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# The Associations between Nutrition, Stress, Infection, and Inflammation and Maternal Health and Pregnancy Outcomes in Rural Malawi

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## Abstract

**Background.** Preterm birth and small birth size are globally important predictors of childhood morbidity, mortality, undernutrition and growth failure, and developmental loss. Maternal infections, inflammation, stress, and malnutrition are thought to cause these adverse birth outcomes, especially in low-income settings. However, there is only limited information on the interaction between these adverse maternal exposures and their relative importance in causing preterm birth and intrauterine growth restriction (IUGR).

**Objective.** To build concept maps to describe the associations between various maternal exposures and shortened pregnancies or smaller birth length, weight, or head circumference in a rural Malawian population.

**Design.** We enrolled 1,391 women with uncomplicated pregnancies (<20 gestation weeks [gw]) in a randomized, controlled trial in Malawi. Nested within this trial, we carried out a prospective cohort study on the determinants of the duration of pregnancy and IUGR (i.e., neonatal weight-for-age, neonatal length-for-age, and neonatal head circumference-for-age). The tested predictors included many variables indicating maternal constitutional factors (e.g., age, parity), nutritional status (e.g., body mass index [BMI], weight gain in pregnancy, blood hemoglobin [Hb] concentration), infection (e.g., HIV, malaria, reproductive and urinary tract infections, chorioamnionitis, periodontitis, dental infections), or stress (self-estimated stress test, salivary cortisol concentration).

We started the analysis by establishing associations between the stated outcome variables and the selected predictors. We then built a matrix of bivariate associations between all variables that were associated with the duration of pregnancy or neonatal weight-for-age, neonatal length-for-age, and neonatal head circumference-for-age. These analyses, including adjustments for possible confounders, allowed us to identify independent associations between various predictors and outcome variables and thus to build an initial concept map about pathways leading to adverse pregnancy outcomes. We then refined this concept map by doing stepwise regression analyses, i.e., by adding the predictor variables into the models one by one, with the most distal variables first and most proximal ones last. Finally, we used structural equation models to estimate correlation coefficients between the variables and to create illustrations of the pathways leading to reduced duration of pregnancy or to reduced newborn length, weight, or head circumference.

**Results.** The duration of pregnancy was predicted by maternal malaria parasitemia at enrollment, severe chorioamnionitis, and the presence of periapical oral infections soon after birth. Additionally, pregnancy duration was predicted by maternal blood Hb concentration at enrollment, salivary cortisol concentration at 36 gw, and placental weight. All newborn size measurements were predicted by the duration of pregnancy, placental weight, and maternal inflammation. In addition, newborn length was independently associated with maternal infections, weight gain during pregnancy, primiparity and height; newborn weight was associated with maternal primiparity, maternal BMI at enrollment, weight gain during pregnancy and newborn length-for-age z-score (LAZ); and newborn head circumference was associated with maternal BMI at enrollment and newborn LAZ. For each outcome, however, there was a complex network of proximal and more distal determinants, so that all dimensions of fetal growth were ultimately associated both with maternal nutritional status and with variables reflecting infection, inflammation, and stress, directly or indirectly.

**Conclusions.** Whereas maternal infections and inflammation seem to be important determinants of the duration of pregnancy and birth length in the studied cohort, the pathways to preterm birth and small birth size also include maternal undernutrition, stress, and certain constitutional factors like age and parity. Because of this complex network of adverse exposures, it is not surprising that single-pronged nutritional or infection-targeted antenatal interventions have had at best modest impacts on fetal growth or duration of pregnancy in low-income settings. For greater impact, it is likely that more-comprehensive multipronged interventions will be needed, to ensure good nutritional status for the mother both before and during pregnancy, prevention and treatment of a wide range of maternal infections, and prevention of maternal stress.

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## Abbreviations and Acronyms

| | |
|---|---|
| AA | arachidonic acid, an omega-6 fatty acid |
| AGP | α-1-acid glycoprotein |
| ALA | α-linolenic acid, an omega-3 fatty acid |
| ANCOVA | analysis of covariance |
| ANOVA | analysis of variance |
| BMI | body mass index |
| CI | confidence interval |
| COM | University of Malawi, College of Medicine |
| CRP | C-reactive protein |
| CV | coefficient of variation |
| DHA | docosahexaenoic acid, an omega-3 fatty acid |
| ELISA | enzyme linked immunosorbent assay |
| FANTA | Food and Nutrition Technical Assistance III Project |
| g | gram(s) |
| G | measurement unit of gravitational force |
| GC | gas chromatography |
| gw | gestation weeks |
| Hb | hemoglobin |
| HCZ | head circumference-for-age z-score |
| HIV | human immunodeficiency virus |
| HPLC | high performance liquid chromatography |
| IDA | iron deficiency anemia |
| IFA | iron and folic acid |
| IGF-1 | insulin like growth factor 1 |
| iLiNS-DYAD-M | A clinical trial with the indicated name (DYAD = mother-child pair, M = Malawi) |
| iLiNS Project | International Lipid-Based Nutrient Supplement Project |
| IOM | Institute of Medicine |
| IPTp | intermittent preventive treatment (of malaria) in pregnancy |
| IQR | interquartile range |
| IUGR | intrauterine growth restriction |
| kcal | kilocalorie(s) |
| kg | kilogram(s) |
| L | liter(s) |
| LAZ | length-for-age z-score |
| LBW | low birth weight |
| LNS | lipid-based nutrient supplement(s) |
| LNS-RTI | Lipid-Based Nutrient Supplement – Reproductive Tract Infections (study) |
| µg | microgram(s) |
| µL | microliter(s) |

| | |
|---|---|
| m | meter(s) |
| MFI | mean fluorescence intensity |
| mg | milligram(s) |
| mL | milliliter(s) |
| mm | millimeter(s) |
| mmHg | millimeter(s) of mercury |
| MMN | multiple micronutrient(s) |
| MUAC | mid-upper arm circumference |
| PCR | polymerase chain reaction |
| pRBC | parasitized red blood cell |
| PSS | Perceived Stress Scale |
| RDT | rapid diagnostic testing |
| RMSEA | root mean square error of approximation |
| SAE | serious adverse event |
| SD | standard deviation |
| SE | standard error |
| SEM | structural equation models |
| SES | socioeconomic status |
| SGA | small for gestational age |
| SP | sulfadoxine-pyrimethamine |
| sTfR | soluble transferrin receptor |
| tHcy | total homocysteine |
| UCD | University of California, Davis |
| UCL | University College London |
| UM | University of Melbourne |
| UNC | University of North Carolina |
| USAID | U.S. Agency for International Development |
| USDA | U.S. Department of Agriculture |
| UTA | University of Tampere |
| UTI | urinary tract infection |
| VSA | variant surface antigens |
| WAZ | weight-for-age z-score |
| WHO | World Health Organization |
| ZPP | zinc protoporphyrin |

# 1. Introduction

## 1.1 Scientific Background to the Study

Worldwide, an estimated 20 million infants are born with a low birth weight (LBW) (<2,500 g) each year, contributing to approximately 10%–15% of the global mortality of children under 5 years old and to a large share of childhood undernutrition, morbidity, and developmental loss (Black et al. 2008, Black et al. 2013, Christian et al. 2013, Espo et al. 2002, Katz et al. 2013, UNICEF/World Health Organization [WHO] 2004). Two factors determine size at birth: the rate of growth during the fetal period and the duration of pregnancy. Thus, LBW may reflect either intrauterine growth restriction (IUGR) or preterm birth or both. While the exact molecular mechanisms leading to early onset of labor or restricted fetal growth are largely unknown, a number of risk factors have been identified for both conditions (Ergaz et al. 2005, Goldenberg et al. 2008). These factors have often been categorized into maternal conditions (e.g., maternal genetics, nutritional status, or overall health), placental pathology (e.g., location in the uterus or vascularization), infant characteristics (e.g., genetics), or environmental or other factors (e.g., use of tobacco, habitat altitude, or others).

Of the identified risk factors, maternal undernutrition and infections have most consistently been associated with both IUGR and preterm birth (Ergaz et al. 2005, Goldenberg et al. 2008). Consequently, there has been wide interest in studying the efficacy of dietary supplements or presumptive treatment of pregnant women with antimicrobial agents as a means to promote fetal growth and prevent preterm birth. Indeed, a recent systematic review concluded that the incidence of IUGR could be markedly reduced by supplementing the maternal diet during pregnancy either with multiple micronutrients (MMN) or with protein and energy (Bhutta et al. 2013). In malaria endemic areas, intermittent preventive treatment (of malaria) in pregnancy (IPTp) has also proven beneficial (Kayentao et al. 2013) and the WHO now recommends its regular use in moderate-to-high malaria transmission areas in Africa (WHO 2012). Some studies have also reported improved birth outcomes after presumptive treatment of pregnant women with antibacterial broad-spectrum antibiotics (Gray et al. 2001, Swadpanich et al. 2008, Luntamo et al. 2010). However, very few trials have evaluated the impact of combined micronutrient and energy/protein supplementation in pregnancy (Kramer and Kakuma 2003). No studies have looked at the interaction between such a dietary intervention, infections, and other possible risk factors with regard to maternal health and pregnancy outcomes.

Lipid-based nutrient supplements (LNS) are versatile and easy-to-use nutritional products that have been successfully applied to the rehabilitation of children with severe acute malnutrition (Bhutta et al. 2013) and that may also offer benefits in the promotion of healthy growth (Adu-Afarwuah et al. 2007, Phuka et al. 2008, Mangani et al. 2013). In the only LNS trial targeting the gestational period and reported before the onset of our own trial, infants born to women who during pregnancy received a relatively large daily dose (72 g) of an LNS called Fortified Food Supplement had a higher mean birth length than infants of women who received MMN (Huybregts et al. 2009). Based on their findings, the authors of the report recommended a targeted nutritional supplementation for pregnant women with suboptimal pre-pregnancy nutritional status, consisting of MMN, protein, and energy, as a means to promote child growth in low-income settings (Huybregts et al. 2009). Subsequently, a modest increase in birth weight was also associated with the provision of a smaller daily LNS dose (20 g/day) to pregnant women in Bangladesh (Mridha et al. 2016) and primiparous pregnant women in Ghana (Adu-Afarwuah et al. 2016).

The International Lipid-Based Nutrient Supplement Project (iLiNS Project) conducted a mother-child dyad trial in Malawi (iLiNS-DYAD-M) that was designed to study the impact on maternal and child health in rural Malawi of an intervention that provided small-quantity LNS both to mothers during

pregnancy and early lactation and to their newly born children from 6 to 18 months of age. In this trial, pregnant women were randomized to receive dietary supplementation during pregnancy with iron and folic acid (IFA), MMN, or LNS. The trial had a large sample size and it included a very detailed clinical follow-up and frequent collection of a wide range of biological samples. Hence, it provided a unique opportunity not only to study the impact of the intervention on a number of maternal and newborn outcomes, but also to understand the possible heterogeneity in the effect of the intervention and to provide clues on other potential pregnancy interventions by carefully analyzing the multiple determinants of IUGR, preterm birth, and small birth size in the same dataset. To take advantage of this opportunity, the iLiNS-DYAD-M study team, with generous support from the U.S. Agency for International Development (USAID)-funded Food and Nutrition Technical Assistance III Project (FANTA), designed and implemented an add-on component to the trial called the Lipid-Based Nutrient Supplement – Reproductive Tract Infection (LNS-RTI) study.

New items studied as part of the LNS-RTI add-on to the iLiNS-DYAD-M trial included variables describing details of maternal nutrition (e.g., plasma concentration of cholesterol or composition of fatty acids) and malaria; maternal periodontitis and dental caries; infections of the reproductive tract, placenta, and amniotic membranes; and maternal stress. These variables were of interest because they had previously been suggested to be related to adverse pregnancy outcomes (Bobetsis et al. 2006, Goldenberg 2003, Goldenberg et al. 2008, Romero et al. 2003, Giurgescu 2009, Allen and Harris 2001, Edison et al. 2007). In the LNS-RTI, we used these variables in two ways: as outcomes from the randomized trial and as predictors for adverse birth outcomes. In this report, we describe the associations between these variables and birth outcomes. The impact of the trial interventions on these variables is described in a separate report (Ashorn et al. 2017).

## 1.2 The Structure of the Report

This report presents the context, methods, and findings for the LNS-RTI add-on study to the iLiNS-DYAD-M trial. The following text gives a brief orientation to the report.

- Chapter 2 describes the study design; the follow-up scheme; and the general approach to data collection, management, and analysis. It provides a description of how outcome variables and their predictors were measured.
- Chapter 3 reports on the general context of the study.
- Chapters 4 and 5 report on the study findings categorized under two themes. Chapter 4 describes the associations between various predictor variables and selected pregnancy outcomes, and Chapter 5 presents an analysis of pathways leading to duration of pregnancy and newborn length-for-age z-score (LAZ), weight-for-age z-score (WAZ), and head circumference-for-age z-score (HCZ).
- Chapter 6 provides a general discussion on the predictive value of various maternal characteristics for the four birth outcomes of focus in the pathway analysis.
- Following Chapter 6 is a list of references mentioned in this report.
- After the references are a number of appendices:
  - Appendix 1: Background characteristics of participants who were included and excluded from the predictor analyses of birth outcomes
  - Appendix 2: Details of data collection, laboratory analyses, and statistical analysis
  - Appendix 3: Additional tables and background to the pathway analysis
  - Appendix 4: Details of biological sample collection
  - Appendix 5: A comprehensive description of variable definitions
  - Appendix 6: A list of reported outcome variables

# 2. Methods

## 2.1 Study Design, Outcomes, and Ethics Statement

The study was a prospective cohort study, nested within the iLiNS-DYAD-M randomized controlled trial that was carried out in Malawi. The hypothesis of the trial was that home fortification of pregnant women's diets with LNS would increase birth size in an African community. The women were provided with one daily IFA capsule; one capsule with 18 micronutrients; or one 20 g sachet of LNS containing 118 kcal, protein, carbohydrates, essential fatty acids, and 22 micronutrients from ≤20 gestation weeks (gw) until delivery. The primary outcome measures were birth weight and child length soon after birth, and secondary outcomes included multiple maternal and child variables. The trial design and main findings are described in Ashorn et al. (2017).

In the study sample, women who received LNS gave birth to infants whose mean birth weight and length were approximately 50 g and 4 mm greater than those of infants born to women who received IFA, respectively. However, the differences were not statistically significant (Ashorn et al. 2015). There were also no significant intergroup differences in the prevalence of maternal malaria parasitemia at various points of pregnancy or soon thereafter or of vaginal trichomoniasis and urinary tract infections (UTIs) after pregnancy. Likewise, the mean maternal saliva concentration of cortisol; the plasma concentrations of inflammation markers C-reactive protein (CRP) and α-1-acid glycoprotein (AGP); and the nutritional markers of cholesterol, triglycerides, folate, and defined fatty acids during the third trimester of pregnancy were similar in the three intervention groups. The groups also did not differ in terms of mean bacterial load in the placenta or amniotic membranes, the prevalence of chorioamnionitis in the placenta, or the development of malaria immunity during pregnancy (Ashorn et al. 2017). Thus, the data do not support a hypothesis that provision of small-quantity LNS or MMN to all pregnant women would increase the mean birth size or markedly affect other pregnancy outcomes in rural Malawi (Ashorn et al. 2016).

The trial setup was used for the presently described nested cohort study where we are interested in associations between maternal nutrition, stress, infection, and inflammation and maternal health and pregnancy outcomes.

The trial was performed according to Good Clinical Practice guidelines and the ethical standards of the Helsinki Declaration. The protocol was approved by the College of Medicine (COM) Research and Ethics Committee, University of Malawi, and the Ethics Committee of Pirkanmaa Hospital District, Finland. Only participants who signed or thumb-printed an informed consent form were enrolled in the study. An independent data safety and monitoring board monitored the incidence of suspected serious adverse events (SAEs) and performed two interim analyses for safety. The data safety and monitoring board members received information about all suspected SAEs on an ongoing basis and met three times during the pregnancies part of the trial.

Key details of the protocol were published at the clinical trial registry of the National Library of Medicine, Bethesda, MD, USA (http://www.clinicaltrials.gov/ct2/show/NCT01239693?term=NCT01239693&rank=1).

## 2.2 Study Hypotheses

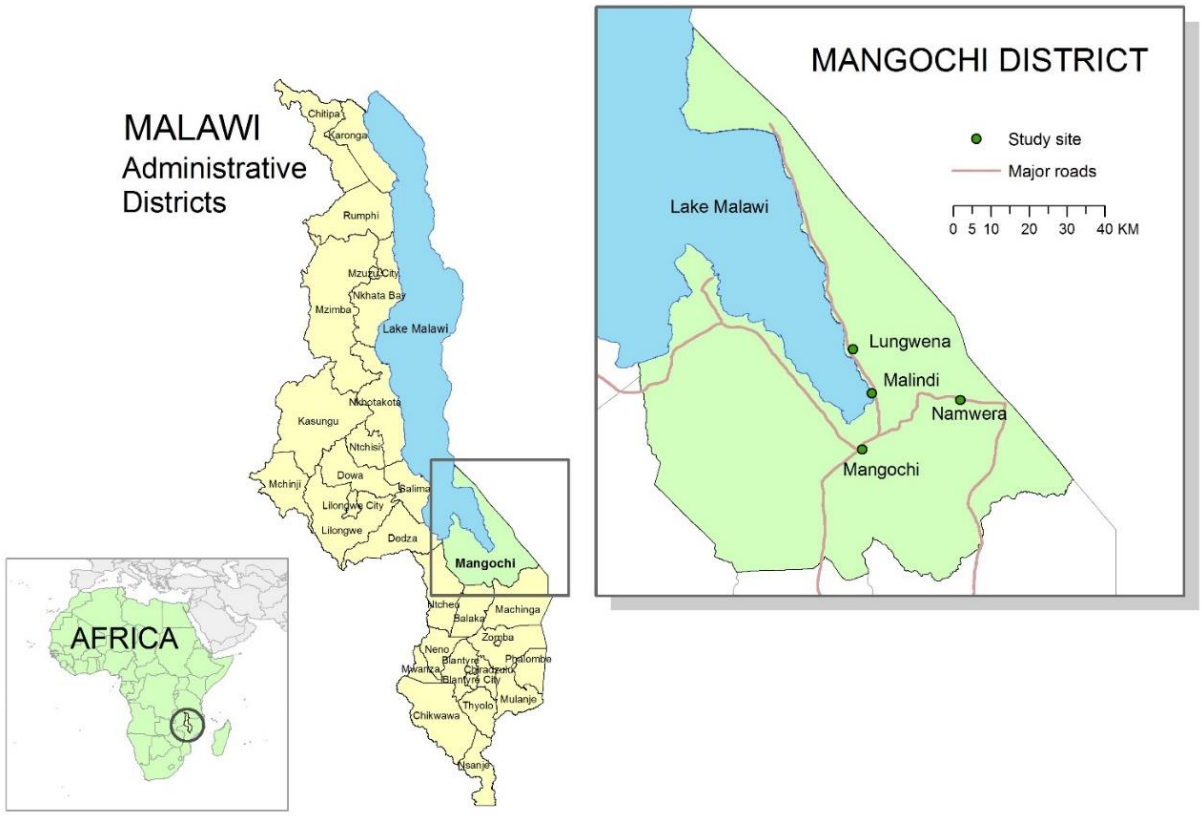
For this observational part of the study, the overall assumption was that maternal infections, inflammation, stress, and poor nutritional status would be associated with lower birth weight, shorter newborn length, shorter duration of pregnancy, and poorer other continuous birth outcomes, as well as

with a higher incidence of LBW and a higher prevalence of newborn stunting and other forms of undernutrition. It was also expected that there would be an association with a higher incidence of preterm birth.

## 2.3 Study Site and Participants

Enrollment in the study took place in one public district hospital (Mangochi), one semi-private hospital (Malindi), and two public health centers (Lungwena and Namwera) in Mangochi District of southern Malawi (Figure 2.3-1). The Mangochi Hospital outpatient clinic served a semi-urban population of 100,000; the other sites provided health care to approximately 30,000 people each. All sites were accessible by all-weather roads. The population subsisted largely on farming and fishing. Prior to commencing the trial, the study team members held numerous discussions with community leaders and organized village meetings to discuss the research objectives and procedures. Pregnant women coming to antenatal visits received further information about the trial.

**Figure 2.3-1. Map of the Study Sites**




Source: Data layer for Africa map downloaded from http://www.thematicmapping.org, 2015 available under a Creative Commons Attribution-Share Alike License 3.0 (borders may not be completely accurate). All other data layers downloaded from Malawi Spatial Data Portal, 2015 (http://www.masdap.mw). Map created with ArcGIS Desktop v.10.3, Environmental Systems Research Institute (ESRI) 2016, Redlands, CA.

The target population was composed of pregnant women who came for antenatal care at any of the study clinics during the enrollment period and met the following inclusion criteria: ultrasound confirmed pregnancy of no more than 20 completed gw, residence in the defined catchment area, availability during the period of the study, and signed or thumb-printed informed consent. Exclusion criteria were: age under 15 years, need for frequent medical attention due to a chronic health condition, diagnosed asthma treated

with regular medication, severe illness warranting hospital referral, history of allergy to peanuts, history of anaphylaxis or serious allergic reaction to any substance, requiring emergency medical care, pregnancy complications evident at enrollment visit (moderate to severe edema, blood hemoglobin [Hb] concentration <50 g/L, systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg), earlier participation in the iLiNS-DYAD-M trial (during a previous pregnancy), or concurrent participation in any other clinical trial.

## 2.4 Study Interventions

Participants in the trial were randomized into three intervention groups.

- Women in the IFA group, the first control group, received standard Malawian antenatal care, including supplementation from enrollment to delivery with one micronutrient capsule per day containing 60 mg of iron and 400 µg folic acid and two doses of IPTp with sulfadoxine-pyrimethamine (SP) (three tablets of 500 mg sulfadoxine and 25 mg pyrimethamine orally).
- Participants in the MMN group, the second control group, received IPTp and micronutrient capsules that contained IFA and 16 additional micronutrients.
- Participants in the LNS intervention group received IPTp and sachets of tailor-made small-quantity LNS formulated for pregnant and lactating women.

The study intervention and group allocation process are described in Ashorn et al. (2016).

## 2.5 Enrollment in the Study

At the enrollment visit, trained anthropometrists measured the participants' weight, height, and mid-upper arm circumference (MUAC). They took all measurements in triplicate, with high-quality scales (SECA 874 flat scale, Seca GmbH & Co., Hamburg, Germany), stadiometers (Harpenden stadiometer, Holtain Limited, Crosswell, Crymych, UK), and non-stretchable plastic tapes (Shorrtape, Weigh and Measure, LLC, Olney, MD, USA), with reading increments of 50 g, 1 mm, and 1 mm, respectively. Research nurses recorded participants' obstetric histories and performed antenatal examinations. They assessed the duration of pregnancy by measuring the fetal biparietal diameter, the femur length, and the abdominal circumference (all in mm, mean of two measurements), with ultrasound imagers that utilized inbuilt Hadlock tables to estimate the duration of gestation (EDAN DUS 3 Digital Ultrasonic Diagnostic Imaging System, EDAN Instruments, Inc., Shekou, Nanshan Shenzhen, China). The same nurses measured the women's peripheral blood malaria parasitemia with rapid tests (Clearview Malaria Combo, British Biocell International Ltd., Dundee, UK) and Hb concentration with on-site cuvette readers (HemoCue AB, Angelholm, Sweden). Health facility nurses gave pretest HIV counseling and tested all participants for HIV infection, except those who opted out or were already known to be HIV infected, using a whole-blood antibody rapid test (Alere Determine HIV-1/2, Alere Medical Co., Ltd., Chiba, Japan). If the result was positive, the test was repeated using another whole-blood antibody rapid test (Uni-Gold HIV, Trinity Biotech plc, Bray, Ireland). If the tests were not available at the health facility on the day of enrollment, the study team arranged the test to be performed as soon as possible thereafter. Participants with a positive test were referred to the antiretroviral clinic for treatment in accordance with Option B+ treatment guidelines for HIV-positive pregnant women.

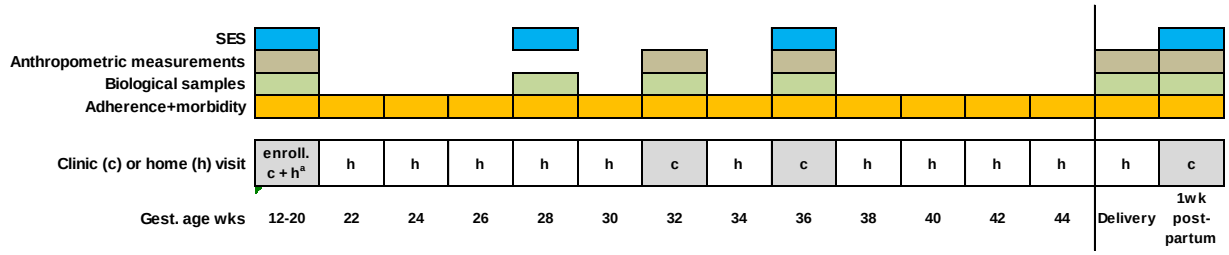
Further description on the enrollment process can be found elsewhere (Ashorn et al. 2015).

## 2.6 Follow-Up

After enrollment, the study team invited the participants to visits at the study clinic: at 32 gw, at 36 gw, and at approximately 1–2 weeks after delivery. Additionally, data collectors visited the participants fortnightly at their homes and soon after delivery either at their home or at the local maternity unit. Larger sets of biological samples were collected at the enrollment and at the 36 gw visits, but some samples were also collected at 28 gw, at delivery, and at 1 week after delivery.

Figure 2-6.1 shows a schematic representation of the study visits, biological sample collection, and clinical outcomes measured at each visit.

**Figure 2-6.1. Study Visits, Biological Sample Collection, and Clinical Outcomes Measured at Each Visit**




SES = socioeconomic status

[a] After the initial enrollment clinic visit, which took place between 12 gw and 20 gw, participants were seen regularly at home or at the clinic every 2 weeks until delivery.

During the antenatal clinical visits, study anthropometrists measured the participants' weight, height, and MUAC with the same methods as at enrollment, and study nurses carried out standardized obstetric examinations.

During the fortnightly home visits, data collectors delivered the supplements and collected information on the participant's adherence to the study intervention. As soon as possible after birth, research assistants visited the mother to record the delivery events, collect a blood sample for malaria tests, measure the placental size and collect samples from it and the amniotic membrane for histological and microbiological analyses, and measure the infant's birth weight. Other anthropometric measurements were not taken since this visit was sometimes completed at home.

A more thorough postnatal visit was completed when the infant was 1–2 weeks old and brought to the study clinic. At this visit, study nurses took anthropometric measurements for both the mother and the newborn infant. A dental therapist made an oral examination and took a panoramic x-ray from the mother's teeth.

If a participant did not come for the scheduled visit within 14 days of the appointment, data collectors made a visit to the participant's home. Information on the participants' hospitalizations and other suspected SAEs was collected actively via interviews at each fortnightly home visit. Study nurses also contacted both hospitals in the study area daily, to obtain information on any hospitalizations or deaths among study participants. Additionally, the study physicians trained health providers at all the known private and public health facilities in the area to identify the study participants from their iLiNS-DYAD-M identification cards and to record information on any nonscheduled visits on structured data collection forms that were collected and reviewed by the study team on a weekly basis. Finally, research assistants made a special home visit at 6 weeks after delivery, to verify the vital status of the participating woman and infant at the end of the primary follow-up period.

The study participants attended antenatal and under-5 clinics according to the same schedule as all other Malawian pregnant women and infants and received all normal preventive services provided by the national health system. Study nurses treated participants with documented peripheral blood malaria parasitemia with lumefantrine/artemether, the nationally recommended antimalarial drug. Other medical conditions were treated in the national health system (in either the public or the private sector). The study team reimbursed the participants for all medical costs that they incurred during the trial participation.

For the clinic visits, participants were compensated for their travel costs according to a local bicycle taxi rate. For visits taking more than 1 hour, there was also a small compensation for participant time, ranging from a reusable diaper (postnatal home visit) or 800 g of rice (antenatal clinic visits and socioeconomic background interviews) to 800 g of rice and 500 g of salt (postpartum clinic visit).

## 2.7 Collection of Biological Samples

As indicated above, biological samples were collected mainly at enrollment and at the 36 gw visits, but some samples were also collected at other visits. Most biological samples were placed in a −20°C freezer within 2 hours of sample collection, then transferred to −80°C within 48 hours of collection and stored at −80°C until analyzed. Placental samples used for histological analysis were fixed in formalin and stored at +4°C until embedded in paraffin and used for histology. Table 2.7-1 presents an overview of the biological samples collected during the visits and which analyses were done. Detailed methods used for sample collection and processing are included in Appendix 4.

**Table 2.7-1. Biological Samples Collected and Analysis Done, by Visit**

| Tissue | Analyses | Timing | | | | | |
|---|---|---|---|---|---|---|---|
| | | Enrollment | 28 gw | 32 gw | 36 gw | Delivery | 1 week postpartum |
| Blood/plasma | AGP, CRP, Hb, sTfR[a], ZPP[b], cholesterol, triglycerides, essential fatty acids, vitamin A, malaria infection, malaria immunity | X | | Only malaria infection | X | | |
| Saliva | Cortisol | X | X | | X | | |
| Placenta | Histology, PCR[c] analysis of bacteria | | | | | X | |
| Amniotic membrane | Histology, PCR[c] analysis of bacteria | | | | | X | |
| Oral swab | PCR[c] analysis of bacteria | | | | | | X |
| Vaginal mucus | Trichomoniasis, PCR[c] analysis of bacteria | | | | | | X |
| Urine | UTIs | | | | | | X |

[a] sTfR = soluble transferrin receptor
[b] ZPP = zinc protoporphyrin
[c] PCR = polymerase chain reaction

## 2.8 Quality Assurance in Data Collection

We ensured data collection quality through regular staff training and monitoring and through the use of written visit guides, instructions about the use of data collection forms, and additional standard operating procedures. Aside from birth weight, anthropometric measurements were taken only by trained personnel whose measurement reliability was verified at the start of the study and at 6-monthly intervals thereafter with methods modified from the procedures used in the WHO Multicenter Growth Reference Study (2006). Birth weight could also be measured by study nurses or study coordinators. The anthropometrists calibrated all equipment with standard weights and length rods on a daily basis. An external monitor appointed by the study team did one site monitoring visit during data collection.

The IFA and MMN interventions were provided using double-masked procedures, i.e., the capsules looked identical and neither the participants nor the research team members were aware of the nutrient contents of the supplement capsules. For the LNS group, we used single-masked procedures, i.e., field workers who delivered the supplements knew which mothers were receiving LNS (but not a difference between IFA and MMN), and the participants were advised not to disclose information about their supplements to anyone other than an iLiNS Project team member. The data collectors who performed the anthropometric measurements or assessed other outcomes were not aware of group allocation. Researchers responsible for the data cleaning remained blind to the trial code until the database was fully cleaned.

## 2.9 Measurement of Outcome Variables and Their Predictors

This section provides general information on the methods of data collection for key outcome variables and their predictors. Detailed information on data collection methods for each subtopic can be found in Appendix 2.

### 2.9.1 Duration of Pregnancy

Gestational age at enrollment was assessed at the enrollment visit by research nurses by measuring the fetal biparietal diameter, femur length, and abdominal circumference (all in mm, mean of two measurements), with ultrasound imagers that utilized inbuilt Hadlock tables to estimate the duration of gestation (EDAN DUS 3 Digital Ultrasonic Diagnostic Imaging System, EDAN Instruments, Inc., Shekou, Nanshan Shenzhen, China).

Duration of pregnancy at birth was calculated by adding the time interval between enrollment and miscarriage or delivery to the ultrasound-determined gestational age at enrollment.

### 2.9.2 Maternal and Child Anthropometrics

Trained anthropometrists measured maternal weight, height, and MUAC. They did all measurements in triplicate, with high-quality scales (SECA 874 flat scale, Seca GmbH & Co., Hamburg, Germany), stadiometers (Harpenden stadiometer, Holtain Limited, Crosswell, Crymych, UK), and non-stretchable plastic tapes (Shorrtape, Weigh and Measure, LLC, Olney, MD, USA), having reading increments of 50 g, 1 mm, and 1 mm, respectively. For measurements that were completed in triplicate, we used the mean of the first two readings if they did not differ by more than a prespecified tolerance limit. If the difference was above the limit, the third measurement was compared with the first and second measurements, and the pair of measurements that had the smallest difference was used to calculate the mean. If there were only one or two repeated measurements, the mean of those was used for the analyses.

Data on birth weight was used as such if measured within 48 hours of delivery, and back-calculated birth weight was used for data collected between 6 and 13 days after delivery using WHO z-scores. If weight was first measured between 2 and 5 days after delivery (when weight loss is typical), we calculated birth weight by multiplying the actual measured weight by a day-specific correction factor (Cheung 2014). We considered birth weight or newborn anthropometric measurements missing if they were collected more than 2 and 6 weeks after delivery, respectively.

Study anthropometrists measured the infant's length with a high-quality length board (Harpenden Infantometer, Holtain Limited, Crosswell, Crymych, UK) and recorded it to the nearest 1 mm, weight with an electronic infant weighing scale with a reading increment of 20 g (SECA 381 baby scale, Seca GmbH & Co., Hamburg, Germany), and head and mid-upper arm circumference with the same plastic tapes that were used for maternal anthropometry.

We calculated age- and sex-standardized anthropometric indices (weight-for-age, length-for-age, weight-for-length, and head circumference-for-age z-scores) using the WHO Child Growth Standards (WHO Multicentre Growth Reference Study Group 2006).

### 2.9.3 Body Mass Index and Weekly Gestational Weight Gain

We calculated body mass index (BMI) from weight and height measurements conducted at the enrollment visit for all women who enrolled in the iLiNS-DYAD-M trial. (See Section 2.9.2 for more information on how weight and height were measured.) Additionally, we measured weight at 32 gw and at 36 gw to estimate weekly gestational weight gain from time of enrollment to 36 weeks gestation. We included in the BMI analyses all participants who had singleton pregnancies and for whom we had weight and height measurements at enrollment, at 32 gw, and at 36 gw. We included in the weekly weight gain analyses those for whom we had at least one weight measurement (this could be just the enrollment measurement), as mixed modeling was used to estimate weekly weight gain even if only one weight measurement was taken. (For further details on the analytic methods used to estimate weekly weight gain, refer to A2.1).

### 2.9.4 Placental Size

Research nurses or laboratory technicians weighed the placentas as soon as possible after the delivery with dietary scales, with a reading increment of 1 g. In addition, they measured the placental diameter with a ruler from two dimensions: one at the largest diameter and the other at a 90° angle to the first one. The average of these two measures was used to calculate the radius and hence the surface area of the placenta.

### 2.9.5 Peripheral Blood Malaria Parasitemia during Pregnancy

Malaria was tested by rapid diagnostic testing (RDT) and by polymerase chain reaction (PCR). A finger prick sample was used for RDT at the study sites at enrollment, at 32 gw, and at delivery. For PCR testing, whole blood was drawn by venipuncture at 36 gw and at delivery. Drops of blood were placed on filter paper to prepare dried blood spots at the study clinic. The dried blood spots samples were then shipped to the University of North Carolina where PCR analyses were conducted.

### 2.9.6 Reproductive Tract and Urinary Tract Infections

At 1 week after delivery, the participants visited the study clinic for reproductive tract infection and UTI testing. A study nurse obtained a blind vaginal swab and immediately sent the sample to the study laboratory. The woman was also asked to provide a urine sample in a screw-top bottle. The study nurse performed a urine dipstick analysis on the urine sample.

### 2.9.7 Inflammatory Response; Maternal Plasma CRP and AGP Concentrations

Clinic nurses collected the blood samples at enrollment and at 36 gw. We analyzed CRP and AGP from those blood samples by immunoturbidimetry on the Cobas Integra 400 system autoanalyzer (F. Hoffmann-La Roche Ltd, Basel, Switzerland) at University of California, Davis (UCD).

### 2.9.8 Blood Hemoglobin, Zinc Protophyrin, and Plasma Transferrin Receptor Concentrations

Clinic nurses measured Hb concentration from whole blood collected from a finger prick at 36 gw. Laboratory technicians isolated red blood cells from the finger prick sample and measured zinc protoporphyrin (ZPP) concentration from washed red blood cells, and soluble transferrin receptor (sTfR) concentration was analyzed by immunoturbidimetry at UCD.

### 2.9.9 Plasma Retinol Concentration

Plasma retinol concentration was measured at enrollment and at 36 gw with high performance liquid chromatography (HPLC) at UCD.

### 2.9.10 Plasma Cholesterol and Triglyceride Concentrations and Plasma Fatty Acid Status

Plasma cholesterol and triglyceride concentrations were determined at the U.S. Department of Agriculture (USDA) Western Human Nutrition Research Center (Davis, CA, USA) using a Cobas Integra 400 plus automatic analyzer (Roche Diagnostic Corp., Indianapolis, IN, USA). Plasma fatty acid composition was analyzed by gas chromatography (GC) with flame ionization detection at OmegaQuant Analytics, LLC (Sioux Falls, SD, USA). Fatty acid composition was expressed as a percent of total identified fatty acids. Measurements were done at enrollment and 36 weeks gestation.

### 2.9.11 Perceived Stress Scale and Salivary Cortisol Concentration

**Perceived Stress Scale (PSS).** We utilized the PSS (Cohen et al. 1983), a 10-item questionnaire that has been used in Brazil (Rondo et al. 2003) and South Africa (Beard et al. 2005), which asks the respondent to rate how frequently she thought or felt a certain way on a scale of 0 to 4 (0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, 4 = very often) in the past month. Specifically, a woman was asked how often, in the last month, she had:

1. Been upset because something had happened unexpectedly
2. Felt unable to control the important things in her life
3. Felt nervous and stressed
4. Felt confident in her ability to handle her personal problems
5. Felt that things were going her way
6. Felt that she could not cope with all the things she had to do
7. Been able to control irritations in her life
8. Felt that she was on top of things
9. Been angered because of things that were outside of her control
10. Difficulties piling up so high she could not overcome them

The PSS was administered to women at enrollment, 28 weeks gestation, and 36 weeks gestation.

**Salivary cortisol concentration.** Saliva samples were collected at enrollment, 28 weeks gestation, and 36 weeks gestation, between 8 am and 4 pm, with a mean collection time at approximately 11 am. Women were instructed not to consume any food or drink besides water for at least 30 minutes before providing the saliva sample. Time of saliva collection, time of waking, and time of last food or drink were recorded. Enrollment and 36-week gestation saliva samples were collected at clinic sites when women came to provide blood and urine samples and have anthropometric measurements taken, while the 28-week gestation saliva samples were collected by field workers during home visits. Saliva collection occurred before any other measurements or sample collection.

Saliva was obtained by having the woman place an inert polymer cylindrical swab (10 mm x 30 mm, Salimetrics Oral Swab [Salimetrics, State College, PA, USA]) under her tongue for approximately 2 minutes, while moving her tongue and jaw as if she were chewing to stimulate saliva. The swab was then placed in a tube with a cap and refrigerated or placed on ice packs. Swabs were brought to room temperature before centrifuging for 15 minutes at 3,000 RPM. Samples were frozen and stored at −20°C within 24 hours of collection.

### 2.9.12 Microbial Communities in the Placenta and Fetal Membranes, the Oral Cavity, and the Vagina

A sample of placenta tissue and the fetal membranes (amnion and chorion) was taken immediately after delivery, and a dental swab and a vaginal swab were taken 1 week after delivery. Inflammation and evidence of malaria infection were both assessed from histological slides taken from the placenta and fetal membranes at the Malawi College of Medicine. A lab technician at the Malawi-Liverpool Wellcome Trust laboratory in Blantyre extracted DNA from the placenta, fetal membranes, oral swabs, and vaginal swabs. The bacterial 16S rRNA gene was selectively amplified as confirmation of the presence of bacteria. Bacterial DNA underwent high-throughput sequencing at Great Ormond Street Hospital in London, UK, to elucidate the entire bacterial community that resided in each sample.

### 2.9.13 Oral Health

We assessed the prevalence of maternal oral diseases soon after delivery.

Two specially trained dental therapists conducted a comprehensive clinical and questionnaire-based oral health assessment and took digital radiographs at the postnatal visit at 1 week after delivery or as soon as possible at the Mangochi central site. The examiners' measurement reliability was assessed and verified at the beginning and regularly during the study. An oral and maxillofacial radiologist and an experienced dentist jointly analyzed the radiographs using structured forms.

### 2.9.14 Malaria Immunity

Malaria immunity was measured in the laboratories of the University of Melbourne (UM) in Melbourne, Australia. Antibody levels to various laboratory-prepared malaria antigens were measured in plasma samples collected at enrollment and at 36 gw. Plasma samples were prepared by heat inactivating at 56˚C for 45 minutes prior to the assays. A number of immunoassays were performed to measure antibody levels against each antigen, and the levels were reported with reference to a positive control.

### 2.9.15 HIV Infection

HIV testing and counseling was conducted at enrollment according to national guidelines. HIV testing was offered to all women attending antenatal clinics at the study sites. Pretest HIV counseling was offered to all the women during the routine antenatal health talk. For the women who expressed interest in taking part in the study and did not opt out of the test, capillary blood was drawn by a finger prick and HIV tests

were performed in the study rooms. Post-test counseling was offered to all women after conducting the test. Women who tested negative or had an indeterminate result were asked to return to the clinic for repeat testing after 3 months. Those who tested positive were referred to the antiretroviral clinic for treatment in accordance with Option B+ treatment guidelines for HIV-positive pregnant women.

### 2.9.16 Socioeconomic and Demographic Background of the Participants

We collected information on the socioeconomic and demographic background of the mothers with structured interviews that took place at the participant's home within 2 weeks of enrollment. We asked questions about the family and household structure, the educational background of the mother and father, and the living environment. Data collectors interviewed the mother, and, if she was not available, they agreed on a later date when she would be available.

The interviews were conducted in Chichewa and Chiyao. The answers given by each respondent were written down in the language of that respondent.

## 2.10 Data Management

**Data collection and review.** Original data collection forms were developed by the researchers. Data collectors received oral instructions on how to fill out the forms from researchers and study monitors. In addition, a user guide was written for each data collection form, with information about the background of the form, specific information on how to conduct the interview and data collection, and instructions on specific form questions.

**Data entry.** All data were initially collected on paper forms from which they were extracted and entered into a tailor-made database through scanning and digital character recognition (TeleForm Desktop Version 10.5, Autonomy, Highland Park, IL, USA). Data entry clerks verified all critical variables or suspicious entries during the data entry process.

**Database management.** After data entry (TeleForm), the checked data were stored in a MySql database. From this database, the data were exported into Excel files that could be used for further data cleaning and analysis. Access to the database was organized through a custom-made access portal (iLiNS suite) with web access. Authorized researchers and personnel could access the data from there.

**Data cleaning.** The data in the database were cleaned by researchers and research assistants. All persons cleaning the data were blinded to the intervention each participant had received. A number of logical checks were performed on the data to identify suspicious values, which were later compared with the original research form used during data collection or after comparison with other data collected for the same participant. In case the suspicious value corresponded with the original collection form, these were mostly left in the data but marked with a cell coloring in the final dataset to be considered for revision during later analysis.

## 2.11 Statistical Notes

### 2.11.1 The Predictive Value of Maternal Characteristics for Birth Outcomes

To analyze the predictive value of maternal characteristics for birth outcomes, we used a standard set of outcome variables, where possible. Included were the duration of pregnancy; birth weight; newborn LAZ; newborn WAZ; newborn HCZ; incidence of preterm birth and LBW; and prevalence of low LAZ, low WAZ, and small HCZ. In addition, the incidence of small for gestational age (SGA) is reported as an outcome for some analyses.

A list of the maternal characteristics that were tested for their predictive value is presented in Appendix 6.

All sections report both unadjusted and adjusted outcomes in their respective analyses. Across the sections, the method for selecting the potential variables to be considered as covariates differed. In some cases, it was based on knowledge of previous literature, on an evaluation of which variables could logically have an association with a certain outcome, and in some cases those variables were predefined in the analysis plan for that section. The method used is described in detail by subtopic in Appendix 2.

### 2.11.2 Pathway Analyses

For the pathway analysis, we built on the results from the analysis of the predictive value of the maternal characteristics for birth outcomes. We were interested in four primary birth outcomes: duration of pregnancy, newborn LAZ, newborn WAZ, and newborn HCZ.

We started the analysis by establishing bivariate associations (as reported in other sections in this report) between all maternal characteristic variables that were associated ($P<0.05$) with any of the above described four primary outcomes (see Table A3-2 for list of variables considered). Next, we created a series of multivariable regression models, in which we defined each of the maternal characteristic variables, one at a time, as the dependent variable in a model and all of the remaining maternal characteristic variables as covariates in the model. This allowed us to determine "predictors of predictors" for our pathway models.

We then built a series of multivariable regression models for each birth outcome of focus by adding the predictor variables into each model one by one, with the most distal predictors for that outcome entered into the model first and the most proximal predictors for that outcome entered into the model last. Distal variables included variables that described maternal characteristics in early pregnancy or direct maternal exposures during the follow-up (e.g., maternal nutrition or infection), whereas the proximal variables were those that would follow from a primary exposure (e.g., maternal weight gain, plasma AGP concentration as a sign of inflammation, or duration of pregnancy). The predictor and intermediate variables were selected either based on earlier literature (Brodsky and Christou 2004) or because our own initial analyses had documented an association between them and newborn size (Harjunmaa et al. 2015, Stewart et al. 2015). By doing this, we were able to see which variables attenuated the effect of others, and this allowed us to determine the pathways through which the independent variables were associated with the dependent variables. In the final regression models, we also included the intervention that the study participants had received during the intervention trial, even if the intervention was not associated with any of the primary outcomes.

We used ordinary least squares regression and logistic regression with multiple imputed data to estimate regression coefficients for continuous variables and odds ratios for dichotomous variables, respectively, when estimating associations between variables in simple (i.e., bivariate analysis) and multivariable regression models. For each outcome, we created pathway models based on the information from the simple and multivariable regression models.

We used multiple imputed data (50 imputations), imputed based on chained equation methods (van Buuren et al. 1999) for all ordinary least squares regression and logistic regression analyses. Of the 1,391 women who were enrolled in the study, 1,379 (99.1%) were included in the pathway analysis for duration of pregnancy and 1,179 (84.8%) were included in the pathway analysis for the newborn size outcomes. For duration of pregnancy, we excluded women with twin pregnancies. We assumed women lost to follow-up before delivery were singleton pregnancies, and we used multiple imputation to impute duration of pregnancy for all participants with singleton pregnancies with missing data. For newborn

anthropometrics, we excluded twins and those who did not have a 1-month measurement taken. We used multiple imputation to impute values for participants who had a measurement at 1 month, but that was actually done more than 6 weeks after delivery. We included in the imputation model variables describing maternal enrollment characteristics (age, parity, height, education, household food insecurity access scale); maternal nutrition (BMI and blood Hb at enrollment, average weekly weight gain during pregnancy); maternal infections (HIV, malaria at enrollment, UTIs and vaginal trichomoniasis at 36 gw, periapical oral infections after delivery); maternal inflammation and stress (maternal plasma AGP concentration at enrollment and at 36 gw; salivary cortisol concentration at enrollment, at 28 gw, and at 36 gw); placental size, infection, and inflammation (placental weight, placental malaria, severe chorioamnionitis); duration of pregnancy; newborn size (birth weight, MUAC, LAZ, WAZ, HCZ); and study intervention group. If any of the maternal characteristics had missing values, those were imputed at the same time, using all variables listed above.

We estimated coefficients for the pathway models using structural equation models (SEM), which allowed us to include variables as both endogenous (outcome) and exogenous (predictor) in the same model. We used the maximum likelihood method with missing values to estimate model parameters in SEM (Allison 2003). In the final SEM, we included pathways that were statistically significant at level $P<0.05$ in simple and multivariable regression models, even though some of the associations in the SEM were $P\geq0.05$ (Kline 2011). We estimated model goodness of fit index root mean square error of approximation (RMSEA), which adjusts for the number of paths estimated in the model (DiLalla 2008). An RMSEA score of 0 indicates perfect fit, scores <0.05 are considered to be a good fit, and scores <0.08 are considered to be adequate fit (Jaccard and Wan 1996). In SEM, we used only continuous endogenous variables because SEM do not allow endogenous variables to be dichotomous. This limited our models a bit because we could not estimate parameters for pathways going to maternal malaria, HIV, and primiparity. We indicate this with dashed lines in our pathway graphs and show the direction of the association, as indicated by the corresponding simple regression model.

When trying to determine pathways between different variables, our aim was to find associations that were independent from the other measurements. When modeling the pathways to newborn LAZ, WAZ, and HCZ, we were primarily interested in fetal growth velocity and hence controlled the models for duration of pregnancy. We were interested in newborn weight as a predictor of childhood wasting and mortality (Katz et al. 2013) and length as a predictor of subsequent childhood growth stunting (Espo et al. 2002). To describe and visualize the predictors of newborn length (linear growth) and "thickness" or "robustness" (ponderal growth) separately, we included LAZ in the models for newborn WAZ. In essence, we were thus modeling newborn weight-for-length, but in a way that separated the contributions of linear and ponderal growth on newborn weight. For consistency, we also controlled for newborn LAZ when we modeled newborn HCZ.

To make it easier to interpret the results of the models, we summarized the models in illustrations showing the pathways leading to each of the four birth outcomes. The arrows going to the most central variable (i.e., the main outcome: duration of pregnancy, LAZ, WAZ, or HCZ) describe the absolute change in that outcome (weeks for duration of pregnancy, z-scores for anthropometric indices). Arrows going to any other continuous variable in the model describe the change in standardized values (mean 0, SD 1). We did not calculate the total effects (sum of direct and indirect effects) of different variables in the model. Since dichotomous variables could not be included in the SEM analysis as outcome variables, we indicated this with a dashed line in our pathway graphs and established the direction of association from regression models.

# 3. Results: General Context

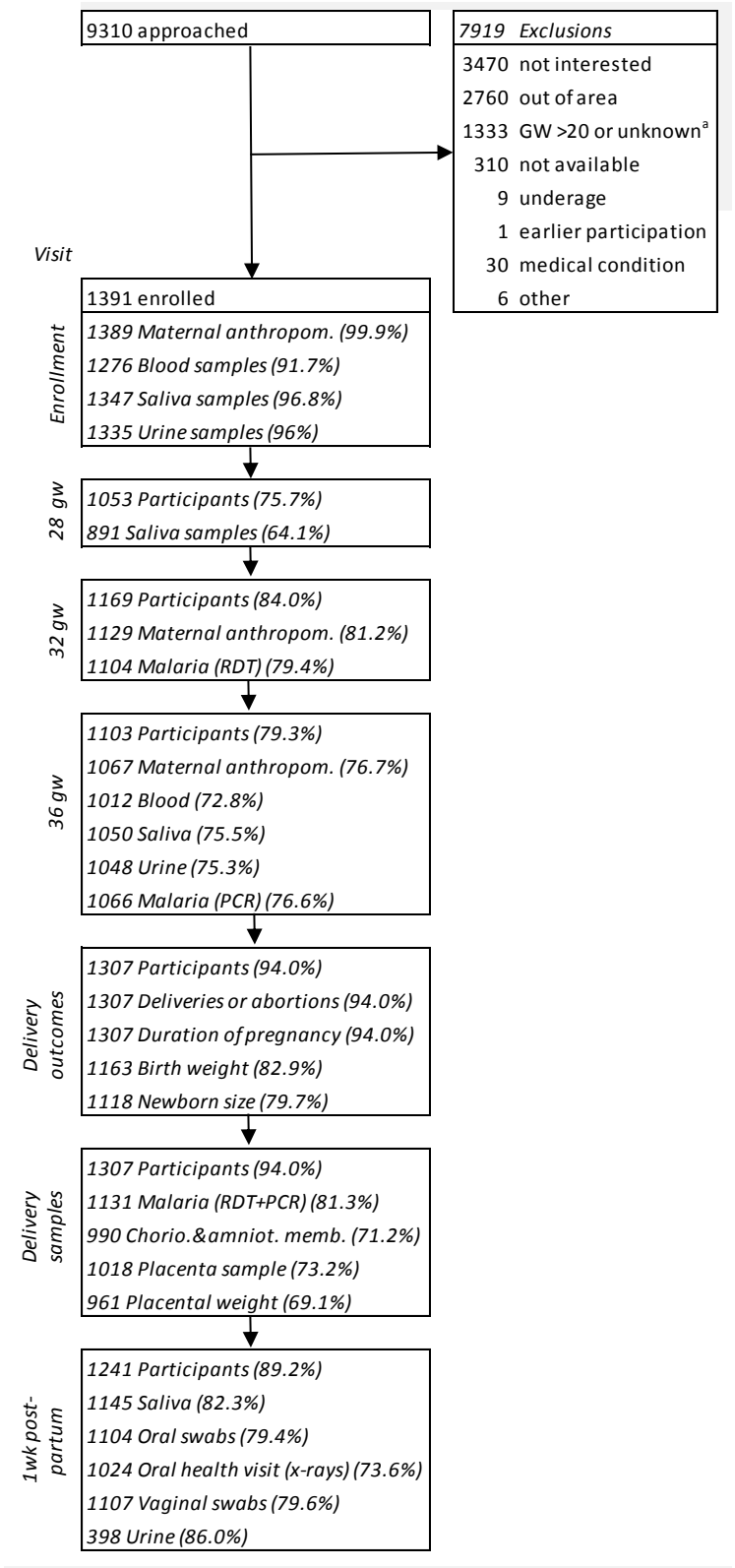
## 3.1 Formation and Characteristics of the Study Sample

Between February 2011 and August 2012, iLiNS Project team members approached a total of 9,310 women at the antenatal clinics of the four study sites. Of these, 1,391 (14.9%) were enrolled in the iLiNS study. The other approached women were excluded because they were not interested, they considered themselves not eligible, or the study team determined that they did not meet all the predefined enrollment criteria (Figure 3.2-1). These non-enrolled women were similar to the enrolled participants in terms of their mean age, number of completed school years, marital status, home building material, and ownership of phones in the household (details not shown).

## 3.2 Participant Follow-Up

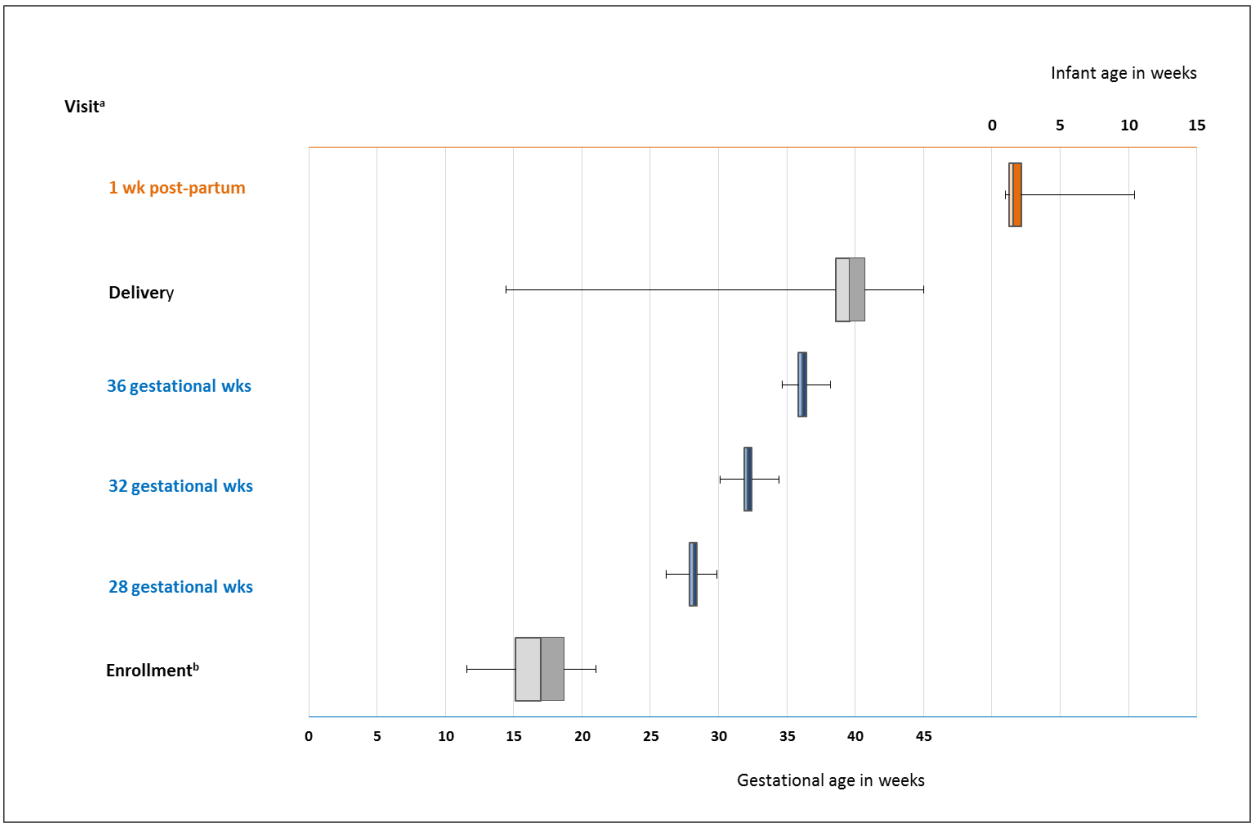
Figure 3.2-1 shows the percent of participants for which the different clinical and biological samples were successfully collected. At the various visits, clinical data were successfully collected from 76%–100% of the enrolled participants. The respective success rate for biological sample collection ranged from 64% to 96%. In general, the main study visits were done within the planned time frame (Figure 3.2-2).

**Figure 3.2-1. Number of Participants, and Samples and Measures Taken at Selected Visits**




[a] In case the approached woman was carrying a twin pregnancy, the duration of pregnancy could not be assessed, and she was excluded from the trial.

## Figure 3.2-2. Selected Maternal Visits by Gestational Age, and Infant Age in Weeks (interquartile range [IQR])




[a] IQR: For enrollment and delivery, all visits are included. For other visits, 95% of visits are included (2.5% on both sides of the visit range left out).
[b] There were four participants enrolled with gestational ages >20 weeks (1@20.1 weeks, 2@20.3 weeks, and 1@21 weeks).

# 4. The Predictive Value of Maternal Characteristics for Birth Outcomes

## 4.1 Maternal BMI at Enrollment and Weight Gain during Pregnancy

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal BMI at enrollment and weight gain during pregnancy on the one hand and birth outcomes on the other.

Of the 1,391 women who were enrolled in the study, we measured BMI at enrollment for 1,382 (99.4%). After excluding 12 women with twin pregnancies, BMI at enrollment was available for 1,370 (98.5%) participants. Weekly gestational weight gain was estimated using mixed modeling, which allowed us to calculate weekly weight gain for all women from whom at least one weight measurement was available at enrollment, 32 gw, or 36 gw (n=1,377; 99.0% of enrolled women). Data on the duration of pregnancy were available for 1,287 (93.9% of those from whom BMI data were available) and birth weight, length, and head circumference were available for 79.7%, 79.0%, and 79.1% of those from whom BMI data were available, respectively.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. There were no differences in enrollment characteristics of women included and excluded from the BMI and weight gain analyses ($P \geq 0.05$) (details not shown because of the small number of excluded participants [1.5% and 1.0%, respectively]).

The mean (SD) BMI of all participants at enrollment was 22.2 (2.8). The percentage of women who were underweight at enrollment (BMI <18.5) was 5.4%, while the prevalence of overweight at enrollment (BMI ≥25.0) was 12.9%. The participants gained on average 292 g per week from enrollment to 36 gw, based on mixed modeling, with 71.8% gaining less than the Institute of Medicine (IOM) recommendations, based on BMI before pregnancy, but because pre-pregnancy BMI was not available to us, we used regression modeling to create a proxy for pre-pregnancy BMI (see Appendix A2.1).

Table 4.1-1,

Table 4.1-2, and Table 4.1-3 show the standardized regression coefficients for the associations between birth outcomes as continuous variables and both maternal BMI at enrollment and weekly gestational weight gain.

In unadjusted models, maternal BMI at enrollment was significantly associated with newborn birth weight (P<0.001), LAZ (P=0.040), HCZ (P=0.003), and WAZ (P=0.001). After adjusting for covariates, there was no significant association between BMI and LAZ (P=0.067), while the other associations remained significant. Weekly gestational weight gain was significantly associated with duration of pregnancy (P=0.002); birth weight; and newborn LAZ, HCZ, and WAZ (P<0.001 for all four). All these associations remained significant after adjusting for covariates.

Table 4.1-4, Table 4.1-5, and Table 4.1-6 show the risks of adverse birth outcomes among women with low and high BMI at enrollment, as well as those with inadequate and excessive weekly gestational weight gain. Women who were underweight at enrollment were at increased risk of having a child who was SGA compared to women in the normal weight category (P=0.028) before adjusting for covariates; after adjusting, there was no increased risk of having a child who was SGA (Table 4.1-4**)**. When compared to women in the normal weight category, there were no significant associations between women being overweight at enrollment and increased risk of adverse birth outcomes in either adjusted or unadjusted models (Table 4.1-5). Compared to women who gained an appropriate amount of weight during pregnancy (within the IOM recommendations), women who gained insufficient weight during pregnancy (i.e., average weight gain per week of pregnancy, as derived from modeling of up to three data points on maternal weight during pregnancy) were at increased risk of giving birth preterm (P=0.033) and to a LBW (P=0.002) or SGA (P=0.008) infant or to an infant with a small head circumference (P=0.022) in unadjusted models (Table 4.1-6). After adjusting for covariates, there was no longer a significant association with risk of preterm birth (P=0.124), but all other associations remained significant. Compared to women who gained an appropriate amount of weight, women who gained excessive weight (above the IOM recommendations) were at decreased risk of giving birth to a LBW (P=0.019), stunted (P<0.001), or SGA (P=0.046) infant in unadjusted models (Table 4.1-7). After adjusting for covariates, the risk of giving birth to a SGA infant was no longer significant (P=0.120).

These results suggest that maternal BMI at enrollment was associated with newborn birth weight, head circumference, and WAZ, but being underweight (BMI <18.5) or overweight (BMI ≥25.0) was not associated with increased risks of adverse birth outcomes. Average weekly gestational weight gain was associated with duration of pregnancy, birth weight, length, head circumference, and WAZ, and inadequate weekly weight gain was associated with increased risks of adverse birth outcomes, including LBW, SGA, and small head circumference.

**Table 4.1-1. Standardized Regression Coefficients for the Associations between Maternal BMI at Enrollment and Weekly Weight Gain, and Duration of Pregnancy and Birth Weight**

| | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Duration of pregnancy | | | | Birth weight | | | |
| | Unadjusted | | Adjusted[a] | | Unadjusted | | Adjusted[a] | |
| Predictor | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| BMI at enrollment (kg/m$^2$) | −0.01 (0.03) | 0.686 | −0.003 (0.03) | 0.926 | 0.11 (0.03) | <0.001 | 0.11 (0.03) | <0.001 |
| Weekly weight gain (g/week) | 0.08 (0.03) | 0.002 | 0.06 (0.03) | 0.031 | 0.22 (0.03) | <0.001 | 0.20 (0.03) | <0.001 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values were determined by linear regression models.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for duration of pregnancy were adjusted for gestational age at enrollment, maternal Hb at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Models for birth weight were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, child sex, season of enrollment, and site of enrollment.

**Table 4.1-2. Standardized Regression Coefficients for the Associations between Maternal BMI at Enrollment and Weekly Weight Gain, and Newborn LAZ and HCZ**

| | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Newborn LAZ | | | | Newborn HCZ | | | |
| | Unadjusted | | Adjusted[a] | | Unadjusted | | Adjusted[b] | |
| Predictor | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| BMI at enrollment (kg/m$^2$) | 0.065 (0.03) | 0.040 | 0.056 (0.03) | 0.067 | 0.09 (0.03) | 0.003 | 0.09 (0.03) | 0.003 |
| Weekly weight gain (g/week) | 0.19 (0.03) | <0.001 | 0.13 (0.03) | <0.001 | 0.15 (0.03) | <0.001 | 0.15 (0.03) | <0.001 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values were determined by linear regression models.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for newborn LAZ were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, child sex, season of enrollment, and site of enrollment. Models for newborn HCZ were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

### Table 4.1-3. Standardized Regression Coefficients for the Associations between Maternal BMI at Enrollment and Weekly Weight Gain, and Newborn WAZ

| | Outcome | | | |
|---|---|---|---|---|
| | Newborn WAZ | | | |
| | Unadjusted | | Adjusted[a] | |
| Predictor | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| BMI at enrollment (kg/m$^2$) | 0.10 (0.03) | 0.001 | 0.07 (0.03) | 0.035 |
| Weekly weight gain (g/week) | 0.21 (0.03) | <0.001 | 0.17 (0.03) | <0.001 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values were determined by linear regression models.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for newborn WAZ were adjusted for maternal BMI at enrollment, maternal height at enrollment, maternal age at enrollment, primiparity, maternal HIV status, maternal Hb at enrollment, proxy for SES, season of enrollment, and site of enrollment.

### Table 4.1-4. Adverse Birth Outcomes among Underweight Women

| Outcome | Participants with BMI ≥18.5 and <25.0 at enrollment | Participants with BMI <18.5 at enrollment | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 108/1058 (10.2%) | 5/71 (7.0%) | 0.7 (0.3 to 1.6) | 0.400 | 0.9 (0.4 to 2.0) | 0.718 |
| Incidence of LBW (<2,500 g) | 117/941 (12.4%) | 11/62 (17.7%) | 1.4 (0.8 to 2.5) | 0.215 | 1.3 (0.6 to 2.4) | 0.500 |
| Prevalence of newborn stunting (LAZ <−2) | 144/893 (16.1%) | 14/60 (23.3%) | 1.4 (0.9 to 2.3) | 0.133 | 1.6 (1.0 to 2.6) | 0.055 |
| Incidence of SGA[c] | 279/941 (29.7%) | 26/62 (41.9%) | 1.4 (1.0 to 1.9) | 0.028 | 1.1 (0.8 to 1.6) | 0.629 |
| Prevalence of newborn small head circumference (HCZ <−2) | 37/894 (4.1%) | 2/61 (3.3%) | 0.8 (0.2 to 3.2) | 0.744 | 0.1 (0.1 to 2.4) | 0.408 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.1-5. Adverse Birth Outcomes among Overweight Women

| Outcome | Participants with BMI ≥18.5 and <25.0 at enrollment[a] | Participants with BMI ≥25.0 at enrollment | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 108/1058 (10.2%) | 16/158 (10.1%) | 1.0 (0.6 to 1.6) | 0.975 | 1.0 (0.6 to 1.6) | 0.926 |
| Incidence of LBW (<2,500 g) | 117/941 (12.4%) | 17/133 (12.8%) | 1.0 (0.6 to 1.7) | 0.909 | 1.2 (0.6 to 2.4) | 0.646 |
| Prevalence of newborn stunting (LAZ <−2) | 144/893 (16.1%) | 15/129 (11.6%) | 0.7 (0.4 to 1.2) | 0.199 | 0.7 (0.5 to 1.2) | 0.244 |
| Incidence of SGA[c] | 279/941 (29.7%) | 30/133 (22.6%) | 0.8 (0.5 to 1.1) | 0.104 | 1.3 (0.8 to 2.0) | 0.237 |
| Prevalence of newborn small head circumference (HCZ <−2) | 37/894 (4.1%) | 4/129 (3.1%) | 0.7 (0.3 to 2.1) | 0.577 | 1.4 (0.3 to 5.6) | 0.675 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment

[c] Defined as having birth weight <10$^{th}$ percentile for infants of the same gestational age from a U.S. population.

### Table 4.1-6. Adverse Birth Outcomes among Women Who Gained Less Weight than the IOM Minimum Average per Week Recommendation

| Outcome | Participants with weight gain within IOM recommendation | Participants with weight gain < IOM recommendation | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 21/302 (7.0%) | 106/916 (11.6%) | 1.7 (1.0 to 2.7) | 0.033 | 1.5 (0.9 to 2.3) | 0.124 |
| Incidence of LBW (<2,500 g) | 21/278 (7.6%) | 124/797 (15.6%) | 2.1 (1.3 to 3.3) | 0.002 | 2.0 (1.2 to 3.2) | 0.006 |
| Prevalence of newborn stunting (LAZ <−2) | 35/257 (13.6%) | 138/767 (18.0%) | 1.3 (0.9 to 1.9) | 0.141 | 1.1 (0.7 to 1.6) | 0.681 |
| Incidence of SGA[c] | 64/278 (23.0%) | 265/797 (24.7%) | 1.4 (1.1 to 1.9) | 0.008 | 1.4 (1.0 to 1.8) | 0.037 |
| Prevalence of newborn small head circumference (HCZ <−2) | 4/261 (1.5%) | 39/765 (5.1%) | 3.3 (1.2 to 9.3) | 0.022 | 3.4 (1.2 to 9.7) | 0.024 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

**Table 4.1-7. Adverse Birth Outcomes among Women Who Gained More Weight than the IOM Maximum Average per Week Recommendation**

| Outcome | Participants with weight gain within IOM recommendation | Participants with weight gain > IOM recommendation | Relative risk (95% CI) | P-value[a,b] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 21/302 (7.0%) | 2/69 (2.9%) | 0.4 (0.1 to 1.8) | 0.237 | 0.4 (0.1 to 1.6) | 0.178 |
| Incidence of LBW (<2,500 g) | 21/278 (7.6%) | 0/61 (0.0%) | n/a | 0.019 | n/a | n/a |
| Prevalence of newborn stunting (LAZ <−2) | 35/257 (13.6%) | 0/58 (0.0%) | n/a | <0.001 | n/a | n/a |
| Incidence of SGA[c] | 64/278 (23.0%) | 6/61 (9.8%) | 0.4 (0.2 to 1.0) | 0.046 | 0.5 (0.2 to 1.2) | 0.120 |
| Prevalence of newborn small head circumference (HCZ <−2) | 4/261 (1.5%) | 0/58 (0.0%) | n/a | >0.999 | n/a | n/a |

[a] P-values for incidence of preterm birth and small for gestational age newborns were obtained from Poisson regression models. P-values for unadjusted incidence of LBW, and prevalence of stunting and small head circumference were obtained by Fisher's exact test, as no women who gained excess weight gave birth to a LBW or stunted infant or an infant with a small head circumference.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

## 4.2 Maternal Asymptomatic Malaria Infections at Enrollment and 32 gw

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal malaria at enrollment and at 32 gw, and birth outcomes.

Of the 1,391 women who were enrolled in the study, 1,388 (99.8%) and 1,117 (80.3%) were tested for malaria by RDT at enrollment and 32 gw, respectively. After excluding twin pregnancies and those with missing data, 1,141 (82.0%) and 1,010 (72.6%) women were included in the malaria at enrollment and malaria at 32 gw analyses, respectively.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of maternal educational achievement, proportion with a low BMI, and proportion with a positive HIV test ($P \geq 0.05$). However, compared to the excluded women, they tended to be older (25 vs. 24, P=0.018) and have a lower proxy for socioeconomic status (SES) (−0.05 vs. 0.29, P<0.001) and a lower BMI (22.0 vs. 22.7, P<0.001) and were less likely to be anemic (19.1% vs. 27.8%, P=0.001), malaria positive (22.3% vs. 27.5%, P=0.042), and primiparous (19.1% vs. 34.5%, P<0.001) (Table A1.2-1).

For the continuous birth outcomes, malaria at enrollment was inversely associated with birth weight and newborn WAZ (P=0.010 and P=0.003, respectively) (Table 4.2-1). However, these associations did not remain significant after adjusting for covariates. On the other hand, malaria at 32 gw was inversely associated with all of the continuous birth outcomes under consideration: duration of pregnancy (P=0.006); birth weight (P=0.011); LAZ (P=0.002), WAZ (P<0.001), and HCZ (P=0.002). All of these associations remained significant after adjusting for covariates (Table 4.2-2).

Malaria at enrollment was not associated with any of the categorical birth outcomes (

Table 4.2-3). But malaria at 32 gw was inversely associated with newborn stunting (P=0.005) in the unadjusted analysis. However, this association was no longer significant after adjusting for covariates. Malaria at 32 gw was inversely associated with LBW after adjusting for covariates (P=0.048) (Table 4.2-4).

These results suggest that malaria at enrollment was not associated with any of the birth outcomes studied. However, malaria at 32 gw was inversely associated with all of the continuous birth outcomes of focus (duration of pregnancy, birth weight, LAZ, WAZ, and HCZ) and was also associated with an increased risk for LBW.

### Table 4.2-1. Malaria at Enrollment and Continuous Birth Outcomes

| Outcome | Participants without malaria (n=887) | Participants with malaria (n=254) | Difference in means (95% CI) | P-value[a] | Adjusted difference in means (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.2 (2.7) | 38.9 (3.5) | −0.4 (−0.7 to 0.0) | 0.078 | −0.2 (−0.5 to 0.2) | 0.427 |
| Mean (SD) birth weight, g | 2,989 (440) | 2,905 (465) | −84 (−147 to −22) | 0.010 | −31 (−95 to 31) | 0.320 |
| Mean (SD) newborn LAZ | −0.92 (0.99) | −1.06 (1.10) | −0.14 (−0.29 to 0.01) | 0.059 | −0.02 (−0.17 to 0.12) | 0.757 |
| Mean (SD) newborn WAZ | −0.49 (0.95) | −0.70 (1.03) | −0.21 (0.35 to −0.07) | 0.003 | −0.03 (−0.17 to 0.10) | 0.623 |
| Mean (SD) newborn HCZ | −0.09 (1.00) | −0.24 (1.09) | −0.15 (−0.30 to −0.01) | 0.066 | −0.09 (−0.23 to −0.06) | 0.261 |

[a] P-values were calculated using analysis of variance (ANOVA) (comparison of means).

[b] P-values were calculated using analysis of covariance (ANCOVA) (comparison of means). Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for duration of pregnancy was adjusted for maternal height at enrollment, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Models for birth weight and LAZ were adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, parity, season of enrollment, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for WAZ was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, proxy for SES, parity, season of enrollment, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for HCZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, parity, season of enrollment, and site of enrollment.

### Table 4.2-2. Malaria at 32 gw and Continuous Birth Outcomes

| Outcome | Participants without malaria (n=905) | Participants with malaria (n=105) | Difference in means (95% CI) | P-value[a] | Adjusted difference in means (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.6 (1.8) | 39.0 (2.3) | −0.5 (−0.9 to −0.1) | 0.006 | −0.4 (−0.7 to −0.1) | 0.043 |
| Mean (SD) birth weight, g | 2,991 (430) | 2,877 (455) | −114 (−201 to −26) | 0.011 | −94 (−180 to −9) | 0.030 |
| Mean (SD) newborn LAZ | −0.89 (1.0) | −1.21 (1.1) | −0.32 (−0.53 to −0.12) | 0.002 | −0.20 (−0.40 to −0.00) | 0.047 |
| Mean (SD) newborn WAZ | −0.47 (0.94) | −0.91 (1.05) | −0.43 (−0.62 to −0.24) | <0.001 | −0.31 (−0.49 to −0.12) | 0.001 |
| Mean (SD) newborn HCZ | −0.08 (1.0) | −0.40 (1.0) | −0.32 (−0.52 to −0.12) | 0.002 | −0.29 (−0.49 to −0.09) | 0.004 |

[a] P-values were calculated using ANOVA (comparison of means).

[b] P-values were calculated using ANCOVA (comparison of means). Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for pregnancy duration was adjusted for intervention group, parity, maternal anemia at enrollment, and site of enrollment. Model for birth weight was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, parity, season of enrollment, maternal malaria status at enrollment, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for LAZ was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, maternal educational achievement, parity, season of enrollment, maternal anemia at enrollment, malaria status at enrollment, maternal HIV status, and site of enrollment. Model for WAZ was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, proxy for SES, parity, season of enrollment, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for HCZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, parity, season of enrollment, maternal malaria status at enrollment, and site of enrollment.

### Table 4.2-3. Malaria at Enrollment and Adverse Birth Outcomes

| Outcome | Participants with no malaria | Participants with malaria | Relative risk (95% CI) | P-value[a] | Relative risk (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 91/991 (9.2%) | 38/301 (12.6%) | 1.37 (0.96 to 1.96) | 0.080 | 1.18 (0.81 to 1.70) | 0.388 |
| Incidence of LBW (<2,500 g) | 106/887 (12.0%) | 40/254 (15.8%) | 1.32 (0.94 to 1.84) | 0.107 | 1.20 (0.82 to 1.75) | 0.343 |
| Prevalence of newborn stunting (LAZ <−2) | 115/833 (13.8%) | 45/239 (18.8%) | 1.36 (1.00 to 1.87) | 0.052 | 1.09 (0.76 to 1.58) | 0.635 |
| Prevalence of newborn small head circumference (HCZ <−2) | 25/837 (3.0%) | 12/244 (4.9%) | 1.65 (0.84 to 3.24) | 0.147 | 1.59 (0.81 to 3.10) | 0.177 |

[a] P-values were obtained from log-binomial models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for LBW was adjusted for maternal height at enrollment, maternal MUAC at enrollment, child sex, parity, and site of enrollment. Model for newborn stunting was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, parity, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for newborn small head circumference was adjusted for intervention group and proxy for SES.

[c] P-values were obtained from log-binomial models or log-Poisson models if the algorithm failed to converge.

### Table 4.2-4. Malaria at 32 gw and Adverse Birth Outcomes

| Outcome | Participants with no malaria | Participants with malaria | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 67/981 (6.8%) | 13/119 (10.9%) | 1.60 (0.91 to 2.81) | 0.102 | 1.43 (0.81 to 2.53) | 0.221 |
| Incidence of LBW (<2,500 g) | 104/905 (11.5%) | 18/105 (17.1%) | 1.49 (0.94 to 2.36) | 0.087 | 1.56 (1.00 to 2.43) | 0.048 |
| Prevalence of newborn stunting (LAZ <−2) | 112/858 (13.1%) | 24/105 (22.9%) | 1.75 (1.18 to 2.59) | 0.005 | 1.42 (0.90 to 2.24) | 0.132 |
| Prevalence of newborn small head circumference (HCZ <−2) | 27/860 (3.1%) | 4/109 (3.7%) | 1.17 (0.42 to 3.28) | 0.767 | 0.98 (0.35 to2.74) | 0.965 |

[a] P-values were obtained from log-binomial models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for intervention group, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for LBW was adjusted for maternal height at enrollment, child sex, parity, maternal HIV status, and site of enrollment. Model for newborn stunting was adjusted for maternal height at enrollment, maternal age at enrollment, child sex, parity, maternal anemia at enrollment, malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn small head circumference was adjusted for maternal HIV status and site of enrollment.

[c] P-values for adjusted models obtained from log-binomial models or log-Poisson models if the algorithm failed to converge.

## 4.3 Maternal HIV, Trichomoniasis, or Urinary Tract Infection at Delivery

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal HIV at enrollment and birth outcomes. The substudy also assessed the association of trichomoniasis and UTI with birth outcomes. The data for the association between trichomoniasis and UTI and birth outcomes were collected as cross-sectional data where both the predictor and outcome variables were collected at the same time, 1 week after delivery.

Of the 1,391 women who were enrolled in the study, 1,334 (95.9%) had a HIV test result at enrollment. After excluding twin pregnancies and those with missing data, 1,132 (81.4%) women were included in the HIV and birth outcomes analyses. For the trichomoniasis and UTI and birth outcome analyses, 1,102 (79.2%) and 1,098 (78.9%) women were included, respectively.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of maternal educational achievement, proportion with low BMI, and proportion with a positive HIV test ($P \geq 0.05$). However, compared to the excluded women, they tended to be older (25 vs. 24 years, P=0.017) and have a lower proxy for SES (−0.05 vs. 0.30, P<0.001) and a lower BMI (22.0 vs. 22.8, P<0.001) and were less likely to be anemic (19.3% vs. 26.7%, P=0.004), malaria positive (22.1% vs. 27.8%, P=0.016), and primiparous (18.6% vs. 36.1%, P<0.001) (Table A1.2-2).

HIV at enrollment was inversely associated with birth weight (P=0.016), LAZ (P=0.004), WAZ (P=0.040) (Table 4.3-1), preterm birth (P=0.005), and stunting (P=0.002) (Table 4.3-4). All of these associations except the one with preterm birth remained significant after adjusting for covariates. Trichomoniasis after delivery was inversely associated with WAZ (P=0.024) (Table 4.3-2) and with a higher risk of stunting (P=0.001) and small head circumference (P=0.021) (Table 4.3-5**)**, but only the association with small head circumference remained significant after adjusting for covariates (P=0.039). UTI after delivery was inversely associated with the duration of pregnancy (P=0.034), LAZ (P=0.003), and WAZ (P=0.002) (Table 4.3-3) and with a higher risk of stunting (P=0.001) (Table 4.3-6). However, after adjusting for covariates, only the associations with LAZ and WAZ remained statistically significant (P=0.030 and P=0.030, respectively).

These results suggest that HIV at enrollment was inversely associated with birth weight, LAZ, and WAZ, and was associated with an increased risk for stunting. Trichomoniasis after delivery was associated with an increased risk for small head circumference, and UTI was inversely associated with LAZ and WAZ.

### Table 4.3-1. HIV at Enrollment and Continuous Birth Outcomes

| Outcome | Participants without HIV (n=979) | Participants with HIV (n=153) | Difference in means (95% CI) | P-value[a] | Adjusted difference in means (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.2 (2.8) | 38.8 (3.4) | −0.4 (−0.9 to 0.5) | 0.079 | −0.2 (−0.7 to 0.2) | 0.312 |
| Mean (SD) birth weight, g | 2,983 (450) | 2,889 (429) | −94 (−170 to −18) | 0.016 | −91 (−166 to −17) | 0.016 |
| Mean (SD) newborn LAZ | −0.97 (1.10) | −1.25 (1.15) | −0.28 (−0.48 to −0.09) | 0.004 | −0.31 (−0.49 to −0.11) | 0.001 |
| Mean (SD) newborn WAZ | −0.52 (0.96) | −0.69 (1.02) | −0.18 (−0.35 to −0.01) | 0.040 | −0.21 (−0.38 to −0.05) | 0.011 |
| Mean (SD) newborn HCZ | −0.11 (1.00) | −0.23 (1.11) | −0.12 (−0.30 to 0.06) | 0.176 | −0.11 (−0.29 to 0.07) | 0.212 |

[a] P-values were obtained from ANOVA (comparison of means).

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for duration of pregnancy was adjusted for maternal height at enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, and site of enrollment. Model for birth weight was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, parity, maternal anemia at enrollment, maternal malaria status at enrollment, and site of enrollment. Model for newborn LAZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, parity, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, and site of enrollment. Model for newborn WAZ was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, parity, season of enrollment, malaria status at enrollment, and site of enrollment. Model for newborn HCZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, parity, season of enrollment, maternal malaria status at enrollment, and site of enrollment.

[c] P-values for adjusted models obtained from ANCOVA (comparison of means).

### Table 4.3-2. Trichomoniasis after Delivery and Continuous Birth Outcomes

| Outcome | Participants without trichomoniasis (n=992) | Participants with trichomoniasis (n=110) | Difference in means (95% CI) | P-value[a] | Adjusted difference in means (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.3 (2.5) | 39.8 (3.6) | 0.48 (0.00 to 0.96) | 0.050 | 0.36 (0.12 to 0.84) | 0.138 |
| Mean (SD) birth weight, g | 2,981 (442) | 2,924 (460) | −57 (−145 to 30) | 0.201 | −18 (−103 to 67) | 0.671 |
| Mean (SD) newborn LAZ | −0.94 (1.01) | −1.04 (1.06) | −0.10 (−0.30 to 0.10) | 0.327 | 0.00 (−0.19 to 0.20) | 0.983 |
| Mean (SD) newborn WAZ | −0.51 (0.95) | −0.73 (1.10) | −0.22 (−0.41 to −0.03) | 0.024 | −0.08 (−0.26 to 0.10) | 0.385 |
| Mean (SD) newborn HCZ | −0.11 (1.01) | −0.22 (1.07) | −0.11 (−0.31 to 0.09) | 0.276 | −0.01 (−0.19 to 0.21) | 0.935 |

[a] P-values were obtained from ANOVA (comparison of means).

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for duration of pregnancy was adjusted for maternal height at enrollment, maternal age at enrollment, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for birth weight was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, parity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn LAZ was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, parity, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn WAZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, proxy for SES, parity, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn HCZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, parity, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, and site of enrollment.

[c] P-values for the adjusted models obtained from ANCOVA (comparison of means).

### Table 4.3-3. Urinary Tract Infection after Delivery and Continuous Birth Outcomes

| Outcome | Participants without UTI (n= 1074) | Participants with UTI (n=24) | Difference in means (95% CI) | P-value[a] | Adjusted difference in means (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.3 (2.5) | 38.3 (4.1) | −0.96 (−1.86 to −0.07) | 0.034 | −0.58 (−1.45 to 0.30) | 0.196 |
| Mean (SD) birth weight, g | 2,977 (444) | 2,858 (382) | −118 (−297 to 61) | 0.196 | −59 (−229 to 111) | 0.495 |
| Mean (SD) newborn LAZ | −0.94 (1.01) | −1.55 (1.15) | −0.61 (−1.01 to −0.21) | 0.003 | −0.42 (−0.80 to −0.04) | 0.030 |
| Mean (SD) newborn WAZ | −0.52 (0.96) | −1.12 (1.08) | −0.59 (−0.98 to −0.21) | 0.002 | −0.40 (−0.76 to −0.04) | 0.030 |
| Mean (SD) newborn HCZ | −0.12 (1.02) | −0.30 (1.02) | −0.19 (−0.59 to 0.22) | 0.369 | −0.06 (−0.45 to 0.34) | 0.775 |

[a] P-values were obtained from ANOVA (comparison of means).

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for duration of pregnancy was adjusted for maternal height at enrollment, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for birth weight was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, parity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn LAZ was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, parity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn WAZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, proxy for SES, parity, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn HCZ was adjusted for intervention group, maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, maternal educational achievement, parity, season of enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment.

[c] P-values for the adjusted models obtained from ANOVA (comparison of means).

### Table 4.3-4. HIV at Enrollment and Adverse Birth Outcomes

| Outcome | Participants with no HIV | Participants with HIV | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 97/1094 (8.9%) | 27/173 (15.6%) | 1.76 (1.19 to 2.61) | 0.005 | 1.46 (0.99 to 2.16) | 0.059 |
| Incidence of LBW (<2,500 g) | 119/979 (12.1%) | 26/153 (17.0%) | 1.40 (0.95 to 2.06) | 0.091 | 1.45 (0.98 to 2.15) | 0.061 |
| Prevalence of newborn stunting (LAZ <−2) | 138/937 (14.7%) | 36/147 (24.5%) | 1.66 (1.20 to 2.30) | 0.002 | 1.84 (1.24 to 2.72) | 0.002 |
| Prevalence of newborn small head circumference (HCZ <−2) | 29/934 (3.1%) | 8/145 (5.5%) | 1.78 (0.83 to 3.81) | 0.140 | 1.94 (0.90 to 4.15) | 0.089 |

[a] P-values were obtained from log-binomial models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for maternal anemia at enrollment and site of enrollment. Model for LBW was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, child sex, parity, maternal malaria status at enrollment, and site of enrollment. Model for newborn stunting was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, parity, maternal anemia at enrollment, maternal malaria status at enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for intervention group and proxy for SES.

[c] P-values for adjusted models obtained from log-binomial models or log-Poisson models if the algorithm failed to converge.

### Table 4.3-5. Trichomoniasis after Delivery and Adverse Birth Outcomes

| Outcome | Participants with no trichomoniasis | Participants with trichomoniasis | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 93/1085 (8.6%) | 13/125 (10.4%) | 1.21 (0.70 to 2.10) | 0.491 | 1.12 (0.66 to 1.90) | 0.682 |
| Incidence of LBW (<2,500 g) | 119/992 (12.0%) | 19/110 (17.3%) | 1.44 (0.93 to 2.24) | 0.106 | 1.48 (0.60 to 3.62) | 0.391 |
| Prevalence of newborn stunting (LAZ <−2) | 149/1032 (14.4%) | 9/25 (36.0%) | 2.49 (1.45 to 4.29) | 0.001 | 1.45 (0.57 to 1.56) | 0.820 |
| Prevalence of newborn small head circumference (HCZ <−2) | 29/962 (3.0%) | 8/109 (7.3%) | 2.43 (1.14 to 5.19) | 0.021 | 2.22 (1.04 to 4.73) | 0.039 |

[a] P-values were obtained from log-binomial models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for parity, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for LBW was adjusted for maternal height at enrollment, child sex, parity, maternal anemia at enrollment, and site of enrollment. Model for newborn stunting was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, parity, maternal anemia, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn small head circumference was adjusted for intervention group and proxy for SES.

[c] P-values for adjusted models obtained from log-binomial models or log-Poisson models if the algorithm failed to converge

### Table 4.3-6. Urinary Tract Infection after Delivery and Adverse Birth Outcomes

| Outcome | Participants without UTI | Participants with UTI | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 101/1174 (8.6%) | 5/33 (15.2%) | 1.76 (0.77 to 4.03) | 0.181 | 1.25 (0.57 to 2.77) | 0.578 |
| Incidence of LBW (<2,500 g) | 134/1074 (12.5%) | 4/24 (16.7%) | 1.34 (0.54 to 3.31) | 0.532 | 1.01 (0.36 to 2.86) | 0.982 |
| Prevalence of newborn stunting (LAZ <−2) | 149/1026 (14.5%) | 9/25 (36.0%) | 2.49 (1.45 to 4.29) | 0.001 | 1.90 (0.96 to 3.75) | 0.060 |
| Prevalence of newborn small head circumference (HCZ <−2) | 35/1041 (3.4%) | 1/25 (4.0%) | 1.19 (0.17 to 8.34) | 0.861 | 1.35 (0.20 to 9.36) | 0.760 |

[a] P-values were obtained from log-binomial models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for parity, maternal anemia at enrollment, maternal HIV status, and site of enrollment. Model for LBW was adjusted for maternal height at enrollment, maternal MUAC at enrollment, child sex, parity, maternal anemia at enrollment, maternal malaria status at enrollment, and site of enrollment. Model for newborn stunting was adjusted for maternal height at enrollment, maternal MUAC at enrollment, maternal age at enrollment, parity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal HIV status, and site of enrollment. Model for newborn small head circumference was adjusted for intervention group and proxy for SES.

[c] P-values for adjusted models obtained from log-binomial models or log-Poisson models if the algorithm failed to converge.

## 4.4 Maternal Plasma CRP and AGP Concentrations

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal plasma CRP and AGP concentrations and birth outcomes. We examined the associations between CRP and AGP at enrollment and at 36 gw and change in CRP or ARP from enrollment to 36 gw, with birth outcomes, and ascertained the relative risk of adverse birth outcomes among women with elevated CRP or AGP.

CRP and AGP data were available for 1,371 (98.6%) participants at the enrollment visit and 1,063 (76.4%) participants at the 36 gw visit. Data on the duration of pregnancy were available for 99.5% of those included in the 36 gw analyses. Birth weight, length, and head circumference data were available for 90%, 89%, and 89% of the included participants, respectively.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of mean maternal BMI at enrollment, maternal educational achievement, proportion with low maternal BMI at enrollment, and proportion with a positive malaria test ($P \geq 0.05$). However, compared to excluded women, they tended to be older (25 vs. 24, $P<0.001$) and have a lower mean proxy for SES (−0.04 vs. 0.19, $P<0.001$) and were less likely to be primiparous (19.8% vs. 28.4%, $P<0.001$), HIV positive (12.7% vs. 17.5%, $P=0.039$), and anemic (18.6% vs. 27.5%, $P<0.001$) (Table A1.2-3).

Table 4.4-1, Table 4.4-2, and Table 4.4-3 show the standardized regression coefficients for the associations between continuous birth outcomes and maternal plasma CRP and AGP concentrations at enrollment and at 36 gw, and associations between birth outcomes and the change in maternal plasma CRP and AGP concentrations from enrollment to 36 gw. Both maternal plasma CRP and AGP concentrations were log-transformed before any analyses were performed. Non-transformed values are presented in the tables. We found all associations to be linear except for the associations between maternal plasma AGP concentration at 36 gw and LAZ, maternal plasma AGP concentration at 36 gw and HCZ, and maternal plasma AGP concentration at 36 gw and WAZ, all of which were inverse U-shaped associations, meaning that both low and high maternal plasma AGP concentrations were associated with lower LAZ, HCZ, and WAZ compared to participants with moderately low to moderately high AGP (see Figure A2-1 in Appendix A2.4 for graphical presentations of these associations). While the values of these non-linear associations are presented below, the results should be interpreted with caution because they were derived using linear models.

In unadjusted models, maternal plasma CRP concentration at enrollment was inversely associated with duration of gestation ($P=0.004$), while an inverse association between maternal plasma CRP concentration at enrollment and birth weight was close to being significant ($P=0.050$). Maternal plasma CRP concentration at 36 gw was inversely associated with LAZ ($P=0.013$) and WAZ ($P=0.046$). After adjusting for covariates, only the association between maternal plasma CRP concentration at enrollment and duration of gestation remained significant ($P=0.013$). Maternal plasma AGP concentration at enrollment and at 36 gw and the change in maternal plasma AGP concentration from enrollment to 36 gw were each inversely associated with duration of pregnancy ($P=0.025$, $P<0.001$, and $P<0.001$, respectively). Maternal plasma AGP concentration at enrollment and at 36 gw were each inversely associated with birth weight ($P<0.001$ for both), but there was not a significant association between change in maternal plasma AGP concentration from enrollment to 36 gw and birth weight ($P=0.779$). Maternal plasma AGP concentration at enrollment and at 36 gw were each inversely associated with LAZ, HCZ, and WAZ ($P<0.001$ for all three), but the change in maternal plasma AGP concentration was not associated with LAZ ($P=0.203$), HCZ ($P=0.197$), or WAZ ($P=0.327$). All of the associations between

maternal plasma AGP concentration and birth outcomes remained significant after adjusting for covariates.

**Table 4.4-4**–Table 4.4-7 show the relative risks of adverse birth outcomes among women with elevated maternal plasma CRP and AGP concentrations at enrollment or 36 gw. Elevated maternal plasma CRP concentration at enrollment was associated with increased risk of LBW, (P=0.013), stunting (P=0.004), and small head circumference (P=<0.001) (Table 4.4-4). At 36 gw, elevated maternal plasma CRP concentration was associated with increased risk of stunting (P=0.003) and small head circumference (P=0.008) (Table 4.4-5). Adjusting for covariates did not change the significance of the associations between elevated maternal plasma CRP concentration and risk of adverse birth outcomes. While elevated maternal plasma AGP concentration at enrollment was related to increased risk of preterm birth (P=0.044), LBW (P=0.041), stunting (P=0.006), and SGA (P=0.005) in unadjusted models, after controlling for covariates, none of the relative risks were significant (Table 4.4-6). At 36 gw, elevated maternal plasma AGP concentration was associated with increased risk of preterm birth (P<0.001), stunting (P<0.001), SGA (P=0.045), and small head circumference (P<0.001) in unadjusted models (Table 4.4-7). After adjusting for covariates, all the associations between elevated maternal plasma AGP concentration at 36 gw and risk of adverse birth outcomes remained significant, except for SGA (P=0.305).

In conclusion, we found that maternal plasma AGP concentration (a marker of inflammation that stays elevated longer than CRP) at enrollment and 36 gw was inversely associated with duration of gestation and birth size outcomes. Additionally, women with elevated maternal plasma AGP concentration at 36 gw were at greater risk of giving birth preterm and to infants who were stunted or had small head circumference. Maternal plasma CRP concentration at enrollment was inversely associated with duration of gestation, and elevated maternal plasma CRP concentration at enrollment was associated with higher risk of LBW. In addition, women with elevated maternal plasma CRP concentration at either enrollment or 36 gw were at increased risk of giving birth to stunted infants and those with a small head circumference.

**Table 4.4-1. Standardized Regression Coefficients for the Associations between Maternal Plasma CRP and AGP Concentrations as Predictors and Duration of Pregnancy and Birth Weight as Outcomes**

| Predictor | Time point | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Duration of pregnancy | | | | Birth weight | | | |
| | | Unadjusted | | Adjusted | | Unadjusted | | Adjusted | |
| | | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| CRP | Enrollment | −0.08 (0.03) | 0.004 | −0.07 (0.03) | 0.013 | −0.06 (0.03) | 0.050 | −0.02 (0.03) | 0.446 |
| | 36 gw | −0.003 (0.02) | 0.858 | −0.001 (0.02) | 0.956 | −0.04 (0.03) | 0.143 | −0.03 (0.03) | 0.303 |
| | Change | 0.01 (0.02) | 0.557 | 0.004 (0.02) | 0.797 | 0.02 (0.03) | 0.422 | 0.02 (0.03) | 0.526 |
| AGP | Enrollment | −0.06 (0.03) | 0.025 | −0.08 (0.03) | 0.004 | −0.15 (0.03) | <0.001 | −0.10 (0.03) | 0.002 |
| | 36 gw | −0.08 (0.02) | <0.001 | −0.07 (0.02) | <0.001 | −0.14 (0.03) | <0.001 | −0.09 (0.03) | 0.002 |
| | Change | −0.10 (0.02) | <0.001 | −0.07 (0.02) | <0.001 | −0.01 (0.03) | 0.779 | −0.02 (0.03) | 0.512 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.

[b] P-values for all models were determined by linear regression.

[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for duration of pregnancy were adjusted for gestational age at enrollment, maternal Hb at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Models for birth weight were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, child sex, season of enrollment, and site of enrollment.

**Table 4.4-2. Standardized Regression Coefficients for the Associations between Maternal Plasma CRP and AGP Concentrations as Predictors and LAZ and HCZ as Outcomes**

| Predictor | Time point | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Newborn LAZ | | | | Newborn HCZ | | | |
| | | Unadjusted | | Adjusted[a] | | Unadjusted | | Adjusted[a] | |
| | | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| CRP | Enrollment | −0.02 (0.03) | 0.447 | 0.01 (0.03) | 0.641 | −0.04 (0.03) | 0.190 | −0.03 (0.03) | 0.389 |
| | 36 gw | −0.08 (0.03) | 0.013 | −0.04 (0.03) | 0.127 | −0.02 (0.03) | 0.406 | −0.02 (0.03) | 0.387 |
| | Change | −0.004 (0.03) | 0.903 | −0.002 (0.03) | 0.937 | 0.01 (0.03) | 0.617 | 0.009 (0.03) | 0.754 |
| AGP | Enrollment | −0.13 (0.03) | <0.001 | −0.07 (0.03) | 0.026 | −0.11 (0.03) | <0.001 | −0.08 (0.03) | 0.011 |
| | 36 gw[d] | −0.18 (0.03) | <0.001 | −0.12 (0.03) | <0.001 | −0.14 (0.03) | <0.001 | −0.11 (0.03) | <0.001 |
| | Change | −0.04 (0.03) | 0.203 | −0.04 (0.03) | 0.178 | −0.04 (0.03) | 0.197 | −0.05 (0.03) | 0.119 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values for all models were determined by linear regression.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for newborn LAZ were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, child sex, season of enrollment, and site of enrollment. Models for newborn HCZ were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.
[d] There were inverse U-shaped relationships between maternal plasma AGP concentration at 36 gw and both newborn LAZ (p(quadratic)<0.001) and HCZ (p(quadratic)=0.002).

**Table 4.4-3. Standardized Regression Coefficients for the Associations between Maternal Plasma CRP and AGP Concentrations as Predictors and Newborn WAZ as an Outcome**

| | | Outcome | | | |
|---|---|---|---|---|---|
| | | Newborn WAZ | | | |
| | | Unadjusted | | Adjusted | |
| Predictor | Time point | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| CRP | Enrollment | −0.05 (0.03) | 0.091 | −0.01 (0.03) | 0.664 |
| | 36 gw | −0.06 (0.03) | 0.046 | −0.03 (0.03) | 0.256 |
| | Change | 0.01 (0.03) | 0.823 | 0.004 (0.03) | 0.871 |
| AGP | Enrollment | −0.16 (0.03) | <0.001 | −0.09 (0.03) | 0.005 |
| | 36 gw[d] | −0.17 (0.03) | <0.001 | −0.11 (0.03) | <0.001 |
| | Change | −0.03 (0.03) | 0.327 | −0.04 (0.03) | 0.209 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values for all models were determined by linear regression.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for newborn WAZ were adjusted for maternal BMI at enrollment, maternal height at enrollment, maternal age at enrollment, primiparity, maternal HIV status, maternal Hb at enrollment, proxy for SES, season of enrollment, and site of enrollment.
[d] There was an inverse U-shaped relationship between maternal plasma AGP concentration at 36 gw and newborn WAZ (p(quadratic)=0.002).

### Table 4.4-4. Adverse Birth Outcomes among Women with Elevated Maternal Plasma CRP Concentration at Enrollment

| Outcome | Participants with CRP ≤5 mg/L at enrollment | Participants with CRP >5 mg/L at enrollment | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 67/754 (8.9%) | 62/534 (11.6%) | 1.3 (0.93 to 1.8) | 0.129 | 1.2 (0.8 to 1.7) | 0.434 |
| Incidence of LBW (<2,500 g) | 71/669 (10.6%) | 75/469 (16.0%) | 1.5 (1.09 to 2.1) | 0.013 | 1.4 (1.02 to 2.0) | 0.035 |
| Prevalence of newborn stunting (LAZ <−2) | 82/635 (12.9%) | 90/448 (20.1%) | 1.6 (1.15 to 2.1) | 0.004 | 1.4 (1.04 to 1.9) | 0.028 |
| Incidence of SGA[c] | 187/669 (28.0%) | 149/469 (31.8%) | 1.1 (0.92 to 1.4) | 0.244 | 1.1 (0.86 to 1.3) | 0.500 |
| Prevalence of newborn small head circumference (HCZ <−2) | 13/635 (2.0%) | 29/450 (6.4%) | 3.1 (1.6 to 6.1) | <0.001 | 3.0 (1.6 to 5.9) | <0.001 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for preterm birth were adjusted for gestational age at enrollment, maternal Hb at enrollment, maternal HIV status, primiparity, and site of enrollment. Models for LBW were adjusted for child sex, maternal Hb at enrollment, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Models for newborn stunting were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Models for SGA were adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Models for newborn small head circumference were adjusted for maternal Hb at enrollment, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.4-5. Adverse Birth Outcomes among Women with Elevated Maternal Plasma CRP Concentration at 36 gw

| Outcome | Participants with CRP ≤5 mg/L at 36 gw[a] | Participants with CRP >5 mg/L at 36 gw | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 19/752 (2.5%) | 9/310 (2.9%) | 1.2 (0.5 to 2.5) | 0.731 | 1.1 (0.5 to 2.4) | 0.894 |
| Incidence of LBW (<2,500 g) | 64/698 (9.2%) | 38/239 (13.2%) | 1.4 (1.0 to 2.1) | 0.078 | 1.3 (0.9 to 2.0) | 0.189 |
| Prevalence of newborn stunting (LAZ <−2) | 74/674 (11.0%) | 53/281 (18.9%) | 1.7 (1.2 to 2.4) | 0.003 | 1.5 (1.0 to 2.1) | 0.041 |
| Incidence of SGA[c] | 192/698 (27.5%) | 100/289 (34.6%) | 1.3 (1.0 to 1.6) | 0.063 | 1.2 (0.2 to 1.5) | 0.179 |
| Prevalence of newborn small head circumference (HCZ <−2) | 10/669 (1.5%) | 13/285 (4.6%) | 3.0 (1.4 to 7.0) | 0.008 | 2.7 (1.2 to 6.3) | 0.021 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for preterm birth were adjusted for gestational age at enrollment, maternal Hb at enrollment, maternal HIV status, primiparity, and site of enrollment. Models for LBW were adjusted for child sex, maternal Hb at enrollment, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Models for newborn stunting were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Models for SGA were adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrolllment, primiparity, season of enrollment, and site of enrollment. Models for newborn small head circumference were adjusted for maternal Hb at enrollment, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.4-6. Adverse Birth Outcomes among Women with Elevated Maternal Plasma AGP Concentration at Enrollment

| Outcome | Participants with AGP ≤1 g/L at enrollment | Participants with AGP >1 g/L at enrollment | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 105/1125 (9.3%) | 24/163 (14.7%) | 1.6 (1.01 to 2.5) | 0.044 | 1.3 (0.83 to 2.1) | 0.225 |
| Incidence of LBW (<2,500 g) | 120/999 (12.0%) | 26/139 (18.7%) | 1.6 (1.02 to 2.4) | 0.041 | 1.3 (0.81 to 2.0) | 0.293 |
| Prevalence of newborn stunting (LAZ <−2) | 139/950 (14.6%) | 33/133 (24.8%) | 1.7 (1.2 to 2.5) | 0.006 | 1.3 (0.90 to 2.0) | 0.155 |
| Incidence of SGA age[c] | 278/999 (27.8%) | 58/139 (41.7%) | 1.5 (1.12 to 2.0) | 0.005 | 1.2 (0.85 to 1.6) | 0.354 |
| Prevalence of newborn small head circumference (HCZ <−2) | 33/953 (3.5%) | 9/132 (6.8%) | 2.0 (0.94 to 4.1) | 0.072 | 1.6 (0.8 to 3.5) | 0.209 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for preterm birth were adjusted for gestational age at enrollment, maternal Hb at enrollment, maternal HIV status, primiparity, and site of enrollment. Models for LBW were adjusted for child sex, maternal Hb at enrollment, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Models for newborn stunting were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Models for SGA were adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Models for newborn small head circumference were adjusted for maternal Hb at enrollment, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.4-7. Adverse Birth Outcomes among Women with Elevated Maternal Plasma AGP Concentration at 36 gw

| Outcome | Participants with AGP ≤1 g/L at 36 gw | Participants with AGP >1 g/L at 36 gw | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 22/1004 (2.2%) | 6/58 (10.3%) | 4.7 (1.9 to 11.6) | <0.001 | 4.1 (1.6 to 10.8) | 0.004 |
| Incidence of LBW (<2,500 g) | 94/938 (10.0%) | 8/49 (16.3%) | 1.6 (0.8 to 3.4) | 0.185 | 1.5 (0.7 to 3.2) | 0.275 |
| Prevalence of newborn stunting (LAZ <−2) | 110/908 (12.1%) | 17/47 (36.2%) | 3.0 (1.8 to 5.0) | <0.001 | 2.2 (1.3 to 3.8) | 0.004 |
| Incidence of SGA[c] | 270/938 (28.8%) | 22/49 (44.9%) | 1.6 (1.01 to 2.4) | 0.045 | 1.3 (0.8 to 2.0) | 0.305 |
| Prevalence of newborn small head circumference (HCZ <−2) | 17/905 (1.9%) | 6/49 (12.2%) | 6.5 (2.6 to 16.5) | <0.001 | 4.6 (1.7 to 12.4) | 0.002 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for preterm birth were adjusted for gestational age at enrollment, maternal Hb at enrollment, maternal HIV status, primiparity, and site of enrollment. Models for LBW were adjusted for child sex, maternal Hb at enrollment, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Models for newborn stunting were adjusted for maternal Hb at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Models for SGA were adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Models for newborn small head circumference were adjusted for maternal Hb at enrollment, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

## 4.5 Maternal Blood Hb, ZPP, and sTfR

This substudy was nested in the main trial as a prospective cohort study to assess the associations between maternal Hb and iron status (as indicated by ZPP and sTfR) at enrollment and at 36 gw, and change from enrollment to 36 gw, with birth outcomes.

Of the 1,391 participants who were enrolled in the trial, 12 women with twin pregnancies were excluded from this analysis. We analyzed Hb from 1,377 (99.0%), ZPP from 1,325 (95.3%), and sTfR from 1,371 (98.6%) of those enrollment visits. At the 36 gw visit, we analyzed Hb from 1,040 (74.8%), ZPP from 1,008 (72.5%), and sTfR from 1,067 (76.7%) participants. Data on the duration of pregnancy were available for 99.5% of those included in the 36 gw analyses, and birth weight, length, and head circumference data for 90.2%, 89.0%, and 89.3% of the included participants, respectively.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of mean maternal BMI at enrollment, maternal educational achievement, proportion with low maternal BMI at enrollment, and proportion with a positive malaria test ($P \geq 0.05$). However, compared to excluded women, they tended to be older (25 vs. 24, $P<0.001$); have a lower proxy SES (−0.04 vs. 0.18, $P<0.002$); and be less often primiparous (19.7% vs. 28.8%, $P<0.001$), HIV positive (12.7% vs. 17.8%, $P=0.029$), and anemic (18.6% vs. 27.6%, $P<0.001$) (Table A1.2-4).

Table 4.5-1,Table 4.5-2, and Table 4.5-3 show the standardized linear regression coefficients for the associations between continuous birth outcomes and Hb, ZPP, and sTfR at enrollment. Hb at enrollment was positively associated with duration of gestation ($P<0.001$), birth weight ($P<0.001$), LAZ ($P<0.001$), HCZ ($P=0.037$), and WAZ ($P<0.001$) in unadjusted models. After adjusting for covariates, the association between Hb at enrollment and HCZ was no longer significant ($P=0.077$), while the other associations remained significant. ZPP at enrollment was inversely associated with duration of gestation ($P=0.029$) and tended to be inversely associated with LAZ ($P=0.063$). These associations did not change after adjusting for covariates. In unadjusted models, sTfR at enrollment was inversely associated with duration of gestation ($P=0.002$), birth weight ($P<0.001$), LAZ ($P<0.001$), HCZ ($P=0.016$), and WAZ ($P<0.001$). After adjusting for covariates, these associations remained significant.

Table 4.5-1,Table 4.5-2, and Table 4.5-3 also show the associations between continuous birth outcomes and Hb, ZPP, and sTfR at 36 gw, and the change in Hb, ZPP, and sTfR from enrollment to 36 gw. Hb at 36 gw was not associated with any of the birth outcomes in unadjusted or adjusted models. However, the change in Hb was inversely associated with duration of gestation ($P<0.001$), birth weight ($P=0.018$), and WAZ ($P<0.001$), and tended to be inversely associated with LAZ ($P=0.053$) in unadjusted models. After adjusting for covariates, the inverse association between change in Hb and duration of gestation remained significant ($P=0.002$), while the inverse associations with birth weight and WAZ were no longer significant ($P=0.060$ and $P=0.151$, respectively), and the association with LAZ no longer tended to be significant ($P=0.178$). ZPP at 36 gw was inversely associated with duration of gestation in both unadjusted ($P=0.015$) and adjusted models ($P=0.029$). Change in ZPP was positively associated with HCZ ($P=0.002$) and tended to be associated with birth weight ($P=0.076$) and LAZ ($P=0.054$) in unadjusted models. After adjusting for covariates, the positive association between change in ZPP and LAZ became significant ($P=0.008$), while the associations between change in ZPP and HCZ remained significant ($P=0.010$) and the association with birth weight still tended to be significant ($P=0.092$).

We examined the risk of adverse birth outcomes among anemic women (Hb <100 g/L) and women with elevated Hb (>130 g/L) compared to women with Hb between 100 and 130 g/L. Before adjusting for covariates, women who were anemic at enrollment were at increased risk of giving birth preterm

(P<0.001) and to a stunted newborn (P<0.001), and tended to have an increased risk of giving birth to a newborn with a small head circumference (P=0.072). The elevated risks of preterm birth and stunting remained significant after adjusting for covariates (P=0.002 and P=0.038, respectively), while there was no longer a trend toward small head circumference (P=0.225). There were no increased risks of adverse birth outcomes among women who were anemic at 36 gw, although there was a trend toward having a SGA infant after adjusting for covariates (P=0.079) (Table 4.5-4 and Table 4.5-5). There were no risks of adverse birth outcomes among women who had elevated Hb at either enrollment or 36 gw (Table 4.5-6 and Table 4.5-7).

Women who were iron deficient at enrollment (ZPP >60 μmol/mol heme or sTfR >6.0 mg/L) were at greater risk of giving birth preterm (P=0.001) and to a stunted newborn (P=0.016), and there was a trend for them to have an increased risk of giving birth to a LBW infant (P=0.081) before adjusting for covariates. Women who were iron deficient at 36 gw were also at greater risk of giving birth preterm (P=0.046) before adjusting for covariates. After adjusting for covariates, women who were iron deficient at enrollment continued to be at greater risk of giving birth preterm and to a stunted newborn (P=0.019 and P=0.024, respectively), but there was no longer a trend toward an increased risk of giving birth to a LBW infant (P=0.103). Also the increased risk of preterm birth among women who were iron deficient at 36 gw was no longer significant (P=0.150). There were no other significant risks of adverse birth outcomes among women with high ZPP or sTfR at enrollment or 36 gw (Table 4.5-8 and Table 4.5-9).

These results suggest that lower maternal Hb and iron status (as indicated by higher ZPP and sTfR) at enrollment were associated with worse birth outcomes, yet decreases in maternal Hb and iron status during pregnancy were associated with improved birth outcomes. Women who were anemic or iron deficient at enrollment were at increased risk of giving birth preterm and to a stunted newborn, yet women with low or high Hb (<100 g/L or >130 g/L) or low iron status (ZPP >60 μmol/mol heme or sTfR >6.0 mg/L) at 36 gw did not have a higher risk of adverse birth outcomes.

**Table 4.5-1. Standardized Regression Coefficients for the Associations between Hb, ZPP, and sTfR and Duration of Pregnancy and Birth Weight**

| | | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Duration of pregnancy | | | | Birth weight | | | |
| | | Unadjusted | | Adjusted[a] | | Unadjusted | | Adjusted[a] | |
| Predictor | Time point | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| Hb | Enrollment | 0.12 (0.03) | <0.001 | 0.10 (0.03) | <0.001 | 0.12 (0.03) | <0.001 | 0.08 (0.03) | 0.008 |
| | 36 gw | −0.008 (0.02) | 0.595 | −0.009 (0.01) | 0.550 | −0.001 (0.03) | 0.960 | −0.02 (0.03) | 0.532 |
| | Change | −0.07 (0.02) | <0.001 | −0.05 (0.02) | 0.002 | −0.08 (0.03) | 0.018 | −0.05 (0.03) | 0.060 |
| ZPP | Enrollment | −0.06 (0.03) | 0.029 | −0.07 (0.03) | 0.020 | −0.04 (0.03) | 0.149 | −0.04 (0.03) | 0.216 |
| | 36 gw | −0.04 (0.02) | 0.015 | −0.04 (0.02) | 0.029 | 0.0001 (0.03) | 0.998 | 0.006 (0.03) | 0.830 |
| | Change | −0.008 (0.02) | 0.661 | 0.009 (0.02) | 0.613 | 0.06 (0.03) | 0.076 | 0.05 (0.03) | 0.092 |
| sTfR | Enrollment | −0.09 (0.03) | 0.002 | −0.07 (0.03) | 0.010 | −0.12 (0.03) | <0.001 | −0.10 (0.03) | <0.001 |
| | 36 gw | −0.03 (0.02) | 0.057 | −0.03 (0.02) | 0.086 | −0.02 (0.03) | 0.613 | −0.006 (0.03) | 0.832 |
| | Change | 0.02 (0.02) | 0.194 | 0.006 (0.02) | 0.726 | 0.09 (0.03) | 0.005 | 0.08 (0.03) | 0.005 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values for all models were determined by linear regression.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for duration of pregnancy were adjusted for gestational age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Additionally, the 36 gw and change from enrollment models were adjusted for intervention group. Models for birth weight were adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, child sex, season of enrollment, and site of enrollment. Additionally, the 36 gw and change from enrollment models were adjusted for intervention group.

**Table 4.5-2. Standardized Regression Coefficients for the Associations between Hb, ZPP, and sTfR and Newborn LAZ and HCZ**

| | | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Newborn LAZ | | | | Newborn HCZ | | | |
| | | Unadjusted | | Adjusted[a] | | Unadjusted | | Adjusted[a] | |
| Predictor | Time point | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| Hb | Enrollment | 0.12 (0.03) | <0.001 | 0.08 (0.03) | 0.006 | 0.06 (0.03) | 0.037 | 0.06 (0.03) | 0.077 |
| | 36 gw | 0.02 (0.03) | 0.444 | 0.01 (0.03) | 0.736 | −0.03 (0.03) | 0.275 | −0.03 (0.03) | 0.265 |
| | Change | −0.06 (0.03) | 0.053 | −0.04 (0.03) | 0.178 | −0.05 (0.03) | 0.134 | −0.04 (0.03) | 0.197 |
| ZPP | Enrollment | −0.06 (0.03) | 0.063 | −0.05 (0.03) | 0.059 | −0.04 (0.03) | 0.183 | −0.03 (0.03) | 0.257 |
| | 36 gw | −0.01 (0.03) | 0.665 | −0.005 (0.03) | 0.848 | 0.04 (0.03) | 0.180 | 0.03 (0.03) | 0.216 |
| | Change | 0.06 (0.03) | 0.054 | 0.08 (0.03) | 0.008 | 0.10 (0.03) | 0.002 | 0.09 (0.03) | 0.010 |
| sTfR | Enrollment | −0.12 (0.03) | <0.001 | −0.10 (0.03) | <0.001 | −0.08 (0.03) | 0.016 | −0.07 (0.03) | 0.023 |
| | 36 gw | −0.02 (0.03) | 0.608 | −0.01 (0.03) | 0.609 | 0.02 (0.03) | 0.545 | 0.02 (0.03) | 0.559 |
| | Change | 0.08 (0.03) | 0.006 | 0.07 (0.03) | 0.014 | 0.07 (0.03) | 0.018 | 0.07 (0.03) | 0.023 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values for all models were determined by linear regression.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for newborn LAZ were adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, child sex, season of enrollment, and site of enrollment. Additionally, the 36 gw and change from enrollment models were adjusted for intervention group. Models for newborn HCZ were adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Additionally, the 36 gw and change from enrollment models were adjusted for intervention group.

### Table 4.5-3. Standardized Regression Coefficients for the Associations between Hb, ZPP, and sTfR and Newborn WAZ

| | | Outcome | | | |
|---|---|---|---|---|---|
| | | Newborn WAZ | | | |
| | | Unadjusted | | Adjusted[a] | |
| Predictor | Time point | Regression coefficient (SE)[a] | P-value[b] | Regression coefficient (SE)[a] | P-value[b,c] |
| Hb | Enrollment | 0.16 (0.03) | <0.001 | 0.11 (0.03) | <0.001 |
| | 36 gw | 0.002 (0.03) | 0.926 | −0.04 (0.03) | 0.151 |
| | Change | −0.10 (0.03) | <0.001 | −0.05 (0.03) | 0.151 |
| ZPP | Enrollment | −0.04 (0.03) | 0.131 | −0.01 (0.03) | 0.801 |
| | 36 gw | 0.001 (0.03) | 0.965 | 0.01 (0.03) | 0.680 |
| | Change | 0.04 (0.03) | 0.267 | 0.03 (0.03) | 0.304 |
| sTfR | Enrollment | −0.13 (0.03) | <0.001 | −0.08 (0.03) | 0.013 |
| | 36 gw | −0.01 (0.03) | 0.837 | 0.01 (0.03) | 0.789 |
| | Change | 0.09 (0.03) | 0.002 | 0.07 (0.03) | 0.022 |

[a] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[b] P-values for all models were determined by linear regression.
[c] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Models for newborn WAZ were adjusted for maternal BMI at enrollment, maternal height at enrollment, maternal age at enrollment, primiparity, maternal HIV status, maternal Hb at enrollment, proxy for SES, season of enrollment, and site of enrollment. Additionally, the 36 gw and change from enrollment models were adjusted for intervention group.

### Table 4.5-4. Adverse Birth Outcomes among Women with Hb <100 g/L at Enrollment

| Outcome | Participants with Hb between 100 and 130 g/L at enrollment | Participants with Hb <100 g/L at enrollment | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 71/881 (8.1%) | 46/264 (17.4%) | 2.2 (1.5 to 3.1) | <0.001 | 1.8 (1.2 to 2.6) | 0.002 |
| Incidence of LBW (<2,500 g) | 97/791 (12.3%) | 35/219 (16.0%) | 1.3 (0.9 to 1.9) | 0.145 | 1.1 (0.8 to 1.6) | 0.465 |
| Prevalence of newborn stunting (LAZ <−2) | 108/743 (14.5%) | 53/213 (24.9%) | 1.7 (1.3 to 2.3) | <0.001 | 1.4 (1.0 to 1.8) | 0.038 |
| Incidence of SGA[d] | 234/791 (29.6%) | 71/219 (32.4%) | 1.1 (0.9 to 1.4) | 0.413 | 1.0 (0.8 to 1.2) | 0.665 |
| Prevalence of newborn small head circumference (HCZ <−2) | 25/745 (3.4%) | 13/213 (6.1%) | 1.8 (0.9 to 3.5) | 0.072 | 1.5 (0.8 to 3.0) | 0.225 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.5-5. Adverse Birth Outcomes among Women with Hb <100 g/L at 36 gw

| Outcome | Participants with Hb between 100 and 130 g/L at 36 gw | Participants with Hb <100 g/L at 36 gw | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 17/734 (2.3%) | 9/211 (4.3%) | 1.8 (0.8 to 4.1) | 0.131 | 1.8 (0.8 to 4.1) | 0.142 |
| Incidence of LBW (<2,500 g) | 68/680 (10.0%) | 20/195 (10.3%) | 1.0 (0.6 to 1.6) | 0.916 | 1.0 (0.6 to 1.6) | 0.978 |
| Prevalence of newborn stunting (LAZ <−2) | 83/658 (12.6%) | 31/187 (16.6%) | 1.3 (0.9 to 1.9) | 0.158 | 1.2 (0.8 to 1.7) | 0.351 |
| Incidence of SGA[c] | 210/680 (30.9%) | 53/195 (27.2%) | 0.9 (0.7 to 1.1) | 0.328 | 0.8 (0.6 to 1.0) | 0.079 |
| Prevalence of newborn small head circumference (HCZ <−2) | 17/657 (2.6%) | 2/188 (1.1%) | 0.4 (0.1 to 1.8) | 0.232 | 0.4 (0.1 to 1.6) | 0.179 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Additionally, the models were adjusted for intervention group.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.5-6. Adverse Birth Outcomes among Women with Hb >130 g/L at Enrollment

| Outcome | Participants with Hb ≤130 g/L at enrollment | Participants with Hb >130 g/L at enrollment | Relative risk (95% CI)[a] | P-value | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 71/881 (8.1%) | 12/149 (8.1%) | 1.0 (0.6 to 1.8) | 0.998 | 1.1 (0.6 to 2.0) | 0.699 |
| Incidence of LBW (<2,500 g) | 97/791 (12.3%) | 14/133 (10.5%) | 0.9 ( 0.5 to 1.5) | 0.572 | 0.9 (0.5 to 1.6) | 0.759 |
| Prevalence of newborn stunting (LAZ <−2) | 108/743 (14.5%) | 13/132 (9.9%) | 0.7 (0.4 to 1.2) | 0.161 | 0.7 (0.4 to 1.3) | 0.269 |
| Incidence of SGA[c] | 234/791 (29.6%) | 33/133 (24.8%) | 0.8 (0.6 to 1.1) | 0.274 | 0.9 (0.6 to 1.2) | 0.466 |
| Prevalence of newborn small head circumference (HCZ <−2) | 25/745 (3.4%) | 5/132 (3.8%) | 1.1 (0.4 to 2.9) | 0.801 | 1.3 (0.5 to 3.3) | 0.622 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.5-7. Adverse Birth Outcomes among Women with Hb >130 g/L at 36 gw

| Outcome | Participants with Hb between 100 and 130 g/L at 36 gw | Participants with Hb >130 g/L at 36 gw | Relative risk (95% CI)[a] | P-value | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 17/734 (2.3%) | 1/87 (1.2%) | 0.5 (0.1 to 3.7) | 0.493 | 0.5 (0.1 to 4.0) | 0.551 |
| Incidence of LBW (<2,500 g) | 68/680 (10.0%) | 11/84 (13.1%) | 1.3 (0.7 to 2.4) | 0.375 | 1.6 (0.9 to 2.8) | 0.136 |
| Prevalence of newborn stunting (LAZ <−2) | 83/658 (12.6%) | 12/82 (14.6%) | 1.2 (0.7 to 2.0) | 0.603 | 1.3 (0.8 to 2.3) | 0.317 |
| Incidence of SGA[c] | 210/680 (30.9%) | 27/84 (32.1%) | 1.0 (0.7 to 1.4) | 0.812 | 1.1 (0.8 to 1.5) | 0.683 |
| Prevalence of newborn small head circumference (HCZ <−2) | 17/657 (2.6%) | 3/81 (3.7%) | 1.4 (0.4 to 4.8) | 0.560 | 1.8 (0.6 to 5.7) | 0.332 |

[a] P-values were obtained from Poisson regression models.
[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Additionally, the models were adjusted for intervention group.
[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

**Table 4.5-8. Adverse Birth Outcomes among Women with Iron Deficiency (ZPP >60 µmol/mol heme or sTfR >6.0 mg/L) at Enrollment**

| Outcome | Participants without iron deficiency at enrollment | Participants with iron deficiency at enrollment | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 68/830 (8.2%) | 59/407 (14.5%) | 1.8 (1.2 to 2.5) | 0.001 | 1.5 (1.1 to 2.1) | 0.019 |
| Incidence of LBW (<2,500 g) | 88/745 (11.8%) | 55/345 (15.9%) | 1.3 (0.96 to 1.9) | 0.081 | 1.3 (0.9 to 1.8) | 0.103 |
| Prevalence of newborn stunting (LAZ <−2) | 97/701 (13.8%) | 68/336 (20.2%) | 1.5 (1.1 to 2.0) | 0.016 | 1.4 (1.0 to 1.8) | 0.024 |
| Incidence of SGA[c] | 215/745 (28.9%) | 108/345 (31.3%) | 1.1 (0.9 to 1.4) | 0.508 | 1.0 (0.9 to 1.3) | 0.645 |
| Prevalence of newborn small head circumference (HCZ <−2) | 25/702 (3.6%) | 15/337 (4.5%) | 1.2 (0.7 to 2.4) | 0.495 | 1.2 (0.6 to 2.3) | 0.549 |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment.

[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

### Table 4.5-9. Adverse Birth Outcomes among Women with Iron Deficiency (ZPP >60 µmol/mol heme or sTfR >6.0 mg/L) at 36 gw

| Outcome | Participants without iron deficiency at 36 gw | Participants with iron deficiency at 36 gw | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 8/515 (1.6%) | 17/469 (3.6%) | 2.3 (1.0 to 5.4) | 0.046 | 1.9 (0.8 to 4.3) | 0.150 |
| Incidence of LBW (<2,500 g) | 45/477 (9.4%) | 47/435 (10.8%) | 1.1 (0.8 to 1.7) | 0.493 | 1.0 (0.7 to 1.5) | 0.961 |
| Prevalence of newborn stunting (LAZ <−2) | 62/464 (13.4%) | 55/420 (13.1%) | 1.0 (0.7 to 1.4) | 0.907 | 0.9 (0.7 to 1.3) | 0.575 |
| Incidence of SGA[c] | 142/477 (29.8%) | 129/435 (29.7%) | 1.0 (0.8 to 1.2) | 0.764 | 1.0 (0.8 to 1.2) | 0.968 |
| Prevalence of newborn small head circumference (HCZ <−2) | 12/466 (2.6%) | 8/419 (1.9%) | 0.7 (0.3 to 1.8) | 0.508 | 0.8 (0.3 to 1.9) | 0.630 |

[a] P-values were obtained from Poisson regression models.
[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for gestational age at enrollment, maternal HIV status, primiparity, and site of enrollment. Model for LBW was adjusted for child sex, maternal HIV status, maternal height at enrollment, primiparity, household food insecurity score, and site of enrollment. Model for newborn stunting was adjusted for maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, and site of enrollment. Model for SGA was adjusted for child sex, gestational age at enrollment, maternal HIV status, maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Model for newborn small head circumference was adjusted for maternal age at enrollment, maternal height at enrollment, primiparity, season of enrollment, and site of enrollment. Additionally, the models were adjusted for intervention group.
[c] Defined as having birth weight <10th percentile for infants of the same gestational age from a U.S. population.

## 4.6 Maternal Plasma Retinol Concentration

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal vitamin A status (as indicated by plasma retinol concentration) at enrollment with birth outcomes.

A subset of 316 women was randomly selected from the 1,391 women enrolled in the iLiNS-DYAD-M trial for assessment of vitamin A status based on plasma retinol concentration. Of these, two women with missing data were left out of the analysis. There were also three women with twin pregnancies. For these mothers, one twin was randomly selected and included in the dataset, resulting in a total sample size of 314 mother-baby pairs.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of maternal age, proportion of anemic or primiparous women, proportion with low maternal BMI at enrollment, and proportion with a positive HIV or malaria test ($P \geq 0.05$). However, compared to the excluded women, they tended to have fewer years of education (3.6 vs. 4.2, $P=0.010$), a lower proxy for SES (−0.17 vs. 0.06, $P<0.001$), and a lower mean BMI (21.8 vs. 22.3, $P=0.012$) (Table A1.2-5).

Plasma retinol concentration at enrollment was not associated with the selected continuous birth outcomes (duration of pregnancy, birth weight, newborn LAZ, newborn WAZ, and newborn HCZ) in either the unadjusted or adjusted models (Table 4.6-1).

Low plasma retinol concentration (<1.05 µmol/L) at enrollment was, however, negatively associated with newborn stunting in an unadjusted and adjusted model ($P=0.046$ and $P=0.015$, respectively; Table 4.6-2) but with none of the remaining dichotomous birth outcomes (preterm birth and LBW) in either an unadjusted or adjusted model. Small head circumference was not analyzed due to a low number of cases.

### Table 4.6-1. Association between Continuous Birth Outcomes and Plasma Retinol Concentration at Enrollment

| | Unadjusted | | Adjusted | |
|---|---|---|---|---|
| **Outcome** | **Regression coefficient (SE)** | **P-value[a]** | **Regression coefficient (SE)** | **P-value[a,b]** |
| Duration of pregnancy (weeks) | −0.037 (0.276) | 0.894 | −0.030 (0.264) | 0.910 |
| Birth weight (g) | 19.80 (85.43) | 0.817 | −16.15 (83.72) | 0.847 |
| Newborn LAZ | 0.045 (0.200) | 0.823 | −0.071 (0.198) | 0.720 |
| Newborn WAZ | 0.150 (0.184) | 0.417 | 0.069 (0.173) | 0.690 |
| Newborn HCZ | −0.125 (0.182) | 0.492 | −0.213 (0.176) | 0.227 |

[a] P-values were obtained from linear regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for duration of pregnancy was adjusted for season of enrollment and site of enrollment. Model for birth weight was adjusted for maternal BMI at enrollment, high maternal plasma AGP concentration and high maternal plasma CRP concentration at enrollment, maternal HIV status, primiparity, maternal malaria status at enrollment, season of enrollment, and site of enrollment. Model for newborn LAZ was adjusted for maternal BMI at enrollment, high maternal plasma AGP concentration and high maternal plasma CRP concentration at enrollment, maternal HIV status, primiparity, maternal malaria status at enrollment, and site of enrollment. Model for newborn WAZ was adjusted for maternal BMI at enrollment, maternal educational achievement, high maternal plasma AGP concentration and high maternal plasma CRP concentration at enrollment, primiparity, and site of enrollment. Model for newborn HCZ was adjusted for maternal BMI at enrollment, high maternal plasma AGP concentration at enrollment, and site of enrollment.

### Table 4.6-2. Adverse Birth Outcomes among Women with Plasma Retinol <1.05 umol/L at Enrollment

| Outcome | Participants with plasma retinol <1.05 umol/L | Participants with plasma retinol ≥1.05 umol/L | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[a,b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 wk) | 4/147 (2.7%) | 2/167 (1.2%) | 2.3 (0.4 to 12.3) | 0.340 | 2.0 (0.4 to 9.6) | 0.385 |
| Incidence of LBW (<2,500 g) | 9/131 (6.9%) | 15/151 (9.9%) | 0.7 (0.3 to 1.5) | 0.363 | 0.5 (0.2 to 1.3) | 0.151 |
| Prevalence of newborn stunting (LAZ <−2) | 14/141 (9.9%) | 28/153 (18.3%) | 0.5 (0.3 to 1.0) | 0.046 | 0.5 (0.3 to 0.9) | 0.015 |
| Prevalence of newborn small head circumference (HCZ <−2) | 0/140 (0.0%) | 3/152 (2.0%) | NA | NA | NA | NA |

[a] P-values were obtained from Poisson regression models.

[b] Models were adjusted for covariates found in bivariate analysis to be associated with the birth outcome (P<0.10). Model for preterm birth was adjusted for high maternal plasma AGP concentration at enrollment, primiparity, maternal malaria status at enrollment, and site of enrollment. Model for LBW was adjusted for high maternal plasma AGP concentration and high maternal plasma CRP concentration at enrollment, maternal HIV status, and primiparity. Model for newborn stunting was adjusted for high maternal plasma AGP concentration and high maternal plasma CRP concentration at enrollment, maternal HIV status, primiparity, and maternal malaria status at enrollment.

## 4.7 Maternal Plasma Cholesterol and Triglyceride Concentrations and Plasma Fatty Acid Status

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal cholesterol, triglycerides, and fatty acid status, as measured in plasma at enrollment and at 36 weeks gestation, with birth outcomes. Previous literature has suggested that low cholesterol (<10th percentile) may be associated with preterm birth (Edison et al. 2007). It has also been suggested that a high proportion of omega-3 fatty acids, particularly docosahexaenoic acid (DHA) and possibly also α-linolenic acid (ALA), and a low omega-6:omega-3 fatty acids ratio may be associated with a lower risk of adverse birth outcomes. The omega-6:omega-3 ratio can be affected not only by the omega-3 fatty acids listed, but also by the omega-6 fatty acid arachidonic acid (AA).

Of the 1,391 women who were enrolled in the study, 12 women were pregnant with twins and were excluded from analyses. As a result, 1,371 (98.6%) had cholesterol and triglyceride measurements at enrollment, and 1,061 (76.3%) also had measurements at 36 gw.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of mean maternal BMI at enrollment, maternal educational achievement, proportion with low maternal BMI at enrollment, and proportion with a positive HIV test or malaria test ($P \geq 0.05$). However, compared to the excluded women, they tended to be older (25 vs. 24, P=0.001) and have a lower proxy for SES (−0.04 vs. 0.16, P=0.004) and were less likely to be primiparous (19.7% vs. 28.6%, P<0.001) or anemic (18.6% vs. 27.4%, P<0.001) (Table A1.2-6).

Table 4.7-1 and Table 4.7-2 show the standardized linear regression coefficients for the association between plasma cholesterol and triglyceride concentration at enrollment and at 36 gw with continuous birth outcomes. The triglyceride concentration variable was log-transformed for this analysis. Non-transformed values are presented in the table. Cholesterol concentration at enrollment was not associated with any birth outcome. Higher cholesterol at 36 gw was associated with higher LAZ (P=0.014), although this became non-significant in adjusted analyses (P=0.372) (Table 4.7-1). Higher triglyceride at enrollment was associated with poorer birth outcome measurements in unadjusted models, but all relationships became non-significant in adjusted models. Triglyceride concentration at 36 gw was not significantly associated with birth outcomes in either adjusted or unadjusted analyses (Table 4.7-2).

Tables 4.7-3 thru 4.7-6 show the standardized linear regression coefficients for the association between plasma fatty acid composition and continuous birth outcomes. All continuous fatty acid variables except for AA were log-transformed for this analysis. Non-transformed values are presented in the table. In an unadjusted model, higher AA at enrollment was associated with a longer duration of gestation (P=0.006), but this association did not remain significant in an adjusted model (P=0.130) (Table 4.7-3). DHA was not significantly associated with any birth outcome (Table 4.7-4), whereas ALA at enrollment was positively associated with every birth outcome, and all of the associations except newborn LAZ remained significant in adjusted analyses. Associations with ALA at 36 gw were less consistent, with higher ALA being associated with a shorter duration of gestation in an adjusted model and with higher LAZ in unadjusted analyses, although this latter association did not remain significant in adjusted analyses (Table 4.7-5). A higher omega-6:omega-3 fatty acids ratio at 36 gw was associated with a higher WAZ in unadjusted analyses (P=0.037), which was attenuated in adjusted analyses (P=0.061) (Table 4.7-6).

Dichotomous birth outcomes were also examined, although, due to a low level of prevalence of both low cholesterol and small head circumference in the given sample size, we were unable to obtain risk ratios

for this association. Both low cholesterol and high fatty acids were defined based on the distribution found in the control group that received IFA in the intervention part of this trial. Low cholesterol was defined as <10th percentile to be consistent with previous literature. High fatty acids were defined as >50th percentile due to the smaller sample size (n=315). Triglycerides were not analyzed as a dichotomous variable because there is no concern that low triglycerides are associated with adverse birth outcomes. While there is a clinical definition of high triglycerides that we could have used to dichotomize the variable, none of the study population actually fell into that category.

An increased risk of newborn stunting was found for women with low cholesterol (<10th percentile) at both enrollment and 36 gw, although when analyzed in adjusted models, these associations became non-significant. No significant associations were seen with LBW, SGA, or preterm birth, although it should be noted that we did not examine the association of preterm birth with cholesterol at 36 gw because a number of preterm births had already occurred before this time point (Table 4.7-7).

For the fatty acids, the only dichotomous birth outcomes examined were LBW, SGA, and newborn stunting, as the prevalences of small head circumference and preterm birth were too low in this subgroup to obtain accurate relative risks given our sample size. High AA (≥50th percentile) and high DHA (≥50th percentile) were not associated with LBW, newborn stunting, or SGA (Table 4.7-8 and Table 4.7-9). Women with high ALA at enrollment, but not at 36 gw, had a lower risk of giving birth to a stunted infant in both unadjusted and adjusted analyses (P=0.015 and P=0.030, respectively) (Table 4.7-10). A high omega-6:omega-3 fatty acids ratio was not associated with LBW, newborn stunting, or SGA (Table 4.7-11).

In summary, cholesterol was not strongly associated with any birth outcome, and low cholesterol (<10th percentile) does not seem to be a cause for concern with respect to adverse birth outcomes in this population. High triglycerides at enrollment showed an association with poorer birth outcomes in unadjusted analyses, but this association was no longer significant in adjusted models. Overall, AA, DHA, and the omega-6:omega-3 fatty acids ratio were not associated with birth outcomes. In contrast, ALA at enrollment (though not at 36 gw) was strongly and consistently associated with a longer duration of gestation; a greater birth weight, WAZ, and head circumference; and a lower risk of newborn stunting.

**Table 4.7-1. Standardized Regression Coefficients for the Associations between Cholesterol at Enrollment and at 36 gw and Continuous Birth Outcomes**

| | Outcome | Enrollment standardized coefficient[c] | P-value[d] | 36 gw standardized coefficient[c] | P-value[d] |
|---|---|---|---|---|---|
| Duration of pregnancy, week | Unadjusted[a] | −0.05 (0.03) | 0.346 | −0.008 (0.03) | 0.663 |
| | Adjusted model[b] | −0.04 (0.03) | 0.127 | −0.02 (0.03) | 0.250 |
| Birth weight, g | Unadjusted[a] | −0.08 (0.03) | 0.615 | 0.03 (0.03) | 0.266 |
| | Adjusted model[b] | −0.05 (0.03) | 0.112 | −0.003 (0.03) | 0.909 |
| Newborn WAZ | Unadjusted[a] | −0.04 (0.03) | 0.067 | 0.05 (0.03) | 0.058 |
| | Adjusted model[b] | −0.04 (0.03) | 0.194 | −0.004 (0.03) | 0.871 |
| Newborn LAZ | Unadjusted[a] | −0.04 (0.03) | 0.089 | 0.07 (0.03) | 0.014 |
| | Adjusted model[b] | −0.02 (0.03) | 0.492 | 0.02 (0.03) | 0.372 |
| Newborn HCZ | Unadjusted[a] | −0.004 (0.03) | 0.891 | −0.01 (0.03) | 0.719 |
| | Adjusted model[b] | −0.06 (0.03) | 0.056 | −0.03 (0.03) | 0.229 |

[a] Unadjusted model.
[b] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.
[c] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[d] P-values were determined by linear regression models.

**Table 4.7-2. Standardized Regression Coefficients for the Associations between Triglycerides at Enrollment and at 36 gw and Continuous Birth Outcomes**

| | Outcome | Enrollment standardized coefficient[c] | P-value[d] | 36 gw standardized coefficient[c] | P-value[d] |
|---|---|---|---|---|---|
| Duration of pregnancy, week | Unadjusted[a] | −0.12 (0.03) | <0.001 | −0.007 (0.02) | 0.702 |
| | Adjusted model[b] | −0.06 (0.03) | 0.058 | 0.006 (0.02) | 0.732 |
| Birth weight, g | Unadjusted[a] | −0.09 (0.03) | 0.003 | −0.01 (0.03) | 0.603 |
| | Adjusted model[b] | −0.04 (0.03) | 0.157 | 0.007 (0.03) | 0.814 |
| Newborn WAZ | Unadjusted[a] | −0.09 (0.03) | 0.004 | 0.02 (0.03) | 0.509 |
| | Adjusted model[b] | −0.05 (0.03) | 0.103 | 0.03 (0.03) | 0.263 |
| Newborn LAZ | Unadjusted[a] | −0.06 (0.03) | 0.053 | 0.03 (0.03) | 0.287 |
| | Adjusted model[b] | −0.04 (0.03) | 0.203 | 0.05 (0.03) | 0.057 |
| Newborn HCZ | Unadjusted[a] | −0.07 (0.03) | 0.012 | −0.01 (0.03) | 0.716 |
| | Adjusted model[b] | −0.04 (0.03) | 0.197 | 0.01 (0.03) | 0.652 |

[a] Unadjusted model.
[b] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.
[c] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[d] P-values were determined by linear regression models.

**Table 4.7-3. Standardized Regression Coefficients for the Associations between Arachidonic Acid at Enrollment and at 36 gw and Continuous Birth Outcomes**

| | Outcome | Enrollment standardized coefficient[c] | P-value[d] | 36 gw standardized coefficient[c] | P-value[d] |
|---|---|---|---|---|---|
| Duration of pregnancy, week | Unadjusted[a] | 0.07 (0.03) | 0.006 | −0.005 (0.03) | 0.843 |
| | Adjusted model[b] | 0.04 (0.03) | 0.130 | −0.03 (0.03) | 0.252 |
| Birth weight, g | Unadjusted[a] | 0.02 (0.05) | 0.694 | −0.01 (0.05) | 0.786 |
| | Adjusted model[b] | −0.006 (0.06) | 0.908 | −0.05 (0.05) | 0.392 |
| Newborn WAZ | Unadjusted[a] | 0.02 (0.05) | 0.767 | 0.002 (0.05) | 0.973 |
| | Adjusted model[b] | −0.006 (0.05) | 0.909 | −0.01 (0.05) | 0.838 |
| Newborn LAZ | Unadjusted[a] | 0.02 (0.05) | 0.638 | 0.03 (0.05) | 0.601 |
| | Adjusted model[b] | 0.002 (0.06) | 0.972 | 0.03 (0.05) | 0.537 |
| Newborn HCZ | Unadjusted[a] | −0.002 (0.05) | 0.970 | 0.03 (0.05) | 0.532 |
| | Adjusted model[b] | −0.01 (0.05) | 0.796 | 0.02 (0.05) | 0.676 |

[a] Unadjusted model.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.

[c] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.

[c] P-values were determined by linear regression models.

**Table 4.7-4. Standardized Regression Coefficients for the Associations between Docosahexaenoic Acid at Enrollment and at 36 gw and Continuous Birth Outcomes**

| | Outcome | Enrollment standardized coefficient[c] | P-value[d] | 36 gw standardized coefficient[c] | P-value[d] |
|---|---|---|---|---|---|
| Duration of pregnancy, week | Unadjusted[a] | −0.01 (0.03) | 0.681 | 0.002 (0.03) | 0.937 |
| | Adjusted model[b] | −0.05 (0.03) | 0.058 | −0.04 (0.03) | 0.157 |
| Birth weight, g | Unadjusted[a] | 0.006 (0.05) | 0.917 | −0.03 (0.05) | 0.626 |
| | Adjusted model[b] | −0.02 (0.05) | 0.657 | −0.04 (0.05) | 0.501 |
| Newborn WAZ | Unadjusted[a] | 0.04 (0.05) | 0.421 | −0.04 (0.05) | 0.433 |
| | Adjusted model[b] | 0.01 (0.05) | 0.779 | −0.04 (0.05) | 0.409 |
| Newborn LAZ | Unadjusted[a] | 0.05 (0.05) | 0.391 | 0.04 (0.05) | 0.459 |
| | Adjusted model[b] | 0.02 (0.05) | 0.623 | 0.05 (0.05) | 0.354 |
| Newborn HCZ | Unadjusted[a] | 0.05 (0.05) | 0.331 | 0.07 (0.05) | 0.137 |
| | Adjusted model[b] | −0.004 (0.05) | 0.929 | 0.03 (0.05) | 0.583 |

[a] Unadjusted model.
[b] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.
[c] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[d] P-values were determined by linear regression models.

**Table 4.7-5. Standardized Regression Coefficients for the Associations between α-Linolenic Acid at Enrollment and at 36 gw and Continuous Birth Outcomes**

| | Outcome | Enrollment standardized coefficient[c] | P-value[d] | 36 gw standardized coefficient[c] | P-value[d] |
|---|---|---|---|---|---|
| Duration of pregnancy, week | Unadjusted[a] | 0.06 (0.03) | 0.019 | −0.008 (0.03) | 0.663 |
| | Adjusted model[b] | 0.06 (0.03) | 0.026 | −0.05 (0.03) | 0.049 |
| Birth weight, g | Unadjusted[a] | 0.14 (0.05) | 0.001 | 0.03 (0.03) | 0.266 |
| | Adjusted model[b] | 0.13 (0.05) | 0.008 | −0.02 (0.05) | 0.676 |
| Newborn WAZ | Unadjusted[a] | 0.17 (0.05) | 0.001 | 0.05 (0.03) | 0.058 |
| | Adjusted model[b] | 0.16 (0.05) | 0.001 | −0.02 (0.05) | 0.650 |
| Newborn LAZ | Unadjusted[a] | 0.12 (0.05) | 0.022 | 0.07 (0.03) | 0.014 |
| | Adjusted model[b] | 0.09 (0.05) | 0.080 | −0.009 (0.05) | 0.848 |
| Newborn HCZ | Unadjusted[a] | 0.13 (0.05) | 0.005 | −0.01 (0.03) | 0.719 |
| | Adjusted model[b] | 0.10 (0.05) | 0.028 | 0.01 (0.05) | 0.765 |

[a] Unadjusted model.
[b] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.
[c] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.
[d] P-values were determined by linear regression models.

**Table 4.7-6. Standardized Regression Coefficients for the Associations between the Omega-6:Omega-3 Fatty Acids Ratio at Enrollment and at 36 gw and Continuous Birth Outcomes**

| | Outcome | Enrollment standardized coefficient[c] | P-value[d] | 36 gw standardized coefficient[c] | P-value[d] |
|---|---|---|---|---|---|
| Duration of pregnancy, week | Unadjusted[a] | 0.02 (0.03) | 0.462 | 0.01 (0.03) | 0.599 |
| | Adjusted model[b] | 0.05 (0.03) | 0.052 | 0.04 (0.03) | 0.139 |
| Birth weight, g | Unadjusted[a] | 0.009 (0.05) | 0.872 | 0.06 (0.05) | 0.249 |
| | Adjusted model[b] | 0.03 (0.05) | 0.539 | 0.06 (0.05) | 0.268 |
| Newborn WAZ | Unadjusted[a] | -0.006 (0.05) | 0.915 | 0.11 (0.05) | 0.037 |
| | Adjusted model[b] | 0.01 (0.05) | 0.804 | 0.10 (0.05) | 0.061 |
| Newborn LAZ | Unadjusted[a] | -0.04 (0.05) | 0.409 | 0.01 (0.05) | 0.777 |
| | Adjusted model[b] | -0.03 (0.05) | 0.550 | -0.04 (0.06) | 0.492 |
| Newborn HCZ | Unadjusted[a] | -0.03 (0.05) | 0.545 | -0.01 (0.05) | 0.829 |
| | Adjusted model[b] | 0.02 (0.05) | 0.644 | 0.03 (0.05) | 0.510 |

[a] Unadjusted model.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.

[c] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.

[d] P-values were determined by linear regression models.

### Table 4.7-7. Adverse Birth Outcomes by Low Cholesterol at Enrollment and at 36 gw

| Outcome[a] | Participants with cholesterol ≥10th percentile | Participants with cholesterol <10th percentile | Relative risk (95% CI) | P-value[c] | Adjusted relative risk (95% CI)[d] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| **Cholesterol, enrollment[a]** | | | | | | |
| Incidence of LBW (<2,500 g) | 136/1041 (13.1%) | 10/97 (10.3%) | 0.79 (0.43 to 1.45) | 0.445 | 0.55 (0.30 to 1.01) | 0.055 |
| Prevalence of newborn stunting (LAZ <−2)[d] | 149/990 (16.6%) | 23/93 (24.7%) | 1.64 (1.12 to 2.41) | 0.011 | 1.01 (0.67 to 1.52) | 0.960 |
| Incidence of SGA | 281/926 (30.4%) | 26/88 (29.6%) | 0.97 (0.70 to 1.36) | 0.877 | 0.68 (0.50 to 0.96) | 0.026 |
| **Cholesterol, 36 weeks[a,b]** | | | | | | |
| Incidence of LBW (<2,500 g) | 92/886(10.4%) | 10/101 (9.9%) | 0.95 (0.51 to 1.17) | 0.880 | 0.82 (0.42 to 1.59) | 0.557 |
| Prevalence of newborn stunting (LAZ <−2) | 107/857 (12.5%) | 20/98 (20.4%) | 1.63 (1.06 to 2.51) | 0.025 | 1.10 (0.69 to 1.75) | 0.686 |
| Incidence of SGA | 237/794 (29.9%) | 29/89 (32.6%) | 1.09 (0.79 to 1.50) | 0.588 | 0.94 (0.68 to 1.30) | 0.696 |

[a] Small head circumference not examined due to low prevalence of small head circumference given the sample size.
[b] Preterm birth outcome not analyzed due to many preterm births occurring before 36 gw.
[c] P-values were obtained from Poisson regression models.
[d] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.

### Table 4.7-8. Adverse Birth Outcomes by High Arachidonic Acid at Enrollment and at 36 gw

| Outcome[a] | Participants with AA <50th percentile | Participants with AA ≥50th percentile | Relative risk (95% CI) | P-value[b] | Adjusted relative risk (95% CI)[c] | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| **AA, enrollment** | | | | | | |
| Incidence of LBW (<2,500 g) | 15/154 (9.7%) | 11/148 (7.4%) | 0.76 (0.36 to 1.61) | 0.477 | 0.68 (0.32 to 1.46) | 0.325 |
| Prevalence of newborn stunting (LAZ <−2) | 22/149 (14.8%) | 20/145 (13.8%) | 0.93 (0.53 to 1.64) | 0.812 | 0.83 (0.45 to 1.54) | 0.556 |
| Incidence of SGA | 43/139 (30.9%) | 40/141 (28.4%) | 0.92 (0.64 to 1.32) | 0.638 | 0.88 (0.60 to 1.27) | 0.484 |
| **AA, 36 gw** | | | | | | |
| Incidence of LBW (<2,500 g) | 12/133 (7.1%) | 14/170 (10.5%) | 1.49 (0.71 to 3.12) | 0.288 | 1.73 (0.82 to 3.67) | 0.152 |
| Prevalence of newborn stunting (LAZ <−2) | 21/163 (12.9%) | 21/131 (16.0%) | 1.24 (0.71 to 2.18) | 0.444 | 1.35 (0.77 to 2.39) | 0.297 |
| Incidence of SGA | 48/160 (30.0%) | 35/121 (28.9%) | 0.96 (0.67 to 1.39) | 0.845 | 1.01 (0.69 to 1.47) | 0.962 |

[a] Small head circumference not examined due to low prevalence of small head circumference given the sample size. Preterm birth outcome not analyzed due to many preterm births occurring before 36 gw.
[b] P-values were obtained from Poisson regression models.
[c] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.

### Table 4.7-9. Adverse Birth Outcomes by High Docosahexaenoic Acid at Enrollment and at 36 gw

| Outcome[a] | Participants with DHA <50th percentile | Participants with DHA ≥50th percentile | Relative risk (95% CI) | P-value[b] | Adjusted relative risk (95% CI)[c] | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| **DHA, enrollment** | | | | | | |
| Incidence of LBW (<2,500 g) | 15/151 (9.9%) | 11/151 (7.3%) | 0.73 (0.35 to 1.54) | 0.414 | 0.74 (0.36 to 1.49) | 0.393 |
| Prevalence of newborn stunting (LAZ <−2) | 24/149 (16.1%) | 18/145 (12.4%) | 0.77 (0.44 to 1.36) | 0.368 | 0.69 (0.39 to 1.22) | 0.201 |
| Incidence of SGA | 36/141 (25.5%) | 47/139 (33.8%) | 1.32 (0.92 to 1.91) | 0.132 | 1.27 (0.90 to 1.81) | 0.178 |
| **DHA, 36 gw** | | | | | | |
| Incidence of LBW (<2,500 g) | 12/152 (7.9%) | 14/151 (9.3%) | 1.17 (0.56 to 2.46) | 0.669 | 1.31 (0.59 to 2.90) | 0.511 |
| Prevalence of newborn stunting (LAZ <−2) | 25/149 (16.8%) | 17/145 (11.7%) | 0.70 (0.39 to 1.24) | 0.220 | 0.60 (0.33 to 1.08) | 0.086 |
| Incidence of SGA | 36/139 (25.9%) | 47/142 (33.1%) | 1.28 (0.89 to 1.84) | 0.189 | 1.20 (0.83 to 1.72) | 0.333 |

[a] Small head circumference not examined due to low prevalence of small head circumference given the sample size. Preterm birth outcome not analyzed due to many preterm births occurring before 36 gw.

[b] P-values were obtained from Poisson regression models.

[c] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.

### Table 4.7-10. Adverse Birth Outcomes by High α-Linolenic Acid, at Enrollment and at 36 gw

| Outcome[a] | Participants with ALA <50th percentile | Participants with ALA ≥50th percentile | Relative risk (95% CI) | P-value[b] | Adjusted relative risk (95% CI)[c] | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| **ALA, enrollment** | | | | | | |
| Incidence of LBW (<2,500 g) | 16/150 (10.7%) | 10/152 (6.7%) | 0.62 (0.29 to 1.31) | 0.211 | 0.64 (0.31 to 1.32) | 0.226 |
| Prevalence of newborn stunting (LAZ <−2) | 28/143 (19.6%) | 14/151 (9.3%) | 0.47 (0.26 to 0.86) | 0.015 | 0.53 (0.30 to 0.94) | 0.030 |
| Incidence of SGA | 47/141 (33.3%) | 36/139 (25.9%) | 0.78 (0.54 to 1.12) | 0.176 | 0.89 (0.62 to 1.27) | 0.513 |
| **ALA, 36 gw** | | | | | | |
| Incidence of LBW (<2,500 g) | 12/149 (8.1%) | 14/154 (9.1%) | 1.13 (0.54 to 2.36) | 0.748 | 1.25 (0.61 to 2.59) | 0.544 |
| Prevalence of newborn stunting (LAZ <−2) | 21/147 (14.3%) | 21/147 (14.3%) | 1.00 (0.57 to 1.75) | 1.000 | 1.00 (0.57 to 1.76) | 1.000 |
| Incidence of SGA | 39/140 (27.9%) | 44/141 (31.2%) | 1.12 (0.78 to 1.61) | 0.539 | 1.21 (0.84 to 1.73) | 0.301 |

[a] Small head circumference not examined due to low prevalence of small head circumference given the sample size. Preterm birth outcome not analyzed due to many preterm births occurring before 36 gw.

[b] P-values were obtained from Poisson regression models

[c] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollmnet, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted for intervention group.

### Table 4.7-11. Adverse Birth Outcomes by High Omega-6:Omega-3 Ratio at Enrollment and at 36 gw

| Outcome[a] | Participants with omega-6:omega-3 <50th percentile | Participants with omega-6:omega-3 ≥50th percentile | Relative risk (95% CI) | P-value[b] | Adjusted relative risk (95% CI)[c] | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| **Omega-6:omega-3, enrollment** | | | | | | |
| Incidence of LBW (<2,500 g) | 11/150 (7.3%) | 15/152 (9.9%) | 1.35 (0.64 to 2.83) | 0.435 | 1.25 (0.58 to 2.68) | 0.566 |
| Prevalence of newborn stunting (LAZ <−2) | 17/144 (11.8%) | 25/150 (16.7%) | 1.41 (0.80 to 2.50) | 0.238 | 1.47 (0.84 to 2.60) | 0.181 |
| Incidence of SGA | 44/139 (31.7%) | 39/141 (27.7%) | 0.87 (0.61 to 1.25) | 0.465 | 0.87 (0.61 to 1.23) | 0.430 |
| **Omega-6:omega-3, 36 gw** | | | | | | |
| Incidence of LBW (<2,500 g) | 15/153 (9.8%) | 11/150 (7.3%) | 0.75 (0.36 to 1.58) | 0.445 | 0.66 (0.30 to 1.46) | 0.303 |
| Prevalence of newborn stunting (LAZ <−2) | 22/151 (14.6%) | 20/143 (14.0%) | 0.96 (0.55 to 1.68) | 0.887 | 1.06 (0.61 to 1.84) | 0.826 |
| Incidence of SGA | 48/141 (34.0%) | 35/140 (25.0%) | 0.73 (0.51 to 1.06) | 0.100 | 0.73 (0.51 to 1.04) | 0.084 |

[a] Small head circumference not examined due to low prevalence of small head circumference given the sample size. Preterm birth outcome not analyzed due to many preterm births occurring before 36 gw.

[b] P-values were obtained from Poisson regression models.

[c] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, site of enrollment, gestational age at enrollment, maternal plasma AGP concentration at enrollment, maternal plasma CRP concentration at enrollment, and maternal HIV status. The 36 gw analyses was also adjusted intervention group.

## 4.8 Maternal Perceived Stress and Salivary Cortisol Concentration

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal perceived stress and salivary cortisol concentration, measured at enrollment, 28 gw, and 36 gw, with birth outcomes.

Of the 1,391 women enrolled in the iLiNS-DYAD-M main study, 12 women had twin pregnancies and were excluded from the analyses. Of the remaining women a total of 1,237 (89.7%) had at least some data on perceived stress or salivary cortisol and were included in this analysis (n = 1237 at enrollment, n = 899 at 28 gw, and n= 1043 at 36 gw).

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of mean BMI, maternal education achievement, maternal age, proportion that were HIV positive, proxy for SES, proportion with a low BMI, and proportion with a positive malaria test (P>0.05). However, compared to the excluded women, they tended to be anemic (19.7% vs. 29.2%, P = 0.008) and were less often primiparous (20.6% vs. 32.6%, P=0.001 (Table A1.2-7).

Salivary cortisol was associated with a number of birth outcomes in this study population. Two adjusted models were used, one with CRP and AGP included and one without these variables, as it is not clear if they are on the causal pathway. Standardized linear regression coefficients were used for analysis of the association of CRP and AGP with continuous birth outcomes. Cortisol was log-transformed due to skewedness of the variable. Non-transformed values are provided in the tables.

Higher cortisol at enrollment was associated with duration of gestation in adjusted models only, and cortisol at 28 gw showed no association. Higher cortisol at 36 gw was associated with a shorter duration of gestation in unadjusted and adjusted models (Table 4.8-1). In the adjusted models, for each SD increase in log cortisol at 36 gw, there was a reduction of 0.04–0.05 SD in the duration of gestation, which translates to roughly 1 day reduced duration of gestation per SD increase in cortisol. In adjusted analysis, using Model 1 (see Appendix A2.8 for more detail), higher cortisol at enrollment, at 28 gw, and at 36 gw was significantly associated with lower birth weight—approximately 36 g, 40 g, and 49 g lower per SD increase in cortisol at those respective time points. Cortisol was not found to be associated with newborn WAZ, LAZ, or HCZ at any time point, however, in the adjusted models (Table 4.8-1).

There was less of a consistent relationship between the PSS and birth outcomes (Table 4.8-2). Perceived stress at 36 gw was significantly associated with lower newborn LAZ (P=0.001) in adjusted analyses. Perceived stress at enrollment was significantly associated with higher birth weight, newborn LAZ, and WAZ in unadjusted analyses, but became non-significant in adjusted analyses. No other associations were found to be significant.

We also examined the association between cortisol categorized into quartiles and dichotomous birth outcomes (Table 4.8-3). Here we found that those with enrollment cortisol in the uppermost quartile experienced a 56% increased risk of newborn stunting compared to those in the lowest quartile (relative risk: 1.56; 95% CI: 1.08 to 2.25, p=0.018) in an adjusted model. However, when further adjusted for CRP and AGP, the effect size attenuated and the P-value was no longer statistically significant (P=0.076, not shown). There was a relatively consistent association between higher cortisol at 28 gw or 36 gw and an increased risk of LBW (Table 4.8-4 and Table 4.8-5). In unadjusted and adjusted models, those with a cortisol concentration in the third or fourth quartile had a roughly twofold increased risk of LBW compared to those in the lowest quartile. Cortisol measured at 28 weeks was not significantly associated with preterm birth. Measured at 36 weeks, cortisol was not significantly associated with preterm birth

either; however, the small number of cases of preterm birth within each of the quartiles limited our ability to fully analyze these models. Nevertheless, the relative risk comparing those in the highest quartile of cortisol at 36 gw to the lowest indicated a strongly elevated risk (relative risk: 6.00; 95% CI: 1.18 to 30.56, p=0.031) in an adjusted model. However, this became non-significant after adjusting for CRP and AGP. There was a significant trend across cortisol quartiles at 36 gw for higher risk of small head circumference (P for trend=0.044) in unadjusted analysis. However, due to the small number of cases in each quartile, adjusted analyses could not be completed. Finally, we also examined the association between PSS dichotomized into high vs. low using a median value cutoff (Table 4.8-6**)**. High PSS at 36 gw was associated with a 71% increase in the risk of newborn stunting in an adjusted model (relative risk: 1.71; 95% CI: 1.22 to 2.41).

We conclude that maternal stress, as measured by either salivary cortisol concentrations or self-reported perceived stress, was associated with poorer birth outcomes in this cohort. Generally, the salivary cortisol measures showed stronger and more-consistent associations with shorter duration of gestation and smaller size at birth compared to the perceived stress scores. Nevertheless, perceived stress in late pregnancy was significantly associated with a smaller newborn LAZ and an elevated risk of newborn stunting, which suggests that this measure may be a useful non-invasive indicator to predict fetal linear growth restriction.

**Table 4.8-1. Standardized Regression Coefficients for Associations between Log Salivary Cortisol Concentration at Enrollment, at 28 gw, and at 36 gw and Birth Outcomes**

| Outcome | Enrollment standardized coefficient (SE)[c] | P-value[d] | 28 gw standardized coefficient (SE)[c] | P-value[d] | 36 gw standardized coefficient (SE)[c] | P-value[d] |
|---|---|---|---|---|---|---|
| **Duration of pregnancy, week** | | | | | | |
| Unadjusted | −0.05 (0.03) | 0.086 | −0.02 (0.02) | 0.528 | −0.05 (0.02) | 0.002 |
| Adjusted model 1[a] | −0.05 (0.02) | 0.015 | −0.02 (0.02) | 0.305 | −0.04 (0.02) | 0.032 |
| Adjusted model 2[b] | −0.05 (0.02) | 0.039 | −0.02 (0.02) | 0.343 | −0.04 (0.02) | 0.037 |
| **Birth weight, g** | | | | | | |
| Unadjusted | −0.08 (0.03) | 0.007 | −0.05(0.04) | 0.189 | −0.10 (0.03) | 0.001 |
| Adjusted model 1[a] | −0.08 (0.03) | 0.013 | −0.09 (0.04) | 0.013 | −0.11 (0.04) | 0.002 |
| Adjusted model 2[b] | −0.06 (0.03) | 0.066 | −0.08 (0.04) | 0.035 | −0.11 (0.04) | 0.003 |
| **Newborn WAZ** | | | | | | |
| Unadjusted | −0.04 (0.03) | 0.143 | −0.03 (0.03) | 0.328 | −0.05 (0.03) | 0.097 |
| Adjusted model 1[a] | −0.04 (0.03) | 0.265 | −0.06 (0.04) | 0.129 | −0.05 (0.04) | 0.174 |
| Adjusted model 2[b] | −0.01 (0.03) | 0.412 | −0.04 (0.04) | 0.319 | −0.04 (0.04) | 0.284 |
| **Newborn LAZ** | | | | | | |
| Unadjusted | −0.04 (0.03) | 0.163 | −0.04 (0.03) | 0.204 | −0.05 (0.03) | 0.100 |
| Adjusted model 1[a] | −0.06 (0.03) | 0.088 | −0.06 (0.04) | 0.111 | −0.02 (0.04) | 0.545 |
| Adjusted model 2[b] | −0.04 (0.03) | 0.255 | −0.04 (0.04) | 0.580 | −0.01 (0.04) | 0.746 |
| **Newborn HCZ** | | | | | | |
| Unadjusted | −0.05 (0.03) | 0.095 | −0.05 (0.04) | 0.130 | −0.07 (0.03) | 0.017 |
| Adjusted model 1[b] | −0.06 (0.03) | 0.089 | −0.07 (0.04) | 0.072 | −0.07 (0.04) | 0.074 |
| Adjusted model 2[c] | −0.04 (0.03) | 0.273 | −0.06 (0.04) | 0.136 | −0.06 (0.04) | 0.089 |

[a] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis and also controlled for time between waking and saliva collection and for time between last food or drink (except for water) and saliva collection. Model 1 was adjusted for primiparity, maternal age at enrollment, child sex, maternal BMI at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, gestational age at enrollment, time between waking and saliva collection, and time between last food or drink (except for water) and saliva collection. Analyses at 28 gw and 36 gw also adjusted for intervention group.

[b] Model 2 was adjusted for everything in model 1, plus maternal plasma AGP concentration at enrollment and maternal plasma CRP concentration at enrollment for cortisol concentration at enrollment and at 28 gw, and maternal plasma AGP concentration at 36 gw and maternal plasma CRP concentration at 36 gw for cortisol concentration at 36 gw.

[c] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.

[d] P-values were determined by linear regression models.

### Table 4.8-2. Standardized Regression Coefficients for Associations between Perceived Stress at Enrollment, at 28 gw, and at 36 gw and Continuous Birth Outcomes

| Outcome | Enrollment standardized coefficient[b] | P-value[c] | 28 gw standardized coefficient[b] | P-value[c] | 36 gw standardized coefficient[b] | P-value[c] |
|---|---|---|---|---|---|---|
| **Duration of pregnancy, week** | | | | | | |
| Unadjusted | 0.005 (0.03) | 0.852 | 0.003 (0.03) | 0.890 | −0.03 (0.02) | 0.081 |
| Adjusted[a] | 0.001 (0.03) | 0.973 | 0.04 (0.02) | 0.087 | −0.03 (0.02) | 0.066 |
| **Birth weight, g** | | | | | | |
| Unadjusted | 0.08 (0.03) | 0.012 | 0.02(0.03) | 0.643 | 0.03 (0.03) | 0.346 |
| Adjusted[a] | 0.04 (0.03) | 0.188 | 0.03 (0.04) | 0.332 | −0.02 (0.03) | 0.536 |
| **Newborn WAZ** | | | | | | |
| Unadjusted | 0.08 (0.03) | 0.010 | 0.02 (0.03) | 0.519 | −0.006 (0.03) | 0.843 |
| Adjusted[a] | 0.04 (0.03) | 0.172 | 0.06 (0.03) | 0.072 | −0.04 (0.03) | 0.156 |
| **Newborn LAZ** | | | | | | |
| Unadjusted | 0.08 (0.03) | 0.017 | 0.03 (0.03) | 0.457 | −0.02 (0.03) | 0.363 |
| Adjusted[a] | 0.03 (0.03) | 0.348 | 0.03 (0.03) | 0.467 | −0.08 (0.03) | 0.001 |
| **Newborn HCZ** | | | | | | |
| Unadjusted | 0.05 (0.03) | 0.091 | −0.002 (0.03) | 0.778 | 0.02 (0.03) | 0.545 |
| Adjusted[a] | 0.03 (0.03) | 0.412 | 0.02 (0.03) | 0.486 | −0.02 (0.03) | 0.460 |

[a] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. Models were adjusted for primiparity, maternal age at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, and gestational age at enrollment. Analyses at 28 wk and 36 gw were also adjusted for intervention group.

[b] Standardized coefficients with standardized SEs. Standardized coefficients are the number of SDs the outcome variable changes per SD change in the predictor variable.

[c] P-values were determined by linear regression models.

### Table 4.8-3. Adverse Birth Outcomes in Participants by Cortisol Concentration at Enrollment Quartile

| Outcome | Q1<br>Relative risk (referent group) | Q2<br>Relative risk (95% CI)[c] | Q3<br>Relative risk (95% CI)[c] | Q4<br>Relative risk (95% CI)[c] | P-trend[c] |
|---|---|---|---|---|---|
| **Preterm birth** | | | | | |
| Unadjusted risk ratio | 1 | 1.26 (0.79 to 2.02) | 1.34 (0.84 to 2.14) | 1.30 (0.80 to 2.12) | 0.257 |
| Adjusted risk ratio, model 1[a] | 1 | 1.31 (0.75 to 2.31) | 1.22 (0.68 to 2.21) | 1.29 (0.76 to 2.20) | 0.384 |
| Adjusted risk ratio, model 2[b] | 1 | 1.33 (0.75 to 2.35) | 1.24 (0.68 to 2.24) | 1.25 (0.74 to 2.14) | 0.546 |
| **LBW** | | | | | |
| Unadjusted risk ratio | 1 | 1.10 (0.72 to 1.70) | 1.21 (0.79 to 1.86) | 1.39 (0.91 to 2.12) | 0.111 |
| Adjusted risk ratio, model 1[a] | 1 | 1.00 (0.64 to 1.55) | 1.01 (0.63 to 1.61) | 1.31 (0.85 to 2.02) | 0.170 |
| Adjusted risk ratio, model 2[b] | 1 | 0.95 (0.60 to 1.49) | 0.92 (0.56 to 1.50) | 1.12 (0.71 to 1.76) | 0.670 |
| **Newborn stunting** | | | | | |
| Unadjusted risk ratio | 1 | 0.98 (0.67 to 1.44) | 1.00 (0.68 to 1.48) | 1.39 (0.96 to 2.01) | 0.098 |
| Adjusted risk ratio, model 1[a] | 1 | 1.17 (0.80 to 1.72) | 1.02 (0.68 to 1.52) | 1.56 (1.08 to 2.25) | 0.072 |
| Adjusted risk ratio, model 2[b] | 1 | 1.15 (0.78 to 1.70) | 0.99 (0.66 to 1.50) | 1.42 (0.96 to 2.09) | 0.170 |
| **Newborn small head circumference** | | | | | |
| Unadjusted risk ratio | 1 | 1.58 (0.66 to 3.81) | 2.19 (0.95 to 5.03) | 1.91 (0.79 to 4.60) | 0.096 |
| Adjusted risk ratio, model 1[a] | 1 | 1.32 (0.58 to 3.02) | 1.96 (0.75 to 5.09) | 1.49 (0.64 to 3.46) | 0.144 |
| Adjusted risk ratio, model 2[b] | 1 | 1.38 (0.52 to 3.68) | 1.92 (0.67 to 5.46) | 1.37 (0.47 to 3.98) | 0.316 |

[a] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis and also controlled for time between waking and saliva collection and for time between last food or drink (except for water) and saliva collection. Model 1 was adjusted for primiparity, maternal age at enrollment, child sex, maternal BMI at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, gestational age at enrollment, time between waking and saliva collection, and time between last food or drink (except for water) and saliva collection.

[b] Model 2 was adjusted for everything in model 1, plus maternal plasma AGP concentration at enrollment and maternal plasma CRP concentration at enrollment.

[c] Relative risks and 95% confidence intervals were obtained from Poisson regression models. P-trend was obtained from the Cochran-Armitage test.

### Table 4.8-4. Adverse Birth Outcomes by Cortisol at 28 gw Quartile

| Outcome | Q1<br>Relative risk (referent group) | Q2<br>Relative risk (95% CI)[c] | Q3<br>Relative risk (95% CI)[c] | Q4<br>Relative risk (95% CI)[c] | P-trend[c] |
|---|---|---|---|---|---|
| **Preterm birth** | | | | | |
| Unadjusted risk ratio | 1 | 1.03 (0.56 to 1.90) | 0.81 (0.43 to 1.54) | 1.16 (0.66 to 2.05) | 0.753 |
| Adjusted risk ratio, model 1[a] | 1 | 0.92 (0.45 to 1.87) | 0.50 (0.26 to 0.99) | 1.00 (0.49 to 2.04) | 0.833 |
| Adjusted risk ratio, model 2[b] | 1 | 0.98 (0.47 to 2.05) | 0.50 (0.25 to 1.00) | 0.95 (0.46 to 1.95) | 0.702 |
| **LBW** | | | | | |
| Unadjusted risk ratio | 1 | 1.05 (0.54 to 2.07) | 1.88 (1.06 to 3.35) | 2.10 (1.20 to 3.67) | 0.002 |
| Adjusted risk ratio, model 1[a] | 1 | 1.13 (0.55 to 2.55) | 2.01 (1.08 to 3.74) | 2.56 (1.31 to 5.00) | 0.001 |
| Adjusted risk ratio, model 2[b] | 1 | 0.87 (0.34 to 2.34) | 1.82 (0.98 to 3.41) | 2.19 (1.09 to 4.42) | 0.004 |
| **Newborn stunting** | | | | | |
| Unadjusted risk ratio | 1 | 1.25 (0.75 to 2.07) | 1.39 (0.86 to 2.25) | 1.47 (0.92 to 2.36) | 0.094 |
| Adjusted risk ratio, model 1[a] | 1 | 1.24 (0.73 to 2.10) | 1.49 (0.89 to 2.51) | 1.16 (0.72 to 1.87) | 0.309 |
| Adjusted risk ratio, model 2[b] | 1 | 1.10 (0.63 to 1.92) | 1.40 (0.82 to 2.40) | 0.97 (0.60 to 1.58) | 0.624 |
| **Newborn small head circumference** | | | | | |
| Unadjusted risk ratio | 1 | 1.43 (0.39 to 5.25) | 2.64 (0.84 to 8.28) | 2.25 (0.70 to 7.19) | 0.100 |
| Adjusted risk ratio, model 1[a] | 1 | n/a | 2.79 (0.82 to 9.45) | n/a | n/a |
| Adjusted risk ratio, model 2[b] | 1 | n/a | 1.93 (0.50 to 7.49) | n/a | n/a |

[a] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis and also controlled for time between waking and saliva collection, time between last food or drink (except for water) and saliva collection, and intervention group. Model 1 was adjusted for primiparity, maternal age at enrollment, child sex, maternal BMI at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, gestational age at enrollment, time between waking and saliva collection, time between last food or drink (except or water) and saliva collection, and intervention group.

[b] Model 2 was adjusted for everything in model 1, plus maternal plasma AGP concentration at enrollment and maternal plasma CRP concentration at enrollment.

[c] Relative risks and 95% confidence intervals were obtained from Poisson regression models. P-trend was obtained from Cochrane-Armitage test.

### Table 4.8-5. Adverse Birth Outcomes by Cortisol at 36 gw Quartile

| Outcome | Q1<br>Relative risk (referent group) | Q2<br>Relative risk (95% CI)[c] | Q3<br>Relative risk (95% CI)[c] | Q4<br>Relative risk (95% CI)[c] | P-trend[c] |
|---|---|---|---|---|---|
| **Preterm birth** | | | | | |
| Unadjusted risk ratio | 1 | 4.75 (1.03 to 21.77) | 3.90 (0.80 to 19.15) | 4.64 (0.99 to 21.65) | 0.087 |
| Adjusted risk ratio, model 1[a] | 1 | n/a | 5.62 (0.61 to 51.84) | 6.00 (1.18 to 30.56) | 0.053 |
| Adjusted risk ratio, model 2[b] | 1 | n/a | 3.70 (0.76 to 17.96) | 5.09 (0.92 to 28.07) | 0.096 |
| **LBW** | | | | | |
| Unadjusted risk ratio | 1 | 1.66 (0.89 to 3.11) | 3.03 (1.71 to 5.39) | 2.13 (1.16 to 3.90) | 0.002 |
| Adjusted risk ratio, model 1[a] | 1 | 2.54 (1.15 to 5.59) | 3.15 (1.46 to 6.78) | 2.14 (1.01 to 4.56) | 0.027 |
| Adjusted risk ratio, model 2[b] | 1 | 2.31 (0.94 to 5.68) | 3.11 (1.41 to 6.88) | 1.90 (0.77 to 4.73) | 0.059 |
| **Newborn stunting** | | | | | |
| Unadjusted risk ratio | 1 | 1.38 (0.88 to 2.16) | 1.34 (0.83 to 2.16) | 1.39 (0.88 to 2.22) | 0.194 |
| Adjusted risk ratio, model 1[a] | 1 | 1.69 (1.03 to 2.77) | 1.47 (0.86 to 2.53) | 1.29 (0.74 to 2.23) | 0.281 |
| Adjusted risk ratio, model 2[b] | 1 | 1.43 (0.85 to 2.41) | 1.33 (0.77 to 2.32) | 1.12 (0.62 to 2.02) | 0.553 |
| **Newborn small head circumference** | | | | | |
| Unadjusted risk ratio | 1 | 1.79 (0.43 to 7.40) | 4.35 (1.21 to 15.63) | 2.90 (0.76 to 11.06) | 0.044 |
| Adjusted risk ratio, model 1[a] | 1 | n/a | n/a | n/a | n/a |
| Adjusted risk ratio, model 2[b] | 1 | n/a | n/a | n/a | n/a |

[a] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis and also controlled for time between waking and saliva collection, time between last food or drink (except for water) and saliva collection, and intervention group. Model 1 was adjusted for primiparity, maternal age at enrollment, child sex, maternal BMI at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, gestational age at enrollment, time between waking and saliva collection, time between last food or drink (except for water) and saliva collection, and intervention group.
[b] Model 2 was adjusted for everything in model 1, plus maternal plasma AGP concentration at 36 gw and maternal plasma CRP concentration at 36 gw.
[c] Relative risks and 95% confidence intervals were obtained from Poisson regression models. P-trend was obtained from the Cochran-Armitage test.

### Table 4.8-6. Adverse Birth Outcomes by High and Low Perceived Stress (median cutoff)

| | Enrollment | | | 28 gw | | | 36 gw | | |
|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **Low PPS** | **High PSS** | **P-value[b]** | **Low PPS** | **High PSS** | **P-value[b]** | **Low PPS** | **High PSS** | **P-value[b]** |
| **Preterm birth** | | | | | | | | | |
| Unadjusted relative risk | 1 | 1.11 (0.79 to 1.56) | 0.557 | 1 | 0.95 (0.63 to 1.43) | 0.808 | 1 | 0.77 (0.36 to 1.75) | 0.502 |
| Adjusted relative risk[a] | 1 | 1.06 (0.72 to 1.58) | 0.763 | 1 | 0.83 (0.54 to 1.28) | 0.401 | 1 | 0.78 (0.36 to 1.71) | 0.538 |
| **LBW** | | | | | | | | | |
| Unadjusted relative risk | 1 | 0.73 (0.54 to 1.00) | 0.050 | 1 | 0.89 (0.62 to 1.29) | 0.544 | 1 | 1.01 (0.70 to 1.45) | 0.967 |
| Adjusted relative risk[a] | 1 | 0.78 (0.57 to 1.06) | 0.113 | 1 | 0.87 (0.60 to 1.28) | 0.484 | 1 | 1.15 (0.80 to 1.67) | 0.448 |
| **Newborn stunting** | | | | | | | | | |
| Unadjusted relative risk | 1 | 0.70 (0.53 to 0.93) | 0.013 | 1 | 1.01 (0.73 to 1.38) | 0.964 | 1 | 1.47 (1.05 to 2.05) | 0.027 |
| Adjusted relative risk[a] | 1 | 0.77 (0.58 to 1.02) | 0.069 | 1 | 0.98 (0.71 to 1.36) | 0.924 | 1 | 1.71 (1.22 to 2.41) | 0.002 |
| **Newborn small head circumference** | | | | | | | | | |
| Unadjusted relative risk | 1 | 1.02 (0.59 to 1.76) | 0.949 | 1 | 0.74 (0.38 to 1.46) | 0.384 | 1 | 1.20 (0.55 to 2.61) | 0.650 |
| Adjusted relative risk[a] | 1 | 1.09 (0.62 to 1.92) | 0.778 | 1 | 0.65 (0.32 to 1.29) | 0.217 | 1 | 1.35 (0.60 to 3.03) | 0.469 |

[a] Models were adjusted for covariates that had a significant association (P<0.10) with any birth outcome in bivariate analysis. All models were adjusted for primiparity, maternal age at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, and gestational age at enrollment. Analyses at 28 gw and 36 gw were also adjusted for intervention group.

[b] P-values were obtained from Poisson regression models.

## 4.9 Histological Signs of Inflammation and Malaria in the Placenta and Fetal Membranes

This substudy was nested in the main trial to assess the histological evidence of chorioamnionitis, intervillositis, and malaria in the placenta and fetal membranes and their associations with birth outcomes. Chorioamnionitis was defined as the presence of inflammation (specifically, the presence of neutrophils) in either the chorion or the amnion (fetal membranes) tissues that surround the fetus. It is an indicator of infection in those tissues, and past studies have identified higher incidences of chorioamnionitis in preterm births. Chorioamnionitis can differ greatly in scale, so we also defined severe chorioamnionitis (specifically, >25 inflammatory cells per 10 high power fields). Intervillositis was defined as the presence of inflammation (specifically, the presence of lymphocytes, monocytes, and neutrophils) in the placental intervillous space. Chronic inflammation of this area has been linked to a higher risk of IUGR. Using the same placental tissue slides, we were also able to stain for malarial parasites to show active infection and record where there was evidence of past malarial infection.

Of the 1,391 participants enrolled in the iLiNS-DYAD-M trial, 12 women with twin pregnancies were excluded from this analysis. A sample of placenta was collected at delivery from 1,030 (74.1%) participants, and a sample from the chorionic and amniotic membrane (fetal membranes) was collected from 1,095 (78.7%) participants for DNA analyses. There were 990 (71.2%) participants who had a viable section of chorionic plate or amniotic membrane to allow for the examination of chorioamnionitis, and 1,008 (72.5%) participants had a section of placental tissue taken with intervillous space identifiable to allow for examination of intervillositis and malarial infection. Data on duration of pregnancy were available for 99.7% of participants, on birth weight for 90.8%, and on neonatal size for 88.2%.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of maternal educational achievement, proportion with anemia or low maternal BMI at enrollment, and proportion with a positive HIV or malaria test ($P \geq 0.05$). However, compared to the excluded women, they tended to have a lower BMI (22.0 vs. 22.5, P=0.006), be older (25 vs. 24, P=0.030), and have a lower proxy for SES (−0.07 vs. 0.25, P<0.001), and were less likely to be primiparous (20.2% vs. 26.7%, P=0.012) (Table A1.2-8).

Table 4.9-1 and Table 4.9-3 show significant associations between both intervillositis and severe chorioamnionitis and duration of pregnancy, but not birth weight, LAZ, or HCZ. Table 4.9-2 shows a significant association in the unadjusted model between chorioamnionitis (moderate or severe) and duration of pregnancy, but this is only marginally significant in the adjusted models (P=0.055).

Table 4.9-4 and Table 4.9-5 show that there was no association between duration of pregnancy, birth weight, LAZ, and HCZ and histological evidence of active or past malarial infection in the placenta. The unadjusted models in Table 4.9-5 show a significant association between past malarial infection in the placenta and birth weight and LAZ, but this was not seen in the adjusted models.

Participants with intervillositis had a significantly higher relative risk for preterm birth (P=0.019) (Table 4.9-6). The same association was also seen in participants with chorioamnionitis (P=0.014) (Table 4.9-7), but there was no association with LBW, stunting, or small head circumference. Severe chorioamnionitis was associated with a higher relative risk for newborn small head circumference (P=0.025) (Table 4.9-8).

Similar to the continuous birth outcome results, histological evidence of active placenta malarial infection was not significantly associated with preterm birth, LBW, newborn stunting, or newborn small head

circumference (Table 4.9-9). However, participants with evidence of past infection had a significantly higher relative risk for newborn stunting (P=0.003) (Table 4.9-10).

Overall, in this study population, placental intervillositis and severe chorioamnionitis were significantly associated with a shorter duration of pregnancy. Intervillositis and chorioamnionitis were also associated with an increased incidence of preterm birth, while severe chorioamnionitis was significantly associated with an increased prevalence of newborn small head circumference. An active malarial infection in the placenta at delivery was not associated with any adverse birth outcome, but evidence of a past infection was associated with an increased prevalence of newborn stunting.

### Table 4.9-1. Continuous Birth Outcomes in Participants with and without Intervillositis

| Outcome | Participants without intervillositis, n=832 | Participants with intervillositis, n=175 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.5 (2.6) | 39.0 (2.0) | −0.5 (−0.8 to −0.1) | 0.008 | −0.4 (−0.7 to −0.0) | 0.031 |
| Mean (SD) birth weight, g | 2,986 (431) | 2,938 (455) | −48 (−122 to 26) | 0.204 | −36 (−110 to 37) | 0.331 |
| Mean (SD) newborn LAZ | −0.98 (1.1) | −1.09 (1.2) | −0.11 (−0.31 to 0.09) | 0.294 | −0.10 (−0.30 to 0.09) | 0.313 |
| Mean (SD) newborn HCZ | −0.12 (1.0) | −0.21 (1.2) | −0.09 (−0.28 to 0.11) | 0.383 | −0.03 (−0.22 to 0.17) | 0.789 |

[a] P-values were calculated using t-test.

[b] Linear regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-2. Continuous Birth Outcomes in Participants with and without Chorioamnionitis

| Outcome | Participants without chorioamnionitis, n=731 | Participants with chorioamnionitis, n=258 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.6 (1.8) | 39.3 (2.4) | −0.3 (−0.6 to −0.0) | 0.047 | −0.3 (−0.6 to 0.0) | 0.055 |
| Mean (SD) birth weight, g | 2,988 (422) | 2,955 (448) | −33 (−96 to 29) | 0.296 | −27 (−91 to 37) | 0.408 |
| Mean (SD) newborn LAZ | −0.99 (1.1) | −1.01 (1.1) | −0.02 (−0.15 to 0.18) | 0.821 | −0.03 (−0.20 to 0.15) | 0.768 |
| Mean (SD) newborn HCZ | −0.13 (11) | −0.16 (1.1) | −0.03 (−0.19 to 0.13) | 0.701 | −0.03 (−0.20 to 0.14) | 0.727 |

[a] P-values were calculated using t-test.

[b] Linear regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-3. Continuous Birth Outcomes in Participants with and without Severe Chorioamnionitis

| Outcome | Participants without severe chorioamnionitis, n=869 | Participants with severe chorioamnionitis, n=120 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.6 (1.9) | 39.0 (2.8) | −0.5 (−0.9 to −0.2) | 0.006 | −0.5 (−0.9 to −0.1) | 0.014 |
| Mean (SD) birth weight, g | 2,987 (424) | 2,927 (460) | −59 (−145 to −27) | 0.176 | −65 (−151 to 22) | 0.141 |
| Mean (SD) newborn LAZ | −0.98 (1.1) | −1.1 (1.2) | −0.15 (−0.38 to 0.08) | 0.210 | −0.19 (−0.42 to 0.05) | 0.114 |
| Mean (SD) newborn HCZ | −0.28 (1.1) | −0.12 (1.1) | 0.16 (−0.05 to0.39) | 0.143 | 0.17 (−0.06 to0.39) | 0.151 |

[a] P-values were calculated using t-test.

[b] Linear regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-4. Continuous Birth Outcomes in Participants with and without an Active Malarial Infection in the Placenta

| Outcome | Participants without active malarial infection, n=43 | Participants with active malarial infection, n=964 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.5 (2.1) | 38.9 (2.4) | −0.5 (−1.1 to 0.2) | 0.168 | −0.1 (−0.77 to 0.56) | 0.766 |
| Mean (SD) birth weight, g | 2,978 (435) | 2,979 (431) | 1 (−144 to 146) | 0.991 | −49 (−97 to 197) | 0.507 |
| Mean (SD) newborn LAZ | −0.98 (1.1) | −1.2 (0.9) | −0.25 (−0.63 to 0.13) | 0.195 | −0.20 (−0.60 to 0.19) | 0.310 |
| Mean (SD) newborn HCZ | −0.12 (1.1) | −0.40 (0.9) | −0.28 (−0.64 to 0.08) | 0.132 | −0.22 (−0.61 to 0.16) | 0.260 |

[a] P-values were calculated using t-test.

[b] Linear regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-5. Continuous Birth Outcomes in Participants with and without Past Malarial Infection in the Placenta

| Outcome | Participants without past malarial infection, n=627 | Participants with past malarial infection, n=380 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.5 (2.3) | 39.4 (1.9) | -0.1 (−0.1 to 0.4) | 0.332 | 0.0 (−0.3 to 0.3) | 0.888 |
| Mean (SD) birth weight, g | 3,017 (426) | 2,916 (442) | −101 (−157 to −43) | <0.001 | −46 (−107 to 15) | 0.137 |
| Mean (SD) newborn LAZ | −0.90 (1.1) | −1.16 (1.1) | −0.26 (−0.41 to −0.10) | <0.001 | −0.16 (−0.32 to 0.00) | 0.056 |
| Mean (SD) newborn HCZ | −0.08 (1.1) | −0.22 (1.1) | −0.14 (−0.28 to 0.01) | 0.067 | −0.09 (−0.25 to 0.07) | 0.253 |

[a] P-values were calculated using t-test.

[b] Linear regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-6. Adverse Birth Outcomes in Participants with and without Intervillositis

| Outcome | Participants without intervillositis | Participants with intervillositis | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 58/832 (6.9%) | 22/175 (12.6%) | 1.8 (1.1 to 2.8) | 0.013 | 1.8 (1.1 to 2.9) | 0.019 |
| Incidence of LBW (<2,500 g) | 87/776 (11.2%) | 23/160 (14.4%) | 1.3 (0.8 to −1.9) | 0.254 | 1.3 (0.9 to 2.1) | 0.190 |
| Prevalence of newborn stunting (LAZ <−2) | 112/735 (15.2%) | 26/143 (18.2%) | 1.2 (0.8 to 1.8) | 0.371 | 1.2 (0.8 to1.8) | 0.418 |
| Prevalence of newborn small head circumference (HCZ <−2) | 23/735 (3.1%) | 9/145 (6.2%) | 1.9 (0.9 to 4.2) | 0.073 | 1.9 (0.9 to 4.4) | 0.092 |

[a] P-values were calculated using Fisher's exact test.

[b] Log-binomial regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-7. Adverse Birth Outcomes in Participants with and without Chorioamnionitis

| Outcome | Participants without chorioamnionitis | Participants with chorioamnionitis | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 48/731 (6.6%) | 26/258 (10.1%) | 1.5 (0.9 to 2.4) | 0.065 | 1.8 (1.1 to 2.9) | 0.014 |
| Incidence of LBW (<2,500 g) | 75/682 (11.0%) | 33/239 (13.8%) | 1.3 (0.9 to 1.8) | 0.243 | 1.2 (0.8 to 1.7) | 0.452 |
| Prevalence of newborn stunting (LAZ <−2) | 97/646 (15.0%) | 38/221 (17.2%) | 1.1 (0.8 to 1.6) | 0.438 | 1.2 (0.8 to 1.7) | 0.410 |
| Prevalence of newborn small head circumference (HCZ <−2) | 20/646 (3.1%) | 12/222 (5.4%) | 1.7 (0.9 to 3.5) | 0.118 | 1.9 (0.9 to 3.9) | 0.109 |

[a] P-values were calculated using Fisher's exact test.

[b] Log-binomial regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-8. Adverse Birth Outcomes in Participants with and without Severe Chorioamnionitis

| Outcome | Participants without severe chorioamnionitis | Participants with severe chorioamnionitis | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 62/869 (7.1%) | 12/120 (10.0%) | 1.4 (0.8 to 2.5) | 0.260 | 1.5 (0.8 to 2.8) | 0.249 |
| Incidence of LBW (<2,500 g) | 90/812 (11.1%) | 18/109 (16.5%) | 1.5 (0.9 to 2.4) | 0.093 | 1.4 (0.9 to 2.4) | 0.154 |
| Prevalence of newborn stunting (LAZ <−2) | 115/765 (15.0%) | 20/102 (19.6%) | 1.3 (0.9 to 2.0) | 0.223 | 1.3 (0.8 to 2.0) | 0.336 |
| Prevalence of newborn small head circumference (HCZ <−2) | 24/765 (3.1%) | 8/103 (7.8%) | 2.5 (1.1 to 5.4) | 0.022 | 2.6 (1.1 to 6.1) | 0.025 |

[a] P-values were calculated using Fisher's exact test.

[b] Log-binomial regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-9. Adverse Birth Outcomes in Participants with and without Active Placenta Malarial Infection

| Outcome | Participants without active malarial infection | Participants with active malarial infection | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 72/964 (7.5%) | 8/43 (18.6%) | 2.5 (1.3 to 4.8) | 0.007 | 1.7 (0.8 to 3.6) | 0.180 |
| Incidence of LBW (<2,500 g) | 105/900 (11.7%) | 5/36 (13.9%) | 1.2 (0.5 to 2.7) | 0.682 | 1.1 (0.4 to 2.8) | 0.831 |
| Prevalence of newborn stunting (LAZ <−2) | 130/844 (15.4%) | 8/34 (25.5%) | 1.5 (0.8 to 2.9) | 0.185 | 1.3 (0.7 to 2.4) | 0.396 |
| Prevalence of newborn small head circumference ( HCZ <−2) | 30/845 (3.6%) | 2/35 (5.7%) | 1.6 (0.4 to 6.5) | 0.502 | 1.8 (0.4 to 7.5) | 0.400 |

[a] P-values were calculated using Fisher's exact test.

[b] Log-binomial regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes based as reported in previous literature. All models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

### Table 4.9-10. Adverse Birth Outcomes in Participants with and without Past Placenta Malarial Infection

| Outcome | Participants without past malarial infection | Participants with past malarial infection | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI) | Adjusted P-value[b] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 47/627 (7.5%) | 33/380 (8.7%) | 1.2 (08 to 1.8) | 0.499 | 0.7 (0.4 to 1.1) | 0.143 |
| Incidence of LBW (<2,500 g) | 57/577 (9.9%) | 53/359 (14.8%) | 1.5 (1.1 to 2.1) | 0.024 | 1.2 (0.8 to 1.9) | 0.298 |
| Prevalence of newborn stunting (LAZ <−2) | 63/546 (11.5%) | 75/332 (22.6%) | 1.9 (1.4 to 2.7) | <0.001 | 1.7 (1.2 to 2.5) | 0.003 |
| Prevalence of newborn small head circumference (HCZ <−2) | 19/545 (3.5%) | 13/335 (3.9%) | 1.1 (0.6 to 2.2) | 0.762 | 0.9 (0.4 to 2.0) | 0.767 |

[a] P-values were calculated using Fisher's exact test.

[b] Log-binomial regression models were adjusted for covariates chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature. All models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.

## 4.10 Placenta and Fetal Membranes and Oral and Vaginal Microbiomes

This substudy was nested in the main trial to assess the role of bacteria found in the placenta and fetal membranes, as well as the microbial communities found in the oral cavity and vagina, and their association with birth outcomes. We defined prevalence of bacteria in the placenta and fetal membranes as detectable presence of 16S rDNA in a sample of placenta or fetal membrane tissue from a participant. This gene is found only in bacteria and so can be used to screen for presence of bacteria. We also analyzed the amount of 16S rDNA present in the tissue as representative of the amount of bacteria found in that tissue, otherwise termed bacterial load.

To represent the diversity of the entire community of bacteria, we calculated the median intra-individual unweighted UniFrac distance for each participant. A higher UniFrac distance means a larger number of different bacteria were found in that participant compared to others. The lower the UniFrac distance, the more similar that participant's microbiota were to other participants. We made pair-wise comparisons of UniFrac distances between each individual in the sample and every other individual in the sample and then took the median value of all pair-wise comparisons related to that individual to represent the UniFrac value for that individual. Therefore, a statistically significant difference between a group's mean unweighted UniFrac value should represent a variation in the species of bacteria found in those communities.

Of the 1,391 participants enrolled in the iLiNS-DYAD-M trial, 12 women with twin pregnancies were excluded from this analysis. A sample of placenta was collected at delivery from 1,018 (73.2%) participants and a sample from the fetal membranes was collected from 1,083 (77.9%) participants for DNA analyses. An oral swab and a vaginal swab were also collected from each participant 1 week after delivery. After excluding any samples that did not produce enough reads after sequencing, 1,104 (79.4%) oral swabs and 1,107 (79.6%) vaginal swabs were included in the analysis. Data on duration of pregnancy were available for 99.7% of participants, on birth weight for 90.8%, and on neonatal size for 88.2%.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of mean maternal BMI at enrollment, proportion with anemia or low maternal BMI at enrollment, and proportion with a positive HIV or malaria test ($P \geq 0.05$). However, compared to the excluded women, they tended to be older (25 vs. 24, P=0.025), have completed fewer years in school (3.9 vs. 4.5, P=0.049), and have a lower proxy for SES (−0.05 vs. 0.30, $P<0.001$) and were less likely to be primiparous (20.7% vs. 29.6%, P=0.001) (Table A1.2-9).

Table 4.10-1 and Table 4.10-3 show associations between bacterial load and diversity in the placenta and fetal membranes, and duration of pregnancy and birth weight, and between bacterial load and diversity and LAZ and HCZ, respectively. There were significant associations between duration of pregnancy and bacterial load and diversity in the unadjusted model. After adjusting for possible covariates, there were no significant associations with duration of pregnancy (Table 4.10-1). An increase in bacterial diversity away from the core microbial community in the placenta, represented by unweighted UniFrac distance, was significantly associated with a lower LAZ in both adjusted and unadjusted analysis (P=0.020) (Table 4.10-3).

Table 4.10-2 and Table 4.10-4 show that bacterial diversity in the oral cavity, represented by an increase in intra-individual unweighted UniFrac distance, was associated with lower birth weight (P=0.014) and smaller newborn head circumference (P=0.027). In contrast, a greater diversity of types of bacteria in the vagina was significantly associated with a higher newborn head circumference (P=0.019) (Table 4.10-4).

Table 4.10-5 shows that, after adjustment for possible covariates, there was a mean increase of 0.4 (0.1, 0.6) weeks in duration of pregnancy if the mother was positive for bacteria presence in the placenta (P=0.009). Detection of bacteria on the fetal membranes was not significantly associated with duration of pregnancy, birth weight, LAZ, or HCZ (Table 4.10-6). When the same predictor values were examined in relation to dichotomous birth outcomes, we found that presence of bacteria in the placenta was not associated with preterm birth, LBW, newborn stunting, or newborn small head circumference (Table 4.10-7). However, participants with presence of bacteria in their fetal membranes were found to have an increased risk of preterm birth (P=0.038) (Table 4.10-8).

Overall, in this study population, presence of bacteria in the placenta was associated with an increase in duration of pregnancy, but presence of bacteria in the fetal membranes was associated with an increased risk of preterm birth. The amount of bacteria found, beyond just presence or absence, did not associate with any of the birth outcomes studied.

Changes in the constituents of the microbial community found in the fetal membranes did not significantly associate with any changes in birth outcome. Shifts in the microbial community found in the placental tissue was found to be associated in a lower LAZ.

Greater bacterial diversity in the oral cavity was associated with lower birth weight and a smaller newborn head circumference. In contrast, greater bacterial diversity in the vagina was associated with a higher newborn head circumference.

**Table 4.10-1. Association between Bacterial Load and Diversity in the Placenta and Fetal Membranes and Duration of Pregnancy and Birth Weight**

| | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Duration of pregnancy | | | | Birth weight | | | |
| | Unadjusted | | Adjusted | | Unadjusted | | Adjusted | |
| Continuous predictor | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] |
| Bacterial load in the placenta ($Log_{10}$ 16S rDNA copies/µL) | −0.3 (−0.5 to −0.0) | 0.043 | −0.2 (−0.5 to 0.0) | 0.104 | −20 (−82 to 42) | 0.524 | 21 (−41 to 82) | 0.508 |
| Bacterial load in the fetal membrane ($Log_{10}$ 16S rDNA copies/µL) | 0.0 (−0.2 to 0.2) | 0.877 | −0.1 (−0.3 to 0.1) | 0.477 | −20 (−60 to 21) | 0.340 | −15 (−56 to 27) | 0.490 |
| Intra-individual unweighted UniFrac distance in the placenta | −0.2 (−0.5 to 0.2) | 0.330 | −0.2 (−0.5 to 0.1) | 0.197 | −31 (−103 to 41) | 0.400 | −32 (−106 to 43) | 0.407 |
| Intra-individual unweighted UniFrac distance in the fetal membranes | −0.4 (−0.7 to −0.1) | 0.005 | −0.2 (−0.5 to 0.0) | 0.120 | −24 (−78 to 31) | 0.390 | −8 (−64 to 48) | 0.770 |

[a] Models were adjusted for intervention group, maternal age, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, caesarean section, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature.

[b] P-values were determined by linear regression models.

**Table 4.10-2. Association between Bacterial Diversity in the Oral Cavity and Vagina and Duration of Pregnancy and Birth Weight**

| | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Duration of pregnancy | | | | Birth weight | | | |
| | Unadjusted | | Adjusted | | Unadjusted | | Adjusted | |
| Continuous predictor | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] |
| Intra-individual unweighted UniFrac distance in the oral cavity | 0.0 (−0.2 to 0.2) | 0.887 | 0.0 (−0.2 to 0.2) | 0.764 | −38 (−68 to 9) | 0.011 | −37 (−67 to 7) | 0.014 |
| Intra-individual unweighted UniFrac distance in the vagina | 0.0 (−0.2 to 0.1) | 0.836 | −0.1 (−0.5 to 0.0) | 0.120 | −24 (−78 to 31) | 0.390 | −8 (−64 to 48) | 0.770 |

[a] Models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature.
[b] P-values were determined by linear regression models.

**Table 4.10-3. Association between Bacterial Load and Diversity in the Placenta and Fetal Membranes and Newborn LAZ and HCZ**

| | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Newborn LAZ | | | | Newborn HCZ | | | |
| | Unadjusted | | Adjusted | | Unadjusted | | Adjusted | |
| Continuous predictor | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] |
| Bacterial load in the placenta ($Log_{10}$ 16S rDNA copies/µL) | −0.08 (−0.23 to 0.08) | 0.340 | −0.04 (−0.19 to 0.12) | 0.657 | −0.04 (−0.19 to 0.11) | 0.568 | −0.02 (−0.18 to 0.15) | 0.841 |
| Bacterial load in the fetal membrane ($Log_{10}$ 16S rDNA copies/µL) | −0.05 (−0.15 to 0.05) | 0.302 | −0.05 (−0.15 to 0.06) | 0.389 | −0.01 (−0.09 to 0.11) | 0.876 | −0.01 (−0.12 to 0.10) | 0.897 |
| Intra-individual unweighted UniFrac distance in the placenta | −0.22 (−0.40 to 0.03) | 0.020 | −0.22 (−0.41 to −0.03) | 0.020 | −0.11 (−0.29 to 0.07) | 0.229 | −0.11 (−0.30 to 0.07) | 0.230 |
| Intra-individual unweighted UniFrac distance in the fetal membranes | −0.08 (−0.22 to 0.06) | 0.248 | −0.01 (−0.16 to 0.13) | 0.841 | −0.03 (−0.16 to 0.11) | 0.629 | 0.03 (−0.11 to 0.18) | 0.637 |

[a] Models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, caesarean section, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature.

[b] P-values were determined by linear regression models.

### Table 4.10-4. Association between Bacterial Diversity in the Oral Cavity and the Vagina and Newborn LAZ and HCZ

| | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Newborn LAZ | | | | Newborn HCZ | | | |
| | Unadjusted | | Adjusted | | Unadjusted | | Adjusted | |
| Continuous predictor | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] | Regression coefficient (95% CI) | P-value[b] | Regression coefficient (95% CI)[a] | P-value[b] |
| Intra-individual unweighted UniFrac distance in the oral cavity | −0.03 (−0.12 to 0.04) | 0.313 | −0.04 (−0.11 to 0.04) | 0.336 | −0.07 (−0.14 to 0.01) | 0.077 | −0.08 (−0.16 to −0.01) | 0.027 |
| Intra-individual unweighted UniFrac distance in the vagina | 0.03 (−0.04 to 0.09) | 0.376 | 0.04 (−0.02 to 0.11) | 0.189 | 0.07 (0.01 to 0.13) | 0.029 | 0.08 (0.01 to 0.15) | 0.019 |

[a] Models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature.
[b] P-values were determined by linear regression models.

### Table 4.10-5. Continuous Birth Outcomes in Participants with and without Bacteria in the Placenta

| Outcome | Participants without bacteria in placenta, n=542 | Participants with bacteria in placenta, n=474 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.1 (2.4) | 39.8 (1.9) | 0.7 (0.4 to 0.9) | <0.001 | 0.4 (0.1 to 0.6) | 0.009 |
| Mean (SD) birth weight, g | 2,948 (451) | 3,001 (432) | 52 (−4 to 109) | 0.069 | 31 (−26 to 89) | 0.286 |
| Mean (SD) newborn LAZ | −1.02 (1.1) | −0.95 (1.1) | 0.07 (−0.07 to 0.21) | 0.339 | −0.02 (−0.16 to 0.13) | 0.822 |
| Mean (SD) newborn HCZ | −0.14 (1.1) | −0.08 (1.1) | 0.06 (−0.08 to 0.19) | 0.429 | 0.05 (−0.09 to 0.19) | 0.514 |

[a] P-values were calculated using t-test.

[b] Models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, caesarean section, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature.

[c] P-values were determined by linear regression models.

### Table 4.10-6. Continuous Birth Outcomes in Participants with and without Bacteria in the Fetal Membranes

| Outcome | Participants without bacteria in fetal membranes, n=345 | Participants with bacteria in fetal membranes, n=737 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.4 (1.9) | 39.4 (2.3) | 0.0 (−0.3 to 0.3) | 0.961 | 0.0 (−0.3 to 0.3) | 0.950 |
| Mean (SD) birth weight, g | 2,964 (423) | 2,980 (448) | 16 (−42 to 74) | 0.590 | 28 (−86 to 29) | 0.336 |
| Mean (SD) newborn LAZ | −1.11 (1.1) | −0.94 (1.1) | 0.17 (0.02 to 0.31) | 0.029 | 0.13 (−0.03 to 0.29) | 0.101 |
| Mean (SD) newborn HCZ | −0.07 (1.0) | −0.17 (1.1) | -0.09 (−0.24 to 0.05) | 0.202 | −0.12 (−0.28 to 0.04) | 0.113 |

[a] P-values were calculated using t-test.

[b] Models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, caesarean section, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature.

[c] P-values were determined by linear regression.

### Table 4.10-7. Adverse Birth Outcomes in Participants with and without Bacteria in the Placenta

| Outcome | Participants without bacteria in placenta | Participants with bacteria in placenta | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 54/542 (9.9%) | 30/474 (6.3%) | 0.6 (0.4 to 0.9) | 0.038 | 0.8 (0.5 to 1.3) | 0.327 |
| Incidence of LBW (<2,500 g) | 62/500 (12.4%) | 54/444 (12.2%) | 0.9 (0.7 to 1.4) | 0.912 | 1.1 (0.8 to 1.5) | 0.660 |
| Prevalence of newborn stunting (LAZ <−2) | 73/460 (15.9%) | 63/423 (14.9%) | 0.9 (0.7 to 1.3) | 0.688 | 1.1 (0.8 to 1.5) | 0.608 |
| Prevalence of newborn small head circumference (HCZ <−2) | 16/461 (3.5%) | 15/424 (3.5%) | 1.0 (0.5 to 2.0) | 0.771 | 0.9 (0.4 to 2.1) | 0.925 |

[a] P-values were calculated using Fisher's exact test.

[b] Models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, caesarean section, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature.

[c] P-values were determined by log-binomial regression.

### Table 4.10-8. Adverse Birth Outcomes in Participants with and without Bacteria in the Fetal Membranes

| Outcome | Participants without bacteria in fetal membranes | Participants with bacteria in fetal membranes | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 20/345 (5.8%) | 68/737 (9.2%) | 1.6 (0.9 to 2.6) | 0.059 | 1.8 (1.0 to 3.1) | 0.038 |
| Incidence of LBW (<2,500 g) | 38/324 (11.7%) | 83/681 (12.2%) | 1.0 (0.7 to 1.5) | 0.834 | 1.0 (0.7 to 1.5) | 0.861 |
| Prevalence of newborn stunting (LAZ <−2) | 47/303 (15.5%) | 99/644 (15.4%) | 0.9 (0.7 to 1.4) | 0.956 | 1.0 (0.7 to 1.4) | 0.896 |
| Prevalence of newborn small head circumference (HCZ <−2) | 7/304 (2.3%) | 27/647 (4.2%) | 1.8 (0.8 to 4.1) | 0.155 | 1.4 (0.6 to 3.6) | 0.425 |

[a] P-values were calculated using Fisher's exact test.

[b] Models were adjusted for intervention group, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia at enrollment, maternal malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, caesarean section, and season of enrollment. Covariates were chosen in a predefined analysis plan based on the logical potential to form an association with one of the outcomes as reported in previous literature

[c] P-values were determined by log-binomial regression

## 4.11 Maternal Oral Health

This substudy was nested in the main trial as a cross-sectional study to assess the association between maternal oral diseases, namely, dental caries, periapical infectious lesions, and periodontitis, and birth outcomes.

Of the 1,391 women who were enrolled in the study, 1,229 (88.4%) completed the oral health examination. After excluding twin pregnancies and those whose oral examination was done more than 6 weeks after delivery, 1,024 (73.6%) participants were included in the analyses. Data on the duration of pregnancy were available for all (100%) and birth weight and neonatal size data for 93% and 94% of the included participants, respectively.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of maternal education achievement, proportion with anemia or low maternal BMI at enrollment, and proportion with a positive HIV test ($P \geq 0.05$). However, compared to the excluded women, they tended to be older (25 vs. 24, $P<0.001$) and less often primiparous (17.8% vs. 33.3%, $P<0.001$) and malaria positive (21.4% vs. 28.1%, $P=0.009$) and had a lower proxy for SES (−0.13 vs. 0.46, $P<0.001$) and mean BMI (22.1 vs. 22.4, $P=0.046$) (Table A1.2-10).

The mean (SD) duration of pregnancy among the groups of healthy participants (n=370), those with dentine caries (grade II, n=354), caries with pulpal exposure but without periapical infection (grade III, n=59), or periapical infection (n=241) was 39.5 gw (2.11), 39.5 gw (2.19), 39.3 gw (2.22), and 39.1 gw (2.72), respectively, and the mean (SD) birth weight was 2,977 g (436), 3,009 g (428), 3,029 g (500), and 2,926 g (400), respectively. The mean (SD) LAZ among the same participants was −0.97 (1.03), −0.91 (1.08), −0.91 (1.44), and −1.15 (1.11), respectively. There were no significant differences between the healthy participants and participants with dental caries in any of the assessed birth outcomes even if a pulpal exposure was present; thus, we collapsed all of these participants into one group. In the subsequent analyses concerning dental infections, we then compared participants with no detectable periapical infections to those who had at least one periapical lesion.

Table 4.11-1 shows the mean duration of pregnancy and neonatal size by maternal periapical infection status using continuous outcomes. After adjusting for possible confounders, those with at least one periapical infectious lesion had on average (95% CI for the mean) 0.4 weeks (0.1 to 0.8) shorter pregnancies and infants with 79 g (13 to 145) smaller birth weight, 0.14 units lower WAZ (0.00 to 0.29), 0.27 units smaller LAZ (0.11 to 0.44), and 0.18 units smaller HCZ (0.01 to 0.35) compared to women without the infection.

The prevalence of all analyzed adverse dichotomous birth outcomes was also higher in the group of women with periapical infection than in the group without the infection, although these were statistically significant only for the prevalence of newborn stunting (adjusted relative risk=1.68, $P=0.007$) and small head circumference (adjusted relative risk=2.52, $P=0.012$) (Table 4.11-2).

There were no statistically significant differences between the groups of participants without or with periodontitis in any of the assessed birth outcomes (Table 4.11-3 and Table 4.11-4).

Periapical infections were associated with shorter pregnancy duration and smaller newborn size in this study population. No significant associations were seen between dental caries only (without periapical infection) or periodontitis and adverse pregnancy outcomes.

### Table 4.11-1. Continuous Birth Outcomes among Participants with and without Periapical Infections

| Outcome | Participants without periapical infection, n=783 | Participants with periapical infection, n=241 | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.5 (2.2) | 39.1 (2.7) | −0.4 (−0.7 to −0.1) | 0.019 | −0.4 (−0.8 to −0.1) | 0.014 |
| Mean (SD) birth weight, g | 2,995 (437) | 2,926 (400) | −69 (−133 to −4) | 0.037 | −79 (−145 to −13) | 0.019 |
| Mean (SD) newborn WAZ | −0.51 (0.99) | −0.63 (0.98) | −0.12 (−0.26 to 0.02) | 0.105 | −0.14 (−0.29 to −0.00) | 0.057 |
| Mean (SD) newborn LAZ | −0.94 (1.08) | −1.15 (1.11) | −0.21 (−0.37 to −0.05) | 0.011 | −0.27 (−0.44 to −0.11) | 0.001 |
| Mean (SD) newborn HCZ | −0.09 (1.06) | −0.25 (1.11) | −0.16 (−0.32 to 0.00) | 0.053 | −0.18 (−0.35 to −0.01) | 0.033 |

[a] P-values were obtained from t-test.

[b] Models were adjusted for all relevant and available covariates that could confound the association between the oral diseases and birth outcomes (based on earlier knowledge) using the forced entry method. The selected covariates were maternal age at enrollment, maternal height at enrollment, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment, maternal anemia at enrollment, parity, site of enrollment, proxy for SES, maternal periodontitis, number of teeth, time between delivery and examination, and intervention group.

[c] P-values were determined by linear regression models.

### Table 4.11-2. Adverse Birth Outcomes among Participants with and without Periapical Infection

| Outcome | Participants without periapical infection | Participants with periapical infection | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[a] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 57/783 (7.3%) | 24/241 (10.0%) | 1.37 (0.87 to 2.16) | 0.178 | 1.52 (0.93 to 2.47) | 0.092 |
| Incidence of LBW (<2,500 g) | 86/735 (11.7%) | 29/221 (13.2%) | 1.12 (0.76 to 1.66) | 0.568 | 1.08 (0.67 to 1.72) | 0.785 |
| Prevalence of newborn stunting (LAZ <−2) | 105/742 (14.2%) | 47/225 (21.0%) | 1.48 (1.08 to 2.01) | 0.014 | 1.68 (1.15 to 2.46) | 0.007 |
| Prevalence of newborn small head circumference (HCZ <−2) | 21/742 (2.8%) | 16/224 (7.2%) | 2.52 (1.34 to 4.75) | 0.004 | 2.52 (1.23 to 5.16) | 0.012 |

[a] P-values were obtained from log-binomial models or if those failed to converge then log-Poisson models.
[b] Models adjusted for all relevant and available covariates that could confound the association between the oral diseases and birth outcome (based on earlier knowledge) using the forced entry method. The selected covariates were maternal age at enrollment, maternal height at enrollment, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment, maternal anemia at enrollment, parity, site of enrollment, proxy for SES, maternal periodontitis, number of teeth, time between delivery and examination, and intervention group.

### Table 4.11-3. Continuous Birth Outcomes among Participants with and without Periodontitis

| Outcome | Participants without periodontitis | Participants with periodontitis | Difference (95% CI) in means | P-value[a] | Adjusted difference in means (95% CI)[b] | Adjusted P-value[c] |
|---|---|---|---|---|---|---|
| Mean (SD) duration of pregnancy, weeks | 39.3 (2.4) | 39.5 (2.1) | 0.2 (−0.1 to 0.5) | 0.198 | 0.2 (−0.1 to 0.5) | 0.174 |
| Mean (SD) birth weight, g | 2,968 (427) | 3,003 (436) | 35 (−23 to 93) | 0.241 | 28 (−32 to 88) | 0.360 |
| Mean (SD) newborn WAZ | −0.55 (1.00) | −0.51 (0.96) | 0.04 (−0.08 to 0.18) | 0.498 | 0.01 (−0.12 to 0.15) | 0.839 |
| Mean (SD) newborn LAZ | −1.01 (1.09) | −0.94 (1.11) | 0.07 (−0.07 to 0.22) | 0.322 | 0.06 (−0.08 to 0.21) | 0.396 |
| Mean (SD) newborn HCZ | −0.11 (1.07) | −0.18 (1.06) | −0.07 (−0.21 to 0.08) | 0.362 | −0.08 (−0.23 to 0.07) | 0.310 |

[a] P-values were obtained from t-test.

[b] Models were adjusted for all relevant and available covariates that could confound the association between the oral diseases and birth outcome (based on earlier knowledge) using the forced entry method. The selected covariates were maternal age at enrollment, maternal height at enrollment, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment, maternal anemia at enrollment, parity, site of enrollment, proxy for SES, maternal periapical infection, number of teeth, time between delivery and examination, and intervention group.

[c] P-values were determined by linear regression models.

### Table 4.11-4. Adverse Birth Outcomes among Participants with and without Periodontitis

| Outcome | Participants without periodontitis | Participants with periodontitis | Relative risk (95% CI) | P-value[a] | Adjusted relative risk (95% CI)[b] | Adjusted P-value[a] |
|---|---|---|---|---|---|---|
| Incidence of preterm birth (<37 gw) | 60/697 (8.6%) | 21/327 (6.4%) | 0.75 (0.46 to 1.20) | 0.227 | 0.74 (0.45 to 1.22) | 0.236 |
| Incidence of LBW (<2,500 g) | 81/652 (12.4%) | 34/304 (11.2%) | 0.90 (0.62 to 1.31) | 0.583 | 0.94 (0.60 to 1.46) | 0.786 |
| Prevalence of newborn stunting (LAZ <−2) | 104/660 (15.8%) | 48/307 (15.6%) | 0.99 (0.72 to 1.36) | 0.961 | 1.03 (0.71 to 1.48) | 0.895 |
| Prevalence of newborn small head circumference (HCZ <−2) | 19/657 (2.9%) | 18/309 (5.9%) | 2.01 (1.07 to 3.78) | 0.027 | 1.95 (0.99 to 3.83) | 0.053 |

[a] P-values were obtained from log-binomial models or if those failed to converge then log-Poisson models.

[b] Models adjusted for all relevant and available covariates that could confound the nutrition intervention effect on the oral diseases (based on earlier knowledge) using the forced entry method. The selected covariates were maternal age at enrollment, maternal height at enrollment, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment, maternal anemia at enrollment, parity, site of enrollment, proxy for SES, maternal periapical infection, number of teeth, time between delivery and examination, and intervention group.

## 4.12 Malaria Immunity in Pregnancy

This substudy was nested in the main trial as a prospective cohort study to assess the association between maternal antibody immunity to malaria and birth outcomes.

Of the 1,391 women who were enrolled in the study, after excluding 12 twin pregnancies from this analysis, 1,009 (72.5%) plasma pairs collected at enrollment and at 36 gw were available for analysis. Data on the duration of pregnancy were available for all (100%) and birth weight and neonatal size data for 93% and 94% of the included participants, respectively.

The participants included and excluded from the analysis were compared on a number of enrollment characteristics. Included participants were similar to those excluded in terms of mean maternal BMI at enrollment, maternal educational achievement, proportion with low maternal BMI at enrollment, and proportion with a positive HIV test or a positive malaria test ($P \geq 0.05$). However, compared to the excluded women, they tended to be older (25 vs. 24, $P<0.001$) and have a lower proxy for SES (−0.04 vs. 0.15, $P=0.007$) and were less likely to be anemic (42.2% vs. 50.0%, $P=0.007$) and primiparous (19.7% vs. 27.6%, $P<0.001$) (Table A1.2-11).

Women participated in the study for varying periods of time, depending on their gestation at enrollment. To adjust for this, we used the rate of change of antibody levels from enrollment to 36 gw as a predictor variable in addition to antibody level at enrollment. Rate of change in antibody level was calculated as the difference in antibody levels between 36 gw and enrollment divided by the number of weeks between enrollment and 36 weeks.

Table 4.12-1 examines the relationship between the rate of change of antibody levels from study enrollment to delivery and the duration of pregnancy. In an adjusted model, the duration of pregnancy was positively associated with total IgG to pregnancy-specific variant surface antigens (VSA) ($P=0.010$), suggesting a protective role of pregnancy-specific malaria immunity. Due to the sample selection process, there were only 25 preterm births for which we had maternal antibody data, and the study lacked power to draw conclusions regarding associations between malaria antibody and preterm birth.

The presence of opsonizing antibodies to pregnancy-specific VSA at enrollment was positively associated with newborn WAZ and HCZ ($P=0.007$ and $P=0.041$, respectively), but not with birth weight or newborn LAZ when adjusting for covariates (Table 4.12-2). Table 4.12-3, Table 4.12-4, Table 4.12-5, and Table 4.12-6 summarize the relationships between antibody levels at enrollment and LBW, newborn stunting, newborn small head circumference, and newborn underweight. For these analyses, antibody levels were divided into tertiles, representing high, medium, and low antibody levels, with low antibody levels serving as the reference group. There were no significant associations between antibody levels and newborn anthropometrics in adjusted analyses, with the exception that women with medium levels of opsonizing antibodies to pregnancy-specific VSA had a higher risk of a stunted baby (Table 4.12-4) or underweight baby (Table 4.12-6). Given that a large number of associations were examined (not all reported here) and the fact that statistically significant associations were not seen in the analysis of high antibody tertiles and newborn anthropometrics, these may represent chance findings, rather than biologically significant associations.

Table 4.12-7 examines the relationship between antibody level (high, medium, or low) and delivery of a SGA infant. In unadjusted analysis, the risk of SGA was significantly lower for women with medium and high levels, compared to low levels, of total IgG to pregnancy-specific VSA, as well as for women with high, compared to low, levels of opsonizing antibodies to pregnancy-specific VSA. After adjustment, these significances disappeared.

The findings indicate that antibodies to pregnancy-specific malaria VSA at enrollment are associated with an increased duration of pregnancy and that the presence of opsonizing antibodies to pregnancy-specific VSA have a positive association with newborn WAZ and HCZ. There were also significant associations between medium (compared to low) levels of opsonizing antibodies to pregnancy-specific VSA and increased risk of stunted and underweight babies, but these could be chance findings.

### Table 4.12-1. Association between the Rate of Change in Antibody Levels and Duration of Pregnancy

| Pregnancy outcome | Total IgG to pregnancy-specific VSA | | | | Opsonizing antibodies to pregnancy-specific VSA | | | |
|---|---|---|---|---|---|---|---|---|
| | Unadjusted[a] | | Adjusted[b] | | Unadjusted[a] | | Adjusted[b] | |
| | Regression coefficient (95% CI) | P-value | Regression coefficient (95% CI) | P-value | Regression coefficient (95% CI) | P-value | Regression coefficient (95% CI) | P-value |
| Duration of pregnancy, weeks | 0.20 (0.11 to 0.30) | <0.001 | 0.17 (0.04 to 0.30) | 0.010 | 0.12 (0.02 to 0.21) | 0.019 | 0.12 (-0.01 to 0.24) | 0.069 |

[a] P-values were determined by linear regression analysis.

[b] P-values were determined by using a linear regression model. Models were adjusted for all relevant and available covariates that could confound the association between the rate of change in antibody levels and the duration of pregnancy (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

### Table 4.12-2. Association between Malaria Antibody Levels at Enrollment and Continuous Birth Outcomes

| Pregnancy outcome | Total IgG to pregnancy-specific VSA | | | | Opsonizing antibodies to pregnancy-specific VSA | | | |
|---|---|---|---|---|---|---|---|---|
| | Unadjusted[a] | | Adjusted[b] | | Unadjusted[a] | | Adjusted[b] | |
| | Regression coefficient (95% CI) | P-value | Regression coefficient (95% CI) | P-value | Regression coefficient (95% CI) | P-value | Regression coefficient (95% CI) | P-value |
| Birth weight (g) | 0.62 (−0.20 to 1.46) | 0.139 | −1.05 (−2.23 to 0.13) | 0.080 | 1.07 (0.31 to 1.83) | 0.006 | 0.14 (−0.95 to 1.23) | 0.802 |
| Newborn LAZ | 0.003 (0.001 to 0.005) | 0.007 | 0.001 (−0.001 to 0.003) | 0.220 | 0.003 (0.001 to 0.005) | 0.003 | 0.002 (−0.001 to 0.004) | 0.086 |
| Newborn WAZ | 0.002 (−0.001 to 0.004) | 0.063 | 0.001 (−0.001 to 0.003) | 0.390 | 0.003 (0.001 to 0.005) | 0.001 | 0.003 (0.001 to 0.004) | 0.007 |
| Newborn HCZ | 0.001 (−0.002 to 0.002) | 0.763 | −0.001 (−0.002 to 0.002) | 0.993 | 0.002 (−0.001 to 0.004) | 0.067 | 0.002 (0.001 to 0.004) | 0.041 |

[a] P-values were determined by linear regression analysis.

[b] P-values were determined by using a linear regression model. Models were adjusted for all relevant and available covariates that could confound the association between malaria antibody levels and birth outcomes (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

**Table 4.12-3. Association between Antibody Levels at Enrollment and the Incidence of LBW (<2,500 g)**

| | Outcome | Low antibody level | Medium antibody level | High antibody level | Relative risk for medium antibody level compared to low antibody level (95% CI) | P-value | Relative risk for high antibody level compared to low antibody level (95% CI) | P-value |
|---|---|---|---|---|---|---|---|---|
| Total IgG to pregnancy-specific VSA | Incidence of LBW[a] | 27/247 (10.9%) | 27/256 (10.5%) | 22/264 (8.3%) | 0.96 (0.58 to 1.6) | 0.889 | 0.76 (0.45 to 1.3) | 0.321 |
| | Adjusted[b] | | | | 1.17 (0.51 to 2.7) | 0.708 | 1.39 (0.61 to 3.15) | 0.431 |
| Opsonizing antibodies to pregnancy-specific VSA | Incidence of LBW[a] | 37/300 (12.3%) | 36/312 (11.5%) | 23/318 (7.2%) | 0.94 (0.61 to 1.44) | 0.762 | 0.59 (0.36 to 0.96) | 0.035 |
| | Adjusted[b] | | | | 1.36 (0.73 to 2.53) | 0.335 | 0.87 (0.42 to 1.79) | 0.701 |

[a] Unadjusted model, P-values were calculated using log-binomial regression analysis.

[b] P-values were calculated using a multinomial logistic regression model. Models were adjusted for all relevant and available covariates that could confound the association between malaria antibody levels and the outcome (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

### Table 4.12-4. Association between Antibody Levels at Enrollment and the Prevalence of Newborn Stunting (LAZ <−2)

| | Outcome | Low antibody level | Medium antibody level | High antibody level | Relative risk for medium antibody level compared to low antibody level (95% CI) | P-value | Relative risk for high antibody level compared to low antibody level (95% CI) | P-value |
|---|---|---|---|---|---|---|---|---|
| Total IgG to pregnancy-specific VSA | Prevalence of LAZ <−2[a] | 39/237 (16.5%) | 28/247 (11.3%) | 27/251 (10.8%) | 0.69 (0.44 to 1.08) | 0.106 | 0.65 (0.41 to 1.03) | 0.069 |
| | Adjusted[b] | | | | 0.99 (0.53 to 1.87) | 0.983 | 1.07 (0.57 to 1.99) | 0.832 |
| Opsonizing antibodies to pregnancy-specific VSA | Prevalence of LAZ <−2[a] | 43/293 (14.7%) | 46/301 (15.3%) | 28/304 (9.2%) | 1.04 (0.71 to 1.53) | 0.836 | 0.63 (0.4 to 0.98) | 0.042 |
| | Adjusted[b] | | | | 2.07 (1.22 to 3.52) | 0.007 | 1.14 (0.6 to 2.16) | 0.696 |

[a] Unadjusted model, P-values were calculated using log-binomial regression analysis.

[b] P-values were calculated using a multinomial logistic regression model. Models were adjusted for all relevant and available covariates that could confound the association between malaria antibody levels and the outcome (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

**Table 4.12-5. Association between Antibody Levels at Enrollment and the Prevalence of Newborn Small Head Circumference (HCZ <−2)**

| | Outcome | Low antibody level | Medium antibody level | High antibody level | Relative risk for medium antibody level compared to low antibody level (95% CI) | P-value | Relative risk for high antibody level compared to low antibody level (95% CI) | P-value |
|---|---|---|---|---|---|---|---|---|
| Total IgG to pregnancy-specific VSA | Prevalence of HCZ <−2[a] | 8/237 (3.4%) | 4/248 (1.6%) | 3/252 (1.2%) | 0.48 (0.15 to 1.57) | 0.223 | 0.35 (0.09 to 1.31) | 0.120 |
| | Adjusted[b] | | | | 0.34 (0.04 to 2.78) | 0.317 | 0.67 (0.11 to 4.05) | 0.665 |
| Opsonizing antibodies to pregnancy-specific VSA | Prevalence of HCZ <−2[a] | 12/291 (4.1%) | 6/304 (2.0%) | 2/304 (0.7%) | 0.48 (0.18 to 1.26) | 0.135 | 0.16 (0.04 to 0.71) | 0.016 |
| | Adjusted[b] | | | | 0.51 (0.12 to 2.06) | 0.342 | 0.34 (0.07 to 1.74) | 0.193 |

[a] Unadjusted model, P-values were calculated using log-binomial regression analysis.

[b] P-values were calculated using a multinomial logistic regression model. Models were adjusted for all relevant and available covariates that could confound the association between malaria antibody levels and the outcome (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

### Table 4.12-6. Association between Antibody Levels at Enrollment and the Prevalence of Newborn Underweight (WAZ <–2)

| | Outcome | Low antibody level | Medium antibody level | High antibody level | Relative risk for medium antibody level compared to low antibody level (95% CI) | P-value | Relative risk for high antibody level compared to low antibody level (95% CI) | P-value |
|---|---|---|---|---|---|---|---|---|
| Total IgG to pregnancy-specific VSA | Prevalence of WAZ <–2[a] | 16/240 (6.7%) | 9/250 (3.6%) | 9/252 (3.6%) | 0.54 (0.24 to 1.2) | 0.130 | 0.54 (0.24 to 1.19) | 0.125 |
| | Adjusted[b] | | | | 0.55 (0.16 to 1.94) | 0.354 | 0.82 (0.25 to 2.69) | 0.742 |
| Opsonizing antibodies to pregnancy-specific VSA | Prevalence of WAZ <–2[a] | 16/296 (5.4%) | 22/304 (7.2%) | 9/305 (3%) | 1.34 (0.72 to 2.5) | 0.359 | 0.55 (0.25 to 1.22) | 0.138 |
| | Adjusted[b] | | | | 3.15 (1.29 to 7.71) | 0.012 | 2.42 (0.76 to 7.68) | 0.134 |

[a] Unadjusted model, P-values were calculated using log-binomial regression analysis.

[b] P-values were calculated using a multinomial logistic regression model. Models were adjusted for all relevant and available covariates that could confound the association between malaria antibody levels and the outcome (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

### Table 4.12-7. Association between Antibody Levels at Enrollment and the Incidence of Small for Gestational Age

| | Outcome | Low antibody level | Medium antibody level | High antibody level | Relative risk for medium antibody level compared to low antibody level (95% CI) | P-value | Relative risk for high antibody level compared to low antibody level (95% CI) | P-value |
|---|---|---|---|---|---|---|---|---|
| Total IgG to pregnancy-specific VSA | Incidence of SGA[a] | 97/261 (37.2%) | 78/270 (28.9%) | 71/271 (26.2%) | 0.68 (0.48 to 0.99) | 0.043 | 0.60 (0.41 to 0.87) | 0.007 |
| | Adjusted[b] | | | | 0.74 (0.42 to 1.31) | 0.301 | 0.91 (0.52 to 1.60) | 0.736 |
| Opsonizing antibodies to pregnancy-specific VSA | Incidence of SGA[a] | 98/276 (35.5%) | 87/278 (31.3%) | 73/283 (25.8%) | 0.83 (0.58 to 1.18) | 0.293 | 0.63 (0.44 to 0.91) | 0.013 |
| | Adjusted[c] | | | | 0.92 (0.54 to 1.57) | 0.757 | 0.78 (0.45 to 1.35) | 0.369 |

[a] Unadjusted model, P-values were calculated using log-binomial regression analysis.

[b] P-values were calculated using a modified Poisson approximation regression model. Models were adjusted for all relevant and available covariates that could confound the association between malaria antibody levels and the outcome (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

[c] P-values were calculated using a multinomial logistic regression model. Models were adjusted for all relevant and available covariates that could confound the association between malaria antibody levels and the outcome (based on earlier knowledge) using the forced entry method. The selected covariates were primiparity, maternal age at enrollment, maternal HIV status, maternal bednet use at enrollment, maternal BMI at enrollment, maternal malaria status at enrollment, proxy for SES, site of enrollment, and intervention group.

# 5. Pathways Leading to Preterm Birth or Intrauterine Growth Restriction

The aim of this sub-study was to assess associations between the predictors of adverse birth outcomes and to build concept maps of pathways by which these predictors could lead to reduced duration of pregnancy or IUGR. Of particular focus were the following outcomes: duration of pregnancy and newborn LAZ, WAZ, and HCZ.

We started the analysis by establishing bivariate associations between the maternal characteristic variables that were associated (P<0.05) with the duration of pregnancy, LAZ, WAZ, or HCZ (as reported in the other sections in this report) (Section 5.1). As part of these analyses, we also established the bivariate association between each of the four outcomes of focus, i.e., duration of pregnancy, LAZ, WAZ, and HCZ. Table A3-2 lists and describes the variables included in this analysis.

We then built a series of multivariable regression models, in which each of the maternal characteristic variables was defined as the outcome variable, and the remaining maternal characteristic variables were included as covariates in the model. This allowed us to determine "predictors of predictors" for the pathway models (Section 5.2).

We then built a series of multivariable regression models for each birth outcome of focus, by adding the predictor variables into each model one by one, with the most distal predictors entered into the model first and the most proximal predictors entered into the model last (Section 5.3). Distal variables included variables that described maternal characteristics in early pregnancy or her direct exposures during the follow-up (e.g., maternal nutrition or infection), whereas the proximal variables were those that would follow from a primary exposure (e.g., maternal weight gain, maternal plasma AGP concentration as a sign of inflammation, or duration of pregnancy). The predictor and intermediate variables were selected either based on earlier literature (Brodsky and Christou 2004) or because our own initial analyses had documented an association between them and newborn size (Harjunmaa et al. 2015, Stewart et al. 2015).

Next, we created illustrations for the pathways leading to each of the four birth outcomes of focus (Section 5.4).

Finally, we made some comparisons on how the various categories of predictor variables were associated with different types of birth outcomes (e.g., whether markers of infection were more associated with newborn length or weight) (Section 5.5).

Of the 1,391 women who were enrolled in the study, 1,379 (99.1%) were included in the analysis of duration of pregnancy and 1,179 (84.8%) in the analysis of newborn size. The reasons for exclusion were twin pregnancy (12 subjects) and missed newborn anthropometric visit (200 subjects). The included and excluded participants in the analysis of newborn size had similar enrollment characteristics, except that, on average, the included participants had a lower maternal BMI at enrollment (22.1 vs. 22.7, P=0.005), tended to be older (25 vs. 24, P=0.002), and had a higher proxy for SES (0.30 vs. −0.04, P<0.001), and a smaller proportion of them were primiparous (Table A3-1).

Among the included participants for regression analyses, the number of originally missing values that were substituted with values obtained by multiple imputation ranged from 0 to 413 (0.0% to 29.9%) per variable (Table A3-2).

## 5.1 Bivariate Associations between Various Predictor Variables

Most of the studied variables were strongly associated with each other. Table 5.1-1 provides a summary of the maternal characteristic and birth outcome variables that were significantly ($P<0.05$) or marginally associated ($0.05\leq P<0.10$) with each of the studied predictors in bivariate analysis. Full analysis details (with regression coefficients or odds ratios and P-values) for all bivariate associations are presented in Appendix A3.3. The studied predictors include both maternal characteristic variables and the birth outcome variables (i.e., duration of pregnancy and newborn LAZ, WAZ, and HCZ). Variables associated with the predictor at the $0.05\leq P<0.10$ level in bivariate analysis are indicated in parentheses.

**Table 5.1-1. Summary of Bivariate Associations among Various Maternal Characteristics and Birth Outcome Variables**

| |
|---|
| **Maternal age**<br>1. **Positive** association with variables: Maternal (height), BMI, blood Hb, weekly weight gain, HIV infection, periapical infections, newborn LAZ, newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, maternal peripheral blood malaria parasitemia, salivary cortisol, plasma AGP, placental malaria |
| **Maternal primiparity**<br>1. **Positive** association with variables: Maternal peripheral blood malaria parasitemia, plasma AGP, placental malaria<br>2. **Negative** association with variables: Maternal age, maternal height, BMI, blood Hb, weekly weight gain, HIV infection, periapical infections, duration of pregnancy, newborn LAZ, newborn WAZ, newborn HCZ |
| **Maternal height**<br>1. **Positive** association with variables: Maternal (age), weekly weight gain, placental weight, duration of pregnancy, newborn LAZ, newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, trichomoniasis |
| **Maternal BMI at enrollment**<br>1. **Positive** association with variables: Maternal age, blood Hb, (HIV infection), placental weight, (newborn LAZ), newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, weekly weight gain, peripheral blood malaria parasitemia, plasma AGP, placental malaria |
| **Maternal Hb concentration at enrollment**<br>1. **Positive** association with variables: Maternal age, BMI, weekly weight gain, duration of pregnancy, newborn LAZ, newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, HIV infection, peripheral blood malaria parasitemia, salivary cortisol, plasma AGP, placental malaria |
| **Maternal weekly weight gain during pregnancy (kg/week)**<br>1. **Positive** association with variables: Maternal age, maternal height, blood Hb, placental weight, duration of pregnancy, newborn LAZ, newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, BMI, HIV infection, peripheral blood malaria parasitemia, (trichomoniasis), salivary cortisol, placental malaria |
| **Maternal HIV infection**<br>1. **Positive** association with variables: Maternal age, (BMI), periapical infections, (UTI), trichomoniasis, plasma AGP<br>2. **Negative** association with variables: Maternal primiparity, blood Hb, weekly weight gain, placental weight, (duration of pregnancy), newborn LAZ |

| |
|---|
| **Maternal peripheral blood malaria parasitemia at enrollment (positive RDT)**<br>1. **Positive** association with variables: Maternal primiparity, trichomoniasis, plasma AGP, placental malaria<br>2. **Negative** association with variables: Maternal age, BMI, blood Hb, weekly weight gain, periapical infections, duration of pregnancy, newborn LAZ, newborn WAZ, newborn HCZ |
| **Maternal periapical oral infections (diagnosed after delivery)**<br>1. **Positive** association with variables: Maternal age, HIV infection, (trichomoniasis), salivary cortisol, (severe chorioamnionitis)<br>2. **Negative** association with variables: Maternal primiparity, peripheral blood malaria parasitemia, (plasma AGP), placental malaria, duration of pregnancy, newborn LAZ, (newborn HCZ) |
| **Maternal UTI (diagnosed after delivery)**<br>1. **Positive** association with variables: Maternal (HIV infection), severe chorioamnionitis<br>2. **Negative** association with variables: Duration of pregnancy, newborn LAZ, newborn WAZ |
| **Maternal trichomoniasis (diagnosed after delivery)**<br>1. **Positive** association with variables: Maternal HIV infection, peripheral blood malaria parasitemia, (periapical infections), plasma AGP, placental malaria<br>2. **Negative** association with variables: Maternal height, (weekly weight gain), (placental weight), duration of pregnancy, newborn LAZ, newborn WAZ, (newborn HCZ) |
| **Maternal salivary cortisol concentration at 36 gw**<br>1. **Positive** association with variables: Maternal periapical infections, (plasma AGP)<br>2. **Negative** association with variables: Maternal age, blood Hb, weekly weight gain, placental weight, duration of pregnancy, newborn WAZ, newborn HCZ |
| **Maternal plasma alpha glycoprotein (AGP) concentration at enrollment**<br>1. **Positive** association with variables: Maternal primiparity, HIV infection, peripheral blood malaria parasitemia, trichomoniasis, (salivary cortisol), placental malaria<br>2. **Negative** association with variables: Maternal age, BMI, blood Hb, (periapical infections), placental weight, severe chorioamnionitis, duration of pregnancy, newborn LAZ, newborn WAZ, newborn HCZ |
| **Placental weight (g)**<br>1. **Positive** association with variables: Maternal height, BMI, weekly weight gain, duration of pregnancy, newborn LAZ, newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal HIV infection, (trichomoniasis), salivary cortisol, plasma AGP |
| **Placental malaria infection**<br>1. **Positive** association with variables: Maternal primiparity, peripheral blood malaria parasitemia, trichomoniasis, plasma AGP<br>2. **Negative** association with variables: Maternal age, BMI, blood Hb, weekly weight gain, periapical infections, newborn LAZ, newborn WAZ, newborn HCZ |
| **Severe chorioamnionitis**<br>1. **Positive** association with variables: Maternal (periapical infections), UTI<br>2. **Negative** association with variables: Maternal plasma AGP, duration of pregnancy |
| **Duration of pregnancy (gestation weeks)**<br>1. **Positive** association with variables: Maternal height, blood Hb, weekly weight gain, placental weight, newborn LAZ, newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, (HIV infection), peripheral blood malaria parasitemia, periapical infections, UTI, trichomoniasis, salivary cortisol, plasma AGP, severe chorioamnionitis |
| **Newborn LAZ**<br>1. **Positive** association with variables: Maternal age, maternal height, (BMI), blood Hb, weekly weight gain, placental weight, duration of pregnancy, newborn WAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, HIV infection, peripheral blood malaria parasitemia, periapical infections, UTI, trichomoniasis, plasma AGP, placental malaria |

| |
|---|
| **Newborn WAZ**<br>1. **Positive** association with variables: Maternal age, maternal height, BMI, blood Hb, weekly weight gain, placental weight, duration of pregnancy, newborn LAZ, newborn HCZ<br>2. **Negative** association with variables: Maternal primiparity, peripheral blood malaria parasitemia, UTI, trichomoniasis, salivary cortisol, plasma AGP, placental malaria |
| **Newborn HCZ**<br>1. **Positive** association with variables: Maternal age, maternal height, BMI, blood Hb, weekly weight gain, placental weight, duration of pregnancy, newborn LAZ, newborn WAZ<br>2. **Negative** association with variables: Maternal primiparity, peripheral blood malaria parasitemia, (periapical infections), (trichomoniasis), salivary cortisol, plasma AGP, placental malaria |

## 5.2 Multivariable Regression Models: Predictors of Predictors

After establishing the bivariate associations, we created a series of multivariable regression models, in which we defined each of the maternal characteristic variables as the dependent variable in the model and all the other variables as the predictor variables. This allowed us to determine "predictors of predictors," to be used in the structural equation models of direct and indirect predictors of the duration of pregnancy or newborn LAZ, WAZ, or HCZ. As expected, most of the maternal characteristics were independently associated with several other variables. Full results from these analyses are shown in Appendix A3.4.

## 5.3 Multivariable Regression Models: Predictors of Reduced Duration of Pregnancy or Intrauterine Growth Restriction

Tables 5.3-1–5.3-4 show the results of a series of multivariable regression models for each birth outcome of focus, i.e., duration of pregnancy, LAZ, WAZ, and HCZ. As described earlier, the models were built by adding the predictor variables into the model for each outcome, one by one, with the most distal predictors entered into the model first and the most proximal predictors entered into the model last. The results from the models are summarized below, by birth outcome.

### 5.3.1 Duration of Pregnancy

Maternal blood Hb concentration at enrollment, peripheral blood malaria parasitemia at enrollment, presence of periapical oral infections, salivary cortisol concentration at 36 gw, placental weight, and severe chorioamnionitis were all independently associated ($P<0.05$) with the duration of pregnancy in a model with all predictor variables included (Table 5.3-1, Model 17).

Maternal height was associated with the duration of pregnancy in initial models with fewer explanatory variables, but the association disappeared when placental weight was included in the model (Table 5.3-1, Models 9–13). UTI and trichomoniasis were associated with the duration of pregnancy in earlier models, but the associations were attenuated when cortisol concentration and chorioamnionitis were added to the model. The association between weekly weight gain and duration of pregnancy became attenuated and finally disappeared when periapical oral infections, UTI, and trichomoniasis were added to the model. Maternal BMI at enrollment was associated with the duration of pregnancy only through placental weight.

Maternal age at enrollment, primiparity, maternal HIV infection, maternal plasma AGP concentration at enrollment, and signs of malaria infection in the placenta were not directly associated with the duration of pregnancy in any of the models, nor was the dietary intervention given to the mother during pregnancy.

## Table 5.3-1. Multivariable Regression Models for the Duration of Pregnancy

| | Model 1 | | Model 2 | | Model 3 | | Model 4 | | Model 5 | | Model 6 | | Model 7 | | Model 8 | | Model 9 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.013 | 0.325 | −0.002 | 0.908 | −0.003 | 0.865 | 0.000 | 0.987 | −0.002 | 0.898 | −0.003 | 0.841 | 0.000 | 0.978 | −0.005 | 0.755 | 0.007 | 0.662 |
| Maternal primiparity | | | −0.406 | *0.077* | −0.388 | *0.090* | −0.384 | *0.093* | −0.222 | 0.335 | −0.193 | 0.401 | −0.216 | 0.351 | −0.167 | 0.472 | −0.186 | 0.423 |
| Maternal height at enrollment | | | | | 0.032 | **0.023** | 0.032 | **0.024** | 0.034 | **0.015** | 0.030 | **0.032** | 0.030 | **0.032** | 0.031 | **0.030** | 0.030 | **0.034** |
| Maternal BMI at enrollment | | | | | | | −0.029 | 0.324 | −0.041 | 0.158 | −0.033 | 0.263 | −0.032 | 0.278 | −0.035 | 0.227 | −0.038 | 0.192 |
| Maternal Hb at enrollment | | | | | | | | | 0.022 | **<0.001** | 0.021 | **<0.001** | 0.020 | **<0.001** | 0.018 | **<0.001** | 0.018 | **0.001** |
| Maternal weekly weight gain | | | | | | | | | | | 1.766 | **0.028** | 1.670 | **0.039** | 1.602 | **0.047** | 1.399 | *0.083* |
| Maternal HIV infection | | | | | | | | | | | | | −0.311 | 0.205 | −0.309 | 0.207 | −0.265 | 0.276 |
| Maternal malaria infection at enrollment | | | | | | | | | | | | | | | −0.344 | *0.053* | −0.405 | **0.024** |
| Maternal periapical infections (diagnosed after delivery) | | | | | | | | | | | | | | | | | −0.778 | **0.005** |
| Maternal UTI (diagnosed after delivery) | | | | | | | | | | | | | | | | | | |
| Maternal trichomoniasis (diagnosed after delivery) | | | | | | | | | | | | | | | | | | |
| Maternal salivary cortisol concentration at 36 gw | | | | | | | | | | | | | | | | | | |
| Maternal plasma AGP concentration at enrollment | | | | | | | | | | | | | | | | | | |
| Placental weight (g) | | | | | | | | | | | | | | | | | | |
| Placental malaria infection | | | | | | | | | | | | | | | | | | |
| Severe chorioamnionitis | | | | | | | | | | | | | | | | | | |
| Intervention group – MMN | | | | | | | | | | | | | | | | | | |
| Intervention group – LNS | | | | | | | | | | | | | | | | | | |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

## Table 5.3-1. Multivariable Regression Models for the Duration of Pregnancy (continued)

| | Model 10 | | Model 11 | | Model 12 | | Model 13 | | Model 14 | | Model 15 | | Model 16 | | Model 17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.006 | 0.686 | 0.005 | 0.752 | −0.003 | 0.871 | −0.003 | 0.863 | −0.001 | 0.970 | 0.001 | 0.939 | 0.002 | 0.898 | 0.002 | 0.897 |
| Maternal primiparity | −0.160 | 0.491 | −0.169 | 0.467 | −0.181 | 0.435 | −0.165 | 0.480 | −0.075 | 0.746 | −0.126 | 0.597 | −0.138 | 0.560 | −0.139 | 0.559 |
| Maternal height at enrollment | 0.029 | **0.039** | 0.026 | *0.067* | 0.024 | *0.088* | 0.024 | *0.090* | 0.007 | 0.595 | 0.007 | 0.592 | 0.006 | 0.656 | 0.006 | 0.656 |
| Maternal BMI at enrollment | −0.035 | 0.235 | −0.036 | 0.219 | −0.033 | 0.255 | −0.033 | 0.248 | −0.056 | **0.047** | −0.053 | *0.060* | −0.048 | *0.089* | −0.048 | *0.089* |
| Maternal Hb at enrollment | 0.017 | **0.001** | 0.018 | **<0.001** | 0.015 | **0.003** | 0.015 | **0.003** | 0.017 | **0.001** | 0.017 | **0.001** | 0.017 | **0.001** | 0.017 | **0.001** |
| Maternal weekly weight gain | 1.340 | *0.096* | 1.281 | 0.111 | 0.920 | 0.249 | 0.949 | 0.237 | 0.302 | 0.698 | 0.366 | 0.640 | 0.386 | 0.620 | 0.387 | 0.620 |
| Maternal HIV infection | −0.227 | 0.351 | −0.181 | 0.459 | −0.164 | 0.497 | −0.148 | 0.547 | 0.020 | 0.933 | 0.010 | 0.967 | 0.013 | 0.956 | 0.014 | 0.954 |
| Maternal malaria infection at enrollment | −0.404 | **0.024** | −0.374 | **0.037** | −0.405 | **0.023** | −0.387 | **0.034** | −0.428 | **0.015** | −0.469 | **0.011** | −0.433 | **0.018** | −0.433 | **0.018** |
| Maternal periapical infections (diagnosed after delivery) | −0.746 | **0.007** | −0.719 | **0.009** | −0.595 | **0.033** | −0.595 | **0.033** | −0.632 | **0.017** | −0.628 | **0.017** | −0.584 | **0.029** | −0.585 | **0.028** |
| Maternal UTI (diagnosed after delivery) | −1.271 | *0.065* | −1.229 | *0.072* | −1.287 | *0.053* | −1.285 | *0.053* | −1.301 | **0.040** | −1.301 | **0.040** | −1.149 | *0.067* | −1.151 | *0.067* |
| Maternal trichomoniasis (diagnosed after delivery) | | | −0.633 | **0.045** | −0.590 | *0.059* | −0.583 | *0.063* | −0.488 | 0.104 | −0.502 | *0.096* | −0.465 | 0.124 | −0.465 | 0.125 |
| Maternal salivary cortisol concentration at 36 gw | | | | | −0.152 | **0.005** | −0.151 | **0.006** | −0.123 | **0.015** | −0.124 | **0.014** | −0.125 | **0.012** | −0.125 | **0.013** |
| Maternal plasma AGP concentration at enrollment | | | | | | | −0.146 | 0.665 | 0.029 | 0.929 | 0.008 | 0.981 | −0.110 | 0.741 | −0.109 | 0.743 |
| Placental weight (g) | | | | | | | | | 0.007 | **<0.001** | 0.007 | **<0.001** | 0.007 | **<0.001** | 0.007 | **<0.001** |
| Placental malaria infection | | | | | | | | | | | 0.208 | 0.388 | 0.206 | 0.392 | 0.206 | 0.392 |
| Severe chorioamnionitis | | | | | | | | | | | | | −0.891 | **0.042** | −0.891 | **0.043** |
| Intervention group – MMN | | | | | | | | | | | | | | | 0.013 | 0.942 |
| Intervention group – LNS | | | | | | | | | | | | | | | 0.008 | 0.963 |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

### 5.3.2 Newborn Length-for-Age Z-Score

Maternal primiparity, height, weight gain during pregnancy, HIV infection (P=0.052), presence of periapical oral infections, maternal plasma AGP concentration at enrollment, placental weight, and duration of pregnancy were all independently associated with newborn LAZ (Table 5.3-2, Model 18).

Maternal age, blood Hb concentration, and peripheral blood malaria parasitemia at enrollment were associated with newborn LAZ in initial models, but these associations disappeared when maternal primiparity or duration of pregnancy was included in the model (Table 5.3-2, Models 2 and 17).

Maternal BMI at enrollment, UTI, trichomoniasis, salivary cortisol concentration, signs of malaria infection in the placenta, severe chorioamnionitis, and the dietary intervention given to the mother during pregnancy were not associated with newborn LAZ in any of the models (Table 5.3-2).

## Table 5.3-2. Multivariable Regression Models for Newborn Length-for-Age Z-Score

| | Model 1 | | Model 2 | | Model 3 | | Model 4 | | Model 5 | | Model 6 | | Model 7 | | Model 8 | | Model 9 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.019 | **0.001** | 0.002 | 0.700 | 0.002 | 0.758 | 0.001 | 0.935 | 0.000 | 0.976 | −0.001 | 0.889 | 0.002 | 0.753 | 0.000 | 0.985 | 0.004 | 0.504 |
| Maternal primiparity | | | −0.486 | **<0.001** | −0.452 | **<0.001** | −0.456 | **<0.001** | −0.405 | **<0.001** | −0.379 | **<0.001** | −0.402 | **<0.001** | −0.368 | **<0.001** | −0.373 | **<0.001** |
| Maternal height at enrollment | | | | | 0.052 | **<0.001** | 0.052 | **<0.001** | 0.053 | **<0.001** | 0.049 | **<0.001** | 0.049 | **<0.001** | 0.049 | **<0.001** | 0.049 | **<0.001** |
| Maternal BMI at enrollment | | | | | | | 0.017 | 0.162 | 0.012 | 0.296 | 0.020 | *0.090* | 0.021 | *0.076* | 0.019 | 0.108 | 0.018 | 0.132 |
| Maternal Hb at enrollment | | | | | | | | | 0.006 | **0.002** | 0.005 | **0.009** | 0.005 | **0.021** | 0.004 | *0.059* | 0.004 | *0.076* |
| Maternal weekly weight gain | | | | | | | | | | | 1.499 | **<0.001** | 1.405 | **<0.001** | 1.361 | **<0.001** | 1.289 | **<0.001** |
| Maternal HIV infection | | | | | | | | | | | | | −0.294 | **0.002** | −0.295 | **0.002** | −0.281 | **0.003** |
| Maternal malaria infection at enrollment | | | | | | | | | | | | | | | −0.176 | **0.015** | −0.196 | **0.007** |
| Maternal periapical infections (diagnosed after delivery) | | | | | | | | | | | | | | | | | −0.264 | **0.002** |
| Maternal UTI (diagnosed after delivery) | | | | | | | | | | | | | | | | | | |
| Maternal trichomoniasis (diagnosed after delivery) | | | | | | | | | | | | | | | | | | |
| Maternal salivary cortisol concentration at 36 gw | | | | | | | | | | | | | | | | | | |
| Maternal plasma AGP concentration at enrollment | | | | | | | | | | | | | | | | | | |
| Placental weight (g) | | | | | | | | | | | | | | | | | | |
| Placental malaria infection | | | | | | | | | | | | | | | | | | |
| Severe chorioamnionitis | | | | | | | | | | | | | | | | | | |
| Duration of pregnancy | | | | | | | | | | | | | | | | | | |
| Intervention group – MMN | | | | | | | | | | | | | | | | | | |
| Intervention group – LNS | | | | | | | | | | | | | | | | | | |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

## Table 5.3-2. Multivariable Regression Models for Newborn Length-for-Age Z-Score (continued)

| | Model 10 | | Model 11 | | Model 12 | | Model 13 | | Model 14 | | Model 15 | | Model 16 | | Model 17 | | Model 18 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.004 | 0.518 | 0.004 | 0.533 | 0.004 | 0.566 | 0.003 | 0.644 | 0.004 | 0.519 | 0.003 | 0.601 | 0.003 | 0.588 | 0.002 | 0.749 | 0.002 | 0.749 |
| Maternal primiparity | −0.368 | **<0.001** | −0.369 | **<0.001** | −0.370 | **<0.001** | −0.331 | **0.001** | −0.307 | **0.001** | −0.283 | **0.004** | −0.287 | **0.004** | −0.279 | **0.002** | −0.280 | **0.002** |
| Maternal height at enrollment | 0.049 | **<0.001** | 0.048 | **<0.001** | 0.048 | **<0.001** | 0.048 | **<0.001** | 0.042 | **<0.001** | 0.042 | **<0.001** | 0.042 | **<0.001** | 0.040 | **<0.001** | 0.040 | **<0.001** |
| Maternal BMI at enrollment | 0.018 | 0.132 | 0.018 | 0.137 | 0.018 | 0.136 | 0.017 | 0.163 | 0.007 | 0.536 | 0.006 | 0.616 | 0.007 | 0.576 | 0.014 | 0.191 | 0.014 | 0.194 |
| Maternal Hb at enrollment | 0.004 | *0.079* | 0.004 | *0.073* | 0.004 | *0.082* | 0.003 | *0.098* | 0.004 | **0.024** | 0.004 | **0.025** | 0.004 | **0.026** | 0.001 | 0.727 | 0.001 | 0.732 |
| Maternal weekly weight gain | 1.272 | **<0.001** | 1.264 | **<0.001** | 1.250 | **<0.001** | 1.332 | **<0.001** | 1.015 | **0.001** | 0.986 | **0.002** | 0.989 | **0.002** | 0.789 | **0.007** | 0.791 | **0.007** |
| Maternal HIV infection | −0.274 | **0.004** | −0.267 | **0.005** | −0.267 | **0.005** | −0.215 | **0.027** | −0.164 | *0.082* | −0.160 | *0.090* | −0.160 | *0.091* | −0.172 | **0.048** | −0.169 | *0.052* |
| Maternal malaria infection at enrollment | −0.196 | **0.007** | −0.193 | **0.008** | −0.194 | **0.007** | −0.144 | *0.051* | −0.163 | **0.023** | −0.145 | **0.047** | −0.139 | *0.057* | −0.086 | 0.201 | −0.087 | 0.197 |
| Maternal periapical infections (diagnosed after delivery) | −0.258 | **0.002** | −0.254 | **0.002** | −0.250 | **0.003** | −0.254 | **0.003** | −0.283 | **0.001** | −0.284 | **0.001** | −0.279 | **0.001** | −0.228 | **0.003** | −0.230 | **0.002** |
| Maternal UTi (diagnosed after delivery) | −0.354 | 0.106 | −0.353 | 0.106 | −0.357 | 0.103 | −0.333 | 0.127 | −0.365 | *0.097* | −0.363 | *0.098* | −0.347 | 0.115 | −0.259 | 0.188 | −0.263 | 0.183 |
| Maternal trichomoniasis (diagnosed after delivery) | | | −0.091 | 0.404 | −0.090 | 0.410 | −0.070 | 0.524 | −0.050 | 0.638 | −0.042 | 0.694 | −0.038 | 0.722 | −0.008 | 0.933 | −0.005 | 0.955 |
| Maternal salivary cortisol concentration at 36 gw | | | | | −0.006 | 0.653 | −0.004 | 0.740 | 0.004 | 0.712 | 0.005 | 0.683 | 0.004 | 0.705 | 0.017 | *0.093* | 0.017 | *0.089* |
| Maternal plasma AGP concentration at enrollment | | | | | | | −0.394 | **0.005** | −0.322 | **0.019** | −0.312 | **0.023** | −0.332 | **0.015** | −0.394 | **0.002** | −0.393 | **0.002** |
| Placental weight (g) | | | | | | | | | 0.003 | **<0.001** | 0.003 | **<0.001** | 0.003 | **<0.001** | 0.002 | **<0.001** | 0.002 | **<0.001** |
| Placental malaria infection | | | | | | | | | | | −0.092 | 0.213 | −0.091 | 0.217 | −0.074 | 0.277 | −0.074 | 0.284 |
| Severe chorioamnionitis | | | | | | | | | | | | | −0.137 | 0.216 | −0.093 | 0.350 | −0.090 | 0.365 |
| Duration of pregnancy | | | | | | | | | | | | | | | 0.222 | **<0.001** | 0.221 | **<0.001** |
| Intervention group – MMN | | | | | | | | | | | | | | | | | 0.048 | 0.486 |
| Intervention group – LNS | | | | | | | | | | | | | | | | | 0.053 | 0.444 |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

### 5.3.3 Newborn Weight-for-Age Z-Score

Maternal primiparity, maternal BMI at enrollment, weight gain during pregnancy, maternal plasma AGP concentration at enrollment, placental weight, duration of pregnancy, and newborn LAZ were all independently associated with newborn WAZ (Table 5.3-3, Model 19).

Maternal age was associated with newborn WAZ in initial models, but the association disappeared when maternal primiparity was included in the model (Table 5.3-3, Model 2). Similar loss of an initially documented association with newborn WAZ was observed for many variables when new ones were entered into the models. For example, the addition of maternal plasma AGP concentration eliminated the association between newborn WAZ and maternal HIV infection (Table 5.3-3, Model 13). The addition of the duration of pregnancy eliminated the association of newborn WAZ with maternal blood Hb concentration at enrollment and weakened that with maternal UTI (Table 5.3-3, Model 17). The addition of newborn LAZ into the model eliminated the association of newborn WAZ with maternal height, peripheral blood malaria parasitemia at enrollment, presence of periapical oral infections, and UTI (Table 5.3-3, Model 18).

Maternal trichomoniasis, salivary cortisol concentration at 36 gw, signs of malaria infection in the placenta, severe chorioamnionitis, and the dietary intervention given to the mother during pregnancy were not associated with newborn WAZ in any of the models (Table 5.3-3).

## Table 5.3-3. Multivariable Regression Models for Newborn Weight-for-Age Z-Score

| | Model 1 | | Model 2 | | Model 3 | | Model 4 | | Model 5 | | Model 6 | | Model 7 | | Model 8 | | Model 9 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.024 | **<0.001** | 0.007 | 0.264 | 0.006 | 0.285 | 0.003 | 0.545 | 0.003 | 0.588 | 0.002 | 0.735 | 0.004 | 0.519 | 0.001 | 0.807 | 0.004 | 0.458 |
| Maternal primiparity | | | −0.508 | **<0.001** | −0.482 | **<0.001** | −0.490 | **<0.001** | −0.429 | **<0.001** | −0.399 | **<0.001** | −0.413 | **<0.001** | −0.372 | **<0.001** | −0.375 | **<0.001** |
| Maternal height at enrollment | | | | | 0.040 | **<0.001** | 0.040 | **<0.001** | 0.040 | **<0.001** | 0.036 | **<0.001** | 0.036 | **<0.001** | 0.036 | **<0.001** | 0.036 | **<0.001** |
| Maternal BMI at enrollment | | | | | | | 0.031 | **0.005** | 0.026 | **0.017** | 0.035 | **0.001** | 0.035 | **0.001** | 0.033 | **0.003** | 0.032 | **0.003** |
| Maternal Hb at enrollment | | | | | | | | | 0.008 | **<0.001** | 0.007 | **<0.001** | 0.006 | **0.001** | 0.005 | **0.007** | 0.005 | **0.009** |
| Maternal weekly weight gain | | | | | | | | | | | 1.689 | **<0.001** | 1.630 | **<0.001** | 1.576 | **<0.001** | 1.526 | **<0.001** |
| Maternal HIV infection | | | | | | | | | | | | | −0.183 | **0.037** | −0.185 | **0.034** | −0.175 | **0.044** |
| Maternal malaria infection at enrollment | | | | | | | | | | | | | | | −0.217 | **0.001** | −0.231 | **<0.001** |
| Maternal periapical infections (diagnosed after delivery) | | | | | | | | | | | | | | | | | −0.185 | **0.014** |
| Maternal UTI (diagnosed after delivery) | | | | | | | | | | | | | | | | | | |
| Maternal trichomoniasis (diagnosed after delivery) | | | | | | | | | | | | | | | | | | |
| Maternal salivary cortisol concentration at 36 gw | | | | | | | | | | | | | | | | | | |
| Maternal plasma AGP concentration at enrollment | | | | | | | | | | | | | | | | | | |
| Placental weight (g) | | | | | | | | | | | | | | | | | | |
| Placental malaria infection | | | | | | | | | | | | | | | | | | |
| Severe chorioamnionitis | | | | | | | | | | | | | | | | | | |
| Duration of pregnancy | | | | | | | | | | | | | | | | | | |
| Newborn LAZ | | | | | | | | | | | | | | | | | | |
| Intervention group – MMN | | | | | | | | | | | | | | | | | | |
| Intervention group – LNS | | | | | | | | | | | | | | | | | | |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

## Table 5.3-3. Multivariable Regression Models for Newborn Weight-for-Age Z-Score (continued)

| | Model 10 | | Model 11 | | Model 12 | | Model 13 | | Model 14 | | Model 15 | | Model 16 | | Model 17 | | Model 18 | | Model 19 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.004 | 0.473 | 0.004 | 0.504 | 0.003 | 0.598 | 0.002 | 0.695 | 0.004 | 0.513 | 0.003 | 0.580 | 0.003 | 0.568 | 0.001 | 0.757 | 0.001 | 0.887 | 0.001 | 0.885 |
| Maternal primiparity | −0.369 | **<0.001** | −0.372 | **<0.001** | −0.374 | **<0.001** | −0.331 | **<0.001** | −0.301 | **<0.001** | −0.284 | **0.001** | −0.287 | **0.001** | −0.280 | **<0.001** | −0.135 | **0.027** | −0.135 | **0.027** |
| Maternal height at enrollment | 0.036 | **<0.001** | 0.035 | **<0.001** | 0.035 | **<0.001** | 0.034 | **<0.001** | 0.027 | **<0.001** | 0.027 | **<0.001** | 0.027 | **<0.001** | 0.024 | **<0.001** | 0.004 | 0.269 | 0.004 | 0.277 |
| Maternal BMI at enrollment | 0.032 | **0.003** | 0.032 | **0.004** | 0.032 | **0.003** | 0.031 | **0.005** | 0.019 | *0.063* | 0.018 | *0.079* | 0.019 | *0.070* | 0.027 | **0.004** | 0.019 | **0.009** | 0.019 | **0.009** |
| Maternal Hb at enrollment | 0.005 | **0.009** | 0.005 | **0.007** | 0.005 | **0.011** | 0.005 | **0.014** | 0.006 | **0.001** | 0.006 | **0.001** | 0.006 | **0.001** | 0.002 | 0.235 | 0.002 | 0.221 | 0.002 | 0.220 |
| Maternal weekly weight gain | 1.506 | **<0.001** | 1.489 | **<0.001** | 1.452 | **<0.001** | 1.543 | **<0.001** | 1.153 | **<0.001** | 1.133 | **<0.001** | 1.135 | **<0.001** | 0.925 | **<0.001** | 0.517 | **0.008** | 0.518 | **0.008** |
| Maternal HIV infection | −0.167 | *0.055* | −0.155 | *0.077* | −0.153 | *0.079* | −0.096 | 0.279 | −0.033 | 0.695 | −0.030 | 0.719 | −0.029 | 0.724 | −0.043 | 0.561 | 0.046 | 0.433 | 0.044 | 0.459 |
| Maternal malaria infection at enrollment | −0.232 | **<0.001** | −0.225 | **0.001** | −0.229 | **0.001** | −0.173 | **0.010** | −0.196 | **0.002** | −0.184 | **0.004** | −0.178 | **0.006** | −0.123 | **0.032** | −0.079 | *0.090* | −0.078 | *0.091* |
| Maternal periapical infections (diagnosed after delivery) | −0.179 | **0.018** | −0.172 | **0.023** | −0.161 | **0.034** | −0.165 | **0.029** | −0.201 | **0.007** | −0.202 | **0.006** | −0.197 | **0.008** | −0.144 | **0.032** | −0.026 | 0.628 | −0.024 | 0.645 |
| Maternal UTI (diagnosed after delivery) | −0.400 | *0.050* | −0.398 | *0.050* | −0.408 | **0.044** | −0.382 | *0.058* | −0.421 | **0.033** | −0.420 | **0.034** | −0.405 | **0.040** | −0.313 | *0.065* | −0.179 | 0.178 | −0.174 | 0.191 |
| Maternal trichomoniasis (diagnosed after delivery) | | | −0.173 | *0.083* | −0.170 | *0.089* | −0.147 | 0.139 | −0.122 | 0.190 | −0.117 | 0.211 | −0.113 | 0.225 | −0.082 | 0.311 | −0.078 | 0.229 | −0.077 | 0.235 |
| Maternal salivary cortisol concentration at 36 gw | | | | | −0.015 | 0.188 | −0.013 | 0.240 | −0.003 | 0.783 | −0.002 | 0.811 | −0.003 | 0.785 | 0.010 | 0.213 | 0.002 | 0.813 | 0.001 | 0.852 |
| Maternal plasma AGP concentration at enrollment | | | | | | | −0.439 | **0.001** | −0.351 | **0.004** | −0.343 | **0.005** | −0.361 | **0.003** | −0.425 | **<0.001** | −0.222 | **0.010** | −0.224 | **0.009** |
| Placental weight (g) | | | | | | | | | 0.004 | **<0.001** | 0.004 | **<0.001** | 0.004 | **<0.001** | 0.003 | **<0.001** | 0.002 | **<0.001** | 0.002 | **<0.001** |
| Placental malaria infection | | | | | | | | | | | −0.066 | 0.321 | −0.065 | 0.327 | −0.048 | 0.424 | −0.009 | 0.852 | −0.009 | 0.858 |
| Severe chorioamnionitis | | | | | | | | | | | | | −0.119 | 0.212 | −0.073 | 0.381 | −0.025 | 0.715 | −0.026 | 0.710 |
| Duration of pregnancy | | | | | | | | | | | | | | | 0.233 | **<0.001** | 0.118 | **<0.001** | 0.118 | **<0.001** |
| Newborn LAZ | | | | | | | | | | | | | | | | | 0.517 | **<0.001** | 0.517 | **<0.001** |
| Intervention group – MMN | | | | | | | | | | | | | | | | | | | −0.030 | 0.517 |
| Intervention group – LNS | | | | | | | | | | | | | | | | | | | 0.004 | 0.932 |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

### 5.3.4 Newborn Head Circumference-for-Age Z-Score

Maternal BMI at enrollment, maternal plasma AGP concentration at enrollment, placental weight, duration of pregnancy, and newborn LAZ were all independently associated with newborn HCZ (Table 5.3-4, Model 19).

Maternal age was associated with newborn HCZ in initial models, but the association disappeared when maternal primiparity was included in the model (Table 5.3-4, Model 2). Similarly, the addition of maternal blood Hb concentration at enrollment and weight gain during pregnancy eliminated the association between newborn HCZ and primiparity (Table 5.3-4, Models 4–6). The addition of newborn LAZ into the model eliminated the association of newborn HCZ with maternal height, maternal weight gain during pregnancy, maternal peripheral blood malaria parasitemia at enrollment, and presence of periapical oral infections (Table 5.3-4, Model 18).

Maternal blood Hb concentration at enrollment, HIV infection, UTI, trichomoniasis, salivary cortisol concentration at 36 gw, signs of malaria infection in the placenta, severe chorioamnionitis, and the dietary intervention given to the mother during pregnancy were not associated with newborn HCZ in any of the models (Table 5.3-4).

## Table 5.3-4. Multivariable Regression Models for Newborn Head Circumference-for-Age Z-Score

| | Model 1 | | Model 2 | | Model 3 | | Model 4 | | Model 5 | | Model 6 | | Model 7 | | Model 8 | | Model 9 | | Model 10 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.014 | **0.011** | 0.007 | 0.272 | 0.007 | 0.289 | 0.004 | 0.544 | 0.004 | 0.562 | 0.003 | 0.664 | 0.004 | 0.565 | 0.001 | 0.820 | 0.005 | 0.474 | 0.005 | 0.476 |
| Maternal primiparity | | | −0.203 | **0.038** | −0.183 | *0.058* | −0.192 | **0.047** | −0.166 | *0.091* | −0.142 | 0.145 | −0.150 | 0.126 | −0.109 | 0.267 | −0.113 | 0.249 | −0.112 | 0.254 |
| Maternal height at enrollment | | | | | 0.029 | **<0.001** | 0.029 | **<0.001** | 0.029 | **<0.001** | 0.026 | **<0.001** | 0.026 | **<0.001** | 0.026 | **<0.001** | 0.026 | **<0.001** | 0.026 | **<0.001** |
| Maternal BMI at enrollment | | | | | | | 0.033 | **0.005** | 0.031 | **0.009** | 0.038 | **0.002** | 0.038 | **0.002** | 0.036 | **0.003** | 0.035 | **0.004** | 0.035 | **0.004** |
| Maternal Hb at enrollment | | | | | | | | | 0.003 | 0.116 | 0.002 | 0.233 | 0.002 | 0.279 | 0.001 | 0.546 | 0.001 | 0.607 | 0.001 | 0.609 |
| Maternal weekly weight gain | | | | | | | | | | | 1.324 | **<0.001** | 1.293 | **<0.001** | 1.241 | **<0.001** | 1.186 | **<0.001** | 1.182 | **<0.001** |
| Maternal HIV infection | | | | | | | | | | | | | −0.097 | 0.320 | −0.098 | 0.312 | −0.088 | 0.365 | −0.086 | 0.374 |
| Maternal malaria infection at enrollment | | | | | | | | | | | | | | | −0.210 | **0.004** | −0.225 | **0.002** | −0.225 | **0.002** |
| Maternal periapical infections (diagnosed after delivery) | | | | | | | | | | | | | | | | | −0.203 | **0.015** | −0.202 | **0.016** |
| Maternal UTI (diagnosed after delivery) | | | | | | | | | | | | | | | | | | | −0.069 | 0.751 |
| Maternal trichomoniasis (diagnosed after delivery) | | | | | | | | | | | | | | | | | | | | |
| Maternal salivary cortisol concentration at 36 gw | | | | | | | | | | | | | | | | | | | | |
| Maternal plasma AGP concentration at enrollment | | | | | | | | | | | | | | | | | | | | |
| Placental weight (g) | | | | | | | | | | | | | | | | | | | | |
| Placental malaria infection | | | | | | | | | | | | | | | | | | | | |
| Severe chorioamnionitis | | | | | | | | | | | | | | | | | | | | |
| Duration of pregnancy | | | | | | | | | | | | | | | | | | | | |
| Newborn LAZ | | | | | | | | | | | | | | | | | | | | |
| Intervention group – MMN | | | | | | | | | | | | | | | | | | | | |
| Intervention group – LNS | | | | | | | | | | | | | | | | | | | | |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

## Table 5.3-4. Multivariable Regression Models for Newborn Head Circumference-for-Age Z-Score (continued)

| | Model 11 | | Model 12 | | Model 13 | | Model 14 | | Model 15 | | Model 16 | | Model 17 | | Model 18 | | Model 19 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.004 | 0.490 | 0.003 | 0.597 | 0.003 | 0.679 | 0.004 | 0.519 | 0.004 | 0.544 | 0.004 | 0.530 | 0.002 | 0.695 | 0.001 | 0.783 | 0.001 | 0.782 |
| Maternal primiparity | −0.114 | 0.248 | −0.117 | 0.235 | −0.076 | 0.442 | −0.046 | 0.629 | −0.039 | 0.690 | −0.043 | 0.662 | −0.035 | 0.695 | 0.092 | 0.245 | 0.092 | 0.246 |
| Maternal height at enrollment | 0.026 | **<0.001** | 0.025 | **<0.001** | 0.025 | **<0.001** | 0.018 | **0.002** | 0.018 | **0.002** | 0.017 | **0.002** | 0.015 | **0.004** | −0.003 | 0.491 | −0.003 | 0.491 |
| Maternal BMI at enrollment | 0.035 | **0.004** | 0.035 | **0.004** | 0.034 | **0.005** | 0.022 | *0.055* | 0.022 | *0.061* | 0.022 | *0.053* | 0.031 | **0.003** | 0.024 | **0.009** | 0.024 | **0.009** |
| Maternal Hb at enrollment | 0.001 | 0.583 | 0.001 | 0.692 | 0.001 | 0.765 | 0.002 | 0.336 | 0.002 | 0.337 | 0.002 | 0.349 | −0.002 | 0.218 | −0.003 | 0.117 | −0.003 | 0.117 |
| Maternal weekly weight gain | 1.173 | **<0.001** | 1.125 | **0.001** | 1.211 | **<0.001** | 0.820 | **0.009** | 0.811 | **0.010** | 0.814 | **0.010** | 0.595 | **0.038** | 0.237 | 0.348 | 0.238 | 0.346 |
| Maternal HIV infection | −0.080 | 0.413 | −0.078 | 0.421 | −0.024 | 0.809 | 0.040 | 0.675 | 0.040 | 0.668 | 0.041 | 0.663 | 0.027 | 0.749 | 0.105 | 0.166 | 0.105 | 0.171 |
| Maternal malaria infection at enrollment | −0.222 | **0.003** | −0.227 | **0.002** | −0.174 | **0.021** | −0.197 | **0.006** | −0.193 | **0.009** | −0.186 | **0.011** | −0.128 | *0.055* | −0.089 | 0.139 | −0.089 | 0.139 |
| Maternal periapical infections (diagnosed after delivery) | −0.199 | **0.018** | −0.184 | **0.029** | −0.188 | **0.025** | −0.224 | **0.007** | −0.224 | **0.007** | −0.218 | **0.008** | −0.162 | **0.029** | −0.058 | 0.363 | −0.058 | 0.366 |
| Maternal UTI (diagnosed after delivery) | −0.068 | 0.754 | −0.081 | 0.711 | −0.056 | 0.796 | −0.096 | 0.647 | −0.095 | 0.650 | −0.076 | 0.715 | 0.020 | 0.915 | 0.137 | 0.400 | 0.140 | 0.394 |
| Maternal trichomoniasis (diagnosed after delivery) | −0.093 | 0.402 | −0.089 | 0.420 | −0.067 | 0.542 | −0.043 | 0.688 | −0.040 | 0.704 | −0.036 | 0.734 | −0.004 | 0.969 | 0.000 | 1.000 | 0.001 | 0.989 |
| Maternal salivary cortisol concentration at 36 gw | | | −0.019 | 0.122 | −0.018 | 0.154 | −0.007 | 0.533 | −0.007 | 0.540 | −0.008 | 0.513 | 0.006 | 0.524 | −0.002 | 0.852 | −0.002 | 0.840 |
| Maternal plasma AGP concentration at enrollment | | | | | −0.414 | **0.003** | −0.325 | **0.016** | −0.322 | **0.017** | −0.345 | **0.011** | −0.412 | **0.001** | −0.233 | **0.033** | −0.235 | **0.032** |
| Placental weight (g) | | | | | | | 0.004 | **<0.001** | 0.004 | **<0.001** | 0.004 | **<0.001** | 0.003 | **<0.001** | 0.002 | **<0.001** | 0.002 | **<0.001** |
| Placental malaria infection | | | | | | | | | −0.026 | 0.730 | −0.025 | 0.741 | −0.007 | 0.924 | 0.027 | 0.667 | 0.028 | 0.662 |
| Severe chorioamnionitis | | | | | | | | | | | −0.153 | 0.150 | −0.105 | 0.263 | −0.063 | 0.447 | −0.062 | 0.450 |
| Duration of pregnancy | | | | | | | | | | | | | 0.243 | **<0.001** | 0.142 | **<0.001** | 0.142 | **<0.001** |
| Newborn LAZ | | | | | | | | | | | | | | | 0.454 | **<0.001** | 0.454 | **<0.001** |
| Intervention group – MMN | | | | | | | | | | | | | | | | | −0.007 | 0.906 |
| Intervention group – LNS | | | | | | | | | | | | | | | | | 0.016 | 0.797 |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

## 5.4 Pathway Analysis: Illustration of the Pathways Leading to Reduced Duration of Pregnancy or Intrauterine Growth Restriction

Illustrations of the pathways leading to reduced duration of pregnancy, newborn LAZ, newborn WAZ, or newborn HCZ are shown in Figure 5.4-1–Figure 5.4-4. These figures indicate the key similarities and differences in the models. A summary of the figures is provided below, by outcome.

The *duration of pregnancy* (Figure 5.4-1) was predicted by maternal blood Hb concentration at enrollment, peripheral blood malaria parasitemia at enrollment, presence of periapical oral infections, salivary cortisol concentration at 36 gw, placental weight, and severe chorioamnionitis. Placental weight, malaria parasitemia, and salivary cortisol concentration at 36 gw had their own predictors that were indirectly associated with the duration of pregnancy. Young maternal age and primiparity were associated with increased risk of malaria but decreased risk of HIV and periapical infections (results from regression models because SEM do not allow dichotomous endogenous variables). Goodness of fit statistic RMSEA for this model was 0.013, which can be considered to be a good fit.

*Newborn LAZ* (Figure 5.4-2) was predicted by the duration of pregnancy, and thus also indirectly by its determinants. Additionally, newborn length was independently predicted by maternal primiparity and height, weight gain during pregnancy, HIV infection, periapical oral infections, and maternal plasma AGP concentration at enrollment, as well as placental weight. Goodness of fit statistic RMSEA for this model was 0.036, which can be considered to be a good fit.

*Newborn WAZ* (Figure 5.4-3) was also predicted by the duration of pregnancy, maternal primiparity, weight gain during pregnancy, maternal plasma AGP concentration at enrollment, and placental weight. Additionally, newborn weight was predicted by maternal BMI at enrollment and newborn LAZ. Maternal height, HIV infection, and periapical oral infections predicted newborn weight, but only if the model excluded newborn LAZ. When LAZ was entered into the model, these associations disappeared, i.e., maternal height, HIV infection, and periapical oral infections were associated with newborn weight only indirectly through their effect on newborn LAZ, but not directly. Goodness of fit statistic RMSEA for this model was 0.063, which can be considered to be an adequate fit.

*Newborn HCZ* (Figure 5.4-4) was directly predicted by newborn LAZ, duration of pregnancy, maternal BMI at enrollment, maternal plasma AGP concentration at enrollment, and placental weight. Maternal height at enrollment, maternal BMI at enrollment, and weight gain during pregnancy were associated with newborn HCZ indirectly, through their association with placental weight. Primiparity, maternal plasma AGP concentration, HIV infection, and periapical oral infections were associated with newborn HCZ through their association with newborn LAZ. Maternal peripheral blood malaria parasitemia at enrollment was associated with newborn HCZ indirectly through its association with the duration of pregnancy and newborn LAZ. In contrast to newborn LAZ and WAZ, newborn HCZ was not directly predicted by maternal age at enrollment or primiparity or by maternal HIV infection, UTI, or vaginal trichomoniasis. Goodness of fit statistic RMSEA for this model was 0.062, which can be considered to be an adequate fit.

The main similarities and differences between the models for newborn LAZ, WAZ, and HCZ were the following (duration of pregnancy is not included here as it is conceptually different from the newborn size measures; and, in the concept maps of newborn size, duration of pregnancy was included as an intermediate outcome):

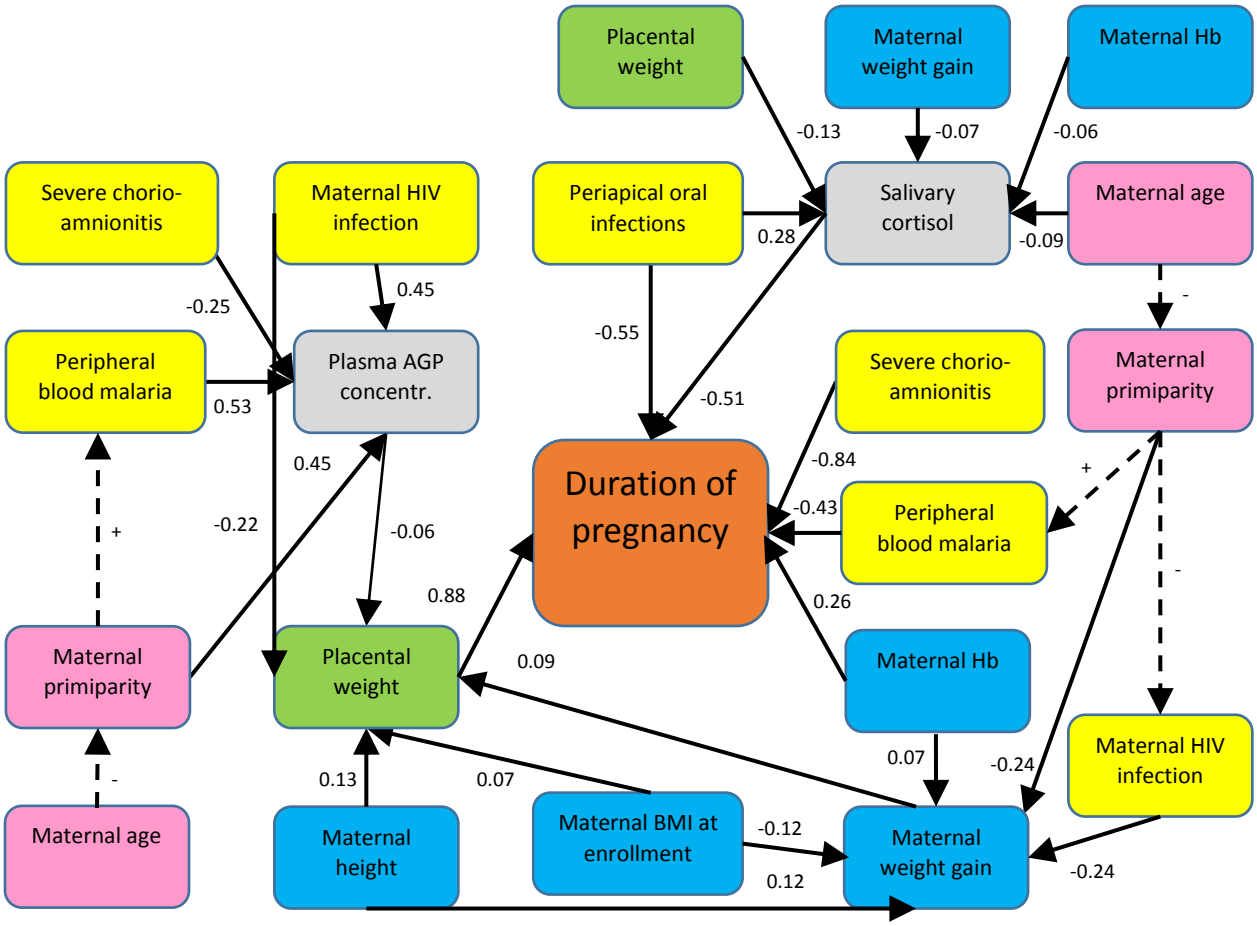
- All anthropometric indices were predicted by the duration of pregnancy, maternal inflammation (measured as maternal plasma AGP concentration), and placental weight.

- Newborn LAZ was additionally predicted by maternal infections (HIV and periapical oral infections), as well as by maternal height, weekly weight gain, and primiparity.
- Newborn WAZ was predicted by newborn LAZ, maternal primiparity and weight gain, and the three common predictors of birth size (duration of pregnancy, maternal inflammation, and placental weight). Additionally, it was predicted by maternal BMI at enrollment, but it was not predicted by maternal height or infections.

Newborn HCZ was predicted only by newborn LAZ, maternal BMI at enrollment, and the three common predictors of birth size. When adjusted for LAZ, newborn HCZ was not associated with maternal infections, height, weekly weight gain, or primiparity.

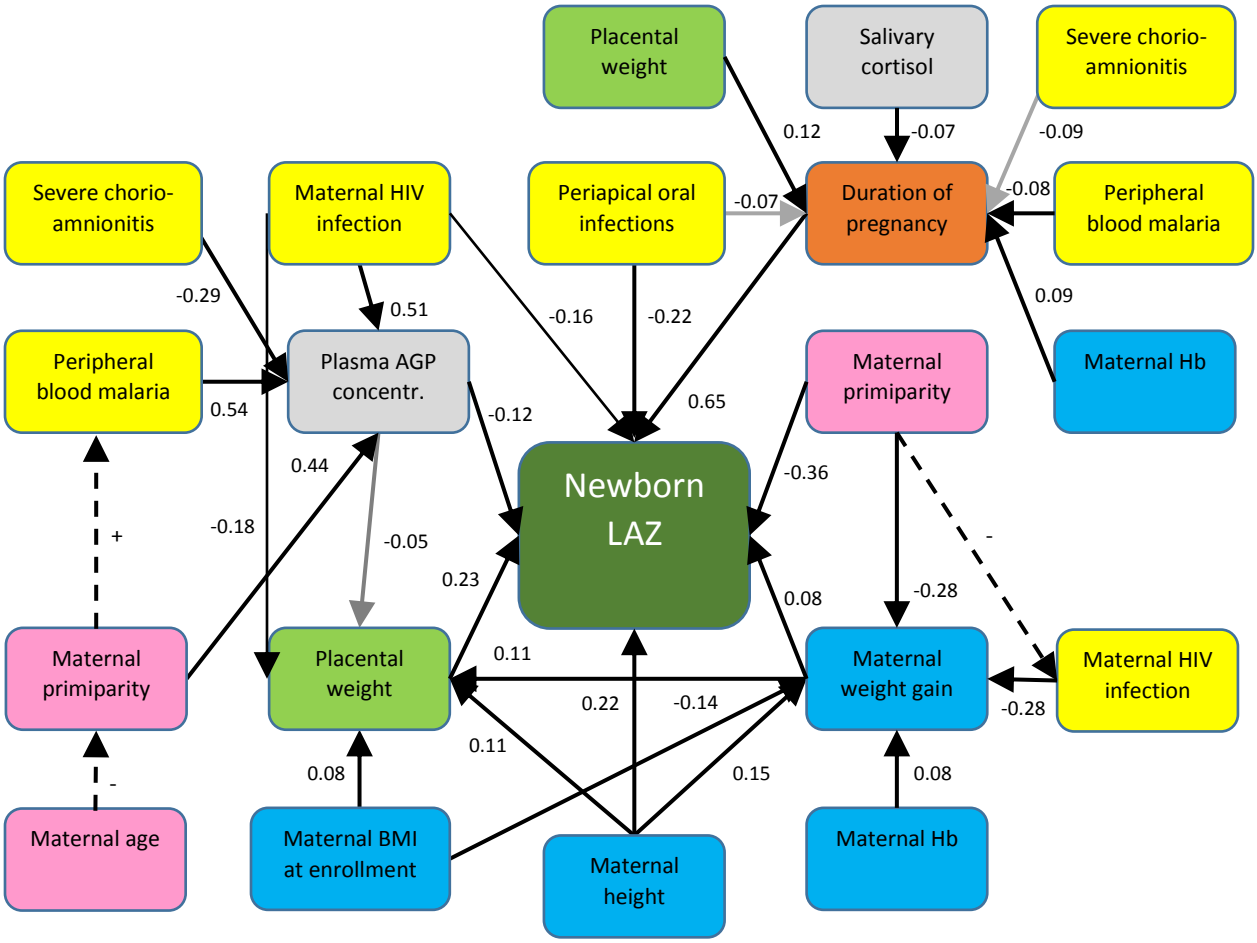
Table 5.4-1 (page 140) shows a summary of the models for the direct predictors of the duration of pregnancy and newborn LAZ, WAZ, and HCZ, which were used for drawing the pathway maps.

**Figure 5.4-1. Pathway Model: Determinants of the Duration of Pregnancy**



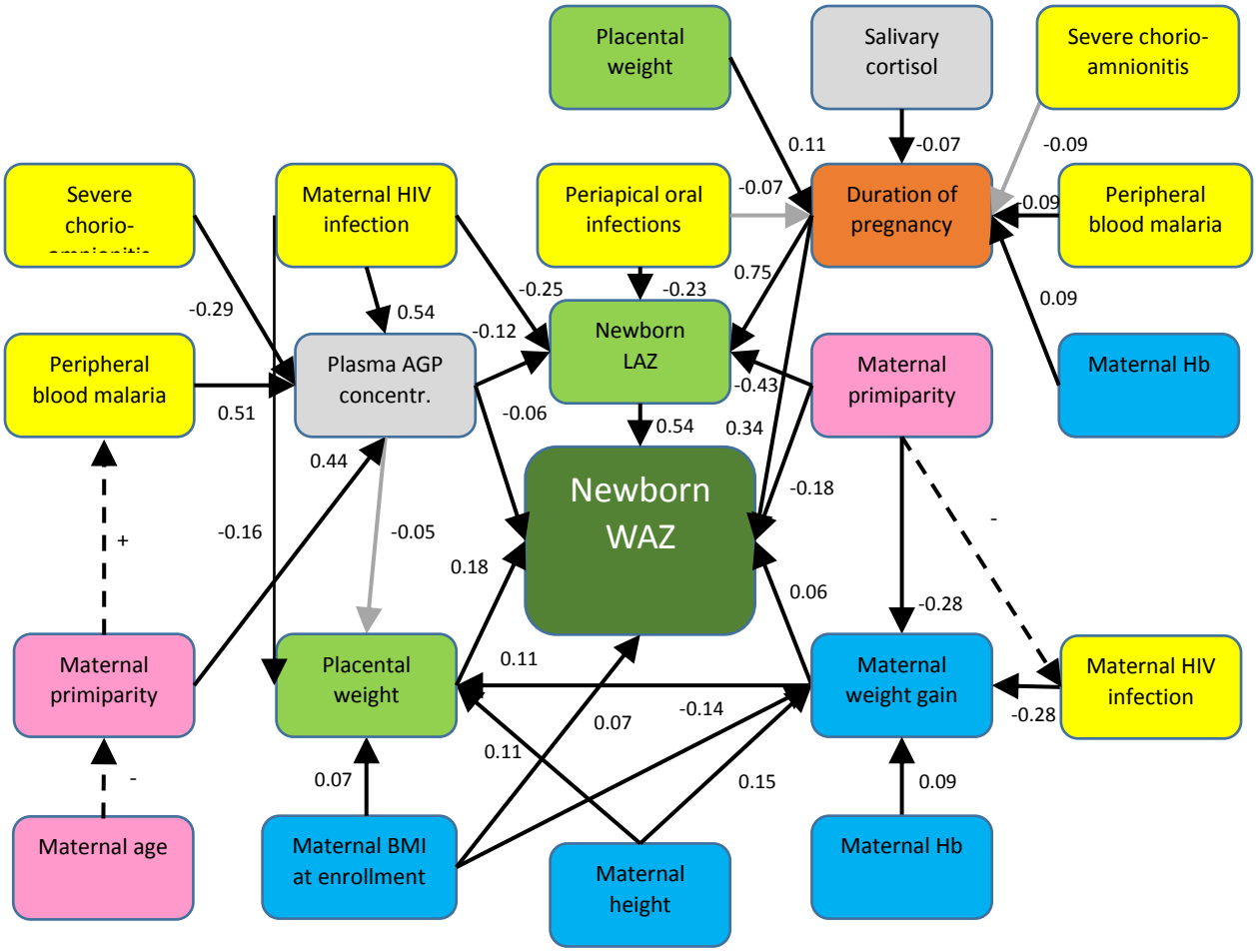

Legend: Number of observations used in the analysis: 1,379. In regression models used to define these pathways, all associations P<0.05. In SEM analyses, thick solid black lines represent P<0.05, thin solid black lines represent 0.05<P<0.1, and gray lines represent P≥0.1. Dashed lines indicate associations to dichotomous outcomes. The figures on the arrows going to the main outcome (duration of pregnancy) describe absolute change in the outcome (weeks), those on the arrows between other continuous variables describe change in standardized value (mean 0, SD 1). SEM do not allow dichotomous endogenous variables, so these associations come from regression models. ± indicates the direction of association.
Box color representation: Blue – maternal nutrition, Yellow – maternal infections, Pink – maternal constitutional variables, Gray – other variables, Green – pregnancy outcome, Orange – main outcome (duration of pregnancy).

**Figure 5.4-2. Pathway Model: Determinants of Newborn Length-for-Age Z-Score**



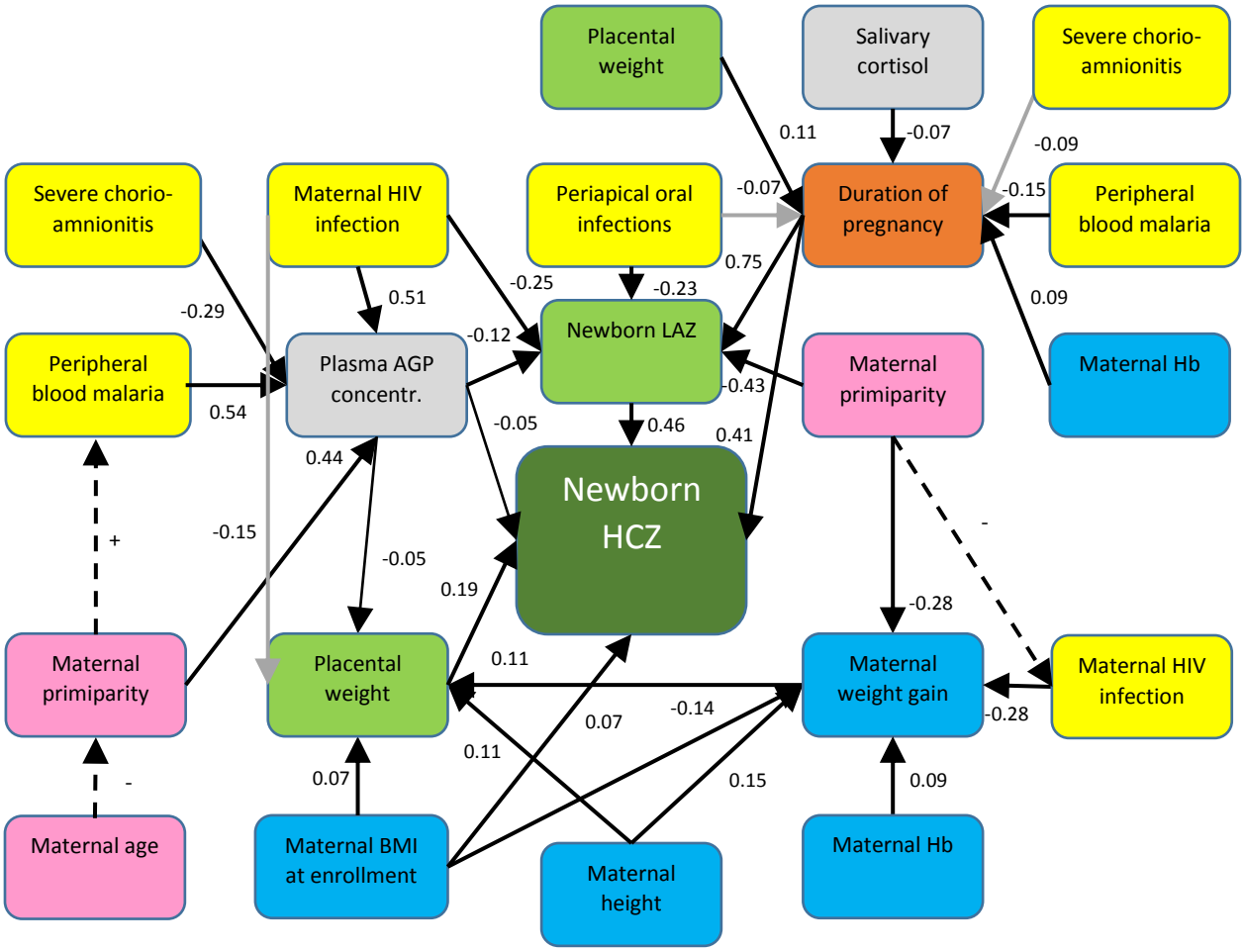

Legend: Number of observations used in the analysis: 1,179. In regression models used to define these pathways, all associations P<0.05. In SEM analyses, thick solid black lines represent P<0.05, thin solid black lines represent 0.05<P<0.1, and gray lines represent P≥0.1. Dashed lines indicate associations to dichotomous outcomes. The figures on the arrows going to the main outcome (newborn LAZ) describe absolute change in the outcome (z-score), those on the arrows between other continuous variables describe change in standardized value (mean 0, SD 1). SEM do not allow dichotomous endogenous variables, so these associations come from regression models. ± indicates the direction of association.
Box color representation: Blue – maternal nutrition, Yellow – maternal infections, Pink – maternal constitutional variables, Gray – other variables, Green – pregnancy outcome, Orange – duration of pregnancy, Dark green – main outcome (newborn LAZ).

### Figure 5.4-3. Pathway Model: Determinants of Newborn Weight-for-Age Z-Score




Legend: Number of observations used in the analysis: 1,179. In regression models used to define these pathways, all associations P<0.05. In SEM analyses, thick solid black lines represent P<0.05, thin solid black line represents 0.05<P<0.1, and gray lines represent P≥0.1. Dashed lines indicate associations to dichotomous outcomes. The figures on the arrows going to the main outcome (newborn WAZ) describe absolute change in the outcome (z-score); those on the arrows between other continuous variables describe change in standardized value (mean 0, SD 1). SEM do not allow dichotomous endogenous variables, so these associations come from regression models. ± indicates the direction of association.
Box color representation: Blue – maternal nutrition, Yellow – maternal infections, Pink – maternal constitutional variables, Gray – other variables, Orange – duration of pregnancy, Green – pregnancy outcome, Dark green – main outcome (newborn WAZ).

### Figure 5.4-4. Pathway Model: Determinants of Newborn Head Circumference-for-Age Z-Score




Legend: Number of observations used in the analysis: 1,179. In regression models used to define these pathways, all associations P<0.05. In SEM analyses, thick solid black lines represent P<0.05, thin solid black lines represent 0.05<P<0.1, and gray lines represent P≥0.1. Dashed lines indicate associations to dichotomous outcomes. The figures on the arrows going to the main outcome (newborn HCZ) describe absolute change in the outcome (z-score); those on the arrows between other continuous variables describe change in standardized value (mean 0, SD 1). SEM do not allow dichotomous endogenous variables, so these associations come from regression models. ± indicates the direction of association.
Box color representation: Blue – maternal nutrition, Yellow – maternal infections, Pink – maternal constitutional variables, Gray – other variables, Orange – duration of pregnancy, Green – pregnancy outcome, Dark green – main outcome (newborn HCZ).

### Table 5.4-1. Final Multivariable Regression Models for the Determinants of Pregnancy Duration and Newborn Size

| | Duration of pregnancy | | Newborn LAZ | | Newborn WAZ | | Newborn HCZ | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** | **Beta** | **P-value** |
| Maternal age at enrollment | 0.002 | 0.897 | 0.002 | 0.749 | 0.001 | 0.885 | 0.001 | 0.782 |
| Maternal primiparity | -0.139 | 0.559 | -0.280 | **0.002** | -0.135 | **0.027** | 0.092 | 0.246 |
| Maternal height at enrollment | 0.006 | 0.656 | 0.040 | **<0.001** | 0.004 | 0.277 | -0.003 | 0.491 |
| Maternal BMI at enrollment | -0.048 | *0.089* | 0.014 | 0.194 | 0.019 | **0.009** | 0.024 | **0.009** |
| Maternal Hb at enrollment | 0.017 | **0.001** | 0.001 | 0.732 | 0.002 | 0.220 | -0.003 | 0.117 |
| Maternal weekly weight gain | 0.387 | 0.620 | 0.791 | **0.007** | 0.518 | **0.008** | 0.238 | 0.346 |
| Maternal HIV infection | 0.014 | 0.954 | -0.169 | *0.052* | 0.044 | 0.459 | 0.105 | 0.171 |
| Maternal malaria infection at enrollment | -0.433 | **0.018** | -0.087 | 0.197 | -0.078 | *0.091* | -0.089 | 0.139 |
| Maternal periapical infections (diagnosed after delivery) | -0.585 | **0.028** | -0.230 | **0.002** | -0.024 | 0.645 | -0.058 | 0.366 |
| Maternal UTI (diagnosed after delivery) | -1.151 | *0.067* | -0.263 | 0.183 | -0.174 | 0.191 | 0.140 | 0.394 |
| Maternal trichomoniasis (diagnosed after delivery) | -0.465 | 0.125 | -0.005 | 0.955 | -0.077 | 0.235 | 0.001 | 0.989 |
| Maternal salivary cortisol concentration at 36 gw | -0.125 | **0.013** | 0.017 | *0.089* | 0.001 | 0.852 | -0.002 | 0.840 |
| Maternal plasma AGP concentration at enrollment | -0.109 | 0.743 | -0.393 | **0.002** | -0.224 | **0.009** | -0.235 | **0.032** |
| Placental weight (g) | 0.007 | **<0.001** | 0.002 | **<0.001** | 0.002 | **<0.001** | 0.002 | **<0.001** |
| Placental malaria infection | 0.206 | 0.392 | -0.074 | 0.284 | -0.009 | 0.858 | 0.028 | 0.662 |
| Severe chorioamnionitis | -0.891 | **0.043** | -0.090 | 0.365 | -0.026 | 0.710 | -0.062 | 0.450 |
| Duration of pregnancy | | | 0.221 | **<0.001** | 0.118 | **<0.001** | 0.142 | **<0.001** |
| Newborn LAZ | | | | | 0.517 | **<0.001** | 0.454 | **<0.001** |
| Intervention group – MMN | 0.013 | 0.942 | 0.048 | 0.486 | -0.030 | 0.517 | -0.007 | 0.906 |
| Intervention group – LNS | 0.008 | 0.963 | 0.053 | 0.444 | 0.004 | 0.932 | 0.016 | 0.797 |

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

## 5.5 Comparison of the Associations: Differential Prediction by Exposure and Outcome

Placental size strongly predicted all the studied outcomes, i.e., the duration of pregnancy and newborn LAZ, WAZ, and HCZ. Based on association analyses alone, it is of course difficult to determine the direction of causality, as placental growth could affect the duration of pregnancy and newborn size, shorter pregnancies could lead to smaller placentas at birth, and placental and newborn size could be driven by the same determinants without any causality between the two. A preliminary analysis indicated, however, that the mean placental weight remained rather constant toward the end of pregnancy, suggesting that at least at this stage the duration of pregnancy was not significantly influencing placental size (Appendix A3.4). Hence, we have built our models on the assumption that duration of pregnancy and newborn size may be a function of placental weight, rather than the opposite.

Maternal plasma AGP concentration at enrollment, used as a proxy for the presence of an inflammatory reaction in the mother, independently predicted newborn LAZ, WAZ, and HCZ, but not the duration of pregnancy. The association was strongest with LAZ, second strongest with WAZ, and weakest with HCZ. Maternal plasma AGP concentration itself was predicted by maternal primiparity, maternal peripheral blood malaria parasitemia, HIV infection, and chorioamnionitis, but not by periapical infections.

Maternal salivary cortisol concentration at 36 gw, used as a proxy for maternal stress reaction during pregnancy, predicted the duration of pregnancy, but not directly newborn size. Maternal salivary cortisol concentration at 36 gw was predicted by maternal age, blood Hb concentration, weight gain during pregnancy, placental size, and periapical infections, but not by other maternal infections.

Of the indicators of maternal nutritional status, maternal height at enrollment (probably mostly a constitutional characteristic, but maybe also an indicator of maternal stunting) was associated with newborn LAZ. Maternal blood Hb concentration at enrollment was associated with the duration of pregnancy, but not directly with any of the birth size indicators. Maternal BMI at enrollment was associated with newborn WAZ and HCZ, but not with the duration of pregnancy or newborn LAZ. Maternal weight gain during pregnancy was associated with newborn LAZ and WAZ (even after adjustment for LAZ), but not with the duration of pregnancy or newborn HCZ.

Of maternal infections, periapical oral infections independently predicted both the duration of pregnancy and newborn LAZ. HIV infection predicted newborn LAZ directly and duration of pregnancy indirectly, through its association with maternal plasma AGP concentration and placental size. Maternal malaria at enrollment and severe chorioamnionitis had a direct association with the duration of pregnancy and an indirect one with newborn LAZ, through both the duration of pregnancy and maternal plasma AGP concentration. Of the infections, HIV was also associated with lower maternal weight gain, which in turn was associated with lower newborn LAZ (and WAZ).

When adjusted for newborn LAZ, none of the studied infections was associated with newborn WAZ or HCZ. There was, however, an indirect association between infections and newborn WAZ and between infections and HCZ, as these latter outcomes were predicted by newborn LAZ, which in turn was strongly associated with newborn WAZ and HCZ.

Of maternal constitutional factors, young age and primiparity were associated with lower weight gain in pregnancy and increased risk of malaria, but decreased risk of HIV infection or periapical oral infections. Because of these opposite associations with variables that were “intermediate” on the pathway to birth outcomes, young maternal age and primiparity were not associated with reduced duration of pregnancy, although primiparity was associated with newborn size.

# 6. The Predictive Value of Various Maternal Characteristics for Birth Outcomes

The goal of the study was to identify variables involved in the pathways leading to reduced duration of pregnancy and IUGR (i.e., LAZ, WAZ, and HCZ) in a rural Malawian setting. In a sample of 1,391 pregnant women, the duration of pregnancy was predicted by maternal peripheral blood malaria parasitemia at enrollment, severe chorioamnionitis, and the presence of periapical oral infections soon after birth. Additionally, pregnancy duration was predicted by maternal blood Hb concentration at enrollment, salivary cortisol concentration at 36 gw, and placental weight. All newborn size measurements were predicted by the duration of pregnancy, placental weight, and maternal inflammation. In addition, newborn LAZ was independently associated with maternal infections and weight gain during pregnancy, newborn WAZ was associated with maternal BMI at enrollment and weight gain during pregnancy, and newborn HCZ was associated with maternal BMI at enrollment. In the study sample, some differences in the pathways predicting linear, ponderal, and head growth in the fetal period were thus observed, with infections being most directly associated with length gain and the duration of pregnancy, maternal BMI at enrollment and weight gain being important predictors of fetal weight gain, and fewer of the predictor variables being linked to head growth. For each outcome, however, there was a complex network of proximal and more distal determinants, so that all dimensions of fetal growth were ultimately associated both with maternal nutritional status and with variables reflecting infection, inflammation, and stress, directly or indirectly.

The methodological strengths of the study included a prospective study design, comprehensive data collection that included both clinical and laboratory variables, and rigorous quality assurance in data collection. Internal validity could have been compromised by missing data, the delay in anthropometric measurements of some participants, and the choice of modeling technique and the variables included in the pathway analyses. We believe these factors did not significantly bias our conclusions because participants with missing values typically had similar enrollment characteristics to those who provided data, we used multiple imputations to estimate the missing values in regression models, we used the maximum likelihood method in SEM to account for the missing data, and the results were robust to several sensitivity analyses. We used SEM to examine relationships among many variables simultaneously and to see how the hypothesized models of relationships from stepwise regression models fit the data (DiLalla 2008). We selected variables used in the models based on results of this report and earlier knowledge. Therefore, we conclude that the findings are valid and representative and can be used to infer causal pathways to adverse pregnancy outcomes in the population from which the sample was drawn.

Maternal nutrition, reproductive tract infections, chorioamnionitis, malaria, inflammation, and stress have been associated with reduced duration of pregnancy in low-income settings in previous studies (Goldenberg et al. 2008). Determinants of IUGR, on the other hand, can be categorized into maternal, placental, and fetal factors (Brodsky and Christou 2004). Within these categories, maternal factors associated with IUGR have included vascular disorders, hypercoagulable states (such as antiphospholipid antibody syndrome), chronic hypoxia, undernutrition, and uterine malformations. Placental factors comprise various pathologies that interfere with placental function, and fetal factors include genetic aberrations; multiple gestation; and infections, such as malaria, HIV, and other congenital viral infections (Resnik 2002, Brodsky and Christou 2004, Hendrix and Berghella 2008). Our results corroborate these earlier findings and provide a concept map of pathways leading to birth outcomes in one rural population in sub-Saharan Africa. Because of the similarity in the identified determinants in our setting and those observed for earlier cohorts, it is likely that maternal nutrition, infection, inflammation, and stress form

some kind of a complex network in other low-income settings as well. However, in the absence of publications reporting similar pathway analyses, it is impossible to draw broader conclusions on whether the processes leading to adverse birth outcomes are similar or vary across settings.

There are several different possible mechanisms underlying the negative associations between maternal infections and duration of pregnancy. First, a local inflammatory response can weaken amniotic membranes and cause their premature rupture (Goldenberg et al. 2008). Additionally, inflammation typically activates prostaglandin synthesis, which may lead to increased contractibility of the uterus and softening and shortening of the cervix, thus inducing preterm labor (Challis et al. 2000). Infection-related restriction of fetal linear growth can, on the other hand, be potentially explained by inflammation-induced reduction in placental vascularization and function and a disturbance in growth-hormone mediated elongation of long bones (Boeuf et al. 2013, Conroy et al. 2013).

Evidence for the infection-related placental insufficiency pathway comes from both human studies and animal experiments. For instance, in a case control study of 492 pregnant Malawian women, placental transfer of selected amino acids was lower among women who had placental malaria and a local inflammatory response than women who had no malaria or no major inflammatory reaction (Boeuf et al. 2013). Women with placental malaria had also a higher mean plasma concentration of complement component C5a, which is an important mediator of human inflammatory response. Maternal plasma C5a concentration was positively associated with the risk of delivering a SGA baby and negatively correlated with maternal plasma concentration of several growth factors involved in the vascularization of the placenta (Conroy et al. 2013). In a murine model, placental malaria was characterized by increased C5a expression and reduced vascularization of the placenta. When C5a binding to its receptor was prevented by antibodies or genetic modification of the host receptor, the mice still developed malaria, but they exhibited fewer disturbances in fetoplacental blood vessel development, reduced placental vascular resistance, and improved fetal growth and survival (Conroy et al. 2013).

In addition to its effects on placental vascularization and function, systemic inflammation has been shown to alter a hormonal pathway that drives the elongation of long bones in human fetuses and children. In a normal situation, the pituitary gland secretes growth hormone, which stimulates the expression of a secondary hormone called insulin like growth factor 1 (IGF-1) in the liver and other target tissues (Le Roith et al. 2001). Although growth hormone also has a direct effect on bone elongation, the major share of linear growth is driven by IGF-1, which stimulates chondrocyte proliferation at the growth plates. Both animal and human studies have indicated that systemic inflammation is characterized by increased plasma concentration of cytokines IL-1, IL-6, and TNF-alpha (Klasing and Johnstone 1991, Walters and Griffiths 2009, Bolton et al. 2012). These molecules can cause chondrocyte death at the growth plates and also interrupt specific steps in the growth hormone-induced JAK/STAT signal transduction pathway in liver cells, leading to reduced expression of IGF-1 and subsequent linear growth failure (Walters and Griffiths 2009). Further evidence for the importance of IGF-1 in fetal growth and growth restriction comes from human genetic studies showing associations between IGF-1 gene polymorphism and birth size, with severe fetal growth restriction being associated with major aberrations in the IGF-1 gene (Woods et al. 1996, Klammt et al. 2011, Netchine et al. 2011). Finally, in murine and rabbit models, overexpression of the IGF-1 gene in placental tissue has prevented IUGR in the fetus (Jones et al. 2013, Keswani et al. 2015).

Whereas maternal infections and inflammation were important determinants of the duration of pregnancy and newborn LAZ in the studied cohort, the pathways to duration of pregnancy and newborn WAZ also always included maternal undernutrition, stress, and certain constitutional factors like age and parity. Because of this complex network of adverse exposures, it is not surprising that single-pronged nutritional

or infection-targeted antenatal interventions have had at best modest impacts on fetal growth or duration of pregnancy in low-income settings (Bhutta et al. 2013, Ashorn et al. 2015). For greater impact, it is likely that more-comprehensive multipronged interventions will be needed, to ensure good nutritional status for the mother both before and during pregnancy, prevention and treatment of a wide range of maternal infections, and reduction of maternal stress. For infection control, the current study suggests that maternal HIV infection, malaria, chorioamnionitis, and also oral infections should be targeted, but there is no reason to believe that this list of infections was exhaustive. Further studies should be conducted to identify other viral, parasitic, or bacterial infections that elicit systemic inflammation in pregnant women and contribute to adverse birth outcomes in low-income settings.

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# Appendix 1. Comparison of the Enrollment Characteristics of Participants Who Were Included and Excluded from the Predictor Analyses of Birth Outcomes

## A1.1. Maternal Enrollment BMI and Weight Gain during Pregnancy

Due to the small number of excluded participants, no table is provided on enrollment characteristics of included and excluded participants for the section on maternal BMI at enrollment and weight gain during pregnancy (Section 4.1).

## A1.2. Maternal Asymptomatic Malaria Infections at Enrollment and 32 gw

### Table A1.2-1. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1141 | 250 | |
| Mean (SD) BMI (kg/m²) | 22.0 (2.7) | 22.7 (3.1) | <0.001 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | 0.018 |
| Mean (SD) maternal educational achievement (completed years at school) | 4.0 (3.0) | 4.0 (4.0) | 1.000 |
| Mean (SD) proxy for SES | −0.05 (0.96) | 0.29 (1.20) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 19.1% | 27.8% | 0.001 |
| Percentage of primiparous women | 19.1% | 34.5% | <0.001 |
| Percentage of women with a low BMI (<18.5) | 5.5% | 4.8% | 1.000 |
| Percentage of women with a positive HIV test | 13.6% | 14.6% | 0.706 |
| Percentage of women with a positive malaria test (RDT) | 22.3% | 27.5% | 0.042 |

[a] Those women who were included in the malaria at enrollment and birth outcome analysis.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.3. Maternal HIV, Trichomoniasis, or UTI at Delivery

### Table A1.2-2. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1132 | 259 | |
| Mean (SD) BMI (kg/m²) | 22.0 (2.7) | 22.8 (3.2) | <0.001 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | 0.017 |
| Mean (SD) maternal educational achievement (completed years at school) | 4.0 (3.0) | 4.0 (4.0) | 1.000 |
| Mean (SD) proxy for SES | −0.05 (0.95) | 0.30 (1.20) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 19.3% | 26.7% | 0.004 |
| Percentage of primiparous women | 18.6% | 36.1% | <0.001 |
| Percentage of women with a low BMI (<18.5) | 5.4% | 5.0% | 1.000 |
| Percentage of women with a positive HIV test | 13.5% | 14.9% | 0.707 |
| Percentage of women with a positive malaria test (RDT) | 22.1% | 27.8% | 0.016 |

[a] Those women who were included in the HIV and birth outcome analysis.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.4. Maternal Plasma CRP and AGP Concentrations

### Table A1.2-3. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1063 | 328 | |
| Mean (SD) BMI (kg/m²) | 22.1 (2.8) | 22.3 (2.8) | 0.181 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | <0.001 |
| Mean (SD) maternal educational achievement (completed years at school) | 4.0 (3.4) | 4.0 (3.7) | 0.883 |
| Mean (SD) proxy for SES | −0.04 (1.0) | 0.19 (1.1) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 18.6% | 27.5% | <0.001 |
| Percentage of primiparous women | 19.8% | 28.4% | <0.001 |
| Percentage of women with a low BMI (<18.5) | 5.5% | 4.9% | 0.671 |
| Percentage of women with a positive HIV test | 12.7% | 17.5% | 0.039 |
| Percentage of women with a positive malaria test (RDT) | 22.7% | 24.7% | 0.463 |

[a] Those included had maternal plasma CRP and AGP concentrations analyzed at 36 gw.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.5. Maternal Blood Hb, ZPP, and sTfR Concentrations

### Table A1.2-4. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1067 | 324 | |
| Mean (SD) BMI (kg/m²) | 22.1 (2.8) | 22.4 (2.8) | 0.123 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | <0.001 |
| Mean (SD) maternal educational achievement (completed years at school) | 4.0 (3.4) | 4.0 (3.6) | 0.914 |
| Mean (SD) proxy for SES | −0.04 (1.0) | 0.18 (1.1) | 0.002 |
| Percentage of anemic women (Hb <100 g/L) | 18.6% | 27.6% | <0.001 |
| Percentage of primiparous women | 19.7% | 28.8% | <0.001 |
| Percentage of women with a low BMI (<18.5) | 5.7% | 4.3% | 0.352 |
| Percentage of women with a positive HIV test | 12.7% | 17.8% | 0.029 |
| Percentage of women with a positive malaria test (RDT) | 22.8% | 24.4% | 0.564 |

[a] Those included had sTfR analyzed at 36 gw, as sTfR had the highest sample size of the three predictor variables.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.6. Maternal Plasma Retinol Concentration

### Table A1.2-5. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 314 | 1077 | |
| Mean (SD) BMI (kg/m²) | 21.8 (2.7) | 22.3 (2.9) | 0.012 |
| Mean (SD) maternal age, years | 25 (6) | 25 (6) | 0.357 |
| Mean (SD) maternal educational achievement (completed years at school) | 3.6 (3.4) | 4.2 (3.5) | 0.010 |
| Mean (SD) proxy for SES | −0.17 (0.83) | 0.06 (1.05) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 20.9% | 20.6% | 0.875 |
| Percentage of primiparous women | 21.5% | 21.9% | 0.938 |
| Percentage of women with a low BMI (<18.5) | 6.3% | 5.0% | 0.393 |
| Percentage of women with a positive HIV test | 11.1% | 13.8% | 0.135 |
| Percentage of women with a positive malaria test (RDT) | 23.4% | 23.1% | 0.879 |

[a] Those included in a randomly selected subset for assessment of vitamin A status based on maternal plasma retinol concentration.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.7. Maternal Plasma Cholesterol and Triglyceride Concentrations and Plasma Fatty Acid Composition

### Table A1.2-6. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1061 | 330 | |
| Mean (SD) BMI (kg/m²) | 22.1 (2.8) | 22.4 (2.8) | 0.095 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | 0.001 |
| Mean (SD) maternal educational achievement (completed years at school) | 4.0 (3.4) | 4.0 (3.6) | 0.836 |
| Mean (SD) proxy for SES | −0.04 (0.96) | 0.16 (1.13) | 0.004 |
| Percentage of anemic women (Hb <100 g/L) | 18.6% | 27.4% | <0.001 |
| Percentage of primiparous women | 19.7% | 28.6% | <0.001 |
| Percentage of women with a low BMI (<18.5) | 5.7% | 4.3% | 0.399 |
| Percentage of women with a positive HIV test | 12.7% | 17.4% | 0.051 |
| Peercentage of women with a positive malaria test (RDT) | 22.9% | 24.2% | 0.602 |

[a] Those with plasma cholesterol and triglycerides measurements at 36 gw.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.8. Maternal Perceived Stress and Salivary Cortisol Concentration

### Table A1.2-7. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1237 | 154 | |
| Mean (SD) BMI (kg/m²) | 22.1 (2.8) | 22.6 (2.9) | 0.405 |
| Mean (SD) maternal age, years | 25 (6) | 23 (6) | 0.139 |
| Mean (SD) maternal educational achievement (completed years at school) | 4.0 (3.8) | 4.1 (3.4) | 0.780 |
| Mean (SD) proxy for SES | –0.02 (0.99) | 0.33 (1.11) | 0.095 |
| Percentage of anemic women (Hb <100 g/L) | 19.7% | 29.2% | 0.008 |
| Percentage of primiparous women | 20.6% | 32.6% | 0.001 |
| Percentage of women with a low BMI (<18.5) | 5.4% | 4.9% | 0.781 |
| Percentage of women with a positive HIV test | 13.4% | 17.0% | 0.297 |
| Percentage of women with a positive malaria test (RDT) | 23.5% | 23.2% | 0.940 |

[a] Those with at least some data on perceived stress or salivary cortisol at enrollment, 28 gw, or 36 gw.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.9. Histological Signs of Inflammation and Malaria in the Placenta and Fetal Membranes

### Table A1.2-8. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1008 | 383 | |
| Mean (SD) BMI (kg/m²) | 22.0 (2.7) | 22.5 (3.1) | 0.006 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | 0.030 |
| Mean (SD) maternal educational achievement (completed years at school) | 3.9 (3.4) | 4.4 (3.7) | 0.051 |
| Mean (SD) proxy for SES | –0.07 (0.9) | 0.25 (1.2) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 19.9% | 22.9% | 0.232 |
| Percentage of primiparous women | 20.2% | 26.7% | 0.012 |
| Percentage of women with a low BMI (<18.5) | 5.4% | 5.4% | 1.000 |
| Percentage of women with a positive HIV test | 13.2% | 14.9% | 0.458 |
| Percentage of women with a positive malaria test (RDT) | 23.3% | 23.1% | 1.000 |

[a] Those participants who had a section of placental tissue taken after delivery with intervillous space identifiable to allow for examination of intervillositis and malarial infection.
[b] P-values were obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.10. Placenta, Fetal Membrane, Oral, and Vaginal Microbiomes

### Table A1.2-9. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1107 | 284 | |
| Mean (SD) BMI (kg/m²) | 22.1 (2.8) | 22.4 (2.9) | 0.126 |
| Mean (SD) maternal age, years | 25 (6) | 24 (7) | 0.025 |
| Mean (SD) maternal educational achievement (completed years at school) | 3.9 (3.4) | 4.5 (3.7) | 0.049 |
| Mean (SD) proxy for SES | −0.05 (0.9) | 0.30 (1.1) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 19.9% | 23.9% | 0.156 |
| Percentage of primiparous women | 20.7% | 29.6% | 0.001 |
| Percentage of women with a low BMI (<18.5) | 4.1% | 5.7% | 0.367 |
| Percentage of women with a positive HIV test | 12.2% | 13.9% | 0.591 |
| Percentage of women with a positive malaria test (RDT) | 22.4% | 23.5% | 0.749 |

[a] Those participants who had a vaginal swab taken after delivery that was processed for DNA extraction and sequencing.
[b] P-values were obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.11. Maternal Oral Health

### Table A1.2-10. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1024 | 367 | |
| Mean (SD) BMI (kg/m²) | 22.1 (2.7) | 22.4 (3.1) | 0.046 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | <0.001 |
| Mean (SD) maternal educational achievement (completed years at school) | 3.9 (3.4) | 4.3 (3.6) | 0.087 |
| Mean (SD) proxy for SES | −0.13 (1.68) | 0.46 (2.12) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 19.8% | 23.0% | 0.208 |
| Percentage of primiparous women | 17.8% | 33.3% | <0.001 |
| Percentage of women with a low BMI (<18.5) | 5.4% | 5.2% | 0.891 |
| Percentage of women with a positive HIV test | 14.1% | 12.5% | 0.460 |
| Percentage of women with a positive malaria test (RDT) | 21.4% | 28.1% | 0.009 |

[a] Those who had singleton pregnancies and completed the oral health visit within 6 weeks of delivery.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A1.12. Malaria Immunity in Pregnancy

### Table A1.2-11. Enrollment Characteristics of the Included and Excluded Participants

| Characteristic | Included[a] | Excluded | P-value[b] |
|---|---|---|---|
| Number of participants | 1009 | 382 | |
| Mean (SD) BMI (kg/m²) | 22.1 (2.8) | 22.4 (2.8) | 0.089 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | <0.001 |
| Mean (SD) maternal educational achievement (completed years at school) | 4.0 (3.4) | 4.0 (3.6) | 0.775 |
| Mean (SD) proxy for SES | −0.14 (0.96) | 0.15 (1.12) | 0.007 |
| Percentage of anemic women (Hb <100 g/L) | 42.2% | 50.0% | 0.007 |
| Percentage of primiparous women | 19.7% | 27.6% | <0.001 |
| Percentage of women with a low BMI (<18.5) | 5.7% | 4.5% | 0.612 |
| Percentage of women with a positive HIV test | 12.8% | 13.6% | 0.195 |
| Percentage of women with a positive malaria test (RDT) | 23.2% | 23.3% | 0.966 |

[a] Those that had plasma pairs collected at enrollment and 36 gw.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

# Appendix 2. Supplementary Details in Data Collection and Statistical Analyses

## A2.1. Maternal BMI and Weight Gain during Pregnancy

**Details of data collection or laboratory analysis.** Study nurses were trained on appropriate methods of measuring maternal height and weight. Nurses measured maternal height (ShorrBoard Infant/Child/Adult, Weigh and Measure, LLC, Olney, MD, USA) and weight (Seca 876, Seca, Hamburg, Germany) in triplicate in the study clinics, with any outlying measurements omitted at the time of statistical analysis. For measurements that were completed in triplicate, we used the mean of the first two readings if they did not differ by more than a prespecified tolerance limit (0.5 cm for length/height measurements and 0.1 kg for infant/child/adult weight). If the difference was above the limit, the third measurement was compared with the first and second measurements, and the pair of measurements that had the smallest difference was used to calculate the mean. If there were only one or two repeated measurements, the mean of those was used for the analyses.

**Details of statistical analysis.** We carried out the statistical analysis with SAS version 9.3 software package (SAS Institute Inc., Cary, NC, USA).

Linear regression models were used to examine the associations between maternal BMI at enrollment and average weekly weight gain and birth outcomes (duration of gestation, birth weight, LAZ, and HCZ). Linear regression coefficients are presented as standardized coefficients, which are the number of SDs a dependent variable will change, per SD increase in the predictor variable. We used standardized coefficients to assess the strength of association between the predictor and birth outcome in a given model in comparison with the association between another predictor and that same birth outcome in a different model. The use of standardized coefficients allows for such comparisons between predictor variables for the same outcome, even though the units of the predictor variables differ (i.e., BMI vs. weekly weight gain). We computed standardized coefficients by multiplying the original regression coefficient by the SD of the predictor variable and then dividing by the SD of the dependent variable.

We used Poisson regression models to estimate the relative risk for dichotomous birth outcomes (preterm birth, LBW, stunting, SGA, and small head circumference) by categorical predictors of maternal BMI at enrollment (<18.5 vs. ≥18.5 and <25.0, and ≥25.0 vs. ≥18.5 and <25.0) and average weekly gestational weight gain (less than the lower limit of the IOM-recommended weight gain [which is based on pre-pregnancy BMI] vs. within the IOM-recommended range, and greater than the IOM-recommended weight gain vs. within the IOM-recommended range). Women with a maternal BMI at enrollment <18.5 were classified as being underweight, while women with a maternal BMI at enrollment ≥25.0 were considered overweight or obese. The cutoff for adequate or inadequate rate of gestational weight gain was the lower limit of the IOM's recommended range of average weekly weight gain during pregnancy, which takes the pre-pregnancy BMI into consideration. Since pre-pregnancy BMI was not available for study participants, we used regression modeling to create a proxy for pre-pregnancy BMI. When inspecting the regression curve, 13.7 gw was the lower limit of the curve at which the 95% CI fit closely to the regression curve. We therefore used the estimated BMI at 13.7 gw as the proxy for pre-pregnancy BMI and assumed that minimal change in BMI occurred between pre-pregnancy and 13.7 gw, based on IOM assumptions of weight gain in the first trimester (0.5–2.0 kg).

A set of variables was selected a priori based on the reported associations with birth outcomes and examined as potential covariates. Those variables included maternal inflammation (AGP and CRP), maternal malaria status at enrollment, maternal HIV status, primiparity, maternal educational achievement, season of enrollment, site of enrollment, maternal Hb concentration at enrollment, maternal

iron status (ZPP and sTfR) at enrollment, maternal age at enrollment, maternal height at enrollment, marital status, household food insecurity score, gestational age at enrollment, proxy for SES, and child sex. Those that were significantly associated with a given birth outcome (P<0.10) in bivariate analysis were included in an adjusted model for that birth outcome to control for possible confounding.

## A2.2. Maternal Asymptomatic Malaria Infections at Enrollment and 32 gw

**Details of data collection or laboratory analysis.** Maternal asymptomatic malaria infections were assessed at enrollment and at 32 gw. Finger prick blood samples were collected for RDT testing at enrollment and 32 gw. RDT was performed using Clearview® Malaria Combo (British Biocell International Ltd., Dundee, UK), which detects the antigens histidine-rich protein 2 and *Plasmodium*-specific aldolase. The test result options were: 1) the presence of *Plasmodium falciparum*, 2) the presence of other non-specified *Plasmodium* species, 3) negative result, or 4) invalid result.

**Details of statistical analysis.** We carried out the statistical analysis with Stata 12.1 (StataCorp, College Station, TX, USA).

We compared enrollment characteristics of women included and excluded from analyses by a t-test to compare means and Fisher's exact test to compare proportions.

We calculated relative risks for comparison of dichotomous end-points and differences in means for comparison of continuous end-points at a single time point. With the central limit theorem, such an analysis of means is robust and valid also in the case of a skewed outcome distribution, due to the large sample size (Cheung 2014).

A set of variables was selected a priori based on the reported associations with birth outcomes and examined as potential covariates. Those that were significantly associated (P<0.10) with a given birth outcome in bivariate analysis were included in an adjusted model for that birth outcome to control for possible confounding. We performed the covariate selection with linear and logistic regression models. Covariate selection was done separately for malaria at enrollment and malaria at 32 gw. The total group of selected covariates were maternal age at enrollment, number of previous pregnancies, proxy for SES, maternal educational achievement, maternal height at enrollment, maternal MUAC at enrollment, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment, parity, child sex, maternal anemia at enrollment, season of enrollment, intervention group, and site of enrollment.

## A2.3. Maternal HIV, Trichomoniasis, or Urinary Tract Infection at Delivery

**Details of data collection or laboratory analysis.** HIV testing was performed using a whole-blood antibody rapid test (Alere Determine HIV-1/2, Alere Medical Co., Ltd., Chiba, Japan). A woman was considered not to have HIV infection if the result of the first test was negative. If the test result came out positive, the test was repeated using a second whole-blood antibody rapid test (Uni-Gold HIV, Trinity Biotech plc, Bray, Ireland). If the second test result was also positive, the woman was determined to have HIV infection. However, if the second test result came out negative, HIV infection was considered to be indeterminate and a third test (SD Biostandard Diagnostics Private Limited, Gurgaon, Haryana, India) was used as a "tiebreaker." All tests were performed as per instructions in the test kit inserts.

Trichomoniasis was diagnosed by direct microscopy. After receiving the vaginal swab sample from the clinic, the laboratory technician immediately smeared the vaginal discharge on a microscope slide without prior cleaning and added a cover slide. Microscopic examination of the sample was performed immediately. Trichomoniasis was diagnosed on the visualization of motile protozoa.

UTI was diagnosed by urine dipstick analysis. The study nurse dipped the urine dipstick into the urine sample and immediately removed it. After a minute, the dipstick was compared to the color code on the dipstick bottle to determine the presence and intensity of nitrite in the sample. The nurse then recorded whether there was nitrite detected or not, and, if present, the intensity of the nitrite was indicated on a scale of 1 to 4 "+" signs. The presence of nitrite regardless of intensity was considered to indicate the presence of a bacterial UTI.

**Details of statistical analysis.** We carried out the statistical analysis with Stata 12.1 (StataCorp, College Station, TX, USA).

We compared enrollment characteristics of women included and excluded from analyses by a t-test to compare means and Fisher's exact test to compare proportions.

We calculated relative risks for comparison of dichotomous end-points and differences in means for comparison of continuous end-points at a single time point. With the central limit theorem, such an analysis of means is robust and valid also in the case of a skewed outcome distribution, due to the large sample size (Cheung 2014).

A set of variables was selected a priori based on the reported associations with birth outcomes and examined as potential covariates. Those that were significantly associated ($P<0.10$) with a given birth outcome in bivariate analysis were included in an adjusted model for that birth outcome to control for possible confounding. We performed the covariate selection with linear and logistic regression models. Covariate selection was done separately for HIV, trichomoniasis, and UTI outcomes. The total group of selected covariates were maternal age at enrollment, number of previous pregnancies, proxy for SES, maternal educational achievement, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment, matenral anemia at enrollment, child sex, maternal height at enrollment, maternal MUAC at enrollment, intervention group, season of enrollment, and site of enrollment.

## A2.4. Maternal Plasma CRP and AGP Concentrations

**Details of data collection or laboratory analysis.** Clinic nurses collected blood from the antecubital vein into a 7.5 mL trace mineral-free polypropylene syringe (Sarstedt Monovette, NH4-heparin, Sarstedt Inc., Newton, NC, USA). The blood tube was inverted 10 times to mix the heparin anticoagulant with the blood to prevent clotting. The tube was placed in an insulated cooler with ice packs until processing. Trained lab staff centrifuged the whole blood at 3,000 RPM for 15 minutes and separated plasma into storage cryovials. The storage vials were placed upright in freezer boxes and put into in a −20°C freezer for temporary storage at the satellite clinics. Within 48 hours, drivers transported the plasma to the main laboratory for long-term storage at −80°C.

Plasma was shipped to UCD on dry ice (via World Courier) for analysis. We analyzed maternal plasma CRP and AGP concentrations from those samples by immunoturbidimetry on the Cobas Integra 400 system autoanalyzer (F. Hoffmann-La Roche Ltd, Basel, Switzerland). All samples were analyzed in singlet, except for 5%, which we randomly selected to be analyzed in duplicate. None of the samples analyzed in duplicate had a coefficient of variation (CV) greater than 5%.

**Details of statistical analysis.** We carried out the statistical analysis with SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

We compared enrollment characteristics of women included and excluded from the 36 gw analyses by a t-test to compare means and Fisher's exact test to compare proportions.

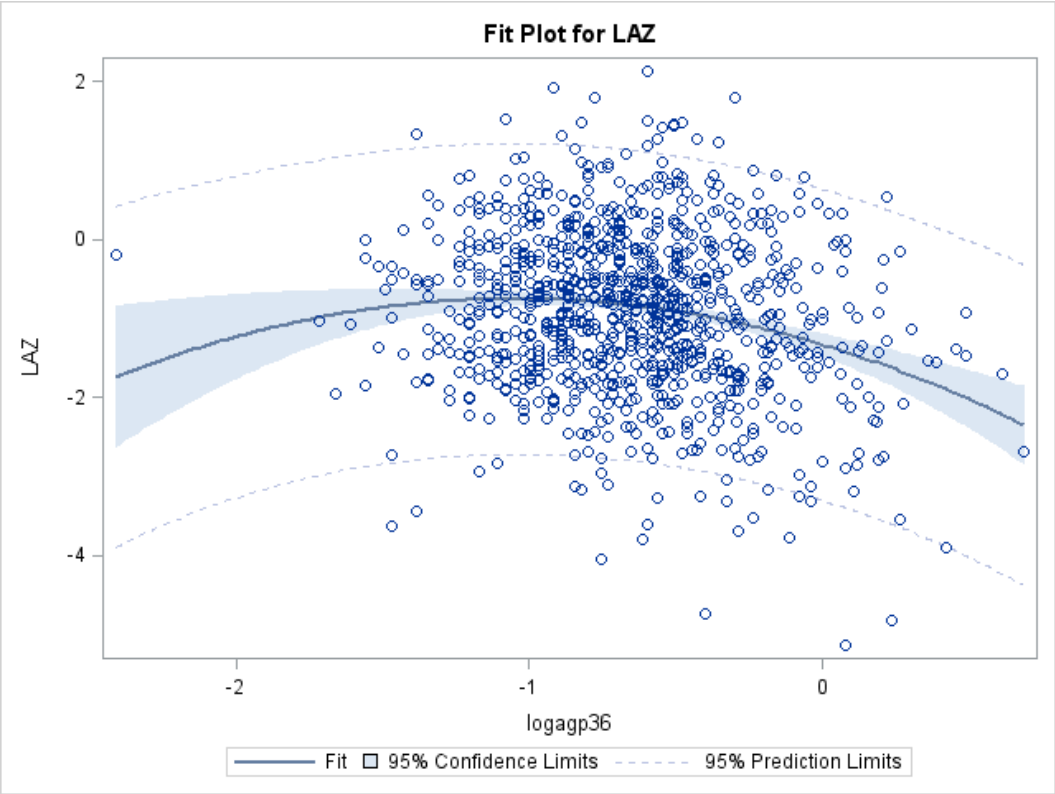
Linear regression models were used to examine the associations between maternal plasma CRP or AGP concentration and continuous birth outcomes (duration of gestation, birth weight, LAZ, WAZ, and HCZ). Both maternal plasma CRP and AGP concentrations were log-transformed before any analyses were performed. Linear regression coefficients are presented as standardized coefficients, which are the number of SDs a dependent variable will change, per SD increase in the predictor variable. We used standardized coefficients to assess the strength of association between the predictor and birth outcome in a given model in comparison with the association between another predictor and that same birth outcome in a different model. The use of standardized coefficients allows for such comparisons between predictor variables for the same outcome, even though the units of the predictor variables differ (e.g., maternal plasma CRP concentration vs. maternal plasam AGP concentration). We computed standardized coefficients by multiplying the original regression coefficient by the SD of the predictor variable and then dividing by the SD of the dependent variable.

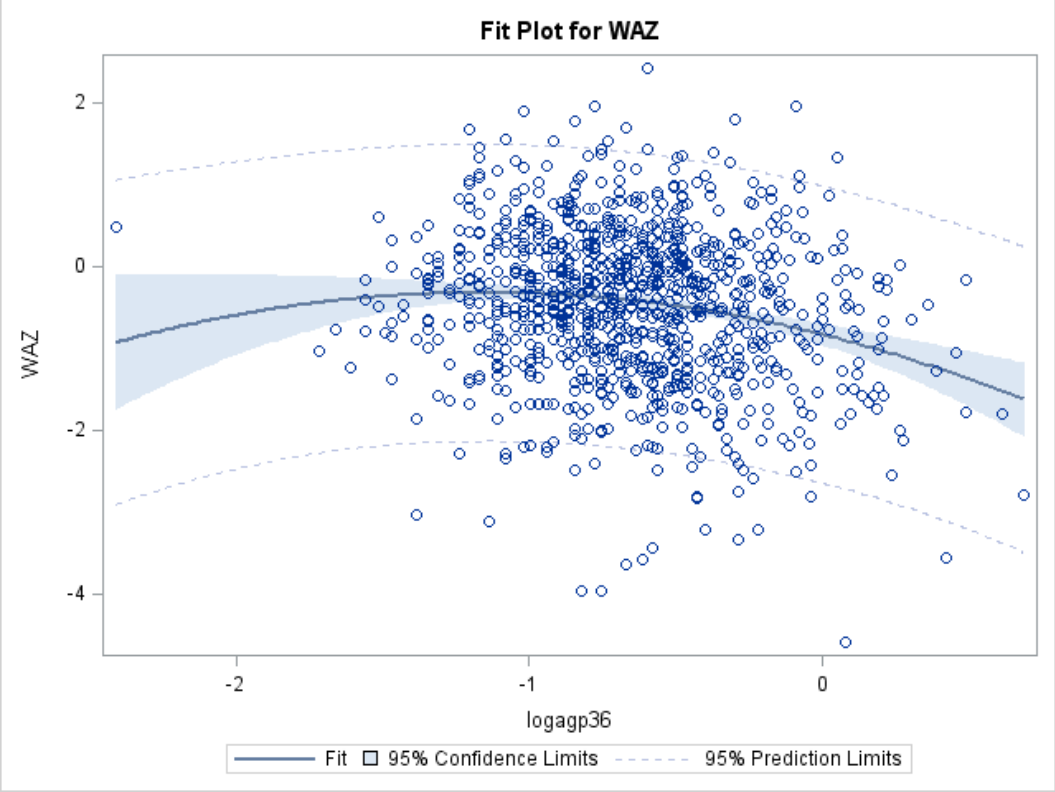
We examined the linearity of all relationships between continuous variables by linear regression models. All relationships were linear except for the associations between maternal plasma AGP concentration at 36 gw and LAZ, between maternal plasma AGP concentration at 36 gw and WAZ, and between maternal plasma AGP concentration at 36 gw and HCZ, all of which were inverted U-shape relationships (Figure A2-1). Relationships between maernal plasma AGP concentration at 36 gw and LAZ, WAZ, and HCZ are shown in Tables 4.4-2 and 4.4-3, but should be interpreted with caution.

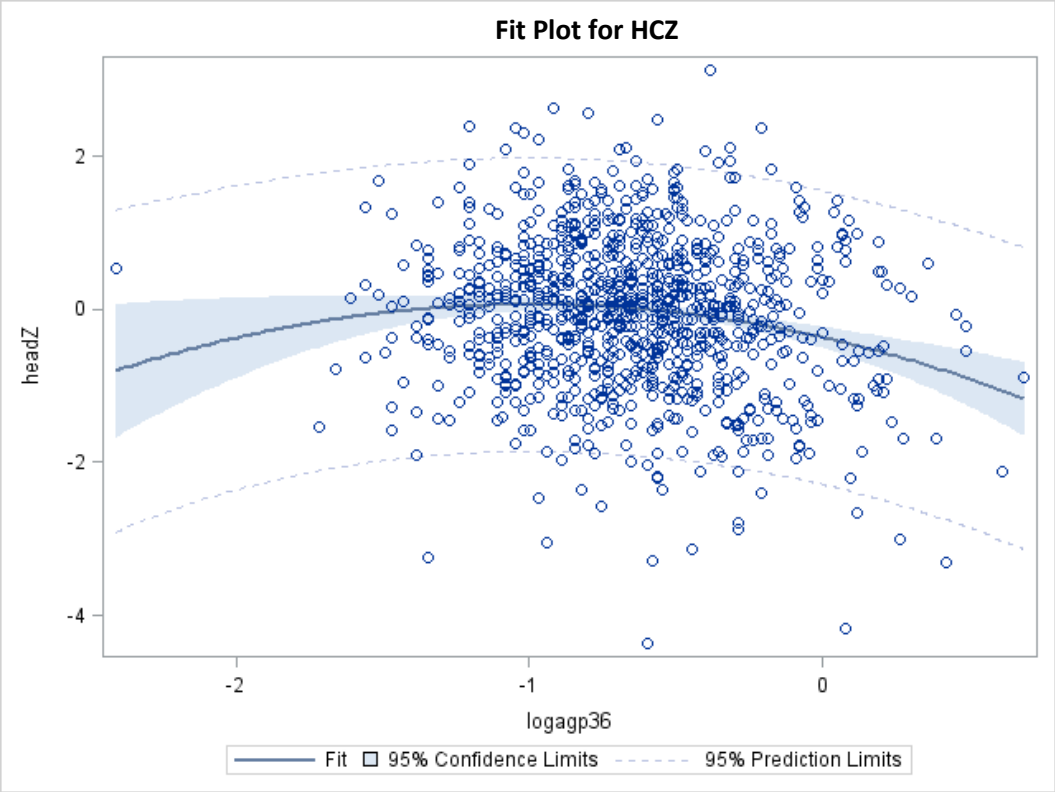
Poisson regression models were used to estimate relative risks for dichotomous birth outcomes (preterm birth, LBW, stunting, SGA, and small head circumference) by categorical predictors of maternal plasma CRP concentration (>5 mg/L) and maternal plasma AGP concentration (>1 g/L).

A set of variables was selected a priori based on the reported associations with birth outcomes and examined as potential covariates. Those that were significantly associated with a given birth outcome ($P<0.10$) in bivariate analysis were included in an adjusted model for that birth outcome to control for possible confounding.

**Figure A2-1. Graphical Representations of the Non-Linear Relationships between log Maternal Plasma AGP Concentration at 36 gw and LAZ, WAZ, and HCZ**






## A2.5. Maternal Blood Hb, ZPP, and sTfR Concentrations

**Details of data collection or laboratory analysis.** Clinic nurses collected blood from the antecubital vein into a 7.5 mL trace mineral-free polypropylene syringe (Sarstedt Monovette, NH4-heparin, Sarstedt Inc., Newton, NC, USA). The blood tube was immediately inverted 10 times to mix the heparin anticoagulant with the blood to prevent clotting. A small aliquot of the whole blood was pipetted out and used to analyze Hb on the Hemocue 201+ system (Hemocue, Brea, CA, USA). The tube containing the remaining whole blood was then placed in an insulated cooler with ice packs until processing. Trained lab staff then aliquotted whole blood into microcuvettes and washed the red cells three times. The washed red cells were used for ZPP analysis (Aviv hematofluorometer, Aviv Biomedical Inc, Lakewood, NJ, USA). Trained lab staff then centrifuged the whole blood at 3,000 RPM for 15 minutes and separated plasma into storage cryovials. The storage vials were placed upright in freezer boxes in a −20°C freezer for temporary storage at the satellite clinics. Within 48 hours, drivers transported the plasma to the main laboratory for long-term storage at −80°C.

Plasma was shipped to UCD on dry ice (World Courier) for analysis. We analyzed sTfR from those samples by immunoturbidimetry on the Cobas Integra 400 system autoanalyzer (F. Hoffmann-La Roche Ltd, Basel, Switzerland). We analyzed all the samples in singlet, except for 5%, which we randomly selected to be analyzed in duplicate. None of those samples had a CV greater than 5%.

**Details of statistical analysis.** We carried out the statistical analyses with SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

We compared enrollment characteristics of women included and excluded from the 36 gw sTfR-analyses (the group with the highest sample size of the three predictor variables) by a t-test to compare means and Fisher's exact test to compare proportions.

We used linear regression models to examine the associations between Hb, ZPP, and sTfR (at enrollment or 36 gw, or for the change between enrollment and 36 gw) and continuous birth outcomes (duration of gestation, birth weight, LAZ, WAZ, and HCZ). We examined the linearity of all relationships before conducting linear regression models and found all models to be linear. Linear regression coefficients are presented as standardized coefficients, which are the number of SDs a dependent variable will change, per SD increase in the predictor variable. We used standardized coefficients to assess the strength of association between the predictor and birth outcome in a given model in comparison with the association between another predictor and that same birth outcome in a different model. The use of standardized coefficients allows for such comparisons between predictor variables for the same outcome, even though the units of the predictor variables differ (e.g., Hb vs. ZPP). We computed standardized coefficients by multiplying the original regression coefficient by the SD of the predictor variable and then dividing by the SD of the dependent variable.

We used Poisson regression models to estimate the relative risk for dichotomous birth outcomes (preterm birth, LBW, stunting, SGA, and small head circumference) using categorical predictor variables (low and high Hb, high ZPP, and high sTfR).

A set of variables was selected a priori based on the reported associations with birth outcomes and examined as potential covariates. Those that were significantly associated with a given birth outcome ($P<0.10$) in bivariate analysis were included in an adjusted model for that birth outcome to control for possible confounding. In addition, the 36 gw and change models adjusted for intervention group. We did not, however, include inflammation as a covariate in the adjusted models, as inflammation is likely on the causal pathway between Hb and iron status and birth outcomes.

## A2.6. Maternal Plasma Retinol Concentration

**Details of data collection or laboratory analysis.** Plasma retinol was analyzed by HPLC. Briefly, 100 µL of plasma was transferred to a screw-top borosilicate vial followed by 1,000 µL HPLC-grade ethanol (Fisher Scientific, Fair Lawn, NJ, USA) containing retinyl acetate as an internal standard. Vials were briefly vortexed and then 4 mL of HPLC-grade hexanes (Fisher Scientific) was added, followed by vortexing for 45 seconds using a Multi-Tube Vortexer (VWR, Radnor, PA, USA). One mL of ultrapure water from a Milli-Q Synthesis A10 (Millipore SAS, Molsheim, France) was then added to each vial and then vials were briefly vortexed and centrifuged for 2 minutes at 2,000 x G. The hexane layer was transferred with a Pasteur pipette to a clean screw-top vial, dried under nitrogen at 40°C using a Pierce ReactiVap III (Thermo Fisher, Waltham, MA, USA), reconstituted in 80 µL of mobile phase (95:05 methanol:water, 0.01% ammonium acetate), briefly vortexed, and transferred to an autosampler vial with glass insert and Teflon-faced septa (SUN-Sri, Rockwood, TN, USA). Sixty microliters of the final extract were injected into an Agilent 1100 HPLC, fitted with an Eclipse Plus C18 5 µM 4.6 x 150 mm column (Agilent Technologies, Santa Clara, CA, USA), with a flow of 1 mL/minute. Peaks for retinol and retinyl acetate were read at 325 nm. The ratio of the area under the curve for retinol and retinyl acetate was used to calculate the retinol concentration in comparison to a plasma pool (UTAK Laboratories, Valencia, CA, USA) calibrated for retinol against NIST 968e reference serum (National Institute of Standards and Technology, Gaithersburg, MD, USA).

**Details of statistical analysis.** We carried out the statistical analysis with SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

We compared enrollment characteristics of women included and excluded from a randomly selected subset for assessment of vitamin A status based on plasma retinol concentration by a t-test to compare means and Fisher's exact test to compare proportions.

The association between plasma retinol concentration at enrollment and continuous birth outcomes (duration of pregnancy, birthweight, newborn LAZ, newborn WAZ, and newborn HCZ) was examined using linear regression analysis. The association between low plasma retinol concentration (<1.05 umol/L) at enrollment and dichotomous birth outcomes (small head circumference, low birth weight, and stunting) was examined using logistic regression. Variables that were predefined in our statistical analysis plan and included as potential covariates in the regression analysis were: high maternal plasma CRP concentration at enrollment, high maternal plasma AGP concentration at enrollment, maternal malaria status at enrollment, maternal HIV status, maternal BMI at enrollment, primiparity, maternal educational achievement, season of enrollment, and site of enrollment. These variables were included as covariates in the regression model if they were associated with the outcome (P<0.10) in binary analysis.

## A2.7. Maternal Plasma Cholesterol and Triglyceride Concentrations and Plasma Fatty Acid Composition

**Details of data collection or laboratory analysis.** Plasma was isolated from blood by centrifuge. Plasma cholesterol and triglycerides concentrations were determined at the USDA Western Human Nutrition Research Center (Davis, CA, USA) using a Cobas Integra 400 plus automatic analyzer (Roche Diagnostic Corp., Indianapolis, IN, USA). Total cholesterol was determined by the Trinder method, an enzymatic colorimetric method using cholesterol esterase and cholesterol oxidase, followed with peroxidase. Triglyceride concentrations were determined with an enzymatic colorimetric method using lipoprotein lipase, glycerol-3-phosphate, and glycerol-3-phosphate-oxidase, followed with peroxidase.

Plasma fatty acid composition was analyzed by GC with flame ionization detection at Omega Quant Analytics, LLC (Sioux Falls, SD, USA). Plasma was transferred to a screw-cap glass vial and BTM (methanol containing 14% boron trifluoride, toluene, methanol; 35:30:35 v/v/v) (Sigma-Aldrich, St. Louis, MO, USA) was added. The vial was briefly vortexed and heated in a hot bath at 100°C for 45 minutes. After cooling, hexane (EMD Chemicals, Gibbstown, NJ, USA) and HPLC-grade water were added and the tubes were recapped, vortexed, and centrifuged to help separate layers. An aliquot of the hexane layer was transferred to a GC vial. GC was carried out using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD, USA) equipped with a SP-2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 um film thickness; Supelco, Bellefonte, PA, USA).

Fatty acids were identified by comparison with a standard mixture of fatty acids (GLC OQ-A, NuCheck Prep, Elysian, MN, USA), which was also used to determine individual fatty acid calibration curves. The following 24 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 20:0, 22:0, 24:0); *cis* monounsaturated (16:1, 18:1, 20:1, 24:1); trans (16:1, 18:1, 18:2); *cis* n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); and *cis* n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). Fatty acid composition was expressed as a percent of total identified fatty acids. The chromatographic conditions used in this study were sufficient to isolate the C16:1 *trans* isomers and the C18:2 Δ 9t-12c, 9t-12t, and 9c-12t isomers (reported as C18:2n6t). However, each individual C18:1 *trans* molecular species (i.e., C18:1 Δ6 thru Δ13) could not be segregated but appeared as two blended peaks that eluted just before oleic acid. The areas of these two peaks were summed and referred to as C18:1 *trans*.

**Details of statistical analysis.** We carried out the statistical analysis with SAS 9.3 (SAS Institute, Cary, NC, USA).

We compared enrollment characteristics of women included and excluded from analyses by a t-test to compare means and Fisher's exact test to compare proportions.

Continuous cholesterol, triglyceride, and fatty acid variables were analyzed for normality using the Shapiro-Wilk test. All continuous fatty acid variables, except for AA, were log-transformed, as was triglyceride concentration. Linear regression models were used to examine associations of plasma cholesterol, triglycerides, and fatty acids with continuous birth outcomes (duration of gestation, birth weight, LAZ, WAZ, and HCZ). Linear regression coefficients are presented as standardized coefficients, which are the number of SDs a dependent variable will change, per SD increase in the predictor variable. We used standardized coefficients to assess the strength of association between the predictor and birth outcome in a given model in comparison with the association between another predictor and that same birth outcome in a different model. The use of standardized coefficients allows for such comparisons between predictor variables for the same outcome, even though the units of the predictor variables differ (e.g., cholesterol vs. triglyceride concentration). We computed standardized coefficients by multiplying the original regression coefficient by the SD of the predictor variable and then dividing by the SD of the dependent variable.

Poisson regression models were used to estimate the relative risk for dichotomous birth outcomes (preterm birth, LBW, stunting, and SGA). Both low cholesterol and high fatty acids were defined based on the distribution found in the control group that received IFA in the intervention part of this trial. Low cholesterol was defined as cholesterol below the 10th percentile, based on cholesterol distribution in the group receiving IFA, since this group was the reference group for the study and there is no clinical definition of low cholesterol, although several previous studies have used the 10th percentile cutoff. AA, DHA, ALA, and the ratio of omega-6:omega-3 fatty acids were analyzed dichotomously, based on a cutoff at the 50th percentile of the IFA group, as this was the reference group for the study.

Covariates were considered for inclusion in the models based on previous literature and were included in the model if significantly ($P<0.10$) associated with any birth outcome. All models included the following covariates: primiparity, gestational age at enrollment, maternal BMI at enrollment, maternal height at enrollment, maternal educational achievement, maternal age at enrollment, maternal HIV status, site of enrollment, maternal plasma CRP concentration at enrollment, and maternal plasma AGP concentration at enrollment. All models for the 36 gw analyses were also controlled for intervention group assignment from the main trial.

## A2.8. Maternal Perceived Stress and Salivary Cortisol Concentration

**Details of data collection or laboratory analysis.** Saliva samples were assayed at the USDA Western Human Nutrition Research Center (Davis, CA, USA). Cortisol concentration was determined by running samples in duplicate using Salimetrics Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, State College, PA, USA), which can detect cortisol concentrations ranging from 0.193 to 82.77 nmol/L (0.007–3.0 µg/dL). The intra- and inter-assay coefficients of variability were 3.5% and 5.1%, respectively. The mean of each duplicate measure was used for analysis.

Salivary cortisol concentration. Saliva samples were collected between 8 am and 4 pm, with a mean collection time of approximately 11 am. Women were instructed not to consume any food or drink besides water for at least 30 minutes before providing the saliva sample. Time of saliva collection, time of waking, and time of last food or drink were recorded. Enrollment and 36 gw saliva samples were collected at clinic sites when women came to provide blood and urine samples and have anthropometric measurements taken, while the 28 gw saliva sample was collected by a field worker during a home visit. Saliva collection occurred before any other measurements or sample collection.

Saliva was obtained by having the woman place an inert polymer cylindrical swab (10 mm x 30 mm, Salimetrics Oral Swab) under her tongue for approximately 2 minutes, while moving her tongue and jaw as if she were chewing to stimulate saliva. The swab was then placed in a tube with a cap and refrigerated or placed on ice packs. Swabs were brought to room temperature before centrifuging for 15 minutes at 3,000 RPM. Samples were frozen and stored at −20°C within 24 hours of collection.

Perceived Stress Scale. We utilized the PSS (Cohen et al. 1983), a 10-item survey that asks the respondent to rate how frequently she thought or felt a certain way on a scale of 0 to 4 (0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, 4 = very often) in the past month. Specifically, a woman was asked how often, in the last month, she had: 1) been upset because something had happened unexpectedly; 2) felt unable to control the important things in her life; 3) felt nervous and stressed; 4) felt confident in her ability to handle her personal problems; 5) felt that things were going her way; 6) felt that she could not cope with all the things she had to do; 7) been able to control irritations in her life; 8) felt that she was on top of things; 9) been angered because of things that were outside of her control; and 10) difficulties piling up so high she could not overcome them. Women were interviewed at enrollment, at 28 gw, and at 36 gw.

**Details of statistical analysis.** We carried out the statistical analysis with SAS 9.3 (SAS Institute, Cary, NC, USA).

We compared enrollment characteristics of women included and excluded from analyses by a t-test to compare means and Fisher's exact test to compare proportions.

Linear regression models were used to examine associations of cortisol and perceived stress score with continuous birth outcomes (duration of gestation, birth weight, WAZ, LAZ, and HCZ). Cortisol was log-transformed due to skewedness of the variable. Linear regression coefficients are presented as standardized coefficients, which are the number of SDs a dependent variable will change, per SD increase in the predictor variable. We used standardized coefficients to assess the strength of association between the predictor and birth outcome in a given model in comparison with the association between another predictor and that same birth outcome in a different model. The use of standardized coefficients allows for such comparisons between predictor variables for the same outcome, even though the units of the predictor variables differ. We computed standardized coefficients by multiplying the original regression coefficient by the SD of the predictor variable and then dividing by the SD of the dependent variable.

Poisson regression models were used to estimate relative risk for dichotomous birth outcomes, including preterm birth, LBW, stunting, and small head circumference. Covariates were considered for inclusion in the models based on previous literature and were included in the model if significantly ($P<0.10$) associated with any birth outcome. In addition, analyses for predictor variables collected at 28 or 36 gw were adjusted for intervention group. All models containing cortisol also included additional adjustments for time since waking and time since last meal. Maternal plasma CRP and AGP concentration measurements were done at enrollment and 36 weeks gestation. While cortisol was significantly associated with the inflammatory markers of CRP and AGP, it is possible that the inflammatory markers are on the causal pathway between cortisol and the various birth outcomes. Therefore, we have presented models both with and without adjustment for CRP and AGP at 36 gw (indicated as adjusted model 1 and adjusted model 2 respectively in Tables 4.8-1, 4.8-3, 4.8-4, and 4.8-5).

## A2.9. Histological Signs of Inflammation and Malaria in the Placenta and Fetal Membranes

### Details of data collection or laboratory analysis.

Sample collection. After delivery, the placenta was transferred to a sterile container to await tissue sampling. In all cases, sampling occurred immediately after delivery, unless delivery occurred overnight, in which case the placenta was sampled the following morning. A study nurse took a 5 cm x 1 cm piece of the chorionic and amniotic membranes from the edge of the rupture site and a 0.5 cm x 0.5 cm piece of placental tissue at full thickness from near the umbilical cord insertion. If the sample collection took place in Mangochi district hospital, the cryovials entered into −80°C storage. In the case that sample collection took place at an outlying health center or Malindi hospital, the samples were kept at −20°C for a maximum of 2 days before being transported to −80°C storage at Mangochi district hospital. One full thickness tissue block (with both maternal and fetal side represented) and a placenta membrane roll were collected and placed into tissue cassettes labeled with the participants' ID numbers. These were then placed in a bottle containing 10% neutral buffered formalin fixative and sent to the histology lab. At the histology lab, the tissue cassettes were processed in a Shandon Citadel tissue processor (Thermo Fisher Scientific, Waltham, MA, USA) and embedded in paraffin wax. Tissue sections 3–5 micron thick were cut from the tissue paraffin blocks and placed onto glass slides labeled with participants' ID numbers. The tissue slides were stained with hematoxylin and eosin stain before being read.

**Details of statistical analysis.** We carried out the statistical analysis with Stata 13 (StataCorp, College Station, TX, USA).

We compared enrollment characteristics of women included and excluded from analyses by a t-test to compare means and Fisher's exact test to compare proportions.

We examined the associations with continuous predictors and birth outcomes (duration of pregnancy, birth weight, newborn LAZ, and newborn HCZ) using linear regression models. We used log-binomial regression models to estimate relative risks for dichotomous birth outcomes (preterm birth, LBW, stunting, and small head circumference).

All analyses were adjusted using multivariable regression models to correct for possible confounding effects. Covariates for each outcome were selected based on previous literature as logically capable of forming an independent association with the outcome being measured. All covariates were then entered in a single step into the equation. The included variables were maternal BMI at enrollment, maternal HIV status, intervention group, maternal malaria status at enrollment, maternal maternal age at enrollment, maternal anemia at enrollment, maternal educational achievement, maternal height at enrollment, primiparity, proxy for SES, season of enrollment, site of enrollment, and time between delivery and collection of placenta sample.

## A2.10. Placenta, Fetal Membrane, Oral, and Vagina Microbiomes

### Details of data collection or laboratory analysis.

Sample collection. After delivery, the placenta was transferred to a sterile container to await tissue sampling. In all cases, sampling occurred immediately after delivery, unless delivery occurred overnight, in which case the placenta was sampled the following morning. A study nurse took a 5 cm x 1 cm piece of the chorionic and amniotic membranes from the edge of the rupture site and a 0.5 cm x 0.5 cm piece of placental tissue at full thickness from near the umbilical cord insertion. The nurse placed the two samples

in separate cryovials. If the sample collection took place in Mangochi district hospital, the cryovials entered into −80°C storage. In the case that sample collection took place at an outlying health center or Malindi hospital, the samples were kept at −20°C for a maximum of 2 days before being transported to −80°C storage at Mangochi district hospital.

Dental swabs were collected at Mangochi central site from all mothers who completed the oral health visit at 1 week after delivery or as soon as possible by specially trained dental therapists. One sterile plastic swab stick with nylon fiber tip, stored in a plain dry tube (microRheologics no. 552, Coban, Brescia, Italy) was used for the sample collection. The dental therapists collected the sample by rubbing the gingival margin of each tooth with the swab. They used a dental mirror to elevate the cheeks so that the teeth were visible and skin contact was avoided. They started the sample collection from the buccal side of the most posterior (farthest) tooth on the right upper jaw. They repeated the procedure for the palatal sites of the same teeth, continuing then to the lower jaw and repeating it for all lower teeth's buccal and lingual side. They immediately placed the tube in a cold box with ice bricks and handed it over to a laboratory technician. The laboratory technician cut the applicator stick with scissors above the fiber tip to fit the swab into cryovials where they were stored at −80°C.

Vaginal swabs were collected at the health centers during the postnatal visit 1 week after delivery. A nurse collected the sample by inserting the swab approximately 7 cm deep past the vaginal introitus, rotated it three times back and forth, and then removed it and placed it back into a storage tube. Once collected, the sample was transferred to the laboratory and stored at −80°C.

Amplification and sequencing of bacteria found in placenta tissue, membrane tissue, dental swabs, and vaginal swabs. In preparation for extraction of genomic DNA, each placenta and membrane sample was cut roughly into smaller pieces using a sterile pair of surgical scissors; 20–50 mg of tissue was then transferred to a sterile 2 ml screw-cap tube and extracted using the QIAmp DNA mini kit (Qiagen, VIC, Australia). Extraction was carried out as per the manufacturer's protocol with an additional cell disruption step after lysis with Proteinase K. In the additional step, 0.1 mm glass beads (Lysing Matrix B, MP Biomedicals, Pittsburgh, PA, USA) were added to each sample and the 2 ml tubes were shaken on a cell disrupter (Vortex Genie 2, Scientific Industries, Bohemia, NY, USA) for 10 minutes at the highest speed. For every 10 extractions, a negative extraction control was included (200 µl buffer AE). The processing of dental and vaginal swabs was the same, except the dental and vaginal swab heads were fully submerged in 200 µl of buffer AE for 1 minute and all liquid was then expressed out of the swab before removal.

All DNA purified from placenta and fetal membrane samples were screened for bacteria using a quantitative PCR SYBR green fluorescent dye assay. The primer set used targeted the V5-7 regions of the 16S rRNA gene, 785F: 5′-GGATTAGATACCCBRGTAGTC-3′, 1175R: 5′-ACGTCRTCCCCDCCTTCCTC-3′. Each PCR reaction was carried out with the following: 1x Power SYBR Green master mix (Life Technologies, Grand Island, NY, USA), 0.4 pmol/µl of forward and reverse primers, 1 µl of template DNA, and molecular grade water (Bioline, Taunton, MA, USA) to give a final volume of 25 µl. Amplification took place in an ABI 7300 Real-Time system (Life Technologies) under the following conditions: 95°C x 10 minutes, 40 cycles of 95°C x 15 seconds, and 60°C x 1 minute. Each PCR run included three negative PCR controls (1 µl buffer AE from QIAmp DNA mini kit) and a serial dilution of a known concentration of positive control from a pure *Escherichia coli* culture for quantification.

Placenta and membrane positive for bacterial DNA by qPCR and all oral and vaginal samples were selected for sequencing. Library preparation was carried out on extracted DNA using dual barcoded primers for each sample to allow multiplexing of samples on a single sequencing run. Each library

preparation PCR was carried out with 1X Molzym PCR Buffer, 200 pmol/µl dNTPs (Bioline), 0.4 pmol/µl forward and reverse primer, 0.025 pmol/µl Moltaq, 5 µL template DNA and molecular grade water (Bioline) to give a final reaction volume of 25 µl. The cycling conditions for placenta and membrane samples were: 94°C x 3 minutes, 32 cycles of 94°C x 30 seconds, 60°C x 40 seconds, and 72°C x 90 seconds, with a final extension cycle of 72°C x 10 minutes. Cycling conditions for oral and vaginal samples were: 94°C x 3 minutes, 30 cycles of 94°C x 30 seconds, 60°C x 40 seconds, and 72°C x 90 seconds, with a final extension cycle of 72°C x 10 minutes. The resulting amplicon was cleaned and pooled using SequalPrep normalization plate kits (Invitrogen, Grand Island, NY, USA) and then AMPure XP beads (Beckman Coulter, Brea, CA, USA), both as per manufacturer's protocol. Each plate was then pooled into a final equimolar library after quantification using a Qubit 2.0 (Life Technologies). The final library underwent a further AMPure XP bead clean-up step at a more stringent 1x ratio of beads to library. 10pM of DNA library was loaded onto a MiSeq (Illumina, San Diego, CA, USA) as per manufacturer's protocol for 500 cycle V2 kits with the addition of custom sequencing primers.

### Details of statistical analysis.

We carried out the statistical analysis with Stata 13 (StataCorp, College Station, TX, USA).

We compared enrollment characteristics of women included and excluded from analyses by a t-test to compare means and Fisher's exact test to compare proportions.

We examined the associations with continuous predictors and birth outcomes (duration of pregnancy, birth weight, newborn LAZ, and newborn HCZ) using linear regression models.

We used log-binomial regression models to estimate relative risks for dichotomous birth outcomes (preterm birth, LBW, stunting, and small head circumference).

Analyses were adjusted using multivariable regression models to correct for possible confounding effects. Covariates for each outcome were selected based on previous literature as logically capable of forming an independent association with the outcome being measured. All covariates were then entered in a single step into the equation. The included variables were maternal BMI at enrollment, caesarean section (for the placenta and fetal membrane analyses), maternal HIV status, intervention group, maternal malaria status at enrollment, maternal age at enrollment, maternal anemia at enrollment, maternal educational achievement, primiparity, proxy for SES, season of enrollment, site of enrollment, and time between delivery and collection of placenta sample (for the placenta and fetal membrane analyses).

## A2.11. Maternal Oral Health

**Details of data collection or laboratory analysis.** Participants were invited and transport was provided to Mangochi district hospital for oral health examination at the postnatal visit 1 week after delivery (for participants from Mangochi site) or as soon as possible (for participants from other sites). Three experienced dental therapists specially trained for this purpose conducted full-mouth dental and periodontal examinations, took digital panoramic radiographs (Planmega Proline XC, Planmega, Finland), and asked multiple choice questions on the participant's oral health care habits and oral health problems and treatments received during the previous 6 months. The participant sat on a chair with back and arm rests, and the examiner used a head lamp for visibility (Pezl Tikka XP$^2$, Pezl, France). The examiners recorded missing teeth and caries lesions extending unambiguously to the dentin. If the extension of the lesion was questionable, they probed gently with a sharp probe (LM-Instruments, Parainen, Finland). They measured periodontal pocket probing depth from six sites of each tooth, excluding third molars, using a WHO periodontal probe (LM-Instruments, Parainen, Finland) (reading increments at 3.5, 5.5, 8.5, and 11.5 mm) and recorded the deepest measurement of each tooth rounded to

the nearest millimeter. They assessed presence of gingivitis as profound bleeding after gentle (20 g weight) probing and recorded it by dental arch sextants (right, mid, and left upper and lower).

Two persons, an oral and maxillofacial radiologist and an experienced dentist, analyzed the radiographs so that they were discussed together and so that the diagnosis was agreed on. For the analyses, they used digital imaging software (Planmega Romexis$^{TM}$, Planmega, Finland) and a good-quality computer screen in a darkened room. The examiners calculated the number of teeth, including impacted teeth and root remnants. They recorded caries as lesions extending to the dentin or to the pulp or as root remnants, and diagnosed periapical infections if an osteolytic finding >1 mm with diffuse margins, surrounding the apex of the root, was present. If the finding was questionable, they recorded it as "not present." They assessed alveolar bone loss by measuring the bone level of each tooth from the dento-enamel junction to the deepest point of the bony pocket (if present) and the mean horizontal bone level by arch sextants, and expressed these measurements relative to the full length of the root (normal level, cervical, mid, or apical third of root length).

The examiners' measurement reliability was assessed and verified at the beginning of the study and approximately every 4 months thereafter against the measurements of an experienced dentist representing the gold standard. Intra-examiner validity examinations were conducted separately so that each data collector reexamined one participant in a week when the participants came to the dental clinic for their treatment appointments. The data monitor assigned the participants to the reexamination so that the examiners were unaware of the purpose of the appointment until the examination was completed. The x-ray analysts reassessed together at least two earlier diagnosed x-rays each week and, if any deviance between the two readings was found, they discussed them until agreement was reached.

**Details of statistical analysis.** We carried out the statistical analysis with Stata 12.1 (StataCorp, College Station, TX, USA).

We compared enrollment characteristics of women included into and excluded from the analyses by a t-test to compare means and Fisher's exact test to compare proportions.

Linear regression models were used to examine associations of periapical infections and periodontitis with continuous birth outcomes (duration of gestation, birthweight, newborn WAZ, newborn LAZ, and newborn HCZ).

Log-binomial models or, in case the algorithm failed to converge, log-Poisson models were used to estimate the relative risk of those with and without periapical infections or periodontitis for dichotomous birth outcomes (preterm birth, LBW, stunting, and small head circumference).

We calculated relative risks for comparison of binary end-points and differences in means for comparison of continuous end-points at a single time point. With the central limit theorem, such an analysis of means is robust and valid also in the case of a skewed outcome distribution, due to the large sample size (Cheung 2014, Rice 1995).

To control for possible confounding, we created multivariable regression models using the forced entry method so that all relevant (based on earlier knowledge) and available covariates that could confound the association between the oral diseases and birth outcomes were included in the model.

## A2.12. Malaria Immunity in Pregnancy

### Details of methods, data collection, or laboratory analysis.

Description of malaria antigens. Antigens expressed by the blood stage parasites have been considered as potential vaccine candidates. These are antigens expressed by VSA expressed by the parasitized red blood cells (pRBCs) (Miller et al. 2002).

Antibody immunity to pregnancy-specific malaria VSA was measured in maternal samples using flow cytometry-based opsonic phagocytosis and VSA recognition assays.

Malaria parasites and cell culture. Pregnancy-specific malaria parasite line CS2 (which binds to placental chondroitin sulfate A) and non-pregnancy-specific isolate E8B (which binds to ICAM-1 and CD36 endothelial receptors in the periphery) were cultured as previously described (Chandrasiri et al. 2014).

THP-1 cells were obtained from ATCC (Manassas, VA, USA; catalogue number TIB-202™) and maintained in RPMI 1640 (GIBCO®, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin-glutamine, and 25 mM HEPES (GIBCO®). These pro-monocytic cells were maintained at 1 to 2 x $10^5$/ml in 80 $cm^2$ cell culture flasks (Nunc™, Thermo Fisher Scientific, Scoresby, VIC, Australia) in the upright position in a humidified 37°C incubator with 5% $CO_2$.

Measuring antibodies to pregnancy-specific VSA. Antibodies that recognize VSA and opsonize parasitized erythrocytes for phagocytic clearance were measured using a flow cytometry based assay previously established in our laboratory (Ataide et al. 2010) with some minor modifications (Teo et al. 2014). These methods were discussed in Chandrasiri et al. (2014). Heat inactivated plasma samples of both enrollment and 36 weeks gestation samples were assayed in duplicate in the same assay on the same day. The percentage phagocytosis was determined relative to the positive control (pRBCs) incubated with a pool of sera from patients with high levels of IgG against VSA).

Measuring total IgG to pregnancy-specific VSA. Using published methods (Chandrasiri et al. 2014, Aitken et al. 2010), we measured total immunoglobulin G (IgG) to pregnancy-specific VSA. Alexa Fluor® 647-conjugated donkey anti-rabbit antibody (Life Technologies) was used as the tertiary antibody, at 4 µg/ml concentration in contrast to the previous methods. We measured geometric mean fluorescence intensity (MFI) for each sample, which is representative of the amount of IgG that recognizes pregnancy-specific VSA in the participant's plasma. The geometric MFI was adjusted for inter- and intra-plate variability and reported as a percentage of the positive control.

**Details of statistical analysis.** We carried out the statistical analyses with Stata version 13.0 (StataCorp LP, College Station, TX, USA).

We compared enrollment characteristics of women included and excluded from these analyses by a t-test to compare means and Fisher's exact test to compare proportions.

Malaria antibody levels at enrollment and rate of change in antibody levels from enrollment to 36 gw were used as the predictor of pregnancy or birth outcomes. Multiple linear regression analysis was performed for determining the association between continuous predictor antibody variables with continuous birth outcomes (duration of pregnancy, birth weight, newborn LAZ, newborn WAZ, and newborn HCZ). The analyses were adjusted for a range of variables that were selected based on published evidence that they influence malaria antibody levels and therefore also likely the birth outcomes of focus. Regression coefficients and 95% CI were reported.

Association with dichotomous birth outcomes (LBW, stunting, small head circumference, newborn underweight, and SGA) were analyzed against maternal antibody tertiles. We compared the association with birth outcomes in medium and high responders compared to the association in low responders. Log-binomial and multinomial logistic regression were performed to estimate unadjusted and adjusted relative risks with 95% CI for each association. In case the algorithm failed to converge in the estimation, we used alternative estimation algorithms with iterated reweighted least squares or modified Poisson approximation, in that order.

Any association between antibody levels and pregnancy outcomes with a reported p-value<0.05 was considered statistically significant.

# Appendix 3. Appendix to the Pathway Analysis

## A3.1. Pathway Analysis: Included and Excluded Participants

### Table A3-1. Comparison of the Enrollment Characteristics of Participants Who Were Included and Excluded from the Pathway Analysis

| Characteristic | Included[a] (n=1179) | Excluded (n=212) | P-value[b] |
|---|---|---|---|
| Mean (SD) BMI, kg/m² | 22.1 (2.8) | 22.7 (2.9) | 0.005 |
| Mean (SD) maternal age, years | 25 (6) | 24 (6) | 0.002 |
| Mean (SD) maternal education, completed years | 4.0 (3.4) | 4.3 (3.7) | 0.340 |
| Mean (SD) proxy for SES | 0.30 (1.17) | −0.04 (0.97) | <0.001 |
| Percentage of anemic women (Hb <100 g/L) | 19.8% | 25.6% | 0.055 |
| Percentage of primiparous women | 19.5% | 35.1% | <0.001 |
| Percentage of women with a BMI <18.5 | 5.5% | 4.7% | 0.667 |
| Percentage of women with a positive HIV test | 13.4 % | 16.1 % | 0.358 |
| Percentage of women with positive malaria test (RDT) | 22.9 % | 25.0 % | 0.500 |

[a] Those participants with singleton pregnancies who completed the 1 week post-partum visit with anthropometric data.
[b] P-values were obtained from t-test (comparison of means) or Fisher's exact test (comparison of proportions).

## A3.2. Pathway Analysis: Included Variables

### Table A3-2. List of Variables Included in the Pathway Analyses

| Category and variable | Variable type | # imputed | % imputed |
|---|---|---|---|
| **Maternal enrollment characteristics** | | | |
| Maternal age | continuous | 0 | 0.0% |
| Maternal primiparity | dichotomous | 3 | 0.2% |
| Maternal height | continuous | 5 | 0.4% |
| **Maternal nutrition** | | | |
| Maternal BMI at enrollment | continuous | 9 | 0.7% |
| Maternal blood Hb concentration at enrollment | continuous | 2 | 0.1% |
| Maternal weekly weight gain (kg/week) | continuous | 2 | 0.1% |
| **Maternal infections** | | | |
| Maternal HIV infection | dichotomous | 57 | 4.1% |
| Maternal peripheral blood malaria parasitemia at enrollment | dichotomous | 25 | 1.8% |
| Maternal periapical oral infection | dichotomous | 353 | 25.6% |
| Maternal UTI | dichotomous | 167 | 12.1% |
| Maternal vaginal trichomoniasis | dichotomous | 165 | 12.0% |
| **Maternal inflammation and stress** | | | |
| Maternal salivary cortisol concentration at 36 gw | continuous | 336 | 24.4% |
| Maternal plasma AGP concentration at enrollment | continuous | 8 | 0.6% |
| **Placental size, infection, and inflammation** | | | |
| Placental weight (g) | continuous | 413 | 29.9% |
| Placental malaria infection | dichotomous | 371 | 26.9% |
| Severe chorioamnionitis | dichotomous | 389 | 28.2% |
| **Duration of pregnancy and newborn size** | | | |
| Duration of pregnancy | continuous | 84 | 6.1% |
| Newborn LAZ | continuous | 80 | 6.8% |
| Newborn WAZ | continuous | 90 | 7.6% |
| Newborn HCZ | continuous | 88 | 7.5% |

## A3.3. Bivariate Associations between Variables Used in the Pathway Analyses

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

| | Maternal age at enrollment | | Maternal primiparity | | Maternal height at enrollment | | Maternal BMI at enrollment | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **B-coefficient** | **P-value** | **Odds ratio** | **P-value** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** |
| Maternal age at enrollment | NA | NA | 0.583 | **<0.001** | 0.046 | *0.064* | 0.077 | **<0.001** |
| Maternal primiparity | −7.852 | **<0.001** | NA | NA | −0.764 | **0.038** | −0.513 | **0.005** |
| Maternal height at enrollment | 0.054 | *0.064* | 0.976 | **0.039** | NA | NA | 0.000 | 0.971 |
| Maternal BMI at enrollment | 0.363 | **<0.001** | 0.933 | **0.006** | 0.002 | 0.971 | NA | NA |
| Maternal blood Hb concentration at enrollment | 0.054 | **<0.001** | 0.969 | **<0.001** | −0.008 | 0.385 | 0.020 | **<0.001** |
| Maternal weekly weight gain | 4.332 | **0.009** | 0.079 | **<0.001** | 7.247 | **<0.001** | −3.199 | **<0.001** |
| Maternal HIV infection | 3.151 | **<0.001** | 0.278 | **<0.001** | −0.057 | 0.901 | 0.446 | *0.058* |
| Maternal peripheral blood malaria parasitemia at enrollment | −3.466 | **<0.001** | 3.444 | **<0.001** | −0.036 | 0.910 | −0.676 | **<0.001** |
| Maternal periapical oral infection (as diagnosed after delivery) | 3.682 | **<0.001** | 0.334 | **<0.001** | −0.099 | 0.814 | 0.207 | 0.333 |
| Maternal UTI (as diagnosed after delivery) | −0.461 | 0.650 | 1.599 | 0.184 | −0.875 | 0.359 | 0.600 | 0.212 |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | −0.539 | 0.353 | 1.021 | 0.931 | −1.755 | **0.001** | −0.132 | 0.616 |
| Maternal salivary cortisol concentration at 36 gw | −0.133 | **0.022** | 1.028 | 0.252 | −0.067 | 0.230 | 0.005 | 0.853 |
| Maternal plasma AGP concentration at enrollment | −4.115 | **<0.001** | 8.291 | **<0.001** | −0.635 | 0.296 | −1.018 | **0.001** |
| Placental weight (g) | 0.002 | 0.341 | 0.999 | 0.119 | 0.007 | **<0.001** | 0.002 | **0.034** |
| Placental malaria infection | −3.682 | **<0.001** | 5.220 | **<0.001** | −0.328 | 0.340 | −0.750 | **<0.001** |
| Severe chorioamnionitis | 0.823 | 0.150 | 0.742 | 0.240 | −0.503 | 0.349 | 0.431 | 0.144 |
| Duration of pregnancy | 0.057 | 0.325 | 0.959 | **0.046** | 0.127 | **0.018** | −0.023 | 0.394 |
| Newborn LAZ | 0.548 | **0.001** | 0.685 | **<0.001** | 1.343 | **<0.001** | 0.124 | *0.096* |
| Newborn WAZ | 0.800 | **<0.001** | 0.619 | **<0.001** | 1.202 | **<0.001** | 0.257 | **0.001** |
| Newborn HCZ | 0.421 | **0.010** | 0.816 | **0.002** | 0.783 | **<0.001** | 0.232 | **0.002** |

| | Maternal blood Hb concentration at enrollment | | Maternal weekly weight gain | | Maternal HIV infection | | Maternal peripheral blood malaria parasitemia at enrollment | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** |
| Maternal age at enrollment | 0.383 | **<0.001** | 0.001 | **0.009** | 1.081 | **<0.001** | 0.899 | **<0.001** |
| Maternal primiparity | −8.264 | **<0.001** | −0.024 | **<0.001** | 0.278 | **<0.001** | 3.444 | **<0.001** |
| Maternal height at enrollment | −0.068 | 0.385 | 0.002 | **<0.001** | 0.998 | 0.901 | 0.999 | 0.910 |
| Maternal BMI at enrollment | 0.676 | **<0.001** | −0.004 | **<0.001** | 1.054 | *0.057* | 0.912 | **<0.001** |
| Maternal blood Hb concentration at enrollment | NA | NA | 0.000 | **0.003** | 0.985 | **0.002** | 0.969 | **<0.001** |
| Maternal weekly weight gain | 13.023 | **0.003** | NA | NA | 0.085 | **0.002** | 0.218 | **0.009** |
| Maternal HIV infection | −4.094 | **0.002** | −0.024 | **0.002** | NA | NA | 0.843 | 0.326 |
| Maternal peripheral blood malaria parasitemia at enrollment | −8.010 | **<0.001** | −0.015 | **0.009** | 0.843 | 0.326 | NA | NA |
| Maternal periapical oral infection (as diagnosed after delivery) | 0.195 | 0.869 | −0.010 | 0.138 | 1.844 | **0.001** | 0.468 | **<0.001** |
| Maternal UTI (as diagnosed after delivery) | −2.855 | 0.284 | −0.025 | 0.103 | 2.132 | *0.055* | 1.092 | 0.794 |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | 1.242 | 0.418 | −0.016 | *0.061* | 1.870 | **0.008** | 1.482 | **0.037** |
| Maternal salivary cortisol concentration at 36 gw | −0.465 | **0.004** | −0.003 | **0.001** | 1.019 | 0.455 | 1.001 | 0.945 |
| Maternal plasma AGP concentration at enrollment | −9.373 | **<0.001** | 0.008 | 0.434 | 3.418 | **<0.001** | 13.281 | **<0.001** |
| Placental weight (g) | −0.001 | 0.907 | 0.000 | **0.001** | 0.998 | **0.016** | 1.000 | 0.806 |
| Placental malaria infection | −4.460 | **<0.001** | −0.020 | **0.001** | 0.938 | 0.732 | 3.756 | **<0.001** |
| Severe chorioamnionitis | −0.263 | 0.863 | −0.005 | 0.538 | 1.141 | 0.607 | 1.054 | 0.797 |
| Duration of pregnancy | 0.686 | **<0.001** | 0.003 | **0.002** | 0.958 | *0.083* | 0.944 | **0.003** |
| Newborn LAZ | 1.707 | **<0.001** | 0.017 | **<0.001** | 0.821 | **0.008** | 0.780 | **<0.001** |
| Newborn WAZ | 2.452 | **<0.001** | 0.021 | **<0.001** | 0.878 | 0.100 | 0.700 | **<0.001** |
| Newborn HCZ | 1.045 | **0.018** | 0.013 | **<0.001** | 0.934 | 0.377 | 0.786 | **<0.001** |

| | **Maternal periapical oral infection** (as diagnosed after delivery) | | **Maternal UTI** (as diagnosed after delivery) | | **Maternal vaginal trichomoniasis** (as diagnosed after delivery) | | **Maternal salivary cortisol concentration at 36 gw** | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** | **B-coefficient** | **P-value** |
| Maternal age at enrollment | 1.100 | **<0.001** | 0.987 | 0.643 | 0.985 | 0.353 | −0.037 | **0.022** |
| Maternal primiparity | 0.334 | **<0.001** | 1.599 | 0.184 | 1.021 | 0.931 | 0.302 | 0.249 |
| Maternal height at enrollment | 0.997 | 0.814 | 0.973 | 0.358 | 0.946 | **0.001** | −0.021 | 0.230 |
| Maternal BMI at enrollment | 1.026 | 0.333 | 1.068 | 0.208 | 0.983 | 0.612 | 0.007 | 0.852 |
| Maternal blood Hb concentration at enrollment | 1.001 | 0.868 | 0.990 | 0.283 | 1.005 | 0.417 | −0.018 | **0.004** |
| Maternal weekly weight gain | 0.349 | 0.139 | 0.074 | 0.102 | 0.184 | *0.062* | −3.087 | **0.001** |
| Maternal HIV infection | 1.844 | **0.001** | 2.132 | *0.055* | 1.870 | **0.008** | 0.209 | 0.452 |
| Maternal peripheral blood malaria parasitemia at enrollment | 0.468 | **<0.001** | 1.092 | 0.794 | 1.482 | **0.037** | 0.016 | 0.940 |
| Maternal periapical oral infection (as diagnosed after delivery) | NA | NA | 1.805 | 0.164 | 1.440 | *0.092* | 0.714 | **0.007** |
| Maternal UTI (as diagnosed after delivery) | 1.805 | 0.164 | NA | NA | 1.894 | 0.190 | −0.104 | 0.864 |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | 1.440 | *0.092* | 1.894 | 0.190 | NA | NA | 0.376 | 0.308 |
| Maternal salivary cortisol concentration at 36 gw | 1.067 | **0.010** | 0.985 | 0.806 | 1.032 | 0.318 | NA | NA |
| Maternal plasma AGP concentration at enrollment | 0.600 | *0.094* | 1.688 | 0.391 | 2.643 | **0.005** | 0.741 | *0.062* |
| Placental weight (g) | 1.000 | 0.962 | 1.000 | 0.892 | 0.998 | *0.051* | −0.004 | **0.004** |
| Placental malaria infection | 0.617 | **0.004** | 1.199 | 0.626 | 1.552 | **0.027** | 0.338 | 0.139 |
| Severe chorioamnionitis | 1.589 | *0.053* | 2.992 | **0.007** | 1.416 | 0.245 | −0.064 | 0.853 |
| Duration of pregnancy | 0.930 | **0.008** | 0.901 | **0.010** | 0.929 | **0.009** | −0.212 | **0.002** |
| Newborn LAZ | 0.856 | **0.019** | 0.685 | **0.013** | 0.820 | **0.019** | −0.159 | 0.133 |
| Newborn WAZ | 0.906 | 0.159 | 0.651 | **0.006** | 0.770 | **0.003** | −0.262 | **0.020** |
| Newborn HCZ | 0.883 | *0.062* | 0.863 | 0.389 | 0.850 | *0.061* | −0.238 | **0.027** |

| | Maternal plasma AGP concentration at enrollment | | Placental weight (g) | | Placental malaria infection | | Severe chorioamnionitis | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** |
| Maternal age at enrollment | −0.007 | **<0.001** | 0.529 | 0.340 | 0.893 | **<0.001** | 1.021 | 0.152 |
| Maternal primiparity | 0.143 | **<0.001** | −13.970 | 0.118 | 5.220 | **<0.001** | 0.742 | 0.240 |
| Maternal height at enrollment | −0.001 | 0.296 | 2.605 | **<0.001** | 0.990 | 0.340 | 0.984 | 0.349 |
| Maternal BMI at enrollment | −0.008 | **0.001** | 2.500 | **0.034** | 0.903 | **<0.001** | 1.052 | 0.139 |
| Maternal blood Hb concentration at enrollment | −0.002 | **<0.001** | −0.024 | 0.907 | 0.983 | **<0.001** | 0.999 | 0.864 |
| Maternal weekly weight gain | 0.054 | 0.434 | 108.739 | **0.001** | 0.129 | **0.001** | 0.574 | 0.538 |
| Maternal HIV infection | 0.087 | **<0.001** | −24.398 | **0.015** | 0.938 | 0.732 | 1.141 | 0.607 |
| Maternal peripheral blood malaria parasitemia at enrollment | 0.156 | **<0.001** | −1.724 | 0.806 | 3.756 | **<0.001** | 1.054 | 0.797 |
| Maternal periapical oral infection (as diagnosed after delivery) | −0.031 | *0.092* | 0.466 | 0.963 | 0.617 | **0.004** | 1.589 | *0.053* |
| Maternal UTI (as diagnosed after delivery) | 0.036 | 0.390 | −3.346 | 0.897 | 1.199 | 0.626 | 2.992 | **0.007** |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | 0.068 | **0.006** | −22.567 | *0.050* | 1.552 | **0.027** | 1.416 | 0.245 |
| Maternal salivary cortisol concentration at 36 gw | 0.005 | *0.061* | −4.117 | **0.003** | 1.033 | 0.146 | 0.992 | 0.821 |
| Maternal plasma AGP concentration at enrollment | NA | NA | −33.006 | **0.022** | 4.815 | **<0.001** | 0.324 | **0.007** |
| Placental weight (g) | 0.000 | **0.021** | NA | NA | 1.000 | 0.946 | 0.999 | 0.394 |
| Placental malaria infection | 0.098 | **<0.001** | 0.502 | 0.945 | NA | NA | 0.869 | 0.480 |
| Severe chorioamnionitis | −0.063 | **0.006** | −10.478 | 0.395 | 0.869 | 0.480 | NA | NA |
| Duration of pregnancy | −0.005 | **0.025** | 11.121 | **<0.001** | 0.996 | 0.882 | 0.906 | **0.014** |
| Newborn LAZ | −0.033 | **<0.001** | 28.962 | **<0.001** | 0.790 | **<0.001** | 0.894 | 0.211 |
| Newborn WAZ | −0.043 | **<0.001** | 39.724 | **<0.001** | 0.748 | **<0.001** | 0.907 | 0.305 |
| Newborn HCZ | −0.030 | **<0.001** | 34.847 | **<0.001** | 0.860 | **0.013** | 0.894 | 0.224 |

| | Duration of pregnancy | | Newborn LAZ | | Newborn WAZ | | Newborn HCZ | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** |
| Maternal age at enrollment | 0.013 | 0.325 | 0.019 | **0.001** | 0.024 | **<0.001** | 0.014 | **0.011** |
| Maternal primiparity | −0.392 | **0.045** | −0.506 | **<0.001** | −0.560 | **<0.001** | −0.258 | **0.002** |
| Maternal height at enrollment | 0.034 | **0.018** | 0.054 | **<0.001** | 0.042 | **<0.001** | 0.030 | **<0.001** |
| Maternal BMI at enrollment | −0.024 | 0.394 | 0.021 | *0.096* | 0.036 | **0.001** | 0.036 | **0.002** |
| Maternal blood Hb concentration at enrollment | 0.022 | **<0.001** | 0.008 | **<0.001** | 0.010 | **<0.001** | 0.005 | **0.018** |
| Maternal weekly weight gain | 2.433 | **0.002** | 2.051 | **<0.001** | 2.129 | **<0.001** | 1.513 | **<0.001** |
| Maternal HIV infection | −0.416 | *0.082* | −0.265 | **0.007** | −0.150 | 0.100 | −0.085 | 0.377 |
| Maternal peripheral blood malaria parasitemia at enrollment | −0.507 | **0.003** | −0.321 | **<0.001** | −0.393 | **<0.001** | −0.294 | **<0.001** |
| Maternal periapical oral infection (as diagnosed after delivery) | −0.702 | **0.008** | −0.205 | **0.019** | −0.112 | 0.159 | −0.155 | *0.061* |
| Maternal UTI (as diagnosed after delivery) | −1.540 | **0.024** | −0.571 | **0.014** | −0.592 | **0.007** | −0.195 | 0.389 |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | −0.811 | **0.011** | −0.270 | **0.019** | −0.316 | **0.003** | −0.210 | *0.062* |
| Maternal salivary cortisol concentration at 36 gw | −0.173 | **0.001** | −0.020 | 0.132 | −0.028 | **0.019** | −0.028 | **0.026** |
| Maternal plasma AGP concentration at enrollment | −0.710 | **0.025** | −0.693 | **<0.001** | −0.771 | **<0.001** | −0.592 | **<0.001** |
| Placental weight (g) | 0.008 | **<0.001** | 0.003 | **<0.001** | 0.004 | **<0.001** | 0.004 | **<0.001** |
| Placental malaria infection | −0.035 | 0.874 | −0.300 | **<0.001** | −0.317 | **<0.001** | −0.184 | **0.013** |
| Severe chorioamnionitis | −1.117 | **0.019** | −0.149 | 0.213 | −0.113 | 0.306 | −0.142 | 0.226 |
| Duration of pregnancy | NA | NA | 0.270 | **<0.001** | 0.288 | **<0.001** | 0.283 | **<0.001** |
| Newborn LAZ | 0.707 | **<0.001** | NA | NA | 0.693 | **<0.001** | 0.611 | **<0.001** |
| Newborn WAZ | 0.878 | **<0.001** | 0.808 | **<0.001** | NA | NA | 0.774 | **<0.001** |
| Newborn HCZ | 0.781 | **<0.001** | 0.646 | **<0.001** | 0.703 | **<0.001** | NA | NA |

## A3.4. Factors Independently Associated with Each of the Predictor Variables Used in the Pathway Analysis: Results from Multivariable Regression Models

Green cells with bold font indicate a regression coeffient with $P < 0.05$; Yellow cells with italic font indicate $0.05 \leq P < 0.10$.

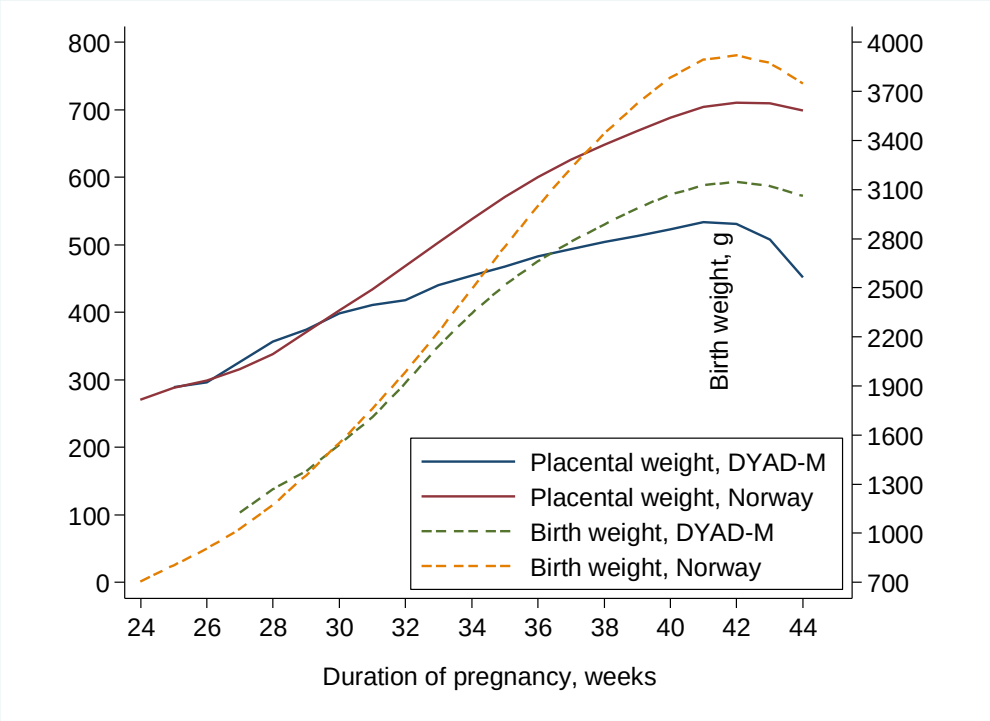
| | Maternal age at enrollment | | Maternal primiparity | | Maternal height at enrollment | | Maternal BMI at enrollment | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **B-coefficient** | **P-value** | **Odds ratio** | **P-value** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** |
| Maternal age at enrollment | NA | NA | 0.616 | **<0.001** | 0.024 | 0.435 | 0.070 | **<0.001** |
| Maternal primiparity | −6.228 | **<0.001** | NA | NA | −0.437 | 0.341 | 0.450 | **0.046** |
| Maternal height at enrollment | 0.019 | 0.435 | 0.994 | 0.752 | NA | NA | 0.002 | 0.867 |
| Maternal BMI at enrollment | 0.229 | **<0.001** | 1.098 | **0.013** | 0.009 | 0.867 | NA | NA |
| Maternal blood Hb concentration at enrollment | 0.004 | 0.657 | 0.978 | **<0.001** | −0.013 | 0.173 | 0.018 | **<0.001** |
| Maternal weekly weight gain | 1.914 | 0.176 | 0.266 | 0.191 | 6.293 | **<0.001** | −3.913 | **<0.001** |
| Maternal HIV infection | 1.867 | **<0.001** | 0.407 | **0.027** | 0.217 | 0.653 | 0.347 | 0.147 |
| Maternal peripheral blood malaria parasitemia at enrollment | −1.131 | **<0.001** | 0.944 | 0.789 | 0.276 | 0.448 | −0.245 | 0.170 |
| Maternal periapical oral infection (as diagnosed after delivery) | 2.366 | **<0.001** | 0.686 | 0.283 | −0.082 | 0.855 | −0.213 | 0.331 |
| Maternal UTI (as diagnosed after delivery) | −0.384 | 0.663 | 1.526 | 0.435 | −0.511 | 0.595 | 0.522 | 0.272 |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | −0.474 | 0.327 | 0.745 | 0.372 | −1.458 | **0.006** | −0.082 | 0.751 |
| Maternal salivary cortisol concentration at 36 gw | −0.118 | **0.014** | 0.982 | 0.603 | −0.017 | 0.756 | 0.026 | 0.332 |
| Maternal plasma AGP concentration at enrollment | −0.371 | 0.529 | 2.903 | **0.007** | −0.393 | 0.551 | −0.358 | 0.268 |
| Placental weight (g) | 0.000 | 0.776 | 0.999 | 0.486 | 0.006 | **<0.001** | 0.002 | **0.007** |
| Placental malaria infection | −1.056 | **0.002** | 2.679 | **<0.001** | −0.030 | 0.939 | −0.529 | **0.012** |
| Severe chorioamnionitis | 0.189 | 0.691 | 0.832 | 0.606 | −0.398 | 0.460 | 0.356 | 0.218 |

| | Maternal blood Hb concentration at enrollment | | Maternal weekly weight gain | | Maternal HIV infection | | Maternal peripheral blood malaria parasitemia at enrollment | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** |
| Maternal age at enrollment | 0.038 | 0.657 | 0.001 | 0.177 | 1.066 | **<0.001** | 0.951 | **<0.001** |
| Maternal primiparity | −6.149 | **<0.001** | −0.016 | **0.043** | 0.258 | **<0.001** | 1.079 | 0.686 |
| Maternal height at enrollment | −0.103 | 0.173 | 0.002 | **<0.001** | 1.005 | 0.739 | 1.009 | 0.440 |
| Maternal BMI at enrollment | 0.566 | **<0.001** | −0.005 | **<0.001** | 1.049 | 0.111 | 0.962 | 0.137 |
| Maternal blood Hb concentration at enrollment | NA | NA | 0.000 | **0.047** | 0.978 | **<0.001** | 0.976 | **<0.001** |
| Maternal weekly weight gain | 8.747 | **0.047** | NA | NA | 0.065 | **0.002** | 0.236 | **0.039** |
| Maternal HIV infection | −5.291 | **<0.001** | −0.024 | **0.003** | NA | NA | 0.740 | 0.150 |
| Maternal peripheral blood malaria parasitemia at enrollment | −5.919 | **<0.001** | −0.012 | *0.051* | 0.806 | 0.287 | NA | NA |
| Maternal periapical oral infection (as diagnosed after delivery) | −1.227 | 0.309 | −0.013 | *0.082* | 1.275 | 0.247 | 0.593 | **0.008** |
| Maternal UTI (as diagnosed after delivery) | −1.632 | 0.531 | −0.014 | 0.369 | 2.119 | *0.090* | 0.868 | 0.729 |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | 2.710 | *0.067* | −0.008 | 0.362 | 1.684 | *0.054* | 1.357 | 0.172 |
| Maternal salivary cortisol concentration at 36 gw | −0.407 | **0.009** | −0.002 | **0.027** | 0.984 | 0.600 | 0.969 | 0.225 |
| Maternal plasma AGP concentration at enrollment | −2.534 | 0.165 | 0.037 | **0.001** | 6.037 | **<0.001** | 8.306 | **<0.001** |
| Placental weight (g) | −0.006 | 0.194 | 0.000 | **0.008** | 0.998 | **0.043** | 1.000 | 0.616 |
| Placental malaria infection | −0.143 | 0.898 | −0.014 | **0.027** | 1.263 | 0.281 | 2.479 | **<0.001** |
| Severe chorioamnionitis | −0.842 | 0.574 | 0.002 | 0.858 | 1.038 | 0.894 | 1.434 | 0.126 |

| | Maternal periapical oral infection (as diagnosed after delivery) | | Maternal UTI (as diagnosed after delivery) | | Maternal vaginal trichomoniasis (as diagnosed after delivery) | | Maternal salivary cortisol concentration at 36 gw | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** | **B-coefficient** | **P-value** |
| Maternal age at enrollment | 1.088 | **<0.001** | 0.986 | 0.715 | 0.979 | 0.302 | −0.048 | **0.014** |
| Maternal primiparity | 0.727 | 0.290 | 1.759 | 0.264 | 0.716 | 0.267 | −0.258 | 0.420 |
| Maternal height at enrollment | 0.998 | 0.903 | 0.981 | 0.537 | 0.954 | **0.007** | −0.006 | 0.756 |
| Maternal BMI at enrollment | 0.974 | 0.369 | 1.072 | 0.245 | 0.989 | 0.753 | 0.035 | 0.332 |
| Maternal blood Hb concentration at enrollment | 0.995 | 0.332 | 0.993 | 0.506 | 1.012 | *0.068* | −0.017 | **0.010** |
| Maternal weekly weight gain | 0.251 | *0.091* | 0.171 | 0.318 | 0.367 | 0.320 | −2.149 | **0.027** |
| Maternal HIV infection | 1.282 | 0.232 | 1.967 | 0.134 | 1.694 | **0.048** | −0.055 | 0.853 |
| Maternal peripheral blood malaria parasiteamia at enrollment | 0.588 | **0.006** | 0.874 | 0.736 | 1.360 | 0.160 | −0.310 | 0.195 |
| Maternal periapical oral infection (as diagnosed after delivery) | NA | NA | 1.804 | 0.208 | 1.489 | *0.095* | 0.835 | **0.003** |
| Maternal UTI (as diagnosed after delivery) | 1.800 | 0.207 | NA | NA | 1.543 | 0.387 | −0.355 | 0.552 |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | 1.515 | *0.081* | 1.527 | 0.401 | NA | NA | 0.182 | 0.619 |
| Maternal salivary cortisol concentration at 36 gw | 1.085 | **0.005** | 0.957 | 0.509 | 1.012 | 0.732 | NA | NA |
| Maternal plasma AGP concentration at enrollment | 1.008 | 0.981 | 1.578 | 0.513 | 2.178 | **0.046** | 0.567 | 0.185 |
| Placental weight (g) | 1.001 | 0.504 | 1.000 | 0.831 | 0.999 | 0.259 | −0.003 | **0.008** |
| Placental malaria infection | 0.886 | 0.547 | 1.022 | 0.961 | 1.449 | 0.114 | 0.240 | 0.338 |
| Severe chorioamnionitis | 1.491 | 0.124 | 2.847 | **0.013** | 1.367 | 0.310 | −0.126 | 0.708 |

| | Maternal plasma AGP concentration at enrollment | | Placental weight (g) | | Placental malaria infection | | Severe chorioamnionitis | |
|---|---|---|---|---|---|---|---|---|
| **Predictor variable** | **B-coefficient** | **P-value** | **B-coefficient** | **P-value** | **Odds ratio** | **P-value** | **Odds ratio** | **P-value** |
| Maternal age at enrollment | −0.001 | 0.529 | −0.194 | 0.776 | 0.956 | **0.002** | 1.007 | 0.707 |
| Maternal primiparity | 0.096 | **<0.001** | −14.827 | 0.183 | 2.893 | **<0.001** | 0.864 | 0.641 |
| Maternal height at enrollment | −0.001 | 0.551 | 2.153 | **<0.001** | 0.999 | 0.937 | 0.987 | 0.464 |
| Maternal BMI at enrollment | −0.003 | 0.268 | 3.249 | **0.008** | 0.926 | **0.014** | 1.048 | 0.204 |
| Maternal blood Hb concentration at enrollment | −0.001 | 0.165 | −0.278 | 0.194 | 1.000 | 0.928 | 0.996 | 0.560 |
| Maternal weekly weight gain | 0.216 | **0.001** | 90.135 | **0.008** | 0.201 | **0.028** | 1.171 | 0.868 |
| Maternal HIV infection | 0.107 | **<0.001** | −22.831 | **0.034** | 1.298 | 0.224 | 1.025 | 0.927 |
| Maternal peripheral blood malaria parasitemia at enrollment | 0.121 | **<0.001** | 3.843 | 0.637 | 2.488 | **<0.001** | 1.423 | 0.141 |
| Maternal periapical oral infection (as diagnosed after delivery) | 0.002 | 0.906 | 6.080 | 0.545 | 0.900 | 0.600 | 1.497 | 0.121 |
| Maternal UTI (as diagnosed after delivery) | 0.023 | 0.559 | 4.355 | 0.860 | 1.022 | 0.960 | 2.786 | **0.016** |
| Maternal vaginal trichomoniasis (as diagnosed after delivery) | 0.045 | *0.051* | −12.905 | 0.250 | 1.398 | 0.152 | 1.341 | 0.345 |
| Maternal salivary cortisol concentration at 36 gw | 0.003 | 0.186 | −3.746 | **0.007** | 1.025 | 0.320 | 0.985 | 0.670 |
| Maternal plasma AGP concentration at enrollment | NA | NA | −25.841 | 0.104 | 1.653 | 0.105 | 0.265 | **0.004** |
| Placental weight (g) | 0.000 | 0.101 | NA | NA | 1.001 | 0.186 | 0.999 | 0.271 |
| Placental malaria infection | 0.028 | *0.091* | 10.901 | 0.189 | NA | NA | 0.974 | 0.902 |
| Severe chorioamnionitis | −0.063 | **0.003** | −12.446 | 0.281 | 0.976 | 0.910 | NA | NA |

## A3.5. Association between the Duration of Pregnancy and Placental Weight and Birth Weight in the Study Cohort (including comparison to a Norwegian reference population)




Source for Norway data (Thompson et al. 2007).

## Appendix 4. Biological Sample Collection

**Blood collection and processing.** Venous blood was collected from the antecubital vein by licensed nurses into a 7.5 mL trace mineral-free polypropylene syringe (Sarstedt Monovette, NH4-heparin, Sarstedt Inc., Newton, NC, USA). The blood tube was inverted 10 times to mix the heparin anticoagulant with the blood to prevent clotting. The tube was covered in aluminum foil and placed in an insulated cooler with ice packs until processing (no longer than 2 hours).

From the whole blood, a pipette was used to collect three drops (3 x 50 µL) onto each of three dried blood spot collection papers that were used to analyze essential fatty acids and PCR diagnosis of malaria. The paper was dried for 15 minutes at room temperature, placed in a zip lock bag with a desiccant pouch, and placed in a −20°C freezer. An additional drop of whole blood was used for malaria microscopy, and another for Hb analysis on the Hemocue 201+ system (Hemocue, Brea, CA, USA). An additional 5 µL of whole blood was used to analyze malaria via a rapid antigen test as per the instructions included with the cassettes.

A pipette was used to transfer 100 µL of whole blood into each of two 0.5 mL microcentrifuge tubes for ZPP analysis. The tubes were stored on ice or in the refrigerator until analysis (within 30 hours of blood draw). ZPP was analyzed from both whole blood and from washed red blood cells. The method of washing red blood cells is as follows. The height of the column of blood in the tube was marked on the side of the tube (to reconstitute later as described below). Normal saline (100 µL) was added to the tube and the tube centrifuged at 1,000 g for 10 minutes. The upper plasma/saline portion was removed and discarded and another 100 µL added. This process was repeated until the red blood cells were washed a total of three times. After the last decant, saline was added to reconstitute to the original volume. ZPP was analyzed from both reconstituted red blood cells and whole blood on a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA).

The remaining whole blood was centrifuged at 3,000 RPM for 15 minutes and plasma separated into storage cryovials. The vials used to analyze vitamin A, and the B vitamins were covered with aluminum foil to protect from UV light. All the storage vials were placed upright in freezer boxes and placed along with the dried blood spot cards into a −20°C freezer for temporary storage at the satellite clinics.

**Urine collection and processing.** Urine was collected during clinic visits. Women were instructed to clean with an alcohol swab and collect urine in a sterile cup. The cup was placed in a cooler with ice packs until processing (within 2 hours). A disposable plastic transfer pipette was used to transfer urine to cryovials for storage.

**Saliva collection and storage.** Saliva was the first specimen collected upon arrival at the clinic. The swab was placed in the participant's mouth under the tongue for 2 minutes. The swab was then placed in the capped storage tube and put into an insulated container with ice until processing (within 2 hours). Saliva was brought to room temperature before processing. Once at room temperature, the storage tube and swab were centrifuged for 15 minutes at 1,500 g. A pipette was used to transfer the saliva to storage cryovials. The vials were placed upright in a −20°C freezer until transferred to −80°C within 48 hours.

**Vaginal swab collection.** At a postnatal visit (approximately 1 week after delivery), a nurse used four cotton swabs to collect samples of vaginal mucus. She inserted the swabs approximately 7 cm deep into the participant's vagina, without a visual control, and rotated the swab three times before withdrawing it. After the sample collection, one swab was used for the so-called sniff test (potassium hydroxide addition), to diagnose bacterial vaginosis, one swab was used for direct microscopy (trichomoniasis and Candida

albicans diagnosis (see Section 2.9.6), and the two others were stored at −80°C for later bacterial analysis with DNA amplification methodology (see Section 2.9.12).

**Placental and amniotic membrane tissue collection.** After delivery, the placenta was transferred to a sterile container to await tissue sampling. In all cases, sampling occurred immediately after delivery, unless delivery occurred overnight, in which case the placenta was sampled the following morning. A 5 cm x 1 cm piece of the chorionic and amniotic membranes was taken from the edge of the rupture site and a 0.5 cm x 0.5 cm piece of placental tissue at full thickness was taken from the near the umbilical cord insertion. The two samples were placed in separate cryovials. If the sample collection took place in Mangochi district hospital, the cryovials were entered into −80°C storage. In the case that sample collection took place at an outlying health center or Malindi hospital, the samples were kept at −20°C for a maximum of 2 days before being transported to −80°C storage at Mangochi district hospital.

**Dental plaque collection and storage.** Dental plaque was collected at the Mangochi central site from all mothers who completed the oral health visit at 1 week after delivery or as soon as possible after delivery, by specially trained dental therapists. One sterile plastic swab stick with a nylon fiber tip, stored in plain dry tube (microRheologics no. 552, Coban, Brescia, Italy), was used for the sample collection.

Prior to the sample collection, the assistant marked the swab tubes with appropriate identification codes. The dental therapists collected the sample by rubbing the gingival margin of each tooth with the swab. They used a dental mirror to elevate the cheeks so that the teeth were visible and skin contact was avoided. They started the sample collection from the buccal side of the most posterior (farthest) tooth on the right upper jaw. They repeated the procedure for the palatal sites of the same teeth, continuing then to the lower jaw and repeating it for all lower teeth's buccal and lingual sides. They then placed the swab back into the tube, closed the cap tightly and immediately placed the tube into a cold box with ice bricks. Immediately after that, they took the cold box to the laboratory and handed it over to a laboratory technician.

The laboratory technician processed the sample as soon as possible after it had arrived at the laboratory. He removed the swab from the tube and cut the applicator stick with scissors above the fiber tip to fit the swab into cryovials where they were stored. Prior to cutting, he wiped the scissors with disinfectant to avoid contamination. He then placed the cryovials in a −20°C freezer and, as soon as possible, moved the swab into a −80°C freezer.

## Appendix 5. Detailed Variable Definitions

**α-1-glycoprotein plasma concentration**
Total AGP (mg/L) measured in plasma. Elevated AGP was defined as AGP >1 mg/L.

**α-linolenic acid (ALA), a fatty acid**
An essential omega-3 polyunsaturated fatty acid, 18:3 (n-3). Chemical name: *all-cis*-9,12,15-octadecatrienoic acid.

**Arachidonic acid (AA), a fatty acid**
An omega-6 polyunsaturated fatty acid, 20:4 (n-6). Chemical name: *all-cis*-5,8,11,14-eicosatetraenoic acid.

**β diversity: bacterial composition in the placenta, fetal membrane, oral cavity, or vagina**
β diversity was measured by unweighted UniFrac distances to create variables that measure how similar or different bacterial communities are between samples.

Unweighted UniFrac distance measures phylogenetic difference at the sequence level rather than just presence/absence of species. As an input, it uses both the relative abundance data of every species in every compared sample, as well as the full phylogenetic tree of each species, and relates it in the entire dataset. The distance between each sample is defined by the equation:

$$u = \sum_{i}^{n} b_i \times \left| \frac{A_i}{A_T} - \frac{B_i}{B_T} \right|$$

where n = the total number of branches in the tree; $b_i$ = the length of branch i; $A_i$ and $B_i$ = the number of descendants of branch i from communities A and B, respectively; and $A_T$ and $B_T$ = the total number of sequences from communities A and B, respectively. To control for unequal sampling effort, $A_i$ and $B_i$ are divided by $A_T$ and $B_T$.

**Bacterial load in the placenta or fetal membrane**
Bacterial load was quantified against a standard curve of extracted DNA from a pure Escherichia coli culture after plate counting. Bacterial 16S rDNA was amplified using broad-range DNA primers in repeated cycles with absolute load defined at what cycle SYBR green fluorescence passed a user-defined threshold compared to the serial dilution of the positive control.

**Birth weight**
Birth weight was defined as a weight measured within 48 hours of delivery, expressed in grams, rounded to the nearest 10 g and with no decimals.

**Birth weight, low birth weight**
LBW was defined as birth weight <2,500 g. The proportion of LBW babies was calculated as the number of babies with a birth weight <2,500 g divided by the number of all babies with valid birth weight data (measured within 48 hours of birth). The values were expressed as a percentage, with one decimal.

**Body mass index**
BMI was calculated by dividing weight by squared height. Underweight was defined as BMI <18.5 and overweight as BMI >25.0.

**Cholesterol**
Total cholesterol concentration (mg/dL) measured in plasma. Low cholesterol was defined as cholesterol <10th percentile of the IFA group, as this was an outcome of interest in previous studies (Edison et al. 2007).

**C-reactive protein, plasma concentration**
Total maternal plasma CRP concentration (g/L) measured in plasma. Elevated CRP was defined as CRP >5 g/L.

**Dental caries, grade II (dentine caries)**
Dentine caries is defined as a carious lesion extending to the dentine but not exposing the pulp of a tooth, diagnosed either in clinical examination or from radiographs. A participant had dentine caries if at least one lesion was recorded.

**Dental caries, grade III (pulpal caries)**
Pulpal caries is defined as a lesion extending to the pulp of the tooth with no bony layer visible in between, diagnosed from radiographs. A participant had pulpal caries if at least one lesion was recorded.

**Docosahexaenoic acid (DHA), a fatty acid**
An omega-3 polyunsaturated fatty acid, 22:6 (n-6). Chemical name: *all-cis*-4,7,10,13,16,19-docosahexaenoic acid

**Duration of pregnancy**
The duration of pregnancy was calculated from gestational age at enrollment, date of enrollment, and date of delivery, using the following formula: The duration of pregnancy at birth = the duration of pregnancy at enrollment + (date of delivery − date of enrollment) ÷ 7.

Women with twin pregnancy were considered not to have valid data on this outcome because ultrasound dating of pregnancy is unreliable for twin pregnancies; therefore, they were excluded from this analysis. The values are expressed as gw, with one decimal.

Preterm birth was defined as one occurring before 37.0 completed gw. The incidence of preterm birth was calculated by dividing the number of women with a preterm birth by the number of all participating women with valid data on the duration of pregnancy. The values are expressed as a percentage, with one decimal.

**Fatty acids**
All fatty acids were measured in plasma as a percentage of total fatty acids. Fatty acids were also defined dichotomously using a median cutoff, with a high fatty acid being >50th percentile of the IFA group.

**Fatty acids, omega-6:omega-3 ratio**
The ratio was defined as the sum of all omega-6 fatty acids divided by the sum of all omega-3 fatty acids.

**Gingivitis**
Gingivitis was diagnosed if periodontal pocket probing resulted in profound bleeding from the gums. A participant had gingivitis if bleeding was seen in at least one dental arch sextant (right, middle and left, upper and lower).

**Hemoglobin concentration, blood, anemia**
Anemia was defined as Hb <100 g/L, which has been suggested as an appropriate cutoff for pregnant women of African descent (Johnson-Spear and Yip 1994, Cao and O'Brien 2013, Chang et al. 2003).

**HCZ**
See Newborn head circumference-for-age z-score.

**Histological chorioamnionitis of the chorionic plate or amniotic membrane**
Chorioamnionitis was defined as ≥5 neutrophil granulocytes on average per 10 high power fields present in either the chorionic plate or the amniotic membrane.

**Histological chorioamnionitis of the chorionic plate or amniotic membrane, severe**
Severe chorioamnionitis was defined as >25 neutrophil granulocytes on average per 10 high power fields present in either the chorionic plate or the amniotic membrane.

**Iron deficiency anemia**
Iron deficiency anemia (IDA) was defined as Hb <100 g/L and either ZPP >60 μmol/mol heme or sTfR >6.0 mg/L.

**LAZ**
See Newborn length-for-age z-score

**Malaria antibody levels, rate of change**
Antibody levels were measured as a percentage of the positive control. The rate of change in antibody levels per week of gestation was measured using the following formula:

$$rate\ of\ change\ in\ antibody\ levels = \frac{antibody\ levels\ at\ 36\ gw - antibody\ levels\ at\ enrollment}{number\ of\ gestation\ weeks\ from\ enrollment\ to\ 36\ gw}$$

**Malaria antibody tertiles (low, medium, and high responders)**
Participants were divided into three groups based on their antibody levels following rearrangement of antibodies from low to high. Low responders were defined as the 33.3% of individuals with the lowest antibody levels and high responders as the 33.3% of individuals with the highest antibody levels.

**Malaria infection, placental malaria, active**
Active malarial infection was defined as presence of malarial parasites in intervillous erythrocytes.

**Malaria infection, placental malaria, past**
Past malarial infection was defined as presence of malarial pigment within fibrin.

**Malaria, peripheral blood parasitemia**
Malaria parasitemia was diagnosed as a positive *P. falciparum* test on RDT using Clearview Malaria Combo (British Biocell International Ltd., Dundee, UK) at enrollment, at 32 gw, and at delivery. PCR was used to diagnose asymptomatic malaria at 36 gw.

**Newborn head circumference-for-age z-score**
HCZ was calculated from age, sex, and head circumference information from the first measurement taken at the study clinic within 6 weeks (42 days) of delivery, using the Stata macro developed by WHO using the WHO 2006 multicenter growth standard. The values are expressed as z-score units.

Small head circumference was defined as HCZ <−2. The prevalence of small head circumference was calculated by dividing the number of babies with HCZ <−2 by the number of all babies with valid data on this outcome.

**Newborn length-for-age z-score**
Length-for-age was calculated from age, sex, and length information from the first measurement taken at the study clinic within 6 weeks (42 days) of delivery, using the Stata macro developed by WHO using the WHO 2006 Multicenter Growth Standard. The values are expressed as z-score units.

Stunting was defined as LAZ <−2. The prevalence of stunting was calculated by dividing the number of babies with LAZ <−2s by the number of all babies with valid data on this outcome. The values are expressed as a percentage.

**Newborn weight-for-age z-score**
Weight-for-age was calculated from age, sex, and weight information from the first measurement taken at the study clinic within 6 weeks (42 days) of delivery, using the Stata macro developed by WHO using the WHO 2006 Multicenter Growth Standard. The values are expressed as z-score units.

Underweight was defined as WAZ <−2. The prevalence of underweight was calculated by dividing the number of babies with WAZ <−2 by the number of all babies with valid data on this outcome. The values are expressed as a percentage.

**Perceived Stress Score**
A score was obtained by reversing the scores on the positive items (0 changes to 4, 1 changes to 3, 2 remains 2, etc.) and then summing all scores (0 = no perceived stress, 40 = high level of perceived stress) (Cohen et al. 1983). Scores at each measurement time point followed a normal distribution and results were analyzed separately at each time point. The scores were also defined dichotomously using a median cutoff value, with high scores being >50th percentile (Cohen et al. 1983).

**Periapical infections**
Pulpal caries was defined as an osteolytic finding >1mm with diffuse margins surrounding the apex of the root, diagnosed from radiographs. A participant had periapical infections if at least one lesion was recorded.

**Periodontitis**
A tooth was defined to have periodontitis if either a ≥4 mm periodontal pocket was diagnosed in clinical examination or a vertical bony pocket extending at least to the cervical third of the root's full length was seen on radiograph. Horizontal bone loss was also assessed from radiographs by measuring it from the dento-enamel junction to the bone margin, and it was reported in comparison to the full root length (normal, cervical, middle, or apical third) by dental arch sextants.

For this study, a participant was defined to have periodontitis if she had at least three teeth with periodontitis or at least one sextant with horizontal bone loss and gingivitis present. Third molars (wisdom teeth) were excluded from the periodontitis diagnosis.

**Placental inflammation, acute intervillositis**
Acute intervillositis was defined as ≥5 neutrophils on average per 10 high power fields in the placental intervillous space.

**Placental inflammation, chronic intervillositis**
Chronic intervillositis was defined as ≥5 lymphocytes/monocytes on average per 10 high power fields in the placental intervillous space.

**Placental weight**
Placental weight was defined as a weight measured after delivery, expressed in grams, rounded to the nearest 1 g and with no decimals.

**Retinol, plasma concentration**
Plasma retinol concentration was expressed as retinol in µmol/L, rounded to two decimal places. Low plasma retinol concentration was defined as plasma retinol <1.05 µmol/L (Sauberlich et al. 1974).

**Salivary cortisol**
The salivary cortisol distribution was highly skewed and was log-transformed for analysis. Cortisol was also classified into quartiles based on the cortisol distributions within the IFA group at each measurement time point.

**Small for gestational age**
Small for gestational age was defined by fetal growth curve developed by Alexander et al. (1996). The incidence of small-for-gestational-age babies was calculated by dividing the number of small-for-gestational-age babies by the number of all babies with valid data on duration of pregnancy and birth weight. The values are expressed as a percentage, with one decimal.

**sTfR, plasma concentration**
**sTfR** was analyzed by immunoturbidimetry on the Cobas Integra 400 system (F. Hoffmann-La Roche Ltd, Basel, Switzerland). The cutoff for iron deficiency was defined at sTfR >6.0 mg/L. A sTfR cutoff of 8.5 mg/L has been used previously when analyzing sTfR by the enzyme linked immunosorbent assay (ELISA) method (Carriaga et al. 1991, Rusia et al. 1999, Vandevijvere et al. 2013). However, Pfeiffer et al. (2007) compared the ELISA and autoanalyzer methods and found that the autoanalyzer gives sTfR estimates approximately 30% lower than the ELISA method. Therefore, we decreased the 8.5 mg/L cutoff by 30%, to approximately 6.0 mg/L.

**Triglycerides**
Total triglyceride concentration (mg/dL) measured in plasma.

**WAZ**
See Newborn weight-for-age z-score

**Weekly gestational weight gain**
Inadequate weekly weight was defined gain as less than the lower cutoff of the IOM recommendations (Rasmussen and Yaktine 2009), which are based on pre-pregnancy BMI as shown in the table below.

| Pre-pregnancy BMI (kg/m$^2$) | Lower limit of recommended weight gain (kg/wk) |
|---|---|
| <18.5 | 0.44 |
| ≥18.5 to <25 | 0.35 |
| ≥25 to <30 | 0.23 |
| ≥30 | 0.17 |

**ZPP, plasma concentration**
ZPP was analyzed from washed erythrocytes on a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA). Iron deficiency was defined as ZPP >60 µmol/mol heme (Walsh et al. 2011).

## Appendix 6. Predictors of Main Birth Outcome Variables

| Chapter | Predictor | Type | Timing |
|---|---|---|---|
| **4.7** | AA, % total fatty acids | continuous, high AA | 36 gw |
| **4.4** | AGP, g/L | continuous, elevated AGP | enrollment, 36 gw |
| **4.7** | ALA, % total fatty acids | continuous, high ALA | 36 gw |
| **4.12** | Antibodies, opsonizing to pregnancy-specific VSA | continuous, low- medium- or high level | 36 gw |
| **4.10** | Bacterial diversity, UniFrac distance in fetal membranes | Continuous | delivery |
| **4.10** | Bacterial diversity, UniFrac distance in placental tissue | Continuous | delivery |
| **4.10** | Bacterial diversity, UniFrac distance in the oral cavity | Continuous | 1 week after delivery |
| **4.10** | Bacterial diversity, UniFrac distance in the vagina | Continuous | 1 week after delivery |
| **4.10** | Bacterial load in the fetal membranes, rDNA copies/µl | continuous, dichotomous | delivery |
| **4.10** | Bacterial load in the placenta, rDNA copies/µl | continuous, dichotomous | delivery |
| **4.5** | Blood Hb, g/L | continuous, Hb <100 or >130 | enrollment, 36 gw |
| **4.1** | BMI at enrollment, kg/m$^2$ | continuous, underweight, overweight | delivery |
| **4.7** | Cholesterol, mg/dL | continuous, low cholesterol | 36 gw |
| **4.9** | Chorioamnionitis | dichotomous | delivery |
| **4.9** | Chorioamnionitis, severe | dichotomous | delivery |
| **4.8** | Cortisol, nmol/L | continuous, quartiles | enrollment, 28 gw, 36 gw |
| **4.7** | DHA, % total fatty acids | continuous, high DHA | 36 gw |
| **4.3** | HIV | dichotomous | enrollment |
| **4.9** | Intervillositis | dichotomous | delivery |
| **4.5** | Iron deficiency anemia (IDA) | dichotomous, low Hb and either high ZPP and/or high sTfR | enrollment, 36 gw, change enrollment to 36 gw |
| **4.9** | Malarial infection, active | dichotomous | delivery |
| **4.9** | Malarial infection, past | dichotomous | delivery |
| **4.2** | Maternal malaria | dichotomous | delivery, 32 gw |
| **4.7** | Omega-6:omega-3 ratio | continuous, high ratio | 36 gw |
| **4.8** | Perceived stress score | continuous, high perceived stress | enrollment, 28 gw, 36 gw |

| Chapter | Predictor | Type | Timing |
|---|---|---|---|
| **4.11** | Periapical infection | dichotomous | 36 gw |
| **4.11** | Periodontitis | dichotomous | 36 gw |
| **4.4** | Plasma CRP, mg/L | continuous, elevated CRP | enrollment, 36 gw |
| **4.6** | Plasma retinol, µmol/L | continuous | enrollment, 36 gw |
| **4.5** | sTfR, mg/L | continuous | enrollment, 36 gw |
| **4.12** | Total IgG to pregnancy-specific VSA | continuous, low- medium- or high level | 36 gw |
| **4.3** | Trichomoniasis | dichotomous | delivery |
| **4.7** | Triglycerides, mg/dL | continuous | 36 gw |
| **4.3** | UTI | dichotomous | delivery |
| **4.1** | Weekly weight gain, g/week | continuous, < IOM recommendation, > IOM recommendation | delivery |
| **4.5** | ZPP, µmol/mol heme | continuous | enrollment, 36 gw |