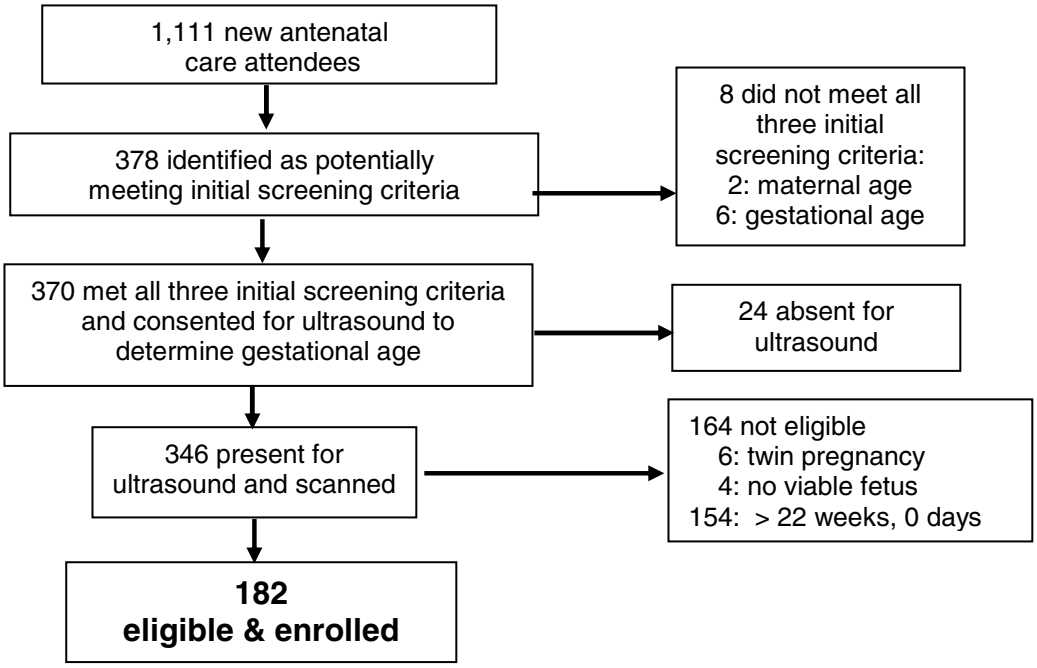
attendees at Binza maternity. Eligibility screening occurred in a two phased process. During routine antenatal registration and clinical evaluation at the maternity, an initial screening was employed that utilized patient reported last menstrual period (LMP) and maternity recorded fundal height information. For the first round of recruitment (May-July, 2005), women determined to be less than 23 weeks gestation via either of these methods were asked to provide written consent to have an ultrasound examination performed to confirm gestational age. During the second round of recruitment (October-November, 2005), a more stringent set of criteria was utilized to minimize costs and time spent screening and consenting women who would likely not be eligible for enrollment in the study. These criteria emphasized LMP dates as we found LMP to be a more reliable indicator of gestational age than the fundal height measurement. For the new criteria, if LMP was known, women determined to be less than 23 weeks gestation were invited for an ultrasound examination. If LMP was not known, then the fundal height cutoff was considered for determination of women to approach for ultrasound screening. Women who met the initial screening criteria were approached by a study coordinator and invited to consent to return to the maternity 3-5 days later for an ultrasound examination to confirm gestational age.

During the second phase of recruitment, women had a baseline ultrasound examination, which assessed fetal biometric measures including BPD, HC, AC, and FL. These measurements were used to estimate gestational age[126] and fetal weight[27] using the Hadlock algorithms. For fetuses in the first trimester, the crown-rump length was used to estimate gestational age. All women who were potentially eligible for enrollment also had amniotic fluid volume (four quadrant method) and placental location recorded. To be eligible for the study, women had to have a singleton pregnancy with an ultrasound derived gestational age of 22 weeks, 0 days or less, be 18 years of age or older and agree to be tested for HIV. Women with high blood pressure at baseline (systolic blood pressure > 140 mmHg and/or diastolic > 90 mmHg), multiple gestations or a detectable fetal abnormality were excluded

from the study. Women with evidence of placenta previa, fetal abnormalities or multiple gestations during ultrasound were referred to the Department of Obstetrics and Gynecology at the Clinque Universitarie in Kinshasa for high risk pregnancy follow-up care. All ultrasounds were conducted by a trained Congolese obstetrician-gynecologist.

Figure 2.1 summarizes the results of recruitment. Of 1,111 new antenatal care attendees, 33% (n=370) met all initial screening criteria and were scanned to determine gestational age. Of those, 182 were eligible and consented to the longitudinal study. Reason for ineligibility included absent for ultrasound (n=24), twin pregnancy (n=6), no viable fetus present (n=4) or gestational age greater than 22 weeks, 0 days (n=154).

Figure 2.1. Recruitment results for longitudinal study, Kinshasa, Democratic Republic of Congo, 2005-2006



Baseline visit: After the ultrasound examination, all eligible women met with a study recruiter who explained the goals and procedures of the study and administered written informed consent to be enrolled in the longitudinal study. After obtaining informed consent, women were interviewed about sociodemographic characteristics, alcohol, tobacco and drug

use, and medical and obstetric history. Current malaria symptoms, recent use of anti-malarial drugs, and use of insecticide treated bed nets were also assessed.

A medical examination was conducted to collect maternal anthropometric measurements, blood pressure, pulse, temperature and physical signs of anemia (Table 2.1). A urine test for albumin and a hematocrit test were conducted in our on-site laboratory. Malaria thick and thin smears and filter paper samples were colleted from fingerprick blood samples. Malaria slides were initially read on site for a gross determination of parasitemia; quality control, assessment of parasite density and identification of parasite sub-type was conducted the following day at the Ecole Sante Publique de Kinshasa.

All women enrolled in the study participated in HIV voluntary counseling and testing services as part of a Glaser Foundation-supported Preventing Mother to Child Transmission of HIV program at Binza maternity. Women identified as HIV positive, and their infants, received Nevirapine treatment per that program's protocol.

In accordance with Congolese National Policy, all enrolled women received two doses of presumptive therapy with sulfadoxine-pyrimethamine (SP: 1500 mg sulfadoxine + 75 mg pyrimethamine) between 16 and 27 weeks, and again between 28-32 weeks, regardless of malaria status. The first dose coincided with the baseline visit for most of the women enrolled in the study (women less than 16 weeks at enrollment received their first dose of SP at the first follow-up visit). All women also received iron supplementation as part of routine antenatal care at Binza maternity.

At the conclusion of the baseline visit, women were provided with a study appointment card, an insecticide treated bed net, and reimbursement for round trip taxi fare. Women were instructed not to take any anti-malarial medication that was not provided through the study and to return to the clinic any time they felt ill or had any symptoms of malaria illness. Women were also interviewed to complete a Participant Locator Form which contained detailed information about her address (street, house number, quartier, and commune), a

reference location near her address (i.e., a popular bar or market), a phone number (if available), as well as similar information for a reference person (i.e., a mother or sister). This form was used to locate participants who missed one of their scheduled appointments.

Table 2.1. Summary of ultrasound, clinical and laboratory measurements, Kinshasa, Democratic Republic of Congo, 2005-2006

Visit	Clinical Measurements	
Baseline	▪ Clinical history ▪ Sociodemographics ▪ Last menstrual period ▪ HIV test and counseling ▪ Ultrasound examination ▪ Fundal height ▪ Peripheral parasitemia and assessment for fever	▪ Filter paper sample ▪ 1st SP dose[1] ▪ Height, weight, mid-upper arm circumference ▪ BP, pulse ▪ Hematocrit ▪ Urine test ▪ Assess for edema
Follow-up (each month)	▪ Ultrasound examination ▪ Fundal height ▪ Peripheral parasitemia and assessment for fever ▪ Filter paper sample ▪ 2nd SP dose[1]	▪ Weight, mid-upper arm circumference ▪ BP, pulse ▪ Hematocrit[2] ▪ Urine test ▪ Assess for edema
Delivery	**Women** ▪ Peripheral parasitemia ▪ Filter paper sample ▪ Placental biopsy ▪ Hematocrit ▪ Maternal mortality	**Infant** ▪ Birth weight ▪ Length (crown-heel, crown-rump) ▪ Head circumference ▪ Abdominal circumference ▪ Gestational age ▪ Infant mortality

[1] SP was given to all women between 16 and 27 weeks, and between 28-32 weeks gestation, regardless of malaria status.
[2] Hematocrit tests performed at every other follow-up visit.

Follow-up: Participants returned to the maternity for follow-up every month until delivery. This resulted in approximately four to five follow-up visits over the course of the study. At each follow-up visit, an ultrasound examination of fetal biometry was conducted to estimate fetal weight (Table 2.1). Amniotic fluid volume (four quadrant method) and placental location were also recorded. Additionally, Doppler assessment of uterine and umbilical artery flow was performed. A medical examination was conducted to collect maternal anthropometric measurements, blood pressure, pulse, temperature, urine test and physical signs of anemia.

Malaria thick and thin smears and filter paper samples were colleted from fingerprick samples. Hematocrit tests were conducted at every other visit.

At all visits, any woman found to have a positive malaria slide was provided with treatment by the study. Treatment determination for malaria positive women was done in collaboration with the attending physician at Binza maternity. Typically, any woman found to have parasitemia was first treated with SP. If a woman had a subsequent positive parasitemia within a month of treatment with SP, another drug was selected. Most often, quinine (along with sulbutamol to control uterine contractions) was prescribed. Depending on a woman's gestational age and her willingness to take quinine, Manalaria (a locally produced herbal drug), Artesunate or Camoquine were also sometimes prescribed. All treatments were provided to patients free of charge. The second presumptive dose of SP was given to women at the visit that coincided with approximately 28-32 gestational weeks. At the conclusion of each follow-up visit, women were given reimbursement for round trip taxi fare.

If a study participant failed to present to the maternity for her regularly scheduled follow-up visit, an active surveillance mechanism utilizing the Participant Locator Form was activated. As a first step, we attempted to contact her, or her reference contact, via phone. If no response was obtained after two phone calls, a study nurse traveled to the participant's home to locate the patient and offer her a ride to the maternity or reschedule her appointment for later that week.

Interim study visits: Women were instructed to return to the maternity if they ever felt ill, had fever or other symptoms of malaria. All interactions with the patient that occurred outside of a regularly scheduled study visit were recorded on an Interim Visit Study Form. At these visits, medical care was provided by the maternity and a member of our study staff assessed the patient for clinical symptoms of malaria and prepared a thick smear and filter paper sample. All medical procedures performed and medications prescribed by the maternity were recorded. The study paid for any medication and laboratory tests

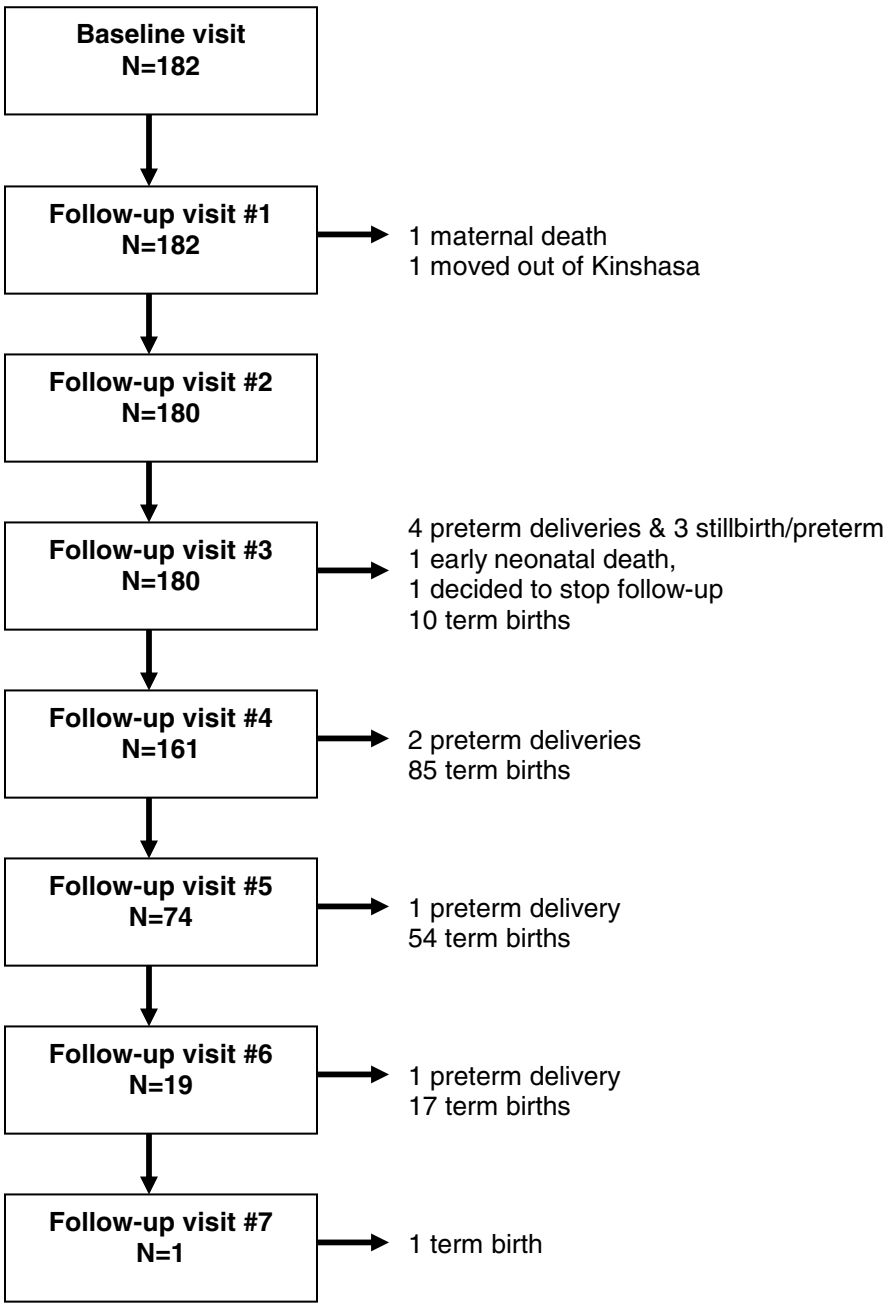
the women required. There was a total of 172 interim study visits during follow-up.

Delivery: All women were encouraged to deliver their infants at Binza maternity and were reimbursed for one-third of the delivery-related fees. Before delivery a hematocrit, thick and thin smear, and filter paper sample were collected (Table 2.1). If these samples were missed before a woman went into labor, we attempted to collect them within 24 hours after delivery. After delivery, a placental thick blood smear and a placental biopsy were taken for assessment of placental malaria. The infant was weighed and infant anthropometrics (crown-heel, crown-rump, head and abdominal circumference) recorded within 24 hours of delivery. Information about the labor and delivery, including complications, maternal and fetal death, were recorded.

For women who were unable or unwilling to delivery at Binza maternity, or who initiated labor at Binza maternity but were later transferred due to complications, our staff would travel to the hospital or clinic where the women delivered as soon after delivery as possible to collect the necessary samples and infant information.

Figure 2.2 summarizes the results of follow-up and delivery. Enrolled women participated in a total of 1,151 study visits (979 regular appointments that included an ultrasound scan and 172 interim visits which did not include a scan). There were a total of 11 regular appointments in the 1st trimester, 423 in the 2nd trimester, and 545 in the 3rd trimester. On average, women participated in five follow-up visits (SD=1 visit) and were enrolled for 18 weeks (SD=3 weeks). Delivery information was collected for 180 women (missing information included one maternal death and one woman with unknown delivery location). There were 167 term deliveries, eight preterm deliveries, three stillbirth/pre-term deliveries, and one preterm/early neonatal death.

Figure 2.2. Follow up and delivery results for longitudinal study, Kinshasa, Democratic Republic of Congo, 2005-2006



Delivery information collected for 180 of 182 women	**Birth outcomes**
• 1 maternal death • 1 women unknown delivery location	• 167 term deliveries • 8 preterm deliveries • 3 stillbirth/pre-term deliveries • 1 preterm/early neonatal death

Ultrasound measurements

All ultrasounds were performed using a GE Logicbook Ultrasound System. All ultrasound images were stored on CD-ROM for blinded reassessment at the University of North Carolina, Chapel Hill. Victor Lokomba, an obstetrician-gynecologist from Clinque Universitarie in Kinshasa received intensive training in ultrasound technique and performed all ultrasounds.

Fetal biometry: Using standard techniques, HC and BPD were measured from an image that displayed the fetal head in an axial plane that included the thalamus and cavum septum pellucidum. The BPD was measured by placing the calipers from leading edge to leading edge (outer to inner skull table) and the HC was measured using an ellipse trace of the outline of the fetal head. AC was measured from an image in which the junction of the umbilical vein and portal sinus were visible. The ellipse function was used to trace the extreme perimeter of the fetal abdomen. FL of the femoral diaphysis was also measured. These measurements were used to estimate gestational age[126] and estimated fetal weight[27] using the formulas of Hadlock.

Measurement of intrauterine environment (fundal height and amniotic fluid volume): Oligohydramnios is a common finding in pregnancies affected by IUGR. The intrauterine environment was assessed clinically by measurements of fundal height and amniotic fluid volume. Amniotic fluid volume was assessed using the four-quadrant technique and the normal values reported by Moore and Cayle.[127]

Assessment of placental location: Placental position was characterized as anterior, posterior, left or right lateral, previa/low lying, or fundal. Lateralization was determined by the side on which the majority of the placenta was located. Low lying/previa was further characterized as Type 1-Type 4 as follows:

Type 1: The placenta is mainly in the upper segment of the uterus but encroaches on the lower segment

Type 2: The placenta extends to, but does not cover, the internal opening into the cervical canal

Type 3: The placenta covers the internal os of the cervical canal during the later stages of pregnancy but does not cover it completely as the cervix dilates during labor

Type 4: The placenta completely covers the internal os of the cervical canal, even when dilated

Laboratory and clinical measurements

Peripheral malaria parasitemia: At baseline, all follow-up visits and delivery, peripheral parasitemia was assessed by thick and thin smears. Giemsa-stained smears were assessed for parasitemia by a laboratory technologist trained at the Institut Superieur de Technologie Medicale in Kinshasa. Parasite sub-type was determined and parasite density was quantified by counting the number of parasites against 200 white blood cells and converted to numbers of parasites per μl under the assumption that there was 6000 WBC per μl.

Filter paper samples: At baseline, all follow-up visits and delivery, a filter paper sample of maternal peripheral blood was taken and stored for future laboratory analysis. From the finger prick puncture, 2-3 large drops of blood were formed and lightly touched onto the filter paper. Five circular blood spot were collected from each woman. The blood spots were dried thoroughly for at least four hours before being stored individually in a sealed plastic storage bag with a desiccant pack. All samples were stored at 4ºC until sent to the University of North Carolina, Chapel Hill for processing.

Placenta malaria parasitemia and histology: Placentas were collected after delivery and stored at 4°C for processing within 24 hours of delivery. An incision was made at a healthy pericentric area of the placenta for collection of placental blood smears and biopsy samples. Several drops of pooled blood were transferred to a glass slide and prepared according to the peripheral malaria blood smear procedures listed above. Following the procedures of

Rogerson,[12] two placental biopsy samples (approximately 1 cm^3) were collected and placed into 10% neutral buffered formalin. Samples were stored at 4°C until sent to the University of Kinshasa Service d'anatomie Pathologique for processing. Samples were embedded in paraffin wax using standard techniques and paraffin sections of approximately 5 μm thick were stained with Gurr's modified Giemsa and/or hematoxylin and eosin (H&E) and examined under light microscopy and polarized light (to assess the deposition of malaria pigment). Intervillous cells were examined under oil immersion for presence of parasites and fibrin. The following classification was used to assess the severity of parasites in the erythrocytes, and hemozoin pigment in fibrin and in monocytes: 1) Absent, 2) Scant, 3) Mild, 4) Abundant.

Hematocrit/anemia: At baseline, alternating follow-up visits and delivery, fingerprick blood was collected into a heparin coated capillary tube and spun for 10 minutes in a hemotocrit centrifuge (Clay Adams, Readacrit). The percent hematocrit was recorded.

Preeclampsia (proteinuria/blood pressure): At each study visit, a urine specimen was tested for the presence of protein and a blood pressure measurement taken as clinical indicators of preeclampsia (sometimes called toxemia or pregnancy-induced hypertension), a disorder often characterized by high blood pressure and large amounts of protein in the urine.

Maternal anthropometric indicators: Using standard techniques,[128] maternal weight was measured on a UNICEF digital scale (SECA Model 890) to the nearest 0.1 kilogram and height (without foot-wear or head cover) was measured to the nearest 0.1 cm. MUAC was measured with cloth tapes on the right arm at the midpoint between the acromial and olecranon processes of the scapula and ulna, respectively. The measurement was made to the nearest 0.1 cm while the arm was hanging freely, with the cloth tape snug to the skin, but not compressing the underlying tissue.

Infant anthropometrics: Using standard techniques,[128] birth crown-heel and crown-rump

length were measured using a pediatric length board within 24 hours of birth. Head and abdominal circumference were measured to the nearest 0.1 cm using a measuring tape. Birth weight was recorded within 24 hours of birth to the nearest gram (LARIO Scale, Soc. Curion & Company, Como, Italy).

Quality control

Malaria parasitemia: For quality assurance, a 10% sample of all malaria thick smears was assessed independently by an experienced laboratory technician at the University of Kinshasa. Of 140 slides examined, there was one discordant positive and one discordant negative between the two technicians for a sensitivity of 92% and a specificity of 99%.

Ultrasound images: All ultrasound images were stored on a CD-ROM and a 10% sample of biometry and Doppler images was assessed for quality by a Maternal-Fetal Medicine physician at the University of North Carolina, Chapel Hill. 92% of the reviewed images were deemed of adequate quality for clinical assessment; 7% of questionable quality and 1% poor quality (i.e., not all biometry landmarks clearly visible, shadowing in the image or poor tracing of the length of circumference).

We conducted a small study comprised of 10 women to assess the intra-operator variability in measuring fetal biometry. The correlation between two independent measurements on the same fetus was r=0.99 for each of biparietal diameter, head circumference, and femur length, and r=0.98 for abdominal circumference.

Data entry and data management

Data entry: All data was entered locally using an EpiInfo database designed specifically for the study. Quality control elements of the data collection process included: (i) routine checking for completion of all data items at the maternity before women are checked out at the end of their appointment; (ii) EpiInfo pre-programmed ranges of plausible values for all

continuous numeric data fields (i.e., temperature, height, weight); (iii) EpiInfo pre-programmed ranges of allowable values for all categorical responses; (iv) EpiInfo pre-programmed skip patterns to ensure that no data is entered for questions that should be skipped; (v) required data entry fields (such as ID numbers and dates) that must be entered by the data entry clerk in order to continue with entry; and vi) re-entry of a 10% sample of forms by an independent data entry clerk for comparison to the larger database using the EpiInfo Data Compare functionality.

Data cleaning: The EpiInfo data base was transferred into SAS and SPSS datasets for data cleaning. Data cleaning steps included: (i) identifying data that is missing from required fields and attempting to locate that data if possible; (ii) checking to ensure that skip patterns were properly followed; (iii) descriptive statistics of all continuous variables to identify outlier values; (iv) ensuring that dates match up on all forms for a given visit; (v) translating dates into American format (DD/MM/YYYY); and (vi) adding descriptive labels and user defined formats to each variable.

Assessment of missing data, drop-outs: Overall, missing data and attrition for this study was extremely low. We minimized "intermittent" missing data (in which a woman misses a follow-up appointment but then returns to complete the study) by our active surveillance of "no-shows." Women were characterized as "lost to follow-up or drop-out" if they left the study before completing all follow-up visits or they did not deliver at Binza maternity and were not able to be located at their delivery location. Follow-up data are missing for one maternal death and one woman who moved out of the study area (both occurred after visit #1). No delivery data was available for the maternal death and for one woman who completed all study visits but could not be located for delivery. Only partial delivery data (date of birth, birth weight and maternal and neonatal vital status only) was available for 10 women who delivered at another maternity and were released before our staff could examine the infant to collect anthropometrics.

This very low rate of missing data is not likely to bias the results significantly. Further, the longitudinal data analysis techniques used in this dissertation will include all available antenatal data for a woman, regardless of the number of follow-up visits that she contributes.

Analyses for Specific Aim 1: Describe the association between maternal malaria infection and intrauterine growth restriction and determine if maternal nutritional status modifies this relationship

Study population: In the full study cohort, 182 women were eligible and consented to the longitudinal study. For this analysis, we excluded five HIV-positive women (3%) to yield a final sample size of 177 women. HIV positive women were similar to HIV negative women with respect to their socio-demographic and obstetrical characteristics as well as the gestational age at enrollment. The frequencies of malaria and IUGR were also similar with those of non-HIV infected women. The 177 included women completed a total of 1,120 study visits. On average, women received five ultrasound scans (range two to eight).

Determination of gestational age: For this analysis, gestational age was defined in rounded weeks according to the ultrasound derived menstrual age algorithm of Hadlock [MA=10.85 + 0.060*HC*FL + 0.6700*BPD + 0.1680*AC].[126] Because an accurate estimate of gestational age is an important factor in the assessment of IUGR, we also considered restricting this analysis to only women who had a “certain” gestational age (defined as an LMP date within ±14 days of the ultrasound derived date). All of the following analytic steps were also run on this sub-population (n=145 women, 608 study visits and 51 episodes of IUGR). Overall, the results were not appreciably different from those obtained using the “full” data set which included all women and utilized the ultrasound derived menstrual age as the anchor for gestational age determination at each follow-up visits. Further, several of the models run on the restricted data set had problems converging due to small sample size when attempting stratified analyses. Thus, we ultimately decided to present findings for the full study population.

Outcome definition*:* Estimated fetal weight was calculated at each ultrasound using the formula by Hadlock [log(EFW)=1.3596 - 0.00386*AC*FL + 0.0064*HC + 0.00061*BPD*AC + 0.0424*AC + 0.174*FL].[27] For this analysis, IUGR was defined at each ultrasound as a binary outcome of <10th percentile of fetal weight for attained gestational age using the Hadlock fetal standard curve.[129]

Exposure and covariate definitions: The following definitions were used to define the main study exposures:

Malaria infection: Among the 177 women included in this analysis, there were a total of 171 positive malaria smears. Before defining the malaria variables for this analysis, we attempted to identify probable recrudescent episodes. To do this, we examined each episode of malaria and determined the number of days between that episode and the most recent prior episode. Any episodes that occurred within 14 days of a previous positive were considered to be probable recrudescent cases, and not representative of a "new" incident infection. In total, 14 probable recrudescent episodes were identified and excluded from analysis.

Using the remaining 157 episodes, we created two malaria variables. The first was a time-dependent measure of incident infection (called malaria parasitemia at visit) which represents the effect of an incident infection that initiated during the interval between a woman's previous visit and the study visit in which the IUGR measurement was taken. The second is a time dependent measure of the cumulative number of positive antenatal parasitemia episodes (called cumulative positive parasitemia) which represents the total number of times that a woman had a positive smear up to and including the study visit in which the IUGR measurement was taken.

Maternal height: Short maternal stature was defined as height <150 cm.

Maternal pre-pregnancy weight or BMI: As pre-pregnancy weight was not available, we utilized maternal weight at enrollment to calculate baseline body-mass index (BMI), with low

baseline BMI defined as <19.8 kg/m^2. Although this proxy of pre-pregnancy weight may have over-estimated true pre-pregnancy weight, any error was likely minimal as women in resource poor countries gain little weight early in pregnancy and these participants were enrolled in the first or early second trimester.[70] Further, the 10th percentile baseline BMI value for this population was nearly identical to the cut point of the standard weight-for-height chart used to define the underweight category in this analysis (≤19.8 kg/m^2 for the standard vs. ≤19.7 kg/m^2 for the Kinshasa population), suggesting that our proxy was a fair estimate of pre-pregnancy BMI.

Pregnancy weight gain: Change in maternal weight was calculated between each monthly visit, and was categorized as low monthly weight gain (<1.5 kg gain per month) or adequate monthly weight gain (≥1.5 kg gain per month). After completion of the study, it was discovered that the digital scale malfunctioned half way through the study period (systematically added an unknown weight to all measurements taken after this time). This error largely involved weight measurements taken during the last follow-up visit for women enrolled early in the study and the first or second follow-up visit for the women recruited later in time. To account for this in data analysis, we removed the suspect data points before calculation of the change in weight variable. Next, to ensure the validity of measurements taken after the malfunction, we ensured that the average weight change between visits before and after the scale malfunction were similar. Due to these statistical adjustments, complete data on weight change is available for 588 observations.

MUAC: Change in MUAC during pregnancy was dichotomized as loss (<0 cm change) or gain (≥0 cm change), over three distinct time periods: (i) monthly change between study visits, (ii) change over the entire second trimester, and (iii) change over the entire third trimester. These time periods were selected so that we could explore whether there were any differential effects of maternal fat accretion during various trimesters as suggested by previous work.[73,105]

Socioeconomic status (SES): SES was defined using a composite variable, with those who were currently employed (women or her partner) and living in a home with toilet facilities, a nearby water source and electricity characterized into the high SES strata.

Anemia: Anemia was defined as a time dependent hematocrit of less than 30%.

Maternal age: Maternal age was defined as a categorical variable (18-24 years, 25-29 years and 30 years and older).

Gravidity: Gravidity was defined as a binary variable (gravida 1-2 and ≥3).

Statistical analysis: A series of descriptive analyses and crude and multivariate modeling analyses were performed on the data.

Descriptive analyses: Frequencies of demographic, socioeconomic, treatment and pregnancy characteristics were calculated for all participants and by IUGR status using routine categorical data analysis techniques.

Basic survival analysis: In this study, the prevalence of malaria was highest during the baseline visit and declined over gestation due in part to provision of presumptive treatment and active case management of all positive malaria smears. The IUGR outcome was, conversely, less prevalent early in pregnancy and increased until near term. Before making assumptions about the overall risk of IUGR associated with malaria over the whole pregnancy, we felt it was important to ensure that the relationship between these two variables did not change appreciable over time. To investigate this, we ran a simple survival analysis using proportional hazards modeling. Details about this analysis can be found in Appendix A. Overall, we found that the relationship between malaria and IUGR did not change over time (the hazards were proportional over gestation) so we felt comfortable moving forward with the longitudinal analyses discussed below.

***Log-binomial models for the binary IUGR outcome*:** The full data set had 1,120 visits in which an ultrasound measurement was conducted. However, because there is

little variation in fetal weight through the first trimester, IUGR is not typically seen until the second trimester. Thus, for these models, we left-truncated all person-time data at 22 weeks gestation, resulting in a total of 758 visits with an IUGR measurement available for analysis. To account for the missing data that resulted from correction of the maternal weight gain variable, a second set of models were fitted for the 588 visits for which complete data were available for all exposures and potential confounders. Maternal characteristics, under-nutrition and malaria status, and gestational age distribution of IUGR for the 170 visits excluded in the complete data analysis were similar to those of the entire study population.

Unadjusted and adjusted risk ratios (RR) and 95% confidence intervals (CI) were derived from log-binomial regression models for the binary IUGR outcome. Generalized estimating equations methods based on the exchangeable "working" correlation structure were used to account for the correlated nature of repeatedly measuring the outcome on fetuses over the course of pregnancy.[130]

As an initial step, crude models were fitted separately for each exposure (incident and cumulative malaria infection, baseline BMI, maternal height, maternal weight gain and MUAC). As there is evidence to suggest that maternal weight gain and MUAC may have differential fetal growth effects depending on pre-pregnancy nutritional status, models for these two exposures were stratified for baseline BMI status.[73,102] As well, models for these two exposures stratified by trimester of pregnancy were also fitted.[73,105 96,97] The trimester specific models are not adjusted for any confounding factors as the log-binomial models for the weight gain exposure variable would not converge with an exchangeable working correlation matrix due to small sample size.

To investigate if poor maternal nutritional status was an effect measure modifier of the relationship between maternal malaria infection and IUGR, a second set of log-binomial models were fitted that contained the malaria variable, the nutrition variable and an

interaction term between the two variables. Risk ratios for the effect of malaria, at both levels of the various nutritional status variables were derived from this model. A P-value of <0.15 for the interaction term was considered significant.[131]

For all exposures, multivariate models were also constructed using a set of candidate confounders identified from a conceptual model and relevant literature. For each covariate, initial categorical analyses were performed to determine which variables met the statistical definition of confounding, that is, were associated with both the IUGR outcome and the malaria (or nutrition) exposure variables. Next, a backwards elimination procedure was used to determine the set of covariates to include in each multivariate model. From a full model that included all potential confounding variables, the variable with the highest Wald Chi-square P-value was dropped from the model. The dropped variable was retained in the model if the RR for the main exposure changed by greater than 10%; otherwise it was removed and the model was refit dropping the variable with the next highest Wald Chi-square. This process was repeated with all candidate confounders until a final model was chosen. Variables may have also been retained in the model if their inclusion significantly improved the precision of the confidence interval for the main exposure RR.

The following variables were assessed as potential confounders of the malaria exposures: maternal age, gravidity, SES, height, baseline BMI, weight gain, and MUAC change. The following variables were assessed as potential confounders of the under-nutrition exposures: maternal age, gravidity, and SES.

Mixed effect models for the continuous estimated fetal weight variable: We undertook a secondary analysis to assess the effects of malaria and maternal under-nutrition on mean fetal weight. Linear mixed effect models were fitted to data for the 758 follow-up visits that occurred after 22 weeks (see Appendix B for a detailed overview of the mixed effect model). Gestational age was modeled as rounded weeks based on the ultrasound derived date. In the mixed effect models, malaria, treatment, anemia, weight gain and MUAC

changes were analyzed as a time dependent variables and maternal sociodemographic factors, height and baseline BMI were modeled as time independent covariates. Models were built utilizing the following steps.

First, various techniques were used to identify a suitable transformation of time to adequately capture the nonlinear relationship between fetal weight and gestational age. The addition of a linear, quadratic and cubic term for gestational age was found to provide similar fit than models utilizing splines or fractional polynomials, so we decided to utilize the standard polynomial for simplicity.

The first model investigated contained only a random intercept that allowed the fetal weight at the baseline gestational age to vary between women. A -2 log likelihood ratio test was used to compare the random intercept model to a model with only fixed effects. This test showed that the addition of the random intercept was highly significant in the model so a random intercept was considered.

In the next step, we tested the need for adding a random slope component to the model (for the time variable) which would allow the slope or growth trajectory of each fetus to differ. These models had problems converging, even after relaxing the convergence criteria. From the few models that would converge, the addition of a random slope did not explain an appreciable amount of the overall model variation and we therefore decided not to include it in the final models. Models for both malaria exposures and the under-nutrition indicators were fitted. Potential effect measure modification of the IUGR-malaria relationship by maternal under-nutrition was also assessed.

The results of these mixed effect model analyses can be found in Appendix C. A negative value for the beta coefficient indicates a lower fetal weight in the malaria group, whereas a positive value for the beta coefficient indicates higher fetal weight in the malaria infected group.

Power calculations: Power calculations appropriate for longitudinal study design were performed separately for the binary IUGR outcome and the continuous EFW outcome using formulas proposed by Diggle *et al*[132] and Twisk,[133] respectively. The following assumptions were made for both power calculations. A fixed sample size of 177 women, an average of 4 follow-up visits, a correlation between repeated IUGR (or EFW) measurements of 0.23 (obtained from the correlation matrix obtained using generalized estimating equations), and alpha equal to 0.05.

For the binary IUGR outcome, we additionally assumed an estimated risk of IUGR in the unexposed of 0.11 based on data from our sample. Under these assumptions, we have 80% power to detect a RR comparing fetuses exposed and unexposed to malaria of approximately 1.9.

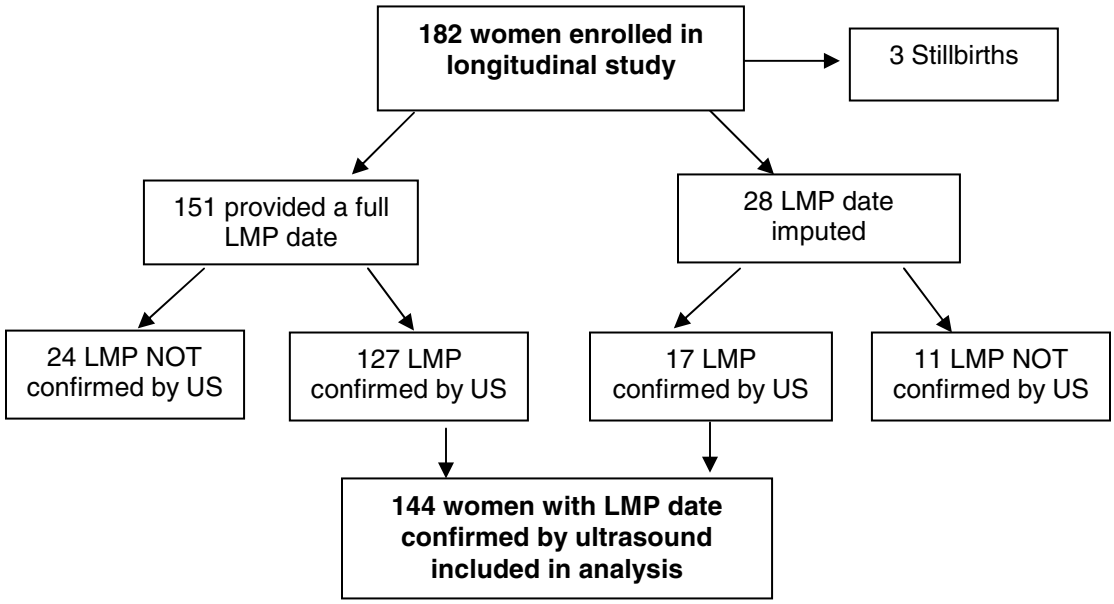
For the continuous EFW outcome, we additionally assumed a ratio of malaria exposed to unexposed women of 0.13 and an average standard deviation of EFW of 500 grams. Under these assumptions, we have 80% power to detect a difference of 202 grams between fetuses exposed and unexposed to malaria.

Sensitivity analyses: We undertook a sensitivity analyses to test the robustness of the RR associations identified in this analysis. We aimed to identify how much the RR and 95% confidence intervals for malaria and under-nutrition would change based on a different definition of IUGR. To do this, we re-analyzed the data using a fetal size nomogram developed specifically from the Congo data (Specific Aim 2) to define IUGR. Details of this analysis can be found in Chapter 5.

Analyses for Specific Aim 2: Utilize prospectively collected ultrasound data to develop a fetal size for gestational age nomogram for a resource poor population with high malaria prevalence

Study population: A total of 182 women participated in the longitudinal study. Two sets of exclusions were made for this analysis (Figure 2.3). First, three stillbirth outcomes were excluded. Next, women who did not have a certain gestational date (defined as LMP date within ±14 days of the ultrasound derived gestational date) were excluded. Of the 182 women enrolled in the study, 151 provided a complete LMP date at enrollment (day, month, year). Of these 151 women, 127 had an ultrasound confirmed LMP date and were included in the analysis. Of the 24 excluded women, nine had an LMP that was <14 days from ultrasound date and 15 had an LMP that was >14 days from the ultrasound date. For the 28 women who did not supply a complete LMP date, we asked them to estimate how many weeks had elapsed since their LMP. Using this information, we imputed an LMP date by multiplying the number of elapsed weeks by seven and subtracting the resultant number of days from the date in which the women was interviewed.

Figure 2.3. Flowchart of exclusion and inclusion criteria for Specific Aim 2



Of these 28 women, 17 had an ultrasound confirmed LMP date and were included in the analysis. Of the 11 excluded women, seven had an LMP that was <14 days from the ultrasound date and 4 had an LMP that was >14 days from the ultrasound date. Thus, the final study sample comprised 144 singleton pregnancies and 755 ultrasound scans. The average number of scan per fetus was four (SD=1) and the average duration between scans was 29 days (SD=4 days).

Initial exploratory analyses were conducted to describe the fetal growth pattern for each woman individually. This helped to provide an idea of the shape of the growth curve in this population and informed which transformations of the estimated fetal weight variable might be needed to achieve good model fit for these data. As well, the distribution of the outcome variable (EFW) at each gestational age was explored to assess normality and variance.

Statistical analysis: In longitudinal studies, the observations collected on one subject over time (often called nested within a subject) are highly correlated. Because of this, some researchers have suggested that reference intervals of fetal size be based on cross-sectional data in which each fetus contributes only a single value to the reference sample.[118] However, advanced statistical techniques, such as the mixed effect model approach used in this analysis, account for the high correlation in longitudinal studies making it possible to utilizing longitudinal data to create a size nomogram (see Appendix B for a detailed overview of the mixed effect model).[133]

For this analysis, we created a fetal size nomogram from longitudinal ultrasound data using the mixed effects model approach for calculating reference intervals suggested by Royston.[125] As a first step, estimated fetal weight (the dependent variable) was log-transformed to ensure normality and reduce heteroscedasticity of the dependent variable residuals. Secondly, gestational age (the independent variable) was modeled using a best fitting second degree fractional polynomial linearizing function.[134] A fractional polynomial is a linear combination that allows for non-negative, negative and fractional powers of a variable

(in this case time *T*). Fractional polynomials are a general class of functions that can capture a variety of shapes to find the "best fit" of the independent variable. A second degree fractional polynomial has the form:

$$f(T;\, p1,\, p2) = \begin{cases} \xi_0 + \xi_1 T^{p1} + \xi_2 T^{p2} & if\ \ p1 \neq p2 \\ \xi_0 + \xi_1 T^{p1} + \xi_2 T^{p2} \log(T) & if\ \ p1 = p2 \end{cases}$$

where $T^{(p)} = T^p$ if $p \neq 0$ and $T^{(p)} = \log(T)$ if $p = 0$.

The SAS procedure PROC MIXED was used to fit a linear mixed effect growth curve for each fetus, which specified both fixed effects and random effects for the intercept and linearized time variable. If Z_{ij} represents the log-transformed estimated fetal weight and X_{ij} represents the factional polynomial transformation of time, then the mean (μ_{ij}) and variance (σ_{ij}^2) of Z_{ij} at transformed time X_{ij} are:

$$\mu_{ij} = E(Z_{ij}) = \beta_{0j} + \beta_{1j}(T_{ij}) + r_{ij}$$

$$\sigma_{ij}^2 = \mathrm{var}(Z_{ij}) = \sigma_{\beta_{0j}}^2 + \sigma_{\beta_{1j}}^2(X_{ij}^2) + 2\sigma_{\beta_{0j},\beta_{1j}}(X_{ij}) + \sigma_{r_{ij}}^2$$

The reference intervals for the untransformed estimated fetal weights, Y_i were calculated from the mean and variance above as $\exp(\mu_{ij} \pm \phi\sigma_{ij})$ where σ_{ij} is the standard error of Z_{ij} and ϕ is the standard distribution function (±1.96 for the 2.5th and 97.5th centile, ±1.645 for the 5th and 95th centiles, ±1.282 for the 10th and 90th centiles, and ±0.674 for the 25th and 75th centiles, and 0 for the 50th centile).

Influence diagnostics: In simple linear regression, influential observations are defined as observations in the data set that appear to have a large influence on the parameter estimates. After the model is fitted, residuals are calculated as the actual value of the response variable minus the model predicted value. The general idea behind influence diagnostics is to quantify the influence of one (or more) observations on the parameter

estimates. This is done by removing each observation from the dataset, refitting the model, and computing statistics based on the change between the full-data and reduced-data estimation. In the case of mixed effect models, an additional level of "influence" must be taken into consideration. Observations can impact not only the fixed effects but also the covariance parameter estimates on which the fixed effects estimates depend.[135]

The mixed effect model described above was fitted using all 144 women eligible for this analysis. Influence diagnostics which iteratively removed each participant from the model were use to identify fetuses who influenced the estimates of the fixed effects and/or precision of the variance and covariance portions of the model. Appendix D provides detailed information about the various influence statistics analyzed as well as tabular and graphical representations of the influence diagnostics for each participant.

As judged by the restricted likelihood distance and the Cook's D statistics, two participants (ID numbers 7192 and 7403) appeared to have the greatest influence on the overall analysis. These participants also influenced the precision of the covariance parameters, as evidenced by a low COVRATIO and high COVTRACE values.

After identifying these two participants as potential influential observations, the model was refitted *excluding* these ID numbers (n=142). This exclusion did slightly improve the normality of the residuals; however, it had very little effect on the parameter estimates (or their standard errors) for the fixed and random portions of the model (Table 2.2).

Accordingly, we found that removing these observations had very little effect on the final mean and percentile values obtained from the model. For example, the difference between the 10^{th} centile values before and after removing the two influential observations were 0 grams for weeks 15 to 23, less than 10 grams for weeks 23 to 30 and 12 to 40 grams from weeks 34 to 40. Thus, we decided to retain those observations in the model and move forward with the full data set of 144 women.

Table 2.2. Beta coefficients and standard errors for the fixed and random components of the model before the after removal of two influential observations

Parameter	Full model		Model after removing influential observations	
	Estimate	Standard Error	Estimate	Standard Error
Fixed effects				
β_{0j}	-0.1195	0.02563	-0.1224	0.02445
β_{1j}	1.0213	0.00404	1.0217	0.00377
Random effects				
$\sigma^2_{\beta_{0j}}$	0.04519	0.01158	0.03592	0.01048
$\sigma^2_{\beta_{1j}}$	0.00132	0.00022	0.00100	0.00025
$\sigma_{\beta_{0j},\beta_{1j}}$	-0.00761	0.00180	-0.00588	0.00159
σ^2_{error}	0.00338	0.00022	0.00335	0.00022

Residual analysis: The mixed effect models used in this analysis are part of the family of generalized linear models, and thus are subject to the statistical assumptions that residual errors are normally distributed with mean zero and a constant variance (homoscadastic). To assess these assumptions, raw and studentized residual errors of the log-transformed outcome were visually inspected by various plots (Figures 2.4 and 2.5). The studentized residuals are the usual residuals divided by their standard errors and always have a mean value of zero. Normality of the errors was determined by visual inspection of normality plots (top right panel shows a histogram of the residuals with Normal density overlay and the bottom left panel shows a Q-Q plot). The data display a normal pattern as evidenced by both plots, with the majority of data falling along the diagonal line of the Q-Q plot. Variance of the errors can be assessed by the scatter plot (top left panel of the figures). The assumption of homoscadasticity also appears to be met as the residuals are spread evenly above and below zero on the plots (constant spread).

Figure 2.4. Raw residuals for the final mixed effect model

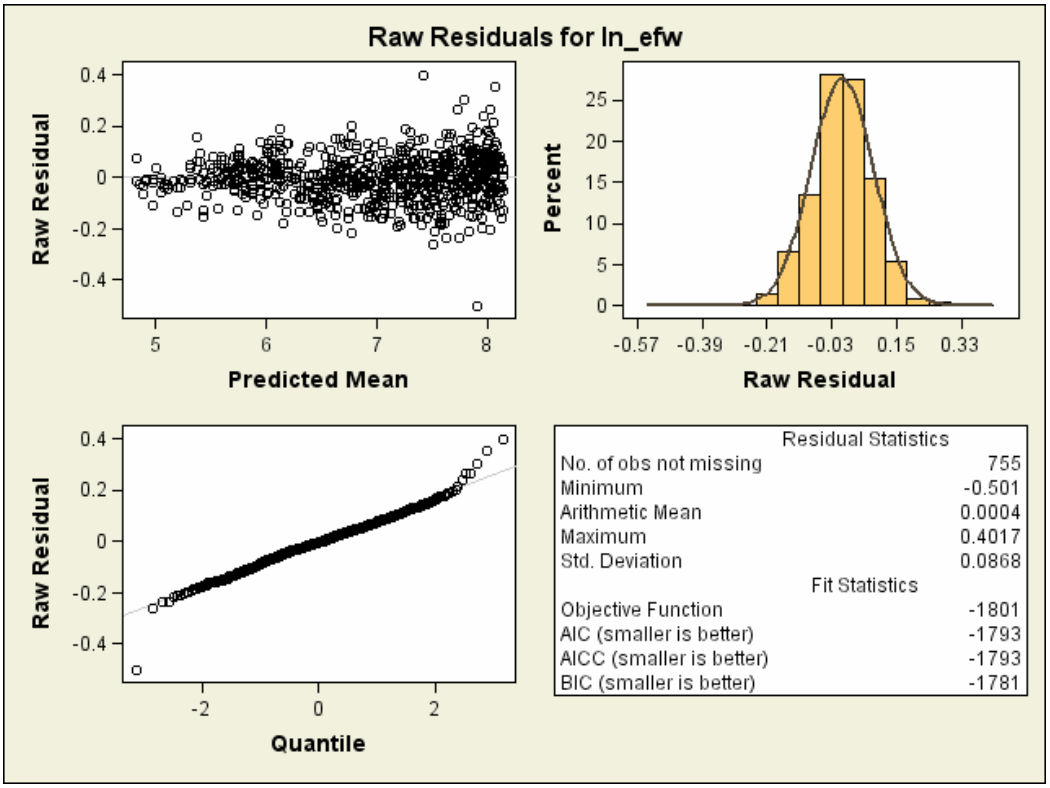
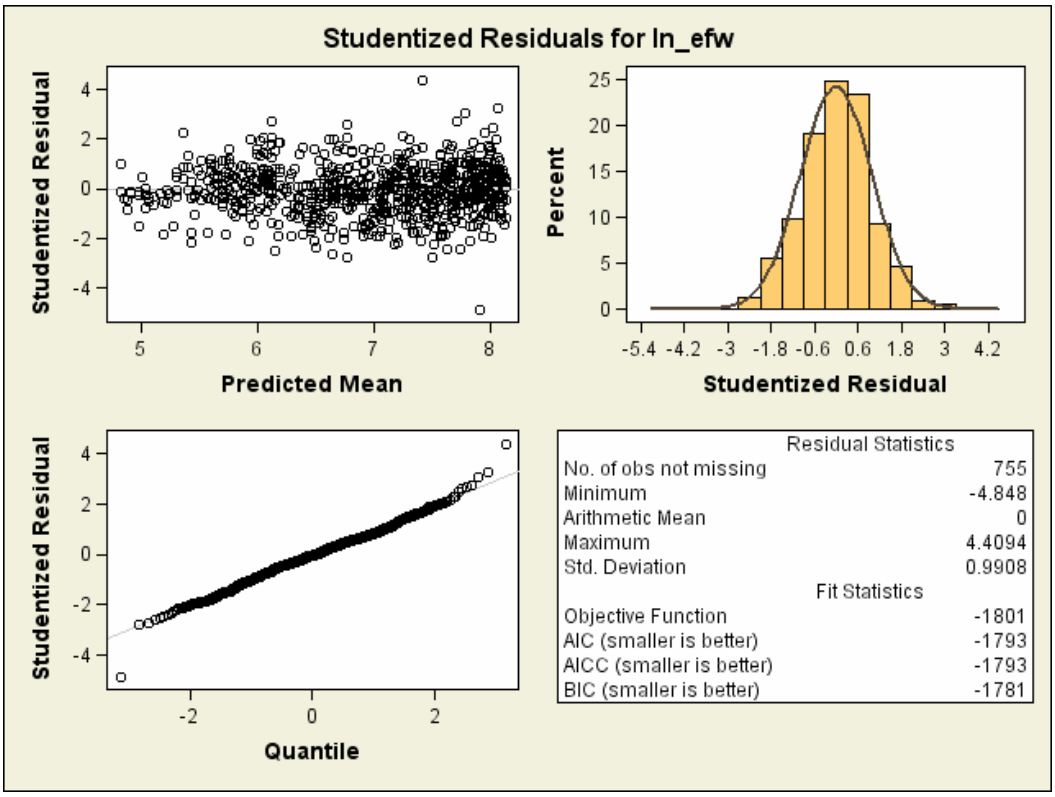


Figure 2.5. Studentized residuals for the final mixed effect model



Comparison with nomograms from industrialized populations: As a final analytic step, we compared our derived reference intervals to nomograms from three industrialized populations (the United States, the United Kingdom and Norway). To compare the 10th, 50th, and 90th percentiles at each gestational age, we calculated the percent difference in estimated fetal weight between the industrialized reference values and the Congo nomogram value as: $[(EFW_{\text{Re}\,ference} - EFW_{Congo} / EFW_{\text{Re}\,ference}) * 100]$. These percent differences were then plotted against gestational age to provide a visual representation of the differences between the curves. Percent differences greater than zero represent a higher EFW value in the industrialized reference compared to the value from the Congo nomogram (overestimation) while percent differences below zero represent lower EFW values in the industrialized nomogram (underestimation).

Sensitivity analysis of the Hadlock formula for estimated fetal weight calculation: Because fetal weight estimation is an important component of a fetal size nomogram, we wanted to explore how the use of another EFW algorithm would affect the centile values of the fetal size nomogram developed for Congo. We re-ran the data analysis and created a new nomogram based upon EFW calculated using the algorithm proposed by Shepard which utilizes two biometric parameters, AC and BPD [log(EFW)= -1.7492 + 0.166*BPD + 0.046*AC - 0.002646*BPD*AC].[136] This formula has been shown to have low systematic and random error in the estimation of fetal weight. Details regarding this sensitivity analysis can be found in Appendix E.

CHAPTER 3:
IMPACT OF MATERNAL MALARIA AND UNDER-NUTRITION ON INTRAUTERINE GROWTH RESTRICTION: A PROSPECTIVE COHORT STUDY IN DEMOCRATIC REPUBLIC OF CONGO

ABSTRACT

Maternal malaria and under-nutrition are established risk factors for small for gestational age (SGA) at delivery; however, a study to investigate their effects on intrauterine growth restriction (IUGR) has never been performed. The authors conducted a prospective, longitudinal ultrasound study of 182 pregnant women in Kinshasa, Democratic Republic of Congo from May 2005 through May 2006. At monthly intervals, malaria infection, maternal anthropometrics, and ultrasound estimated fetal weight were measured. All positive malaria cases were treated and intermittent presumptive therapy with sulphadoxine-pyrimethamine was provided. IUGR was defined as estimated fetal weight below the 10^{th} percentile of a standardized fetal weight curve. Log-binomial models were fitted separately for malaria and maternal anthropometric exposures, accounting for statistical clustering due to repeat IUGR measurements. Variation in the relationship between malaria and IUGR by under-nutrition was also examined. Incident malaria infection was not significantly associated with an increased risk of IUGR (Risk ratio (RR)=1.2, 95% confidence interval (CI): 0.7, 2.2). The risk of IUGR associated with malaria infection was 2 to 7-fold higher among women with poor nutrition. Frequent monitoring and case management of antenatal malaria infections may prevent IUGR, suggesting that antenatal malaria screening policies and nutrient supplementation in malaria endemic areas should be bolstered.

INTRODUCTION

Each year, over 20 million infants worldwide are born with low birth weight (LBW), placing them at significantly increased risk of neonatal mortality and other childhood morbidities.[137] The major contributor of LBW in resource poor settings is small-for-gestational-age at delivery (SGA).[1,15,138] Although some SGA is constitutionally (genetically) determined, most results from intrauterine growth restriction (IUGR), an underlying pathological condition characterized by insufficient transfer of nutrients and oxygen to the fetus and impaired growth of fetal organs and tissues. IUGR may result from limited availability of maternal micro- and macro-nutrients (maternal under-nutrition), or from medical conditions, including hypertension or infection, that impede proper vascularization of the placenta and restrict the transfer of essential nutrients from mother to fetus.[15,16,139]

In resource poor settings such as sub-Saharan Africa, pregnant women are frequently under-nourished and at increased risk of malaria infection, making them particularly vulnerable to delivering an SGA infant. Malaria infection[28,30, 54,140,141,142,143] and maternal anthropometric indicators of under-nutrition, including short stature,[15,80] low pre-pregnancy weight[80,144] or body mass index (BMI),[15,145] inadequate pregnancy weight gain,[97,146] and low maternal upper arm fat mass[15] are independently associated with an increased risk of SGA at delivery.

To date, studies of fetal growth in sub-Saharan Africa have been limited to describing the size of the fetus at birth (SGA). Studies describing *in utero* fetal growth are limited, due largely to a lack of ultrasound resources necessary to diagnose IUGR. The objective of this study was to prospectively describe IUGR in an urban, low-income African population to assess the unique and combined effects of maternal malaria and under-nutrition on the risk of IUGR.

MATERIALS AND METHODS

Study population and recruitment

This prospective longitudinal cohort study was conducted between May 2005 and May 2006 among pregnant women seeking antenatal care at Binza Maternity Hospital in Kinshasa, Democratic Republic of Congo. Binza Maternity Hospital is one of the oldest maternities in Kinshasa and serves a predominately urban population. During routine antenatal registration, all women identified as aged ≥18 years old with a fundal height or last menstrual period derived gestational age of <23 weeks were invited to receive an ultrasound examination to confirm gestational age. All women with an ultrasound confirmed gestational age ≤22 weeks were invited to participate in the longitudinal study. Women with high blood pressure at baseline (systolic >140 mmHg and/or diastolic >90 mmHg), multiple gestations, or a detectable fetal abnormality were excluded. All enrolled women participated in an existing HIV voluntary counseling and testing program at Binza Maternity Hospital. Written informed consent was obtained for all participants, and the protocol was approved by the Institutional Review Boards at the University of North Carolina, Chapel Hill and the University of Kinshasa.

Baseline and follow-up visits

During a baseline interview, sociodemographic characteristics, alcohol, tobacco and drug use, medical and obstetric history, malaria symptoms, and current use of anti-malarial drugs were collected. Using standard techniques,[128] maternal weight was measured to the nearest 0.1 kilogram (SECA digital scale Model 890), and maternal height (without footwear or head cover) and mid upper arm circumference (MUAC) to the nearest 0.1 cm. Blood pressure and body temperature were recorded and a malaria thick smear and hematocrit were prepared from a finger-prick blood sample. All women received an insecticide treated bed net.

Participants returned for monthly follow-up visits until delivery during which the ultrasound

examination and the medical and laboratory examinations (including malaria thick smears) were repeated. In accordance with Congolese National Policy, presumptive therapy with sulfadoxine-pyrimethamine (SP) was provided to all women between 16-27 weeks and 28-32 weeks gestation, regardless of malaria status. In addition, all women with positive parasitemia were treated; SP was the first line treatment; however, quinine, artesunate or camoquine was prescribed if the women had received SP within the preceding month.

Women were instructed to return to the maternity hospital between follow-up visits if they experienced any pregnancy complications or symptoms of malaria. At these visits, a malaria thick smear was prepared and medical care was provided by Binza Maternity's outpatient clinic staff.

Ultrasound measurements

Fetal biometric measurement of the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) were taken to estimate gestational age and fetal weight. In the first trimester (gestational age <14 weeks), crown-rump length was used to estimate gestational age. All ultrasounds were performed by a single ultrasonographer using a GE Logiqbook System. HC and BPD were measured using standard techniques.[147] BPD was measured by placing the calipers from leading edge to leading edge (outer to inner skull) and HC using an ellipse trace of the outline of the fetal head. AC was measured where the junction of the umbilical vein and portal sinus was visible. The ellipse function was used to trace the extreme perimeter of the fetal abdomen. FL was measured along the long axis of the femur from outer to outer margin, including the femoral diaphysis and excluding the epiphyses. Gestational age in weeks and days (first ultrasound scan only) and estimated fetal weight in grams (all ultrasound scans) were calculated using formulas proposed by Hadlock.[126,27]

Laboratory methods

A single microscopist read all malaria thick smears on site during study visits. Smears were stained with Giemsa and read counting the number of asexual parasites against 200 white blood cells and converted to numbers of parasites per μl under the assumption that there were 6000 white blood cells per μl. Anemia was assessed from finger-prick blood samples and the percent hematocrit was recorded (Clay Adams, Readacrit).

Quality control

For quality assurance, a 10% sample of all malaria thick smears was assessed independently by a second laboratory technician. Of 140 slides examined, there was one discordant positive and one discordant negative between the two technicians (sensitivity: 92%, specificity: 99%). In a similar fashion, a 10% sample of ultrasound images was assessed for quality by a maternal-fetal medicine physician at the University of North Carolina, Chapel Hill. Ninety-two percent of reviewed images were deemed adequate for clinical assessment; 7% of questionable quality and 1% poor quality (i.e., not all biometry landmarks clearly visible, shadowing in the image, or poor tracing of the length of circumference).

Definitions

IUGR was defined as a binary outcome of $<10^{th}$ percentile of fetal weight for attained gestational age using the Hadlock fetal weight nomogram.[129] Socioeconomic status (SES) was defined as a composite variable, with those who were currently employed (participant or her partner) and living in a home with toilet facilities, a nearby water source, and electricity characterized as high SES. Anemia was defined as a time-dependent hematocrit of <30%.

The “malaria parasitemia at visit” variable represents a time-dependent measure of an incident antenatal infection that began during the interval between a woman’s previous

visit and the study visit in which the IUGR measurement was obtained. The "cumulative positive parasitemia" variable represents a time-dependent measure of the number of times that a woman had a positive smear up to and including that visit. Short stature was defined as height <150 cm. Maternal weight and height at enrollment were used to calculate baseline body-mass index (BMI), with low BMI defined as <19.8 kg/m^2. Change in MUAC during pregnancy was dichotomized as loss (<0 cm change) versus no change or gain (≥0 cm change), over three distinct periods: (i) monthly change between study visits; (ii) change over the entire second trimester; and (iii) change over the entire third trimester. Change in maternal weight was calculated between each monthly visit, and categorized as low (<1.5 kg) or adequate (≥1.5 kg) monthly weight gain.

Statistical methods

All analyses were performed in SAS, version 8.2 (SAS, Cary, NC). Log-binomial regression models were fitted to estimate risk ratio (RR) and 95% confidence intervals (CI) for IUGR before and after adjusting for potential confounders. To account for repeat outcome measures over the course of pregnancy, the regression models were estimated based on the method of generalized estimating equations with an exchangeable working correlation structure.[130] Owing to reduced variability in fetal weight during the first trimester, IUGR is not typically seen until the second trimester; we therefore left-truncated all person-time at 22 weeks gestation, resulting in 758 IUGR measurements available for analysis.

We discovered a malfunction in the scale used to weigh mothers that affected measurements taken during a three to four week period. All suspect data points were removed before calculation of the weight gain variable leaving 588 visits with complete data for all covariates. A second set of log-binomial models was fitted for these 588 visits; the distribution of maternal socio-demographics, under-nutrition, malaria status, and

gestational age of IUGR for the excluded visits were similar to those of the entire study population (data not shown).

Models were fitted separately for the incident and cumulative malaria exposure variables and for several maternal anthropometric indicators of under-nutrition. As there is evidence that maternal weight gain and MUAC may have differential fetal effects depending on pre-pregnancy nutritional status and timing during pregnancy,[73,102] models for these exposures were stratified for baseline BMI status and trimester of pregnancy, respectively.

To evaluate potential interaction between maternal malaria infection and under-nutrition on the risk of IUGR, an interaction term for malaria and each anthropometric indicator was added to the models. A P-value of <0.15 for the interaction term was considered significant. For all analyses, maternal age, SES, and gravidity were assessed as confounders using a backward elimination procedure with 10% change in estimate criterion; additionally maternal nutritional factors were assessed as confounders in malaria exposure models.

RESULTS

Recruitment and follow-up

Of 1,111 new antenatal care attendees, 33% (n=370) met all initial screening criteria and were scanned to determine gestational age. Of those, 182 were eligible and consented to the longitudinal study (reasons for ineligibility included: absent for dating ultrasound (n=24); twin pregnancy (n=6); no viable fetus present (n=4); and gestational age greater than 22 weeks (n=154)). Five HIV-positive women (3%) were excluded from all analyses for a final sample size of 177 women. These 177 women completed a total of 1,120 study visits. On average, women received five ultrasound scans (range two to eight). One maternal death and one loss to follow-up occurred before delivery. Mean gestational age at enrollment was

18 weeks (standard deviation (SD)=3).

Antenatal malaria

Of 1,120 thick smears, 14% (n=157) were incident positive malaria infections. (Fourteen probable recrudescent episodes that occurred within 14 days of a previous positive, despite receiving treatment, were excluded from analysis). Sixty percent of women had at least one positive smear during follow-up: 38% had a single incident infection, 15% had two incident infections, and 8% were infected three or more times. Baseline malaria prevalence was 27% and generally declined with increasing gestational age (Figure 3.1). The majority of infections were *P. falciparum* (98%). The parasite density ranged from 29 to 13,380 with a mean of 525 (SD 1,734) parasites per μl.

Maternal under-nutrition

At baseline, mean BMI was 23.7 kg/m^2 (SD 3.6). Eleven percent of women were underweight (BMI <19.8 kg/m^2), 66% were normal weight (19.8-26 kg/m^2), 14% overweight (26-29 kg/m^2) and 8% obese (≥ 30 kg/m^2). Three percent of women had short stature (mean height 161.4 cm, SD 6.6). Mean monthly weight gain was 1.6 kg (SD 1.5). Participants generally gained more upper arm fat during the second trimester (mean 0.2 cm, SD 0.8) than the third trimester (mean 0.1 cm, SD 0.8).

IUGR

IUGR was measured at the 758 visits after 22 weeks gestation. A total of 52 fetuses (29%) experienced 76 episodes of IUGR. Of these, 17% were IUGR at only one scan, 8% were IUGR at two scans and 4% at three or more ultrasound scans. Eighty two percent of the IUGR episodes occurred in the third trimester, with peak prevalence between 28 and 33 weeks gestation. Receiving antimalarial treatment at the previous

visit was significantly associated with a reduced risk of IUGR (RR=0.5, 95% CI: 0.3, 0.7). Maternal anemia, younger (18-24) and older (≥30) maternal age and low gravidity were not associated with IUGR in unadjusted analyses (Table 3.1).

IUGR and malaria

We observed no significant effect of a single incident malaria infection on IUGR either in the unadjusted analysis (RR=1.2, 95% CI: 0.7, 2.2) or after adjustment for maternal age and weight gain in the past month (RR=1.6, 95% CI: 0.9, 2.8) (Table 3.2). Compared to fetuses with no antenatal malaria exposure, a three-fold increase in the risk of IUGR was observed among women infected three or more times throughout pregnancy, despite treatment (RR=3.3, 95% CI: 1.3, 8.2).

IUGR and under-nutrition

Associations between anthropometric indicators of under-nutrition and IUGR are shown in Tables 3.3 and 3.4. Inadequate maternal weight gain (defined as <1.5 kg per month) was more strongly associated with an increased risk of IUGR in women with low baseline BMI (RR=2.7, 95% CI: 0.9, 8.5) compared to women with adequate baseline BMI (RR=1.4, 95% CI: 0.9, 2.2). Associations between MUAC loss and IUGR varied by trimester (Table 4), with increased risk seen in the second (RR=2.7, 95% CI: 1.0, 7.7), but not in the third trimester (RR=1.1, 95% CI: 0.6, 1.9). Similarly, low monthly weight gain during the second trimester (RR=5.7, 95% CI: 1.3, 25.0), but not the third (RR=1.1, 95% CI: 0.7, 1.7) was significantly associated with an increased risk of IUGR.

Combined effects of malaria and under-nutrition on IUGR

A detrimental effect of malaria infection on IUGR risk was significantly stronger among under-nourished women (Table 3.5), regardless of which anthropometric indicator was

examined. For example, among women with low baseline BMI, an incident malaria infection increased the risk of IUGR over four-fold (RR=4.5, 95% CI: 1.0, 19.9) compared to those unexposed to malaria. However, at normal baseline BMI levels, there was no observed association between malaria and IUGR (RR=1.1, 95%CI: 0.6, 2.1). A similar pattern was seen among shorter women, women with monthly MUAC loss, and women with low monthly weight gain. Analyses of cumulative malaria resulted in a similar pattern, with the joint effect of low baseline BMI and cumulative malaria associated with the largest risk (RR=7.0, 95% CI: 3.2, 15.3).

DISCUSSION

A longitudinal study of IUGR has never previously been carried out in a malaria-endemic area. We measured fetal growth *in utero* and identified IUGR in nearly a third of fetuses in this urban, sub-Saharan Africa population. This analysis focused on two component causes of IUGR that have heightened relevance in resource poor settings, malaria infection and maternal under-nutrition. In this Congolese population, we found that malaria infection alone was only modestly associated with an increased risk of IUGR, and that a significant independent effect of malaria was seen only among women with three or more incident infections during gestation. We also found that the effect of maternal malaria varied significantly by maternal nutritional status, and that the highest risks of IUGR were evident among the most under-nourished women.

Antenatal malaria may lead to IUGR through accumulation of *P. falciparum* infected erythrocytes, and immunity related monocytes and pro-inflammatory cytokines in the placental intervillious space. Hemozoin, a byproduct of parasite hemoglobin digestion, can also be found in phagocytic leucocytes and within fibrin deposits in the intervillous space.[12] This build-up can lead to thickening of the trophoblast basement membrane and effect uteroplacental arterial development, thus decreasing maternal-fetal nutrient exchange.[42,45]

Previous studies conducted in areas of high *P. falciparum* transmission have consistently reported associations between SGA and both antenatal[30,54,140] and placental malaria infection.[30,54,140,141,142]

Our findings are at variance with these earlier studies, with differences most likely stemming from the fact that we screened for malaria at monthly intervals and treated all positive antenatal parasitemia. Further, virtually all women received two presumptive doses of SP. Routine screening and treatment may have eliminated parasites before they had adequate time to sequester in the placenta and cause damage to the placental vasculature, potentially minimizing the effect of malaria infection. In this study, treatment was independently protective against both incident malaria infection and IUGR, and led to higher attained fetal weight (data not shown), further supporting this hypothesis. Our findings are consistent with two studies of low malaria transmission areas (the Thai-Burmese border and highlands of Ethiopia) that also had frequent monitoring and treatment of antenatal parasitemia.[148,149] Collectively, these findings suggest that even in areas of high malaria transmission, prompt identification and treatment of sub-clinical malaria infections may prevent fetal growth restriction from occurring.

Maternal under-nutrition was both an independent risk factor for IUGR and a significant modifier of the association between malaria and IUGR. Chronic pre-pregnancy under-nutrition, low weight gain and inadequate accumulation of fat stores during pregnancy can render a woman incapable of meeting the substantial metabolic demands of pregnancy.[8] The mean monthly weight gain of 1.6 kg for these Congolese women was similar to weight gain reported in other resource poor settings.[70] As suggested in previous studies, we found that maternal weight gain was more strongly associated with IUGR in women with low baseline BMI, [102,146] and that low weight gain in the second trimester increased IUGR risk.[93,96] Our data also corroborate previous findings that failure to accrue arm fat during the second trimester, but not the third trimester, is associated with lower fetal weight.[73]

The association between malaria and IUGR was consistently two- to seven-fold higher among women with evidence of under-nutrition. In resource poor settings, it has long been recognized that childhood malnutrition and attendant sequellae influence susceptibility to and severity of malaria infection.[31] Repercussions of childhood under-nutrition and malaria infection, such as stunting and low BMI, place pregnant women at increased risk of poor birth outcomes. Further, the joint effects of adult under-nutrition and malaria infection may act on similar physiologic pathways to reduce uteroplacental blood flow[8] and decrease maternal-fetal oxygen transfer.[34]

Limitations and strengths

Although malaria and under-nutrition are common causes of IUGR, other risk factors, such as chromosomal abnormalities, preeclampsia or substance use may have played a role. We attempted to minimize the effects of other medical factors through our exclusion criteria and found that reported tobacco, alcohol and drug use were minimal. The extent to which fetuses in our cohort were constitutionally small-for-age versus truly pathological IUGR cases remains unknown, thereby leading to the possibility of some misclassification of IUGR. Moreover, our IUGR definition utilized a fetal weight-for-age nomogram created from an industrialized country, which may have overestimated the proportion of IUGR fetuses in this resource poor population. We may have also overestimated true pre-pregnancy weight by using maternal weight at enrollment as a proxy; however, any resultant bias is likely minimal because participants were enrolled early in pregnancy, before women in resource poor settings tend to gain significant pregnancy weight.[70] Lastly, this study was designed as a pilot to prepare laboratory, ultrasound and clinical operating procedures for a larger subsequent trial, and thus the sample size was selected for convenience, rather than to maximize power. A larger longitudinal study to replicate these findings is warranted.

Despite these limitations, our findings suggest that active antenatal screening and

effective treatment of maternal malaria infections, regardless of symptoms, may reduce the prevalence of IUGR and consequently the burden of infant mortality. The heightened risk of IUGR seen among women who were both under-nourished and malaria-infected underscores the importance of incorporating maternal anthropometric screening and nutritional supplementation into routine antenatal care in malaria endemic areas.

Figure 3.1. Prevalence of parasitemia by gestational age, Kinshasa, Democratic Republic of Congo, 2005-2006

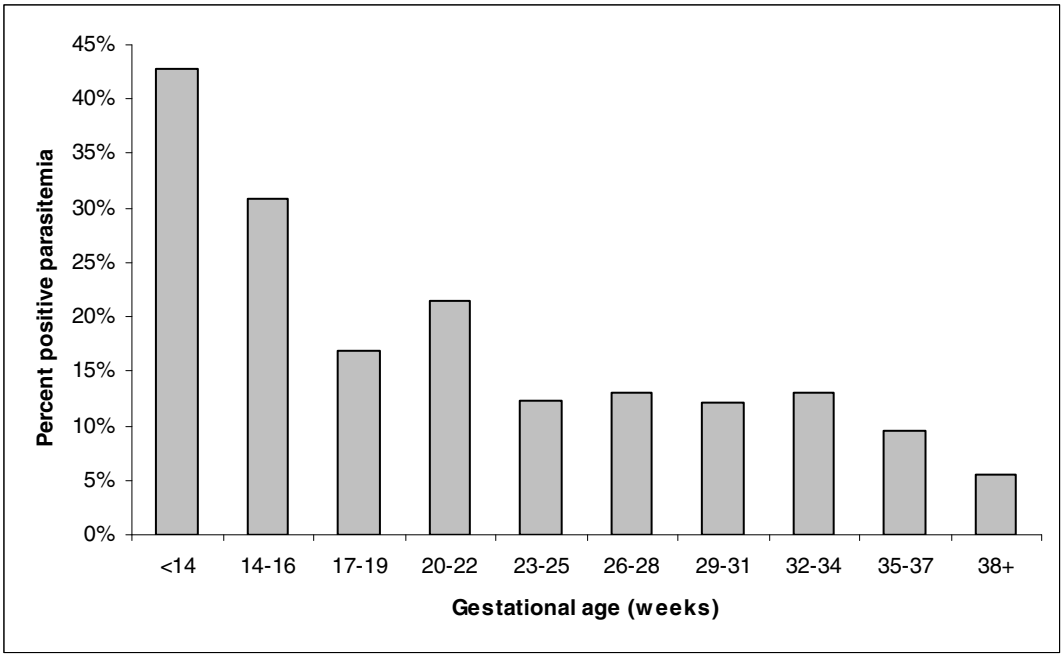


Table 3.1. Baseline and visit-specific characteristics of pregnant women and risk ratios (RR) and 95% confidence intervals (CI) for IUGR, Kinshasa, Democratic Republic of Congo, 2005-2006

	Percent*	RR†	95% CI
Maternal age (years)			
18-24	32%	1.7	0.8, 3.4
25-29	32%	1.0	Ref
≥30	36%	1.4	0.7, 2.9
Socioeconomic status			
High	14%	1.1	0.5, 2.4
Low	86%	1.0	Ref
Gravida			
1-2	41%	1.5	0.8, 2.6
≥3	59%	1.0	Ref
Fetal gender			
Male	47%	0.6	0.3, 1.0
Female	53%	1.0	Ref
Treated in previous month			
Yes	49%	0.5	0.3, 0.7
No	51%	1.0	Ref
Hematocrit at visit			
<30	11%	0.9	0.4, 2.1
≥30	89%	1.0	Ref

* Maternal age, socioeconomic status and gravidity recorded at baseline only (n=177); treatment recorded at baseline and each follow-up visit (n=758); hematocrit recorded at baseline and every other follow-up visit (n=388).
† Unadjusted. Model included 758 study visits and 76 episodes of IUGR.

Table 3.2. Risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and incident and cumulative malaria infection, Kinshasa, Democratic Republic of Congo, 2005-2006

	Percent*	RR†	95% CI	RR‡	95% CI	RR§	95% CI
Malaria parasitemia at visit							
Positive	11%	1.2	0.7, 2.2	1.2	0.7, 2.2	1.6	0.9, 2.8
Negative	89%	1.0	Ref	1.0	Ref	1.0	Ref
Cumulative malaria parasitemia up to visit							
0	52%	1.0	Ref	1.0	Ref	1.0	Ref
1	34%	0.9	0.5, 1.7	0.9	0.5, 1.6	0.8	0.4, 1.5
2	10%	1.2	0.5, 2.6	1.2	0.5, 2.6	1.6	0.8, 3.3
≥3	4%	2.4	0.9, 6.5	2.3	0.8, 6.3	3.3	1.3, 8.2
		$P_{Trend} = 0.31$		$P_{Trend} = 0.33$		$P_{Trend} = 0.11$	
Cumulative malaria parasitemia up to visit							
≥2 positive	14%	1.5	0.8, 2.9	1.5	0.8, 2.8	2.1	1.2, 3.6
<2 positive	86%	1.0	Ref	1.0	Ref	1.0	Ref
≥3 positive	4%	2.4	0.9, 6.3	2.3	0.9, 6.0	3.2	1.3, 7.7
<3 positive	96%	1.0	Ref	1.0	Ref	1.0	Ref

* Malaria status recorded at baseline and each follow-up visit (n=758).
† Unadjusted. Model included 758 study visits and 76 episodes of IUGR.
‡ Adjusted for age. Model included 758 study visits and 76 episodes of IUGR.
§ Adjusted for age and weight gain. Model included 588 visits with complete data for weight gain and 66 episodes of IUGR.

Table 3.3. Risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and maternal anthropometric indicators, Kinshasa, Democratic Republic of Congo, 2005-2006

	Percent*	RR†	95% CI	RR‡	95% CI	RR§	95% CI
Time-independent variables							
Baseline BMI							
<19.8 kg/m^2	11%	1.1	0.6, 2.2	1.0	0.5, 2.0	1.0	0.5, 2.0
≥19.8 kg/m^2	89%	1.0	Ref	1.0	Ref	1.0	Ref
Short stature							
<150 cm	3%	1.5	0.4, 5.4	1.4	0.4, 4.7	1.7	0.5, 5.5
≥150 cm	97%	1.0	Ref	1.0	Ref	1.0	Ref
Time-dependent variables							
MUAC change per month #							
Baseline BMI <19.8 kg/m^2	11%	1.5	0.5, 4.2	1.5	0.5, 4.2	1.1	0.3, 3.6
Baseline BMI ≥19.8 kg/m^2	89%	1.0	0.7, 1.5	1.0	0.7, 1.5	0.9	0.6, 1.4
Maternal weight gain per month**							
Baseline BMI <19.8 kg/m^2	13%	2.7	0.9, 8.5	--	--	2.7	0.9, 8.5
Baseline BMI ≥19.8 kg/m^2	87%	1.3	0.8, 2.0	--	--	1.4	0.9, 2.2

* Baseline BMI and height recorded at baseline only (n=177); MUAC data reflects percent of women with MUAC change <0 cm at each level of BMI (n=711 visits); weight gain data reflects percent of women with <1.5 kg of weight gain per month at each level of BMI (n=588 visits).
† Unadjusted. Model included 758 study visits and 76 episodes of IUGR for BMI, stature, and MUAC; 588 visits and 66 episodes of IUGR for weight gain.
‡ Adjusted for age. Model included 758 study visits and 76 episodes of IUGR.
§ Adjusted for age. Model included 588 visits with complete data for weight gain and 66 episodes of IUGR.
# Comparing MUAC change in the previous month of <0 cm vs. ≥0 cm.
** Comparing maternal weight gain in the previous month of <1.5 kg per month vs. ≥1.5 kg per month.

Table 3.4. Trimester specific risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and change in MUAC and weight gain, Kinshasa, Democratic Republic of Congo, 2005-2006

	IUGR in second trimester		IUGR in third trimester	
	RR*	95% CI	RR†	95% CI
MUAC change*				
Over entire 2nd trimester	2.7	1.0, 7.7	--	--
Over entire 3rd trimester	--	--	1.1	0.6, 1.9
Weight gain per month†				
Second trimester	5.7	1.3, 25.0	--	--
Third trimester	--	--	1.1	0.7, 1.7

* Unadjusted. Models included 217 study visits and 14 episodes of IUGR in the second trimester and 526 study visits and 62 episodes of IUGR in the third trimester. Comparing MUAC change over the whole trimester of < 0 cm vs. ≥ 0 cm.
† Unadjusted. Models included 160 study visits and 12 episodes of IUGR in the second trimester and 428 study visits and 54 episodes of IUGR in the third trimester. Comparing maternal weight gain in the previous month of <1.5 kg per month vs. ≥1.5 kg.

Table 3.5. Risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and malaria, stratified by maternal anthropometrics, Kinshasa, Democratic Republic of Congo, 2005-2006

	RR*	95% CI	RR†	95% CI	RR‡	95% CI
Malaria parasitemia at visit (Positive vs. negative)						
Baseline BMI <19.8 kg/m^2	**4.8**	**2.0, 12.1**	**4.9**	**2.0, 11.9**	**7.0**	**2.9, 17.1**
Baseline BMI ≥19.8 kg/m^2	**0.8**	**0.4, 1.7**	**0.8**	**0.4, 1.7**	**0.9**	**0.4, 1.8**
Height <150 cm	**5.1**	**1.1, 22.9**	**4.5**	**1.0, 19.9**	3.3	0.8, 13.8
Height ≥150 cm	**1.1**	**0.6, 2.1**	**1.1**	**0.6, 2.1**	1.3	0.7, 2.5
MUAC gain <0 cm in past month	**2.4**	**1.2, 5.0**	**2.4**	**1.2, 5.0**	**3.2**	**1.8, 5.9**
MUAC gain ≥0 cm in past month	**0.5**	**0.1,1.9**	**0.5**	**0.1,1.9**	**0.3**	**0.1,1.5**
Weight gain <1.5 kg in past month	1.7	0.8, 3.6	--	--	1.8	0.9, 3.8
Weight gain ≥1.5 kg in past month	1.4	0.6, 3.0	--	--	1.4	0.6, 3.0
Cumulative malaria parasitemia (≥ 2 positive vs. < 2 positive)						
Baseline BMI <19.8 kg/m^2	**7.3**	**3.3, 15.9**	**7.0**	**3.2, 15.3**	**9.2**	**4.1, 20.7**
Baseline BMI ≥19.8 kg/m^2	**1.1**	**0.5, 2.3**	**1.1**	**0.5, 2.3**	**1.3**	**0.6, 2.9**
Height <150 cm	2.1	0.2, 24.3	2.6	0.4,19.2	2.0	0.3,12.8
Height ≥150 cm	1.5	0.7, 2.9	1.4	0.7, 2.8	1.9	1.0, 3.6

Table 3.5., continued

	RR*	95% CI	RR†	95% CI	RR‡	95% CI
MUAC gain <0 cm in past month	2.0	0.9, 4.3	2.0	0.9, 4.4	**3.2**	**1.5, 6.7**
MUAC gain ≥0 cm in past month	1.1	0.5, 2.5	1.1	0.5, 2.4	**1.3**	**0.5, 3.0**
Weight gain <1.5 kg in past month	2.4	1.2, 4.5	--	--	2.3	1.3, 4.3
Weight gain ≥1.5 kg in past month	1.7	0.8, 4.0	--	--	1.8	0.8, 4.0

* Unadjusted. Model included 758 study visits and 76 episodes of IUGR for BMI, stature, and MUAC; 588 visits and 66 episodes of IUGR for weight gain.
† Adjusted for age. Model included 758 study visits and 76 episodes of IUGR.
‡ Adjusted for age. Model included 588 visits with complete data for weight gain and 66 episodes of IUGR.

Note: RR pairs highlighted in bold indicate a significant P-value for the interaction term between malaria and the anthropometric indicator (P-value < 0.15).

CHAPTER 4:
AN ULTRASOUND DERIVED FETAL SIZE NOMOGRAM FOR A SUB-SAHARAN AFRICAN POPULATION: A LONGITUDINAL STUDY

ABSTRACT

We created a fetal size nomogram for use in low resource settings and compared the derived centiles to reference intervals from industrialized countries. Fetal biometric measurements were obtained monthly from pregnant women enrolled in a longitudinal ultrasound study in Kinshasa, Democratic Republic of Congo. Women with a singleton pregnancy and certain gestational dates (ultrasound derived gestational age within 14 days of LMP estimate) were included in the analysis (n=144). A total of 755 monthly ultrasound scans were included with an average of four scans per fetus (SD=1). Estimated fetal weight (EFW) was calculated at each ultrasound using the Hadlock algorithm. A linear mixed effect model that incorporated random effects for the intercept and slope was fitted to log-transformed estimated fetal weight as a function of gestational age. Reference intervals (5^{th}, 10^{th}, 50^{th}, 90^{th} and 95^{th} centiles) were then derived from these models. The 50^{th} centile EFW for this low resource sub-Saharan Africa population were on average, consistently lower than fetuses born in industrialized populations. Differences observed in the outer centiles were largely due to variation in study design and statistical techniques. This fetal size nomogram should improve diagnosis of IUGR in resource poor settings with a high incidence of maternal malaria infection.

INTRODUCTION

Ultrasound assessment of intrauterine growth can be used as a clinical tool to identify abnormally growing fetuses at risk of poor birth outcome and to evaluate fetal response to maternal interventions. Fetal size nomograms are used to assess the estimated fetal weight (EFW) of a fetus of known gestational age against a reference standard at a certain point in gestation. Conventionally, fetal weight estimates below the 10th centile are suggestive of intrauterine growth restriction (IUGR).[150] This definition, however, is highly dependent on the origin of the reference population and most currently available nomograms were derived from industrialized, primarily Caucasian, populations. Some studies have demonstrated racial and ethnic variation in fetal growth patterns[151-153] and maternal and environmental factors are also likely to play a role.[154] For example, in low resource sub-Saharan Africa populations, maternal HIV and malaria infection, chronic under-nutrition, and micronutrient deficiency are often endemic and are highly associated with lower birth weight.[2,15]

Although minimal data exist regarding *in utero* fetal growth patterns in sub-Saharan Africa, mean birth weights (2,900-3,200 grams) are lower than industrialized countries (3,300-3,500 grams) and rates of SGA are two to three fold higher (15% in sub-Saharan Africa versus 4-8% in the United States and Europe).[15,1] It is likely that *in utero* growth patterns also vary between these populations, and fetal weight nomograms created from industrialized countries may not serve as appropriate benchmarks for identifying growth restricted fetuses in these underserved populations. If a nomogram identifies an inappropriately large proportion of fetuses as IUGR, the clinical usefulness of this tool to distinguish fetuses that are truly growth compromised and would benefit from maternal interventions such as nutritional supplementation or malaria treatment, is vastly reduced.

The purpose of this study was to develop a fetal size nomogram for use in resource poor settings with a high prevalence of maternal malaria infection and under-nutrition. The derived

reference intervals are also compared to commonly used nomograms from industrialized countries to assess the applicability of such nomograms for low resource populations.

MATERIALS AND METHODS

Study population

The study population consisted of 182 women enrolled in a prospective longitudinal cohort study conducted between May 2005 and May 2006 at the Binza Maternity Hospital in Kinshasa, Democratic Republic of Congo. Binza Maternity Hospital is one of the oldest maternities in Kinshasa and serves a predominately urban low-income population. The purpose of this study was to understand the effects of maternal malaria and nutritional status on fetal growth. At baseline, all participants had a singleton pregnancy and no evidence of high blood pressure (systolic >140 mmHg and/or diastolic >90 mmHg) or ultrasound detected fetal abnormality. Women were enrolled before 22 weeks gestation and returned to the maternity hospital for monthly follow-up visits during which malaria status and maternal anthropometrics were assessed and fetal biometry measured by ultrasound. Delivery information is available for 98% of enrolled women. All participants provided written informed consent to participate in the study and the protocol was approved by the Institutional Review Boards of the University of North Carolina, Chapel Hill and the University of Kinshasa.

We excluded three stillbirths and 35 women with uncertain gestational dates (LMP date differed from ultrasound derived date by more then ±14 days) leaving 144 fetuses in this analysis. Women who developed complications during pregnancy or at delivery were not excluded in order to obtain a representative population. Five newborns with structural malformations identified at delivery (one cleft palate/eye orbit deformity, one mild nuchal hump, one club foot/lower limb deformity, and two infants with polydactyly) were also included.

Sixty one percent of women had at least one positive malaria smear. Of those, 61% were positive only once, 30% had two positives and 9% had three or more positive smears. All enrolled women received two courses of presumptive malaria therapy with sulfadoxine-pyrimethamine between 16-27 weeks and 28-32 weeks gestation, regardless of malaria status. In addition, all antenatal positive parasitemias were treated. Five women (3%) were HIV positive. Maternal anthropometrics, hematocrit and prevalence of positive malaria parasitemia were similar to other sub-Saharan African populations,[153,28,92] however the HIV prevalence is among the lowest for antenatal populations.[155] Tobacco and alcohol use during pregnancy was minimal.

There were 11 cesarean sections (8%) and 14 women (10%) with delivery complications (two premature rupture of the membrane, eight breech deliveries and/or prolonged or obstructed labor, four post-partum hemorrhages). Six infants (4%) were delivered at <37 weeks gestation and there were three early neonatal deaths (2%). Infant anthropometrics and length of gestation were similar to delivery outcomes in other areas with endemic malaria.[156] The prevalence of low birth weight (<2,500 grams) and preterm delivery were lower than reported for other populations receiving presumptive malaria treatment.[53,157] Overall, this population of mothers and fetuses could be considered an adequate representation of a typical sub-Saharan African population.

Ultrasound measurements

All ultrasounds were performed using a GE Logiqbook System by a single Congolese obstetrician-gynecologist. Biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) were measured using standard techniques.[147] Estimated fetal weight in grams was calculated using the Hadlock algorithm[27].

All ultrasound images were saved onto CD-ROM as *jpeg* files. A 10% sample of ultrasound images was assessed for quality by a maternal-fetal medicine physician at the

University of North Carolina, Chapel Hill. Ninety-two percent of reviewed images were deemed adequate for clinical assessment; 7% of questionable quality and 1% of poor quality (i.e., not all biometry landmarks clearly visible, shadowing in the image or poor tracing of the length of circumference). Intra-operator variability in measuring fetal biometry was assessed in ten patients. The correlation between two independent measurements on the same fetus was r=0.99 for each of BPD, HC, and FL, and r=0.98 for AC.

Statistical analysis

All analyses were performed in SAS, version 9.1 (SAS, Cary, NC). Due to sparse data in the first trimester and post-term, analyses were limited to 15 and 40 weeks gestation. Gestational age was calculated in days according to the ultrasound derived dates. The 50th centile and outer reference centiles (i.e., 5th, 10th, 90th, and 95th) were derived using a linear mixed effect model approach.[125] This method accounts for variability in estimated fetal weight at both the between-subject and within-subject levels, by incorporating subject-specific effects for the intercept and growth (slope) component. Briefly, estimated fetal weight (the dependent variable) was log-transformed to ensure normality and reduce heteroscedasticity of the dependent variable residuals. The independent (time) variable was a best fitting second degree fractional polynomial linearizing function of gestational age in days.[134] If Z_{ij} represents the log-transformed estimated fetal weight and X_{ij} represents the factional polynomial transformation of time, then the mean (μ_{ij}) and variance (σ_{ij}^2) of Z_{ij} at transformed time X_{ij} are:

$$\mu_{ij} = E(Z_{ij}) = \beta_{0j} + \beta_{1j}(T_{ij}) + r_{ij}$$

$$\sigma_{ij}^2 = \mathrm{var}(Z_{ij}) = \sigma_{\beta_{0j}}^2 + \sigma_{\beta_{1j}}^2(X_{ij}^2) + 2\sigma_{\beta_{0j},\beta_{1j}}(X_{ij}) + \sigma_{r_{ij}}^2$$

where $\sigma^2_{\beta_{0j}}$ represents the (between-women) variance of the random intercepts, $\sigma^2_{\beta_{1j}}$ represents the (between-women) variance of the random slopes, $\sigma_{\beta_{0j},\beta_{1j}}$ is the covariance between them and σ^2_{error} is the estimated within-women variance.

The reference intervals for the untransformed estimated fetal weights, Y_i are calculated from the mean and variance above as $\exp(\mu_{ij} \pm \phi\sigma_{ij})$ where σ_{ij} is the standard error of Z_{ij} and ϕ is the standard distribution function (±1.96 for the 2.5th and 97.5th centile, ±1.645 for the 5th and 95th centiles, ±1.282 for the 10th and 90th centiles, and ±0.674 for the 25th and 75th centiles, and 0 for the 50th centile).

The raw and studentized residual errors were visually inspected by plots of the errors against gestational age. Normality in the distribution of the errors was determined by visual inspection of plots and subsequently confirmed using the Shapiro-Wilks test. Influence diagnostics, based on iteratively deleting each subject from the model, was used to identify fetuses that over influenced the estimates of the fixed effects and/or precision of the variance estimates on overall model fit.

We compared our derived reference intervals to those developed from three industrialized populations (see Table 4.3 for details about each study). For each gestational week, we calculated the percent difference in estimated fetal weight between the industrialized reference value and the Congo nomogram as $[(EFW_{\text{Re}ference} - EFW_{Congo} / EFW_{\text{Re}ference}) * 100]$. Thus, percent differences greater than zero represent a higher EFW value in the industrialized reference compared to the value from the Congo nomogram (overestimation) while percent differences below zero represent lower EFW values in the industrialized nomogram (underestimation).

RESULTS

The reference intervals were based on 144 singleton fetuses that underwent 755 ultrasound scans. The average number of scans per fetus was four (SD=1) and the average duration between scans was 29 days (SD=4 days). Maternal and fetal characteristics of the study population are provided in Table 4.1. The mean maternal age at enrollment was 27.4 years (SD=5.5) and 29% of women were primigravid. The distribution of ultrasound examinations and descriptive statistics of the estimated fetal weight variables by gestational age are provided in Table 4.2. The best fitting fractional polynomial of time T_{ij} was a quadratic polynomial defined as $X_{ij} = 4.70794 + 0.03148T_{ij} - 0.0007T_{ij}^2$. The mean and variance of the mean were estimated with the following fitted regression models:

$$\mu_{ij} = E(Z_{ij}) = -0.1195 + 1.0213(X_{ij})$$

$$\sigma_{ij}^2 = \text{var}(Z_{ij}) = 0.04519 + 0.001323(X_{ij}^2) + 2(-0.00761)X_{ij} + 0.003371$$

Figure 4.1 shows the predicted centiles for gestational ages 15 to 40 weeks superimposed on the raw estimated fetal weight data. Growth is continuously linear through term, with variance increasing with advancing gestational age. Raw and studentized residuals of estimated fetal weight were obtained from the mixed model regression equations presented above and plotted against gestational age (Figure 4.2). The residuals are evenly dispersed above and below zero at all gestational ages suggesting that the logarithmic transformation of estimated fetal weight was adequate to meet the assumption of constant variance of the residual errors.

Comparison to nomograms from industrialized countries

Figure 4.3 shows the percent difference in estimated fetal weight comparing the reference intervals from Congo to three nomograms of industrialized populations. For the 50th centile, all 3 nomograms overestimated the 50th centile value for Congolese fetuses by roughly 5% to

12%, and the difference tended to be highest at earlier gestational ages.

The Hadlock 10th centiles slightly underestimated the Congo 10th centile at early gestational ages whereas the 90th centile of consistently overestimated fetal weight early in gestation; both differences became less pronounced near term. Both the 10th and 90th centiles derived by Gallivan consistently overestimated the corresponding Congo centiles. The Johnsen nomogram also consistently overestimated the inner and outer centiles, however for both, the overestimation gradually decreased with advancing gestation.

DISCUSSION

This analysis shows that the 50th centile estimated fetal weights in this low resource sub-Saharan Africa population are, on average, consistently lower than fetuses born in industrialized populations. Previous ultrasound studies of fetal biometry conducted in Africa in the late 1980's found similar results. In an investigation of 200 cross-sectional ultrasound measurements, Ayangade and Okonofau[110] found that the BPD of Nigerian fetuses were consistently lower between 20 to 40 weeks when compared to a European standard. These findings were similar to other studies from Nigeria[113] and Zimbabwe.[112] A study of AC based upon a combination of cross-sectional and longitudinal ultrasound scans of 558 women also found consistently lower mean AC measurements in Nigerian fetuses compared to a European standard.[111]

We hypothesize that environmental factors including maternal nutritional deficiencies and infections such as malaria and HIV, may explain some of the difference in achieved fetal size in this low resources population. Because a nomogram serves as reference data, it should relate to normal fetuses and be derived from as unselected a population as possible.[118] In order to be most useful as a clinical tool, the nomogram should adequately represent the range of maternal characteristics and environmental conditions relevant to the population being screened. In the context of sub-Saharan Africa, excluding women with poor nutritional

status or antenatal malaria infections would create an “artificially healthy” source population, which is likely to produce fetuses with higher in utero weights. This would lead to reference intervals that are shifted upward and consequently incorrectly over diagnose IUGR. Our population was comprised of women living in an urban, low income setting with a high prevalence of malaria and evidence of maternal under-nutrition. This population provides a reasonable representation of normative environmental conditions for women living in similar sub-Saharan African communities. However, it should be noted that the prevalence of poor birth outcomes including LBW and PTD were lower in this population compared to other countries in the region. These differences are likely related to the lower prevalence of HIV in this Congo population as well as the high level of antimalarial prophylaxis and treatment coverage achieved in the parent study.

Inconsistencies in study design and statistical methodologies used in past studies are also likely to explain some of the observed difference. For example, the choice of algorithm used to estimate fetal weight could influence the centile values of a nomogram. A recent study that compared 25 different ultrasonographic algorithms for estimating fetal weight demonstrated a range of mean absolute error between estimated fetal weight and birth weight from 263 to 646 grams.[158] We chose the Hadlock algorithm for this analysis to facilitate comparability to other nomograms and because composite algorithms that combine several biometric parameters together provide more accurate weight estimates than those that use fewer parameters.[27,159] The Hadlock algorithm provided a reasonable estimate of fetal weight in our population, with absolute differences between the predicted 50th centile EFW value and the post-natal actual birth weight measurement of 3.7%, 7.1%, and 6.9% for 38, 39 and 40 weeks, respectively. We also found the Hadlock algorithm to be fairly robust to misspecification of gestational dates. A sensitivity analysis of the Hadlock algorithm that compared the modeled 50th centile fetal weight values obtained from a subset of women with and without certain gestational dates, demonstrated a difference of only 6 to 20 grams, with

the greatest difference occurring between 30 and 35 weeks.

Secondly, varying methods used to characterize gestational dates may effect fetal weight estimation and IUGR diagnosis. For example, utilizing ultrasound derived gestational age, as opposed to LMP derived age, often shifts the mean gestational age of the population to the left (earlier) by approximately two weeks, consequently lowering the proportion of fetuses classified as IUGR and inflating the preterm delivery rate.[160,161] We only included women in our analysis with ultrasound confirmed LMP dates. The definition of gestational age (i.e., completed weeks, exact weeks, or days) can also lead to discrepancies in EFW estimation. Completed week definitions may introduce systematic errors for most biometric parameters that rapidly increase during gestation. Our nomogram was based on gestational age classified in days as previously recommended,[162] thereby avoiding the "averaging" of fetal weights within six-day intervals.

In clinical practice, values at the extremes of a nomogram are typically of more interest and greater importance than mean values. Variation in the lower centile values between different populations can lead to over- or under diagnosis of IUGR, and highlight the importance of utilizing nomograms created from a relevant source population with rigorous statistical techniques. In this analysis, comparisons of outer centile values between the local population and industrialized population nomograms revealed inconsistent patterns. The differences observed are likely due in large part to differing statistical approaches used to create the reference intervals. Early attempts to develop fetal size nomograms from longitudinal ultrasound studies were based on methods applicable for cross-sectional data.[119,120,121] This approach ignores the high correlation amongst biometric parameters over time (gestational age) and assumes that the fetal growth velocity, and the estimated model residual errors, are constant over time. These assumptions are not appropriate for fetal growth data, which generally demonstrates a pattern of increased variability in estimated fetal weight with increasing gestational age. Several studies, including the Gallivan study

used as a comparison here, attempted to improve the statistical methods for analyzing longitudinal data by fitting a separate regression curve to each fetus and using the average variation (i.e., average of the individual regression coefficients) among these curves to derive the size centiles.[122,123] This method, however, is also flawed and leads to outer centiles that are too narrow because it only accounts for between-fetus variation.[124] The mixed effect model approach utilized in our study overcomes the above limitations by considering both the between- and within-fetus variation in the calculation of the reference intervals. This method should result in more accurate estimation of reference intervals and better IUGR diagnosis. The nomogram developed by Johnsen utilized a similar statistical approach and as expected, provided the most consistent comparison to the local nomogram.

A fetal size nomogram is used to determine if a particular fetus has attained an appropriate weight at a particular gestational age. This tool can be a helpful diagnostic addition to obstetric care even in resource poor settings in which it is only possible to scan a woman one time during pregnancy. For example, the nomogram can be used to identify fetuses that appear to be faltering in growth and are likely to benefit from maternal interventions, such as presumptive antimalarial treatment regimens, hypertension management, bednet programs, and nutritional supplementation. In the context of intervention research, longitudinal ultrasound studies that scan women before and after implementation of an intervention can be used to identify time points during pregnancy in which the intervention has maximum impact.

In summary, this fetal size nomogram was developed from an unselected antenatal population that adequately represents a typical urban, resource poor sub-Saharan African population. We utilized advanced statistical techniques that address statistical clustering of the longitudinal data to produce valid reference intervals. Our findings lend support to the hypothesis that maternal characteristics including malaria infection and under-nutrition likely lead to lower fetal weight when compared to 50th centile values from industrialized

populations. This customized nomogram should improve the diagnosis of IUGR in resource poor populations.

Figure 4.1. Estimated fetal weight centiles by gestational age with raw fetal weight values superimposed on the plot, Kinshasa, Democratic Republic of Congo, 2005-2006

(Dotted line=5th and 95th centiles, Solid thin line=10th and 90th centiles, Solid thick line=50th centile)

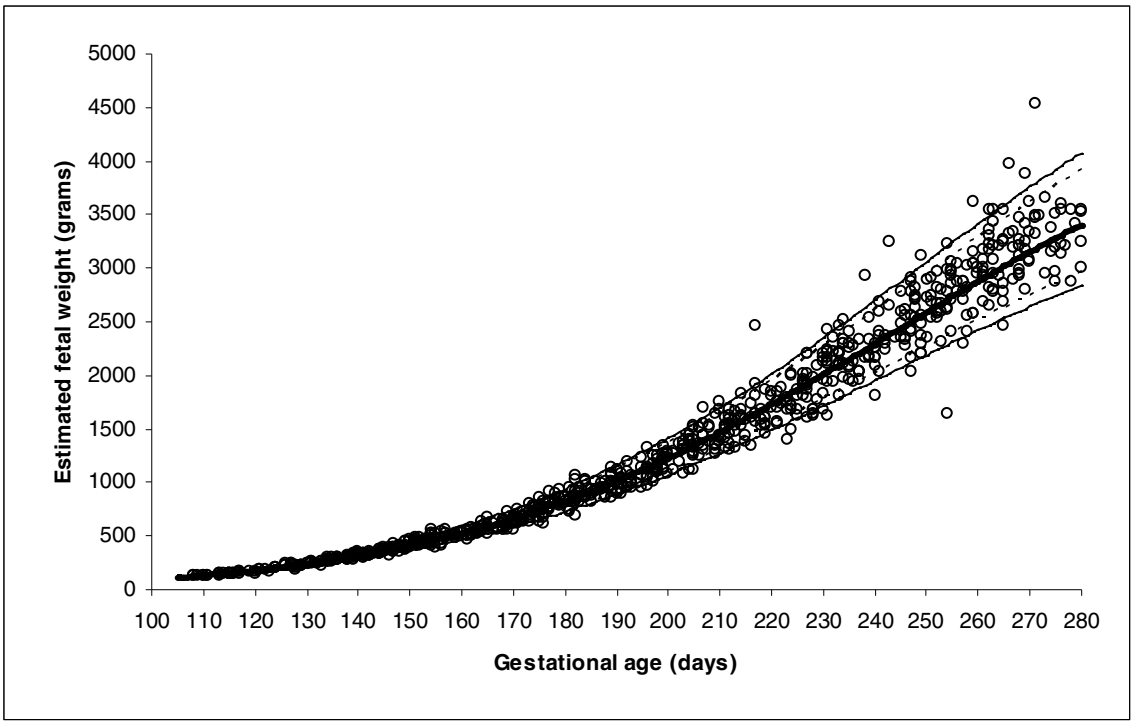


Figure 4.2. Studentized residuals across gestational age from the fit of the regression model

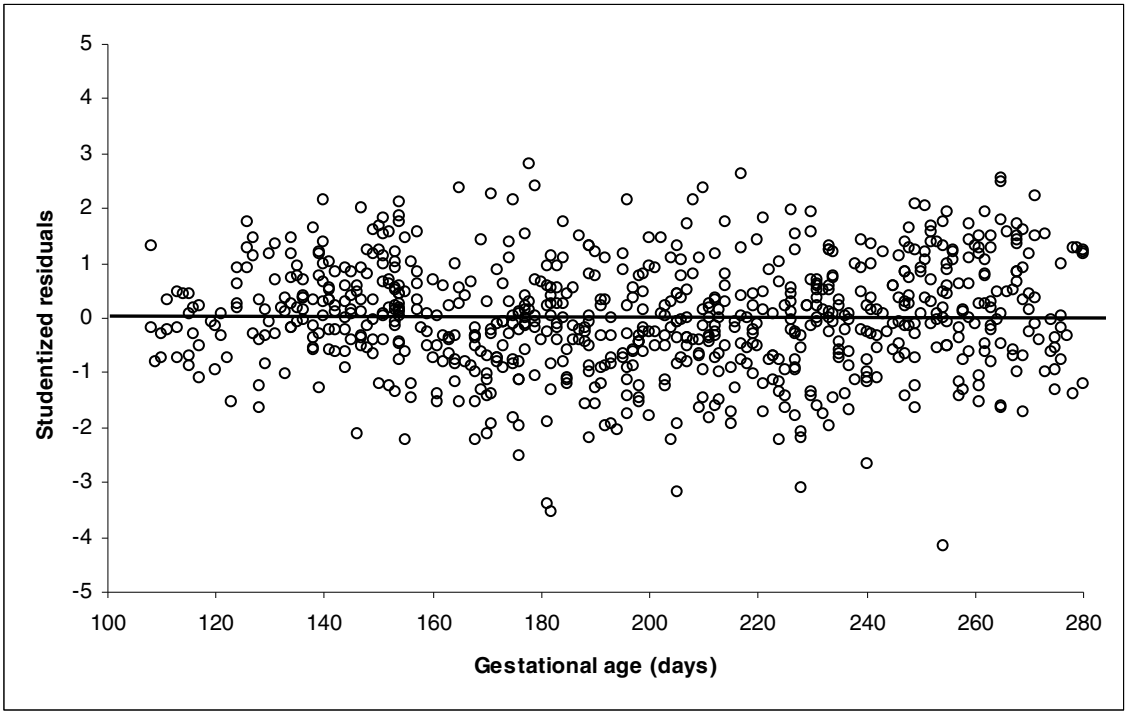


Figure 4.3. Percent difference comparing 10th, 50th and 90th centiles
(Dotted line=Hadlock; Solid thin line=Gallivan; Solid thick line=Johnsen)

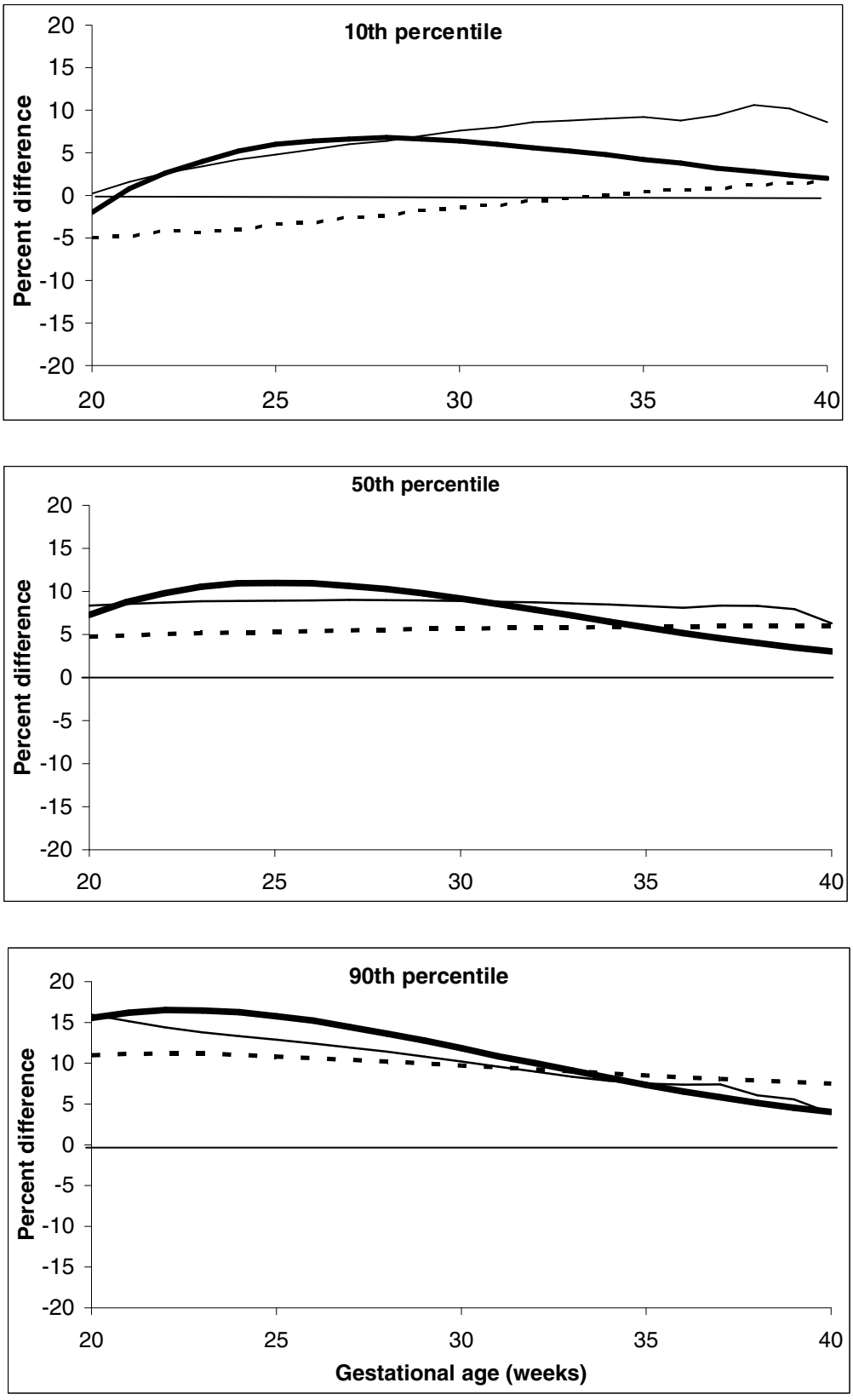


Table 4.1. Maternal and fetal characteristics of the study population (N=144), Kinshasa, Democratic Republic of Congo, 2005-2006

	Mean/Percent	SD
Maternal characteristics		
Age at enrollment (years)	27.4	5.5
BMI at enrollment (kg/m^2)	23.7	3.7
Height (cm)	161.4	7.4
MUAC at enrollment	26.5	3.2
Weight gain per month (kg)	1.6	1.5
Hematocrit at enrollment (%)	33.7	3.8
Primigravid	29%	--
Malaria parasitemia at enrollment	30%	--
HIV positive	3%	--
Infant characteristics		
Birth weight (g)*	3041	413
Birth length (cm)†	49.7	1.9
Birth head circumference (cm) †	34.1	2.4
Gestational age at birth (days)*	275	11
Low birth weight (<2,500 grams)	6%	--
Preterm delivery (<37 weeks)	4%	--
Female gender	56%	--

* Data available for 142 infants
† Data available for 137 infants

Table 4.2. Distribution of ultrasound examinations by gestational week and descriptive statistics of the estimated fetal weight variable by gestational age, Kinshasa, Democratic Republic of Congo, 2005-2006

Gestational age (weeks)	# observations	Estimated fetal weight (grams)				
		Mean	SD	Minimum	Maximum	CV
15	2	128	7.9	122	134	6.2
16	13	140	11.6	124	155	8.3
17	11	166	12.5	152	187	7.5
18	18	217	18.7	176	251	8.6
19	25	271	19.3	231	295	7.1
20	29	319	20.2	271	353	6.3
21	35	382	36.5	320	470	9.5
22	49	464	37.7	390	560	8.1
23	22	535	38.7	472	643	7.2
24	30	617	52.3	520	742	8.5
25	40	745	69.1	625	907	9.3
26	38	874	80.1	700	1062	9.2
27	32	988	85.0	856	1198	8.6
28	36	1119	101.8	944	1341	9.1
29	33	1314	125.2	1076	1528	9.5
30	40	1480	126.2	1275	1761	8.5
31	29	1661	210.5	1351	2466	12.7
32	36	1813	181.3	1402	2203	10.0
33	40	2073	239.7	1615	2524	11.6
34	32	2226	233.9	1814	2929	10.5
35	30	2536	259.3	2034	3245	10.2
36	38	2666	308.3	1639	3227	11.6
37	34	2935	293.4	2301	3622	10.0
38	34	3152	324.4	2466	3975	10.3
39	21	3360	357.3	2875	4529	10.6
40	8	3296	254.9	2875	3549	7.7
Total	755					

SD=Standard deviation
CV=Coefficient of variation (SD/mean), expressed as percent

Table 4.3. In utero fetal weight centiles by week of gestation Kinshasa, Democratic Republic of Congo, 2005-2006

Gestational age (weeks)	2.5th	5th	10th	25th	50th	75th	90th	95th	97.5th
15	96	99	102	106	**112**	118	124	128	131
16	121	124	127	133	**140**	147	154	158	162
17	150	154	158	165	**173**	182	190	195	200
18	186	190	195	203	**213**	223	233	239	245
19	227	232	238	248	**260**	273	284	292	298
20	275	281	289	301	**315**	330	345	353	361
21	331	339	347	362	**380**	398	415	426	435
22	395	404	415	433	**454**	476	497	509	521
23	468	479	491	513	**539**	565	591	606	620
24	550	563	578	604	**635**	667	698	717	733
25	641	656	675	706	**743**	782	819	842	862
26	742	760	782	820	**864**	911	955	982	1007
27	852	874	900	945	**997**	1053	1106	1138	1167
28	971	997	1028	1081	**1143**	1209	1271	1310	1345
29	1100	1130	1166	1228	**1301**	1378	1452	1498	1539
30	1237	1272	1313	1386	**1471**	1561	1647	1700	1748
31	1381	1421	1469	1552	**1651**	1755	1854	1917	1972
32	1532	1578	1632	1727	**1840**	1959	2074	2145	2209
33	1687	1739	1801	1909	**2036**	2172	2302	2384	2457
34	1846	1904	1973	2094	**2238**	2391	2538	2630	2713
35	2006	2070	2147	2282	**2442**	2613	2778	2881	2974
36	2165	2236	2321	2470	**2647**	2836	3019	3133	3236
37	2321	2398	2491	2655	**2848**	3056	3257	3383	3496
38	2471	2556	2656	2833	**3044**	3270	3488	3626	3749
39	2614	2705	2813	3004	**3230**	3474	3709	3858	3991
40	2747	2844	2959	3162	**3404**	3664	3916	4075	4218

Table 4.4. Comparison of estimated fetal weight reference intervals comparing Kinshasa, Democratic Republic of Congo and industrialized population nomograms

	Present study			**Hadlock[129]**			**Gallivan[123]**			**Johnsen[163]**		
Population	Dem Rep. of Congo, African			United States, Caucasian			United Kingdom, Caucasian			Norway, 98% of European origin		
Study design	Longitudinal			Cross-sectional			Longitudinal			Longitudinal		
N (women)	144			392			67			635		
N (scans)	755			392			434			1795		
Avg. scan/women	5			1			6			3		
EFW formula	Hadlock[27]			Hadlock[27]			Hadlock[27]			Combs[164]		
GA weeks	10th	50th	90th	10th	50th	90th	10th	50th	90th	10th	50th	90th
20	289	315	345	275	331	387	289	344	410	283	340	408
25	675	743	819	652	785	918	709	816	940	717	835	972
30	1313	1471	1647	1294	1559	1824	1421	1614	1834	1403	1619	1868
35	2147	2442	2778	2154	2595	3036	2362	2663	3003	2242	2593	2998
40	2959	3404	3916	3004	3619	4234	3240	3633	4074	3021	3511	4081

CHAPTER 5:
SENSITIVITY ANALYSIS OF MALARIA AND UNDER-NUTRITION ANALYSIS UTILIZING THE CONGO FETAL SIZE NOMOGRAM AS THE REFERENCE FOR IUGR DIAGNOSIS

PURPOSE

We conducted a sensitivity analysis to assess the robustness of the associations between IUGR, malaria and under-nutrition (Chapter 3) using the Congo fetal size nomogram as the standard for defining IUGR.

MATERIALS AND METHODS

A total of 757 observations after 22 weeks gestation were used in this sensitivity analysis (one fetus with an ultrasound at 42 weeks gestation was not included as the Congo derived nomogram covers only 15-40 weeks). We began by assigning an IUGR diagnosis to each observation utilizing the 10th centile of the Congo nomogram values (Table 5.1). Next, we compared the distribution and characteristics of the IUGR cases diagnosed using the Hadlock[129] and Congo nomograms and re-fitted the log-binomial models to determine if the resultant Risk ratios (RR) and 95% confidence intervals (CI) differed by IUGR definition (Tables 5.2-5.7).

RESULTS

The Congo 10th centile is higher than the Hadlock tenth centile on average by 20 grams early in gestation and then drops below the Hadlock values from weeks 35 to 40 (Table 5.1). More fetuses were categorized as IUGR using the Congo nomogram (n=107) as compared to the Hadlock nomogram (N=75). The gestational age distribution of IUGR cases is shown

in Figure 5.1. As expected based on the gestational age specific pattern of the absolute and percent differences, the Congo nomogram characterized more fetuses as IUGR until week 30, after which the two nomograms performed equally.

The concordance in IUGR diagnosis between the Hadlock nomogram and the Congo nomogram was 95.5%. The Kappa statistic is often used to compare the actual agreement in how two algorithms (or observers) classify a particular outcome, against the agreement which might be expected by chance. This index ranges of from positive one (perfect agreement) to negative one (complete disagreement), with zero indicating no agreement above that expected by chance. Kappa can be thought of as the chance-corrected proportional agreement. The Kappa value was 0.789 with a 95% confidence interval of (0.721, 0.857). A kappa in this range is considered to have a substantial level of agreement.

No appreciable differences were observed among the associations between IUGR and maternal sociodemographic factors, fetal gender or anemia (Table 5.3). The RR for antimalarial treatment was slightly attenuated and no longer statistically significant using the Congo IUGR definition. Results for the independent associations of incident and cumulative malaria remained largely unchanged (Table 5.4). The risk ratio and 95% CI for the continuously coded cumulative malaria variable were slightly higher for one and two malaria infections utilizing the Congo nomogram, and the trend test P-values became lower and highly significant (0.02) for the model that adjusted for both age and maternal weight gain. Risk ratios for the binary ≥3 positives (vs. <3 positives) malaria definition were slightly attenuated using the Congo IUGR model, however conclusion regarding statistical significance remained the same.

Among the various maternal anthropometric indicators, results for the MUAC change and weight gain indicators were the most divergent. When utilizing the Hadlock IUGR definition, both MUAC change and weight gain suggested a stronger effect among women with low baseline BMI, although these differences were not statistically significant. In contrast, when

using the Congo IUGR definition, the effect of both variables on IUGR risk was essentially null at either level of baseline BMI (Table 5.5). Similarly, the statistically significant strong second trimester effect demonstrated in the original analysis was also attenuated when the Congo definition was used (Table 5.6).

The strong effect measure modification patterns identified using the Hadlock IUGR definition largely held when the data were re-analyzed (Table 5.7). Although the RRs are slightly attenuated, conclusions regarding the statistical significance of the effect measure modification were essentially the same. The only exception was the MUAC change indicator, which was no longer a significant effect measure modifier in the cumulative malaria analysis. As well, the overall precision of the estimates improved when utilizing the Congo definition.

DISCUSSION

In general, the results of the malaria analysis appear very robust to changes in the definition of IUGR. The RRs and conclusions regarding statistical significance were nearly identical in all analyses with malaria as the main exposure variable. The maternal anthropometric exposures were more sensitive to changes in the IUGR definition, with the MUAC change and maternal weight gain variables showing the most contradictory results. The loss of effect for these two variables likely has to do with the differing gestational age distribution of the IUGR cases using the two definitions. The Congo nomogram characterized a much higher proportion of fetuses as IUGR in the second trimester and as a result, the strong trimester effects of MUAC and weight gain essentially disappeared. The loss of a strong second trimester effect may explain, at least in part, the attenuation in the RRs for the independent and modifying effects of these two variables over the whole pregnancy.

Figure 5.1. Distribution of IUGR cases by gestational age using the Congo and Hadlock nomograms to define IUGR

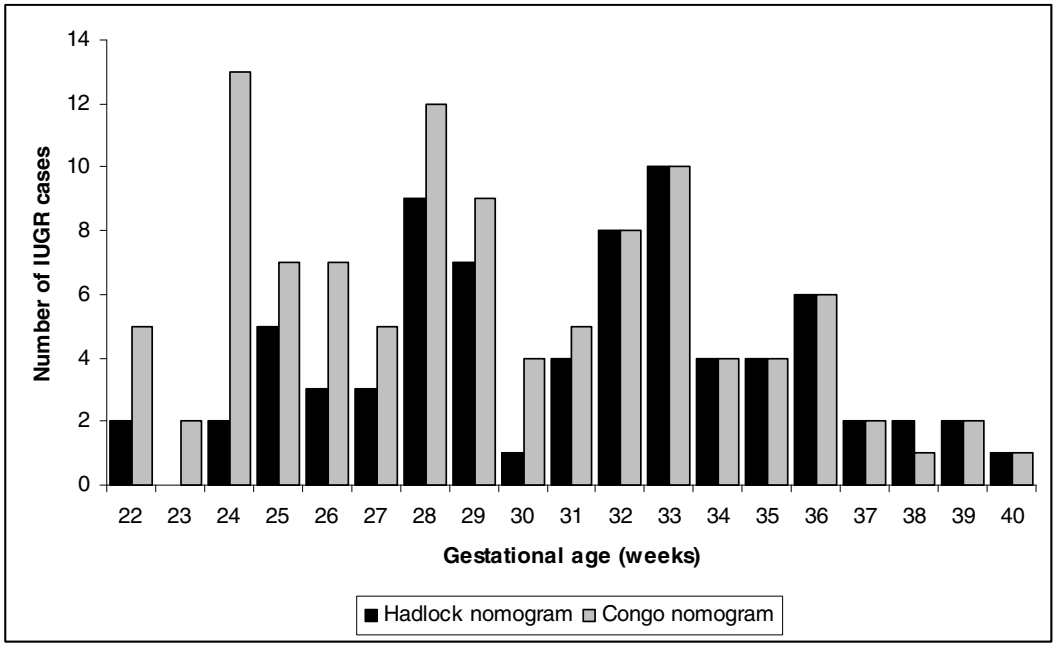


Table 5.1. Comparison of the 10th centile values of the Hadlock nomogram and the Congo nomogram, 22 – 40 weeks gestation

Gestational age (weeks)	Congo 10th centile	Hadlock 10th centile	Absolute difference	Percent difference
22	415	398	17	4
23	491	471	20	4
24	578	556	22	4
25	675	652	23	3
26	782	758	24	3
27	900	876	24	3
28	1028	1004	24	2
29	1166	1145	21	2
30	1313	1294	19	1
31	1469	1453	16	1
32	1632	1621	11	1
33	1801	1794	7	0
34	1973	1973	0	0
35	2147	2154	-7	0
36	2321	2335	-14	-1
37	2491	2513	-22	-1
38	2656	2686	-30	-1
39	2813	2851	-38	-1
40	2959	3004	-45	-2

Absolute difference = Congo 10th centile – Hadlock 10th centile
Percent difference = (Congo 10th centile – Hadlock 10th centile) / Congo 10th centile) * 100

Table 5.2. Concordance between the Congo and Hadlock nomograms

	Hadlock nomogram		
Congo nomogram	**IUGR**	**Not IUGR**	
IUGR	74	33	107
Not IUGR	1	649	650
	75	682	757

Percent concordant = (74 + 649) / 757 = 95.5%

Kappa statistic = 0.789 (95% confidence interval: 0.721, 0.857)

Table 5.3. Baseline and visit-specific characteristics of pregnant women and risk ratios (RR) and 95% confidence intervals (CI) for IUGR using Congo nomogram

	RR†	95% CI
Maternal age (years)		
18-24	1.3	0.7, 2.4
25-29	1.0	Ref
≥30	1.2	0.6, 2.2
Socioeconomic status		
High	1.0	0.5, 2.0
Low	1.0	Ref
Gravida		
1-2	1.3	0.8, 2.1
≥3	1.0	Ref
Fetal gender		
Male	0.7	0.4, 1.2
Female	1.0	Ref
Treated in previous month		
Yes	0.8	0.6, 1.0
No	1.0	Ref
Hematocrit at visit		
<30	0.6	0.2, 1.5
≥30	1.0	Ref

† Unadjusted. Model included 757 study visits and 107 episodes of IUGR.

Table 5.4. Risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and incident and cumulative malaria infection using Congo nomogram

	RR†	95% CI	RR‡	95% CI	RR§	95% CI
Malaria parasitemia at visit						
Positive	1.2	0.8, 2.0	1.2	0.8, 2.0	1.4	0.8, 2.4
Negative	1.0	Ref	1.0	Ref	1.0	Ref
Cumulative malaria parasitemia up to visit						
0	1.0	Ref	1.0	Ref	1.0	Ref
1	1.0	0.6, 1.7	1.0	0.6, 1.7	1.1	0.6, 1.7
2	1.4	0.7, 2.6	1.4	0.8, 2.6	1.6	0.9, 3.0
≥3	2.1	0.7, 6.5	2.1	0.7, 6.5	3.6	1.5, 8.4
	$P_{Trend} = 0.17$		$P_{Trend} = 0.19$		$P_{Trend} = 0.02$	
Cumulative malaria parasitemia up to visit						
≥2 positive	1.5	0.9, 2.7	1.5	0.9, 2.7	1.9	1.1, 3.2
<2 positive	1.0	Ref	1.0	Ref	1.0	Ref
≥3 positive	1.9	0.6, 6.2	1.9	0.6, 6.2	3.2	1.4, 7.4
<3 positive	1.0	Ref	1.0	Ref	1.0	Ref

† Unadjusted. Model included 757 study visits and 107 episodes of IUGR.
‡ Adjusted for age. Model included 757 study visits and 107 episodes of IUGR.
§ Adjusted for age and weight gain. Model included 587 visits with complete data for weight gain and 92 episodes of IUGR.

Table 5.5. Risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and maternal anthropometric indicators using Congo nomogram

	RR†	95% CI	RR‡	95% CI	RR§	95% CI
Time-independent variables						
Baseline BMI						
<19.8 kg/m^2	1.3	0.7 2.3	1.2	0.7, 2.2	1.0	0.5, 2.0
≥19.8 kg/m^2	1.0	Ref	1.0	Ref	1.0	Ref
Short stature						
<150 cm	1.3	0.5, 3.5	1.3	0.5, 3.4	1.2	0.3, 4.1
≥150 cm	1.0	Ref	1.0	Ref	1.0	Ref
Time-dependent variables						
MUAC change per month #						
Baseline BMI <19.8 kg/m^2	0.8	0.3, 2.2	0.8	0.3, 2.2	0.8	0.2, 4.5
Baseline BMI ≥19.8 kg/m^2	1.1	0.8, 1.6	1.1	0.8, 1.6	1.0	0.7, 1.4
Maternal weight gain per month**						
Baseline BMI <19.8 kg/m^2	--	--	--	--	1.3	0.5, 3.6
Baseline BMI ≥19.8 kg/m^2	--	--	--	--	1.3	1.0, 1.9

† Unadjusted. Model included 757 study visits and 107 episodes of IUGR.
‡ Adjusted for age. Model included 757 study visits and 107 episodes of IUGR.
§ Adjusted for age. Model included 587 visits with complete data for weight gain and 92 episodes of IUGR.
# Comparing MUAC change in the previous month of <0 cm vs. ≥ 0cm.
** Comparing maternal weight gain in the previous month of <1.5 kg per month vs. ≥1.5 kg per month.

Table 5.6. Trimester specific risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and change in MUAC and weight gain using Congo nomogram

	IUGR in second trimester		IUGR in third trimester	
	RR	95% CI	RR	95% CI
MUAC change*				
Over entire 2nd trimester	1.1	0.6, 1.9	--	--
Over entire 3rd trimester	--	--	1.1	0.6, 2.0
Weight gain per month†				
Second trimester	1.4	0.7, 2.8	--	--
Third trimester	--	--	1.3	0.8, 1.9

* Unadjusted. Models included 217 study visits and 36 episodes of IUGR in the second trimester and 525 study visits and 71 episodes of IUGR in the third trimester. Comparing MUAC change over the whole trimester of <0 cm vs. ≥0 cm.
† Unadjusted. Models included 160 study visits and 29 episodes of IUGR in the second trimester and 427 study visits and 63 episodes of IUGR in the third trimester. Comparing maternal weight gain in the previous month of <1.5 kg per month vs. ≥1.5 kg.

Table 5.7. Risk ratios (RR) and 95% confidence intervals (CI) for the association between IUGR and malaria, stratified by maternal anthropometrics using Congo nomogram

	RR*	95% CI	RR†	95% CI	RR‡	95% CI
Malaria parasitemia at visit (Positive vs. negative)						
Baseline BMI <19.8 kg/m^2	**3.3**	**1.8, 5.7**	**3.2**	**1.8, 5.7**	**5.0**	**2.6, 9.6**
Baseline BMI ≥19.8 kg/m^2	**0.9**	**0.5, 1.7**	**0.9**	**0.5, 1.7**	**0.9**	**0.5, 1.8**
Height <150 cm	**3.9**	**1.4, 10.9**	**3.7**	**1.2, 10.8**	**3.9**	**0.9, 16.2**
Height ≥ 150 cm	**1.2**	**0.7, 1.9**	**1.2**	**0.7, 1.9**	**1.2**	**0.7, 2.2**
MUAC gain <0 cm in past month	**2.0**	**1.1, 3.8**	**2.0**	**1.1, 3.8**	**2.2**	**1.2, 3.8**
MUAC gain ≥0 cm in past month	**0.8**	**0.3,1.9**	**0.8**	**0.3,2.0**	**0.7**	**0.3,1.8**
Weight gain <1.5 kg in past month	--	--	--	--	1.4	0.7, 2.8
Weight gain ≥1.5 kg in past month	--	--	--	--	1.4	0.7, 2.7
Cumulative malaria parasitemia (≥ 2 positive vs. < 2 positive)						
Baseline BMI <19.8 kg/m^2	**3.3**	**1.6, 6.4**	**3.2**	**1.6, 6.4**	**5.0**	**2.8, 8.7**
Baseline BMI ≥19.8 kg/m^2	**1.3**	**0.7, 2.6**	**1.3**	**0.7, 2.5**	**1.6**	**0.8, 2.9**
Height <150 cm	3.3	0.5, 20.4	3.6	0.7,18.9	2.3	0.3,16.5
Height ≥150 cm	1.4	0.8, 2.6	1.4	0.7, 2.6	1.9	1.1, 3.2

Table 5.7., continued

	RR*	95% CI	RR†	95% CI	RR‡	95% CI
MUAC gain <0 cm in past month	1.4	0.6, 3.1	1.4	0.6, 3.2	2.2	1.0, 4.5
MUAC gain ≥0 cm in past month	1.5	0.9, 2.7	1.5	0.8, 2.7	1.7	1.0, 2.9
Weight gain <1.5 kg in past month	--	--	--	--	1.7	0.9, 3.3
Weight gain ≥1.5 kg in past month	--	--	--	--	2.1	1.1, 3.8

* Unadjusted. Model included 757 study visits and 107 episodes of IUGR.
† Adjusted for age. Model included 757 study visits and 107 episodes of IUGR.
‡ Adjusted for age. Model included 587 visits with complete data for weight gain and 92 episodes of IUGR.

Note: RR pairs highlighted in bold indicate a significant P-value for the interaction term between malaria and the anthropometric indicator (P-value <0.15).

CHAPTER 6:
DISCUSSION

Summary of findings

This dissertation provides one of the first longitudinal studies of antenatal malaria infection. It provides a unique contribution to the pregnancy malaria literature because we utilized ultrasound to study *in utero* fetal growth, rather than relying on SGA as a proxy measure of antenatal growth.

The first analysis focused on two component causes of IUGR, which have heightened relevance in low resource settings, maternal malaria infection and under-nutrition. In the case of *P. falciparum* infection, a causal link to IUGR is primarily mediated though processes which affect placental function and nutrient transport.[42] Whereas under-nutrition and low energy intake work through metabolic pathways that affect a women's ability to support the increasing nutrient demands of the developing fetus, including insufficient plasma volume expansion, reduction in glucose plasma levels and inadequate deposition of subcutaneous fat stores.[67]

In the first analysis, we found that 60% of women had at least one antenatal malaria infection and we identified IUGR in nearly one third of the fetuses studied (Chapter 3). Malaria infection was only modestly associated with an increased risk of IUGR in this population. A single incident malaria infection was not significantly associated with IUGR in unadjusted or adjusted analyses. The data suggest a trend effect associated with cumulative malaria exposure. When compared to fetuses with no antenatal malaria exposure, there was no increased risk of IUGR after just one positive smear (RR=0.9, 95% CI: 0.5 1.6, a slight increase in risk after two positives (RR=1.2, 95% CI: 0.5, 2.5) and a two fold increase in risk

among fetuses who were exposed ≥ 3 times *in utero* (RR=2.3, 95% CI: 0.8, 6.3). Data from a secondary analysis to investigate the effects of malaria infection on mean fetal weight (Appendix C) support the IUGR findings, with significantly lower attained fetal weight in only those fetuses exposed ≥ 3 times.

We demonstrated that the effect of malaria on IUGR was significantly modified by maternal nutritional status, such that the highest associations between malaria and IUGR were among women with poor nutritional status. This pattern was observed in all four of the anthropometric indicators studied; however, the weight gain indicator did not reach statistical significance. Repercussions of childhood malnutrition and malaria infection, such as stunting and low young adult BMI place reproductive age women at increased risk of poor birth outcome.[31-33,165] In women who have become pregnant, the joint effects of these conditions may act on similar physiologic pathways to reduce uteroplacental blood flow. Further, these conditions both contribute to maternal anemia, which independently contributes to LBW through decreased maternal-fetal oxygen transfer.[34,35]

Analogous findings of a modifying effect of maternal nutritional status have been demonstrated in other studies of IUGR risk factors. For example, Cliver and colleagues showed a differential effect of psychosocial profile during pregnancy over different BMI levels.[166] Among women with a poor psychosocial profile during pregnancy, those with low BMI were at higher risk of IUGR compared to women of normal BMI. Conversely, findings from LBW prevention trials of aspirin[167] and zinc supplementation[168] showed the any *benefit* of the intervention is largely present only in women with a healthy pre-pregnancy BMI.

In regard to the independent effects of maternal nutritional status, we found that maternal weight gain was more strongly associated with an increased risk of IUGR in women with low baseline BMI. A similar association has been demonstrated in studies of LBW and SGA,[76,90,97,102] with risk generally decreasing with increasing BMI values. We demonstrated that failure to gain weight or accrue arm fat during the second trimester was associated with

lower fetal weight. Because the fetus grows most rapidly in the third trimester, we might have expected that third trimester maternal weight or MUAC gain would have a greater impact. However, many studies have shown that second trimester weight gain[93,96,98,99-101] and MUAC gain[73,99,105] are more important. These findings suggest that maternal physiologic changes that occur earlier in pregnancy, such as increased plasma volume and maternal fat deposition, may have the greatest impact on fetal growth.

The second analysis consisted of an application of the mixed effects model technique to develop a fetal size nomogram appropriate for use in low resources settings with a high prevalence of malaria infection (Chapter 4). Linear mixed effect models appropriately handle highly correlated longitudinal data by modeling both between- and within-women variation in the outcome (in this case EFW). The model consisted of a log-transformation of EFW and a fractional polynomial of gestational age; random effects for both the intercept and slope were included. Reference intervals (5^{th}, 10^{th}, 50^{th}, 90^{th} and 95^{th} centiles) were derived from the model and compared to centiles derived from industrialized populations.

We demonstrated that the 50^{th} centile for fetuses in Congo were, on average, consistently lower than fetuses born in industrialized populations. In contrast, the outer centiles showed considerable variation, with some reference standards over-estimating the Congo centile values while others under-estimated them. We believe these differences were largely determined by variation in study design and statistical techniques used to derive the centiles. A nomogram developed using a similar mixed effect model approach showed the most similar pattern to our data.[163]

Public health implications

Our findings are at variance with previously published sub-Saharan Africa studies, that found significant associations between malaria and SGA or LBW.[28,140,141,142,143,169] In our study, malaria was only associated with IUGR after repeated infections (≥ 3) and among

women with evidence of under-nutrition. These null findings were robust to the use of different fetal size nomograms to define IUGR (Chapter 5) as well as varying definitions of gestational age. A central dissimilarity between our study and those previously conducted is that our protocol called for presumptive treatment, routine malaria screening and prompt treatment of all positive cases. We hypothesize that rapid case detection and prompt treatment may have eliminated parasites before they had adequate time to sequester in the placenta and damage the placental vasculature. This is supported by our findings that treatment was independently protective against both malaria (data not shown) and IUGR (Chapter 3), and resulted in higher attained fetal weight (Appendix C).

There have only been two previous studies that have had similar frequent monitoring and treatment of antenatal parasitemia, both of which were conducted in areas of low malaria transmission. In these populations, we do see similar null effects for SGA or LBW (note IUGR measurements are not available as ultrasound was not utilized). For example, a study from the Thai-Burmese border found no increased risk of SGA associated with antenatal *P. falciparum* infection (10.1% SGA prevalence among infected women vs. 10.7% among uninfected women).[148] In the highland areas of Ethiopia where over 80% of placental infections were due to *P. falciparum,* there was no increased risk of LBW associated with placental blood parasitemia at delivery (RR=1.0, 95%CI: 0.2-6.9).[149] Thus, our study is the first to show that even in areas of high *P. falciparum* malaria transmission, prompt identification and treatment of sub-clinical malaria infections may prevent fetal growth restriction from occurring.

The findings from this dissertation have important implications for malaria control policies in antenatal populations in sub-Saharan Africa. In areas of high malaria transmission, WHO recommends a three pronged approach to prevention of malaria in pregnancy: (i) use of insecticide treated bednets, (ii) intermittent presumptive therapy, and (iii) case management of confirmed positives.[170]

In regard to the insecticide treated bednet recommendation, our study did not directly measure the impact of bednet utilization on malaria infection or IUGR, however all of the women in our study were provided a free bednet and self reported use of the nets was high during pregnancy (data not shown). Thus, the findings of our study should be interpreted within the context of high bed net utilization.

All women in our study received two doses of presumptive treatment with SP. Our findings add to the body of literature about the beneficial effects of intermittent presumptive treatment and underscore the importance of scaling up this important intervention. Providing two courses of presumptive treatment with an inexpensive antimalarial to all pregnant women is a safe, cost effective intervention[171,172] that has been shown to significantly reduce the prevalence of antenatal peripheral malaria,[53,173] placental malaria,[53,173-175] malaria associated anemia,[175,176] and birth weight[53,173-175,177] in both HIV positive and negative women. Despite the proven efficacy of this intervention, coverage in sub-Saharan Africa remains below 50% in many areas, with use generally lower in rural areas.[178]

Our data also lend support to the WHO recommendations regarding therapeutic management of positive cases and suggest that routine malaria screening and treatment be extended to all women, not just women with clinical signs of malaria. In many areas of sub-Saharan Africa, scarce resources need to be balanced with benefits to patients. We recognize that it in many resource poor settings, lack of laboratory infrastructure, microscopists, and high patient volume may make it impractical to routinely screen pregnant women at every antenatal care visit. At a minimum, however, routine screening and prompt treatment should be targeted to women at greatest risk of malaria associated poor birth outcome, including primi- and secundigravida and women with evidence of under-nutrition. Further, roll out of rapid malaria tests, which have proven to be cost effective and substantially decrease inappropriate treatment, may also improve our ability to implement routine screening.[179]

To date, most presumptive treatment programs have utilized SP as the drug of choice. This drug is highly cost-effective at an average cost of about $1-$2 per pregnancy including service overhead.[178] However, the efficacy of SP is starting to wane, as drug resistance develops throughout sub-Saharan Africa.[180] Long term success of this prevention strategy thus requires the continuous availability of new proven regimens, including artemesinin-based combination therapies, for presumptive treatment. This has significant economic implications, however, as these drugs can cost up to ten times more than SP. Thus, it is important that we conduct research to maximize the efficacy of these new drug regimens by identifying intervals during pregnancy when the drugs provide the most benefit. For example, does presumptive treatment early in pregnancy provide the same benefit as waiting until later in pregnancy? When is the latest possible time during pregnancy that you can give a drug and still see some fetal benefit? What is the minimum number of presumptive doses that still offers adequate protection?

The fetal size nomogram we developed is a helpful research tool for this purpose. Prospective studies that routinely monitor fetuses with ultrasound can be used to identify intervals during pregnancy when exposures are most harmful and treatments have the greatest impact. By studying growth achievement before and after an intervention has been implemented, we can identify when during pregnancy the treatment seems to provide the greatest benefit. Similar research uses can be extended to other interventions as well, including therapeutic treatment of clinical malaria, treatment of other infections such as syphilis, and provision of nutrition supplementation.

Another advantage of using ultrasound and fetal size nomograms in longitudinal studies such as these is improved efficiency in study design. First, ultrasound can provide more accurate estimation of gestational age that improves classification of study endpoints. As well, the use of IUGR endpoints measured during the antenatal period provides direct assessment of fetal growth and provides more timely results than waiting for birth to measure

surrogate growth markers such as LBW or SGA.

At present, research purposes such as those discussed above are probably the most practical uses of fetal size nomograms in low resources settings. In industrialized countries, these nomograms are often utilized to identify fetuses in distress and who may be candidates for early induction of labor. Clinical applications such as this are limited in low resources settings, however, due to a lack of neonatal intensive care facilities to care for premature infants or infants with under-developed lung capacity.

When utilizing nomograms in any population, certain considerations should be noted. First, an accurate estimate of gestational age is an essential component of correctly utilizing a nomogram. In low resource settings, estimates of gestational age are most often determined through either LMP or post-delivery physical and neurological evaluations such as the Dubowitz method. Last menstrual period dating can lead to inaccurate results due to variation in length of menstrual cycles and irregular menses.[181] This method is also subject to recall bias, which may be even more prominent in the African setting where women do not typically seek antenatal care until early in the second trimester.[161,182] Secondly, not all fetuses categorized as “small” based on the nomogram are necessarily at increased risk of neonatal morbidity and mortality. This is because not all fetuses that are at or below the 10th percentile are pathologically growth restricted (i.e., some are constitutionally small), and not all fetuses that have not met their genetic growth potential are in less than the 10th percentile for weight. Utilizing a nomogram that has been developed from a relevant reference population can help to minimize some of the misclassification of this outcome.

Future research directions

The longitudinal study utilized for the aims of this dissertation is a rich source of research data that can be used to address other clinically important questions related to malaria and risk factors for IUGR and poor birth outcome in low resource settings. A summary of those

questions is included below.

The association between malaria and birth outcomes: This dissertation focused on *in utero* measurement of fetal growth. An analysis investigating the association between antenatal and placental malaria on birth outcomes including mean birth weight, LBW and PTD would provide a nice companion analysis. If the birth outcome analyses also found no or minimal association with malaria, this would further support the null results we identified for IUGR.

The association between antenatal and placental malaria infection: This dissertation focused upon antenatal malaria infections; however, data on placental infection was also collected. Analyses on the associations between antenatal and placental malaria infection would be an important contribution to the literature. Many studies of pregnancy malaria are conducted only at the time of delivery. In these studies, placental histology is often used to suggest potential timing of infection during pregnancy, for example if the placenta was chronically infected or just subjected to an insult near the time of delivery. Few studies exist, however, in which the accuracy of placental malaria to predict "true" antenatal malaria can be assessed. A study in Thailand which compared the sensitivity of pathology to repeat measures of parasitemia during pregnancy found that pathology alone my miss up to one-quarter of antenatal infections.[148] They showed that pathology was more reflective of infections that occurred close to the time of delivery. Our longitudinal study design with frequent measurement of malaria provides an ideal study design to assess the accuracy of histology in predicting presence and timing of antenatal infection.

Placental malaria and uteroplacental blood flow: Doppler ultrasound data were also collected at each ultrasound examination. Evaluating the shape of the flow velocity waveform of pulsed-wave Doppler ultrasound of the umbilical and uterine artery allows a non-invasive method for studying placental transformation and fetal hemodynamics. Analysis of Doppler data would allow us to determine if and when malaria increases the risk of abnormal uterine

and umbilical artery blood flow and also study whether placental histopathological changes are associated with increases in uterine or umbilical artery resistance. We could also assess the relationship between uterine artery blood flow, umbilical artery blood flow, and IUGR.

The association between malaria and asymmetric vs. symmetric growth restriction: Assessing ratios of individual biometric values can be helpful in identifying a fetus that is growing asymmetrically. Measures of proportionality can be interpreted as evidence of the aspects of growth that are compromised by prenatal insults. Menendez *et al* reported chronic placental malaria to be associated with a decrease in both length and head circumference, suggesting a chronic insult.[141] By combining accurate timing of gestation (by early ultrasound), IUGR measurement (by serial ultrasound) and malaria exposure data (by repeat microscopy), we can ascertain the pattern of malaria infections that are associated with asymmetric vs. symmetric IUGR.

Nomograms for fetal growth determination: The ultrasound biometry data can be utilized to develop other types of fetal nomograms for obstetrical use in resource poor settings. For example, because the ultrasound data was collected longitudinally, it is perfectly suited for the development for conditional fetal growth nomograms, which can be utilized to track fetal growth progress over time.

Improving IUGR diagnosis: The sensitivity of IUGR diagnosis can often be enhanced using other criteria that were collected as part of this study, including amniotic fluid volume (oliogohydriaminos), fundal height, and abnormal Doppler ultrasound of the umbilical artery.[4] It would be interesting to assess how accurately these factors predict IUGR, either individually or in combination with other factors.

In conclusion, this dissertation addressed clinically important questions concerning the pathogenesis of malaria infection and under-nutrition on *in utero* fetal growth and aimed to

improve the identification of IUGR in resource poor settings. Utilizing longitudinal data analysis techniques, we sought to determine the association between malaria infection, maternal under-nutrition and IUGR. We concluded that frequent antenatal monitoring and prompt treatment of malaria might prevent IUGR, especially in women with evidence of under-nutrition.

Secondly, we developed a fetal size nomogram for use in resource poor settings and compared this nomogram with reference intervals derived from industrialized countries. We found that the 50th centile EFW for Congo fetuses was consistently lower than fetuses born in industrialized populations; there was large variation in the outer centiles, owing primarily to differing statistical techniques. We feel that this fetal size nomogram should improve diagnosis of IUGR in resource poor settings with endemic malaria.

APPENDIX A:
BASIC SURVIVAL ANALYSIS OF IUGR AND MALARIA

In our study, the prevalence of malaria was highest during the baseline visit and declined over gestation due in part to provision of presumptive treatment and active case management of all positive malaria smears. The IUGR outcome was, conversely, less prevalent early in pregnancy and increased until near term. Before making assumptions about the overall risk of IUGR associated with malaria over the whole pregnancy, we felt it was important to ensure that the relationship between these two variables did not change appreciable over time. To investigate this, we utilized a proportional hazards modeling process.

To compare survival curves between the malaria exposed and unexposed groups, we produced a Kaplan-Meier curve and tested for homogeneity in the survival curves using the log-rank chi-square and the Wilcoxon test. The Kaplan-Meier curve showed the survival was nearly identical between the two groups until week 34, after which the survival function for the malaria group decreased slightly faster than the non-malaria group (Figure A.1). Despite these difference, P-values for the log-rank (p = 0.13) and Wilcoxon tests (p=0.37) were not statically significant, suggesting that the survival curves were not different from each other over time.

We tested the proportional hazards assumption to assess if the hazard ratio was constant over time using two techniques: 1) plots of log-log survival, and 2) test for interaction between malaria and categorical time. In the plots, parallelism in the log hazards suggests that the proportional hazards assumption was satisfied; lines that cross one another violate the proportional hazards assumption. From the log-negative log plots, we can see that the lines are roughly parallel over all gestational ages (Figure A.2). In the model with a time interaction, the P-value for the interaction term can provide a statistical test of the proportional hazards assumption; high P-values indicate that the hazards are not significantly

different from each other. The P-value for the interaction term in our model was 0.30 indicating that we would accept the null hypotheses of equivalent hazards.

Lastly, we ran a proportional hazards regression model using a robust variance estimator to account for our repeated measures study design. The hazard ratio for malaria was elevated but not statistically significant [hazard ratio=1.7, 95% confidence interval (0.8, 3.4]. A 1 degree of freedom test of the malaria variable also indicated that malaria was not a significant predictor of time to development of IUGR in the proportional hazards regression model (p=0.16)

Figure A.1. Kaplan Meier curves comparing malaria positive and negative women.
(Dotted line=malaria positive, solid line=malaria negative)

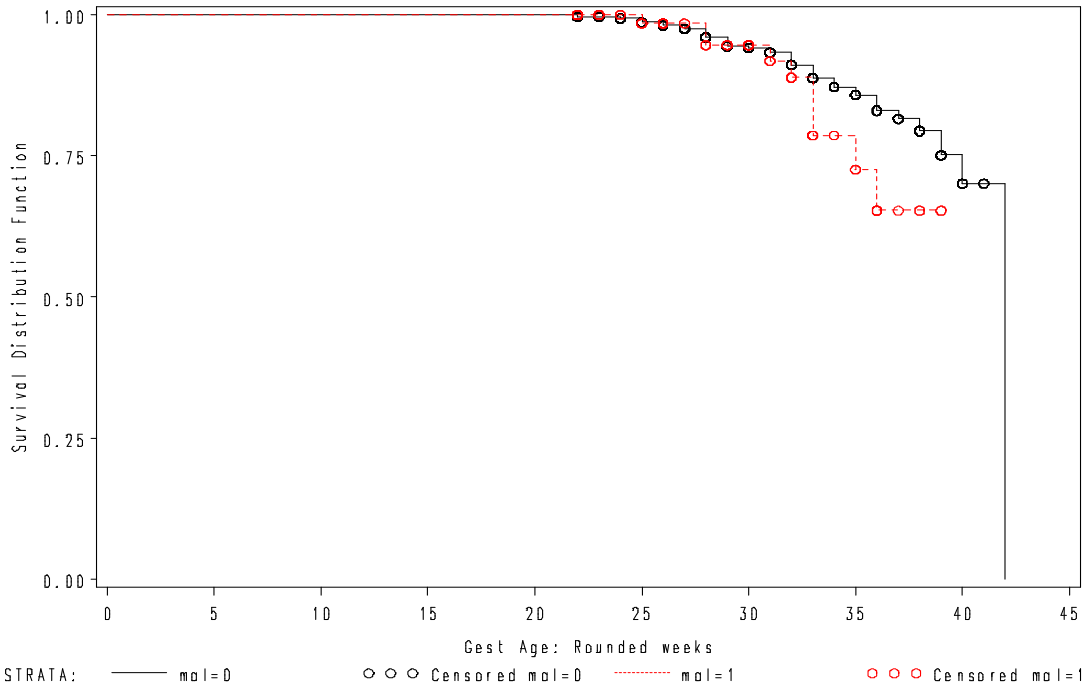
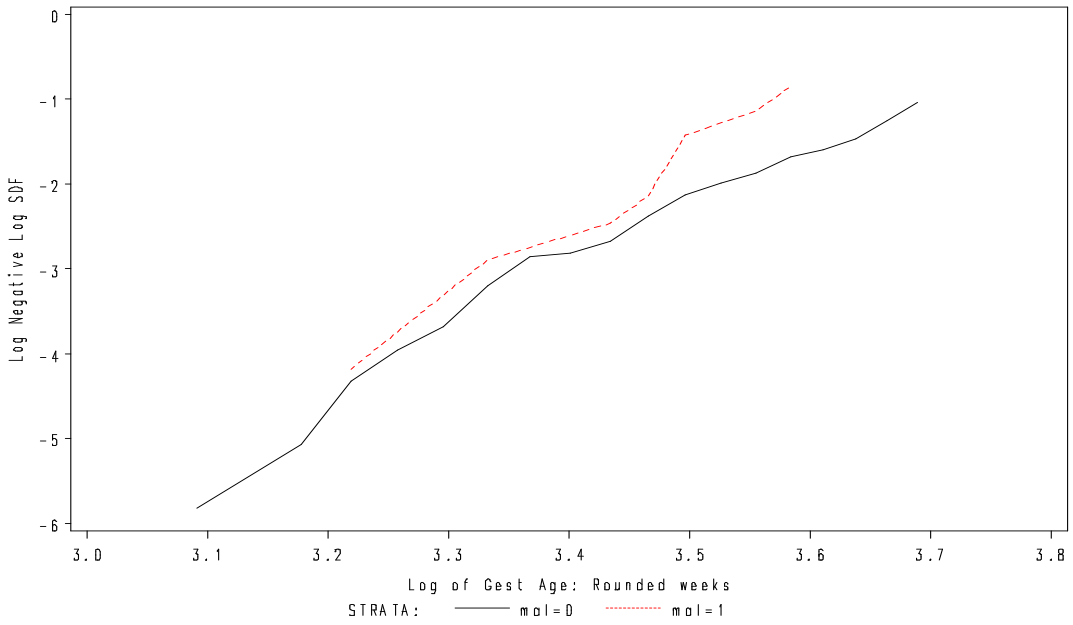


Figure A.2. Log-negative log survival curves comparing malaria positive and negative women
(Dotted line=malaria positive, solid line=malaria negative)



APPENDIX B:
AN OVERVIEW OF MIXED EFFECT MODELS

In longitudinal studies, the observations collected on a single subject over time (often called nested within a subject) are highly correlated. Mixed effect models account for the correlation in longitudinal studies by allowing the regression coefficients to differ between subjects.[133] The simplest form of a mixed effect model is one in which only the intercept can vary between subjects; more complex forms also allow the time (or slope) variable to vary. In this type of analysis, the unexplained variance in the outcome variable is divided into different components; one for the random intercept and another for the random slope.

Mixed effect model techniques are especially relevant in fetal growth analysis as they can be used to model heteroscadastic data, or data in which the variance in the outcome variable changes over time (in this case, variance increases with gestational age). Other advantages of mixed effect models is that they can accommodate un-equal spacing of time intervals, use all data that is available on a subject (i.e., will not eliminate the whole case for some missing data), and allows for the modeling of time-varying covariates such as malaria infection or anemia status.

If Y_{ij} denotes the estimated fetal weight for fetus *i* at measurement occasion *j* and T_{ij} denotes the gestational age for fetus *i* at measurement occasion *j*, then the level 1 and level 2 mixed models can be expressed as:

Level 1: Visit level equation (within-person)

$$Y_{ij} = \beta_{0j} + \beta_{1j} T_{ij} + r_{ij} \text{ where } r_{ij} \sim N(0, \sigma^2_{r_{ij}})$$

Level 2: Women level equations (between-person)

$$\beta_{0j} = \gamma_{00} + \mu_{0j} \text{ (random intercept)}$$
$$\beta_{1j} = \gamma_{10} + \mu_{1j} \text{ (random slope)}$$

where $\begin{pmatrix} \mu_{0j} \\ \mu_{1j} \end{pmatrix} \sim MVN\left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma^2_{\beta_{0j}} & \sigma_{\beta_{0j};\beta_{1j}} \\ \sigma_{\beta_{0j},\beta_{1j}} & \sigma^2_{\beta_{1j}} \end{pmatrix}\right]$, and γ_{00} and γ_{01} are the average of the women level intercepts and slopes, respectively. μ_{0j} represents the between-women variation in intercepts and is ~ $N(0, \sigma^2_{\beta_{0j}})$ and μ_{1j} represents the between-women variation in slopes and is ~ $N(0, \sigma^2_{\beta_{1j}})$. The covariance between the random slopes and random intercepts is denoted as $\sigma_{\beta_{0j},\beta_{1j}}$.

Substituting the Level 2 equations into the Level 1 equation yields the mixed effects model:

$$Y_{ij} = (\gamma_{00} + \mu_{oj}) + (\gamma_{10} + \mu_{1j})T_{ij} + r_{ij} \qquad \text{or alternatively as}$$

$$Y_{ij} = (\gamma_{00} + \gamma_{10}T_{ij}) + (\mu_{oj} + \mu_{1j}T_{ij}) + r_{ij}$$

The above example demonstrates a situation in which there are both random intercepts and slopes. At times, only one of these may be necessary or of interest to the research question. In those situations, the other component would simply be interpreted as a fixed effect. As well, additional level 1 and level 2 covariates can be added to the model as main exposures or confounding factors.

APPENDIX C:
LINEAR MIXED EFFECT MODELS FOR MEAN ESTIMATED FETAL WEIGHT BY MALARIA AND NUTRITIONAL STATUS

This appendix contains the results of a secondary analysis to assess the effects of malaria and maternal under-nutrition on mean fetal weight. These data were derived from linear mixed effect models fitted to data from 758 follow-up visits that occurred after 22 weeks. Gestational age was modeled as rounded weeks based on the ultrasound derived date. All final models contained a random intercept term and time was modeled using a quadratic and cubic polynomial. In the tables below, a negative value for the beta coefficient indicates a lower fetal weight in the exposed group, whereas a positive value for the beta coefficient indicates higher fetal weight in the unexposed group.

In general, the direction of the associations for mean fetal weight were similar to the risk ratio (RR) results demonstrated in Chapter 3, such that exposures with a RR value above one correspond to a decreased fetal weight in grams. The pattern of effect measure modification by maternal under-nutrition was also evident in the mean fetal weight data. Fetuses exposed to malaria were significantly smaller among women with low anthropometric measures compared to women with normal values.

Table C.1. Beta coefficients and 95% confidence intervals (CI) for mean fetal weight by sociodemographic and pregnancy characteristics, Kinshasa, Democratic Republic of Congo, 2005-2006

	Beta coefficient*	95% CI
Maternal age (years)		
18-24	-59	-108,-10
25-29	Ref	Ref
≥ 30	-32	-79, 15
Socioeconomic status		
High	24	-34, 82
Low	Ref	Ref
Gravida		
1-2	-27	-67, 13
≥ 3	Ref	Ref
Fetal gender		
Male	34	-6, 73
Female	Ref	Ref
Treated in previous month		
Yes	34	11, 57
No	Ref	Ref
Hematocrit at visit		
< 30	37	-26, 99
≥ 30	Ref	Ref

* Unadjusted. Model included 758 study visits. Difference in mean fetal weight for a fetus exposed to the covariate of interest. Positive EFW values indicate higher attained weight among the exposed while negative values indicated lower fetal weight.

Table C.2. Beta coefficients and 95% confidence intervals (CI) for mean fetal weight by incident and cumulative malaria infection, Kinshasa, Democratic Republic of Congo, 2005-2006

	Beta coefficient†	95% CI	Beta coefficient‡	95% CI	Beta coefficient§	95% CI
Malaria parasitemia at visit						
Positive	-5	-43, 33	-5	-42, 33	7	-34, 48
Negative	Ref	Ref	Ref	Ref	Ref	Ref
Cumulative malaria parasitemia up to visit						
0	Ref	Ref	Ref	Ref	Ref	Ref
1	13	-22, 48	16	-19, 50	27	-8, 62
2	31	-22, 84	33	-20, 86	27	-32, 86
≥3	-45	-128, 38	-42	-125, 41	-74	-170, 22
Cumulative malaria parasitemia up to visit						
≥2 positive	-8	-54, 38	8	-38, 53	-8	-59, 43
<2 positive	Ref	Ref	Ref	Ref	Ref	Ref
≥3 positive	-63	-141, 15	-61	-139, 17	-92	-185, 1
<3 positive	Ref	Ref	Ref	Ref	Ref	Ref

† Unadjusted. Model included 758 study visits.
‡ Adjusted for age. Model included 758 study visits.
§ Adjusted for age and weight gain. Model included 588 visits with complete data for weight gain.
Difference in mean fetal weight for a fetus exposed to the covariate of interest. Positive EFW values indicate higher attained weight among the exposed while negative values indicated lower fetal weight.

Table C.3. Beta coefficients and 95% confidence intervals (CI) for mean fetal weight by maternal anthropometric indicators, Kinshasa, Democratic Republic of Congo, 2005-2006

	Beta coefficient†	95% CI	Beta coefficient‡	95% CI	Beta coefficient§	95% CI
Time-independent variables						
Baseline BMI						
<19.8 kg/m^2	-48	-110, 14	-33	-96, 30	-9	-71, 53
≥19.8 kg/m^2	Ref	Ref	Ref	Ref	Ref	Ref
Short stature						
<150 cm	-79	-188, 30	-68	-177, 40	-57	-167, 52
≥150 cm	Ref	Ref	Ref	Ref	Ref	Ref
Time-dependent variables						
MUAC change per month #						
Baseline BMI <19.8 kg/m^2	-34	-104, 36	-34	-104, 36	-37	-104, 30
Baseline BMI ≥19.8 kg/m^2	6	-20, 32	6	-20, 31	20	-6, 47
Maternal weight gain per month**						
Baseline BMI <19.8 kg/m^2	--	--	--	--	-68	-140, 3
Baseline BMI ≥19.8 kg/m^2	--	--	--	--	-22	-49, 5

† Unadjusted. Model included 758 study visits.
‡ Adjusted for age. Model included 758 study visits.
§ Adjusted for age. Model included 588 visits with complete data for weight gain.
# Comparing MUAC change in the previous month of < 0 cm vs. ≥ 0 cm.
**Comparing maternal weight gain in the previous month of < 1.5 kg per month vs. ≥ 1.5 kg per month.
Difference in mean fetal weight for a fetus exposed to the covariate of interest. Positive EFW values indicate higher attained weight among the exposed while negative values indicated lower fetal weight.

Table C.4. Beta coefficients and 95% confidence intervals (CI) for mean fetal weight by malaria status, stratified by maternal anthropometrics, Kinshasa, Democratic Republic of Congo, 2005-2006

	Beta coefficient*	95% CI	Beta coefficient†	95% CI	Beta coefficient‡	95% CI
Malaria parasitemia at visit (Positive vs. negative)						
Baseline BMI <19.8 kg/m^2	-70	-172, 32	-69	-173, 35	**-95**	**-211, 21**
Baseline BMI ≥19.8 kg/m^2	6	-34, 46	5	-35, 45	**27**	**-17, 71**
Height <150 cm	-132	-382, 118	-124	-374, 125	-146	-386, 94
Height ≥150 cm	-2	-40, 36	-2	-40, 36	16	-26, 58
MUAC gain <0 cm in past month	-19	-80, 42	-19	-80, 41	-17	-79, 44
MUAC gain ≥0 cm in past month	20	-35, 75	19	-36, 74	40	-16, 95
Weight gain <1.5 kg in past month	--	--	--	--	-39	-29, 107
Weight gain ≥1.5 kg in past month	--	--	--	--	-10	-61, 41
Cumulative malaria parasitemia (≥ 2 positive vs. < 2 positive)						
Baseline BMI <19.8 kg/m^2	**-138**	**-272, -4**	**-135**	**-268, -1**	**-117**	**-272, 39**
Baseline BMI ≥19.8 kg/m^2	**25**	**-24, 73**	**25**	**-24, 73**	**7**	**-47, 62**
Height <150 cm	-49	-246, 148	-66	-262, 131	-70	-264, 124
Height ≥150 cm	12	-35, 59	13	-34, 60	1	-53, 54

Table C.4., continued

	Beta coefficient*	95% CI	Beta coefficient†	95% CI	Beta coefficient‡	95% CI
MUAC gain <0 cm in past month	-5	-68, 58	-6	-69, 56	7	-61, 75
MUAC gain ≥0 cm in past month	12	-45, 69	14	-42, 71	-27	-90, 36
Weight gain <1.5 kg in past month	--	--	--	--	0	-71, 71
Weight gain ≥1.5 kg in past month	--	--	--	--	-13	-73, 47

* Unadjusted. Model included 758 study visits and 76 episodes of IUGR.
† Adjusted for age. Model included 758 study visits and 76 episodes of IUGR.
‡ Adjusted for age. Model included 588 visits with complete data for weight gain and 66 episodes of IUGR.
Difference in mean fetal weight for a fetus exposed to the covariate of interest. Positive EFW values indicate higher attained weight among the exposed while negative values indicated lower fetal weight.

Note: Mean fetal weight pairs highlighted in bold indicate a significant P-value for the interaction term between malaria and the anthropometric indicator (P-value <0.15).

APPENDIX D:
INFLUENCE DIAGNOSTICS FOR SPECIFIC AIM 2

The following influence statistics were used to identify fetuses that influenced the estimates of the fixed effects and/or precision of the variance and covariance portions of the model.

Restricted likelihood distance: A global, summary measure of the influence of removing an observation jointly on all parameters. Calculated as twice the difference between the (restricted) log-likelihood evaluated at the full-data set and the reduced-data set.

PRESS statistic (Prediction Sum of Squares Statistic)**:** Measures the change in the predicted value of the response (Y_{ij}) variable caused by removal of an observation. Calculated as the sums of squares of the prediction residuals (calculated as the difference between the predicted response variable including the observation and the predicted response variable after removing the observation).

Cook's D**:** Measures the change in the parameter estimates caused by removal of an observation.

MDFFITS: Measures the change in parameter estimates due to removal of an observation (Closely related to Cook's D).

COVRATIO and COVTRACE**:** Measures the effect on the precision of the covariance matrix of the parameter estimates due to removal of an observation.

Table D.1. Influence diagnostics for log transformed estimated fetal weight

	INFLUENCE DIAGNOSTICS FOR THE FIXED EFFECTS					INFLUENCE DIAGNOSTICS FOR THE RANDOM EFFECTS			
Id	**Number of Observations in Level**	**Restricted Likelihood Distance**	**PRESS Statistic**	**Cook's D**	**COVRATIO**	**Cook's D CovParms**	**MDFFITS CovParms**	**COVRATIO CovParms**	**COVTRACE CovParms**
7100	6	0.0080	0.01472	0.00029	1.0304	0.00764	0.00746	1.0806	0.0784
7103	5	0.0648	0.04476	0.00291	1.0221	0.05971	0.06090	1.0308	0.0311
7108	6	0.0147	0.04414	0.00443	1.0214	0.00568	0.00557	1.0570	0.0560
7114	6	0.0092	0.01379	0.00077	1.0298	0.00740	0.00724	1.0814	0.0791
7118	5	0.0125	0.01947	0.00368	1.0224	0.00602	0.00594	1.0466	0.0459
7123	5	0.0157	0.00987	0.00144	1.0224	0.01291	0.01267	1.0710	0.0692
7124	5	0.0072	0.02215	0.00142	1.0228	0.00418	0.00411	1.0577	0.0566
7125	5	0.0149	0.01837	0.00255	1.0213	0.00984	0.00967	1.0643	0.0628
7127	5	0.0114	0.01309	0.00334	1.0201	0.00465	0.00456	1.0490	0.0482
7129	4	0.0131	0.02726	0.00337	1.0189	0.00656	0.00648	1.0383	0.0380
7130	5	0.0161	0.00816	0.00336	1.0194	0.00865	0.00850	1.0500	0.0493
7132	5	0.0516	0.07524	0.01288	0.9978	0.02632	0.02707	0.9834	0.0159
7136	5	0.0103	0.02697	0.00207	1.0199	0.00552	0.00546	1.0456	0.0449
7143	5	0.0198	0.04461	0.00637	1.0152	0.00683	0.00676	1.0356	0.0353
7145	6	0.0124	0.01587	0.00121	1.0288	0.01008	0.00988	1.0820	0.0797
7146	6	0.0123	0.03273	0.00235	1.0260	0.00828	0.00815	1.0608	0.0596
7150	5	0.0339	0.02749	0.00943	1.0048	0.01717	0.01746	0.9869	0.0124
7151	5	0.0090	0.00509	0.00006	1.0240	0.00894	0.00879	1.0749	0.0729
7154	6	0.0211	0.00655	0.00280	1.0313	0.01568	0.01536	1.0849	0.0823
7155	6	0.1889	0.03999	0.01706	0.9833	0.12686	0.12924	0.9823	0.0155
7157	6	0.0513	0.04493	0.00514	1.0293	0.04340	0.04388	1.0468	0.0466
7158	6	0.0335	0.03050	0.00896	1.0113	0.01265	0.01268	1.0180	0.0183

	INFLUENCE DIAGNOSTICS FOR THE FIXED EFFECTS					INFLUENCE DIAGNOSTICS FOR THE RANDOM EFFECTS			
Id	**Number of Observations in Level**	**Restricted Likelihood Distance**	**PRESS Statistic**	**Cook's D**	**COVRATIO**	**Cook's D CovParms**	**MDFFITS CovParms**	**COVRATIO CovParms**	**COVTRACE CovParms**
7159	5	0.0220	0.04062	0.00702	1.0118	0.00810	0.00806	1.0126	0.0128
7163	6	0.0660	0.08653	0.01360	1.0101	0.03887	0.03937	1.0215	0.0224
7171	6	0.0157	0.03277	0.00378	1.0292	0.00730	0.00712	1.0669	0.0655
7174	5	0.0081	0.00592	0.00001	1.0261	0.00815	0.00801	1.0777	0.0756
7179	5	0.0170	0.05201	0.00535	1.0151	0.00661	0.00656	1.0283	0.0281
7181	6	0.0155	0.02295	0.00076	1.0310	0.01481	0.01462	1.0717	0.0701
7189	5	0.0491	0.08151	0.01195	0.9994	0.02337	0.02404	0.9792	0.0202
7192	**5**	**1.2339**	**0.35792**	**0.06751**	**0.8958**	**0.93784**	**1.20143**	**0.6721**	**0.3583**
7195	5	0.0090	0.01946	0.00039	1.0244	0.00835	0.00825	1.0615	0.0603
7196	6	0.0630	0.06414	0.01568	0.9978	0.03465	0.03556	0.9900	0.0092
7204	5	0.0148	0.00980	0.00129	1.0214	0.01240	0.01217	1.0696	0.0679
7205	6	0.0686	0.08849	0.01297	1.0063	0.04293	0.04349	0.9822	0.0171
7207	5	0.0558	0.04347	0.01458	0.9975	0.02996	0.03139	0.9643	0.0348
7211	5	0.0141	0.00288	0.00047	1.0226	0.01338	0.01312	1.0742	0.0722
7214	5	0.0114	0.02741	0.00348	1.0158	0.00412	0.00407	1.0334	0.0331
7215	5	0.0648	0.03343	0.01579	0.9954	0.03787	0.03968	0.9553	0.0439
7217	6	0.0185	0.02582	0.00685	1.0242	0.00549	0.00540	1.0519	0.0510
7218	5	0.0056	0.01117	0.00045	1.0226	0.00457	0.00449	1.0654	0.0639
7221	5	0.0091	0.02758	0.00303	1.0207	0.00294	0.00287	1.0531	0.0521
7224	6	0.0361	0.04557	0.00935	1.0106	0.01868	0.01861	1.0280	0.0281
7228	6	0.1041	0.12847	0.01861	0.9984	0.06532	0.06802	0.9810	0.0175
7236	5	0.0260	0.02730	0.00852	1.0073	0.01086	0.01103	1.0037	0.0042
7237	5	0.0886	0.04090	0.02029	0.9896	0.05422	0.05806	0.9357	0.0635

	INFLUENCE DIAGNOSTICS FOR THE FIXED EFFECTS					INFLUENCE DIAGNOSTICS FOR THE RANDOM EFFECTS			
Id	**Number of Observations in Level**	**Restricted Likelihood Distance**	**PRESS Statistic**	**Cook's D**	**COVRATIO**	**Cook's D CovParms**	**MDFFITS CovParms**	**COVRATIO CovParms**	**COVTRACE CovParms**
7243	4	0.0080	0.00894	0.00224	1.0194	0.00353	0.00347	1.0507	0.0498
7244	6	0.4435	0.09805	0.02722	0.9544	0.32610	0.36132	0.8663	0.1364
7247	6	0.0179	0.02192	0.00346	1.0292	0.01142	0.01114	1.0760	0.0740
7248	6	0.0515	0.09191	0.01030	1.0129	0.03174	0.03187	1.0111	0.0116
7249	6	0.0662	0.02824	0.01771	0.9998	0.03764	0.03846	0.9967	0.0024
7251	5	0.0072	0.02191	0.00225	1.0197	0.00252	0.00248	1.0521	0.0512
7258	5	0.0070	0.00774	0.00013	1.0245	0.00679	0.00667	1.0735	0.0716
7260	7	0.0188	0.02556	0.00314	1.0328	0.01220	0.01189	1.0855	0.0830
7261	4	0.0191	0.04081	0.00547	1.0087	0.00780	0.00780	1.0056	0.0057
7272	6	0.0134	0.01057	0.00274	1.0234	0.00839	0.00823	1.0684	0.0667
7275	5	0.0608	0.06067	0.01350	0.9954	0.03472	0.03600	0.9559	0.0438
7276	4	0.1497	0.05185	0.00146	1.0242	0.14130	0.14703	1.0180	0.0195
7278	6	0.0407	0.05240	0.01006	1.0071	0.01807	0.01829	1.0077	0.0083
7284	5	0.0102	0.01133	0.00128	1.0252	0.00782	0.00769	1.0726	0.0707
7285	5	0.0097	0.01328	0.00295	1.0209	0.00390	0.00382	1.0511	0.0502
7289	5	0.0496	0.03682	0.00265	1.0168	0.04184	0.04246	1.0165	0.0169
7290	6	0.0098	0.01127	0.00100	1.0269	0.00804	0.00789	1.0771	0.0750
7295	5	0.0239	0.03129	0.00596	1.0128	0.01293	0.01272	1.0394	0.0390
7296	6	0.0142	0.01569	0.00265	1.0311	0.00958	0.00937	1.0805	0.0782
7297	5	0.0135	0.01472	0.00181	1.0220	0.01005	0.00988	1.0683	0.0667
7302	6	0.0135	0.02232	0.00037	1.0305	0.01204	0.01187	1.0713	0.0697
7308	5	0.0147	0.01187	0.00270	1.0223	0.00922	0.00905	1.0627	0.0614
7309	5	0.0162	0.01916	0.00370	1.0178	0.00927	0.00910	1.0538	0.0528

	INFLUENCE DIAGNOSTICS FOR THE FIXED EFFECTS					INFLUENCE DIAGNOSTICS FOR THE RANDOM EFFECTS			
Id	**Number of Observations in Level**	**Restricted Likelihood Distance**	**PRESS Statistic**	**Cook's D**	**COVRATIO**	**Cook's D CovParms**	**MDFFITS CovParms**	**COVRATIO CovParms**	**COVTRACE CovParms**
7313	5	0.0212	0.01755	0.00655	1.0106	0.01003	0.01001	1.0221	0.0223
7314	5	0.0109	0.01173	0.00165	1.0205	0.00775	0.00761	1.0617	0.0603
7317	6	0.0413	0.09362	0.01051	1.0117	0.01998	0.02006	1.0174	0.0179
7322	5	0.0145	0.02303	0.00444	1.0231	0.00662	0.00654	1.0462	0.0455
7325	4	0.0718	0.04606	0.00472	1.0180	0.05880	0.06011	1.0164	0.0169
7328	5	0.0135	0.00276	0.00013	1.0297	0.01338	0.01312	1.0865	0.0838
7331	6	0.2769	0.05913	0.02046	0.9667	0.19324	0.21657	0.8771	0.1234
7332	5	0.0115	0.02363	0.00265	1.0193	0.00620	0.00611	1.0579	0.0567
7333	6	0.0705	0.04425	0.00672	1.0278	0.05994	0.06108	1.0384	0.0385
7346	4	0.0058	0.01603	0.00119	1.0185	0.00327	0.00322	1.0464	0.0457
7349	5	0.0282	0.04882	0.00787	1.0084	0.01144	0.01147	1.0150	0.0153
7350	5	0.0166	0.00114	0.00056	1.0245	0.01554	0.01523	1.0777	0.0755
7351	5	0.0059	0.01585	0.00109	1.0229	0.00413	0.00406	1.0622	0.0608
7352	6	0.0187	0.03272	0.00572	1.0274	0.00824	0.00802	1.0634	0.0622
7354	5	0.0908	0.06359	0.01903	0.9887	0.05630	0.05937	0.9509	0.0482
7357	2	0.0042	0.00661	0.00135	1.0114	0.00152	0.00149	1.0279	0.0277
7358	6	0.0114	0.02289	0.00147	1.0314	0.00925	0.00906	1.0744	0.0726
7360	5	0.0100	0.01642	0.00330	1.0204	0.00366	0.00358	1.0481	0.0474
7362	5	0.0407	0.06132	0.01061	1.0093	0.01965	0.01981	1.0160	0.0165
7366	5	0.0162	0.00392	0.00180	1.0242	0.01251	0.01228	1.0704	0.0686
7367	4	0.0223	0.02325	0.00031	1.0225	0.02159	0.02167	1.0480	0.0475
7370	5	0.0067	0.01277	0.00118	1.0240	0.00481	0.00471	1.0635	0.0621
7375	5	0.0073	0.01512	0.00180	1.0201	0.00371	0.00364	1.0538	0.0528

	INFLUENCE DIAGNOSTICS FOR THE FIXED EFFECTS					INFLUENCE DIAGNOSTICS FOR THE RANDOM EFFECTS			
Id	**Number of Observations in Level**	**Restricted Likelihood Distance**	**PRESS Statistic**	**Cook's D**	**COVRATIO**	**Cook's D CovParms**	**MDFFITS CovParms**	**COVRATIO CovParms**	**COVTRACE CovParms**
7380	5	0.0449	0.06817	0.01170	1.0021	0.02127	0.02181	0.9843	0.0152
7383	6	0.0283	0.02110	0.00446	1.0261	0.01973	0.01926	1.0765	0.0745
7386	2	0.0253	0.02105	0.00723	1.0110	0.01223	0.01227	0.9984	0.0010
7387	6	0.0123	0.02657	0.00125	1.0314	0.01003	0.00983	1.0735	0.0717
7389	5	0.0098	0.02529	0.00266	1.0212	0.00387	0.00381	1.0503	0.0494
7390	6	0.3388	0.24526	0.03084	0.9661	0.24901	0.27776	0.9015	0.0967
7391	5	0.0189	0.04640	0.00426	1.0193	0.01045	0.01039	1.0374	0.0371
7394	6	0.0168	0.02703	0.00586	1.0199	0.00521	0.00511	1.0397	0.0392
7395	4	0.0086	0.00184	0.00006	1.0210	0.00853	0.00839	1.0674	0.0658
7396	5	0.0099	0.03243	0.00367	1.0162	0.00244	0.00240	1.0384	0.0379
7397	6	0.1279	0.06556	0.01758	0.9898	0.07879	0.08329	0.9557	0.0431
7400	6	0.0183	0.01101	0.00396	1.0262	0.01009	0.00990	1.0701	0.0684
7403	**6**	**1.1555**	**0.44762**	**0.05373**	**0.9131**	**0.87581**	**1.09354**	**0.7817**	**0.2207**
7404	5	0.0085	0.01616	0.00162	1.0222	0.00522	0.00514	1.0636	0.0622
7407	6	0.0522	0.08065	0.00951	1.0189	0.03467	0.03476	1.0234	0.0238
7410	5	0.0154	0.02155	0.00559	1.0157	0.00495	0.00489	1.0382	0.0378
7412	5	0.0734	0.07220	0.01549	0.9981	0.04283	0.04413	0.9914	0.0075
7413	5	0.0114	0.02384	0.00174	1.0240	0.00726	0.00717	1.0548	0.0539
7415	5	0.0329	0.04260	0.00268	1.0241	0.02669	0.02683	1.0432	0.0430
7419	5	0.0221	0.04056	0.00698	1.0131	0.00762	0.00758	1.0277	0.0277
7420	5	0.0106	0.01014	0.00225	1.0193	0.00648	0.00637	1.0544	0.0534
7423	6	0.0368	0.06890	0.00869	1.0172	0.01871	0.01857	1.0428	0.0427
7424	5	0.0815	0.04306	0.00288	1.0264	0.07698	0.07881	1.0376	0.0380

	INFLUENCE DIAGNOSTICS FOR THE FIXED EFFECTS					INFLUENCE DIAGNOSTICS FOR THE RANDOM EFFECTS			
Id	**Number of Observations in Level**	**Restricted Likelihood Distance**	**PRESS Statistic**	**Cook's D**	**COVRATIO**	**Cook's D CovParms**	**MDFFITS CovParms**	**COVRATIO CovParms**	**COVTRACE CovParms**
7425	5	0.0175	0.01198	0.00473	1.0184	0.00906	0.00890	1.0524	0.0515
7426	7	0.0435	0.06981	0.01000	1.0136	0.02188	0.02168	1.0413	0.0411
7427	5	0.0211	0.01778	0.00493	1.0254	0.01144	0.01119	1.0638	0.0625
7428	5	0.0149	0.02143	0.00371	1.0239	0.00870	0.00863	1.0482	0.0476
7430	5	0.0464	0.02318	0.01280	1.0008	0.02561	0.02646	0.9825	0.0164
7433	5	0.0430	0.02981	0.01198	1.0010	0.02306	0.02373	0.9860	0.0131
7435	5	0.0118	0.00501	0.00035	1.0259	0.01120	0.01100	1.0792	0.0770
7437	6	0.3676	0.09486	0.04903	0.9584	0.27158	0.30023	0.8913	0.1075
7438	5	0.0175	0.02214	0.00337	1.0225	0.01102	0.01081	1.0647	0.0632
7439	5	0.0984	0.05754	0.00673	1.0194	0.08693	0.08940	1.0112	0.0120
7440	5	0.0084	0.02152	0.00181	1.0264	0.00496	0.00484	1.0664	0.0650
7441	5	0.0194	0.02774	0.00666	1.0189	0.00599	0.00590	1.0404	0.0399
7442	5	0.0135	0.00957	0.00139	1.0282	0.01091	0.01070	1.0789	0.0767
7444	5	0.0187	0.00438	0.00279	1.0205	0.01405	0.01376	1.0631	0.0617
7448	6	0.0337	0.07829	0.00974	1.0121	0.01461	0.01463	1.0237	0.0239
7449	6	0.1075	0.06271	0.01991	0.9902	0.07068	0.07374	0.9466	0.0532
7451	4	0.0086	0.00836	0.00143	1.0196	0.00573	0.00564	1.0564	0.0553
7452	5	0.0573	0.07304	0.01263	1.0054	0.03196	0.03252	1.0095	0.0103
7456	5	0.0175	0.00656	0.00254	1.0210	0.01315	0.01289	1.0652	0.0637
7458	5	0.0712	0.10232	0.01471	0.9977	0.03944	0.04079	0.9744	0.0248
7459	4	0.0445	0.04196	0.01215	0.9990	0.02180	0.02256	0.9753	0.0241
7460	4	0.0112	0.01681	0.00288	1.0164	0.00539	0.00532	1.0471	0.0463
7464	5	0.0149	0.00666	0.00209	1.0324	0.01143	0.01118	1.0821	0.0797

	INFLUENCE DIAGNOSTICS FOR THE FIXED EFFECTS					INFLUENCE DIAGNOSTICS FOR THE RANDOM EFFECTS			
Id	**Number of Observations in Level**	**Restricted Likelihood Distance**	**PRESS Statistic**	**Cook's D**	**COVRATIO**	**Cook's D CovParms**	**MDFFITS CovParms**	**COVRATIO CovParms**	**COVTRACE CovParms**
7465	5	0.0149	0.02085	0.00288	1.0233	0.00914	0.00897	1.0662	0.0647
7467	5	0.0079	0.00922	0.00126	1.0218	0.00541	0.00532	1.0620	0.0606
7470	5	0.0337	0.03183	0.00864	1.0084	0.01406	0.01420	1.0076	0.0081
7472	6	0.2075	0.06738	0.02030	0.9765	0.14013	0.15333	0.8975	0.1030
7474	5	0.0295	0.03520	0.00199	1.0247	0.02399	0.02411	1.0463	0.0459
7476	7	0.0373	0.06329	0.00538	1.0229	0.02429	0.02431	1.0481	0.0476
7477	7	0.0162	0.01044	0.00017	1.0394	0.01616	0.01572	1.0987	0.0953

Figure D.1. Influence diagnostics for log transformed estimated fetal weight variable: effects on the fixed portion of the mixed effects model

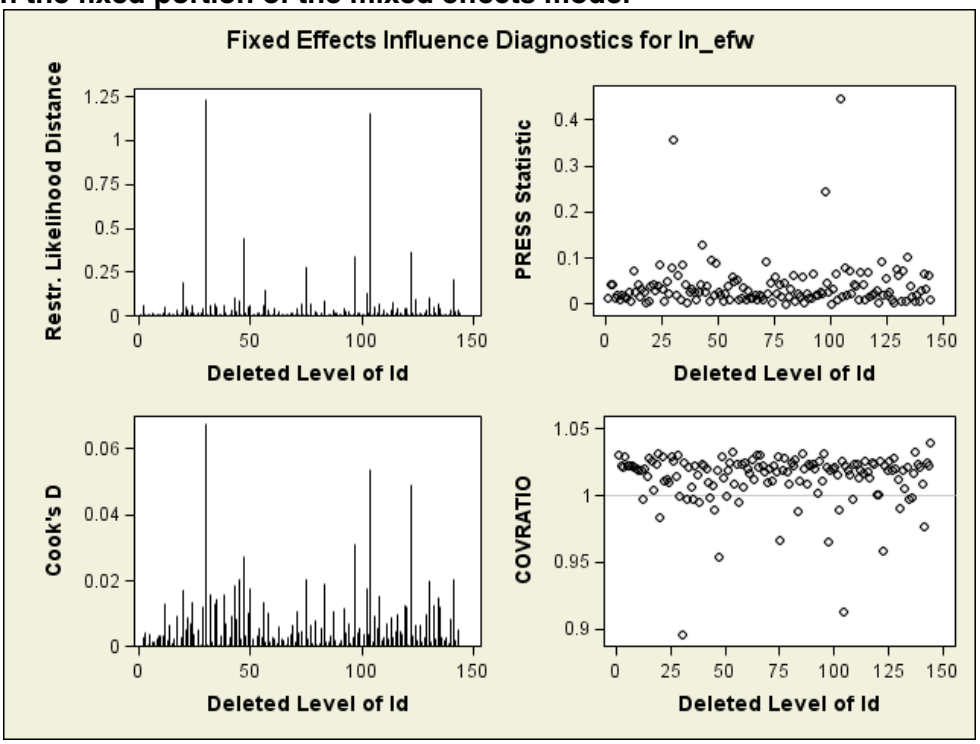
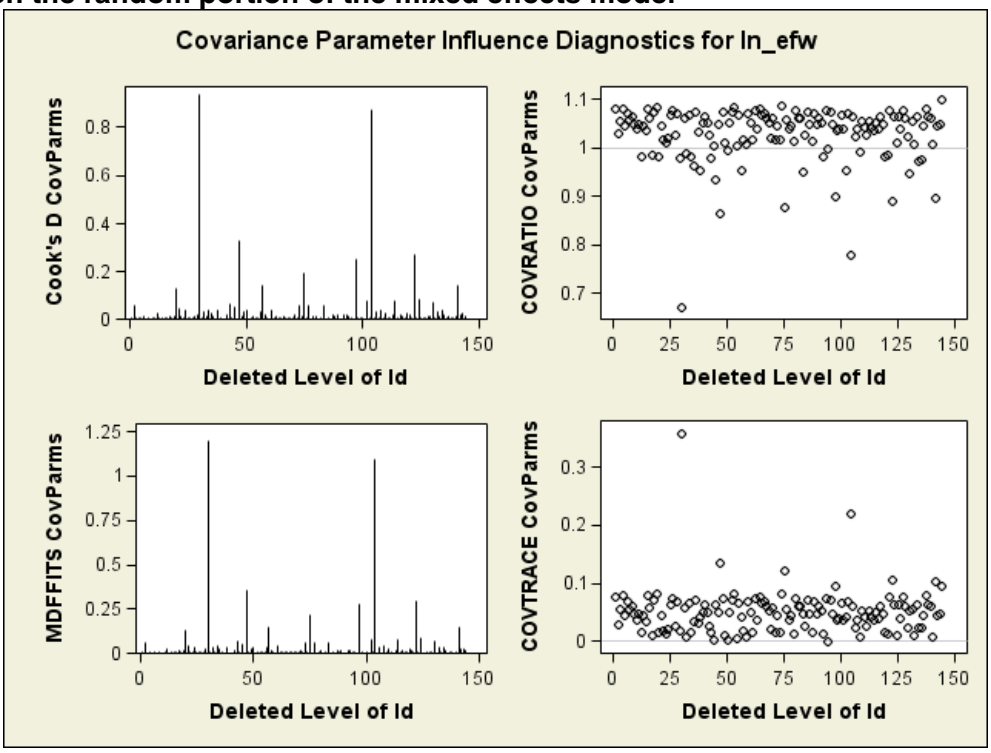


Figure D.2. Influence diagnostics for log transformed estimated fetal weight variable: effects on the random portion of the mixed effects model



APPENDIX E:
SENSITIVITY ANALYSIS FOR DEVELOPMENT OF THE FETAL SIZE NOMOGRAM UTILIZING A DIFFERENT ESTIMATED FETAL WEIGHT FORMULA

Measurement of fetal biometry by ultrasound is the gold standard for estimating the size or weight of a fetus and is assumed to be more accurate than clinical methods including palpation or measurement of fundal height. Many researchers have generated algorithms where log weight is calculated as a polynomial function of various biometric parameters.[159] A recent analysis comparing the accuracy of 25 ultrasound derived EFW algorithms found that correlation between actual birth weight and predicted birth weight ranged from 0.44 to 0.79, with mean absolute errors ranging from ± 263 grams to 646 grams (± 7.5% to 18.5%).[158]

Because fetal weight estimation is an important component of a fetal size nomogram, we wanted to explore how the use of another EFW algorithm would affect the centile values of the fetal size nomogram developed for Congo. We re-ran the data analysis and created a new nomogram based upon EFW calculated using the algorithm proposed by Shepard which utilizes two biometric parameters, AC and BPD [log(EFW)= -1.7492 + 0.166*BPD + 0.046*AC - 0.002646*BPD*AC].[136] This formula has been shown to have low systematic and random error in the estimation of fetal weight.

Table E.1 displays the 10th, 50th, and 90th centile values for the nomograms utilizing the Hadlock and Shepard EFW formulas. Generally, the centiles derived from the two algorithms are quite similar, with the exception of late in gestation when the centiles derived using the Shepard EFW are increasingly larger than the Hadlock derived values. Unlike the curve derived from the Hadlock data, the Shepard derived EFW values do not show a leveling off near term, and hence overestimate the Hadlock values after about 35 weeks gestation.

We also ran a small analysis to assess the accuracy of the EFW measurements against actual post-term birth weights (Table E.2). The percent difference between the 50th centile predicted fetal weight and the actual term birth weight at 38, 39 and 40 weeks gestation was 3.7%, 7.1% and 6.9% for the Hadlock EFW algorithm, and 4.8%, 9.1% and 9.9% for the

Shepard algorithm, respectively. Thus, the Hadlock EFW algorithm provided a better estimate of fetal weight than the Shepard algorithm in this population.

In conclusion, the centile values of the Congo derived nomogram appear fairly robust to differing EFW algorithms, except near term when the differences varied by as much as 200 grams. The Hadlock EFW algorithm appears to provide a more accurate estimation of fetal weight as evidenced by lower percent differences between predicted and actual weight for infants born at term. Thus, we feel that the nomogram based upon the Hadlock algorithm is the better choice for presentation.

Table E.1. Comparison of the predicted 10th, 50th and 90th centiles utilizing the estimated fetal weight algorithms by Hadlock and Shepard

	Using Hadlock EFW formula[27]			Using the Shepard EFW formula[136]		
GA weeks	10th	50th	90th	10th	50th	90th
20	289	315	345	306	338	373
21	347	380	415	362	400	442
22	415	454	497	426	472	523
23	491	539	591	499	554	614
24	578	635	698	580	645	718
25	675	743	819	670	748	836
26	782	864	955	770	863	967
27	900	997	1106	880	990	1112
28	1028	1143	1271	1000	1128	1273
29	1166	1301	1452	1130	1279	1449
30	1313	1471	1647	1269	1442	1639
31	1469	1651	1854	1418	1617	1845
32	1632	1840	2074	1575	1803	2064
33	1801	2036	2302	1740	1999	2296
34	1973	2238	2538	1912	2204	2540
35	2147	2442	2778	2089	2416	2794
36	2321	2647	3019	2270	2634	3055
37	2491	2848	3257	2454	2855	3322
38	2656	3044	3488	2637	3078	3591
39	2813	3230	3709	2820	3299	3860
40	2959	3404	3916	2998	3517	4124

Table E.2. Percent difference between post-natal birth weight measurements and predicted 50th centile values utilizing the estimated fetal weight algorithms by Hadlock and Shepard

	50th centile predicted EFW				Percent difference*	
Gestational age (weeks)	Hadlock EFW Formula	Shepard EFW Formula	Post-natal mean birth weight	N	Hadlock	Shepard
38	3044	3078	2930	17	3.7	4.8
39	3230	3299	3000	43	7.1	9.1
40	3404	3517	3169	46	6.9	9.9

Percent difference = (Predicted 50th centile – Postnatal birth weight) / Predicted 10th centile) * 100

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