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The Impact of Dietary Supplementation with Lipid-Based Nutrient Supplements on Maternal Health and Pregnancy Outcomes in Rural Malawi

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Abstract

Background. Small birth size, often associated with insufficient maternal nutrition, contributes to a large share of global child undernutrition, morbidity, and mortality. We developed a small-quantity lipid-based nutrient supplement (LNS) to enrich the diets of pregnant women.

Objective. To test the effect of home fortification of pregnant women's diets in an African community with LNS on the duration of pregnancy and birth size, as well as on multiple maternal outcomes.

Design. We enrolled 1,391 women with uncomplicated pregnancies (<20 gestation weeks [gw]) in a randomized, controlled trial in rural Malawi. The women were provided with one daily iron and folic acid (IFA) capsule, one capsule containing 18 micronutrients, or one 20 g sachet of LNS (containing 118 kcal, protein, carbohydrates, essential fatty acids [EFA], and 22 micronutrients). Primary measured outcomes were birth weight and newborn length. Secondary measured outcomes included newborn weight, head and mid-upper arm circumferences, pregnancy duration, and maternal weight gain. As inflammatory and stress outcomes, we measured maternal plasma concentration of C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) and salivary concentration of cortisol. For maternal nutrition, we analyzed two markers of iron status (soluble transferrin receptor [sTfR] and erythrocyte zinc protoporphyrin [ZPP] concentrations); plasma concentrations of vitamin A, folate, vitamin B12, defined fatty acid, cholesterol and triglycerides; and blood hemoglobin (Hb) concentrations. As infection markers, we studied maternal malaria and malaria immunity, vaginal trichomoniasis and urinary tract infections (UTIs), periodontitis, dental infections, and histological evidence of chorioamnionitis, as well as presence of bacteria and microbiota composition of samples collected from the placenta, amniotic membranes, vaginal mucus, and dental swabs. Analysis was by intention-to-treat.

Results. The mean [standard deviation (SD)] birth weight and newborn length were 2,948 g (432), 2,964 g (460), and 3,000 g (447) (P=0.258) and 49.5 cm (2.4), 49.7 cm (2.2), and 49.9 cm (2.1) (P=0.104) in the IFA, multiple micronutrient (MMN), and LNS groups, respectively. For newborn weight-for-age and head and arm circumferences, the point estimate for the mean was also highest in the LNS group, intermediate in the MMN group, and lowest in the IFA group, but, except for mid-upper arm circumference (MUAC) (P=0.024), the differences were not statistically significant. There were no significant intergroup differences in the mean plasma concentrations of CRP or AGP or salivary concentrations of cortisol, nor in plasma concentrations of cholesterol, triglycerides, folate, and defined fatty acid during the third trimester of pregnancy. At 36 gw of pregnancy, the proportion of women with iron deficiency anemia or low plasma concentration of vitamin A was higher in the LNS group than in the IFA group.

The prevalences of maternal malaria parasitemia at various points of pregnancy or soon thereafter, vaginal trichomoniasis, and UTIs after pregnancy were similar in the three groups, as were the mean bacterial load in the placenta and amniotic membranes, the prevalence of chorioamnionitis in the placenta, and the development of malaria immunity during pregnancy. Mean plasma vitamin B12 showed less decline during pregnancy in the LNS group compared to the IFA group, but there were no differences in the proportions of participants with low plasma vitamin B12 concentration at 36 gw.

Conclusions. The study findings do not support hypotheses that LNS would promote maternal health and fetal growth or increase mean birth size, if provided to an unselected group of pregnant women in the study area.

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Abbreviations and Acronyms

AA	arachidonic acid, an omega-6 fatty acid
AGP	α-1-acid glycoprotein
AI	adequate intake(s)
ALA	α-linolenic acid, an omega-3 fatty acid
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BMI	body mass index
CI	confidence interval
cm	centimeter(s)
COM	University of Malawi, College of Medicine
CRP	C-reactive protein
CV	coefficient of variation
DHA	docosahexaenoic acid, an omega-3 fatty acid
dL	deciliter(s)
DSMB	data safety and monitoring board
EBA	erythrocyte binding antigen
EFA	essential fatty acid(s)
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
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
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)
MFI	mean fluorescence intensity
µg	microgram(s)
m	meter(s)
mg	milligram(s)
mm	millimeter(s)
mmHg	millimeter(s) of mercury
MMN	multiple micronutrient(s)
MUAC	mid-upper arm circumference
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pRBC	parasitized red blood cell
PSS	Perceived Stress Scale
RDA	recommended dietary allowance(s)
RDT	rapid diagnostic testing
RE	retinol equivalent(s)
SAE	serious adverse event
SD	standard deviation
SE	standard error
SES	socioeconomic status
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
USDA-ARS	U.S. Department of Agriculture Agriculture Research Service
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 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)[1] 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

[1] "LNS" refers to a broad set of nutritional products used for rehabilitating malnourished individuals or promoting healthy growth. The majority of the energy provided by LNS products comes from fats or lipids (including essential fatty acids). LNS products also provide protein, vitamins, and minerals. They vary along a number of dimensions, most notably in energy dose and concentration of micronutrients. The dosage of LNS products ranges from large quantity LNS (usually around 180–200 g/day), which is used primarily for treatment of severe acute malnutriton; to medium quantity LNS (usually around 45–90 g/day), which is used primarily for treatment of moderate acute malnutrition; to small-quantity LNS (usually around 20 g/day), which is used primarily to support the promotion of healthy growth (Arimond et al. 2013). Although the term, "LNS", as used here, refers to the whole spectrum of lipid based nutrient supplement products, elsewhere in this report, unless otherwise noted, "LNS" is used to refer to small quantity LNS, specifically.

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 provides 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 study called Lipid-Based Nutrient Supplement – Reproductive Tract Infections (LNS-RTI). New variables studied as part of this add-on to the iLiNS-DYAD-M trial included maternal nutrition and malaria; maternal periodontitis and dental caries; infections of women's reproductive tract, placenta, and amniotic membranes; and maternal stress and plasma concentrations of fatty acids and cholesterol. These variables were used in two ways: as outcomes from the randomized trial and as predictors for adverse birth outcomes. In this report, we describe the impact of the trial interventions on these outcome variables. The associations between these variables and birth outcomes are described in a separate report (Ashorn et al. 2017).

1.2 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 intervention, the follow-up scheme, and the general approach to data collection and analysis. Details of the various methods used for the collection of data and laboratory and statistical analyses, along with the complete list of itemized study hypotheses, are not included in Chapter 2, but can be found in appendices at the end of the report.
- The study findings are reported in Chapters 3 and 4. Chapter 3 describes the general context of the study and Chapter 4 covers the intervention effects on various birth outcomes.
- Chapter 5 provides a general discussion on the reliability and validity of the findings, as well as their possible public health implications.
- A list of references mentioned in this report follows Chapter 5.
- The references are followed by a number of appendices: comparison tables on the enrollment characteristics of participants by intervention group (Appendix 1); supplementary details on data collection, laboratory analyses, and statistical analysis (Appendix 2); details on biological sample collection (Appendix 3); a full list of study hypotheses (Appendix 4); a comprehensive description of variable definitions (Appendix 5); and a list of reported outcome variables (Appendix 6).

2. Methods

2.1 Study Design, Outcomes, and Ethics Statement

The LNS-RTI study was based on a randomized, controlled, outcome assessor-blinded clinical trial in rural Malawi. In this trial, one-third of the enrolled women received LNS during pregnancy, while the rest of the enrolled women, who made up the control groups, received either IFA or MMN capsules. We used IFA as the first control because it was the national practice at the study site and MMN as a second control because of the existing evidence of its possible benefits over IFA (Fall et al. 2009).

The iLiNS-DYAD-M trial also included a postnatal component. However, since the LNS-RTI add-on concerned only the pregnancy intervention, the postnatal component is not described in detail in this report.

The primary outcome measures for the pregnancy component of the iLiNS-DYAD-M trial were birth weight and newborn length. Secondary outcomes included a wide range of maternal and child variables. The outcome variables included in this report can be found in Appendix 6.

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 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 (DSMB) monitored the incidence of suspected serious adverse events (SAEs) and performed two interim analyses for safety. The DSMB members received information about all suspected SAEs on an ongoing basis and met three times during the pregnancy 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, trial identification NCT01239693).

2.2 Study Hypotheses

For the intervention trial, the broad study hypothesis was that birth outcomes would be better among women supplemented with LNS than among women supplemented with IFA or MMN. For the primary outcomes (birth weight and newborn length), this was formulated as follows:

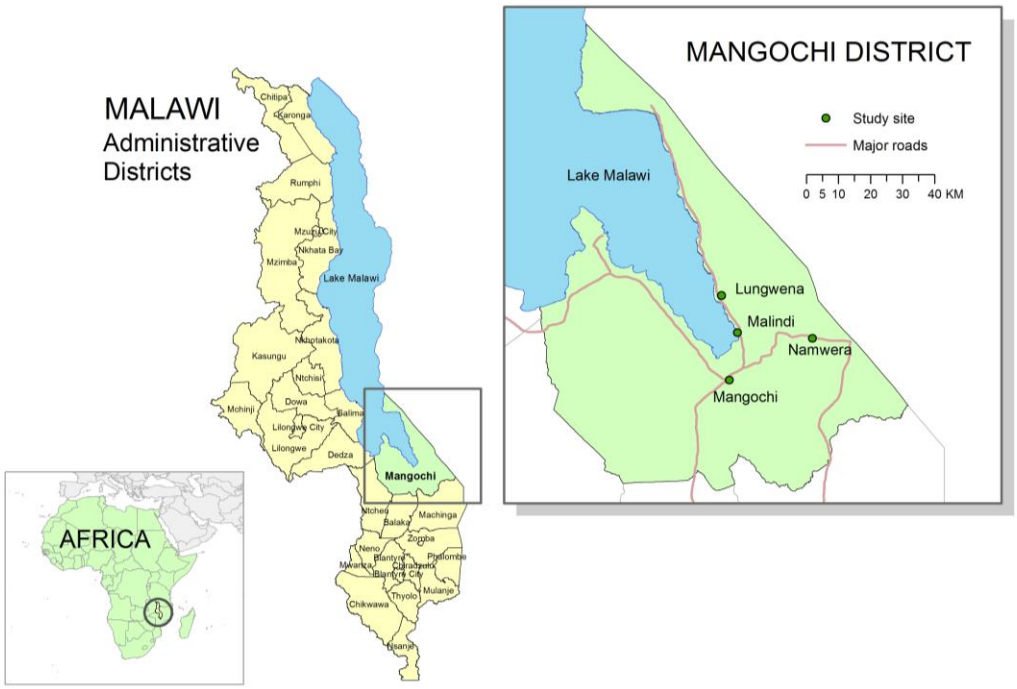
- Hypothesis 1: The mean birth weight among infants whose mothers are provided with LNS during pregnancy is higher than among infants whose mothers receive either IFA or MMN supplementation.
- Hypothesis 2: The proportion of LBW infants is lower among women who are provided with LNS during pregnancy than among women who receive either IFA or MMN supplementation.
- Hypothesis 3: The mean newborn length-for-age z-score (LAZ) is higher among infants whose mothers are provided with LNS during pregnancy than among infants whose mothers receive either IFA or MMN supplementation.
- Hypothesis 4: The prevalence of newborn stunting (LAZ <-2) is lower among infants whose mothers are provided with LNS during pregnancy than among infants whose mothers receive either IFA or MMN supplementation.

Similar hypotheses were formulated using the other outcome variables described in this report. A full list of hypotheses is provided in Appendix 4.

2.3 Study Site and Participants

Enrollment into 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 gestation weeks (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 of folic acid and two doses of IPTp with sulfadoxine-pyrimethamine (SP) (three tablets of 500 mg sulfadoxine and 25 mg pyrimethamine orally). One SP dose was given at enrollment and the other between the 28th week and the 34th week of gestation.
- Participants in the MMN group, the second control group, received IPTp and micronutrient capsules that contained IFA and 16 additional micronutrients (Arimond et al. 2015). Because of earlier positive results in Guinea-Bissau with higher-dose micronutrient supplementation (Fall et al. 2009) and failure to reach desired tissue concentrations among pregnant women provided with the recommended dietary allowances (RDAs) of micronutrients (Christian et al. 2006), the MMN capsules we used contained twice the amount used in most previous prenatal MMN trials for several micronutrients (thiamine, riboflavin, niacin, vitamin B6, vitamin B12, vitamin D, vitamin E, zinc, copper, and selenium) (Arimond et al. 2015).
- Participants in the LNS intervention group received IPTp and sachets of tailor-made small-quantity LNS. The daily dose (20 g) was designed to contain the same micronutrients as the MMN capsules, four additional minerals (calcium, phosphorous, potassium, and magnesium), protein, and fat. It also provided 118 kcal of energy (Table 2.4-1). The fat content of the LNS was optimized to provide high amounts of selected essential fatty acids (EFA) that were thought to be important in pregnancy (Coletta et al. 2010).

The iron dose was lower for participants in the MMN and LNS groups (20 mg/day) than for those in the IFA group (60 mg), because the MMN and LNS supplementation was continued during the first 6 months postpartum, when the recommended nutrient intake for lactating women is much lower than the standard antenatal dose (Arimond et al. 2015). Based on a literature review and our estimates of the normal dietary iron intakes among pregnant women in the study area, we considered 20 mg/day a safe and adequate dose to prevent iron deficiency anemia (IDA) during pregnancy (even for women who would be iron deficient at entry) (Arimond et al. 2015, Milman et al. 2006, Zhou et al. 2009).

The manufacturers packed the IFA and MMN capsules in plastic 10-capsule blister packs and the LNS in individual 20 g foil-sachets. Data collectors delivered 15 supplement doses (capsules or sachets) fortnightly to each participant until birth and advised them to consume daily either one capsule, to be taken with water after a meal (IFA and MMN groups), or one sachet of LNS, mixed with a small quantity of any food and consumed as one morning dose. At each visit, the data collectors counted and recovered any unused supplement doses from the participants.

The IFA and MMN capsules were custom-made at and purchased from DSM Nutritional Products South Africa (Pty) Ltd (Isando, South Africa). The LNS was produced and packed by Nutriset S.A.S. (Malaunay, France). Raw ingredients for the LNS included soybean oil, dried skimmed milk, peanut paste, mineral and vitamin mix, and sugar (Arimond et al. 2015). At the project office, the research team stored all supplements at a temperature between 20°C and 40°C, in cardboard boxes that protected the supplements from light. At participant homes (maximum storage time 2 weeks), the dietary supplements were recommended to be stored indoors, in as dry and cool a place as possible.

During the trial implementation, international organizations involved in medium-quantity LNS distribution to children with acute malnutrition made a recommendation on a new quality assurance procedure for all such products. The recommendation involved testing the LNS for the presence of *Cronobacter sakazakii* bacteria and in clinical practice withholding the use of untested products or those that were found to contain any *C. sakazakii*. After consultation with members of the trial's DSMB, the study team decided to withhold further distribution of LNS to the iLiNS-DYAD-M trial participants until the recommended testing had been completed. Because of this episode, a total of 160 pregnant women in the LNS group missed their study supplement for a period ranging from 1 to 20 days between August 1 and August 21, 2012. Of these women, 127 were provided with IFA capsules (one capsule per day) during the period when the LNS was on hold; the other 33 were not located at their homes during the IFA distribution.

Table 2.4-1. Nutrient and Energy Contents of the Dietary Supplements

	Daily Ration			U.S. Dietary Reference Intakes[a]		
	IFA	MMN	LNS			
Nutrient	1 tablet	1 tablet	20 g sachet	AI[b]/RDA Pregnancy (19–50 years)	AI[b]/RDA Lactation (19–50 years)	Tolerable Upper Intake Level[c]
Total energy (kcal)	0	0	118			
Protein (g)	0	0	2.6			
Fat (g)	0	0	10			
Linoleic acid (g)	0	0	4.59	13*	13*	--
α-linolenic acid (g)	0	0	0.59	1.4*	1.3*	--
Vitamin A (µg RE)[e]	0	800	800	770	1300	3000
Vitamin C (mg)	0	100	100	85	120	2000
Vitamin B1 (mg)	0	2.8	2.8	1.4	1.4	--
Vitamin B2 (mg)	0	2.8	2.8	1.4	1.6	--
Niacin (mg)	0	36	36	18	17	35
Folic acid (µg)	400	400	400	600	500	1000
Pantothenic acid (mg)	0	7	7	6*	7*	--
Vitamin B6 (mg)	0	3.8	3.8	1.9	2.0	100
Vitamin B12 (µg)	0	5.2	5.2	2.6	2.8	--
Vitamin D (µg)	0	10	10	15	15	100
Vitamin E (mg)	0	20	20	15	19	1000
Vitamin K (µg)	0	45	45	90*	90*	--
Iron (mg)	60	20	20	27	9	45
Zinc (mg)	0	30	30	11	12	40
Copper (mg)	0	4	4	1	1.3	10
Calcium (mg)	0	0	280	1000*	1000*	2500
Phosphorus (mg)	0	0	190	700	700	3500/4000
Potassium (mg)	0	0	200	4700*	5100*	--
Magnesium (mg)	0	0	65	350/360[d]	310/320[d]	350
Selenium (µg)	0	130	130	60	70	400
Iodine (µg)	0	250	250	220	290	1100
Manganese (mg)	0	2.6	2.6	2.0*	2.6*	11

[a] U.S. dietary reference intakes accessed at http://www.iom.edu/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx, May 19, 2014. Historical vitamin D and calcium dietary reference intakes are from Otten et al. 2006.
[b] Adequate intakes (AI) are denoted with an "*".
[c] "–" indicates not determinable or data insufficient; where two values are given, the first is for pregnancy and the second is for lactation.
[d] Values for ages 19–30 years/31–50 years.
[e] RE = retinol equivalents.

2.5 Randomization and Enrollment

A study statistician not involved in data collection generated four randomization code lists (one list for each of the four enrollment sites) in blocks of nine. In the pre-randomization phase, each participant number was allocated one of nine possible letter codes (A, B, C, D, E, H, J, K, M). Each letter code corresponded to one of the three interventions, i.e., each intervention matched with three different letter codes. Another researcher, not involved with the iLiNS-DYAD-M trial, then created individual randomization slips, each containing one unique identification number and the corresponding letter code. The researcher sealed the slips into individual opaque randomization envelopes, marked each envelope with the trial name and an individual participant number, and sorted the envelopes in four stacks (one for each site), each ordered by the participant number shown on the envelope.

Study nurses and their assistants screened for possible participants among pregnant women who started antenatal care at any of the four study sites. They gave a brief introduction on the trial procedures, recorded data on selected "routine antenatal" background variables from all women, and invited the interested women to a more thorough information session about participant eligibility and trial assessment. Women who gave a written or thumb print consent then underwent a full eligibility assessment that included a test dose of LNS, to rule out allergic reactivity. Afterward, a study randomizer summarized each person's eligibility and provided further information on the trial implementation to women who were eligible for participation. The women were also given written information on the study and encouraged to discuss possible participation with their family members, prior to their final decision. Before enrollment, the randomizer verified that appropriate information had been provided and obtained the participant's signature or thumb print on a second consent form.

For the actual enrollment and group allocation, a randomizer picked and shuffled the randomization envelopes for the six lowest participant numbers that had not yet been assigned to any participant. S/he then asked the potential participant to choose one, without showing her the envelope identifiers. The number on the envelope chosen by the woman became her participant number and the contents of the envelope indicated her group allocation (in letter codes). The randomizer then matched the letter code with the actual intervention, gave the participant her first 2-week ration of trial supplements, instructed her on their use, briefed her about subsequent study visits and supplement delivery, and made her a study identification card. The five unused randomization envelopes were returned to the original stack and used (together with a sixth envelope) in the group allocation for the next participant from the same site. The enrollment information that included participant details, her randomization number, and the letter code corresponding to her group allocation (but not the actual intervention) was sent to a study coordinator, who recorded the information in an electronic file that he used to plan subsequent study visits for all participants.

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,

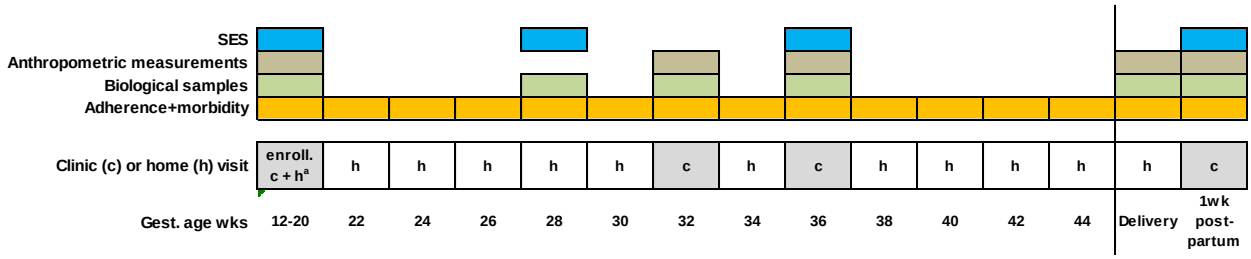
British Biocell International Ltd., Dundee, UK) and Hb concentration with on-site cuvette readers (HemoCue AB, Angelholm, Sweden). Health facility nurses gave pretest HIV counseling and tested all participants for HIV infection, except those who opted out or were already known to be HIV infected, using 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.

2.6 Follow-Up

After enrollment, the study team invited the participants to three 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 each participant's adherence to the study intervention. The first home visit was conducted within 2 weeks of enrollment by data monitors, who recorded the home location with a global positioning system (to facilitate subsequent visits) and interviewed the participants about their demographic, social, and economic background. Data on participant adherence to the study intervention were collected by counting the numbers of delivered and recovered capsules or sachets. For each participant, we calculated an adherence index, using the following formula:

$$adherence\ index\ = \frac{number\ of\ delivered\ supplement\ doses - number\ of\ returned\ doses}{total\ number\ of\ days\ between\ enrollment\ and\ delivery} * 100$$

For each participant we also calculated the percent of visits when supplements were returned, using the following formula:

$$\frac{number\ of\ visits\ when\ supplements\ were\ returned}{number\ of\ visits\ conducted} * 100$$

Each of these indices provided one data point per participant for the analysis.

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 of 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.

Data on suspected SAEs were recorded on structured adverse event forms. Upon the first notification, a study physician reviewed each suspected SAE, decided whether the participant could continue receiving the trial intervention, and reported the event to members of the trial's DSMB. After the outcome of the SAE was known, a study physician also made the judgment on the adverse event type, outcome category, and possible relationship to the trial interventions.

The study participants were advised to attend 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 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 clinical 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 provides an overview of the samples and measurements collected. Detailed methods used for sample collection and processing are included in Appendix 3.

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

Tissue	Analyses	Visit Timing					
		Enrollment	28 gw	32 gw	36 gw	Delivery	1 Week Postpartum
Blood/plasma	AGP[a], CRP[b], Hb, sTfR[c], ZPP[d], cholesterol, triglycerides, EFA, vitamin A, vitamin B12, folate, tHcy[e], malaria infection, malaria immunity	X		Only malaria infection	X		
Saliva	Cortisol	X	X		X		
Placenta	Histology, PCR[f] analysis of bacteria					X	
Amniotic membrane	Histology, PCR[f] analysis of bacteria					X	
Oral swab	PCR[f] analysis of bacteria						X
Vaginal mucus	Trichomoniasis, PCR[f] analysis of bacteria						X
Urine	Urinary tract infection						X

[a] AGP = α-1-acid glycoprotein
[b] CRP = C-reactive protein
[c] sTfR = soluble transferrin receptor
[d] ZPP = zinc protoporphyrin
[e] tHcy = total homocysteine
[f] 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-month 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 women 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

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

2.9.1 Duration of Pregnancy

Duration of pregnancy 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 (refer to Section A2.2 for more details on analytic methods used).

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 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 urinary tract infection (UTI) testing. A study nurse obtained a blind vaginal swab and immediately sent the sample to the study laboratory. Participants were 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 C-reactive protein (CRP) and α-1-acid glycoprotein (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 Vitamin B12, Folate, and Total Homocysteine Concentrations

Plasma vitamin B12, folate, and total homocysteine (tHcy) were assessed at enrollment and at 36 gw by Cobas e411 immunoassay analyzer using electrochemiluminescence (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) at the U.S. Department of Agriculture Agriculture Research Service (USDA-ARS) Western Human Nutrition Research Centre (Davis, CA, USA).

2.9.11 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.

2.9.12 Perceived Stress Scale and Salivary Cortisol Concentration

Perceived Stress Scale (PSS). We utilized the Perceived Stress Scale (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 that 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.

2.9.13 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.

2.9.14 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.

2.9.15 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.

2.9.16 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.

2.9.17 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 into 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

Once the database was considered clean, we broke the code and carried out statistical analyses (with Stata versions 12.1/13.0 (StataCorp, College Station, TX, USA), or SAS version 9.3 software package (SAS Institute, Cary, NC, USA) according to a detailed statistical analysis plan written and published after the onset of the trial, but before the code was opened (www.ilins.org). All presented analyses on the intervention effects were prespecified either in the trial protocol or in the statistical analysis plan.

2.11.1 Sample Size and Power

The target sample size for the trial was 467 per group and 1,400 in total. This was based on the following assumptions:

- The initial sample size calculation was based on being able to detect differences between the three groups equivalent to an effect size of 0.30 (difference between groups divided by the pooled standard deviation [SD]) for most continuous outcomes, assuming 80% power and a 2-sided α=0.05. This would have required 216 participants per group, for a total of 648 subjects. Allowing for up to 25% loss to follow-up, we estimated needing to recruit 864 subjects.
- For the study of an interaction between the maternal intervention (LNS vs. control) and her reproductive tract or other infections on the length of gestation and other continuous birth outcomes, we assumed a combined prevalence (i.e., prevalence of any of the studied infections) of 25% at delivery and an interaction effect size of 0.47, with P<0.10 (2-sided test) and 80% power. With these assumptions, we would have needed 373 participants per group or a total of 1,119 participants. We expected missing data (because of loss to follow-up or missing biological specimen) for approximately 20% of participants for the reproductive tract infection analyses and hence estimated needing 1,400 participants for the study to have appropriate power for this substudy.
- For the effect of the intervention on selected biochemical outcomes (micronutrient concentration and EFA status), we assumed an effect size of at least 0.50, which would have required a subsample of 79 per group. Allowing for 25% attrition for loss to follow-up for blood collection, the biochemical analyses were estimated to require a minimum sample size of 315 enrolled subjects (105 per group).
- Given the calculations above, we chose to enroll 1,400 participants to the trial. Selected biochemical analyses were planned to be carried out among 315 participants, selected randomly, but stratified so that there were 105 participants from each intervention group. The selected sample size provided 80% power and 95% confidence to detect an approximately 30%–35% reduction (among the participants receiving LNS as compared to the control women) in the prevalence of maternal reproductive tract infections or maternal malaria parasitemia in the placenta or peripheral blood.

2.11.2 Analysis of the Intervention Effects

We based the analysis of the intervention effects on the principle of modified intention-to-treat, i.e., we included all randomized participants in the analyses, with the exceptions that participants with missing data on an outcome variable were excluded for the analysis of that outcome and that two participants whose group allocation was incorrectly transcribed and assigned during enrollment were included in the group corresponding to the actual intervention they received throughout the trial.

Twelve twin pregnancies were excluded from all main analyses because estimation of the duration of pregnancy is unreliable for twins with ultrasound assessment and because twins and singletons follow different intrauterine growth patterns. However, for birth size and the duration of pregnancy, we carried out sensitivity analyses that included the twins and used the number of fetuses as a covariate (results not reported here). For these same outcomes, we carried out a second, "per protocol" sensitivity analysis that was confined to the most "adherent" participants (participants who received and did not return supplements for more than 80% of the follow-up days).

We estimated relative risks and odds ratios for comparison of dichotomous end-points at a single time point. To prevent inflated type I errors due to multiple group comparisons, we employed the close testing

procedure (Cheung 2014), i.e., the null hypotheses for pair-wise comparisons could be rejected only if the global null hypotheses of all three groups being equivalent had also been rejected. We did not adjust for multiplicity in any analyses of SAEs, as this should err on the safe side. In general, we tested the global null hypotheses either with Fisher's exact test (for dichotomous end-points) or with analysis of variance (ANOVA) (for continuous end-points) and the pair-wise hypotheses with a log-binomial regression model (for dichotomous end-points) or with ANOVA (for continuous end-points), although for some analyses other methods were used.

We performed likelihood ratio tests for the interaction between the intervention and maternal characteristics specified in the statistical analysis plan prior to data analysis. In case of a statistically significant interaction ($P<0.10$), we stratified the analyses by the potential effect modifier. Variables tested for interaction typically included maternal HIV status, malaria at enrollment, maternal educational achievement (years of completed education), maternal age at enrollment, number of previous pregnancies, maternal height at enrollment, maternal BMI at enrollment, maternal anemia at enrollment, site of enrollment, exposure to the cessation of supplement provision (delivery before or after the temporary suspension of LNS distribution), season of enrollment, gestational age at enrollment, proxy for socioeconomic status (SES), and child sex. For some analyses, the potential effect modifiers consisted of a slightly different set of variables, selected on the basis of related literature for the defined outcomes.

Covariates used in the adjusted models were derived from the list of variables that were tested for interaction with the intervention. In general, we performed the covariate selection with linear and logistic regression models and variables that showed a statistically significant association with tested outcomes ($P<0.10$) in bivariate analysis were included, although for some analyses other methods were used.

Further details of the statistical analyses undertaken by study subtopic are reported in the footnotes of results tables in Section 4 and described in Appendix 2.

We recorded and analyzed all SAEs for the mothers and infants during pregnancy and until 6 weeks after delivery. The analysis unit was a participant, with no adjustment for time in follow-up. Maternal mortality ratio, perinatal mortality rate, and neonatal mortality rate were calculated using standard definitions.

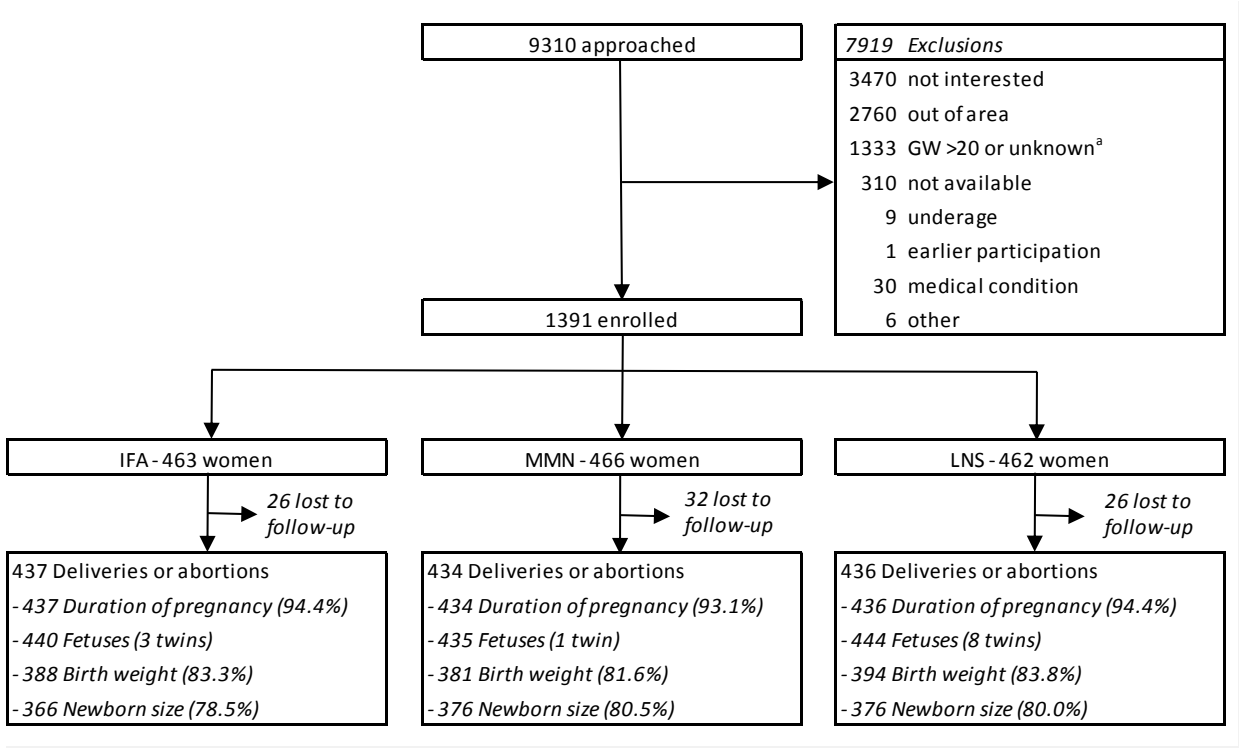
3. Results: General Context

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 and randomized into one of the three intervention groups. 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).

The women in each of the three intervention groups were similar at enrollment in terms of their average demographic and socioeconomic characteristics and nutritional and health status (see Table A1-1 in Appendix 1).

Figure 3.1-1. Participant Flow in CONSORT Recommended Format



[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.

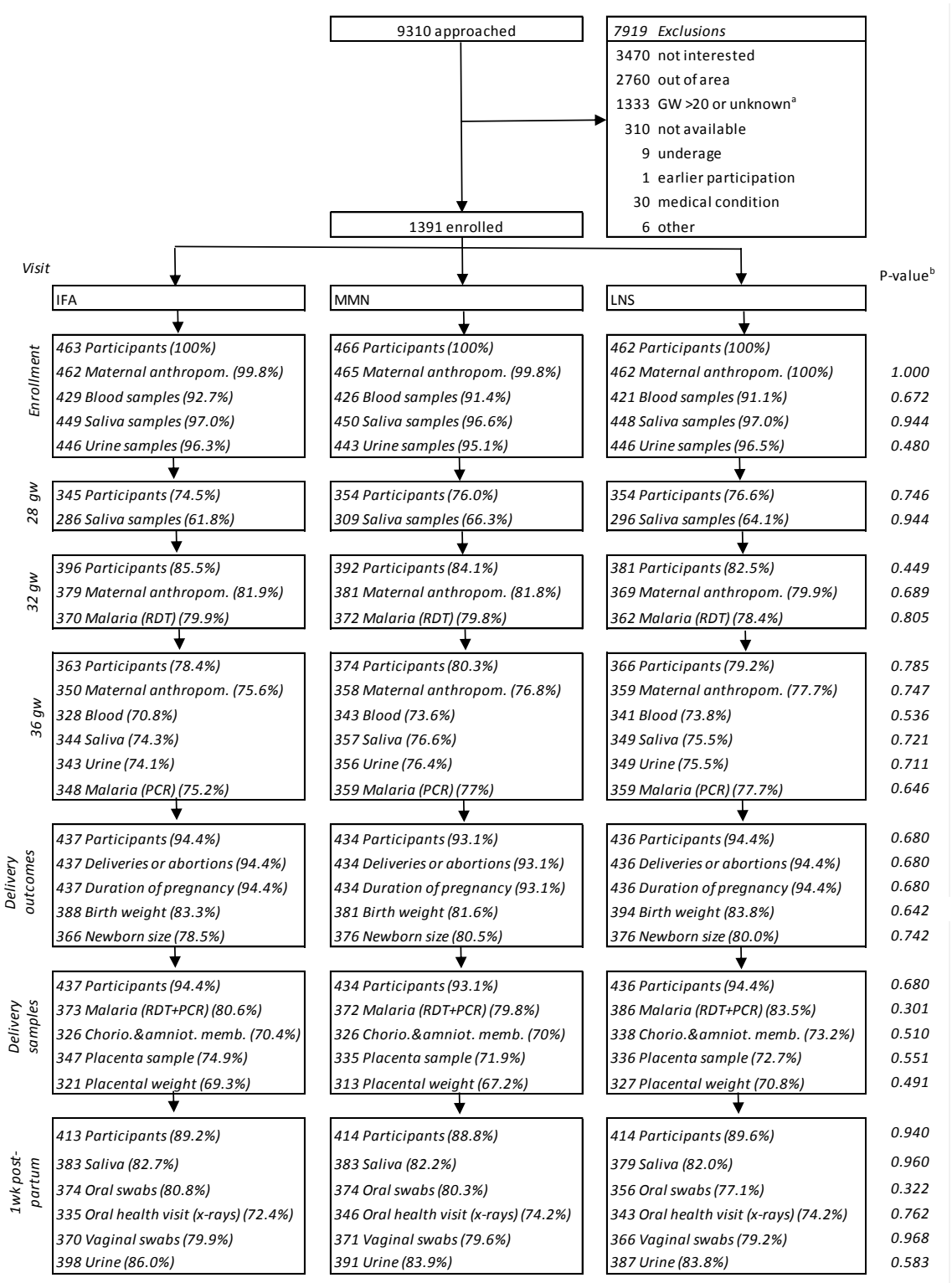
3.2 Intervention Adherence and Participant Follow-Up

The percentage of biweekly home visits in which the study team recovered any unused supplements was higher for the IFA and MMN groups than for the LNS group (40.5%, 41.2%, and 27.9%, respectively, P<0.001). Because of a concern of supplement safety, there was, however, a temporary suspension in supplement delivery for the LNS group. (See Section 2.4 for further information.) The adherence data reported here have not specifically been adjusted for that suspension. Based on the length of follow-up

and the number of delivered and returned supplement doses, we estimated that the mean adherence to the intervention (proportion of days when the supplements were consumed) was 84.1%, 83.4%, and 85.7% in the IFA, MMN, and LNS groups, respectively (P=0.145). Within each group, the proportion of returned supplements did not change during any of the follow-up visits (P=0.343 for the IFA group, 0.281 for the MMN group, and 0.951 for the LNS group).

Figure 3.2-2 shows the percent of participants for which the different clinical and biological samples and anthropometric measures were successfully collected. At the various visits, clinical data were successfully collected from 76% to 100% of the enrolled participants. The respective success rate for biological sample collection ranged from 62% to 96%. There were no statistically significant differences between the groups in the success of follow-up or clinical or biological sample collection ($P \geq 0.05$) (Figure 3.2-2). In general, the main study visits were done within the planned time frame (Figure 3.2-3).

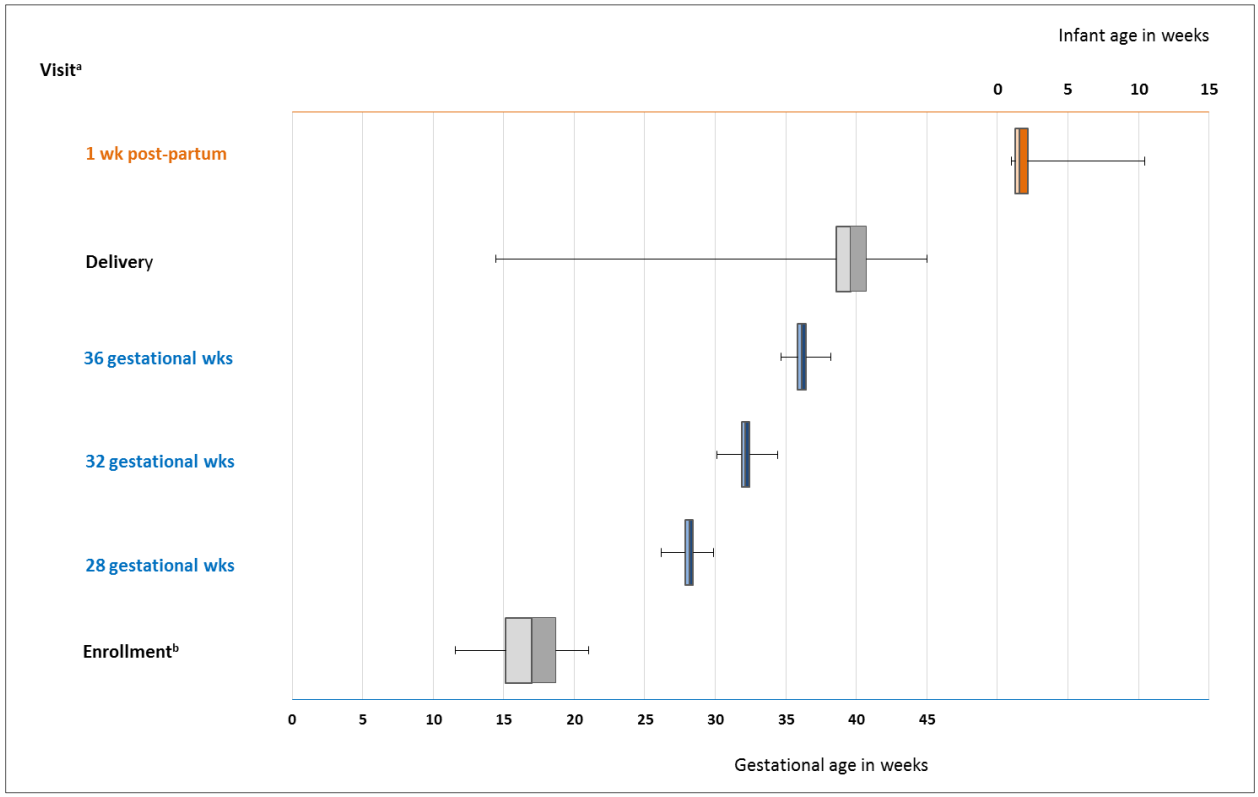
Figure 3.2-1. Number and Percentages of Participants, and Samples and Measures Taken at Selected Visits



[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.

[b] P-value obtained from Fisher's exact test.

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 included (2.5% on both sides of the visit range excluded).
[b] There were four participants enrolled with gestational ages >20 weeks (1@20.1 weeks, 2@20.3 weeks, and 1@21 weeks).

We recorded and analyzed all SAEs (defined as hospitalizations, life-threatening events, deaths, congenital malformations, or other) for the mothers and infants during pregnancy and until 6 weeks after delivery. We categorized deaths of the fetus or child as abortions (fetal loss before 22 completed gw), stillbirths (fetal death at or after 22 gw), early neonatal deaths (death to a live-born infant within 7 days of birth), late neonatal death (death at 8–28 days after birth), and infant deaths (death at 29–42 days after birth). Analysis unit was participant, with no adjustment for time in follow-up. Maternal mortality ratio, perinatal mortality rate, and neonatal mortality rate were calculated using standard definitions.

We recorded a total of 162 SAEs for 141 women and 259 SAEs for 247 infants by 6 weeks after delivery. In total, there were 8 maternal deaths, 8 spontaneous abortions (before 22 gw), 23 stillbirths, and 41 neonatal or infant deaths within 6 weeks of birth, resulting in a maternal mortality ratio of 629/100,000 live births, a perinatal mortality rate of 45/1,000 births, and a neonatal mortality rate of 31/1,000 live births. The number of SAEs and the proportion of participants who experienced at least one SAE during the follow-up were roughly equally distributed among the three study groups, although the number of stillbirths was higher in the LNS than in the MMN group (Table 3.2-1). The perinatal mortality rate was 53 deaths per 1,000 births in the IFA group, 33 in the MMN group, and 49 in the LNS group (P=0.300). Of the fetal or infant deaths, 29 were considered of unknown etiology, followed by infection (14), preterm birth (12), intrapartum asphyxia (11), maternal bleeding (3), maternal eclampsia (1), fetal malpresentation

(1), and cephalopelvic disproportion (1). The trial physicians considered none of the reported SAEs likely to be caused by the trial interventions.

Table 3.2-1. Incidence of Maternal and Infant SAEs by Intervention Group, from Enrollment to 6 Weeks after Delivery

Variable	Result by Intervention Group			
	IFA	MMN	LNS	P-value[a]
Number (%) of women who experienced SAEs[b]	42/460 (9.1%)	45/465 (9.7%)	54/454 (11.9%)	0.353
Number (%) of women who were hospitalized[b]	40/460 (8.7%)	41/465 (8.8%)	53/454 (11.7%)	0.242
Number (%) of women who died[b]	3/460 (0.7%)	4/465 (0.9%)	1/454 (0.2%)	0.550
Number (%) of infants who experienced SAEs[c]	79/460 (17.2%)	79/465 (17.0%)	89/454 (19.6%)	0.519
Number (%) of infants who were hospitalized[c]	51/460 (11.1%)	59/465 (12.7%)	64/454 (14.1%)	0.389
Number (%) of fetal or infant losses (abortion, stillbirth, death)[c]	28/460 (6.1%)	20/465 (4.3%)	25/454 (5.5%)	0.461
Number (%) of spontaneous abortions[d]	1/434 (0.2%)	4/433 (0.9%)	3/428 (0.7%)	0.420
Number (%) of stillbirths[d]	8/434[f,g] (1.8%)	2/433[f] (0.5%)	14/428[g] (3.3%)	0.006
Number (%) of early neonatal deaths (0–7 days)[e]	16/425 (3.8%)	12/427 (2.8%)	7/411 (1.7%)	0.193
Number (%) of late neonatal deaths (8–28 days)[e]	2/425 (0.5%)	1/427 (0.2%)	1/411 (0.2%)	0.849
Number (%) of infant deaths (29–42 days)[e]	1/425 (0.2%)	1/427 (0.2%)	0/411 (0.0%)	1.000

[a] P-value obtained from Fisher's exact test.
[b] The denominator includes all enrolled women with singleton pregnancy.
[c] Includes also fetal losses.
[d] The denominator includes women with singleton pregnancy from whom the date of spontaneous abortion or delivery is known.
[e] The denominator includes singleton, live-born infants with a known birth date.
[f,g] Proportions with a different superscript letter are significantly different from each other at P<0.05 level by log-binomial regression.

4. The Impact of LNS Supplementation on Various Outcomes

4.1 The Duration of Pregnancy and Child Size at Birth

In this section, we report on the impact of LNS or MMN supplementation on the duration of pregnancy and child size at birth. Specifically, we test the hypotheses that home fortification of pregnant women's diets with LNS increases mean birth size and duration of pregnancy in the target population. The detailed hypotheses can be found in Appendix 4.

Of the 1,391 participants who were enrolled in the study, 12 women with twin pregnancies were excluded from the analysis. Of the remaining 1,379 participants, we used data on the duration of pregnancy from 1,295 women (93.9%) and on birth weight from 1,144 (83.0%). And we had at least one of the newborn anthropometric measurements from 1,100 (79.0%) newborns. Loss to follow-up was similar among the intervention groups (P=0.638 for birth weight, P=0.739 for newborn length and other anthropometrics, and P=0.655 for duration of pregnancy). There were 434, 433, and 428 participants included in the duration of pregnancy analysis; 385, 379, and 380 newborns in the birth weight analysis; 360, 372, and 357 newborns in the LAZ analysis; 362, 374 and 363 newborns in the weight-for-age z-score (WAZ) analysis; 362, 373, and 363 newborns in the MUAC analysis, and 360, 372, and 359 newborns in the head circumference-for-age z-score (HCZ) analysis in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-1).

The mean (SD) age of the infants at birth weight and newborn size measurements was 30 hours (50) and 13 days (6), respectively. Of the recorded birth weights, 89.1% were measured within 48 hours; the rest were back-calculated from a measurement within 14 days (when measured between 2 and 5 days after delivery, weight was calculated as a percentage of the measured weight; and when measured between 6 and 13 days after delivery, weight was calculated using WHO z-scores; see Appendix 2.1 for more details). The mean (SD) recorded birth weight of singleton infants born to the study participants was 2,970 g (447), the mean (SD) newborn length was 49.7 cm (2.3), and the mean (SD) duration of pregnancy was 39.1 gw (2.9).

The incidence of LBW (<2500g) was 12.8% and of preterm birth (<37 weeks) 10.0%; the prevalence of newborn stunting (LAZ <−2) was 16.0%, underweight (WAZ <−2) 7.7%, and small head circumference (HCZ <−2) 3.9%. The mean (SD) newborn WAZ, LAZ, and HCZ were −0.56 (1.03), −1.00 (1.11), and −0.15 (1.09), respectively.

For the primary continuous outcomes birth weight and newborn length, as well as for the other newborn outcomes, the point estimate for the mean was highest in the LNS group, intermediate in the MMN group, and lowest in the IFA group (Table 4.1-1). Except for the MUAC results, however, the differences were not statistically significant. The mean (95% confidence interval [CI]) MUAC was 0.2 cm (0.1 to 0.3) higher in the LNS group than in the IFA group (P=0.006). The respective difference between the LNS and MMN groups was 0.1 cm (0.0 to 0.2) (P=0.175).

There were no statistically significant differences between the groups in the incidence of LBW or preterm birth or in the prevalence of newborn stunting, underweight, or small head circumference (Table 4.1-2).

Covariates were selected a priori for inclusion in an adjusted model based on their expected association with the outcome variables. Covariates used in the adjusted models were derived from the same list of variables that were tested for interaction. To control for possible confounding between variables (Cheung 2014), all variables were put into the model at once, and those that showed statistically significant association ($P<0.10$) with any of the outcomes were included in the models as covariates. Adjustment of the analyses for the selected covariates did not markedly change the results. An analysis that included the twins and used the number of fetuses as a covariate also gave essentially similar results, as did an analysis to assess if loss to follow-up might have biased the results based on Heckman's selection models. Finally, an analysis that was confined to the most adherent participants (>80% adherence to the intervention) also indicated no statistically significant intergroup differences in the continuous (except for the same difference in MUAC noted earlier between the IFA and LNS group) (Table 4.1-3) or dichotomous (details not shown) outcomes.

Tests for interaction with predefined variables indicated that maternal parity, age, maternal BMI at enrollment, exposure to the cessation of supplement provision (delivery before or after the temporary suspension of LNS distribution), and other variables did not modify the associations between the intervention and the study outcomes ($P\geq0.10$). Maternal educational achievement modified the association for newborn stunting ($P=0.018$). Additional, exploratory tests for interaction suggested effect modification on newborn stunting by maternal malaria-test positivity at enrollment ($P=0.018$) and on small newborn head circumference by maternal malaria-test positivity at enrollment ($P=0.077$) and HIV infection ($P=0.011$).

Among women with a positive malaria test at enrollment or low educational achievement, stratified analyses suggested statistically significant differences in stunting prevalence between the MMN and IFA groups, but not between the LNS and IFA groups or between the LNS and MMN groups (Table 4.1-4). Statistically significant differences in the prevalence of small head circumference between the LNS and IFA groups were observed among women with HIV or a positive malaria test at enrollment (Table 4.1-5). Adjustment of the stratified analyses for the other effect modifier variables or the use of Heckman models (to account for the missing data) did not change the analysis results (data not shown).

The study findings do not support a hypothesis that provision of LNS to pregnant women would increase the mean birth size or duration of pregnancy in rural Malawi. There may, however, be a modest impact of LNS among selected subgroups of women with infections, such as HIV or malaria.

Table 4.1-1. Continuous Birth Outcomes by Intervention Group

Outcome	Intervention group			P-value[b]	Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS		Difference in means (95% CI)	P-value[j]	Difference in means (95% CI)	P-value[j]	Difference in means (95% CI)	P-value[j]
Mean (SD) birth weight (g)[a,d]	2,948 (432)	2,964 (460)	3,000 (447)	0.258	52 (−11 to 115)	0.108	36 (−28 to 99)	0.269	16 (−47 to 80)	0.617
Birth weight (g), adjusted model[c]				0.229	52 (−8 to 112)	0.089	32 (−28 to 93)	0.293	20 (−41 to 80)	0.519
Mean (SD) newborn length (cm)[a,e]	49.5 (2.4)	49.7 (2.2)	49.9 (2.1)	0.104	0.4 (0.1 to 0.7)	0.036	0.1 (−0.2 to 0.4)	0.459	0.2 (−0.1 to 0.6)	0.169
Newborn length (cm), adjusted model[c]				0.130	0.3 (0.0 to 0.6)	0.044	0.1 (−0.2 to 0.4)	0.397	0.2 (−0.1 to 0.5)	0.237
Mean (SD) newborn LAZ[a,e]	−1.10 (1.21)	−0.98 (1.10)	−0.93 (1.02)	0.104	0.17 (0.01 to 0.33)	0.039	0.05 (−0.11 to 0.21)	0.538	0.12 (−0.04 to 0.28)	0.141
Newborn LAZ, adjusted model[c]				0.144	0.15 (−0.00 to 0.31)	0.051	0.06 (−0.09 to 0.21)	0.449	0.09 (−0.06 to 0.25)	0.226
Mean (SD) duration of pregnancy (weeks)[a,f]	39.0 (2.9)	39.2 (3.0)	39.2 (2.9)	0.550	0.2 (−0.2 to 0.6)	0.295	0.0 (−0.3 to 0.4)	0.802	0.2 (−0.2 to 0.5)	0.425
Duration of pregnancy (weeks), adjusted model[c]				0.439	0.2 (−0.1 to 0.5)	0.203	0.1 (−0.2 to 0.3)	0.614	0.1 (−0.2 to 0.4)	0.442
Mean (SD) newborn WAZ[a,g]	−0.64 (1.08)	−0.57 (1.02)	−0.48 (0.99)	0.092	0.17 (0.02 to 0.32)	0.029	0.09 (−0.06 to 0.24)	0.236	0.08 (−0.07 to 0.23)	0.310
Newborn WAZ, adjusted model[c]				0.085	0.16 (0.02 to 0.30)	0.028	0.10 (−0.04 to 0.24)	0.158	0.06 (−0.08 to 0.20)	0.427
Mean (SD) newborn HCZ[a,h]	−0.24 (1.12)	−0.14 (1.11)	−0.06 (1.02)	0.091	0.18 (0.02 to 0.34)	0.029	0.08 (−0.08 to 0.24)	0.334	0.10 (−0.06 to 0.26)	0.216
Newborn HCZ, adjusted model[c]				0.141	0.16 (0.00 to 0.31)	0.048	0.08 (−0.07 to 0.24)	0.285	0.07 (−0.08 to 0.23)	0.356
Mean (SD) newborn MUAC (cm)[a,i]	10.5 (1.0)	10.6 (0.9)	10.7 (0.9)	0.024	0.2 (0.1 to 0.3)	0.006	0.1 (0.0 to 0.2)	0.175	0.1 (0.0 to 0.2)	0.162
Newborn MUAC (cm), adjusted model[c]				0.036	0.2 (0.0 to 0.3)	0.011	0.1 (0.0 to 0.2)	0.119	0.1 (−0.1 to 0.2)	0.311

[a] Unadjusted model.
[b] P-value obtained from ANOVA, for adjusted models by analysis of covariance (ANCOVA).
[c] Models were adjusted for maternal height at enrollment, maternal BMI at enrollment, maternal age at enrollment, duration of pregnancy at enrollment, number of previous pregnancies, maternal anemia at enrollment, proxy for SES, child sex, and site of enrollment.
[d] Number of participants: IFA n=385, MMN n=379, LNS n=380.
[e] Number of participants: IFA n=360, MMN n=372, LNS n=357.
[f] Number of participants: IFA n=434, MMN n=433, LNS n=428.
[g] Number of participants: IFA n=362, MMN n=374, LNS n=363.
[h] Number of participants: IFA n=360, MMN n=372, LNS n=359.
[i] Number of participants: IFA n=362, MMN n=373, LNS n=363.
[j] P-values for pair-wise comparisons were obtained from ANOVA; for adjusted models by ANCOVA.

Table 4.1-2. Dichotomous Birth Outcomes by Intervention Group

Outcome	Result by Intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[b]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Incidence of LBW[a]	49/385 (12.7%)	51/379 (13.5%)	46/380 (12.1%)	0.856	0.95 (0.65 to 1.39)	0.794	0.90 (0.62 to 1.31)	0.577	1.06 (0.73 to 1.52)	0.765
Incidence of LBW, adjusted model[c]				0.868	0.91 (0.63 to 1.32)	0.636	0.92 (0.63 to 1.33)	0.646	1.00 (0.70 to 1.43)	0.990
Prevalence of newborn stunting[a]	69/360 (19.2%)	52/372 (14.0%)	53/357 (14.9%)	0.130	0.77 (0.56 to 1.07)	0.125	1.06 (0.75 to 1.51)	0.739	0.73 (0.52 to 1.01)	0.060
Prevalence of newborn stunting, adjusted model[c]				0.150	0.79 (0.58 to 1.08)	0.146	1.06 (0.75 to 1.50)	0.735	0.75 (0.54 to 1.03)	0.075
Incidence of preterm birth[a]	49/434 (11.3%)	41/433 (9.5%)	39/428 (9.1%)	0.528	0.81 (0.54 to 1.20)	0.292	0.96 (0.63 to 1.46)	0.857	0.84 (0.57 to 1.24)	0.380
Incidence of preterm birth, adjusted model[c]				0.550	0.78 (0.50 to 1.22)	0.279	0.84 (0.53 to 1.33)	0.466	0.93 (0.60 to 1.42)	0.731
Prevalence of newborn underweight[a]	34/362 (9.4%)	29/374 (7.8%)	22/363 (6.1%)	0.250	0.65 (0.39 to 1.08)	0.096	0.78 (0.46 to 1.33)	0.367	0.83 (0.51 to 1.33)	0.428
Prevalence of newborn underweight, adjusted model[c]				0.314	0.68 (0.41 to 1.12)	0.130	0.81 (0.48 to 1.37)	0.428	0.84 (0.53 to 1.33)	0.450
Prevalence of newborn small head circumference[a]	21/360 (5.8%)	11/372 (3.0%)	11/359 (3.1%)	0.099	0.53 (0.26 to 1.07)	0.077	1.04 (0.45 to 2.36)	0.932	0.51 (0.25 to 1.04)	0.063
Prevalence of newborn small head circumference, adjusted model[c]				0.100	0.53 (0.26 to 1.08)	0.079	1.00 (0.44 to 2.26)	0.994	0.53 (0.26 to 1.08)	0.080

[a] Unadjusted model.
[b] P-value obtained from Fisher's exact test for unadjusted models and from log-binomial regression model for adjusted models.
[c] Models were adjusted for maternal height at enrollment, maternal BMI at enrollment, maternal age at enrollment, duration of pregnancy at enrollment, number of previous pregnancies, maternal anemia at enrollment, proxy for SES, child sex, and site of enrollment.
[d] P-values for pair-wise comparisons were obtained from log-binomial regression models for both unadjusted and adjusted models.

Table 4.1-3. Continuous Birth Outcomes by Intervention Group among Participants with >80% Adherence to the Intervention

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[b]	Difference in means (95% CI)	P-value[j]	Difference in means (95% CI)	P-value[j]	Difference in means (95% CI)	P-value[j]
Mean (SD) birth weight (g)[a,d]	2,944 (437)	2,976 (454)	3,022 (432)	0.117	78 (4 to 153)	0.040	46 (−28 to 121)	0.223	32 (−44 to 108)	0.410
Birth weight (g), adjusted model[c]				0.106	76 (5 to 146)	0.035	43 (−28 to 114)	0.238	33 (−39 to 105)	0.366
Mean (SD) newborn length (cm)[a,e]	49.6 (2.4)	49.7 (2.1)	49.8 (2.1)	0.336	0.3 (−0.1 to 0.7)	0.144	0.1 (−0.3 to 0.5)	0.605	0.2 (−0.2 to 0.6)	0.351
Newborn length (cm), adjusted model[c]				0.356	0.3 (−0.1 to 0.6)	0.154	0.1 (−0.3 to 0.5)	0.603	0.2 (−0.2 to 0.5)	0.374
Mean (SD) newborn LAZ[a,e]	−1.05 (1.18)	−0.93 (1.03)	−0.94 (1.04)	0.347	0.12 (−0.07 to 0.30)	0.222	−0.01 (−0.20 to 0.18)	0.914	0.13 (−0.06 to 0.32)	0.191
Newborn LAZ, adjusted model[c]				0.329	0.13 (−0.05 to 0.31)	0.152	0.03 (−0.15 to 0.21)	0.746	0.10 (−0.08 to 0.28)	0.277
Mean (SD) duration of pregnancy (weeks)[a,f]	39.0 (3.0)	39.2 (3.0)	39.3 (3.1)	0.435	0.3 (−0.2 to 0.8)	0.232	0.0 (−0.4 to 0.5)	0.878	0.3 (−0.2 to 0.8)	0.305
Duration of pregnancy (weeks), adjusted model[c]				0.151	0.3 (−0.0 to 0.7)	0.064	0.1 (−0.3 to 0.4)	0.715	0.3 (−0.1 to 0.6)	0.145
Mean (SD) newborn WAZ[a,g]	−0.61 (1.07)	−0.55 (0.95)	−0.48 (0.98)	0.285	0.14 (−0.03 to 0.31)	0.113	0.07 (−0.10 to 0.24)	0.418	0.07 (−0.11 to 0.24)	0.445
Newborn WAZ, adjusted model[c]				0.145	0.16 (−0.00 to 0.32)	0.055	0.10 (−0.06 to 0.26)	0.204	0.05 (−0.11 to 0.22)	0.522
Mean (SD) newborn HCZ[a,h]	−0.27 (1.11)	−0.11 (1.03)	−0.06 (1.03)	0.069	0.21 (0.03 to 0.39)	0.024	0.06 (−0.12 to 0.24)	0.541	0.15 (−0.03 to 0.34)	0.107
Newborn HCZ, adjusted model[c]				0.064	0.21 (0.03 to 0.39)	0.019	0.09 (−0.09 to 0.26)	0.342	0.13 (−0.06 to 0.31)	0.173
Mean (SD) newborn MUAC (cm)[a,i]	10.5 (1.0)	10.6 (0.9)	10.7 (0.8)	0.049	0.2 (0.0 to 0.3)	0.014	0.1 (−0.1 to 0.2)	0.280	0.1 (−0.0 to 0.3)	0.176
Newborn MUAC (cm), adjusted model[c]				0.131	0.16 (0.00 to 0.31)	0.044	0.07 (−0.08 to 0.23)	0.331	0.08 (−0.07 to 0.23)	0.306

[a] Unadjusted model.
[b] P-value obtained from ANOVA, for adjusted models by ANCOVA.
[c] Models were adjusted for maternal height at enrollment, maternal BMI at enrollment, maternal age at enrollment, duration of pregnancy at enrollment, number of previous pregnancies, maternal anemia at enrollment, proxy for SES, child sex, and site of enrollment.
[d] Number of participants: IFA n=262, MMN n=260, LNS n=278.
[e] Number of participants: IFA n=247, MMN n=253, LNS n=271.
[f] Number of participants: IFA n=285, MMN n=287, LNS n=308.
[g] Number of participants: IFA n=249, MMN n=254, LNS n=276.
[h] Number of participants: IFA n=247, MMN n=252, LNS n=273.
i Number of participants: IFA n=248, MMN n=254, LNS n=276.
[j] P-values for pair-wise comparisons were obtained from ANOVA, for adjusted models by ANCOVA.

Table 4.1-4. Effect Modification: Prevalence of Newborn Stunting (LAZ <–2) by Intervention Group, Stratified Analyses

Outcome	Interaction test P-value	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[a]	Relative risk (95% CI)	P-value[b]	Relative risk (95% CI)	P-value[b]	Relative risk (95% CI)	P-value[b]
LAZ <–2											
Negative malaria test at enrollment	0.018	43/281 (15.3%)	42/283 (14.8%)	37/276 (13.4%)	0.802	0.88 (0.58 to 1.32)	0.524	0.90 (0.60 to 1.36)	0.627	0.97 (0.66 to 1.44)	0.878
Positive malaria test at enrollment		26/79 (32.9%)	10/88 (11.4%)	16/79 (20.3%)	0.003	0.62 (0.36 to 1.06)	0.078	1.78 (0.86 to 3.70)	0.120	0.35 (0.18 to 0.67)	0.002
LAZ <–2											
Maternal educational achievement above median (≥4 years)	0.018	27/176 (15.3%)	33/194 (17.0%)	26/174 (14.9%)	0.853	0.97 (0.59 to 1.60)	0.917	0.88 (0.55 to 1.41)	0.590	1.11 (0.70 to 1.77)	0.664
Maternal educational achievement below median (<4 years)		41/182 (22.5%)	18/175 (10.3%)	27/181 (14.9%)	0.007	0.66 (0.43 to 1.03)	0.066	1.45 (0.83 to 2.54)	0.192	0.46 (0.27 to 0.76)	0.003

[a] P-value obtained from Fisher's exact test.
[b] P-values for pair-wise comparisons were obtained from log-binomial regression models.

Table 4.1-5. Effect Modification: Prevalence of Small Newborn Head Circumference (HCZ <–2) by Intervention Group, Stratified Analyses

Outcome	Interaction test P-value	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[a]	Relative risk/ ddds ratio (95% CI)	P-value	Relative risk/ odds ratio (95% CI)	P-value	Relative risk/ odds ratio (95% CI)	P-value
HCZ < –2											
Negative HIV test at enrollment	0.011	15/303 (5.0%)	8/328 (2.4%)	11/308 (3.6%)	0.243	0.72 (0.34 to 1.55)[b]	0.401[b]	1.46 (0.60 to 3.59)[b]	0.405[b]	0.49 (0.21 to 1.15)[c]	0.100[c]
Positive HIV test at enrollment		6/56 (10.7%)	3/41 (7.3%)	0/50 (0.0%)	0.043	0.13 (0.00 to 0.91)[b]	0.028[b]	0.20 (0.00 to 1.95)[b]	0.088[b]	0.66 (0.10 to 3.33)[b]	0.729[b]
HCZ < –2											
Negative malaria test at enrollment	0.077	12/280 (4.3%)	6/283 (2.1%)	10/278 (3.6%)	0.319	0.84 (0.37 to 1.91)[c]	0.676[c]	1.70 (0.63 to 4.61)[c]	0.299[c]	0.49 (0.19 to 1.30)[c]	0.153[c]
Positive malaria test at enrollment		9/80 (11.3%)	5/88 (5.7%)	1/79 (1.3%)	0.030	0.11 (0.01 to 0.87)[c]	0.036[c]	0.22 (0.03 to 1.87)[c]	0.166[c]	0.51 (0.18 to 1.44)[c]	0.202[c]

[a] P-value obtained from Fisher's exact test.
[b] P-values and odds ratios were obtained from exact logistic regression (used due to 0-value in LNS group).
[c] P-values and relative risks were obtained by log-binomial regression.

4.2 Maternal Weight Gain during Pregnancy and Placental Size

In this section, we test hypotheses that the provision of LNS increases maternal weight gain in pregnancy and reduces the incidence of abnormally small placental size. The detailed hypotheses can be found in Appendix 4 (13–15). Since the pre-pregnancy BMIs were not available, we used regression modeling to create a proxy for pre-pregnancy BMI as modeled by BMI at 13.7 weeks gestation (see Appendix 2.2). We assumed minimal change in BMI occurred between pre-pregnancy and 13.7 gw, based on Institute of Medicine (IOM) assumptions of weight gain in the first trimester (0.5–2.0 kg) (Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines 2009).

Of the 1,391 women who were enrolled in the study, 12 women with twin pregnancies were excluded from all analyses in this section. Of the remaining 1,379 participants, 1,377 women (99.9%) with at least one weight measurement available at any of the three time points (enrollment, 32 gw, or 36 gw) were included in the pregnancy weight gain analyses, and 961 women (69.7%) who provided placentas at delivery were included in the analyses involving placental weight. Loss to follow-up was similar in all of the intervention groups (P=0.298 for placental weight analysis). There were 459, 464, and 454 participants included in the pregnancy weight gain analyses and 321, 313, and 327 in the placental weight analyses, in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the maternal weight gain analyses in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-2).

The prevalence of low maternal BMI at enrollment (BMI <18.5) was 5.4% and 5.5% among participants included in the maternal weight gain analysis and placental weight analysis, respectively.

The modeled mean (SD) maternal pregnancy weight gain between enrollment and 36 weeks gestation was 292 g/week (99) and the mean (SD) placental weight was 512 g (106). Table 4.2-1 shows average weekly maternal weight gain and placental weight by intervention group, using continuous outcomes. There was no statistically significant group-level difference in maternal weight gain. The mean placental weight was 20 g higher in the LNS than in the IFA group, but the group-level difference was not statistically significant (P=0.057).

The following variables were selected a priori based on their expected association with the outcome variables and examined as potential covariates: maternal height at enrollment, maternal BMI at enrollment, duration of pregnancy at enrollment, maternal age at enrollment, child sex (for placental weight analysis), proxy for SES, number of previous pregnancies, maternal anemia at enrollment, and study site. Those with significant associations (P<0.10) in bivariate analyses with either maternal weight gain during pregnancy or placental size were included in all models as covariates. Adjustment of the analyses for the selected covariates did not markedly change the association between the intervention and the continuous outcomes (Table 4.2-1)**.**

Table 4.2-2 shows the prevalence of pregnancy weight gain below the recommended lower range of pregnancy weight gain, placental weight by gestational age <10th percentile, placental weight by birth weight <10th percentile, and placental-weight-to-birth-weight ratio <10th percentile by intervention group. The prevalence of maternal weight gain below the recommended gain was 71.8%, low placental weight by gestational age (<10th percentile) 49.3%, low placental weight by birth weight (<10th percentile) 49.4%, and placental-weight-to-birth-weight ratio <10th percentile 37.3% (Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines 2009).

The prevalences of low average weekly weight gain, placental weight by gestational age, placental weight by birth weight, and placental-weight-to-birth-weight ratio were not statistically significantly different between the intervention groups.

To control for potential confounding, the analyses for these outcomes were adjusted for the same set of variables as for the continuous outcomes of maternal weight gain and placental weight. Adjustment of the analyses for maternal height at enrollment, maternal BMI at enrollment, duration of pregnancy at enrollment, maternal age at enrollment, child sex, proxy for SES, number of previous pregnancies, maternal anemia at enrollment, and site of enrollment did not markedly change the results for these dichotomous outcomes (Table 4.2-2).

Tests for interaction with predefined variables indicated that maternal height at enrollment, maternal BMI at enrollment, duration of pregnancy at enrollment, maternal age at enrollment, maternal educational achievement, number of previous pregnancies, parity, season of enrollment, Hb at enrollment, HIV status, and household food insecurity score did not modify the associations between the intervention and the outcomes ($P \geq 0.10$).

Child sex ($P=0.026$), proxy for SES ($P=0.008$), site of enrollment ($P=0.076$), and maternal malaria at enrollment ($P=0.019$) modified the association for placental weight. Among girls, stratified analyses suggested statistically significant differences in placental weight between the LNS and IFA groups, but not between the LNS and MMN groups or between the MMN and IFA groups (Table 4.2-3). Statistically significant differences in the placental weight between the LNS and IFA groups were observed among women with higher proxy for SES or a negative malaria test at enrollment but not between the LNS and MMN groups (Table 4.2-3). On the other hand, statistically significant differences in placental weight were observed between the MMN and IFA groups among women who had a higher proxy for SES and those enrolled from the Lungwena site. Among those enrolled from the Lungwena site, placental weight was also significantly different between the LNS and MMN groups.

The study findings do not support a hypothesis that provision of LNS to pregnant women increases the mean maternal weight gain during the second and third trimesters of pregnancy or placental size in rural Malawi.

Table 4.2-1. Weight Gain and Placental Weight by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[c]	Difference in means (95% CI)	P-value[c]	Difference in means (95% CI)	P-value[c]	Difference in means (95% CI)	P-value[c]
Mean (SD) maternal weight gain (g/week)[a,d]	295 (96)	293 (99)	289 (102)	0.631	−6 (−19 to 7)	0.347	−4 (−17 to 9)	0.523	−2 (−15 to 11)	0.759
Maternal weight gain (g/week), adjusted model[b]				0.859	−3 (−17 to 11)	0.650	−3 (−17 to 11)	0.617	0 (−13 to 14)	0.963
Mean (SD) placental weight (g)[a,e]	503 (104)	510 (101)	523 (111)	0.057	20 (3 to 36)	0.018	13 (−4 to 29)	0.125	7 (−10 to 23)	0.419
Placental weight (g), adjusted model[b]				0.219	14 (−2 to 30)	0.090	10 (−6 to 26)	0.238	4 (−12 to 20)	0.616

[a] Unadjusted model.
[b] Models were adjusted for covariates that had a significant association (P<0.10) with either outcome (maternal weight gain or placental weight) in bivariate analysis. All models were adjusted for maternal height at enrollment, maternal BMI at enrollment, duration of pregnancy at enrollment, maternal age at enrollment, child sex, proxy for SES, number of previous pregnancies, maternal anemia at enrollment, and site of enrollment.
[c] P-value obtained from ANOVA, for adjusted models by ANCOVA.
[d] Number of participants: IFA n=459, MMN n=464, LNS n=454.
[e] Number of participants: IFA n=321, MMN n=313, LNS n=327.

Table 4.2-2. Pregnancy Weight Gain and Placental Weight below Specified Cutoff Points by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[f]	Relative risk (95% CI)	P-value[g]	Relative risk (95% CI)	P-value[g]	Relative risk (95% CI)	P-value[g]
Pregnancy weekly weight gain below recommended gain[a]	327/457 (71.6%)	335/461 (72.7%)	322/452 (71.2%)	0.880	1.00 (0.92 to 1.08)	0.916	0.98 (0.90 to 1.06)	0.631	1.02 (0.94 to 1.10)	0.707
Pregnancy weekly weight gain below recommended gain, adjusted model[b]				0.991[c]	1.01 (0.85 to 1.19)	0.935[c]	1.00 (0.85 to 1.17)	0.961[c]	1.01 (0.86 to 1.19)	0.896[c]
Placental weight by gestational age below 10th percentile[a,e]	163/318 (51.3%)	160/312 (51.6%)	149/327 (45.6%)	0.224	0.89 (0.76 to 1.04)	0.149	0.88 (0.75 to 1.04)	0.127	1.01 (0.86 to 1.17)	0.931
Placental weight by gestational age below 10th percentile, adjusted model[b,e]				0.328[c]	0.92 (0.74 to 1.16)	0.487[c]	0.89 (0.71 to 1.12)	0.328[c]	1.03 (0.83 to 1.29)	0.776[c]
Placental weight by birth weight below 10th percentile[a,e]	166/313 (53.0%)	154/302 (51.0%)	141/319 (44.2%)	0.066	0.83 (0.71 to 0.98)	0.027	0.87 (0.73 to 1.02)	0.091	0.96 (0.83 to 1.12)	0.613
Placental weight by birth weight below 10th percentile, adjusted model[b,e]				0.322[c]	0.85 (0.67 to 1.06)	0.150[c]	0.87 (0.69 to 1.10)	0.255[c]	0.97 (0.77 to 1.21)	0.774[c]
Placental-weight-to-birth-weight ratio below 10th percentile[a,e]	123/313 (39.3%)	114/302 (37.8%)	111/319 (34.8%)	0.493	0.89 (0.72 to 1.09)	0.242	0.92 (0.75 to 1.14)	0.444	0.96 (0.79 to 1.17)	0.693
Placental-weight-to-birth-weight ratio below 10th percentile, adjusted model[b,e]				0.535[d]	0.89 (0.73 to 1.09)	0.261[d]	0.93 (0.76 to 1.14)	0.495[d]	0.96 (0.79 to 1.16)	0.664[d]

[a] Unadjusted model.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with either maternal weight gain or placental weight in bivariate analysis. All models were adjusted for maternal height at enrollment, maternal BMI at enrollment, duration of pregnancy at enrollment, maternal age at enrollment, child sex, proxy for SES, number of previous pregnancies, maternal anemia at enrollment and site of enrollment.

[c] Calculated using modified Poisson approximation.

[d] Calculated using iterated reweighted least squares.

[e] The choice for the 10th percentile as a cutoff point for these analyses was arbitrary.

[f] P-value obtained by Fisher's exact test.

[g] P-value obtained from Log-binomial regression.

Table 4.2-3. Effect Modification: Placental weight by Intervention Group, Stratified Analyses

Outcome	Interaction test P-value	Result by intervention group Mean (SD)				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[a]	Difference in means (95% CI)	P-value[a]	Difference in means (95% CI)	P-value[a]	Difference in means (95% CI)	P-value[a]
Placental weight											
Boy[b]	0.026	522 (95)	512 (102)	517 (110)	0.687	−5 (−28 to 18)	0.661	5 (−18 to 29)	0.658	−10 (−34 to 13)	0.387
Girl[c]		490 (104)	509 (100)	528 (112)	0.004	38 (16 to 61)	0.001	19 (−3 to 42)	0.093	19 (−4 to 42)	0.099
Placental weight											
Proxy for SES above median (>−0.39)[d]	0.008	490 (93)	518 (103)	527 (112)	0.007	37 (13 to 61)	0.002	9 (−14 to 33)	0.433	28 (4 to 51)	0.021
Proxy for SES at or below median (≤−0.39)[e]		515 (112)	503 (99)	519 (111)	0.386	4 (−19 to 26)	0.757	16 (−7 to 39)	0.184	−12 (−36 to 11)	0.310
Placental weight											
Site of enrollment Lungwena[f]	0.076	537 (102)	507 (88)	542 (106)	0.024	6 (−21 to 32)	0.675	36 (9 to 62)	0.010	−30 (−57 to −2)	0.033
Site of enrollment Malindi[g]		496 (124)	529 (95)	530 (114)	0.101	34 (−2 to 70)	0.063	1 (−35 to 37)	0.968	33 (−2 to 69)	0.065
Site of enrollment Namwera[h]		473 (95)	480 (93)	497 (106)	0.430	24 (−13 to 61)	0.208	17 (−20 to 54)	0.370	7 (−30 to 44)	0.713
Site of enrollment Mangochi[i]		487 (80)	515 (120)	507 (115)	0.205	20 (−12 to 52)	0.220	−8 (−41 to 24)	0.605	28 (−4 to 61)	0.083
Placental weight											
Negative malaria test at enrollment[j]	0.019	497 (106)	515 (102)	526 (113)	0.010	29 (10 to 47)	0.003	11 (−8 to 30)	0.278	18 (−1 to 37)	0.058
Positive malaria test at enrollment[k]		528 (90)	493 (96)	512 (107)	0.111	−16 (−49 to 17)	0.337	19 (−13 to 51)	0.233	−35 (−69 to −2)	0.037

[a] P-value obtained from ANOVA.
[b] Number of participants: IFA n=153, MMN n=143, LNS n=159.
[c] Number of participants: IFA n=165, MMN n=169, LNS n=168.
[d] Number of participants: IFA n=145, MMN n=154, LNS n=145.
[e] Number of participants: IFA n=171, MMN n=156, LNS n=179.
[f] Number of participants: IFA n=106, MMN n=98, LNS n=114.
[g] Number of participants: IFA n=77, MMN n=77, LNS n=73.
[h] Number of participants: IFA n=53, MMN n=54, LNS n=54.
[i] Number of participants: IFA n=85, MMN n=84, LNS n=86.
[j] Number of participants: IFA n=258, MMN n=239, LNS n=249.
[k] Number of participants: IFA n=63, MMN n=74, LNS n=76.

4.3 Maternal Asymptomatic Malaria Infections at 32 and 36 Gestation Weeks and at Delivery

In this section, we examine the impact of LNS supplementation on malaria parasitemia at 32 gw (RDT), at 36 gw (PCR), and at delivery (RDT and PCR). Our main hypothesis stated that the prevalence of maternal malaria parasitemia at each time point would be lower among women who received LNS than among women who received either IFA or MMN. The detailed hypotheses can be found in Appendix 4 (16–18).

Of the 1,391 women who were enrolled in the study, 12 women had twin pregnancies and were excluded from these analyses. Of the remaining women, the number of women included in the analysis was 1,108 (80.3%) at 32 gw, 1,070 (77.6%) at 36 gw, and 1,131 (82.0%) at delivery (RDT). Loss to follow-up at delivery (RDT) was similar in all of the intervention groups (P=0.306). There were 373, 372, and 386 participants included in the malaria at delivery (RDT) analysis in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the malaria at delivery (RDT) analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-3).

The overall prevalence of malaria parasitemia was 23.3% (n=1,376) at enrollment (RDT), 10.8% (n=1,108) at 32 gw (RDT), 9.0% (n=1,070) at 36 gw (PCR), 8.3% (n=1,131) at delivery (RDT), and 20.2% (n=1,095) at delivery (PCR).

Table 4.3-1 shows malaria parasitemia by intervention group. There was no difference in the proportion of malaria parasitemia between the three intervention groups at the different time points. Adjustment of the analyses for the enrollment variables predefined in the analysis plan that were significantly associated (P<0.10) with malaria parasitemia in bivariate analysis did not markedly change the association between the intervention and the corresponding malaria outcome variable.

Variables tested for effect modification were maternal age at enrollment, maternal parity, gestational age at enrollment, proxy for SES, maternal educational achievement, maternal BMI at enrollment, maternal HIV status, maternal malaria parasitemia and anemia at enrollment, season of enrollment, and site of enrollment. These variables were predefined in the analysis plan. Tests for interaction were significant for gestational age at enrollment (P=0.024, for malaria by RDT at 32 gw), maternal educational achievement, malaria at enrollment (P=0.048 and P=0.058, respectively, for malaria by RDT at delivery), and malaria at enrollment (P=0.078, for malaria by PCR at delivery).

Table 4.3-2 shows stratified analyses for effect modifiers that were significant after performing the likelihood ratio test. None of the interaction effects that were significant showed significant effects in pair-wise comparisons in stratified analysis.

The study findings do not support the hypothesis that there is a beneficial effect of gestational LNS supplementation on the prevalence of malaria parasitemia. However, there was a lower prevalence of malaria parasitemia at delivery (PCR) among women in the LNS group compared to those in the MMN group for the subgroup of women who had malaria at enrollment. For women who enrolled in the study earlier in pregnancy, the prevalence of malaria was higher in the IFA group compared to the MMN group at 32 weeks gestation.

Table 4.3-1. Malaria Parasitemia by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Malaria parasitemia at enrollment (RDT)[a]	105/463 (22.6%)	112/465 (24.1%)	105/460 (22.8%)	0.957	1.01 (0.79 to 1.28)	0.957	0.95 (0.75 to 1.20)	0.651	1.06 (0.84 to 1.34)	0.613
Malaria parasitemia at 32 gw (RDT)[a]	45/374 (12.0%)	36/377 (9.6%)	39/357 (10.9%)	0.621	0.91 (0.61 to 1.36)	0.639	1.14 (0.74 to 1.76)	0.539	0.79 (0.52 to 1.20)	0.274
Malaria parasitemia at 32 gw (RDT), adjusted model[b]				0.782	0.93 (0.63 to 1.37)	0.700	1.17 (0.77 to 1.78)	0.461	0.79 (0.53 to 1.18)	0.256
Malaria parasitemia at 36 gw (PCR)[a]	38/352 (10.8%)	31/363 (8.5%)	27/355 (7.6%)	0.139	0.70 (0.44 to 1.13)	0.145	0.89 (0.54 to 1.46)	0.646	0.79 (0.50 to 1.24)	0.309
Malaria parasitemia at 36 gw (PCR), adjusted model[b]				0.186	0.71 (044 to 1.13)	0.144	0.90 (0.55 to 1.96)	0.659	0.79 (0.50 to 1.53)	0.298
Malaria parasitemia at delivery (RDT)[a]	34/373 (9.1%)	29/372 (7.8%)	31/386 (8.0%)	0.592	0.88 (0.55 to 1.40)	0.594	1.03 (0.63 to 1.67)	0.904	0.86 (0.53 to 1.37)	0.518
Malaria parasitemia at delivery (RDT), adjusted model[b]				0.561	0.88 (0.55 to 1.40)	0.584	1.02 (0.63 to 1.65)	0.938	0.86 (0.54 to 1.38)	0.538
Malaria parasitemia at delivery (PCR)[a]	76/369 (20.6%)	80/357 (22.4%)	65/369 (17.6%)	0.313	0.86 (0.63 to 1.15)	0.304	0.79 (0.59 to 1.05	0.108	1.09 (0.82 to 1.44)	0.552
Malaria parasitemia at delivery (PCR), adjusted model[b]				0.363	0.87 (0.64 to 1.67)	0.343	0.75 (0.56 to 1.00)	0.051	1.16 (0.88 to 1.52)	0.300

[a] Unadjusted model.
[b] Models were adjusted for covariates that had significant association (P<0.10) with the outcome in bivariate analysis. Malaria parasitemia at 32 gw (RDT) models were adjusted for parity, maternal age at enrollment, maternal anemia at enrollment, and site of enrollment. Malaria parasitemia at 36 gw (PCR) models were adjusted for parity, maternal age at enrollment, and site of enrollment. Malaria parasitemia at delivery (RDT) models were adjusted for site of enrollment. Malaria parasitemia at delivery (PCR) models were adjusted for maternal age at enrollment, gestational age at enrollment, maternal BMI at enrollment, maternal educational achievement, proxy for SES, and site of enrollment.
[c] The global P-value for the unadjusted and adjusted results was obtained by logistic regression.
[d] The P-value for the pair-wise comparisons for unadjusted and adjusted results was obtained by log-binomial regression models reporting relative risk.

Table 4.3-2. Effect Modification: Malaria Parasitemia by Intervention Group, Stratified Analysis

Outcome	Interaction test P-value	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[a]	Relative risk (95% CI)	P-value[b]	Relative risk (95% CI)	P-value[b]	Relative risk (95% CI)	P-value[b]
Malaria Parasitemia at 32 gw (RDT)											
Gestation age at enrollment <16.9 (weeks)	0.024	24/184 (13.0%)	11/182 (6.0%)	17/169 (10.1%)	0.320	0.77 (0.43 to 1.38)	0.384	1.66 (0.80 to 3.45)	0.171	0.46 (0.23 to 0.92)	0.027
Gestation age at enrollment ≥16.9 (weeks)		21/190 (11.0%)	25/195 (12.8%)	22/188 (11.7%)	0.844	1.06 (0.60 to 1.86)	0.842	0.91 (0.53 to 1.56)	0.739	1.16 (0.67 to 2.00)	0.593
Malaria Parasitemia at Delivery (RDT)											
Maternal educational achievement <4 years	0.048	16/187 (8.6%)	20/170 (11.8%)	14/190 (7.4%)	0.685	0.86 (0.43 to 1.71)	0.670	0.63 (0.33to 1.20)	0.159	1.38 (0.74 to 2.57)	0.317
Maternal educational achievement ≥4 years		18/186 (9.7%)	9/202 (4.5%)	17/196 (8.7%)	0.734	0.90 (0.48 to 1.69)	0.734	1.95 (0.89 to 4.26)	0.096	0.46 (0.21 to 1.00)	0.050
Malaria Parasitemia at Delivery (RDT)											
No malaria (RDT) at enrollment	0.058	28/299 (9.4%)	15/284 (5.3%)	22/297 (7.4%)	0.360	0.79 (0.46 to 1.35)	0.390	1.40 (0.74 to 2.65)	0.297	0.56 (0.31 to 1.03)	0.064
Malaria (RDT) at enrollment		6/74 (8.1%)	14/87 (16.1%)	9/87 (10.3%)	0.716	1.28 (0.48 to 3.42)	0.628	0.64 (0.29to 1.41)	0.269	1.98 (0.80 to 4.90)	0.138
Maternal Parasitemia at Delivery (PCR)											
No malaria (RDT) at enrollment	0.078	59/288 (20.5%)	51/272 (18.8%)	50/278 (18.0%)	0.448	0.88 (0.63 to 1.23)	0.451	0.96 (0.67 to 1.36)	0.817	0.92 (0.65 to 1.28)	0.606
Malaria (RDT) at enrollment		17/81 (21.0%)	28/84 (33.3%)	15/90 (16.7%)	0.458	0.79 (0.42 to 1.49)	0.470	0.50 (0.29 to 0.87)	0.817	1.59(0.94 to 2.67)	0.081

[a] The global P-value was obtained by logistic regression.
[b] The P-value for the pair-wise comparisons was obtained by log-binomial regression models reporting relative risks.

4.4 Maternal Reproductive Tract Infections and Urinary Tract Infections after Delivery

In this section, we examine the impact of LNS supplementation on the prevalence of trichomoniasis and UTIs after delivery. Our main hypothesis stated that the prevalence of these infections would be lower among women who received LNS than among women who received either IFA or MMN. The detailed hypotheses can be found in Appendix 4 (19 and 20).

Of the 1,391 women who were enrolled in the study, 12 were excluded because they had twin pregnancies. After removing participants with unknown or missing data, 1,210 (87.0%) and 1,212 (87.9%) women were included in the trichomoniasis and UTI analyses, respectively. Loss to follow-up was similar in all of the intervention groups (P=0.846 and P=0.927 in the trichomoniasis and UTI analyses, respectively). The number of women included was 405, 407, and 398 in the trichomoniasis analysis and 406, 405, and 401 in the UTI analysis in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the trichomoniasis analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-4).

The overall prevalence of trichomoniasis and UTI 1 week after delivery was 10.2% (n=1,210) and 3.1% (n=1,212), respectively.

Table 4.4-1 shows the prevalence of trichomoniasis and UTI by intervention group. There was no difference in the prevalence of either trichomoniasis or UTI between the three intervention groups. Adjustment of the analyses for the enrollment variables predefined in the analysis plan that were significantly associated (P<0.10) with the outcome in bivariate analysis did not change the association between the intervention and trichomoniasis or UTI.

Variables tested for effect modification were maternal age at enrollment, maternal BMI at enrollment, parity, maternal HIV status, maternal malaria status at enrollment, maternal educational achievement, and gestational age at enrollment. These variables were predefined in the analysis plan. Tests for interaction between the intervention and maternal BMI at enrollment were significant (P=0.039) for trichomoniasis. The median maternal BMI at enrollment, 21.6, was chosen as the cutoff point for high and low BMI.

Table 4.4-2 shows stratified analyses for the effect modifiers that were significant after performing the likelihood ratio test.

The study findings do not support the hypothesis that there is a beneficial effect of gestational LNS supplementation on the prevalence of trichomoniasis and UTI after delivery.

Table 4.4-1. Trichomoniasis and UTI by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Prevalence of trichomoniasis[a]	47/405 (11.6%)	44/407 (10.8%)	33/398 (8.3%)	0.123	0.71 (0.47 to 1.09)	0.119	0.77 (0.50 to 1.18)	0.226	0.93 (0.63 to 1.37)	0.720
Prevalence of trichomoniasis, adjusted model[b]				0.154	0.75 (0.49 to 1.14)	0.178	0.74 (0.48 to 1.13)	0.163	1.02 (0.69 to 1.50)	0.939
Prevalence of UTI[a]	9/406 (2.2%)	15/405 (3.7%)	13/401 (3.2%)	0.397	1.46 (0.63 to 3.38)	0.374	0.86 (0.42 to 1.82)	0.721	1.67 (0.74 to 3.77)	0.217
Prevalence of UTI, adjusted model[b]				0.380	1.48 (0.64 to 3.42)	0.357	0.90 (0.43 to 1.89)	0.783	1.64 (0.72 to 3.76)	0.238

[a] Unadjusted model.
[b] Models were adjusted for covariates that had significant association (P<0.10) with the outcome in bivariate analysis. Trichomoniasis models were adjusted for maternal educational achievement, proxy for SES, and maternal HIV status. UTI models were adjusted for maternal HIV status.
[c] The global P-value for unadjusted and adjusted results was obtained using logistic regression.
[d] The P-value for pair-wise comparisons for adjusted and unadjusted results was obtained using log-binomial regression models.

Table 4.4-2. Effect Modification: Trichomoniasis by Intervention Group, Stratified Analysis

Outcome	Interaction test P-value	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[a]	Relative risk (95% CI)	P-value[b]	Relative risk (95% CI)	P-value[b]	Relative risk (95% CI)	P-value[b]
Trichomoniasis											
Maternal BMI at enrollment <21.6	0.039	26/204 (12.8%)	27/201 (13.4%)	14/190 (7.4%)	0.098	0.58 (0.31 to 1.07)	0.116	0.55 (0.30 to 1.01)	0.079	1.05 (.0.64 to 1.74)	0.837
Maternal BMI at enrollment ≥21.6		21/201 (10.5%)	17/206 (8.3%)	19/208 (9.1%)	0.652	0.87 (0.48 to 1.58)	0.655	1.02 (0.59 to 2.07)	0.750	0.79(0.43 to 1.45)	0.448

[a] The global P-value for unadjusted and adjusted results was obtained using logistic regression.
[b] The P-value for pair-wise comparisons for unadjusted and adjusted results was obtained using log-binomial regression models.

4.5 Maternal Plasma CRP and AGP Concentrations

In this section, we examine the impact of LNS supplementation on maternal inflammation. Our main hypothesis stated that maternal inflammation, as measured by plasma concentration of CRP and AGP at 36 gw, would be lower among women who received LNS than among women who received either IFA or MMN. The detailed hypothesis can be found in Appendix 4 (21).

Of the 1,391 women who were enrolled in the study, 12 were excluded because they had twin pregnancies. Of the remaining participants, CRP and AGP data were available for 1,371 (99.4%) participants at the enrollment visit and 1,063 (77.1%) participants at the 36 gw visit. Loss to follow-up was similar across the intervention groups (P=0.931). There were 350, 361, and 352 participants in the IFA, MMN, and LNS groups, respectively, included in the analyses for 36 gw.

There were no clinically meaningful differences between the three intervention groups of participants included in the maternal plasma CRP and AGP analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-5).

Overall, the mean (SD) CRP was 8.7 mg/L (17.8) at enrollment and 6.5 mg/L (14.0) at 36 gw. The mean (SD) AGP was 0.7 g/L (0.3) at enrollment and 0.6 g/L (0.2) at 36 gw.

Table 4.5-1 shows comparisons between groups in the proportion of participants with elevated CRP or AGP at each time point. There were no differences between groups in the proportion of women with elevated CRP or AGP at enrollment or at 36 gw in unadjusted models.

The following variables were selected a priori based on their expected association with the outcome variables and were examined as potential covariates: maternal BMI at enrollment; maternal malaria status at enrollment; maternal HIV status; primiparity; maternal educational achievement; site of enrollment; season of enrollment; and maternal Hb, ZPP, and sTfR at enrollment. Those variables with significant associations (P<0.10) with the outcome in bivariate analysis were included as covariates in the model for the corresponding outcome and are listed in Table 4.5-1. In addition, the model for elevated CRP and the model for AGP were each adjusted for the corresponding enrollment value for the respective outcome. After adjusting for covariates, there were no differences between groups in the proportion of women with elevated CRP or AGP.

The same enrollment variables as listed above were tested for effect modification. Tests did not indicate a modification of the intervention effect on change in CRP or AGP by any of the tested variables.

The study findings do not support a hypothesis that provision of LNS to pregnant women decreases maternal inflammation, as measured by her plasma concentration of CRP and AGP in pregnancy.

Table 4.5-1. Elevated CRP or AGP by Intervention Group

Outcome	Time point	Number of outcomes/women with outcome data				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
CRP >5 mg/L	Enrollment[a]	195/456 (42.8%)	176/462 (38.1%)	202/453 (44.6%)	0.120	1.04 (0.86 to 1.27)	0.677	1.17 (0.96 to 1.43)	0.127	0.89 (0.73 to 1.09)	0.266
	36 gw[a]	108/350 (30.9%)	97/361 (26.9%)	107/352 (30.4%)	0.428	0.99 (0.76 to 1.29)	0.946	1.13 (0.86 to 1.49)	0.358	0.87 (0.66 to 1.14)	0.323
	36 gw, adjusted model[b]				0.625	0.97 (0.74 to 1.27)	0.842	1.12 (0.84 to 1.47)	0.436	0.87 (0.66 to 1.15)	0.330
AGP >1 g/L	Enrollment[a]	64/456 (14.0%)	50/462 (10.8%)	61/453 (13.5%)	0.288	0.96 (0.67 to 1.36)	0.817	1.24 (0.86 to 1.81)	0.252	0.77 (0.53 to 1.12)	0.169
	36 gw[a]	26/350 (7.4%)	17/361 (4.7%)	15/352 (4.3%)	0.152	0.58 (0.31 to 1.09)	0.090	0.91 (0.45 to 1.82)	0.790	0.63 (0.34 to 1.17)	0.144
	36 gw, adjusted model[b]				0.244	0.65 (0.34 to 1.24)	0.188	0.85 (0.42 to 1.71)	0.651	0.76 (0.41 to 1.42)	0.393

[a] Unadjusted model.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding outcome value at enrollment. CRP models were adjusted for maternal CRP at enrollment, maternal sTfR at enrollment and maternal HIV status. AGP models were adjusted for maternal AGP at enrollment, maternal Hb at enrollment, primiparity, maternal educational achievement, maternal HIV status, and site of enrollment.

[c] P-value for enrollment and unadjusted analyses were calculated using Fisher's exact test. Adjusted analyses at 36 gw were calculated using logistic regression.

[d] Unadjusted and adjusted pair-wise comparisons were calculated using log-Poisson regression.

4.6 Maternal Blood Hb, ZPP, and sTfR Concentrations

In this section, we examine the impact of LNS supplementation on maternal Hb, ZPP, and sTfR concentration. Our general hypotheses for the effect of the intervention on Hb and iron status were that the mean blood Hb concentration would be lower and mean red blood cell ZPP and sTfR concentrations would be higher at 36 gw among women in the LNS and MMN groups compared to the IFA group. (There is a reciprocal relationship between both ZPP and sTfR and iron status, i.e., high ZPP and sTfR mean low iron status.) We hypothesized that the Hb and iron status would be higher in the IFA group because of the higher iron content in the IFA than the LNS or MMN supplements (60 mg/day vs. 20 mg/day). The detailed hypotheses can be found in Appendix 4 (22–26).

Of the 1,391 women who were enrolled in the study, 12 were excluded because they had twin pregnancies. Of the remaining 1,379 women, we analyzed Hb from 1,377 (99.9%), ZPP from 1,325 (96.1%), and sTfR from 1,371 (99.4%) of those women. There were no differences between groups in the proportion of women who were lost to follow up (P=0.882). At the 36 gw visit, we analyzed Hb from 1,041 (75.5% of the original 1,379 women who completed the enrollment visit), ZPP from 1,008 (73.1%), and sTfR from 1,067 (77.4%). There were 342, 353, and 346 participants included in the Hb analyses at 36 gw; 330, 343, and 335 participants in the ZPP analyses; and 352, 363, and 352 participants included in the sTfR analysis in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the sTfR analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-6).

At enrollment, the mean (SD) Hb level of all participants included in these analyses was 111.5 g/L (16.3). The prevalence of anemia (Hb <100 g/L) was 20.8%, while 11.1% had high Hb (>130 g/L) (Pena-Rosas et al. 2012). The mean (SD) ZPP at enrollment was 54.5 µmol/mol heme (41.6), with 24.5% of participants having high ZPP (>60 µmol/mol heme). The mean (SD) sTfR was 4.8 mg/L (2.7), with 19.7% having high sTfR (>6 mg/L).

At 36 gw, the mean (SD) Hb was 110.8 g/L (15.2), 20.4% of women were anemic, and 8.5% had high Hb. The mean (SD) ZPP at 36 gw was 60.2 µmol/mol heme (40.9), with 34.0% of participants having high ZPP. The mean (SD) sTfR was 5.6 mg/L (3.0), with 33.5% of participants having high sTfR.

Table 4.6-1 shows the difference in mean Hb, ZPP, and sTfR between the intervention groups. At enrollment, there were no differences in any of the markers between intervention groups. Before adjusting for covariates, Hb at the 36 gw visit was lower in both the LNS (P=0.041) and MMN (P=0.044) groups compared to the IFA group. Also in unadjusted models, mean ZPP concentration was greater in the LNS (P=0.006) and MMN (P=0.043) groups than in the IFA group at 36 gw, while the change (increase) in ZPP from enrollment to 36 gw was greater in the LNS group than in the IFA and MMN groups (P=0.004 for both). sTfR was greater in the LNS and MMN groups than in the IFA group at 36 gw (P=0.005 and P=0.010, respectively) before adjusting for covariates, and the change (increase) in sTfR from enrollment to 36 gw tended to be greater in the LNS and MMN groups compared to the IFA group (P=0.055 and P=0.085, respectively). Again, because of the reciprocal nature of both ZPP and sTfR and iron status, higher ZPP and sTfR at 36 gw among women in the MMN and LNS groups means that their iron status was lower.

The following variables were selected a priori based on their expected association with the outcome variables and were examined as potential covariates: maternal BMI at enrollment, maternal malaria status at enrollment, maternal HIV status, primiparity, maternal educational achievement, site of enrollment, season of enrollment, maternal Hb (for ZPP and sTfR analyses), and ZPP and sTfR (for Hb analyses) at enrollment. Those with significant associations (P<0.10) with the outcome variable in bivariate analysis were included as covariates in the model for the corresponding outcome and are listed in the tables below. After adjusting for covariates, the difference between MMN and IFA groups in Hb at 36 gw in unadjusted models was no longer significant (P=0.069). The difference in the change (decrease) in Hb in the IFA group was not significantly greater than in the LNS group before adjusting for covariates (P=0.130), yet became significant

(P=0.030) after adjusting. Similarly, the change (increase) in sTfR that tended to be greater in the LNS and MMN groups compared to the IFA group in unadjusted models (P=0.055 and P=0.085, respectively) became significant after adjusting for covariates (P=0.010 and P=0.022, respectively). After adjusting for covariates, mean ZPP at 36 gw was no longer significantly greater in the MMN group compared to the IFA group (P=0.383). For other comparisons, the significance of the difference between groups did not change after adjusting for covariates.

The same enrollment variables examined as covariates were tested for effect modification. There were significant interactions (P<0.05) between intervention group assignment and Hb at enrollment, CRP at enrollment, and sTfR at enrollment in the change in Hb from enrollment to 36 gw, as well as an interaction between intervention group assignment and Hb at enrollment in the change in sTfR from enrollment to 36 gw. Regression modeling was used to predict the change in Hb and sTfR from enrollment to 36 gw at the 10th, 50th, and 90th percentiles of the effect modifiers at enrollment. Essentially, the change in outcome variable at the 10th, 50th, and 90th percentile of the effect modifier was predicted and compared between intervention groups. This method allowed us to look at the modifying effect at the extremes of the modifier (i.e., the 10th and 90th percentiles), rather than simply using a cutoff and creating a dichotomous variable. The results are shown in Tables 4.6-2–4.6-5**.**

Table 4.6-2 **s**hows that the predicted change in Hb in the IFA group was more positive than in the LNS group at the 10th and 50th percentiles of Hb at enrollment (P<0.001 and P=0.020, respectively) and tended to be more positive in the IFA compared to MMN group (P=0.058). In other words, those in the IFA group with a low to average Hb at enrollment would be predicted to have a greater increase in Hb from enrollment to 36 gw than those in the LNS group. The predicted change in Hb was also more positive in the IFA group compared to the LNS group at the 90th percentile of CRP at enrollment (P=0.008) (

Table 4.6-3**)**, and the predicted change in Hb tended to be more positive in the IFA group compared to the MMN group at the 90th percentile of CRP at enrollment (P=0.090). At the 90th percentile of sTfR at enrollment, the predicted change in Hb was more positive in the IFA group compared to the LNS group (P<0.001), while the predicted change in Hb tended to be more positive in the MMS group compared to the LNS group (P=0.053) and in the IFA group compared to the MMN group (P=0.064) (

Table 4.6-4). The predicted change in sTfR was more positive in the MMN group compared to the IFA group at the 90th percentile of Hb at enrollment (P=0.003) (

Table 4.6-5) and tended to be more positive in the LNS group compared to the IFA group at the 50th and 90th percentiles of Hb at enrollment (P=0.090 and P=0.071, respectively).

Table 4.6-6 shows the difference between intervention groups in the prevalence of abnormal Hb, ZPP, and sTfR values. At enrollment, there were no differences between groups in the prevalence of abnormal values. In unadjusted models at 36 gw, the risk of elevated ZPP was greater among women in the LNS group compared to the IFA group (P=0.008) and tended to be greater among women in the LNS group compared to the MMN group (P=0.088). The risk of IDA (Hb <100 g/L and either ZPP >60 μmol/mol heme or sTfR >6.0 mg/L) was greater in both the LNS and MMN groups compared to the IFA group (P=0.023 and P=0.031, respectively). After adjusting for covariates as described above, there was a greater risk of anemia (Hb <100 g/L) at 36 gw among women in the LNS group compared to the IFA group (P=0.036) and the risk of elevated ZPP among women in the LNS group compared to the IFA group remained significant (P=0.013). The trend toward increased ZPP in the LNS group compared to the MMN group became significant after adding covariates to the model (P=0.021). After adjusting for covariates, the global P-value for the difference between intervention groups in the prevalence of IDA was no longer significant (P=0.079). There were no differences in proportion with abnormal markers between groups.

Because of the described influence of inflammation on Hb (Weiss and Goodnough 2005), we examined the effect of the intervention on Hb among women without inflammation (CRP <5 mg/L and AGP <1 g/L) (data not shown). After excluding cases with inflammation at 36 gw (n=368), there were no differences between intervention groups in mean Hb at 36 gw (P=0.122) or in the change in Hb from enrollment to 36 gw (P=0.331) before adjusting for covariates. There was a greater proportion of anemic women (Hb <100 g/L) in the LNS group compared to the IFA group (global P=0.044; LNS vs. IFA, P=0.028) at 36 gw, while there were no differences between MMN and IFA groups (P=0.096) or between LNS and MMN groups (P=0.555). There were no differences between groups in the proportion of women with elevated Hb at 36 gw before adjusting for covariates (P=0.846). After adjusting for covariates as described above, there were no differences between groups in mean Hb or change in Hb from enrollment to 36 gw (P=0.079 for both). After adjusting for covariates, there remained a greater proportion of anemic women in the LNS group compared to the IFA group (global P=0.034; LNS vs. IFA, P=0.026) and continued to be no differences between MMN and IFA groups (P=0.109) or between LNS and MMN groups (P=0.484). There also continued to be no differences between groups in the proportion of women with elevated Hb at 36 gw after adjusting for covariates (P=0.918).

The study findings support the hypothesis that the IFA group would have higher Hb and markers of iron status than the LNS group after supplementation from enrollment to 36 gw. The differences between the MMN group and the IFA group were not as consistent. The MMN group had a lower change in ZPP from enrollment to 36 gw and was at decreased risk of elevated ZPP at 36 gw compared to the LNS group. Hb, iron status, and inflammation (as indicated by CRP) modified the effect of intervention, such that Hb tended to increase more in the IFA group among those with low Hb, low iron status, or high inflammation at enrollment.

Table 4.6-1. Hb, ZPP, and sTfR at Enrollment, 36 gw, and Change from Enrollment to 36 gw by Intervention Group

Outcome	Time point	Result by intervention group[d]				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[c]	Difference in means (95% CI)	P-value[d]	Difference in means (95% CI)	P-value[d]	Difference in means (95% CI)	P-value[d]
Hb (g/L)	Mean (SD) Enrollment[a,f]	111.3 (16.7)	111.4 (16.2)	111.7 (16.1)	0.923	0.4 (−2.1 to 2.9)	0.930	0.4 (−2.2 to 2.9)	0.942	0.0 (−2.5 to 2.6)	0.999
	Mean (SD) 36 gw[a,f]	112.8 (14.8)	110.1 (14.3)	110.0 (15.4)	0.021	−2.7 (−5.4 to −0.1)	0.041	−0.1 (−2.7 to 2.6)	0.999	−2.7 (−5.3 to −0.1)	0.044
	36 gw, adjusted model[b]				0.022	−2.8 (−5.3 to −0.2)	0.030	−0.4 (−2.9 to 2.2)	0.943	−2.4 (−5.0 to 0.1)	0.069
	Mean (SD) change[a,e]	0.03 (18.5)	−1.9 (17.5)	−2.5 (15.9)	0.134	−2.6 (−5.7 to 0.6)	0.130	−0.7 (−3.7 to 2.4)	0.872	−1.9 (−5.0 to 1.2)	0.318
	Change, adjusted model[b,e]				0.022	−2.8 (−5.3 to −0.2)	0.030	−0.4 (−2.9 to 2.2)	0.943	−2.4 (−5.0 to 0.1)	0.069
ZPP (µmol/mol heme)	Mean (SD) Enrollment[,f]	53.7 (40.8)	55.4 (43.4)	54.3 (40.6)	0.829	0.5 (−6.0 to 7.1)	0.979	−1.1 (−7.7 to 5.5)	0.915	1.7 (−4.9 to 8.2)	0.819
	Mean (SD) 36 gw[a,f]	54.3 (34.2)	61.9 (45.6)	64.0 (41.1)	0.006	9.7 (2.3 to 17.1)	0.006	2.2 (−5.2 to 9.5)	0.769	7.5 (0.2 to 14.9)	0.043
	36 gw, adjusted model[b]				0.002	9.5 (3.2 to 15.9)	0.001	6.0 (−0.3 to 12.3)	0.069	3.6 (−2.8 to 9.9)	0.383
	Mean (SD) change[a,e]	3.7 (34.9)	3.7 (41.0)	12.8 (30.2)	0.001	9.1 (2.5 to 15.8)	0.004	9.0 (2.4 to 15.6)	0.004	0.1 (−6.5 to 6.7)	>0.999
	Change, adjusted model[b,e]				<0.001	9.5 (3.4 to 15.6)	<0.001	7.3 (1.2 to 13.4)	0.015	2.2 (−3.9 to 8.3)	0.676
sTfR (mg/L)	Mean (SD) Enrollment[a,f]	4.8 (2.4)	4.8 (2.9)	4.9 (2.8)	0.642	0.1 (−0.3 to 0.6)	0.693	0.1 (−0.3 to 0.6)	0.692	0.0 (−0.4 to 0.4)	>0.999
	Mean (SD) 36 gw[a,f]	5.2 (2.1)	5.8 (3.7)	5.9 (2.9)	0.002	0.7 (0.2 to 1.2)	0.005	0.1 (−0.5 to 0.6)	0.960	0.7 (0.1 to 1.2)	0.010
	36 gw, adjusted model[b]				0.006	0.5 (0.1 to 0.9)	0.010	0.1 (−0.4 to 0.5)	0.957	0.5 (0.1 to 0.9)	0.022
	Mean (SD) change[a,e]	0.7 (1.8)	1.1 (2.8)	1.1 (2.5)	0.038	0.4 (−0.0 to 0.9)	0.055	0.0 (−0.4 to 0.5)	0.979	0.4 (−0.0 to 0.8)	0.085
	Change, adjusted model[b,e]				0.006	0.5 (0.1 to 0.9)	0.010	0.1 (−0.4 to 0.5)	0.957	0.5 (0.1 to 0.9)	0.022

[a] Unadjusted model.

[b] Adjusted models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment. Models for Hb at 36 gw and change in Hb were adjusted for intervention group, maternal Hb at enrollment, maternal BMI at enrollment, maternal ZPP at enrollment, maternal CRP at enrollment, primiparity, maternal malaria at enrollment, season of enrollment, and site of enrollment. Models for ZPP at 36 gw and change in ZPP were adjusted for intervention group, maternal ZPP at enrollment, maternal BMI at enrollment, maternal Hb at enrollment, maternal AGP at enrollment, maternal educational achievement, season of enrollment, and site of enrollment. Models for sTfR at 36 gw and change in sTfR were adjusted for intervention group, maternal sTfR at enrollment, maternal Hb at enrollment, maternal CRP at enrollment, maternal AGP at enrollment, maternal educational achievement, and site of enrollment.

[c] Enrollment and unadjusted P-values were calculated using ANOVA; adjusted P-values were calculated using ANCOVA.

[d] Unadjusted and adjusted pair-wise comparisons were calculated using log-Poisson regression.

[e] Change from enrollment to 36 gw is reported as the mean change in values.

[f] Sample sizes:
Hb at enrollment: IFA n=460, MMN n=463, LNS n=454.
Hb at 36 gw: IFA: n=342, MMN: n=353, LNS: n=346.
ZPP at enrollment: IFA: n=446, MMN: n=445, LNS: n=434.
ZPP at 36 gw: IFA: n=330, MMN: n=343, LNS: n=335.
sTfR at enrollment: IFA: n=456, MMN: n=462, LNS: n=453.
sTfR at 36 gw: IFA: n=352, MMN: n=363, LNS: n=352.

Table 4.6-2. Effect Modification: Predicted Change from Enrollment to 36 gw in Hb at the 10th, 50th, and 90th Percentiles of Enrollment Hb by Intervention Group

Outcome	Interaction test P-value	Predicted change in Hb (g/L) by intervention group[a] Mean (SE)			Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA (n=342)	MMN (n=353)	LNS (n=346)	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]
Change in Hb from enrollment to 36 gw										
At enrollment Hb=91 g/L (10th percentile)	0.007	17.4 (1.3)	14.4 (1.3)	10.5 (1.4)	−6.9 (−11.2 to −2.6)	<0.001	−4.0 (−8.3 to 0.3)	0.078	−2.9 (−7.1 to 1.3)	0.238
At enrollment Hb=112 g/L (50th percentile)		1.6 (0.9)	−0.8 (0.9)	−1.3 (0.9)	−2.9 (−5.5 to −0.4)	0.020	−0.4 (−3.0 to 2.1)	0.913	−2.5 (−5.0 to −0.1)	0.058
At enrollment Hb=132 g/L (90th percentile)		−13.3 (1.3)	−15.4 (1.4)	−12.5 (1.4)	0.9 (−3.1 to 4.9)	0.861	2.9 (−1.1 to 7.0)	0.209	−2.5 (−6.0 to 1.9)	0.444

[a] Predicted least squares mean (SE) estimated at the 10th, 50th, and 90th percentile of enrollment Hb by using ANOVA.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment. Models for change in Hb were adjusted for maternal Hb at enrollment, maternal ZPP at enrollment, maternal CRP at enrollment, maternal BMI at enrollment, primiparity, maternal malaria status at enrollment, season of enrollment, and site of enrollment. Pair-wise comparisons were analyzed by ANCOVA with a Tukey-Kramer adjustment. Global P-values not calculated as a Global F test cannot be calculated for specified values of a continuous variable.

Table 4.6-3. Effect Modification: Predicted Change from Enrollment to 36 gw in Hb at the 10th, 50th, and 90th Percentiles of Enrollment CRP by Intervention Group

Outcome	Interaction test P-value	Predicted change in Hb (g/L) by intervention group[a] Mean (SE)			Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA (n=342)	MMN (n=353)	LNS (n=346)	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]
Mean (SE) Change in Hb from enrollment to 36 gw										
At enrollment CRP=0.91 mg/L (10th percentile)	0.020	2.3 (1.2)	0.5 (1.1)	0.5 (1.2)	−1.8 (−5.2 to 1.6)	0.437	−0.02 (−3.4 to 3.3)	>0.999	−1.8 (−5.2 to 1.6)	0.437
At enrollment CRP=4.04 mg/L (50th percentile)		2.6 (1.1)	0.5 (1.1)	0.2 (1.1)	−2.3 (−5.5 to 0.9)	0.212	−0.2 (−3.4 to 2.9)	0.982	−2.1 (−5.2 to 1.1)	0.283
At enrollment CRP=17.43 mg/L (90th percentile)		3.8 (1.2)	0.5 (1.2)	−0.7 (1.1)	−4.5 (−8.0 to −1.0)	0.008	−1.2 (−4.7 to 2.3)	0.689	−3.3 (−6.9 to 0.4)	0.090

[a] Predicted least squares mean (SE) estimated at the 10th, 50th, and 90th percentile of enrollment CRP by using ANOVA.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment. Models for change in Hb were adjusted for maternal Hb at enrollment, maternal CRP at enrollment, maternal ZPP at enrollment, maternal BMI at enrollment, primiparity, maternal malaria status at enrollment, season of enrollment, and site of enrollment. Pair-wise comparisons were analyzed by ANCOVA with a Tukey-Kramer adjustment. Global P-values not calculated as a Global F test cannot be calculated for specified values of a continuous variable.

Table 4.6-4. Effect Modification: Predicted Change from Enrollment to 36 gw in Hb at the 10th, 50th, and 90th Percentiles of Enrollment sTfR by Intervention Group, Stratified Analysis

Outcome	Interaction test P-value	Predicted change in mean Hb (g/L) by intervention group[a] Mean (SE)			Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA (n=342)	MMN (n=353)	LNS (n=346)	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]
Change in Hb from enrollment to 36 gw										
At enrollment sTfR=2.65 mg/L (10th percentile)	0.030	−0.7 (1.4)	−2.1(1.3)	−0.4 (1.3)	0.3 (−3.7 to 4.3)	0.984	1.7 (−2.1 to 5.5)	0.533	−1.4 (−5.4 to 2.4)	0.666
At enrollment sTfR=4.12 mg/L (50th percentile)		2.1 (1.1)	−0.3 (1.1)	−0.4 (1.1)	−2.5 (−5.6 to 0.7)	0.154	0.0 (−3.2 to 3.1)	0.999	−2.4 (−5.5 to 0.7)	0.162
At enrollment sTfR=7.45 mg/L (90th percentile)		8.4 (1.8)	3.7 (1.4)	−0.3 (1.4)	−8.7 (−13.7 to −3.8)	<0.001	−4.1 (−8.2 to −0.0)	0.053	−4.6 (−9.5 to 0.2)	0.064

[a] Predicted least squares mean (SE) estimated at the 10th, 50th, and 90th percentile of enrollment sTfR by using ANOVA.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome on bivariate analysis, in addition to the corresponding values at enrollment. Models for change in Hb were adjusted for maternal Hb at enrollment, maternal sTfR at enrollment, materanl ZPP at enrollment, maternal CRP at enrollment, maternal BMI at enrollment, primiparity, maternal malaria status at enrollment, season of enrollment, and site of enrollment. Pair-wise comparisons were analyzed by ANCOVA with a Tukey-Kramer adjustment. Global P-values not calculated as a Global F test cannot be calculated for specified values of a continuous variable.

Table 4.6-5. Effect Modification: Predicted Change from Enrollment to 36 gw in sTfR at the 10th, 50th, and 90th Percentiles of Enrollment Hb by Intervention Group, Stratified Analysis

Outcome	Interaction test P-value	Predicted change in mean sTfR by intervention group[a] Mean (SE)			Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA (n=352)	MMN (n=363)	LNS (n=352)	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]	Difference in least squares means (95% CI)	P-value[b]
Change in sTfR from enrollment to 36 gw										
At enrollment Hb=91 g/L (10th percentile)	0.030	0.6 (0.2)	0.4 (0.2)	0.8 (0.2)	0.1 (−0.6 to 0.8)	0.924	0.3 (−0.4 to 1.1)	0.532	−0.2 (−0.9 to 0.5)	0.757
At enrollment Hb=112 g/L (50th percentile)		0.8 (0.1)	1.2 (0.1)	1.2 (0.1)	0.4 (−0.0 to 0.8)	0.090	0.0 (−0.4 to 0.4)	>0.999	0.4 (−0.0 to 0.8)	0.093
At enrollment Hb=132 g/L (90th percentile)		0.9 (0.2)	1.9 (0.2)	1.6 (0.2)	0.6 (−0.0 to 1.3)	0.071	−0.3 (−1.0 to 0.4)	0.544	1.0 (0.3 to 1.6)	0.003

[a] Predicted least squares mean (SE) estimated at the 10th, 50th, and 90th percentile of enrollment Hb by using ANOVA.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome on bivariate analysis, in addition to the corresponding values at enrollment. Models for change in sTfR were adjusted for maternal sTfR, CRP and AGP at enrollment, maternal educational achievement, and site of enrollment. Pair-wise comparisons were analyzed by ANCOVA with a Tukey-Kramer. Global P-values not calculated as a Global F test cannot be calculated for specified values of a continuous variable.

Table 4.6-6. Abnormal Hb, ZPP, and sTfR by Intervention Group

Outcome	Time point	Number of outcomes/women with outcome data				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Hb <100 g/L	Enrollment[a]	97/460 (21.1%)	92/463 (19.9%)	97/454 (21.4%)	0.840	1.01 (0.76 to 1.34)	0.927	1.07 (0.80 to 1.43)	0.618	0.94 (0.71 to 1.25)	0.683
	36 gw[a]	56/342 (16.4%)	76/353 (21.5%)	80/346 (23.1%)	0.068	1.41 (1.00 to 1.99)	0.048	1.07 (0.78 to 1.47)	0.656	1.31 (0.93 to 1.86)	0.120
	36 gw, adjusted model[b]				0.049	1.48 (1.04 to 2.11)	0.036	1.13 (0.82 to 1.55)	0.487	1.32 (0.92 to 1.88)	0.155
Hb >130 g/L	Enrollment[a]	55/460 (12.0%)	48/463 (10.4%)	50/454 (11.0%)	0.748	0.92 (0.62 to 1.35)	0.674	1.06 (0.71 to 1.57)	0.765	0.87 (0.59 to 1.28)	0.470
	36 gw[a]	36/342 (10.5%)	26/353 (7.4%)	26/346 (7.5%)	0.262	0.71 (0.43 to 1.18)	0.190	1.02 (0.59 to 1.76)	0.942	0.70 (0.42 to 1.16)	0.165
	36 gw, adjusted model[b]				0.247	0.75 (0.45 to 1.25)	0.261	1.04 (0.60 to 1.81)	0.937	0.72 (0.43 to 1.21)	0.226
ZPP >60 µmol/mol heme	Enrollment[a]	103/446 (23.1%)	114/445 (25.6%)	108/434 (24.9%)	0.666	1.08 (0.82 to 1.41)	0.588	0.97 (0.75 to 1.26)	0.829	1.08 (0.82 to 1.41)	0.446
	36 gw[a]	94/330 (28.5%)	112/343 (32.8%)	136/335 (40.6%)	0.004	1.43 (1.10 to 1.85)	0.008	1.24 (0.96 to 1.60)	0.088	1.15 (0.87 to 1.51)	0.329
	36 gw, adjusted model[b]				<0.001	1.41 (1.07 to 1.85)	0.013	1.36 (1.05 to 1.77)	0.021	1.04 (0.78 to 1.38)	0.802
sTfR >6.0 mg/L	Enrollment[a]	88/456 (19.3%)	92/462 (19.9%)	90/453 (19.9%)	0.970	1.03 (0.77 to 1.38)	0.846	1.00 (0.75 to 1.33)	0.988	1.03 (0.77 to 1.38)	0.833
	36 gw[a]	103/352 (29.3%)	126/363 (34.7%)	128/352 (36.4%)	0.110	1.24 (1.01 to 1.41)	0.046	1.04 (0.86 to 1.25)	0.644	1.13 (0.95 to 1.46)	0.120
	36 gw, adjusted model[b]				0.164	1.16 (0.96 to 1.40)	0.072	1.11 (0.92 to 1.34)	0.487	1.05 (0.86 to 1.36)	0.260
IDA[e]	Enrollment[a]	47/456 (10.3%)	56/458 (12.2%)	54/450 (12.0%)	0.611	1.16 (0.81 to 1.68)	0.446	0.98 (0.69 to 1.39)	0.922	1.18 (0.82 to 1.71)	0.388
	36 gw[a]	30/335 (9.0%)	50/349 (14.3%)	50/340 (14.7%)	0.036	1.62 (1.07 to 2.52)	0.023	1.03 (0.71 to 1.47)	0.888	1.60 (1.04 to 2.45)	0.031
	36 gw, adjusted model[b]				0.079	1.62 (1.05 to 2.48)	0.029	1.12 (0.78 to 1.61)	0.535	1.44 (0.94 to 2.21)	0.096

[a] Unadjusted model.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment. Models for Hb were adjusted for maternal Hb at enrollment, maternal BMI at enrollment, maternal ZPP at enrollment, maternal CRP at enrollment, primiparity, maternal malaria status at enrollment, season of enrollment, and site of enrollment. Models for sTfR were adjusted for maternal sTfR at enrollment, maternal Hb at enrollment, maternal CRP at enrollment, maternal AGP at enrollment, maternal educational achievement, and site of enrollment. Models for ZPP were adjusted for maternal ZPP at enrollment, maternal BMI at enrollment, maternal Hb at enrollment, maternal AGP at enrollment, maternal educational achievement, season of enrollment, and site of enrollment.

[c] Unadjusted P-values were calculated using Fisher's exact test; adjusted P-values were calculated using logistic regression models.

[d] Unadjusted and adjusted P-values were calculated using log-Poisson regression models.

[e] Hb <100 and either ZPP >60 µmol/mol heme or sTfR >6.0 mg/L.

4.7 Maternal Plasma Retinol Concentration

In this section, we test the hypotheses that provision of LNS or MMN during pregnancy increases maternal plasma retinol concentration at 36 gw. The detailed hypotheses can be found in Appendix 4 (27–30).

Of the 1,391 women who were enrolled in the study, 12 were excluded because they had twin pregnancies. From the remaining 1,379 participants, a subset of 316 women was randomly selected for assessment of vitamin A status. Plasma retinol concentration was measured in 314 women at enrollment and in 313 women at 36 weeks gestation. There were 103, 105, and 106 participants included in the analyses at enrollment, and 103, 105, and 105 participants included in the analyses at 36 gw in the IFA, MMN, and LNS groups, respectively. Plasma samples were missing for two women (0.6%) at enrollment and for three women at 36 gw (0.6%).

There were no clinically meaningful differences between the three intervention groups of participants included in the maternal plasma retinol analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-7).

The overall mean (SD) plasma retinol concentration at enrollment and at 36 weeks of gestation was 1.11 µmol/L (0.29) and 1.02 µmol/L (0.31), respectively. Mean plasma retinol concentrations did not differ by intervention group at enrollment (P=0.365; Table 4.7-1) or at 36 gw (unadjusted model, P=0.381, adjusted model P=0.122; Table 4.7-1).

The overall prevalence of low plasma retinol concentrations (<1.05 µmol/L) at enrollment and at 36 gw was 46.8% (95% CI: 37.1, 56.5) and 56.5% (95% CI: 46.9, 66.1), respectively. The proportion of women with low plasma retinol concentration values at 36 gw tended to be lower in the IFA group (unadjusted model, P=0.065). After adjusting for selected covariates, the proportion of women with low plasma retinol concentrations at 36 gw differed by group (adjusted model, P=0.024; Table 4.7-2). The relative risk of low plasma retinol concentration (<1.05 µmol/L) at 36 gw was greater in the LNS group than in the IFA group (P=0.018; Table 4.7-2).

The following variables were selected a priori based on their expected association with the outcome variables and were examined as potential covariates: maternal plasma retinol at enrollment (for plasma retinol concentration), maternal low plasma retinol at enrollment (for plasma retinol <1.05 µmol/L), maternal BMI at enrollment, maternal inflammatory markers (high plasma CRP, >5 mg/L; and high plasma AGP, >1.0 g/L) at enrollment and at 36 gw, maternal malaria status at enrollment, maternal HIV status, primiparity, maternal educational achievement, site of enrollment, and season of enrollment. Those with a significant association (P<0.10) with the outcome in bivariate analysis were included as covariates in the model for the corresponding outcome.

The variables that were tested for effect modification were predefined in our statistical analysis plan and included maternal plasma retinol concentration at enrollment, maternal BMI at enrollment, maternal educational achievement, maternal high plasma CRP (>5 mg/L) at enrollment, maternal high plasma AGP (>1.0 g/L) at enrollment, maternal malaria status at enrollment, maternal HIV status at enrollment, primiparity, site of enrollment, and season of enrollment. Results were stratified by variables that had a significant interaction with intervention group (P<0.05) in an adjusted model. A significant interaction was found for maternal malaria status at enrollment in the adjusted model (P=0.024; Table 4.7-3).

Stratified results from the adjusted model showed that among women who tested positive for malaria status at enrollment, plasma retinol concentration at 36 weeks gestation was lower among women in the MMN group than in the IFA group (P=0.007; Table 4.7-3).

The study findings do not support a hypothesis that provision of LNS or MMN to pregnant women increases maternal plasma retinol concentration at 36 gw in rural Malawi.

Table 4.7-1. Plasma Retinol Concentration by Intervention Group

Outcome	Time point	Plasma retinol concentration by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[b]	Difference in means (95% CI)	P-value[b]	Difference in means (95% CI)	P-value[b]	Difference in means (95% CI)	P-value[b]
Mean (SD) plasma retinol (µmol/L)	Enrollment[a,d]	1.08 (0.27)	1.14 (0.27)	1.10 (0.32)	0.365	0.02 (−0.12 to 0.07)	0.812	−0.03 (−0.06 to 0.12)	0.698	0.06 (−0.04 to 0.15)	0.333
	36 gw[a,e]	1.05 (0.33)	1.03 (0.28)	0.99 (0.33)	0.381	−0.06 (−0.16 to 0.04)	0.357	−0.04 (−0.14 to 0.06)	0.649	−0.02 (−0.12 to 0.08)	0.876
Plasma retinol (µmol/L)	36 gw, adjusted model[c]				0.122	−0.08 (−0.16 to 0.01)	0.118	−0.02 (−0.11 to 0.07)	0.868	−0.06 (−0.15 to 0.03)	0.307

[a] Unadjusted model, plasma retinol concentrations are mean (SD).
[b] Unadjusted mean plasma retinol concentrations at enrollment and 36 gw were compared using ANOVA; adjusted values using ANCOVA; the Tukey-Kramer test was used for pair-wise comparisons.
[c] Model was adjusted for predefined covariates that were associated with the outcome (P<0.10) in bivariate analysis; covariates included in the model were maternal plasma retinol at enrollment and primiparity.
[d] Number of participants: IFA n=103, MMN n=105, LNS n=106.
[e] Number of participants: IFA n=103, MMN n=105, LNS n=105.

Table 4.7-2. Plasma Retinol <1.05 µmol/L by Intervention Group

Outcome	Time point	Percentage of women with plasma retinol <1.05 µmol/L by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[b]	Risk Ratio (95% CI)	P-value[c]	Risk Ratio (95% CI)	P-value[c]	Risk Ratio (95% CI)	P-value[c]
Plasma retinol <1.05 µmol/L	Enrollment[a]	48/103 (46.6%)	45/105 (42.9%)	54/106 (50.9%)	0.500	1.09 (0.78 to 1.53)	0.530	1.19 (0.89 to 1.59)	0.239	0.92 (0.64 to 1.32)	0.850
	36 gw[a]	53/103 (51.4%)	56/105 (53.3%)	69/105 (65.7%)	0.065	1.28 (0.98 to 1.73)	0.070	1.23 (0.94 to 1.62)	0.164	1.04 (0.77 to 1.45)	0.911
	36 gw, adjusted model[d]				0.024	1.32 (1.02 to 1.70)	0.018	1.17 (0.91 to 1.50)	0.226	1.13 (0.84 to 1.51)	0.478

[a] Unadjusted model.
[b] P-value generated by Fisher's exact test.
[c] Fisher's exact test was used to compare the proportion of women with plasma retinol <1.05 µmol/L at enrollment and 36 gw in unadjusted analyses; logistic regression was used for adjusted analyses.
[d] Model was adjusted for predefined covariates that were associated with the outcome (P<0.10) in bivariate analysis; covariates included in the model were maternal plasma retinol concentration <1.05 µmol/L at enrollment, maternal educational achievement, and primiparity.

Table 4.7-3. Effect Modification: Plasma Retinol Concentration (µmol/L) at 36 gw by Intervention Group, Stratified Analysis

Outcome	Interaction test P-value	Plasma retinol at 36 gw by intervention group Mean (SD)				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[e]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]
Plasma retinol concentration µmol/L											
No malaria at enrollment[a,c]	0.088	1.00 (0.30)	1.03 (0.29)	0.99 (0.37)	0.725	−0.01 (−0.13 to 0.11)	0.970	−0.04 (−0.16 to 0.08)	0.715	0.03 (−0.09 to 0.14)	0.850
Malaria at enrollment[a,d]		1.20 (0.38)	1.04 (0.24)	0.98 (0.22)	0.042	−0.22 (−0.43 to −0.01)	0.038	−0.06 (−0.28 to 0.15)	0.763	−0.16 (−0.37 to −0.06)	0.200
Plasma retinol concentration µmol/L											
No malaria at enrollment, adjusted models[b]	0.024				0.319	−0.05 (−0.16 to 0.05)	0.415	−0.06 (−0.16 to 0.04)	0.367	0.00 (−0.10 to 0.10)	0.997
Malaria at enrollment, adjusted models[b]					0.009	−0.15 (−0.33 to 0.03)	0.121	0.09 (−0.10 to 0.28)	0.498	−0.24 (−0.43 to −0.06)	0.007

[a] Unadjusted model, plasma retinol concentrations are mean (SD).
[b] Models adjusted for covariates maternal plasma retinol concentration at enrollment and primiparity.
[c] Number of participants: IFA n=79, MMN n=81, LNS n=79.
[d] Number of participants: IFA n=24, MMN n=23, LNS n=25.
[e] Unadjusted P-values for the interaction are from the group by malaria interaction in the ANOVA model, adjusted from the ANCOVA model.
[f] The Tukey-Kramer test was used for comparison of the groups within each malaria category, within the context of the full-sample model.

4.8 Maternal Plasma Vitamin B12, Folate, and tHcy Concentrations

In this section, we test hypotheses that provision of LNS or MMN during pregnancy increases maternal plasma concentration of vitamin B12, decreases maternal plasma concentration of tHcy, and does not influence maternal plasma concentration of folate at 36 gw. These hypotheses were based on the nutrient composition of LNS, MMN, and IFA. The daily dose of LNS and MMN used in the study provided 5.2 µg B12 (twice the RDA) and 400 µg folic acid. The IFA used in the study also contained 400 µg folic acid, but no B12. Deficiency of either vitamin B12 or folic acid results in an increase in plasma tHcy; thus, a decrease in this metabolite would indicate an improved status of these vitamins. The detailed hypotheses can be found in Appendix 4 (31–33).

Of the 1,391 women who were enrolled in the study, 12 were excluded because they had twin pregnancies. From the remaining 1,379 participants, a subset of 314 women was randomly selected for assessment of plasma vitamin B12, folate, and tHcy concentrations at enrollment and 36 weeks gestation. At both enrollment and 36 gw, there were 104, 105, and 105 women in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-8).

There were no differences among intervention groups in enrollment values of plasma B12, folate, or tHcy (Table 4.8-1). The prevalence of both vitamin B12 and folate deficiency was low. For B12, using the traditional cutoff for deficiency of <150 pmol/L, one woman was deficient, while 9.9% fell below the 225 pmol/L concentration that defines marginal status. Folate deficiency (<10 µmol/L) was present in only 4.4% and elevated tHcy (>10 mmol/L) in only 3.2%.

tHcy concentrations were significantly correlated with plasma folate at enrollment ($r=-0.145$, $P=0.010$), but not with plasma B12 ($r=-0.074$, $P=0.191$). This suggests that at enrollment folate status was a stronger determinant of tHcy than was vitamin B12 status.

At 36 weeks of pregnancy, there were no significant differences in folate or tHcy among the intervention groups (Table 4.8-1). The unadjusted means for vitamin B12 did not differ significantly among groups, but when adjusted for enrollment vitamin B12 (and other covariates), the mean for the LNS group and the MMN group was higher than for the IFA group.

The same was evident when examining the change in vitamin B12 concentration between enrollment and 36 weeks gestation, which was significantly less in both the LNS group and the MMN group compared to the IFA group (−9.3 pmol/L vs. −87.7 pmol/L and −61.4 pmol/L vs. −87.7 pmol/L, $P=0.001$, respectively) in an unadjusted model. These findings persisted when adjusting for enrollment vitamin B12 (and other covariates). The prevalence of low B12, low folate, and elevated tHcy was not different across intervention groups at 36 gw (

Table 4.8-2).

As at enrollment, tHcy concentrations at 36 gw were significantly correlated with plasma folate at 36 gw (r=−0.228, P<0.001), but not with plasma B12 (r=−0.058, P<0.311), suggesting that folate status was the main determinant of tHcy.

Although the fortification level of vitamin B12 was the same in the MMN and LNS supplements, MMN had no significant effect on plasma concentrations of the vitamin at 36 weeks gestation, whereas LNS prevented most of the decline in plasma B12 during gestation that was observed in those who received IFA. These results thus confirm our hypothesis for LNS, but not for MMN. It is likely that the daily dose of LNS is sometimes divided into small amounts several times a day, while the MMN is taken once a day; since the efficiency of absorption of vitamin B12 is substantially lower from a 5 µg dose than smaller doses, this could explain the apparently greater efficacy of LNS for improving B12 status. Alternatively, it is possible that the slightly greater adherence in the LNS group resulted in women receiving a higher percentage of the intended dose of vitamin B12. It is noteworthy that the prevalence of vitamin B12 deficiency at enrollment was virtually zero and marginal plasma concentrations were present in only about 9%–10% of women. We conjecture that the key source of dietary B12 for these women is small fish, but confirmation of this awaits analysis of the dietary intake data. We did not carry out any effect modification testing.

As hypothesized, there was no difference among intervention groups in plasma folate at 36 gw (all three of the supplements contained the same amount of folic acid). The prevalence of folate deficiency was equal in the IFA and LNS groups (both 8.7%) and higher in the MMN group (16.2%), but the difference was not statistically significant (P=0.213).

Mean plasma tHcy was not significantly different across the three groups at 36 weeks gestation. The prevalence of elevated tHcy was significantly lower in the LNS group compared to the IFA group (relative risk 0.11 (0.01, 0.84), P=0.034) (

Table 4.8-2).

Enrollment concentrations of B12, folate, and tHcy correlated strongly with values at 36 gw: for B12, r=0.577, P<0.001; for folate, r=0.266, P<0.001; and for tHcy, r=0.863, P<0.001. This suggests that enrollment values are strong determinants of concentrations later in pregnancy.

Table 4.8-1. Plasma B12, Folate, and tHcy by Intervention Group

Outcome	Time point	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[h]	Difference in means (95% CI)	P-value[i]	Difference in means (95% CI)	P-value[i]	Difference in means (95% CI)	P-value[i]
Plasma B12 (pmol/L)[j]	Mean (SD) enrollment[a]	439.0 (191.9)	413.0 (206.6)	393.5 (159.9)	0.212	−45.5 (−106.5 to 15.4)	0.185	−19.6 (−80.4 to 41.3)	0.729	−26.0 (−87.0 to 35.0)	0.575
	Mean (SD) 36 gw[a]	353.5 (143.8)	351.6 (141.8)	385.5 (173.7)	0.205	32.0 (−18.4 to 82.4)	0.294	33.9 (−16.2 to 84)	0.251	−1.9 (−52.1 to 48.4)	0.996
	36 gw, adjusted model[b]				0.004	54.8 (21.7 to 87.9)	0.001	37 (4.2 to 69.8)	0.027	17.8 (−15.1 to 50.7)	0.288
	Mean (SD) change[a]	−87.7 (167.9)	−61.4 (140.1)	−9.3 (161.8)	0.001	78.4 (27 to 129.8)	0.001	52.1 (0.9 to 103.2)	0.045	26.4 (−24.9 to 77.6)	0.448
	Change, adjusted model[c]				0.007	53.1 (13.4 to 92.8)	0.005	34.2 (−5.2 to 73.6)	0.103	18.9 (−20.6 to 58.4)	0.498
Plasma folate (µmol/L)[k]	Mean (SD) enrollment[a]	19.9 (7.6)	21.7 (7.9)	20.8 (8.2)	0.241	0.9 (−1.7 to 3.5)	0.691	−0.9 (−3.5 to 1.6)	0.660	1.8 (−0.7 to 4.4)	0.210
	Mean (SD) 36 gw[a]	18.5 (7.7)	18.3 (7.8)	19.1 (7.2)	0.733	0.6 (−1.9 to 3)	0.857	0.8 (−1.7 to 3.3)	0.722	−0.2 (−2.7 to 2.2)	0.969
	36 gw, adjusted model[d]				0.692	0.4 (−2 to 2.8)	0.923	0.9 (−1.5 to 3.3)	0.667	−0.5 (−2.9 to 1.9)	0.883
	Mean (SD) change[a]	−1.3 (9.5)	−3.4 (9.7)	−1.6 (8.9)	0.226	−0.3 (−3.4 to 2.8)	0.975	1.8 (−1.3 to 4.8)	0.355	−2.1 (−5.1 to 1.0)	0.251
	Change, adjusted model[e]				0.767	0.5 (−1.9 to 2.9)	0.880	0.7 (−1.7 to 3.1)	0.756	−0.2 (−2.7 to 2.2)	0.972
Plasma tHcy (mmol/L)[l]	Mean (SD) enrollment[a]	7.9 (2.3)	1.9 (1.9)	1.7 (2.5)	0.216	−1.0 (−2.6 to 0.5)	0.266	0.0 (−1.6 to 1.5)	0.997	−1.0 (−2.5 to 0.6)	0.299
	Mean (SD) 36 gw[a]	5.3 (2.5)	2.0 (2.0)	1.7 (2.6)	0.181	−0.8 (−1.9 to 0.3)	0.190	−0.2 (−1.2 to 0.9)	0.943	−0.7 (−1.8 to 0.4)	0.331
	36 gw, adjusted model[f]				0.731	−0.2 (−0.8 to 0.4)	0.708	−0.1 (−0.7 to 0.5)	0.912	−0.1 (−0.7 to 0.5)	0.921
	Mean (SD) change[a]	0.0 (3.6)	0.3 (1.8)	0.2 (1.6)	0.661	0.2 (−0.6 to 1)	0.824	−0.1 (−0.9 to 0.7)	0.950	0.3 (−0.5 to 1.1)	0.643
	Change, adjusted model[g]				0.855	−0.1 (−0.7 to 0.4)	0.842	−0.1 (−0.6 to 0.5)	0.960	−0.1 (−0.6 to 0.5)	0.955

[a] Unadjusted model.

Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment:

[b] Adjusted for maternal B12 at enrollment, maternal folate at enrollment, and site of enrollment.

[c] Adjusted for maternal B12 at enrollment, maternal folate at enrollment, maternal malaria status at enrollment, and site of enrollment.

[d] Adjusted for maternal folate at enrollment, maternal age at enrollment, primiparity, and season of enrollment.

[e] Adjusted for maternal folate at enrollment, maternal tHcy at enrollment, maternal educational achievement, maternal HIV status, and season of enrollment.

[f] Adjusted for maternal tHcy at enrollment, maternal folate at enrollment, and season of enrollment.

[g] Adjusted for maternal tHcy at enrollment, maternal folate at enrollment, maternal B12 at enrollment, maternal educational achievement, and maternal high AGP at enrollment.

[h] Unadjusted and adjusted p--values from Wald Test after linear regression.

[i] Unadjusted and adjusted pair-wise comparisons and P-values from linear regression. The Tukey-Kramer test was used for pair-wise comparisons.

[j] Plasma B12: at enrollment IFA n=104, MMN n=105, LNS n=105; at 36 gw IFA n=103, MMN n=105, IFA=104; change IFA n=103, MMN n=105, IFA=104.

[k] Plasma folate: at enrollment IFA n=104, MMN n=105, LNS n=104; at 36 gw IFA n=103, MMN n=105, IFA=104; change IFA n=103, MMN n=105, IFA=103.

[l] Plasma tHcy: at enrollment IFA n=103, MMN n=105, LNS n=105; at 36 gw IFA n=103, MMN n=105, IFA=105; change IFA n=103, MMN n=105, IFA=105.

Table 4.8-2. Low Plasma B12 or Folate or High tHcy by Intervention Group, at 36 gw

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[e]	Relative risk (95% CI)	P-value[f]	Relative risk (95% CI)	P-value[f]	Relative risk (95% CI)	P-value[f]
Plasma B12 <225 pmol/L[a]	11/103 (10.7%)	15/105 (14.3%)	10/104 (9.6%)	0.734	0.90 (0.4 to 2.03)	0.800	0.67 (0.32 to 1.43)	0.303	1.34 (0.65 to 2.77)	0.434
Plasma B12 <225 pmol/L, adjusted model[b]				0.795	0.86 (0.54 to 1.36)	0.513	0.92 (0.55 to 1.55)	0.767	0.93 (0.62 to 1.4)	0.721
Plasma folate <10 µmol/L[a]	9/103 (8.7%)	17/105 (16.2%)	9/104 (8.7%)	0.213	0.99 (0.41 to 2.39)	0.983	0.53 (0.25 to 1.14)	0.107	1.85 (0.87 to 3.97)	0.112
Plasma folate <10 µmol/L, adjusted model[c]				0.217	1.11 (0.46 to 2.65)	0.817	0.61 (0.29 to 1.29)	0.198	1.82 (0.85 to 3.88)	0.123
Plasma tHcy>10 mmol/L[a]	9/103 (8.7%)	3/105 (2.9%)	1/105 (1.0%)	0.017	0.11 (0.01 to 0.84)	0.034	3.06 (0.85 to 10.98)	0.086	0.33 (0.09 to 1.17)	0.086
Plasma tHcy>10 mmol/L, adjusted model[d]				0.099	0.13 (0.02 to 1.05)	0.056	0.32 (0.03 to 3.04)	0.323	0.42 (0.11 to 1.56)	0.193

[a] Unadjusted model.
Log-binomial regression models adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment:
[b] Adjusted for maternal B12 at enrollment, maternal folate at enrollment, maternal age at enrollment, and site of enrollment.
[c] Adjusted for maternal folate at enrollment, primiparity, and season of enrollment.
[d] Adjusted for maternal tHcy at enrollment and maternal folate at enrollment.
[e] Unadjusted P-value generated by Fisher's exact test, adjusted P-value from Wald Test after log-binomial regression models.
[f] Unadjusted and adjusted pair-wise comparisons were obtained from log-binomial regression models; P-values were obtained from Wald Test after log-binomial regression models.

4.9 Maternal Plasma Cholesterol and Triglyceride Concentrations and Plasma Fatty Acid Status

In this section, we test a hypothesis that provision of LNS during pregnancy increases maternal plasma concentration of cholesterol and/or triglycerides, increases maternal plasma arachidonic acid (omega-6 AA), α-linolenic acid (omega-3 ALA), and docosahexaenoic acid (omega-3 DHA) (measured as percent of total fatty acids) and lowers proportion of women with low total cholesterol in plasma at 36 weeks gestation. The detailed hypothesis can be found in Appendix 4 (34).

Of the 1,391 women who were enrolled in the study, 12 women had twin pregnancies and were excluded from these analyses. Of the remaining women, 1,371 (99.4%) women had a measurement at enrollment for plasma cholesterol and triglyceride concentrations. After excluding those without plasma cholesterol and triglyceride measurements at 36 weeks gestation, 1,061 (76.9%) participants were included in the analyses. Loss to follow-up was similar across the intervention groups (P=0.824). There were 350, 360, and 351 included participants in the IFA, MMN, and LNS groups, respectively. After excluding twin pregnancies from the 1,391 women enrolled in the study, a subset of 315 women was randomly selected for assessment of fatty acid status. There were 103, 106, and 106 participants included in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-9).

At enrollment there was no significant difference in cholesterol, triglycerides, or any of the measurements of fatty acid status across groups, except for mean AA (P=0.024) (Table 4.9-1). In adjusted analyses, models were adjusted for covariates that had a significant association (P<0.10) with any of the outcomes of focus in this section in bivariate analysis. In addition, the adjusted models controlled for the corresponding value of the outcome variable at enrollment. After adjusting the analysis for gestational age at enrollment, primiparity, maternal BMI at enrollment, maternal age at enrollment, site of enrollment, season of enrollment, maternal malaria status at enrollment, maternal HIV status, proxy for SES, and maternal AGP at enrollment, this finding was no longer significant (results not shown). All fatty acids, except AA, were log-transformed for analysis, but non-log-transformed units are presented in the tables. At 36 gw, there was no significant difference in cholesterol, triglycerides, or any of the measurements of fatty acid status across groups in either unadjusted or adjusted analysis (Table 4.9-1).

We also looked at the effect of the intervention on the proportion of women with a high fatty acid measurement (≥50th percentile of the IFA group) or a low total cholesterol (<10th percentile of the IFA group). High fatty acid status is possibly a positive health indicator, while extremely low cholesterol is potentially an adverse health indicator. At 36 weeks gestation, the cutoffs used for low cholesterol and high AA, ALA, and DHA were 80.19 mg/dL, 7.99% total fatty acids, 0.45% total fatty acids, and 4.39% total fatty acids, respectively. Cutoffs were used based on the IFA group, as this was the reference group for the study and there are no clinically established cutoffs for high fatty acids or low total cholesterol. There was no significant difference in proportion of women with low cholesterol or high fatty acid measurement across intervention groups in unadjusted or adjusted models (

Table 4.9-2).

Interactions were tested between intervention group and maternal age at enrollment, primiparity, and maternal BMI at enrollment on cholesterol, triglycerides, and fatty acids, and none were found to be significant. These interactions were selected based on previous literature and were stated in a predefined analysis plan.

The study findings do not support a hypothesis that provision of LNS to pregnant women would increase maternal plasma concentration of cholesterol, triglycerides, AA, ALA, and DHA at 36 gw in rural Malawi.

Table 4.9-1. Plasma Fatty Acids, Cholesterol, and Triglycerides at Enrollment and 36 Weeks Gestation, by Intervention Group

Outcome	Time point	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA (n=350)[c]	MMN (n=360)[c]	LNS (n=351)[c]	P-value[d]	Difference in means (95% CI)	P-value[d]	Difference in means (95% CI)	P-value[d]	Difference in means (95% CI)	P-value[d]
Cholesterol, mg/dL	Mean (SD) enrollment[a]	122.1 (32.4)	121.3 (30.3)	120.9 (30.7)	0.837	−1.21 (−6.07 to 3.64)	0.827	−0.43 (−5.26 to 4.41)	0.977	−0.79 (−5.62 to 4.04)	0.922
	Mean (SD) 36 gw[a]	149.6 (36.1)	151.0 (39.0)	150.9 (37.2)	0.864	1.26 (−5.36 to 7.88,)	0.896	−0.11 (−6.68 to 6.46)	0.999	1.37 (−5.20 to 7.94)	0.876
	36 gw, adjusted model[b]				0.198	4.17 (−1.27 to 9.61)	0.170	2.32 (−3.08 to 7.71,)	0.572	1.85 (−3.54 to 7.25)	0.699
Triglycerides, mg/dL	Mean (SD) enrollment[a]	99.8 (43.1)	94.1 (34.6)	94.5 (35.8)	0.084	−5.32 (−11.24 to 0.60)	0.088	0.35 (−5.55 to 6.25)	0.989	−5.67 (−11.56 to 0.22)	0.062
	Mean (SD) 36 gw[a]	150.8 (62.6)	147.4 (61.4)	150.0 (63.6)	0.620	−0.71 (−11.8 to 10.4)	0.992	2.63 (−8.35 to 13.6)	0.716	−3.34 (−14.3 to 7.64)	0.641
	36 gw, adjusted model[b]				0.453	4.37 (−5.93 to 14.7)	0.516	3.35 (−6.85 to 13.5)	0.523	1.02 (−9.17 to 11.2)	1.000
AA, % total fatty acids	Mean (SD) enrollment[a]	8.31 (1.41)	7.79 (1.21)	8.06 (1.45)	0.024	−0.22 (−0.66 to 0.22,)	0.461	0.30 (−0.14 to 0.74)	0.246	−0.52 (−0.96 to −0.08)	0.016
	Mean (SD) 36 gw[a]	6.76 (1.18)	6.55 (0.98)	6.42 (1.06)	0.073	−0.34 (−0.69 to 0.01,)	0.060	−0.13 (−0.48 to 0.22)	0.655	−0.21 (−0.56 to 0.14)	0.338
	36 gw, adjusted model[b]				0.119	−0.23 (−0.54 to 0.08)	0.178	−0.24 (−0.55 to 0.08)	0.175	0.00 (−0.31 to 0.32)	0.999
ALA, % total fatty acids	Mean (SD) enrollment[a]	0.50 (0.18)	0.53 (0.20)	0.48 (0.18)	0.156	−0.02 (−0.08 to 0.04)	0.713	−0.05 (−0.11 to 0.01)	0.137	0.03 (−0.03 to 0.09)	0.507
	Mean (SD) 36 gw[a]	0.48 (0.16)	0.47 (0.18)	0.51 (0.18)	0.264	0.03 (−0.02 to 0.09)	0.448	0.03 (−0.02 to 0.09)	0.267	−0.01 (−0.06 to 0.05)	0.940
	36 gw, adjusted model[b]				0.058	0.03 (−0.02 to 0.08)	0.295	0.04 (−0.01 to 0.10)	0.049	0.02 (−0.07 to 0.04)	0.643
DHA, % total fatty acids	Mean (SD) enrollment[a]	4.49 (1.04)	4.35 (1.01)	4.41 (0.97)	0.595	−0.10 (−0.42 to 0.23)	0.772	0.06 (−0.27 to 0.38)	0.916	−0.15 (−0.48 to 0.18)	0.529
	Mean (SD) 36 gw[a]	3.63 (0.82)	3.61 (0.92)	3.61 (0.79)	0.898	−0.02 (−0.30 to 0.25)	0.985	0.00 (−0.27 to 0.28)	0.953	−0.02 (−0.30 to 0.25)	0.891
	36 gw, adjusted model[b]				0.865	0.05 (−0.17 to 0.27)	0.853	−0.01 (−0.24 to 0.21)	0.974	0.06 (−0.17 to 0.29)	0.949
Omega−6: omega-3[a]	Mean (SD) enrollment[a]	6.20 (1.67)	6.46 (1.89)	6.25 (1.57)	0.481	0.07 (−0.48 to 0.63)	0.948	−0.20 (−0.75 to 0.35)	0.664	0.28 (−0.28 to 0.83)	0.476
	Mean (SD) 36 gw[a]	7.52 (1.79)	7.82 (2.46)	7.46 (1.82)	0.630	−0.06 (−0.73 to 0.60)	0.960	−0.37 (−1.03 to 0.30)	0.618	0.30 (−0.37 to 0.97)	0.960
	36 gw, adjusted model[b]				0.704	0.14 (−0.66 to 0.38)	0.765	0.28 (−0.81 to 0.25)	0.735	0.14 (−0.39 to 0.66)	0.998

[a] Unadjusted model.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with any of the outcomes of focus in this section in bivariate analysis. All models were adjusted for gestational age at enrollment, primiparity, maternal BMI at enrollment, maternal age at enrollment, site of enrollment, season of enrollment, maternal malaria status at enrollment, maternal HIV status, proxy for SES, and maternal AGP at enrollment. In addition, the adjusted models controlled for the corresponding value of the outcome variable at enrollment.

[c] Fatty acid data available only for subgroup of participants: IFA n=103, MMN n=106, LNS n=106.

[d] Enrollment and unadjusted P-values were calculated using ANOVA; adjusted P-values were calculated using ANCOVA. The Tukey-Kramer test was used for pair-wise comparisons.

Table 4.9-2. Prevalence of Low Cholesterol and High Plasma Fatty Acids at 36 wk Gestation, by Intervention Group

	Number of outcomes/women with outcome data				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
Outcome	IFA	MMN	LNS	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Low cholesterol[a,b]	35/350 (10.0%)	39/360 (10.8%)	39/351 (11.1%)	0.865	1.01 (0.96 to 1.07)	0.632	1.00 (0.95 to 1.06)	0.906	1.01 (0.96 to 1.06)	0.716
Low cholesterol, adjusted model[a,c]				0.939	1.01 (0.96 to 1.06)	0.719	1.00 (0.95 to 1.05)	0.848	1.01 (0.96 to 1.06)	0.671
High AA[a,b]	52/103 (50.5%)	47/106 (44.3%)	40/106 (37.7%)	0.180	0.80 (0.62 to 1.02)	0.067	0.89 (0.71 to 1.12)	0.330	0.89 (0.69 to 1.15)	0.375
High AA, adjusted model[a,c]				0.193	0.83 (0.66 to 1.04)	0.104	0.95 (0.76 to 1.18)	0.642	0.91 (0.71 to 1.17)	0.457
High DHA[a,b]	51/103 (49.5%)	54/106 (50.9%)	52/106 (49.1%)	0.960	0.99 (0.76 to 1.29)	0.947	0.96 (0.74 to 1.26)	0.784	1.03 (0.78 to 1.35)	0.836
High DHA, adjusted model[a,c]				0.919	1.07 (0.83 to 1.38)	0.602	0.97 (0.74 to 1.26)	0.824	1.12 (0.87 to 1.44)	0.393
High ALA[a,b]	51/103 (49.5%)	47/106 (44.3%)	61/106 (57.6%)	0.155	1.19 (0.89 to 1.59)	0.246	1.31 (0.99 to 1.73)	0.057	0.91 (0.70 to 1.17)	0.455
High ALA, adjusted model[a,c]				0.073	1.22 (0.92 to 1.62)	0.158	1.36 (1.04 to 1.77)	0.026	0.88 (0.68 to 1.13)	0.306
High omega-6:omega-3 ratio[a,b]	52/103 (50.5%)	51/106 (48.1%)	51/106 (48.1%)	0.925	0.95 (0.73 to 1.25)	0.732	1.00 (0.77 to 1.30)	1.000	0.95 (0.73 to 1.25)	0.732
High omega-6:omega-3 ratio, adjusted model[a,c]				0.938	0.93 (0.71 to 1.21)	0.578	0.97 (0.76 to 1.25)	0.822	0.96 (0.74 to 1.25)	0.767

[a] Low cholesterol defined as <10th percentile in the IFA group, high fatty acids defined as ≥50th percentile of the IFA group.
[b] Unadjusted model.
[c] Models were adjusted for covariates that had a significant association (P<0.10) with any of the outcomes of focus in this section in bivariate analysis. All models were adjusted for gestational age at enrollment, primiparity, maternal BMI at enrollment, maternal age at enrollment, site of enrollment, season of enrollment, maternal malaria status at enrollment, maternal HIV status, proxy for SES, and AGP at enrollment. In addition, the adjusted models controlled for the corresponding value for the outcome variable at enrollment.
[d] Unadjusted P-values were calculated using Fisher's exact test; adjusted P-values were calculated using log-Poisson regression models.

4.10 Maternal Salivary Cortisol Concentration

In this section, we test hypotheses that provision of LNS during pregnancy decreases maternal stress at 28 gw or 36 gw, as measured by a perceived stress scale or salivary cortisol concentration. The detailed hypotheses can be found in Appendix 4 (35 and 36).

Of the 1,391 women enrolled in the 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. Loss to follow-up was similar across the intervention groups (P=0.662). There were 411, 414, and 412 participants in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-10).

Women's perceived stress scores at enrollment were a mean (SD) of 14.4 (5.6) and this did not differ by group. Mean cortisol concentrations at enrollment also did not differ between groups (P=0.080), with mean (SD) cortisol concentrations of 6.0 µmol/L (4.4), 5.4 µmol/L (3.1), and 5.6 µmol/L (3.4) in the IFA, MMN, and LNS groups, respectively.

Mean (SD) maternal cortisol values for the whole study group increased during pregnancy, from 5.7 µmol/L (3.7) at enrollment to 5.9 µmol/L (3.4) at 28 gw and 8.1 µmol/L (3.2) at 36 gw. Perceived stress scores were normally distributed and did not change substantially during pregnancy. There was not a significant correlation between maternal perceived stress and salivary cortisol at any point in pregnancy. Cortisol response to stressors is generally blunted during pregnancy (de Weerth and Buitelaar 2005), which is in line with our finding of no correlation between the PSS and cortisol in this cohort.

There were no differences in salivary cortisol concentration between any of the groups at 28 weeks gestation or at 36 weeks gestation in either unadjusted or adjusted models accounting for maternal salivary cortisol at enrollment; maternal age at enrollment; maternal educational achievement; proxy for SES; site of enrollment; season of enrollment; maternal Hb at enrollment, maternal ZPP at enrollment, or maternal sTfR at enrollment; time between waking and saliva collection; and time between last food or drink and saliva collection (Table 4.10-1). Variables selected as potential covariates were based on previous literature and stated in a predefined analysis plan. Variables were included in the model for a given outcome if they were significantly (P<0.10) associated with the outcome in bivariate analysis.

We also examined the risk of low (<25th percentile of IFA group at time point being analyzed) or high (>75th percentile of IFA group at time point being analyzed) cortisol and found there to be no significant between-group differences in unadjusted or adjusted analyses at 28 gw or at 36 gw (Table 4.10-2). Cutoffs were based on the IFA group because this was the reference group for the study and there is not an established definition of low or high cortisol during pregnancy.

There were also no differences in maternal perceived stress between groups in either unadjusted analyses or models adjusted for maternal perceived stress at enrollment, maternal age at enrollment, maternal educational achievement, site of enrollment, season of enrollment, and proxy for SES at either 28 or 36 gw.

Interactions were tested between intervention group and maternal age at enrollment, parity, maternal BMI at enrollment, and infant sex on salivary cortisol and perceived stress and none were found to be

significant at 28 gw and 36 gw. Variables were selected for interaction testing based on previous literature and were stated in a predefined analysis plan.

The study findings do not support a hypothesis that provision of LNS to pregnant women decreases maternal stress at 28 gw or 36 gw in rural Malawi.

Table 4.10-1. Cortisol and Perceived Stress at 28 Weeks Gestation and 36 Weeks Gestation, by Intervention Group

Outcome	Time point	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS and MMN group		Comparison between MMN and IFA group	
		IFA	MMN	LNS	P-value[c]	Difference in means (95% CI)	P-value[c]	Difference in means (95% CI)	P-value[c]	Difference in means (95% CI)	P-value[c]
Cortisol, µmol/L	Mean (SD) enrollment[a,d]	6.01 (4.36)	5.39 (3.08)	5.58 (3.36)	0.080	−0.43 (−1.09 to 0.14)	0.165	0.19 (−0.48 to 0.75)	0.865	−0.62 (−1.23 to −0.01)	0.053
	Mean (SD) 28 gw[a,e]	5.68 (2.72)	5.72 (2.82)	6.23 (4.28)	0.275	0.55 (−0.10 to 1.20)	0.414	0.52 (−0.12 to 1.15)	0.295	0.04 (−0.60 to 0.68)	0.979
	28 gw, adjusted model[b]				0.260	0.40 (−0.34 to 1.13)	0.608	0.47 (−0.25 to 1.19)	0.233	−0.08 (−0.81 to 0.66)	0.797
	Mean (SD) 36 gw[a,f]	8.35 (3.47)	7.84 (2.67)	8.16 (3.33)	0.088	−0.19 (−0.76 to 0.38)	0.582	0.32 (−0.25 to 0.88)	0.455	−0.51 (−1.07 to 0.05)	0.071
	36 gw, adjusted model[b]				0.218	−0.21 (−0.84 to 0.41)	0.584	0.25 (−0.37 to 0.86)	0.756	−0.46 (−1.07 to 0.15)	0.191
Perceived stress score	Mean (SD) enrollment[a,g]	14.6 (5.7)	14.3 (5.5)	14.2 (5.6)	0.517	−0.37 (−1.27 to 0.52)	0.592	−0.04 (−0.93 to 0.85)	0.993	−0.33 (−1.21 to 0.56)	0.657
	Mean (SD) 28 gw[a,h]	14.9 (5.6)	15.1 (6.0)	14.1 (5.8)	0.073	−0.75 (−1.84 to 0.34)	0.236	−1.03 (−2.11 to 0.06)	0.069	0.27 (−0.81 to 1.36)	0.825
	28 gw, adjusted model[b]				0.139	−0.81 (−1.90 to 0.28)	0.191	0.79 (−1.87 to 0.29)	0.196	0.01 (−1.08 to 1.05)	1.000
	Mean (SD) 36 gw[a,i]	13.7 (5.1)	13.9 (5.4)	13.7 (4.9)	0.835	0.01 (−0.91 to 0.93)	1.000	−0.20 (−1.12 to 0.72)	0.869	0.21 (−0.71 to 1.12)	0.854
	36 gw, adjusted model[b]				0.941	−0.00 (−0.90 to 0.90)	1.000	−0.11 (−1.01 to 0.78)	0.951	0.11 (−0.77 to 1.00)	0.951

[a] Unadjusted model.
[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment. Models for cortisol were adjusted for cortisol at enrollment, maternal age at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, maternal Hb at enrollment, maternal ZPP at enrollment, maternal sTfR at enrollment, time between waking and saliva collection, and time between last food or drink (besides water) and saliva collection. Models for PSS were adjusted for perceived stress score at enrollment, maternal age at enrollment, maternal educational achievement, proxy for SES, site of enrollment, and season of enrollment.
[c] Unadjusted P-values were obtained by ANOVA, adjusted P-values were calculated using ANCOVA. The Tukey-Kramer test was used for unadjusted and adjusted pair-wise comparisons.
[d] Cortisol enrollment: IFA n=411, MMN n=414, LNS n=412.
[e] Cortisol 28 gw: IFA n=289, MMN n=314, LNS n=296.
[f] Cortisol 36 gw: IFA n=345, MMN n=357, LNS n=341.
[g] Perceived stress score at enrollment: IFA n=411, MMN n=414, LNS n=412.
[h] Perceived stress score 28 gw: IFA n=317, MMN n=321, LNS n=314.
[i] Perceived stress score 36 gw: IFA n=349, MMN n=346, LNS n=341.

Table 4.10-2. Low and High Cortisol at 28 Weeks and 36 Weeks Gestation, by Intervention Group

Outcome	Number of outcomes/women with outcome data				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA (n=411)	MMN (n=414)	LNS (n=412)	P-value[c]	Odds ratio[d] (95% CI)	P-value	Odds ratio[d] (95% CI)	P-value	Odds ratio[d] (95% CI)	P-value
Low cortisol at 28 gw[a]	72/289 (24.9%)	87/314 (27.7%)	75/296 (25.3%)	0.698	1.01 (0.92 to 1.10)	0.901	0.97 (0.88 to 1.07)	0.508	1.04 (0.94 to 1.14)	0.436
Low cortisol at 28 gw, adjusted model[b]				0.222	1.01 (0.91 to 1.12)	0.843	0.93 (0.84 to 1.04)	0.202	1.08 (0.97 to 1.20)	0.176
High cortisol at 28 gw[a]	72/289 (24.9%)	83/314 (26.4%)	88/296 (29.7%)	0.405	1.07 (0.97 to 1.18)	0.192	1.05 (0.95 to 1.15)	0.366	1.02 (0.93 to 1.12)	0.669
High cortisol at 28 gw, adjusted model[b]				0.475	1.07 (0.95 to 1.20)	0.226	1.05 (0940 to 1.17)	0.357	1.01 (0.91 to 1.13)	0.789
Low cortisol at 36 gw[a]	86/345 (24.9%)	110/357 (30.8%)	95/341 (27.9%)	0.221	1.04 (0.95 to 1.14)	0.384	0.96 (0.87 to 1.06)	0.392	1.09 (0.99 to 1.19)	0.083
Low cortisol at 36 gw, adjusted model[b]				0.262	1.03 (0.93 to 1.13)	0.593	.97 (0.88 to 1.08)	0.573	1.07 (0.97 to 1.18)	0.198
High cortisol at 36 gw[a]	86 (24.9%)	74 (20.7%)	91 (26.7%)	0.166	1.02 (0.94 to 1.12)	0.599	1.08 (0.99 to 1.18)	0.066	0.95 (0.87 to 1.03)	0.186
High cortisol at 36 gw, adjusted model[b]				0.056	1.01 (0.92 to 1.12)	0.767	1.09 (0.99 to 1.19)	0.064	0.93 (0.85 to 1.01)	0.084

[a] Unadjusted model. Low cortisol was defined as <25th percentile of the IFA group and high cortisol was defined as >75th percentile of the IFA group. Low and high cortisol were defined at 28 weeks and 36 weeks gestation.

[b] Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment. All models were adjusted for maternal cortisol at enrollment, maternal age at enrollment, maternal educational achievement, proxy for SES, site of enrollment, season of enrollment, maternal Hb at enrollment, maternal ZPP at enrollment, maternal sTfR at enrollment, time between waking and saliva collection, and time between last food or drink (besides water) and saliva collection.

[c] P-value obtained by Fisher's exact test; adjusted P-values were calculated using log-Poisson regression models.

[d] Pair-wise comparisons for both unadjusted and adjusted analyses are based on binomial logistic regression.

4.11 Histological Signs of Inflammation and Malaria in the Placenta and Fetal Membranes

We did not have any predefined hypotheses concerning the effect of LNS on the placental histological findings attached to this substudy at inception. However, we were still interested in any potential impact on inflammation and postulated that LNS might decrease the prevalence of chorioamnionitis, intervillositis, and histological evidence of malaria in the placenta tissue and fetal membranes. 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 a higher incidence of chorioamnionitis in preterm deliveries. 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 during pregnancy.

Of the 1,391 participants enrolled in the study, 12 women with twin pregnancies were excluded from this analysis. Of the remaining women, a sample of placenta was collected at delivery from 1,030 (74.7%) participants, and a sample from the chorionic and amniotic membranes was collected from 1,095 (79.4%) participants. After being sent for histological examination, 990 (71.8%) participants had a viable section of chorionic plate or amniotic membrane to allow for the examination of chorioamnionitis. There were 326, 326, and 338 participants in the IFA, MMN, and LNS groups, respectively. Loss to follow-up was similar in all of the intervention groups (P=0.233). A section of placental tissue taken with intervillous space identifiable to allow for examination of intervillositis and malarial infection was taken from 1,008 (73.1%) participants. There were 330, 333, and 345 participants in the IFA, MMN, and LNS groups, respectively. Loss to follow-up was similar in all of the supplement groups (P=0.269).

There were no clinically meaningful differences between the three intervention groups of participants with a placental tissue slide included in the analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-11).

Prevalence of intervillositis across all participants included in the analysis was 17.5% (n=176). Chorioamnionitis was recorded in 26.1% (n=258) of participants, whereas 12.1% (n=120) of participants had severe chorioamnionitis. Active malarial infection was observed in 4.3% (n=43) of participants' placenta at delivery, and 37.7% (n=380) of participants showed evidence of a past malarial infection in their placenta.

Table 4.11-1 and Table 4.11-2 show the analysis of dichotomous histological signs of malarial infection and inflammation variables by intervention group. There were no significant differences between intervention groups for the dichotomous variables in either the unadjusted values or the adjusted models.

Multiple variables were tested for interactions with the intervention, with respect to inflammatory and placental malaria outcomes. These variables were preselected in the analysis plan due to the logical possibility of forming an association with the outcome. The following variables tested positive for effect modification with at least one outcome: primiparity, gestational age at enrollment, sex of the child, season of enrollment, maternal anemia at enrollment, and site of enrollment (details not shown). However, when

stratified by the dichotomous enrollment variable, there were no significant differences between the groups.

The study findings suggest that provision of LNS to pregnant women does not have an impact on the prevalence of histological evidence of malaria in the placenta or on the prevalence of chorioamnionitis or intervillositis.

Table 4.11-1. Prevalence of Inflammation in the Placenta and Fetal Membranes by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Intervillositis[a]	54/330 (16.4%)	64/333 (19.2%)	58/345 (16.8%)	0.580	1.03 (0.72 to 1.44)	0.876	0.87 (0.63 to 1.21	0.415	1.17 (0.84 to 1.63)	0.337
Intervillositis, adjusted model[b]				0.264	1.02 (0.72 to 1.46)	0.909	0.79 (0.56 to 1.1)	0.173	1.26 (0.90 to 1.77)	0.173
Chorioamnionitis[a]	89/326 (27.3%)	85/326 (26.1%)	84/338 (24.9%)	0.180	0.91 (0.70 to 1.17)	0.473	0.955 (0.73 to 1.24)	0.718	0.96 (074 to 1.23)	0.723
Chorioamnionitis, adjusted model[b]				0.486	0.83 (0.64 to 1.07)	0.245	0.91 (0.69 to 0.85)	0.509	0.91 (0.64 to 1.17)	0.450
Severe chorioamnionitis[a]	45/326 (13.8%)	38/326 (11.7%)	37/338 (10.9%)	0.511	0.79 (0.53 to 1.19)	0.265	0.94 (0.61 to 1.43)	0.773	0.84 (0.53 to 1.19)	0.412
Severe chorioamnionitis, adjusted model[b]				0.476	0.77 (0.51 to 1.18)	0.230	0.91 (0.69 to 1.19)	0.509	0.76 (0.49 to 1.15)	0.197

[a] Unadjusted model.
[b] Adjusted P-values were calculated using log-binomial regression models. Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis. All models were adjusted for maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia and 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.
[c] P-value obtained by Fisher's exact test, and log-binomial regression model for adjusted model.
[d] P-values for unadjusted and adjusted pair-wise comparisons were calculated using log-binomial regression models.

Table 4.11-2. Prevalence of Histological Signs of Malaria Infection by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA (n=330)	MMN (n=333)	LNS (n=345)	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Active malarial infection in placenta[a]	9 (2.7%)	16 (4.8%)	18 (5.2%)	0.222	1.91 (0.82 to 4.12)	0.106	1.02 (0.53 to 1.99)	0.806	1.76 (0.79 to 3.93)	0.337
Active malarial infection in placenta, adjusted model[b]				0.441	1.61 (0.72 to 3.61)	0.248	0.95 (0.47 to 1.91)	0.892	1.69 (0.75 to 3.79)	0.205
Past malarial infection in placenta[a]	127 (38.5%)	129 (38.7%)	124 (35.9%)	0.708	0.93 (0.77 to 1.14)	0.494	0.93 (0.76 to 1.13)	0.452	1.01 (0.83 to 1.22)	0.946
Past malarial infection in placenta, adjusted model[b]				0.444	0.95 (0.80 to 1.12)	0.558	0.88(0.74 to 1.05)	0.154	1.08 (0.91 to 1.28)	0.558

[a] Unadjusted model.
[b] Adjusted P-values were calculated using log-binomial regression models. Models were adjusted for covariates that had a significant association (P<0.10) with the outcome in bivariate analysis. All models were adjusted for maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, anemia at enrollment, maternal educational achievement, proxy for SES, site of enrollment, time between delivery and collection of placenta sample, and season of enrollment.
[c] P-value obtained by Fisher's exact test, and log-binomial regression model for adjusted model.
[d] P-values for unadjusted and adjusted pair-wise comparisons were calculated using log-binomial regression models.

4.12 Placental, Fetal Membrane, Oral, and Vaginal Microbiomes

We did not have any predefined hypotheses concerning the effect of LNS on participants' resident microbiomes at inception. However, we were still interested in any potential impact of LNS on the microbiome and postulated that LNS might change the prevalence and amount of bacteria recovered from the placenta and fetal membranes and the diversity of bacteria found in the oral cavity and vagina.

Prevalence of bacteria in the placenta and fetal membrane was defined as detectable presence of 16S rDNA in a sample of placenta or fetal membrane tissue from a participant. This DNA is found only in bacteria and so can be used to screen for its presence. 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 how an entire community of bacteria varies between the different intervention groups, we calculated the median intra-individual unweighted UniFrac distance for each participant. A higher UniFrac distance means an increased 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 calculated pair-wise comparisons of UniFrac distances between each individual and then took the median value of each pair-wise comparison related to that individual. Therefore, a statistically significant shift in a group's mean unweighted UniFrac value should represent a difference in the species of bacteria found in those communities.

Of the 1,391 participants enrolled in the study, 12 women with twin pregnancies were excluded from this analysis. Of the remaining women, a sample of placenta was collected at delivery from 1,030 (74.7%) participants and a sample from the fetal membranes was collected from 1,095 (79.4%) participants. For DNA analysis, this left 1,018 (73.8%) participants with a placenta sample analyzed. There were 347, 335, and 336 in the IFA, MMN, and LNS groups, respectively. Loss to follow-up was similar in all of the supplement groups (P=0.535). There were 1,083 (78.5%) participants with fetal membrane samples analyzed. There were 358, 364, and 361 participants in the IFA, MMN, and LNS groups, respectively. Loss to follow-up was similar in all of the supplement groups (P=0.811).

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 (80.1%) oral swabs and 1,107 (80.3%) vaginal swabs were included in the analysis. There were 374, 374, and 356 participants with oral swabs and 370, 371, and 366 participants with vaginal swabs in the IFA, MMN, and LNS groups, respectively. Loss to follow-up was similar in all of the supplement groups for both analyses (P=0.531 and P=0.952 for the oral microbiome and vaginal microbiome analyses, respectively).

There were no clinically meaningful differences between the three intervention groups of participants with a vaginal swab taken included in the analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-12).

Bacteria were identified in 46.8% (n=476) of participants' placental tissue and 60.4% (n=654) of fetal membranes. The mean (SD) bacterial load was 4.8 Log_{10} 16S rDNA copies/µl (0.7) in the placental tissue and 5.2 Log_{10} 16S rDNA copies/µl (0.8) in the fetal membranes. Bacterial load had a J-shaped distribution in both the placenta and the fetal membranes, with most tissues having very low bacterial loads.

Table 4.12-1 shows the analysis of bacterial load in the placenta and fetal membranes by intervention group. There was an association seen in the unadjusted values, with the bacterial load in the placenta being lower in the LNS group. The difference in means (95% CI) was −0.17 (−0.31, −0.02) between the LNS and IFA groups (P=0.023) and −0.21 (−0.35, −0.06) between the LNS and MMN groups (P=0.006).

This difference was also seen in the adjusted model at the global level and between the LNS and MMN groups, but not between the LNS and IFA groups.

Table 4.12-2, Table 4.12-3, and Table 4.12-4 show the analysis of bacterial diversity in placenta and fetal membranes, bacterial diversity in the oral cavity and vagina, and presence of bacteria in the placenta and fetal membranes by intervention group, respectively. There were no significant differences between intervention groups for these variables at the global level in either the unadjusted values or the adjusted models.

Maternal height at enrollment, maternal BMI at enrollment, gestational age at enrollment, maternal age at enrollment, proxy for SES, maternal educational achievement, primiparity, season of enrollment, maternal anemia at enrollment, maternal malaria status at enrollment, site of enrollment, and maternal HIV status were all tested for interactions with the intervention, with respect to the microbiota. The variables were tested using the likelihood ratio test and were selected in a predefined analysis plan as likely to be able to modify the effect of the nutritional intervention. Only primiparity tested positive for effect modification with at least one outcome.

Table 4.12-5 shows bacterial load by intervention group, stratified by primiparity. Among multiparous (but not primiparous) mothers, those in the LNS group had a lower bacterial load compared to the MMN group (−0.24 [−0.39, −0.09], P=0.002), but not compared to the IFA group.

The study findings suggest that provision of LNS to pregnant women may have an impact on lowering placental bacteria load when compared against the MMN group, but only in mothers who have delivered more than once. There were no other differences seen between intervention groups in presence of bacteria in the fetal membranes or bacterial diversity in the placenta, fetal membranes, oral cavity, or vagina.

Table 4.12-1. Bacterial Load in the Placenta and Fetal Membranes by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[e]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]
Mean (SD) bacterial load in the placenta (Log_{10} 16S rDNA copies/µl)[a,c]	4.85 (0.7)	4.89 (0.7)	4.68 (0.6)	0.014	−0.17 (−0.31 to −0.02)	0.023	−0.21 (−0.35 to −0.06)	0.006	0.04 (−0.10 to 0.18)	0.583
Placenta bacterial load comparison, adjusted model[b]				0.049	−0.14 (−0.28 to 0.01)	0.071	−0.18 (−0.34 to −0.03)	0.019	0.05 (−0.10 to 0.20)	0.536
Mean (SD) bacterial load in the fetal membrane (Log_{10} 16S rDNA copies/µl)[a,d]	5.21 (0.8)	5.22 (0.9)	5.24 (0.8)	0.896	0.03 (−0.12 to 0.17)	0.736	0.03 (−0.12 to 0.19)	0.650	0.01 (−0.14 to 0.16)	0.901
Fetal membrane bacterial load comparison, adjusted model[b]				0.761	0.04 (−0.11 to 0.19)	0.588	0.05 (−0.09 to 0.20)	0.478	−0.01 (−0.16 to 0.13)	0.865

[a] Unadjusted model.

[b] Model was adjusted for maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia and 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 on the logical potential to form an association with the outcomes based on previous literature.

[c] Number of participants: IFA n=168, MMN n=151, LNS n =157.

[d] Number of participants: IFA n=247, MMN n=260, LNS n =231.

[e] Unadjusted P-value obtained by one-way ANOVA, adjusted by ANCOVA.

[f] P-values for unadjusted and adjusted pair-wise comparisons were calculated using linear regression models.

Table 4.12-2. Bacterial Diversity in the Placenta and Fetal Membranes by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[e]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]
Mean (SD) intra-individual unweighted UniFrac distance in placental tissue[a,c]	0.69 (0.1)	0.69 (0.1)	0.69 (0.1)	0.869	0.00 (−0.01 to 0.02)	0.627	0.00 (−0.01 to 0.01)	0.947	0.00 (−0.01 to 0.01)	0.680
Placenta unweighted UniFrac distance comparison adjusted model[b]				0.872	0.00 (−0.01 to 0.02)	0.700	0.00 (−0.01 to 0.01)	0.903	0.00 (−0.01 to 0.02)	0.620
Mean (SD) intra-individual unweighted UniFrac distance in fetal membranes[a,d]	0.72 (0.1)	0.72 (0.1)	0.71 (0.1)	0.156	−0.01 (−0.02 to 0.00)	0.141	−0.01 (−0.02 to 0.00)	0.067	0.00 (−0.01 to 0.01)	0.730
Fetal membrane unweighted UniFrac distance comparison adjusted model[b]				0.219	−0.01 (−0.02 to 0.00)	0.136	−0.01 (−0.02 to 0.00)	0.124	0.00 (−0.01 to 0.01)	0.962

[a] Unadjusted model.

[b] Model was adjusted for maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia and 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 on the logical potential to form an association with the outcomes based on previous literature.

[c] Number of participants: IFA n=167, MMN n=150, LNS n =157.

[d] Number of participants: IFA n=247, MMN n=260, LNS n =231.

[e] Unadjusted P-value obtained by one-way ANOVA, adjusted by ANCOVA.

[f] P-values for unadjusted and adjusted pair-wise comparisons were calculated using linear regression models.

Table 4.12-3. Bacterial Diversity in the Oral Cavity and Vagina by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[e]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]	Difference in means (95% CI)	P-value[f]
Mean (SD) intra-individual unweighted UniFrac distance in the oral cavity[a,c]	0.49 (0.1)	0.49 (0.1)	0.51 (0.1)	0.342	0.01 (−0.01 to 0.02)	0.347	0.01 (−0.01 to 0.02)	0.148	0.00 (−0.02 to 0.01)	0.609
Oral cavity unweighted UniFrac distance comparison adjusted model[b]				0.326	0.01 (−0.01 to 0.02)	0.366	0.01 (−0.00 to 0.02)	0.137	0.00 (−0.02 to 0.01)	0.553
Mean (SD) intra-individual unweighted UniFrac distance in the vagina[a,d]	0.59 (0.1)	0.59 (0.1)	0.60 (0.1)	0.510	0.01 (−0.01 to 0.02)	0.295	0.00 (−0.02 to 0.02)	0.923	0.01 (−0.01 to 0.02)	0.340
Vaginal unweighted UniFrac distance comparison adjusted model[b]				0.438	0.01 (−0.01 to 0.03)	0.264	0.00 (−0.02 to 0.02)	0.990	0.01 (−0.01 to 0.03)	0.270

[a] Unadjusted model.

[b] Model was adjusted for maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia and 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 on the logical potential to form an association with the outcomes based on previous literature.

[c] Number of participants: IFA n=370, MMN n=371, LNS n =366.

[d] Number of participants: IFA n=374, MMN n=374, LNS n =356.

[e] Unadjusted P-value obtained by one-way ANOVA, adjusted by ANCOVA.

[f] P-values for unadjusted and adjusted pair-wise comparisons were calculated using linear regression models.

Table 4.12-4. Prevalence of Bacteria in the Placenta and Fetal Membranes by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Bacteria in the placenta[a]	167/347 (48.1%)	150/335 (44.8%)	157/336 (46.7%)	0.679	0.97 (0.83 to 1.14)	0.714	1.04 (0.88 to 1.23)	0.612	0.93 (0.79 to 1.09)	0.381
Bacteria in the placenta comparison, adjusted model[b]				0.400	1.00 (0.86 to 1.17)	0.967	1.11 (0.94 to 1.30)	0.235	0.91 (0.77 to 1.07)	0.246
Bacteria in the fetal membrane[a]	247/358 (68.9%)	260/364 (71.4%)	231/361 (63.9%)	0.094	0.93 (0.84 to 1.03)	0.156	0.89 (0.81 to 0.99)	0.033	1.04 (0.94 to 1.14)	0.475
Bacteria in the fetal membranes comparison, adjusted model[b]				0.112	0.93 (0.84 to 1.04)	0.200	0.89 (0.81 to 0.99)	0.036	1.04 (0.95 to 1.14)	0.414

[a] Unadjusted model.

[b] Model was adjusted for maternal height at enrollment, maternal age at enrollment, maternal BMI at enrollment, maternal HIV status, primiparity, maternal anemia and 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 on the logical potential to form an association with the outcomes based on previous literature.

[c] P-value obtained by Fisher's exact test, for adjusted results from Log-Poisson regression.

[d] P-values for unadjusted and adjusted pair-wise comparisons were calculated using log-binomial regression models.

Table 4.12-5. Effect Modification: Placenta Bacterial Load by Intervention Group, Stratified Results

Outcome	Interaction test P-value[a]	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
		IFA	MMN	LNS	P-value[b]	Difference in means (95% CI)	P-value[c]	Difference in means (95% CI)	P-value[c]	Difference in means (95% CI)	P-value[c]
Mean (SD) placenta bacteria load											
Multiparous[d]	0.035	4.8 (0.6)	4.9 (0.7)	4.7 (0.5)	0.010	−0.12 (−0.27 to 0.03)	0.127	−0.24 (−0.39 to −0.09)	0.002	0.12 (−0.03 to 0.27)	0.113
Primiparous[e]		5.1 (0.9)	4.7 (0.6)	4.6 (0.6)	0.084	−0.42 (−0.84 to −0.01)	0.045	−0.03 (−0.45 to 0.39)	0.881	−0.39 (−0.81 to 0.03)	0.066

[a] P-values were calculated using likelihood ratio test using a linear regression model.
[b] P-values were calculated using ANOVA comparison of means.
[c] P-values for pair-wise comparisons were calculated using linear regression models.
[d] Number of participants: IFA n=141, MMN n=125, LNS n =131.
[e] Number of participants: IFA n=26, MMN n=25, LNS n =26.

4.13 Maternal Oral Health

We did not formulate any written, predefined hypotheses as to the effect that we would expect provision of LNS during pregnancy to have on maternal oral health. We assumed, however, that provision of MMN or LNS might improve periodontal health of the participants, but that provision of LNS might have detrimental effects on dental caries, given that it contains sucrose.

Periodontal health relates to the periodontium—tooth-supporting tissues that consist mainly of gingiva (the gums), the alveolar bone surrounding the teeth, and the ligaments that attach the teeth to the bone. Gingivitis is a superficial infection in the gums that usually precedes periodontitis—deeper infection in the alveolar bone and the ligaments. Periapical infection is formed mostly when a deep dental caries in the crown of a tooth exposes the pulp (the nerve chamber), allowing access of oral microflora to the dental root canals, from where infection can then spread to the root-surrounding tissues. Periodontal diseases and dental caries (which also sometimes lead to periapical infection) have different etiology and the microbes involved are partly different.

Of the 1,391 participants enrolled in the study, 12 women with twin pregnancies were excluded from this analysis. Of the remaining women, 1,229 (89.1%) completed the oral health examination after delivery. After excluding those whose examination was done more than 6 weeks after delivery, 1,024 (74.3%) participants were included in the analyses. Loss to follow-up was similar in all of the supplement groups (P=0.762). There were 335, 346, and 343 included participants in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-13).

Overall, the included participants had an average (SD) of 31.3 teeth (1.6) and 2.1 carious teeth (2.8). Thirty women had supernumerous teeth (1, 2, or 5 extra teeth per woman). At least one caries lesion (grade II to grade III) was diagnosed in 63.1% (n=646) of the participants. In 27.8% (n=285) of the participants, at least one of the caries lesions exposed the pulp (grade III), and 23.5% (n=241) of the participants had at least one periapical infectious lesion. Fifteen participants had a periapical lesion without carious pulpal exposure. Gingivitis was diagnosed in 85.8% (n=879) and periodontitis in 31.9% (n=327) of the participants. Only five participants had received any restorative treatment (fillings).

Table 4.13-1 shows the oral health variables by intervention groups, using continuous outcomes. There was a between-group difference in the mean number of grade II–III (P=0.006) and grade II (P=0.001) caries lesions. Compared to women in the IFA group, those in the LNS group (95% CI) had 0.59 (0.17 to 1.01) more grade II–III caries lesions and 0.51 (0.21 to 0.80) more grade II caries lesions. The women in the MMN group (95% CI) had 0.60 (0.18 to 1.02) more grade II–III caries legions and 0.47 (0.18 to 0.77) more grade II caries lesions than women in the IFA group. Signs of gingivitis, periodontal disease, and periapical lesions were roughly equally distributed between the groups.

To control for possible confounding and to maximize power by reducing the variance of the outcomes, regression models were created using the forced entry method. All relevant and available enrollment variables that could confound the nutrition intervention effect on the oral diseases (based on earlier knowledge) were included in the model as covariates. Adjustment for these selected enrollment variables did not markedly change the association between the intervention and the continuous oral health outcomes (Table 4.13-1).

Table 4.13-2 shows the prevalence of caries, periapical infections, and periodontal diseases by intervention group. The prevalence of grade II–III caries lesions was 56.7% in the IFA group, 69.1% in the MMN group, and 63.3% in the LNS group (P=0.004). Compared to the IFA control, the relative risk (95% CI) of grade II–III caries was 1.12 (0.99 to 1.26) in the LNS group (P=0.083) and 1.22 (1.08 to 1.37) in the MMN group (P=0.001). There were similar differences in the prevalence of grade II and grade III caries lesions when analyzed separately and in the prevalence of periapical infections. The differences in the prevalence of grade III caries lesions and periapical infections were, however, not statistically significant. The prevalence of clinically or radiologically diagnosed periodontitis was slightly higher in the IFA group than in the MMN and LNS groups, but the differences were statistically insignificant. The prevalence of gingivitis was highest in LNS group, but the differences were again small and statistically insignificant.

After adjusting the analyses for the selected enrollment variables, there were no statistically significant differences between the intervention groups in any of the oral health outcomes.

Before using covariates in the model, we performed tests for interaction between the intervention and selected (based on earlier literature) variables from the covariate list using the likelihood ratio test. Enrollment variables tested for effect modification were maternal age at enrollment, maternal BMI at enrollment, parity, maternal HIV status, maternal malaria status at enrollment, proxy for SES, site of enrollment, and maternal educational achievement. Tests for interaction did not indicate a modification of the intervention effect on oral health outcomes by any of the tested variables.

The study findings do not support a beneficial effect of gestational MMN or LNS supplementation on periodontal health; rather, they were consistent with the possibility of a detrimental effect on caries development or progression.

Table 4.13-1. Continuous Oral Health Outcomes by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA (n=335)	MMN (n=346)	LNS (n=343)	P-value[c]	Difference in means (95% CI)	P-value[d]	Difference in means (95% CI)	P-value[d]	Difference in means (95% CI)	P-value[d]
Mean (SD) no. of any (grade II–III) caries lesions[a]	1.73 (2.32)	2.32 (2.83)	2.32 (3.16)	0.006	0.59 (0.17 to 1.01)	0.006	0.00 (−0.42 to 0.41)	0.989	0.60 (0.18 to 1.02)	0.006
No. of any (grade II–III) caries lesions, adjusted model[b]				0.008	0.53 (0.13 to 0.94)	0.010	0.04 (−0.45 to 0.36)	0.830	0.57 (0.17 to 0.98)	0.005
Mean (SD) no. of grade II caries lesions[a]	1.19 (1.65)	1.66 (1.98)	1.69 (2.21)	0.001	0.51 (0.21 to 0.80)	0.001	0.03 (−0.26 to 0.33)	0.815	0.47 (0.18 to 0.77)	0.002
No. of grade II caries lesions, adjusted model[b]				0.002	0.47 (0.18 to 0.76)	0.001	0.04 (−0.25 to 0.32)	0.800	0.43 (0.15 to 0.72)	0.003
Mean (SD) no. of grade III caries lesions[a]	0.54 (1.25)	0.66 (1.37)	0.62 (1.65)	0.529	0.08 (−0.13 to 0.30)	0.448	−0.04 (−0.25 to 0.18)	0.729	0.12 (−0.09 to 0.34)	0.269
No. of grade III caries lesions, adjusted model[b]				0.417	0.06 (−0.15 to 0.27)	0.577	−0.08 (−0.29 to 0.13)	0.447	0.14 (−0.07 to 0.35)	0.188
Mean (SD) no. of periapical lesions[a]	0.41 (0.97)	0.52 (1.20)	0.50 (1.41)	0.442	0.09 (−0.09 to 0.28)	0.321	−0.02 (−0.20 to 0.16)	0.839	0.11 (−0.07 to 0.29)	0.231
No. of periapical lesions, adjusted model[b]				0.347	0.07 (−0.11 to 0.25)	0.433	−0.06 (−0.24 to 0.12)	0.502	0.13 (−0.05 to 0.31)	0.146
Mean (SD) no. of sextants with bleeding on probing[a]	3.50 (2.08)	3.47 (2.17)	3.33 (2.12)	0.538	−0.17 (−0.49 to 0.15)	0.293	−0.14 (−0.45 to 0.18)	0.402	−0.04 (−0.36 to 0.28)	0.824
No. of sextants with bleeding on probing, adjusted model[b]				0.665	−0.13 (−0.43 to 0.18)	0.407	−0.11 (−0.41 to 0.19)	0.469	−0.02 (−0.32 to 0.29)	0.912
Mean (SD) no. of periodontal pockets ≥4 mm (clinical)[a]	1.70 (3.54)	1.38 (3.05)	1.43 (3.39)	0.409	−0.27 (−0.77 to 0.23)	0.291	0.04 (−0.45 to 0.54)	0.853	−0.32 (−0.82 to 0.18)	0.214
No. of periodontal pockets ≥4 mm (clinical), adjusted model[b]				0.386	−0.25 (−0.74 to 0.24)	0.310	0.07 (−0.41 to 0.56)	0.762	−0.33 (−0.81 to 0.16)	0.187
Mean (SD) periodontal pocket depth (mm)[a]	2.32 (0.54)	2.29 (0.52)	2.32 (0.53)	0.654	0.00 (−0.08 to 0.08)	0.950	0.03 (−0.05 to 0.11)	0.444	−0.03 (−0.11 to 0.05)	0.410
Periodontal pocket depth (mm), adjusted model[b]				0.663	0.00 (−0.08 to 0.07)	0.920	0.03 (−0.05 to 0.10)	0.465	−0.03 (−0.11 to 0.04)	0.408

[a] Unadjusted models.

[b] Models were adjusted using forced entry method for all relevant and available covariates that could potentially confound the nutrition intervention effect on the oral diseases (based on earlier knowledge). The selected covariates were time after delivery for oral health examination, maternal age at enrollment, maternal BMI at enrollment, number of previous pregnancies, maternal anemia at enrollment, maternal HIV and malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, number of teeth, toothbrush usage, and daily toothpaste usage.

[c] P-values for unadjusted values were obtained by ANOVA and for adjusted values by Wald Test after linear regression modeling.

[d] P-values for unadjusted values by ANOVA and adjusted values were obtained by pair-wise comparison using linear regression models.

Table 4.13-2. Prevalence of Oral Disease by Intervention Group

Outcome	Number of outcomes/women with outcome data				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA (n=335)	MMN (n=346)	LNS (n=343)	P-value[c]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]	Relative risk (95% CI)	P-value[d]
Prevalence of any (grade II–III) caries[a]	190 (56.7%)	239 (69.1%)	217 (63.3%)	0.004	1.12 (0.99 to 1.26)	0.083	0.92 (0.82 to 1.02)	0.108	1.22 (1.08 to 1.37)	0.001
Prevalence of any (grade II–III) caries, adjusted model[b]				0.182	1.10 (0.90 to 1.34)	0.342	0.92 (0.76 to 1.11)	0.371	1.20 (0.99 to 1.46)	0.065
Prevalence of grade II caries[a]	173 (51.6%)	218 (63.1%)	202 (58.9%)	0.010	1.14 (1.00 to 1.31)	0.059	0.93 (0.83 to 1.05)	0.269	1.22 (1.07 to 1.39)	0.003
Prevalence of grade II caries, adjusted model[b]				0.203	1.13 (0.92 to 1.39)	0.244	0.94 (0.77 to 1.14)	0.543	1.20 (0.98 to 1.47)	0.077
Prevalence of grade III caries[a]	86 (25.7%)	106 (30.6%)	93 (27.1%)	0.330	1.06 (0.82 to 1.36)	0.670	0.89 (0.70 to 1.12)	0.308	1.19 (0.94 to 1.52)	0.151
Prevalence of grade III caries, adjusted model[b]				0.343	0.99 (0.73 to 1.33)	0.941	0.83 (0.62 to 1.10)	0.195	1.19 (0.89 to 1.59)	0.232
Prevalence of periapical infections[a]	73 (21.8%)	87 (25.1%)	81 (23.6%)	0.584	1.08 (0.82 to 1.43)	0.571	0.94 (0.72 to 1.22)	0.640	1.15 (0.88 to 1.52)	0.303
Prevalence of periapical infections, adjusted model[b]				0.592	1.01 (0.73 to 1.39)	0.968	0.87 (0.64 to 1.19)	0.386	1.15 (0.84 to 1.58)	0.376
Prevalence of gingivitis[a]	289 (86.3%)	291 (84.1%)	299 (87.2%)	0.499	1.01 (0.90 to 1.14)	0.887	1.04 (0.98 to 1.11)	0.211	0.83 (0.80 to 0.87)	0.312
Prevalence of gingivitis, adjusted model[b]				0.883	1.01 (0.86 to 1.19)	0.884	1.01 (0.86 to 1.19)	0.873	0.97 (0.82 to 1.15)	0.744
Prevalence of clinical periodontitis[a]	68 (20.3%)	62 (17.9%)	58 (16.9%)	0.505	0.83 (0.61 to 1.14)	0.258	0.94 (0.68 to 1.31)	0.727	0.88 (0.65 to 1.20)	0.430
Prevalence of clinical periodontitis, adjusted model[b]				0.642	0.85 (0.60 to 1.21)	0.373	0.96 (0.67 to 1.39)	0.837	0.88 (0.62 to 1.26)	0.493
Prevalence of periodontitis (clinical+x-ray)[a]	117 (34.9%)	103 (29.8%)	107 (31.2%)	0.338	0.89 (0.72 to 1.11)	0.302	1.05 (0.84 to 1.31)	0.684	0.85 (0.69 to 1.06)	0.151
Prevalence of periodontitis (clinical+x-ray), adjusted model[b]				0.649	0.93 (0.71 to 1.21)	0.574	1.05 (0.80 to 1.38)	0.725	0.88 (0.67 to 1.15)	0.358

[a] Unadjusted models.

[b] Models were adjusted for relevant and available covariates that could potentially confound the nutrition intervention effect on the oral diseases (based on earlier knowledge) using forced entry method. The selected covariates were time after delivery for oral health examination, maternal age at enrollment, maternal BMI at enrollment, number of previous pregnancies, maternal anemia at enrollment, maternal HIV and malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, number of teeth, toothbrush usage, and daily toothpaste usage.

[c] P-values for unadjusted values were obtained by Fisher's exact test and for adjusted values by Wald Test after log-Poisson regression models.

[d] P-values were obtained by pair-wise comparison using Wald Test after log-Poisson regression models.

4.14 Malaria Immunity in Pregnancy

In this section, we test the hypothesis that provision of LNS to pregnant women increases maternal humoral immunity toward malaria parasites at 36 gw. The more detailed hypothesis can be found in Appendix 4 (37).

Of the 1,391 participants who were enrolled in the study, 12 women with twin pregnancies were excluded from this analysis. Of the remaining participants, 1,008 (73.1%) had maternal antibody levels measured in plasma samples collected at both enrollment and at 36 gw and were included in the analysis. There were 325, 347, and 336 participants in the IFA, MMN, and LNS groups, respectively.

There were no clinically meaningful differences between the three intervention groups of participants included in the analysis in terms of their demographic and socioeconomic characteristics or nutritional and health status at enrollment (Table A1-14).

Antibody levels were compared between intervention groups in samples collected at 36 gw. In unadjusted analyses, opsonizing antibodies to non-pregnancy-specific variant surface antigens (VSA) were significantly different (P=0.038), with pregnant women in the MMN group having the highest mean antibody levels, 38.4% (SD=27.74) of positive control, compared to the women in the other two groups; in pair-wise comparison, these antibodies were significantly higher in women receiving MMN than in those receiving IFA (Table 4.14-1). An adjusted analysis was performed, adjusting for variables known from the literature to influence levels of one or more of the antibodies measured. In the global analysis, only levels of antibody to Rh2A9 differed across intervention groups. In adjusted pair-wise analyses, women in the MMN group had significantly higher antibodies to Rh2A9 compared to the women in the IFA group (adjusted mean difference 6.00, P=0.019) (Table 4.14-1). No other differences in antibody levels were observed in adjusted analyses.

We further assessed whether participants had levels of antibodies (measured by PCR) above the range for unexposed controls. Women with levels above controls were defined as seropositive. The proportions of participants who were seropositive to each malaria antigen at 36 gw were comparable between the supplementation groups in unadjusted and adjusted analyses (

Table 4.14-2).

The potential effect modifiers of the association between antibody levels and supplementation type were tested using likelihood ratio tests. There was no effect modification by the variables tested, which were selected based on literature, indicating that they can influence levels of one or more of the malaria antibodies measured.

The study findings do not support a hypothesis that provision of LNS to pregnant women increases maternal humoral immunity toward malaria parasites at 36 gw in rural Malawi.

Table 4.14-1. Antibody Levels at 36 gw by Intervention Group

Outcome	Mean (SD) antibody concentration by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value	Difference in means (95% CI)	P-value	Difference in means (95% CI)	P-value	Difference in means (95% CI)	P-value
Mean (SD) total IgG to pregnancy-specific VSA[a,e]	44.65 (34.70)	41.39 (34.89)	41.60 (34.47)	0.415[b]	−3.05 (−8.46 to 2.35)	0.267[c]	0.21 (−5.11 to 5.54)	0.937[c]	−3.27 (−8.65 to 2.11)	0.234[c]
Total IgG to pregnancy-specific VSA, adjusted model[d]				0.148[d]	−4.78 (−10.91 to 1.35)	0.126[d]	0.38 (−5.55 to 6.31)	0.900[d]	−4.25 (−10.50 to 2.00)	0.182[d]
Mean (SD) opsonizing antibodies to pregnancy-specific VSA[a,f]	49.48 (36.28)	51.27 (36.76)	47.30 (36.26)	0.363[b]	−2.18 (−7.72 to 3.36)	0.440[c]	−3.97 (−9.46 to 1.52)	0.156[c]	1.79 (−3.75 to 7.33)	0.526[c]
Opsonizing antibodies to pregnancy-specific VSA, adjusted model[d]				0.879[d]	−0.84 (−6.82 to 5.15)	0.783[d]	−2.15 (−7.54 to 3.25)	0.434[d]	0.23 (−6.03 to 6.49)	0.942[d]
Mean (SD) opsonizing antibodies to non-pregnancy-specific VSA[a,g]	33.16 (25.71)	38.44 (27.74)	35.34 (26.77)	0.038[b]	2.18 (−1.84 to 6.21)	0.287[c]	−3.10 (−7.10 to 1.00)	0.139[c]	5.28 (1.20 to 9.36)	0.011[c]
Opsonizing antibodies to non-pregnancy-specific VSA, adjusted model[d]				0.881[d]	0.45 (−3.07 to 3.96)	0.802[d]	−0.003 (−3.18 to 3.17)	0.999[d]	−0.39 (−4.16 to 3.38)	0.839[d]
Mean (SD) MSP-1 19kD[a,g]	16.15 (21.03)	16.50 (21.18)	15.37 (19.01)	0.765[b]	−0.77 (−3.86 to 2.31)	0.622[c]	−1.13 (−4.17 to 1.91)	0.467[c]	0.35 (−2.87 to 3.58)	0.829[c]
MSP-1 19kD, adjusted model[d]				0.738[d]	−0.32 (−3.71 to 3.08)	0.854[d]	2.23 (−0.88 to 5.34)	0.160[d]	0.87 (−2.21 to 3.95)	0.580[d]
Mean (SD) MSP-2[a,g]	20.56 (20.0)	23.71 (24.15)	20.68 (19.86)	0.099[b]	0.12 (−2.95 to 3.18)	0.941[c]	−3.03 (−6.37 to 0.32)	0.076[c]	3.14 (−0.26 to 6.55)	0.070[c]
MSP-2, adjusted model[d]				0.108[d]	−2.38 (−6.10 to 1.34)	0.208[d]	−2.79 (−6.69 to 1.11)	0.160[d]	0.84 (−2.46 to 4.14)	0.617[d]
Mean (SD) MSP-3[a,g]	19.29 (20.09)	18.82 (21.33)	19.31 (20.46)	0.942[b]	0.02 (−3.10 to 3.15)	0.988[c]	0.49 (−2.67 to 3.64)	0.763[c]	−0.46 (−3.63 to 2.71)	0.775[c]
MSP-3, adjusted model[d]				0.712[d]	−3.05 (−7.57 to 1.46)	0.184[d]	−0.81 (−5.38 to 3.76)	0.727[d]	−0.01 (−4.57 to 4.56)	0.998[d]
Mean (SD) EBA-175[a,g]	12.66 (13.84)	13.78 (15.29)	12.54 (12.89)	0.452[b]	−0.12 (−2.17 to 1.94)	0.911[c]	−1.24 (−3.37 to 0.90)	0.257[c]	1.12 (−1.11 to 3.35)	0.326[c]
EBA-175, adjusted model[d]				0.725[d]	0.15 (−2.37 to 2.68)	0.905[d]	−0.60 (−3.41 to 2.21)	0.674[d]	1.23 (−1.73 to 4.19)	0.414[d]
Mean (SD) Rh2A9[a,g]	17.19 (19.29)	19.83 (24.06)	18.57 (19.76)	0.280[b]	1.38 (−1.63 to 4.39)	0.368[c]	−1.26 (−4.59 to 2.07)	0.459[c]	2.64 (−0.71 to 5.98)	0.123[c]
Rh2A9, adjusted model[d]				0.046[d]	−0.37 (−4.55 to 3.80)	0.860[d]	−3.67 (−8.30 to 0.96)	0.120[d]	6.00 (0.98 to 11.02)	0.019[d]
Mean (SD) schizont extract[a,h]	33.77 (26.92)	36.07 (29.51)	32.13 (28.19)	0.278[b]	−1.64 (−6.46 to 3.18)	0.505[c]	−3.94 (−8.91 to 1.03)	0.120[c]	2.30(−2.60 to 7.20)	0.357[c]
Schizont extract, adjusted model[d]				0.745[d]	1.69 (−5.38 to 8.75)	0.638[d]	−0.84 (−8.11 to 6.43)	0.820[d]	2.73 (−4.05 to 9.51)	0.428[d]

[a] Unadjusted models.
[b] P-value calculated using ANOVA (comparison of means).
[c] ANOVA followed by linear regression analysis performed to determine the unadjusted mean difference and P-value.
[d] ANOVA followed by linear regression analysis performed to determine the adjusted mean difference and P-value while adjusting for primiparity, maternal age at enrollment, maternal HIV status, maternal bed net use at enrollment, maternal BMI at enrollment, maternal malaria status (PCR) at enrollment, proxy for SES, site of enrollment, and corresponding antibody level at enrollment.
[e] Number of participants: IFA n=312, MMN n=334, LNS n=321.
[f] Number of participants: IFA n=325, MMN n=347, LNS n=336.
[g] Number of participants: IFA n=321, MMN n=345, LNS n=336.
[h] Number of participants: IFA n=249, MMN n=266, LNS n=256.

Table 4.14-2. Antibody Seropositivity at 36 gw by Intervention Group

Outcome	Result by intervention group				Comparison between LNS group and IFA group		Comparison between LNS group and MMN group		Comparison between MMN group and IFA group	
	IFA	MMN	LNS	P-value[c]	Odds ratio (95% CI)	P-value[d]	Odds ratio (95% CI)	P-value[d]	Odds ratio (95% CI)	P-value[d]
Total IgG to pregnancy-specific VSA[a]	273/312 (87.5%)	307/334 (91.9%)	286/321 (89.1%)	0.171	1.17 (0.72 to 1.90)	0.532	0.72 (0.42 to 1.22)	0.219	1.62 (0.97 to 2.72)	0.066
Total IgG to pregnancy-specific VSA, adjusted model[b]				0.269	1.92 (0.73 to 5.09)	0.188	0.92 (0.35 to 2.38)	0.861	2.09 (0.79 to 5.52)	0.136
Opsonizing antibodies to pregnancy-specific VSA[a]	307/325 (94.5%)	327/347 (94.2%)	313/336 (93.2%)	0.773	0.80 (0.42 to 1.51)	0.487	0.83 (0.45 to 1.55)	0.561	0.96 (0.50 to 1.85)	0.899
Opsonizing antibodies to pregnancy-specific VSA, adjusted model[b]				0.574	1.32 (0.50 to 3.49)	0.569	1.66 (0.65 to 4.25)	0.292	0.80 (0.32 to 2.00)	0.631
Opsonizing antibodies to non-pregnancy-specific VSA[a]	286/321 (89.1%)	320/345 (92.8%)	306/336 (91.1%)	0.265	1.25 (0.75 to 2.09)	0.397	0.80 (0.46 to 1.39)	0.421	1.57 (0.92 to 2.68)	0.102
Opsonizing antibodies to non-pregnancy-specific VSA, adjusted model[b]				0.548	1.53 (0.66 to 3.59)	0.325	1.04 (0.42 to 2.57)	0.939	1.48 (0.62 to 3.56)	0.382
MSP-1 19kD[a]	193/318 (60.7%)	217/343 (63.3%)	208/333 (62.5%)	0.789	1.08 (0.79 to 1.48)	0.642	0.97 (0.71 to 1.32)	0.829	1.12 (0.81 to 1.53)	0.496
MSP-1 19kD, adjusted model[b]				0.836	1.04 (0.62 to 1.74)	0.882	0.90 (0.54 to 1.49)	0.673	1.16 (0.70 to 1.93)	0.565
MSP-2[a]	220/318 (69.2%)	248/343 (72.3%)	229/333 (68.8%)	0.543	0.98 (0.70 to 1.37)	0.909	0.84 (0.61 to 1.17)	0.314	1.16 (0.83 to 1.63)	0.378
MSP-2, adjusted model[b]				0.243	0.77 (0.45 to 1.33)	0.346	0.63 (0.36 to 1.08)	0.093	1.23 (0.71 to 2.11)	0.460
MSP-3[a]	217/318 (68.2%)	223/343 (65%)	233/333 (70%)	0.379	1.08 (0.78 to 1.51)	0.633	1.25 (0.91 to 1.73)	0.170	0.86 (0.63 to 1.2)	0.380
MSP-3, adjusted model[b]				0.759	1.92 (0.73 to 5.09)	0.188	0.92 (0.35 to 2.38)	0.861	2.09 (0.79 to 5.52)	0.136
EBA-175[a]	130/318 (40.9%)	159/343 (46.4%)	139/333 (41.7%)	0.309	1.04 (0.76 to 1.42)	0.823	0.83 (0.61 to 1.12)	0.227	1.25 (0.92 to 1.7)	0.156
EBA-175, adjusted model[b]				0.396	0.99 (0.61 to 1.60)	0.952	0.75 (0.46 to 1.20)	0.229	1.32 (0.82 to 2.13)	0.253
Rh2A9[a]	186/318 (58.5%)	197/343 (57.4%)	198/333 (59.5%)	0.869	1.04 (0.76 to 1.42)	0.802	1.09 (0.80 to 1.48)	0.593	0.96 (0.70 to 1.30)	0.783
Rh2A9, adjusted model[b]				0.760	1.01 (0.65 to 1.58)	0.950	0.87 (0.56 to 1.36)	0.542	1.16 (0.75 to 1.82)	0.503
Schizont extract[a]	213/249 (85.5%)	236/266 (88.7%)	215/256 (84.0%)	0.278	0.89 (0.55 to 1.44)	0.626	0.67 (0.40 to 1.11)	0.116	1.33 (0.79 to 2.23)	0.282
Schizont extract, adjusted model[b]				0.618	1.04 (0.51 to 2.10)	0.920	0.73 (0.34 to 1.57)	0.422	1.42 (0.67 to 2.98)	0.359

[a] Unadjusted models.
[b] P-value calculated using logistic regression reporting odds ratios while adjusting for primiparity, maternal age at enrollmnet, maternal HIV status, maternal bed net use at enrollment, maternal BMI at enrollment, maternal malaria status (PCR) at enrollment, proxy for SES, site of enrollment, and corresponding antibody level at enrollment.
[c] Unadjusted P-value calculated Fisher's exact test, adjusted p-values from logistic regression.
[d] P-value calculated using logistic regression reporting odds ratios.

5. Discussion

The current study tested a series of hypotheses that provision of LNS rather than IFA or MMN to pregnant women would improve maternal nutritional status, decrease stress, reduce inflammation, lower bacterial load in the placenta and amniotic membranes, decrease the prevalence of certain infections, strengthen maternal immune response toward malaria, and increase the mean duration of pregnancy and birth size in rural Malawi.

Among study participants who were followed up within 6 weeks of delivery/birth, the mean birth weight and newborn length were approximately 50 g and 4 mm larger in the LNS group than in the IFA group. These differences were, however, not statistically significant. 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 UTIs after pregnancy. Likewise, the mean maternal saliva concentration of cortisol; plasma concentrations of inflammation markers CRP and AGP; and nutritional markers of cholesterol, triglycerides, folate, and defined fatty acids during the third trimester of pregnancy were similar between the three arms. Mean plasma vitamin B12 showed less decline during pregnancy in the LNS group than in the IFA group, but there were no differences in the proportions of participants with low plasma vitamin B12 concentration at 36 gw. The groups 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. Hence, the study findings generally do not support the hypotheses that LNS would promote maternal health and fetal growth or increase mean birth size, if provided to an unselected group of pregnant women in the study area.

The methodological strengths of the trial included random group allocation that led to similarity of the intervention groups at enrollment, rigorous quality assurance in data collection, and blinding of the outcome assessors to group allocation. Internal validity could have been compromised by the relatively large number of missing data, the delay in anthropometric measurements of some participants, the temporary discontinuation of the LNS distribution during the trial, and our inability to directly observe the consumption of the study supplements. Since the results were robust to several sensitivity analyses, we believe these factors did not significantly bias our conclusions. However, the smaller than originally intended sample size (due to budget reduction) limited the statistical power of the study. Therefore, while the results do not support the study hypotheses, they also do not rule out a modest intervention effect on birth size or other pregnancy outcomes.

We could identify only three other published trials on provision of LNS to pregnant women. In a Ghanaian trial that had an identical design to the one reported here, infant babies born to women who received 20 g/day LNS during pregnancy had on average 85 g (95% CI 3 g to 166 g) and 3 mm (−1 mm to 6 mm) higher birth weight and length than infants born to IFA-supplemented women (Adu-Afarwuah et al. 2015). In Burkina Faso, women who received a much larger daily dose of LNS (72 g vs. 20 g in our study) gave birth to infants whose mean (95% CI) birth weight and length were approximately 10 g (−43 g to 67 g) and 4 mm (1 mm to 7 mm) greater than those of infants whose mothers had received MMN as UNIMMAP capsules (Huybregts et al. 2009). There was no IFA control group in that trial, but an earlier publication from the same investigators in the same area suggested that MMN increased mean birth weight and length by 40 g and 3 mm, respectively, compared to IFA (Roberfroid et al. 2008). A modest increase in birth weight has also been associated with the provision of a smaller daily dose of LNS (20 g/day) to pregnant women in Bangladesh (Mridha et al. 2016). Our point-estimates of a 50 g and

4 mm difference in the mean birth weight and length between LNS and IFA groups are thus in a similar range to those from these trials, which further supports the possibility of a modest intervention effect on birth size.

One common finding from nutrient supplementation trials is the heterogeneity of the treatment effect in different target populations (Roberfroid et al. 2008, Ramakrishnan et al. 2003, Christian et al. 2003, Friis et al. 2004, Kaestel et al. 2005, Osrin et al. 2005, Zagre et al. 2007, Supplementation with Multiple Micronutrients Intervention Trial Study Group et al. 2008). Besides geographic heterogeneity, several authors have also suggested variation in the effect of dietary supplements on various subpopulations of the target group. For instance, in the trial from Burkina Faso, the treatment effect of LNS was observed among multigravid women, those with anemia or low BMI, and in some seasons, but not, for example, among primigravid participants or in other seasons (Huybregts et al. 2009, Toe et al. 2015). In the Ghana trial, an effect on birth size was observed among primigravid, but not among multigravid women (Adu-Afarwuah et al. 2015). In some energy supplementation trials, a larger effect has been noted in the most undernourished population (Winkvist et al. 1998, de Onis et al. 2013), whereas in others this has not been documented (Kramer 1993, Kramer and Kakuma 2003). With MMN interventions, a larger treatment effect has actually been documented among the better-nourished pregnant women (Fall et al. 2009). While it thus seems likely that selected pregnant women will benefit from dietary supplementation with products like LNS, the inconsistent findings and the importance of the context make it hard to predict the most-responsive target group in advance (Manary 2015).

In our own trial, maternal age at enrollment, parity, nutritional status (low BMI), season of enrollment, or relative wealth did not appear to modify the association between the intervention and the birth size outcomes. Maternal educational achievement modified the association for newborn stunting, but not for other studied birth outcomes. For the association between the intervention and other outcomes, we observed occasional modification by some sociodemographic or maternal characteristics, but there was no systematic pattern to it. This and the lack of main effect of the intervention on numerous outcome variables suggest that dietary insufficiency may not have been the main determinant of poor pregnancy outcomes in this target population. Alternative explanations include maternal infections, stress, and inflammation that were all quite prevalent in this population and that may lead to fetal growth restriction through several pathways (Ashorn et al. 2015). These hypotheses are supported by findings that the duration of pregnancy and birth size were strongly predicted by maternal infections and inflammation in this cohort (Ashorn et al. 2017). Maternal malaria, HIV infection, and inflammatory response also seemed to modify some of the associations between the intervention and birth size (Ashorn et al. unpublished). Finally, results from several controlled trials suggest that in sub-Saharan Africa the duration of pregnancy and birth size may be influenced by interventions addressing maternal malaria or bacterial infections (Gray et al. 2001, Kayentao et al. 2013). Hence, multipronged interventions that also address maternal infections are likely to be needed to promote maternal and fetal well-being in the sub-Saharan setting.

Our observations on maternal iron, vitamin A, and oral health status warrant some further discussion. At 36 gw, the mean maternal blood Hb concentration was slightly lower and the mean concentrations of ZPP and sTFR reciprocally higher in the LNS and MMN than in the IFA groups. Similarly, the proportion of women with a low plasma retinol concentration was highest in the LNS group. The Hb, ZPP, and sTFR results were consistent with each other and hence probably truly causal to the intervention and related to the lower dose of iron in the LNS and MMN supplements (20 mg/day) than in the IFA supplements (60 mg/day). The retinol result may have been a random finding and related to multiple comparisons, as the differences became non-significant 6 months after pregnancy (Haskell et al. unpublished observation) and there were no intergroup differences in mean plasma retinol concentration. Alternatively, the

difference may be related to iron status, which is known to be critical in vitamin metabolism (Jang et al. 2000, Oliveira et al. 2008).

The choice of a lower iron content in LNS than IFA supplements was motivated by our desire to have the iron content identical in the MMN and LNS supplements and to use the same MMN preparations in pregnancy and lactation (when less iron is recommended); our calculations and previous evidence that 20 mg/day would be sufficient, given all other dietary intake of iron in the study area; and our unwillingness to change the iron dose for the control group from the nationally recommend 60 mg/day (Zhou et al. 2009, Arimond et al. 2015). The study findings suggest that the lower dose was not sufficient for maintaining a "high" iron status among pregnant women in the study area. However, it is uncertain how this translates into maternal and child health. In our preliminary analyses on the determinants of birth outcomes, a reduction in blood Hb concentration during pregnancy and a "worsening" iron status was associated with longer duration of pregnancy and larger birth size (see Ashorn et al. 2016). Hence, the lower dose, although apparently worse in terms of laboratory values late in the third trimester, may actually have been better in promoting child health. This would not contradict earlier findings, as LBW has been associated with low blood Hb concentration or anemia in the first or second trimester but not in the third trimester of pregnancy (Murphy et al. 1986, Allen 2000).

Women in the LNS and MMN groups also had a slightly higher prevalence of dental caries after birth than women in the IFA group. In the absence of enrollment oral health data, firm conclusions on causality cannot be drawn. However, the results do come from a randomized prospective trial, with strict quality assurance and reasonably low loss to follow-up. Hence, although not confirmatory, the findings are consistent with a possibility that provision of MMN or LNS may have increased the caries incidence in this target population (Harjunmaa et al. 2015). Again, one possible mechanism is the reduced dose of iron, as iron may suppress caries development, for instance, by reducing enamel demineralization (Alves et al. 2011, Torell 1988). Alternatively, other nutrients in LNS and MMN may have changed the oral microbiota (Blais and Lavoie 1990), possibly leading to increasing bacterial amounts or virulence and thus caries development. Such an effect might be aggravated due to pregnancy-related changes in the oral cavity and suppression in immune responses (Laine 2002).

Taken together, the study findings generally do not support hypotheses that small-quantity LNS would promote maternal health and fetal growth or increase mean birth size, if provided to an unselected group of pregnant women in the study area. Further studies are needed to understand and identify group-level predictors of preterm birth and fetal growth restriction and to assess the longer-term effect of prenatal LNS provision on subsequent child growth and development.

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Appendix 1. Enrollment Characteristics of Participants by Intervention Group

A1.1. Duration of Pregnancy and Child Size at Birth

Table A1-1. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,391)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	463	466	462	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	22.2 (2.9)	22.2 (3.0)	0.794
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.768
Mean (SD) maternal educational achievement (completed years at school)	3.9 (3.4)	4.1 (3.4)	4.1 (3.6)	0.704
Mean (SD) proxy for SES	−0.02 (0.99)	0.01 (0.99)	0.02 (1.03)	0.850
Percentage of anemic women (Hb <100 g/L)	21.0%	19.8%	21.2%	0.856
Percentage of primiparous women	20.4%	23.0%	22.1%	0.607
Percentage of women with a low BMI (<18.5)	5.9%	4.6%	5.7%	0.626
Percentage of women with a positive HIV test	15.6%	11.1%	14.4%	0.125
Percentage of women with a positive malaria test (RDT)	22.7%	24.1%	22.8%	0.861

[a] Those participants enrolled in the iLiNS-DYAD-M trial.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.2. Maternal Weight Gain during Pregnancy and Placental Size

Table A1-2. Enrollment Characteristics of Included Participants, by Intervention Group (n=961)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	321	313	327	
Mean (SD) BMI (kg/m²)	22.1 (2.9)	22.1 (2.9)	22.1 (2.8)	0.975
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.410
Mean (SD) maternal educational achievement (completed years at school)	4.1 (3.3)	4.3 (3.4)	4.1 (3.5)	0.516
Mean (SD) proxy for SES	−0.05 (0.93)	0.01 (0.97)	−0.01 (0.98)	0.691
Percentage of anemic women (Hb <100 g/L)	18.4%	16.9%	20.2%	0.579
Percentage of primiparous women	17.2%	21.8%	20.2%	0.332
Percentage of women with a low BMI (<18.5)	6.0%	5.5%	5.2%	0.922
Percentage of women with a positive HIV test	16.4%	10.3%	14.9%	0.069
Percentage of women with a positive malaria test (RDT)	19.6%	23.6%	23.4%	0.392

[a] Those participants who provided placentas at delivery.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.3. Maternal Asymptomatic Malaria Infections at 32 gw and 36 gw and at Delivery

Table A1-3. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,131)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	373	372	386	
Mean (SD) BMI (kg/m²)	22.0 (2.5)	22.1 (3.0)	22.1 (2.9)	0.834
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.734
Mean (SD) maternal educational achievement (completed years at school)	3.9 (3.4)	4.2 (3.4)	4.1 (3.5)	0.360
Mean (SD) proxy for SES	−0.08 (0.9)	0.02 (1.0)	0.00 (1.0)	0.369
Percentage of anemic women (Hb <100 g/L)	19.6%	17.5%	20.75%	0.531
Percentage of primiparous women	18.0%	21.3%	20.2%	0.523
Percentage of women with a low BMI (<18.5)	5.7%	5.2%	6.2%	0.805
Percentage of women with a positive HIV test	13.8%	10.9%	14.7%	0.127
Percentage of women with a positive malaria test (RDT)	22.0%	23.5%	22.7%	0.454

[a] Those participants who were included in the malaria (RDT) at delivery analysis.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.4. Maternal Reproductive Tract Infections and Urinary Tract Infections at Delivery

Table A1-4. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,210)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	405	407	398	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	22.1 (2.9)	22.1 (2.8)	0.978
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.821
Mean (SD) maternal educational achievement (completed years at school)	4 (3.4)	4 (3.4)	40 (3.5)	0.647
Mean (SD) proxy for SES	−0.07 (0.96)	−0.01 (0.98)	−0.02 (1.00)	0.647
Percentage of anemic women (Hb <100 g/L)	21.2%	19.5%	20.9%	0.804
Percentage of primiparous women	19.3%	20.9%	20.1%	0.837
Percentage of women with a low BMI (<18.5)	5.5%	5.0%	6.3%	0.723
Percentage of women with a positive HIV test	15.9%	10.6%	14.7%	0.070
Percentage of women with a positive malaria test (RDT)	22.2%	23.9%	22.5%	0.830

[a] Those participants who were included in the trichomoniasis 1 week after delivery analysis.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.5. Maternal Plasma CRP and AGP Concentrations

Table A1-5. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,063)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	350	361	352	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	22.2 (3.0)	22.1 (2.8)	0.908
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.797
Mean (SD) maternal educational achievement (completed years at school)	4.0 (3.4)	4.0 (3.4)	4.1 (3.5)	0.931
Mean (SD) proxy for SES	−0.06 (1.0)	−0.05 (0.9)	−0.02 (1.0)	0.832
Percentage of anemic women (Hb <100 g/L)	18.3%	17.8%	19.6%	0.818
Percentage of primiparous women	19.5%	19.2%	20.7%	0.851
Percentage of women with a low BMI (<18.5)	5.2%	4.8%	6.6%	0.558
Percentage of women with a positive HIV test	14.7%	10.4%	13.1%	0.209
Percentage of women with a positive malaria test (RDT)	21.3%	24.0%	23.1%	0.671

[a] Those participants who had CRP and AGP analyzed at 36 gw.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.6. Maternal Blood Hb, ZPP, and sTfR Concentrations

Table A1-6. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,067)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	352	363	352	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	22.2 (3.0)	22.1 (2.8)	0.906
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.764
Mean (SD) maternal educational achievement (completed years at school)	4.0 (3.4)	4.0 (3.4)	4.1 (3.5)	0.927
Mean (SD) proxy for SES	−0.06 (1.0)	−0.04 (0.9)	−0.02 (1.0)	0.873
Percentage of anemic women (Hb <100 g/L)	18.2%	18.0%	19.6%	0.832
Percentage of primiparous women	19.4%	19.1%	20.7%	0.835
Percentage of women with a low BMI (<18.5)	5.4%	5.0%	6.6%	0.657
Percentage of women with a positive HIV test	14.7%	10.3%	13.1%	0.207
Percentage of women with a positive malaria test (RDT)	21.3%	24.0%	23.1%	0.673

[a] Those participants who had sTfR analyzed at 36 gw, as sTfR had the largest sample size of the three analyses.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.7. Maternal Plasma Retinol Concentration

Table A1-7. Enrollment Characteristics of Included Participants, by Intervention Group (n=314)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	103	105	106	
Mean (SD) BMI (kg/m²)	21.8 (2.6)	21.8 (2.7)	21.8 (2.9)	0.999
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (7)	0.920
Mean (SD) maternal educational achievement (completed years at school)	3.5 (3.3)	3.6 (3.4)	3.6 (3.5)	0.966
Mean (SD) proxy for SES	−0.25 (0.83)	−0.18 (0.79)	−0.09 (0.86)	0.396
Percentage of anemic women (Hb <100 g/L)	17.5%	20.0%	25.2%	0.377
Percentage of primiparous women	20.4%	22.9%	21.5%	0.912
Percentage of women with a low BMI (<18.5)	5.8%	3.8%	9.3%	0.257
Percentage of women with a positive HIV test	14.6%	8.7%	10.3%	0.409
Percentage of women with a positive malaria test (RDT)	23.3%	22.9%	24.5%	0.958

[a] Those participants included in a randomly selected subset for assessment of plasma retinol concentration at enrollment.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.8. Maternal Plasma Vitamin B Concentrations

Table A1-8. Enrollment Characteristics of Included Participants, by Intervention Group (n=314)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	104	105	105	
Mean (SD) BMI (kg/m²)	21.8 (2.6)	21.9 (2.7)	21.9 (2.9)	0.982
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (7)	0.939
Mean (SD) maternal educational achievement (completed years at school)	3.5 (3.3)	3.6 (3.4)	3.6 (3.5)	0.986
Mean (SD) proxy for SES	−0.25 (0.83)	−0.18 (0.80)	−0.10 (0.87)	0.411
Percentage of anemic women (Hb <100 g/L)	18.3%	20.2%	24.8%	0.533
Percentage of primiparous women	21.2%	22.1%	21.0%	0.985
Percentage of women with a low BMI (<18.5)	5.8%	2.9%	9.5%	0.141
Percentage of women with a positive HIV test	14.4%	8.7%	10.5%	0.431
Percentage of women with a positive malaria test (RDT)	23.1%	23.1%	25.0%	0.957

[a] Those participants included in a randomly selected subset for assessment of plasma vitamin B12 status, folate, and tHcy concentrations at enrollment and 36 gw.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.9. Maternal Plasma Cholesterol and Triglycerides Concentrations and Plasma Fatty Acid Composition

Table A1-9. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,061)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	350	360	351	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	22.2 (3.0)	22.1 (2.8)	0.935
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.764
Mean (SD) maternal educational achievement (completed years at school)	4.0 (3.4)	4.0 (3.4)	4.1 (3.5)	0.927
Mean (SD) proxy for SES	−0.06 (0.96)	−0.04 (0.95)	−0.02 (0.98)	0.873
Percentage of anemic women (Hb <100 g/L)	17.1%	18.7%	19.1%	0.775
Percentage of primiparous women	18.9%	19.6%	20.5%	0.863
Percentage of women with a low BMI (<18.5)	5.3%	4.4%	6.3%	0.530
Percentage of women with a positive HIV test	14.8%	9.9%	13.1%	0.145
Percentage of women with a positive malaria test (RDT)	21.2%	22.7%	23.7%	0.739

[a] Those participants with plasma cholesterol and triglycerides measurements at 36 weeks gestation.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.10. Maternal Salivary Cortisol Concentration

Table A1-10. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,237)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	411	414	412	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	22.2 (2.9)	22.1 (2.9)	0.908
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.957
Mean (SD) maternal educational achievement (completed years at school)	3.9 (3.4)	4.0 (3.4)	4.1 (3.6)	0.647
Mean (SD) proxy for SES	−0.05 (0.98)	−0.02 (0.97)	0.008 (1.00)	0.711
Percentage of anemic women (Hb <100 g/L)	17.1%	18.7%	19.1%	0.775
Percentage of primiparous women	18.9%	19.6%	20.5%	0.863
Percentage of women with a low BMI (<18.5)	5.3%	4.4%	6.3%	0.530
Percentage of women with a positive HIV test	14.8%	9.9%	13.1%	0.145
Percentage of women with a positive malaria test (RDT)	21.2%	22.7%	23.7%	0.739

[a] Those participants with at least some data on perceived stress or salivary cortisol at 28 or 36 weeks gestation.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.11. Histological Signs of Inflammation and Malaria in the Placenta and Fetal Membranes

Table A1-11. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,008)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	330	333	345	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	21.9 (2.8)	22.0 (2.8)	0.886
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.848
Mean (SD) maternal educational achievement (completed years at school)	3.8 (3.3)	4.1 (3.4)	3.9 (3.4)	0.617
Mean (SD) proxy for SES	−0.10 (0.9)	−0.04 (0.9)	−0.07 (0.9)	0.714
Percentage of anemic women (Hb <100 g/L)	21.2%	17.5%	21.2%	0.377
Percentage of primiparous women	18.8%	21.1%	20.9%	0.734
Percentage of women with a low BMI (<18.5)	5.2%	5.4%	5.5%	0.984
Percentage of women with a positive HIV test	15.7%	10.9%	12.9%	0.201
Percentage of women with a positive malaria test (RDT)	21.5%	24.7%	23.6%	0.612

[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-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions)

A1.12. Placenta, Fetal Membrane, Oral, and Vaginal Microbiomes

Table A1-12. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,107)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	370	371	366	
Mean (SD) BMI (kg/m²)	22.1 (2.6)	22.1 (3.0)	22.1 (2.8)	0.908
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.824
Mean (SD) maternal educational achievement (completed years at school)	3.9 (3.4)	3.9 (3.4)	4.0 (3.5)	0.809
Mean (SD) proxy for SES	−0.08 (0.9)	−0.04 (0.9)	−0.03 (0.9)	0.817
Percentage of anemic women (Hb <100 g/L)	21.6%	18.1%	20.2%	0.480
Percentage of primiparous women	19.7%	20.8%	19.7%	0.916
Percentage of women with a low BMI (<18.5)	5.4%	5.5%	6.3%	0.860
Percentage of women with a positive HIV test	16.0%	10.9%	14.8%	0.097
Percentage of women with a positive malaria test (RDT)	21.9%	24.6%	23.8%	0.699

[a] Those participants who had a vaginal swab collected 1 week after delivery and were processed for DNA extraction and sequencing.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.13. Maternal Oral Health

Table A1-13. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,024)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	335	346	343	
Mean (SD) BMI (kg/m²)	22.1 (2.5)	22.1 (2.9)	22.0 (2.7)	0.966
Mean (SD) maternal age, years	25 (6)	25 (6)	25 (6)	0.986
Mean (SD) maternal educational achievement (completed years at school)	3.9 (3.4)	4.0 (3.4)	3.9 (3.4)	0.805
Mean (SD) proxy for SES	−0.22 (1.59)	−0.08 (1.72)	−0.10 (1.72)	0.509
Percentage of anemic women (Hb <100 g/L)	21.5%	18.3%	19.8%	0.578
Percentage of primiparous women	18.0%	18.6%	16.9%	0.857
Percentage of women with a low BMI (<18.5)	4.8%	5.0%	6.4%	0.623
Percentage of women with a positive HIV test	15.0%	11.6%	15.7%	0.246
Percentage of women with a positive malaria test (RDT)	21.2%	20.9%	22.2%	0.909

[a] Those participants who completed the oral health examination within 6 weeks of delivery.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

A1.14. Malaria Immunity in Pregnancy

Table A1-14. Enrollment Characteristics of Included Participants, by Intervention Group (n=1,008)

Characteristic	IFA	MMN	LNS	P-value[b]
Number of participants[a]	325	347	336	
Mean (SD) BMI (kg/m²)	22.0 (2.6)	22.2 (3.0)	22.2 (2.9)	0.612
Mean (SD) maternal age, years	24 (6)	25 (6)	25 (6)	0.535
Mean (SD) maternal educational achievement (completed years at school)	4.0 (3.3)	4.1 (3.3)	4.1 (3.7)	0.891
Mean (SD) proxy for SES	−0.08 (0.95)	−0.03 (0.96)	−0.03 (0.95)	0.725
Percentage of anemic women (Hb <100 g/L)	44.3%	40.2%	42.4%	0.554
Percentage of primiparous women	19.7%	19.6%	19.9%	0.463
Percentage of women with a low BMI (<18.5)	5.6%	5.3%	6.3%	0.965
Percentage of women with a positive HIV test	14.6%	10.8%	13.7%	0.298
Percentage of women with a positive malaria test (RDT)	25.7%	23.4%	25.7%	0.722

[a] Those participants with maternal antibody levels measured in plasma samples at both enrollment and 36 gw.
[b] P-value obtained from ANOVA (comparison of means) or Fisher's exact test (comparison of proportions).

Appendix 2. Supplementary Details in Data Collection and Statistical Analyses

A2.1. The Duration of Pregnancy and Child Size at Birth

Details of data collection or laboratory analysis. This topic is covered in Section 2.

Details of statistical analysis. We carried out the statistical analysis with Stata 12.1 (StataCorp, College Station, TX, USA) according to a detailed statistical analysis plan written and published after the onset of the trial, but before the code was opened (http://www.ilins.org). All presented analyses were prespecified either in the trial protocol or in the statistical analysis plan. We based the analysis on the principle of modified intention-to-treat, i.e., we included all randomized participants in the analyses, with the exceptions that participants with missing data on an outcome variable were excluded for the analysis of that outcome and that two participants whose group allocation was incorrectly transcribed and assigned during enrollment were included in the group corresponding to the actual intervention that they received throughout the trial.

The target sample size of 1,400 participants was based on two separate calculations. Our first aim was to detect differences between the three groups, assuming an effect size of 0.30 (difference between groups, divided by the pooled SD) for each continuous outcome, assuming power of 80% and a 2-sided type I error rate of 5%. This would require 216 participants per group, for a total of 648 subjects. Allowing for up to 25% loss to follow-up, we would have needed to recruit 864 subjects. Our secondary aim was to study the interaction between the maternal intervention and a total of 14 potential effect modifiers. Because the interaction analyses were considered exploratory, the multiple comparisons were not taken into account in determining the sample size. For each interaction, we assumed a total infection prevalence of 25% and an interaction effect size of approximately 0.3, with $P<0.10$ (2-sided test) and 80% power. With these assumptions, we would have needed approximately 640 participants per group in the analysis. Allowing for 20% missing values, we planned to recruit 2,400 participants, which would have given the study 80% power to detect main effects of >0.18 SD. The sample size was, however, subsequently reduced to 1,400 due to unexpected budgetary difficulties. The revised final sample size of 370 per group provided the study with 80% power to detect main effects of ≥0.23 SD and an interaction effect of ≥0.47 SD with a 2-sided type I error rate of 5% (corresponding to a difference of approximately 100 g in birth weight or 0.5 cm in newborn length).

All data were initially collected on paper forms from which they were extracted and entered into a tailor-made database through scanning, digital character recognition, and manual verification of critical variables and all suspicious entries. Researchers and research assistants cleaned all data through a number of logic checks. Once the database was considered clean, we broke the code and carried out statistical analysis.

We used data on birth weight as such if measured within 48 hours of delivery, and back-calculated birth weight from 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. Twelve twin pregnancies were excluded from all main analyses, but we carried out sensitivity analyses that included the twins and used the number of fetuses as a covariate. We carried out a second, "per protocol" sensitivity analysis that was confined to the most "adherent" participants (participants who received and did not return supplements for more than 80% of the follow-up days). As a third sensitivity analysis, we built Heckman's selection models to explore if loss to follow-

up might have biased the results (Cheung 2001, Greene 2012). In these models, we included maternal height at enrollment, maternal BMI at enrollment, gestational age at enrollment, maternal age at enrollment, child sex, proxy for SES, number of previous pregnancies, maternal anemia at enrollment, and site of enrollment as potential factors that might have affected loss to follow-up.

We calculated the duration of pregnancy by adding the time interval between enrollment and miscarriage or delivery to the ultrasound-determined gestational age at enrollment and defined preterm birth as one occurring before 37 completed gw (259 days), and calculated LBW as <2,500 g. 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) and considered values <−2.0 as indicative of underweight, stunting, wasting, or small head circumference, respectively. For all the anthropometric 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 (for length/height measurements 0.5 cm, for circumferences [head, MUAC] 0.5 cm, for infant/child/adult weight 0.1 kg, and for skinfold thickness 2.0 mm). 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.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

We estimated relative risks for comparison of dichotomous end-points at a single time point and differences in means for comparison of continuous end-points at a single time point. To prevent inflated type I errors due to multiple group comparisons, we employed the close testing procedure (Cheung 2014), i.e., null hypotheses for pair-wise comparisons could be rejected only if the global null hypotheses of all three groups being identical was also rejected. We did not adjust for multiplicity in safety analysis, as this should err on the safe side. We tested the global null hypotheses with either Fisher's exact test (for dichotomous end-points) or ANOVA (continuous end-points) and the pair-wise hypotheses with either log-binomial regression models (for dichotomous end-points) or ANOVA (continuous end-points). With the log-binomial regression models for the dichotomous end-points, we used the software's default setting of Newton-Raphson maximization of the log likelihood. 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 this order (Zou 2004, Filmer and Pritchett 2001a). In case the prevalence of the outcome was 0% in some group, we used exact logistic regression to calculate odds ratios.

We performed likelihood ratio tests for interaction between the intervention and maternal characteristics specified in the statistical analysis plan prior to data analysis, and stratified analyses (in case of a positive interaction test [P<0.10]) by maternal HIV infection, malaria status at enrollment, and maternal educational achievement for the most important outcomes. Additional variables tested for interaction (per the analysis plan) included maternal age at enrollment, number of previous pregnancies, maternal height at enrollment, maternal BMI at enrollment, maternal anemia at enrollment, study site, exposure to the cessation of supplement provision (delivery before or after the temporary suspension of LNS distribution), season of enrollment, gestational age at enrollment, proxy for SES (Filmer and Pritchett 2001b), and child sex.

Covariates used in the adjusted models were derived from the list of variables that were tested for interaction with the intervention but not selected as effect modifiers. All variables that showed a statistically significant association with birth weight, LBW, LAZ, or newborn stunting (P<0.10) in multivariable regression were included in all the models, i.e., all the models were adjusted for the same

set of covariates. We performed the covariate selection with linear and logistic regression models. All the models were adjusted for the same set of covariates: maternal height at enrollment, maternal BMI at enrollment, gestational age at enrollment, maternal age at enrollment, child sex, proxy for SES, number of previous pregnancies, maternal anemia at enrollment, and site of enrollment. In one stratified analysis model, there were no events in one intervention group. Because standard regression analysis was not applicable in this case, we used exact logistic regression to make the comparison, with and without covariate adjustment.

A2.2. Maternal Weight Gain during Pregnancy and Placental Size

Details of data collection or laboratory analysis. This topic is covered in Section 2.

Details of statistical analysis. We carried out the statistical analysis with Stata 12.1 (StataCorp, College Station, TX, USA). We conducted the statistical analysis according to an analysis plan written and published at the project-wide website before the intervention code was broken. We based the analysis on the principle of modified intention-to-treat.

We calculated maternal weight gain during pregnancy using a mixed model with maternal weight calculated for each time point (enrollment, 32 gw, and 36 gw). We included a random intercept and random slope, which were allowed to correlate with each other. We determined whether participants gained insufficient or sufficient weight by using the IOM guidelines on appropriate gestational weight gain, which vary based on the pre-pregnancy BMI. We used the lower limit of the range because the weight gains in this population were well below the IOM cutoff points for appropriate weight gain (Drehmer et al. 2013). We established a proxy for pre-pregnancy BMI by use of regression modeling because pre-pregnancy BMI was not directly available. We first transformed maternal BMI at enrollment to achieve normal distribution by regressing maternal BMI at enrollment on gestational age, log BMI at enrollment, and inverse BMI at enrollment. Secondly, we regressed BMI, log BMI, and inverse BMI on gestational age, gestational age squared, and gestational age cubed, and chose the regression with the highest r-square as the best model for predicting maternal pre-pregnancy BMI.

We estimated the prevalence of low placental weight for gestation age, low placental weight for birth weight, and low placental-weight-to-birth-weight ratio by dividing the number of placentas <10th percentile by the number of all babies with valid data on this outcome.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

We estimated relative risks for comparison of dichotomous end-points and risk difference for comparison of continuous end-points at a single time point. To prevent inflated type I errors caused by multiple comparisons, we tested global null hypotheses of all three groups being identical before doing pair-wise comparisons. We tested the global null hypothesis with either Fisher's exact test (for dichotomous end-points) or ANOVA (for continuous end-points) and the pair-wise hypotheses with log-binomial regression model (for dichotomous end-points) or ANOVA (for continuous end-points). By the central limit theorem, such an analysis of means is robust and valid also in the case of skewed outcome distribution, due to the large sample size (Cheung 2014).

To control for possible confounding we used generalized linear models. We performed the covariate selection with linear regression models and generalized linear models. Those that were significantly associated with maternal weight gain during pregnancy or placental size ($P<0.10$) in bivariate regression analysis were included in the adjusted models. All the models used the same set of covariates.

We performed likelihood ratio tests for interaction between the intervention and maternal characteristics specified in the statistical analysis plan prior to data analysis and stratified analyses by maternal malaria status at enrollment, child sex, proxy for SES, and site of enrollment. Additional variables tested for interaction included maternal HIV status, maternal age at enrollment, number of previous pregnancies, height at enrollment, maternal BMI at enrollment, maternal anemia at enrollment, season of enrollment, gestational age at enrollment, and household food security index.

A2.3. Maternal Asymptomatic Malaria Infections at 32 gw, at 36 gw, and at Delivery

Details of data collection or laboratory analysis. Maternal asymptomatic malaria infections were assessed at 32 gw by RDT, at 36 gw by PCR, and at delivery by both RDT and PCR. Finger-prick blood samples were collected for RDT testing at 32 gw, whereas at 36 gw and at delivery venous blood samples were collected for both RDT and PCR testing. 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 *P. falciparum*, 2) the presence of other non-specified *Plasmodium* species, 3) negative result, or 4) invalid result.

PCR testing for *P. falciparum* was performed on dried blood spot samples collected from whole blood. Genomic DNA was extracted from dried blood spots stored on filter paper. From three 0.3 cm punches, genomic DNA was extracted using Chelex-100 and then tested in a real-time PCR assay targeting the *P. falciparum* lactate dehydrogenase gene (Rantala et al. 2010). Genomic DNA samples were tested on 384-well reaction plates that included a dilution series of 10 templates with *P. falciparum* strain 3D7 at a range of concentrations; these results were used to compute a standard curve, with which parasite densities were estimated in the clinical samples.

Details of statistical analysis. We carried out the statistical analysis with Stata 12.1 (StataCorp, College Station, TX, USA). We conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

We estimated relative risks for comparison of dichotomous end-points at a single time point. To prevent inflated type I errors caused by multiple comparisons, we tested global null hypotheses of all three groups being identical before doing pair-wise comparisons. We tested the global null hypothesis with logistic regression and the pair-wise hypotheses with log-binomial regression model.

Covariates used in the adjusted models were prespecified in the statistical analysis plan and were significantly associated ($P<0.10$) with the malaria parasitemia in bivariate analysis. We performed the covariate selection with logistic regression models. The selected covariates were parity, maternal age at enrollment, maternal anemia at enrollment, and site of enrollment for malaria parasitemia at 32 gw (RDT); parity, maternal age at enrollment, and site of enrollment for malaria parasitemia at 36 gw (PCR); site of enrollment for malaria parasitemia at delivery (RDT); maternal age at enrollment, gestational age at enrollment, maternal BMI at enrollment, maternal educational achievement, proxy for SES, and site of enrollment for malaria parasitemia at delivery (PCR).

We performed likelihood ratio tests for interaction between the intervention and maternal characteristics specified in the statistical analysis plan prior to data analysis. The variables tested for interaction (per the analysis plan) included maternal age at enrollment, number of previous pregnancies, maternal educational

achievement, maternal height at enrollment, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment, maternal anemia at enrollment, site of enrollment, season of enrollment, gestational age at enrollment, proxy for SES, and child sex.

A2.4. Maternal Reproductive Tract Infections and Urinary Tract Infections 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 60 seconds, 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 conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

We estimated risk ratios for comparison of dichotomous end-points at a single time point. To prevent inflated type I errors caused by multiple comparisons, we tested global null hypotheses of all three groups being identical before doing pair-wise comparisons. We tested the global null hypothesis with logistic regression and the pair-wise hypotheses with a log-binomial regression model.

Covariates used in the adjusted models were prespecified in the statistical analysis plan and were significantly associated ($P<0.10$) with the outcome in bivariate analysis. We performed the covariate selection with logistic regression models. The selected covariates were maternal educational achievement, proxy for SES, and HIV status at enrollment for trichomoniasis and HIV status at enrollment for UTI.

We performed likelihood ratio tests for interaction between the intervention and maternal characteristics specified in the statistical analysis plan prior to data analysis. The variables tested for interaction (per the analysis plan) included maternal age at enrollment, number of previous pregnancies, maternal height at enrollment, maternal BMI at enrollment, maternal HIV status, maternal malaria status at enrollment,

maternal anemia at enrollment, site of enrollment, season of enrollment, gestational age at enrollment, proxy for SES, and child sex.

A2.5. 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 CRP and AGP 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 performed statistical analysis with the SAS version 9.3 software package (SAS Institute Inc., Cary, NC, USA). We conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

We analyzed the differences in the percentage of women in each group who had abnormal CRP or AGP at either the enrollment or the 36 gw visit by Fisher's exact test. We analyzed the relative risks of elevated CRP or AGP between intervention groups by log-Poisson regression models, adjusting for covariates. We included those covariates in the model based on whether these variables have been shown in prior work to influence the outcome and, on bivariate analysis, were significantly associated ($P<0.10$) with the outcome. In addition, we included the corresponding enrollment value for the outcome variable as a covariate in each adjusted model. Variables considered as potential covariates were selected a priori for their expected associations with inflammation.

Two-way interactions between group assignment and Hb at enrollment, ZPP at enrollment, sTfR at enrollment, CRP at enrollment, AGP at enrollment, and BMI at enrollment; maternal educational achievement; HIV status; malaria status at enrollment; and season of and site of enrollment were included separately in the ANCOVA models for change in CRP and AGP. There were no significant effect modifiers ($P<0.05$).

A2.6. 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 performed statistical analysis with the SAS version 9.3 software package (SAS Institute Inc., Cary, NC, USA). We conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat. We did not inspect variables for skewedness, as by the central limit theorem analyses of means are robust and valid even in the case of skewed outcome distribution, due to the large sample size (Cheung 2014).

We compared the enrollment characteristics of the three intervention groups by ANOVA for comparison of means or by Fisher's exact test for comparison of proportions.

We analyzed the differences between groups in mean Hb, ZPP, and sTfR at 36 gw and change from enrollment to 36 gw by ANCOVA, with the main effect being intervention group and controlling for enrollment status of each variable and chosen covariates. For all analyses, when the overall model was significant ($P<0.05$), we compared groups by using the Tukey post hoc multicomparison test. The differences in proportion of women in each group who had low or high Hb, who had high ZPP or sTfR, or who had IDA (Hb <100 g/L and either ZPP >60 μmol/mol heme or sTfR >6.0 mg/L) at either the enrollment or the 36 gw visit were compared by log-Poisson regression. We examined whether inflammation at 36 gw was interfering with the ability to assess impact of the intervention on Hb at 36 gw by comparing the group-wise differences in mean Hb at 36 gw and proportion of women who were anemic at 36 gw after excluding cases with elevated CRP or AGP.

The covariates in the ANCOVA and log-Poisson regression models were included in the model based on whether these variables have been shown in prior work to influence the outcome and on bivariate analysis were significantly associated ($P<0.10$) with the outcome.

To examine effect modification, variables were selected a priori based on their expected associations with Hb, ZPP, and sTfR. Two-way interactions between group assignment and Hb, ZPP, sTfR, CRP, AGP, and BMI at enrollment; maternal educational achievement; HIV status; malaria status at enrollment; and season of and site of enrollment were included separately in the ANCOVA models for change in Hb, ZPP, and sTfR. Global p-values for the interactions could not be calculated as that is not possible on a specified value of a continuous covariate. We found significant interactions ($P<0.05$) between group assignment and Hb at enrollment, sTfR at enrollment, and CRP at enrollment for the change in Hb, and for group assignment and Hb at enrollment for the change in sTfR. For each of these interactions, the predicted least squares mean change in Hb and sTfR were estimated at the 10th, 50th, and 90th percentiles of the effect modifier at enrollment by using linear modeling. We compared the differences between groups in the predicted change in Hb or sTfR after adjusting for the covariates mentioned above.

A2.7. 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 1000 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, Fair Lawn, NJ, USA) 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. Statistical analysis was carried out with the SAS version 9.3 software package (SAS Institute Inc., Cary, NC, USA). We conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANCOVA and the Tukey-Kramer test for comparisons of means and Fisher's exact test for comparison of proportions.

Data were tested for normality of distribution by using the Shapiro-Wilk test. Bivariate analysis was used to examine associations between plasma retinol concentration at 36 weeks gestation and the following variables (which were predefined in the analysis plan): plasma retinol concentration at enrollment, maternal plasma CRP and AGP concentrations 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. Those with a significant association ($P<0.10$) with the outcome in bivariate analysis were included as covariates in the model for the corresponding outcome.

Plasma retinol concentration at enrollment was compared by intervention group by using ANOVA. Mean plasma retinol concentration at 36 weeks gestation was compared by group by using ANCOVA with intervention group as the main effect, controlling for plasma retinol concentration at enrollment and selected covariates. The difference in the proportion of women in each group that had low plasma retinol concentration (<1.05 µmol/L) at the enrollment and at 36 weeks gestation time points was compared by logistic regression, controlling for plasma retinol concentration at enrollment and selected covariates. Vitamin A deficiency was defined as plasma retinol concentration <1.05 µmol/L (Sauberlich et al. 1974). The Tukey-Kramer post-hoc multicomparison test was done for pair-wise comparisons for both continuous and dichotomous outcomes.

To examine effect modification, interactions between group assignment and plasma retinol concentration at enrollment, maternal BMI at enrollment, maternal plasma CRP and AGP concentrations at enrollment, maternal malaria status at enrollment, maternal HIV status, primiparity, maternal educational achievement, site of enrollment, and season of enrollment were included separately in the ANCOVA

models for plasma retinol concentration at 36 weeks gestation. Variables tested for effect modification were predefined in the analysis plan.

A2.8. Maternal Plasma Vitamin B Concentration

Details of data collection or laboratory analysis. The samples to assess plasma vitamin B concentration were analyzed simultaneously with control and standards provided by Roche, as well as an internal control, which was used to assess the intra- and inter-assay variations. Plasma homocysteine was determined by high performance liquid chromatography with fluorescence detector (Agilent 1200, Santa Clara, CA, USA). The same internal control and commercial controls were used to monitor the analyses for accuracy and precision. The CVs of 15% for folate, 10% for B12, and 10% for tHcy were deemed acceptable based on multiple test runs prior to the sample analyses given the limitations of the analyzer and the variability in biological samples. Samples with values outside of these ranges (>15% for folate; >10% for B12 and tHcy) were reanalyzed.

Details of statistical analysis. Statistical analysis was carried out with Stata 13.1 (StataCorp, College Station, TX, USA). We conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. The analysis was based on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

The group means and SDs for B12, folate, and tHcy were calculated at enrollment and 36 gw and for the change from enrollment to 36 gw. An overall ANOVA P-value was estimated for comparison across intervention groups and pair-wise differences denoted. The mean change in biomarkers in each of the intervention groups from enrollment to 36 gw was compared across intervention groups using ANOVA (ANCOVA for adjusted p-values). Linear regression was used for pair-wise outcomes, both unadjusted and adjusted models, with the Tukey-Kramer test used for the adjusted pair-wise comparisons

In addition, the percentage of abnormal values (as defined in Table 4.8-2) at enrollment and at 36 gw was calculated and the relative risks compared at each time point. The global test for dichotomous outcomes was carried out with Fisher's exact test and the pair-wise comparisons using log-binomial regression models for binary outcomes. We used Newton-Raphson maximization of the log-likelihood. In case the algorithm failed to converge in the estimation, we used alternative estimation algorithms with iterated reweighted least squares. Adjusted global P-values were obtained from Wald Test after log-binomial regression models.

Models were adjusted for covariates that had a significant association ($P<0.10$) with the outcome in bivariate analysis, in addition to the corresponding value for the outcome variable at enrollment.

A2.9. Maternal Plasma Cholesterol and Triglyceride Concentrations and Plasma Fatty Acid Composition

Details of data collection or laboratory analysis. To determine plasma cholesterol and triglyceride concentrations and fatty acid composition, plasma was isolated from blood by centrifuge. Plasma cholesterol and triglyceride 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. Statistical analysis was carried out with the SAS version 9.3 software package (SAS Institute Inc., Cary, NC, USA). We conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

The primary outcomes for this analysis were maternal cholesterol, triglycerides, AA, DHA, ALA, and omega-6:omega-3 fatty acids ratio, all measured in plasma at 36 weeks gestation. All fatty acid variables, except for AA, were log-transformed, as was triglyceride concentration. Group-wise differences for continuous outcomes were tested using ANOVA and ANCOVA models, using the Tukey-Kramer adjustment for multiple comparisons, and P-values <0.05 were considered statistically significant. Group-wise differences for dichotomous outcomes were tested using Fisher's exact test and log-Poisson models, using binomial logistic regression for pair-wise comparisons. Potential covariates selected from previous literature and stated in a predefined analysis plan included gestational age at enrollment, primiparity, maternal BMI at enrollment, maternal age at enrollment, site of enrollment, season of enrollment, maternal malaria status at enrollment, maternal HIV status, proxy for SES, maternal AGP at enrollment, and the corresponding enrollment value for the outcome variable. Covariates were included in the model if they were significantly (P<0.10) associated with one of the above outcomes in bivariate analysis. All outcomes were adjusted for the same set of covariates.

Interactions were tested between the intervention group and maternal age at enrollment, parity, and maternal BMI at enrollment. Interactions were selected based on previous literature and stated in a predefined analysis plan.

A2.10. 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 µmol/L (0.007 to 3.0 µg/dL). The intra- and inter-assay coefficient of variability is 3.5% and 5.1%, respectively. The mean of each duplicate measure was used for analysis.

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.

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) (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.

Details of statistical analysis. All analyses were performed using the SAS version 9.3 software package (SAS Institute, Cary, NC, USA). We conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

For all analyses, participants were included if they had non-missing data on either cortisol or the perceived stress score at any time point. We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions. Maternal report of perceived stress during the previous month was analyzed using the 10-item PSS, scored using standard methods. Cortisol and PSS were checked for normality using the Shapiro-Wilk test and cortisol was log-transformed. We examined the correlation between log cortisol and the perceived stress score at each time point by calculating Pearson's correlation coefficients.

The primary outcomes for this analysis were maternal salivary cortisol concentrations measured at 28 gw and 36 gw and perceived stress scores at these same time points. Group-wise differences were tested using ANOVA and ANCOVA models, using the Tukey-Kramer adjustment for multiple comparisons, and P-values <0.05 were considered statistically significant. Group-wise differences for dichotomous outcomes were tested using Fisher's exact test and log-Poisson regression models, and pair-wise comparisons were tested using log-binomial regression. Covariates were included in the model if they were significantly (P<0.10) associated with salivary cortisol for the cortisol model or if they were significantly (P<0.10) associated with PSS for the PSS model. Potential covariates selected from previous literature and stated in a predefined analysis plan included maternal cortisol at enrollment, maternal age at enrollment, gestational age at enrollment, maternal educational achievement, maternal BMI at enrollment, maternal height at enrollment, season of enrollment, maternal malaria status at enrollment, maternal HIV status, maternal Hb at enrollment, maternal iron status at enrollment, maternal inflammatory markers at enrollment, household food insecurity, proxy for SES, primiparity, infant sex, site of enrollment, and maternal PSS at enrollment (PSS considered in cortisol model only). From this list, the adjusted cortisol models at 28 weeks gestation and 36 weeks gestation included maternal cortisol at enrollment; maternal age at enrollment; maternal educational achievement; proxy for SES; study site; season of enrollment; and maternal Hb at enrollment, maternal ZPP at enrollment, and maternal sTfR at enrollment, as well as time between waking and saliva collection and time between last food or drink (besides water) and saliva collection. Time since waking and time since last meal were included in the adjusted cortisol models, regardless of their association with the outcome variables. Adjusted PSS models at 28 weeks gestation and 36 weeks gestation included perceived stress score at enrollment, maternal age at enrollment, maternal educational achievement, proxy for SES, site of enrollment, and season of enrollment.

Interactions were selected based on previous literature and stated in a predefined analysis plan. Interactions were tested between the intervention group and maternal age at enrollment, parity, maternal BMI at enrollment, and infant sex.

A2.11. 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. 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 transported to −80°C storage at Mangochi district hospital. 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. 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, 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 conducted the statistical analysis according to the analysis plan written and

published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

The global null hypothesis was tested between intervention groups using Fisher's exact test. Pair-wise comparisons between different intervention groups were only considered significant if the global null hypothesis was rejected with statistical significance. Log-binomial regression models were used to do pair-wise comparisons.

All analyses were also adjusted for possible confounding effects. Covariates were selected based on whether they had a significant association ($P<0.10$) with the outcome in bivariate analysis. All covariates were then entered in a single step into the equation. The outcomes were modeled using log-binomial regression.

We tested variables predefined in the analysis plan as effect modifiers using the likelihood ratio tests for interaction between the intervention and maternal characteristics. None of the effect modifiers tested were statistically significant.

A2.12. 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 membrane 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 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 transported to −80°C storage at Mangochi district hospital.

Dental swabs were collected by specially trained dental therapists at Mangochi central site from all mothers who completed the oral health visit, at 1 week after delivery or as soon as possible thereafter. 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 sides. 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 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 as for the placenta and membrane samples, 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, 1µl 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 membranes 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. 10 pM 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 conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

The global null hypothesis was tested between intervention groups using Fisher's exact test for dichotomous outcomes and one-way ANOVA for continuous outcomes. Pair-wise comparisons between different intervention groups were only considered significant if the global null hypothesis was rejected with statistical significance. For continuous variables pair-wise comparisons were carried out using linear regression models and for dichotomous variables log-binomial regression models were used.

All analyses were also adjusted using regression models to correct for possible confounding effects. Covariates selected for inclusion in the models were chosen based on previous literature as logically capable of forming an independent interaction with the outcome being measured. All covariates were then entered in a single step into the equation. Continuous outcomes were modeled using linear regression, and dichotomous outcomes were modeled using log-Poisson regression.

We tested variables predefined in the analysis plan as effect modifiers using the likelihood ratio test for interaction between the intervention and maternal characteristics. Maternal variables tested were maternal age at enrollment, maternal BMI at enrollment, number of previous pregnancies, maternal anemia at enrollment, maternal HIV and malaria status at enrollment, maternal educational achievement, proxy for SES, and site of enrollment. Stratified analysis was then produced for any effect modifiers that were statistically significant.

A2.13. 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 specifically 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™, 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 conducted the statistical analysis according to the analysis plan written and published before the intervention code was opened. We based the analysis on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

We estimated relative risks for comparison of dichotomous end-points and risk differences for comparison of continuous end-points at a single time point. To prevent inflated type I errors caused by multiple comparisons, we tested global null hypotheses of all three groups being identical before doing pair-wise comparisons. We tested the unadjusted global null hypothesis either with Fisher's exact test (for dichotomous end-points) or ANOVA (for continuous end-points) and the adjusted global hypothesis either with Wald test after log-Poisson regression (for dichotomous end-points) or Wald test after linear regression (for continuous end-points). The pair-wise comparisons were made with a log-Poisson regression model (for dichotomous end-points) or linear regression (for continuous end points). By the central limit theorem, such an analysis of means is robust and valid also in the case of skewed outcome distribution, due to the large sample size (Cheung 2014, Rice 1995).

To control for possible confounding and to maximize power by reducing the variance of the outcomes, we created regression models using the forced entry method so that all relevant and available covariates that could confound the nutrition intervention effect on the oral diseases (based on earlier knowledge, same covariates for all oral diseases) were included. All the models were adjusted for the same set of variables: time after delivery for oral health examination, maternal age at enrollment, maternal BMI at enrollment, number of previous pregnancies, maternal anemia at enrollment, maternal HIV and malaria status at enrollment, maternal educational achievement, proxy for SES, site of enrollment, number of teeth, tooth brush usage, and daily toothpaste usage. We included all prespecified variables as covariates in the model (even though they could have also been effect modifiers). Log-Poisson models were used for adjusted analysis of dichotomous outcomes and linear regression models for adjusted analysis of continuous outcomes.

Before using covariates in the model, we performed tests for interaction between the intervention, oral infection outcomes, and variables from the covariate list using the likelihood ratio test. Toothbrush and toothpaste usage and number of teeth were excluded from interaction testing. If any variable had been found to be an effect modifier, we would have conducted a stratified analysis for that variable.

A2.14. 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 include antigens expressed by the merozoites and VSA expressed by the parasitized red blood cells (pRBCs) (Miller et al. 2002).

Antibody immunity to both pregnancy-specific and non-pregnancy-specific malaria VSA was measured in maternal samples using flow cytometry-based opsonic phagocytosis and VSA recognition assays. In addition, immunity to merozoite antigens merozoite surface protein-1 (MSP-1 19kD), MSP-2, MSP-3, reticulocyte binding homologue 2A9 (Rh2A9), erythrocyte binding antigen-175 (EBA-175), and schizont extract was measured using enzyme linked immunosorbent assays (ELISAs). These selected antigens have been previously reported as potential vaccine candidates.

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 1–2 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.

Preparation of recombinant malaria antigens and schizont extract. Recombinant merozoite surface protein-1, MSP-1 19kD (3D7 clone) and full-length MSP-2 (FC27 clone) antigens, were kindly provided by Paul Gilson (Burnet Institute, VIC, Australia) and Robin Anders (La Trobe University, Australia), respectively. Region III-V of EBA 175 was expressed as a His-tagged protein and region 6088bp-7584bp of the full length protein for Rh2A9 was expressed in pGEX-4-T3 vector (GE Health Care, Australia). The full-length MSP-3 protein was expressed in pMAL-c2X vector (New England Biolabs® Inc., Ipswich, MA, USA) in BL21(DE3)-RIL cells (derivative of *E.coli* BL21-(DE3) cells) by Christine Langer from the Burnet Institute. Once expressed, the proteins were purified using nickel-nitrilotriacetic acid resin (Qiagen, VIC, Australia) following manufacturer's protocol. Proteins were then dialyzed overnight in phosphate-buffered saline (PBS) and run on SDS-PAGE and Coomassie stained gels to determine their purity and integrity. If the purity was low, the proteins were repurified using nickel-nitrilotriacetic acid resin.

The schizont extract was prepared by extracting schizonts from a 60% Percoll gradient followed by multiple freeze-thaw cycles with cold PBS and disruption of the parasites via sonication to release parasite antigens. The sonicated parasites were centrifuged at 1,300 RPM for 1 minute and the extract in the supernatant stored at −80°C till use.

Measuring antibodies to pregnancy-specific and non-pregnancy-specific VSA. Antibodies that recognize VSA and opsonize parasitized erythrocytes (pRBCs) 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. (Chandrasiri et al. 2014). Heat inactivated plasma samples of both enrollment and 36 weeks gestation samples were assayed in duplicates 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 VSAs).

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 Corporation, Carlsbad, CA92008, USA) 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.

Measuring antibodies to merozoite antigens and schizont extract. Preparation of merozoite recombinant proteins and schizont extract is described above. In brief, 0.5–2.0 µg/ml of each recombinant antigen and 1:8,000 diluted crude schizont extract were coated onto 384 well high protein binding black Optiplates (PerkinElmer Inc., Waltham, MA, USA) and left overnight at 4°C. The plates were washed and non-specific binding blocked with 0.1% casein on the following day. The plates were then incubated with participant plasma samples diluted at 1:1,000 in 0.1% casein, which were assayed in triplicate. Secondary antibody Alexa fluor 488-conjugated goat anti-human IgG (Life Technologies, Australia) was used at 1:2,000 dilution to capture IgG. The fluorescence intensity of each well, which is proportional to the amount of IgG, was measured using BMG POLARstar Omega fluorimeter (BMG Labtech, Germany) in the 485-1 excitation and 520 emission spectra range. Serial samples were run on the same assay and the MFIs were reported as a percentage of the positive control standard curve following adjustment for inter- and intra-plate variability.

Determination of seropositivity. A participant is considered seropositive for an antigen if the percentage of the sample's MFI was greater than the sum of the average and 3 SD of the percentage MFI of the negative controls.

Details of statistical analysis. All statistical analyses were performed using Stata 13.0 (StataCorp LP, College Station, TX, USA). Statistical analysis was conducted according to the analysis plan written and published before the intervention code was opened. The analysis was based on the principle of modified intention-to-treat.

We compared enrollment characteristics between intervention groups by ANOVA for comparisons of means and Fisher's exact test for comparison of proportions.

To determine the mean difference in antibody levels at 36 weeks gestation between intervention groups, ANOVA was performed, followed by pair-wise group comparisons with a linear regression analysis. For the adjusted analysis, the association between antibody levels and intervention group was adjusted by a number of variables selected based on published evidence that they influence malaria antibody levels. The selected variables, along with the corresponding antibody level at enrollment, were included in the model for each antibody measure.

To compare the proportion of participants seropositive for malaria antigens between intervention groups we used Fisher's exact test. Pair-wise comparisons were made using logistic regression analysis. Unadjusted and adjusted odds ratios were reported with 95% CI and P-value.

We performed likelihood ratio tests for interaction between the intervention and maternal characteristics specified in the statistical analysis plan prior to data analysis, and would have provided stratified analyses in case of a positive interaction test ($P<0.10$). Variables tested were maternal age at enrollment, parity, maternal HIV status, maternal bed net use at enrollment, site of enrollment, and maternal malaria status at enrollment.

An association between the intervention group and antibody level with a reported P-value <0.05 was considered statistically significant.

Appendix 3. 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 EFA 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 to the clinic. The swab was placed into the participant's mouth under the tongue for 2 minutes. The swab was then placed into 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), and the two others were stored at −80°C for later bacterial analysis with DNA amplification methodology.

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 membrane 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 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 ID 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 cyrovials into a −20°C freezer and, as soon as possible, moved the swab into a −80°C freezer.

Appendix 4. Study Hypotheses

The duration of pregnancy and child size at birth:

1. The mean birth weight among infants whose mothers were provided with LNS during pregnancy is higher than among infants whose mothers received either IFA or MMN supplementation.
   - As a secondary analysis (for this and to all other items below), we will also test hypotheses about differences between the MMN and IFA groups.
2. The mean placental weight among women who were provided with LNS during pregnancy is higher than among women who received either IFA or MMN supplementation.
3. The proportion of LBW infants is lower among women who are provided with LNS during pregnancy than among women who receive either IFA or MMN supplementation.
4. The mean newborn LAZ is higher among infants whose mothers are provided with LNS during pregnancy than among infants whose mothers received either IFA or MMN supplementation.
5. The prevalence of newborn stunting (LAZ <−2) is lower among infants whose mothers are provided with LNS during pregnancy than among infants whose mothers received either IFA or MMN supplementation.
6. The mean duration of pregnancy among women who are provided with LNS during pregnancy is longer than among women who receive either IFA or MMN supplementation.
7. The incidence of preterm birth is lower among pregnant women who are provided with LNS during pregnancy than among pregnant women who receive either IFA or MMN supplementation.
8. The incidence of being a small-for-gestational-age baby is lower among infants whose mothers are provided with LNS during pregnancy than among infants whose mothers receive either IFA or MMN supplementation.
9. The mean newborn WAZ is higher among infants whose mothers were provided with LNS during pregnancy than among infants whose mothers received either IFA or MMN supplementation.
10. The mean newborn MUAC is higher among infants whose mothers were provided with LNS during pregnancy than among infants whose mothers received either IFA or MMN supplementation.
11. The mean newborn HCZ is higher among infants whose mothers were provided with LNS during pregnancy than among infants whose mothers received either IFA or MMN supplementation.
12. The prevalence of various forms of malnutrition (underweight, acute malnutrition, small head circumference) is lower among infants whose mothers were provided with LNS during pregnancy than among infants whose mothers received either IFA or MMN supplementation.

Maternal weight gain during pregnancy and placental size:

13. Women who receive LNS during pregnancy will have higher mean weekly change in weight compared to the IFA and MMN groups.
14. The proportion of women with placental weight below the 10th percentile of a reference population's placental weight for gestational age and birth weight will be lower among women who received LNS than among women who received either MMN or IFA.
15. The proportion of women with placental weight below the 10th percentile of a reference population's placental-weight-to-birth-weight ratio will be lower among women who received LNS than among women who received either MMN or IFA.

Maternal asymptomatic malaria infections at 32 and 36 gw and at delivery:

16. The prevalence of maternal malaria parasitemia at 32 gw (RDT) will be lower among women who received LNS than among women who received either IFA or MMN.
17. The prevalence of maternal malaria parasitemia at 36 gw (PCR) will be lower among women who received LNS than among women who received either IFA or MMN.
18. The prevalence of maternal malaria parasitemia at delivery (RDT and PCR) will be lower among women who received LNS than among women who received either IFA or MMN.

Maternal reproductive tract infections and urinary tract infections at delivery:

19. The prevalence of trichomoniasis at 1 week after delivery will be lower among women who received LNS than among women who received either IFA or MMN.
20. The prevalence of UTIs will be lower among women who received LNS than among women who received either IFA or MMN.

Maternal plasma CRP and AGP concentrations:

21. At 36 gw, the prevalence of elevated plasma concentration of CRP or AGP will be greater in the IFA group than in the MMN or LNS group.

Maternal blood Hb, ZPP, and sTfR concentrations:

22. Women who receive IFA during pregnancy will have higher mean blood Hb and lower plasma sTfR and ZPP at 36 gw compared to the MMN and LNS groups.
23. The percentage of women with Hb <100 g/L will be lower in the IFA group at 36 gw compared to the MMN and LNS groups.
24. The percentage of women with Hb >130 g/L will be higher in the IFA group at 36 gw compared to the MMN and LNS groups.
25. The percentages of women with elevated ZPP will be lower in the IFA group compared to the MMN and LNS groups.
26. The percentages of women with elevated sTfR will be lower in the IFA group compared to the MMN and LNS groups.

Maternal plasma retinol concentration:

27. Women receiving LNS during pregnancy will have higher plasma retinol concentration at 36 weeks gestation compared with those receiving either MMN or IFA.
28. Women receiving MMN during pregnancy will have higher plasma retinol concentration at 36 weeks gestation compared with those receiving IFA.
29. Women receiving LNS during pregnancy will have a lower prevalence of plasma retinol <1.05 µmol/L at 36 weeks gestation compared with those receiving either MMN or IFA.
30. Women receiving MMN during pregnancy will have lower prevalence of plasma retinol <1.05 µmol/L at 36 weeks gestation compared with those receiving IFA.

Maternal plasma vitamin B12, folate, and tHcy concentrations:

31. Plasma B12 will be higher at 36 weeks gestation in women who received either MMN or LNS compared to those who received IFA.

32. There will be no difference among intervention groups in plasma folate at 36 gw.
33. Plasma tHcy will be lower at 36 weeks gestation in women who received either MMN or LNS compared to those who received IFA.

Maternal plasma cholesterol and triglyceride concentrations and plasma EFA composition:

34. Women who receive LNS during pregnancy will have higher mean total cholesterol and triglyceride concentrations, higher fatty acid compositions (AA, ALA, and DHA), and a lower prevalence of low total cholesterol (<10th percentile of IFA group) in plasma at 36 weeks gestation compared to the IFA and MMN groups.

Maternal salivary cortisol concentration:

35. Women who receive LNS during pregnancy will have a lower mean salivary cortisol concentration at 28 weeks and 36 weeks gestation compared to the MMN and IFA groups.
36. Women who receive LNS during pregnancy will have a lower prevalence of high salivary cortisol at 28 weeks and 36 weeks gestation compared to the MMN and IFA groups.

Histological signs of inflammation and malaria in the placenta and fetal membranes:

Not applicable; we did not have predefined hypotheses for this substudy.

Placental, fetal membrane, oral, and vaginal microbiomes:

Not applicable; we did not have predefined hypotheses for this substudy.

Maternal oral health:

Not applicable; we did not have predefined hypotheses for this substudy.

Malaria immunity in pregnancy:

37. Lipid-based nutrient supplementation during pregnancy increases antibody responses to malaria at 36 weeks gestation compared to MMN supplementations and IFA supplements.

Appendix 5. Detailed Variable Definitions

α-1-glycoprotein (AGP), 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.

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.

Bacterial prevalence in the placental or fetal membrane tissue
Presence of bacteria was defined by a positive result after 16S rDNA broad-range SYBR green quantitative PCR assay. A positive result was classed as a level of fluorescence above the lower limit of detection (50 cfu/µl) of the assay.

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 two decimals.

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.

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
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 infection, placental malaria, active-acute
Active-acute malarial infection was defined as presence of malarial parasites in intervillous erythrocytes.

Malaria infection, placental malaria, active-chronic
Active-chronic malarial infection was defined as presence of malarial pigment in free macrophages.

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, with two decimals.

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. The proportion is expressed with one decimal point.

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, with two decimals.

Stunting was defined as LAZ <−2. The prevalence of stunting was calculated by dividing the number of babies with LAZ <−2 by the number of all babies with valid data on this outcome. The values are expressed as a percentage, with one decimal.

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, with two decimals.

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, with one decimal.

Omega-6:omega-3 ratio, fatty acids
The ratio was defined as the sum of all omega-6 fatty acids divided by the sum of all omega-3 fatty acids.

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.

Periodontitis, clinical periodontitis
Clinical periodontitis was diagnosed independently from radiological data. For this study, a participant was defined to have clinical periodontitis if at least three teeth with periodontitis were diagnosed in clinical examination. Third molars (wisdom teeth) were excluded from the periodontitis diagnosis.

Periodontitis, mean periodontal pocket depth and mean number of periodontal pockets
Periodontal pocket depth was measured clinically from six sites of each tooth and the deepest measurement for each tooth was recorded, rounded to the nearest millimeter. The mean periodontal pocket depth for each participant was then calculated and expressed in millimeters. When calculating the mean number of periodontal pockets in a participant, a periodontal pocket was defined as one that was ≥4 mm. 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.

Rate of change in antibody levels
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:

$$rate\ of\ change\ in\ antibody\ levels = \frac{antibody\ levels\ at\ 36\ gw - antibody\ levels\ at\ enrollment}{number\ of\ gestation\ weeks\ from\ enrollment\ to\ 36\ gw}$$

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.

Seroprevalence to malaria antigens
Number of individuals with seropositivity to a malaria antigen (details on calculating seropositivity included in general methods).

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 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. Outcome Variables

Section	Subject	Variable	Reported statistics	Timing
4.9	mother	AA, % total fatty acids	mean, SD, prevalence of high AA	36 gw
4.5	mother	AGP, g/L	mean, SD, proportion >1 g/L	enrollment, 36 gw
4.9	mother	ALA, % total fatty acids	mean, SD, prevalence of high ALA	36 gw
4.14	mother	Antibodies, opsonizing to non-pregnancy-specific VSA	mean concentration, SD, seropositivity	36 gw
4.14	mother	Antibodies, opsonizing to pregnancy-specific VSA	mean concentration, SD, seropositivity	36 gw
4.14	mother	Antibody, EBA-175	mean concentration, SD, seropositivity	36 gw
4.14	mother	Antibody, MSP-1 19k	mean concentration, SD, seropositivity	36 gw
4.14	mother	Antibody, MSP-2	mean concentration, SD, seropositivity	36 gw
4.14	mother	Antibody, MSP-3	mean concentration, SD, seropositivity	36 gw
4.14	mother	Antibody, Rh2A9	mean concentration, SD, seropositivity	36 gw
4.12	placenta	Bacterial diversity, UniFrac distance in fetal membranes	mean, SD	delivery
4.12	placenta	Bacterial diversity, UniFrac distance in placental tissue	mean, SD	delivery
4.12	mother	Bacterial diversity, UniFrac distance in the oral cavity	mean, SD	1 wk after delivery
4.12	mother	Bacterial diversity, UniFrac distance in the vagina	mean, SD	1 wk after delivery
4.12	placenta	Bacterial load in the fetal membranes, rDNA copies/µl	mean, SD, prevalence	delivery
4.12	placenta	Bacterial load in the placenta, rDNA copies/µl	mean, SD, prevalence	delivery
4.1	child	Birth weight, g	mean, SD, incidence of LBW, prevalence of newborn underweight	delivery
4.6	mother	Blood Hb, g/L	mean, SD, change, prevalence of abnormal Hb	enrollment, 36 gw
4.9	mother	Cholesterol, mg/dL	mean, SD, prevalence of low cholesterol	36 gw
4.11	placenta	Chorioamnionitis of the amniotic membrane	prevalence	delivery
4.11	placenta	Chorioamnionitis of the amniotic membrane, severe	prevalence	delivery
4.11	placenta	Chorioamnionitis of the chorionic plate	prevalence	delivery
4.11	placenta	Chorioamnionitis of the chorionic plate, severe	prevalence	delivery
4.10	mother	Cortisol, µmol/L	mean, SD, prevalence of low and high cortisol	28 gw, 36 gw
4.9	mother	DHA, % total fatty acids	mean, SD, prevalence of high DHA	36 gw
4.1	child	Duration of pregnancy, weeks	mean, SD, incidence of preterm	delivery

Section	Subject	Variable	Reported statistics	Timing
4.13	mother	Gingivitis	prevalence	36 gw
4.6	mother	IDA	prevalence	enrollment, 36 gw
4.11	placenta	Intervillositis, acute	prevalence	delivery
4.11	placenta	Intervillositis, chronic	prevalence	delivery
4.11	mother	Malarial infection, active-acute	prevalence	delivery
4.11	mother	Malarial infection, active-chronic	prevalence	delivery
4.11	mother	Malarial infection, past	prevalence	delivery
4.3	mother	Maternal malaria parasitemia	prevalence	32 gw, 36 gw, delivery
4.2	mother	Maternal weight gain, kg/week	mean, SD, proportion of weight gain below recommended gain	enrollment, 32 gw, 36 gw
4.1	child	Newborn HCZ	mean, SD, prevalence of small head circumference	delivery
4.1	child	Newborn length, cm	mean, SD	delivery
4.1	child	Newborn LAZ	mean, SD, prevalence of newborn stunting	delivery
4.1	child	Newborn MUAC	mean, SD	delivery
4.1	child	Newborn WAZ	mean, SD, incidence of small for gestational age	delivery
4.13	mother	No. of any (grade II–III) caries lesions	mean, SD, prevalence	36 gw
4.13	mother	No. of grade II caries lesions	mean, SD, prevalence	36 gw
4.13	mother	No. of grade III caries lesions	mean, SD, prevalence	36 gw
4.13	mother	No. of periapical lesions	mean, SD, prevalence	36 gw
4.13	mother	No. of periodontal pockets ≥4 mm (clinical)	mean, SD	36 gw
4.13	mother	No. of sextants with bleeding on probing	mean, SD	36 gw
4.9	mother	Omega-6:omega-3 ratio	mean, SD, prevalence of high omega-6:omega-3 ratio	36 gw
4.10	mother	Perceived Stress Score	mean, SD	28 gw, 36 gw
4.13	mother	Periodontal pocket depth, mm	mean, SD	36 gw
4.13	mother	Periodontitis	prevalence	36 gw
4.13	mother	Periodontitis, clinical	prevalence	36 gw
4.2	placenta	Placental weight by birth weight	number, percent below 10th percentile	delivery
4.2	placenta	Placental weight by gestation age	number, percent below 10th percentile	delivery
4.2	placenta	Placental-weight-to-birth-weight ratio	number, percent below 10th percentile	delivery

Section	Subject	Variable	Reported statistics	Timing
4.2	placenta	Placental weight, g	mean, SD	delivery
4.8	mother	Plasma B12, pmol/L	mean, SD, proportion <150 pmol/L, proportion <225 pmol/L	enrollment, 36 gw
4.5	mother	Plasma CRP, mg/L	mean, SD, proportion >5 mg/L	enrollment, 36 gw
4.8	mother	Plasma folate, µmol/L	mean, SD, proportion <10 µmol/L	enrollment, 36 gw
4.7	mother	Plasma retinol, µmol/L	mean, SD, proportion <1.05 µmol/L	enrollment, 36 gw
4.8	mother	Plasma tHcy, mmol/L	mean, SD, proportion >10 mmol/L	enrollment, 36 gw
4.14	mother	Schizont extract	mean concentration, SD, seropositivity	36 gw
4.6	mother	sTfR, mg/L	mean, SD, change, prevalence of abnormal sTfR	enrollment, 36 gw
4.14	mother	Total IgG to pregnancy-specific VSA	mean concentration, SD, seropositivity	36 gw
4.4	mother	Trichomoniasis	prevalence	delivery
4.9	mother	Triglycerides, mg/dL	mean, SD	36 gw
4.4	mother	UTIs	prevalence	delivery
4.6	mother	ZPP, µmol/mol heme	mean, SD, change, prevalence of abnormal ZPP	enrollment, 36 gw