MALARIA AND MALNUTRITION DURING PREGNANCY:
AN INVESTIGATION OF INTERACTIONS AND INTERVENTIONS

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ABSTRACT

Jordan Emile Cates: Malaria and Malnutrition during Pregnancy: An Investigation of
Interactions and Interventions.
(Under the direction of Daniel J. Westreich)

Malnutrition and malaria infection commonly co-exist, afflicting pregnant women in
resource-poor settings and increasing the risk of a low birthweight (LBW) infant. By 2025,
WHO targets a reduction in the incidence LBW by 30%. Previously, four studies indicated that
the effect of malaria infection on the risk of LBW may depend upon maternal nutritional status.

We evaluated the interaction between maternal malaria infection and maternal
anthropometric status on the risk of LBW using data from 14,633 pregnancies from 13 studies
conducted in Africa and the Western Pacific. Study-specific adjusted effect estimates were
calculated using inverse probability of treatment-weighted linear and log-binomial regression
models and pooled using a random effects model. Using parametric g-formula, we estimated
population-attributable effects and generalized intervention effects for differences in the
incidence of LBW expected under hypothetical malaria and malnutrition interventions.

The adjusted risk ratio (aRR) for delivering a baby with LBW was 1.14 (95% CI: 0.91,
1.42) among women with malaria infection at antenatal enrollment, 1.32 (95% CI: 1.08, 1.62)
among women with malaria infection at delivery, and 1.60 (95% CI: 1.36, 1.87) among women
with low mid-upper arm circumference at enrolment (MUAC <23cm). The joint aRR for women
with both malaria infection and low MUAC at enrollment was 2.13 (95% CI: 1.21, 3.73;

N=8,152). There was no evidence of synergism between malaria infection and MUAC on the multiplicative (p=0.5) or additive scale (p=0.9). We estimated that, compared to the current patterns of IPTp use in the study population, increasing every woman's dosage of IPTp to at least three doses would result in a relative decrease in the incidence of LBW of 34% (95% CI: 25%, 43%). The intervention effects for malaria at delivery, low MUAC in early pregnancy, and bed nets were all modest.

Pregnant women with malnutrition and malaria infection are at increased risk of LBW, but malaria and malnutrition do not act synergistically. Scale up of IPTp, alone or in tandem with other antenatal interventions, could help achieve the WHO's Global Nutrition Target of a 30% reduction in LBW by 2025.

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LIST OF ABBREVIATIONS

BMI	Body mass index
BW	Birth weight
CI	Confidence interval
DAG	Directed acyclic graph
DRC	Democratic Republic of the Congo
DNA	Deoxyribonucleic acid
EMM	Effect measure modification
FGR	Fetal growth restriction
IPTW	Inverse probability of treatment weights
HIV	Human immunodeficiency virus
LBW	Low birthweight
LM	Light microscopy
LMIC	Low- and middle-income countries
M3	Maternal Malaria and Malnutrition
MUAC	Mid-upper arm circumference
PCR	Polymerase chain reaction
PEI	Population effects interval
PNG	Papua New Guinea
RDT	Rapid diagnostic test
RR	Risk ratio
SGA	Small-for-gestational age
WHO	World Health Organization.

CHAPTER I: SPECIFIC AIMS

In malaria endemic countries, up to one in four pregnant women are infected with malaria during pregnancy (1). **A major consequence of maternal malaria infection is low birth weight** (1–3). Malaria doubles the risk of low birth weight (LBW) and may contribute to up to 25% of LBW infants in endemic countries (2,4). LBW can have a profound impact on neonatal mortality and childhood morbidity, affecting multiple aspects of mental, metabolic, and anthropometric development (5). These risks persist despite scaling up of malaria prevention programs targeted towards pregnant women, such as intermittent preventive therapy during pregnancy (IPTp) and bed-net campaigns (1,2,5,6). Consequently, there is an urgent need to understand the effects of malaria on the developing fetus and how malaria prevention during pregnancy can improve pregnancy outcomes (7).

In resource-poor settings, malaria infection and malnutrition commonly co-occur in pregnant women. Recent evidence indicates that the harmful impact of malaria on fetal growth and LBW may depend upon the nutritional status of the mother (8–10). In a few small studies conducted in malaria endemic areas, malaria increased the risk of LBW only among malnourished women, and not among well-nourished women (8,9), however two additional studies found contradictory results (11,12). One study found that the risk of intrauterine growth restriction associated with malaria was consistently two to eight times higher among women with evidence of macronutrient malnutrition (8). Not only are these studies somewhat inconsistent in their findings, but our confidence in their interpretation is hindered by

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their small sample size and potential lack of generalizability. Understanding the potential synergistic relationship between malaria and malnutrition could identify possible pregnancy interventions that improve fetal growth and prevent infant morbidity and mortality.

Furthermore, it is unclear how hypothetical, realistic interventions to improve both maternal nutrition and prevent malaria could impact population-level pregnancy outcomes. State-of-the-art epidemiologic methods exist that allow us to model the impact of plausible targeted antimalarial and malnutrition interventions during pregnancy could have on the number of LBW infants in malaria endemic areas (13–15).

We conducted a pooled analysis utilizing data from 14,633 singleton, live birth pregnancies across thirteen completed studies conducted in malaria-endemic areas (Africa and Western Pacific) (Aim 1). We also used this pooled data to implement statistical modeling to explore the impact of hypothetical targeted antimalarial and nutritional interventions on population-level pregnancy outcomes (Aim 2).

Specific Aim 1: To investigate the joint effects of malaria infection and maternal malnutrition during pregnancy on the risk of LBW. We hypothesized that there would be a synergistic interaction between malaria infection and malnutrition in causing LBW, such that the observed joint effect of being both infected with malaria and malnourished would be greater than expected if considering each exposure independently.

Specific Aim 2: To explore the impact of realistic interventions to improve maternal nutritional status and targeted antimalarial interventions on population-level pregnancy outcomes. We simulated multiple plausible interventional contrasts using the parametric g-formula to assess the impact of targeted antimalarial and nutrition interventions.

This work has *significant* impact on public health by providing evidence on the malaria-malnutrition-LBW relationship and the effect of hypothetical interventions on this relationship. Our study is *innovative* in its utilization of robust extant data and exploration of the effect of realistic interventions. These results identified effective interventions to help address the burden of adverse pregnancy outcomes and improve clinical practice among this population.

CHAPTER II: BACKGROUND AND SIGNIFICANCE

Low Birthweight

Annually, over 20 million infants are born with low birthweight (LBW), defined as birth weight less than 2,500 grams (16). This constitutes 15% to 20% of all births worldwide. LBW is of global health concern, but low- and middle-income countries (LMICs) are disproportionately affected, with estimates as high as 28% in south Asia and 13% in sub-Saharan Africa (16). One recent study that accounted for potential underestimation of LBW due to deliveries in homes or smaller health clinics estimated that approximately 32.4 million babies were born small-for-gestational age (SGA) in 2010, representing 27% of all births in LMICs (17).

Low birthweight (LBW) is associated with a nine-fold increase in neonatal mortality (death within the first month) and a three-fold increase in infant mortality (death within the first year) (18,19). Thus, even though the prevalence of LBW is only 15-20% worldwide, it is estimated that LBW babies account for 60-80% of neonatal deaths (20). In addition to the contribution to infant mortality, LBW leads to long-term morbidities, specifically affecting mental, metabolic, and anthropometric development (16). Behavioral development, demonstrated by school performance and academic achievement, has been negatively associated with LBW (19). Because of these negative health associations, the World Health Organization (WHO) has included a 30% reduction in LBW as one of the 2025 Global Nutrition Targets (16). As of 2014, the Global Nutrition Report found that there was little progress towards this target (21). Interventions in LMICs to improve fetal growth have the potential to produce substantial public

health effects, improving a wide array of factors ranging from cognitive development to enhanced neonatal survival, assuming causality between LBW and these factors.

Birth weight is easily measured with maximal precision, making it a feasible marker of high-risk infants in low-resource settings. However, birth weight is not without its own limitations. Many LMICs use Salter Scales with variable precision, and infants born at home are often not weighed at all. Historically LBW has been dichotomized at less than 2500 grams based on evidence of marked mortality among this high-risk group of infants (3). LBW does not correct for gestational age at birth, and can result from premature delivery of the infant, intrauterine growth restriction (IUGR), or both (3,22,23). Although IUGR and preterm birth share common risk factors, they are two distinct phenomena with differing etiology, and LBW does not differentiate between the two (3). One recent study estimated that approximately half of LBW babies in low- to middle-income countries are preterm (23), higher than previously assumed (24). When reliable estimates of gestational age are available, SGA is often used as a proxy for IUGR. SGA is defined as a birth weight below the 10th compared to other infants at the same gestational age using a published and validated referent (3,25,26). However, diagnosis of SGA, as well as preterm birth, requires reliable gestational age assessment, preferably using ultrasound pregnancy dating, which limits its feasibility as a measure in most resource-limited settings.

Malaria during pregnancy

Extent and impact of malaria infection during pregnancy

Malaria infection during pregnancy affects more than 25 million pregnant women annually, producing detrimental effects on maternal, newborn, and infant health (1–3). Over 125 million pregnant women are at risk for malaria in areas of stable and unstable P. falciparum

and/or P. vivax malaria transmission in most parts of Africa and select areas of Central and South America, Asia, and Western Pacific regions (4).

A major consequence of maternal malaria infection is LBW (1–3). The most predominant malaria parasite species, *Plasmodium falciparum,* has the unique capability of sequestering in the placenta, causing adverse sequelae (27). In low transmission settings, pregnant women typically present with severe, symptomatic disease when infected with malaria, resulting in maternal and infant mortality. However, in malaria-endemic high-transmission areas, maternal mortality due to malaria is less frequent (although still exists) due to increased maternal immunity, but infection remains frequent and deleterious for fetal growth and preterm delivery (27,28). In malaria-endemic settings, 20-50% of LBW has been attributed to malaria infection during pregnancy (1,2,19). Malaria doubles the risk of LBW, and that effect is even greater among women who are pregnant for the first time (primigravid) or second time (secundigravid) (1). Immunological studies have identified that sera from the placenta of multigravid women, but not primi- or secundigravid women, are able to bind/aggregate parasites (19). This physiological phenomenon of parity-specific immunity supports the epidemiologic studies identifying greater susceptibility and harmful impact of malaria among primi- and secundigravid women. In addition to gravidity, HIV infection also modifies the effect of malaria on LBW, with a larger impact of malaria on LBW found among HIV-infected women (1,29). HIV further increases the risk of placental malaria, febrile illness, and higher parasitic densities (1,29). However, there are conflicting results as to whether or not placental malaria increases the risk of mother to child transmission of HIV (1).

There are five species of malaria-causing *Plasmodium* parasites (*falciparum, vivax, ovale, malariae, and knowlesi*) that can infect pregnant women. *P. falciparum* is the most virulent

because it cytoadheres (sticks) to capillary endothelium and the placental syncytiotrophoblast (27). Most of the severe effects on maternal and neonatal outcomes are associated with *P. falciparum* infection, although some research has also assessed the adverse consequences of *P. vivax* infection (1). While *P vivax* found in peripheral blood of pregnant women has been associated with LBW, the magnitude of effect is not as great as with *P. falciparum* (1). It has been postulated that this reduced harmful effect is due to a lack of cytoadherence of *P. vivax* in the placental (1). However, more research is needed regarding the differential effects of malaria infection on adverse birth outcomes by *Plasmodium* species.

Malaria prevention programs during pregnancy

The knowledge that malaria infection during pregnancy induces negative maternal and fetal sequelae is not new, with research dating back to 1899 (30). The first trial aimed at preventing malaria during pregnancy using chemoprevention was in 1957, comparing monthly pyrimethamine to placebo (31). Since then, there have been over fifty trials assessing the effectiveness of various chemoprevention drugs, regimens, and strategies (31). The current WHO Policy Recommendations for malaria prevention during pregnancy involve three separate evidence-based strategies: insecticide treated bed nets (ITN), intermittent preventive therapy during pregnancy (IPTp), and effective case management (32). The current strategy for IPTp involves monthly doses of sulfadoxine/pryimethamine (SP) starting in the second trimester (32). However, the IPTp regimen was only recently changed in 2007, when monthly IPTp-SP replaced the prior WHO recommended IPTp schedule of one curative dose of SP in the second trimester and then a second dose in the third trimester (with a third dose recommended for HIV-infected women) (33). The recommendation change in 2007 which increased the dosage to monthly doses

was spurred by a meta-analysis showing added benefit of additional doses (34). An updated 2014 Cochrane Review summarized data from eight randomized and quasi-randomized trials that effective chemoprevention of malaria with chloroquine prophylaxis or IPT reduced the risk of LBW by 27% (RR 0.73, 95% CI 0.61 to 0.87) in primi- and secundigravid women (31). This evidence is consistent with other observational and trial data (9,35,36). Another malaria prevention strategy, replacing IPTp- with intermittent screening for malaria infection (ISTp) at ANC visits using rapid diagnostic tests (RDTs) and treating infected women with dihydroartemisin-piperaquine, is currently being investigated(37,38). However, analyses from these ISTp studies suggest that ISTp is not a good alternative to IPTp (37).

Despite an increase in the proportion of women receiving IPTp, the level of coverage remains inadequate (39–41). In 2013, an estimated 43% of the 35 million eligible pregnant women did not receive even a single dose of IPTp (41). Emerging parasitic resistance to important antimalarial medications, and particularly multi-drug resistance, presents another major concern for malaria prevention and treatment during pregnancy (42). The risk of infection in between IPTp doses may still be high, particularly in areas of low ITN usage and high sulphadoxine-pryimethamine resistance (28). In light of these limitations to malaria prevention during pregnancy, there is a need to explore alternative approaches to improving pregnancy outcomes, particularly in areas where malaria is endemic (43).

Malnutrition during pregnancy

Extent and impact of malnutrition during pregnancy

Another modifiable risk factor for impaired fetal growth and subsequent LBW is maternal malnutrition (44,45). The prevalence of low body mass index (BMI), a marker of

malnutrition, reaches as high as 20% among African women of reproductive age (17,46).

Malnutrition is a heterogeneous condition dependent upon macronutrient deficiency (carbohydrates, fats, proteins), micronutrient deficiencies (vitamins, minerals, trace elements, phytochemicals, and antioxidants), or both (46). The relationship between malnutrition and adverse birth outcomes is complex. Optimal fetal growth is dependent upon sufficient maternal dietary intake of macro- and micronutrients (particularly iron and folate) (44,47). However, micronutrient deficiencies are often difficult to measure in resource-limited settings, thus anthropometric measures of body composition are often assessed as approximate indicators of maternal malnutrition. Typical maternal anthropometric measures of malnutrition in resource-limited settings are BMI or mid-upper-arm-circumference (MUAC). BMI and MUAC are indicators of maternal fat stores, which are reflective of maternal energy balance, and low values are a risk factor for poor pregnancy outcomes (17,44,48,49). Additionally, BMI and MUAC are correlated with maternal leptin levels, an adipose-tissue secreted protein, which may regulate fetal growth, although the effects of low levels of leptin remain unknown (50,51). Henceforth, we will use the term 'malnutrition' to indicate approximate nutrient deficiencies measured using anthropometric measures.

A WHO collaborative study in 1996 reported an association between measures of maternal body size, such as height, weight, and BMI, and LBW, SGA, and preterm birth (48). A systematic review and meta-analysis in 2011 reported that underweight women have increased risks of preterm birth (aRR: 1.29 [95% CI: 1.15-1.46]) and LBW (aRR: 1.64 [95% CI: 1.38-1.94]), although the definitions of underweight varied depending on the original study definitions (52). More recently, results from studies conducted in LMICs suggest that maternal malnutrition may be an independent risk factor for LBW and SGA. A 2015 study in Papua New Guinea

(PNG) found that maternal malnutrition, as measured by MUAC, negatively affected fetal growth (11). Additionally, in the Democratic Republic of the Congo (DRC) low MUAC was associated with IUGR; in Thailand a low BMI was negatively associated with biparietal diameter z-scores in univariate analyses; and in Tanzania low MUAC was associated with lower infant anthropometric measurements (53–55). Due to the difficulties of accurately measuring malnutrition during pregnancy, some researchers have used pre-pregnancy anthropometrics as a proxy indicator of gestational malnutrition. A systematic review and meta-analysis of pre-pregnancy anthropometrics found that pre-pregnancy underweight, compared with normal-weight mothers, were at increased risk of SGA (OR: 1.81 [95% CI: 1.76-1.87) and LBW (OR: 1.47 [95% CI: 1.27-1.71]) (56). Taken collectively, this information suggests that adequate maternal nutrition is integral for fetal health and development in LMICs (57).

Malnutrition interventions during pregnancy

Due to this association between maternal anthropometric measures and fetal development, a number of nutritional interventions targeted at pregnant women have been explored to optimize birth outcomes. Studies assessing actual dietary consumption have found that insufficient maternal protein intake is associated with LBW, especially in early pregnancy (44). A meta-analysis in 2011 summarized that balanced protein energy supplementation lead to a 31% relative reduction in the risk of SGA, and that this effect was even more pronounced among women malnourished at baseline (58). Most notably, a randomized control trial (RCT) in rural Gambia in the early 90s found that prenatal dietary supplementation targeting chronically undernourished pregnant women was associated with substantially lower odds of a LBW infant (OR=0.61 [95% CI: 0.47-0.79]) (59).

However, debate remains regarding whether dietary supplementation during pregnancy is beneficial overall and should be scaled up in LMICs. Recently, two RCTS in Ghana and Malawi, as part of the International Lipid-Based Nutrient Supplements Project (iLiNS), assessed the effect of lipid-based nutrient supplements (LNS) for pregnant women on fetal growth. These two studies, with harmonized study designs, had contradictory results. In Ghana, but not in Malawi, prenatal LNS supplementation had a positive effect on fetal growth among first-time mothers (birth weight 237 grams greater in LNS arm among primigravid) (60).

Malaria and malnutrition during pregnancy

Background studies

Recent evidence indicates that the relationship between malaria and LBW may depend upon the nutritional status of the mother (51). Two studies addressing the harmful effect of malaria on IUGR and subsequent LBW found a significant association between malaria and LBW among malnourished pregnant women, but no-to-minimal association among well-nourished women (8,9), however two additional found inconsistent results (11). First, a small study published in 2009 of 177 Congolese women by Sarah Landis and colleagues showed that the risk of IUGR was two to eight times higher among women with evidence of malnutrition (8). In this study, the magnitude of the difference in effect sizes depended on the measurement for malnutrition (BMI vs MUAC vs short stature vs weight gain) and the measurement for malaria (cross-sectional versus cumulative); however, with all measurements of malnutrition and malaria the pattern and directionality of the modification was consistent. In a second study of 477 Kenyan pregnant women published by Elizabeth McClure and colleagues in 2014, there was no statistically significant association between malaria and birth weight among pregnant women

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with normal BMI, but among those with low BMI the mean difference in birth weight associated with malaria infection was -370 g (95% CI: -728, -12) (9). However, there could have been potential publication bias in these studies if they did not report findings when there was a lack of modification or interaction.

A third cohort study published by Holger Unger and colleagues among Papua New Guinean women found contrary results. In this study, there was no modification of the effect of peripheral malaria on SGA by maternal anthropometric measures (N=671) (11). However, a subsequent unpublished analysis by the same group did find modification such that that the risk of LBW associated with active placental infection was highest among women with low BMI (Holger Unger, personal communication, June 15, 2015). However, modification went in the other direction when using MUAC (risk of LBW due to placental malaria was increased among women with normal MUAC and not among undernourished women). In a fourth study in Benin, the effect of malaria infection on fetal growth velocity was greatest among women with low anthropometric status, but there was no modification of the effect of malaria infection on birthweight z-scores (N=735) (12). Collectively, these studies suggest that there may be a synergistic relationship between malnutrition and malaria with regards to the risk of LBW, but further investigation is warranted.

The biologic rationale surrounding malaria, malnutrition, and low birthweight

The interplay between malaria and malnutrition with regards to LBW is supported by well-defined biologic rationale. The adverse intrauterine environment caused by malaria infection restricts sufficient placental delivery of nutrients to the fetus (2). *P falciparum* expresses a pregnancy-specific antigen variant, VAR2CSA, which enables the sequestration of

malaria-infected erythrocytes in the placenta (28). *P falciparum* infected red blood cells adhere predominately to the molecule chondroitin sulphate A in the intervillous area of the placenta (27). While *P vivax* has not been found to adhere to the placenta, it is still associated with increased placental inflammation and altered placental function (2,27). It is hypothesized that malaria-infected red blood cells and inflammatory products obstruct the placenta, alter uterine vascular function, and cause dysregulation of pregnancy-associated growth-regulating hormones (2,3,61). These sequelae of malaria infection can cause both IUGR and preterm birth (27). Furthermore, malaria can also cause anemia, which itself can have deleterious effects on fetal development (2).

While IUGR and preterm birth may both result from infection, they may also result from limited availability of maternal nutrients (4). Maternal nutritional status influences the availability and supply of nutrients to the developing fetus (62). Malaria and malnutrition may act along similar physiological pathways by decreasing maternal-fetal oxygen and nutrient transfer and reducing uteroplacental blood flow, interfering with optimal fetal growth (2,4). The preliminary findings from the above-mentioned epidemiologic studies are thus further supported through research on the biologic mechanisms causing adverse birth outcomes. However, it still remains necessary to corroborate these findings with larger, robust, epidemiologic analysis. Further evidence of true synergism could provide insight into the pathogenesis of malaria in pregnancy.

Overview, limitations of the current evidence

The major limitation of prior work is that it comes from only three small cohorts from three countries, with two studies reporting a strong synergistic interaction and the third reporting no interaction. Not only are these studies somewhat inconsistent in their findings, but our

confidence in their interpretation is hindered by their small sample size and potential lack of generalizability: none of the prior studies included HIV-infected pregnant women. The prevention and treatment of both malaria and HIV is complicated by immunologic, clinical, and therapeutic interactions between these two, potentially highly prevalent, infections (63). HIV-infected women represent an important group to assess the interaction between malaria and malnutrition with regards to LBW.

A potential synergistic relationship between malaria and malnutrition could have important implications for possible pregnancy interventions to improve fetal growth and prevent infant morbidity and mortality. Malnutrition, while highly prevalent in malaria-endemic countries, is modifiable and thus represents an ideal candidate for potential public health interventions, especially in early pregnancy. Mathematical modeling results assessing the effect of targeted ITN interventions and food supplementation for undernourished children suggests these targeted interventions could have substantial impact on malaria mortality and morbidity in children (64). However, assessments of hypothetical targeted interventions among pregnant women have not been addressed. Presently, over 20 million LBW infants annually are born (4). Improving our understanding of the joint effects of nutrition and malaria would help tackle this overwhelming health issue from a new, and actionable, perspective.

Aim 1: Exposure Question

Specifically, in this dissertation I investigated the joint effects of malaria infection and malnutrition during pregnancy with regards to increasing the risk of LBW. This aim focuses on the etiologic interaction between these two risk factors for LBW, and I hypothesized that there will be a net increase in the average estimated risk of LBW associated with joint exposure to

malaria and malnutrition compared to the sum of the average risks for each exposure independently (synergism).

Aim 2: Intervention Question

Furthermore, the main decision that public health officials may need to make is whether implementing or scaling up nutritional and antimalarial interventions during pregnancy can have a substantial public health impact on pregnancy outcomes in malaria-endemic countries. Specifically, in this dissertation I investigated the impact of implementing targeted antimalarial and nutritional interventions on population-level pregnancy outcomes. To accomplish this aim, I simulated several hypothetical interventions using the parametric g-formula.

Interventional estimates

State-of-the-art epidemiologic methods allow us to use complex, real-world data to estimate the effects of plausible public health interventions (13). Combining nutritional interventions with antimalarial interventions or targeting antimalarial interventions to malnourished women to reduce the risk of LBW has not been assessed (2), but could be highly effective and impactful for reducing fetal morbidity and mortality (1). To our knowledge, no one has calculated and reported interventional effect estimates for the impact of hypothetical malnutrition interventions in the context of malaria prevention programs or hypothetical targeted antimalarial interventions on LBW. Additionally, no one has calculated interventional effects for the expansion of existing antimalarial interventions, such as intermittent prophylaxis therapy during pregnancy (IPTp).

Calculation of interventional effects is rarely done in epidemiologic studies, despite the utility of such results (13,65). Most epidemiologic studies exclusively estimate the effects of specific exposures. However, these exposure effect estimates may be less valuable for policy decision-makers when considering interventions or changes in clinical practice, protocols, or guidelines (13). Instead of merely providing the exposure (etiologic) effects of malnutrition and malaria with regards to their effect on LBW, we also provided estimates of the effect of potential public health interventions.

Interventional estimates can be calculated from longitudinal data by stochastically simulating different intervention scenarios (13,14,66). We used an imputation-based causal inference method called the parametric g-formula (15,67) to estimate a range of population intervention effects of plausible nutritional and antimalarial interventions. Specifically we estimated a series of generalized intervention risk differences, comparing the risk under the *observed* distribution of maternal malnutrition and malaria to the risk under a hypothetical, counterfactual setting in which specific and evidence-based candidate interventions reduce the extent of maternal malnutrition and/or malaria (13–15,66,67). The g-formula can be used to impute the missing outcomes under the unobserved, counterfactual exposure distribution that might be expected under the proposed interventions. We likewise compared the observed distribution of IPTp receipt and dosage to a counterfactual population in which these existing interventions are scaled-up.

Policy and public health implications

Provision of interventional effects in addition to exposures effects provides results that may be more immediately useful to policy-makers. For example, outputs from this work could be

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directly used in cost-effectiveness models created by health policy professionals for understanding the impact of implementing potential joint interventions on malaria and malnutrition, integrating malnutrition interventions with existing malaria prevention programs, or targeting antimalarial interventions towards women with evidence of malnutrition. Risk factors for, and interventions to prevent, LBW are commonly studied in isolation and without cross-discipline collaboration. The interventional effects could provide valuable information to policy markers regarding the scale-up of both malaria prevention and malnutrition interventions during pregnancy.

CHAPTER III: RESEARCH METHODS

Approach: Pooled Analysis

Overview

We conducted a pooled analysis of thirteen pregnancy studies conducted in malaria-endemic settings to assess the interaction between malaria infection and maternal nutritional status during pregnancy in causing LBW. Secondly, we used advanced epidemiologic methods to further analyze the pooled data and provide interventional effect estimates translatable for policy implementation. We stochastically simulate different intervention scenarios using the parametric g-formula to estimate the impact of plausible targeted antimalarial and malnutrition interventions on LBW.

Pooled data overview-study population

The data for this work came from the Maternal Malaria and Malnutrition (M3) initiative (68). The M3 initiative comprised thirteen cohort studies and RCTs conducted among pregnant women in malaria-endemic countries. Studies were selected for M3 based on the availability of a predetermined set of essential variables for each woman, adequate ethical institutional review board (IRB) clearance for this secondary analysis, and willingness of collaborators to share data. Essential variables included the assessment of malariometric indices at enrollment/first antenatal visit, assessment of anthropometric indicators of nutritional status at enrollment (MUAC and/or BMI), gravidity, type of malaria prevention used, and age. Essential delivery outcomes included birthweight and sex. With birthweight being the primary focus, we opted to restrict inclusion to women who delivered live singleton infants without congenital abnormalities, as recommended

by Rijken et al. (69). Miscarriages and stillbirths were included in the shared data from four studies for sensitivity analyses (see page 127) (37).

We identified 14,633 women who had singleton live births with no congenital abnormalities and participated in one of thirteen M3 pregnancy studies (Table 3.1). In total, seven RCTs and six prospective cohort studies performed from 1996 to 2015 in Sub-Saharan Africa (eight countries) and the Western Pacific (PNG) were included (Table 3.1, Figure 3.1).

Included studies had initially been conducted with a range of objectives. Of the seven RCTs, four (37,38,70,71) assessed the effectiveness of different pharmacological malaria prevention regimens, one additionally evaluated nutritional supplementation (72), one was designed to solely assess the effectiveness of nutritional supplementation (60), and one measured the impact of ITNs on birthweight (73). Details of antimalarials and nutritional supplementation in these studies can be found in Table 3.1. Most women had hemoglobin measurements done at first antenatal visits, and most received routine iron/folate supplements, although information on iron/folate supplements was not collected for most studies.

For ten of the thirteen studies, pregnant women were identified and recruited at one of their self-initiated antenatal care visits, usually the booking visit (8,60,70,71,74–76) but sometimes at a subsequent visit (77). The EMEP study enrolled women early in pregnancy, but a subset of these same women were also enrolled in a cross-sectional study at delivery, the IPTp-MON study (78,79). The remaining two studies comprised slightly different study populations due to broader enrollment efforts (72,73). The Asembo Bay/ITN cohort (Kenya) identified pregnant women through monthly community census, and the FSP/MISAME study recruited through a community-based network of home visitors (72,73). Three studies excluded HIV-infected pregnant women (37,60,80), three studies did not assess HIV status (70,72,74), and the

remaining seven studies included both HIV-infected and HIV-negative pregnant women (8,71,73,75–79).

Locations

The thirteen studies covered seven countries in Africa and one in the Western Pacific (Figure 3.1, Table 3.1). *P. falciparum* was the predominant malaria species observed in areas where included studies were conducted. The two PNG studies also reported *P. vivax* infections (70,74). Some studies detected *P. ovale* and *P. malariae* infections but prevalence was low. Studies not differentiating between species (n=4) were conducted in areas where *P. falciparum* is the principal malaria species, and *P. vivax* is thought to be largely absent. Eleven studies reported high and perennial malaria transmission in the study area during the study period,(8,37,71–80) while two studies reported medium to high perennial malaria transmission (60,70).

Available data/measurements

Women in all studies had at least two study visits, at enrollment (at a gestational age that varied both between and within studies, Table 3.1) and delivery, but the frequency of follow-up visits during pregnancy varied by study. Given logistical and computational intensity required in pooling longitudinal measures with vast amounts of heterogeneity in frequency and timing of measurements, only data at enrollment and at delivery were pooled from every study. To address this limitation of the data, six studies (STOPPAM-Tanzania, STOPPAM-Benin, ISTp-Malawi, PNG-IPTp, PNG-Sek, ISTp-Malawi, STOPMIP-Kenya) provided repeat malaria diagnostics throughout pregnancy, which was not part of the original data request for the M3 cohort initiative.

Table 3.2 and Table 3.3 present available enrollment and delivery measurements for each included cohort.

Outcome assessment

Delivery weight (used to define LBW)

The main outcome of interest for this work was birth weight. We hypothesized based on current understanding of the biologic processes of malnutrition and malaria infection that these factors influence both intrauterine growth and preterm birth in malaria-endemic settings (2,3,45). However, both IUGR and preterm birth are difficult to accurately assess in resource-limited settings. Intrauterine growth restriction is more challenging to measure than SGA or LBW, as it requires frequent ultrasounds throughout pregnancy. Only eight of the twelve studies used ultrasounds to assess gestational age (Table 3.3).Thus, given our confidence in the accuracy and availability of birth weight measurements, we have chosen LBW as our primary endpoint. While LBW is only a proxy for insufficient transfer of nutrients and does not differentiate between IUGR and preterm birth, the implications of this adverse birth outcome for subsequent risk of infant death and poor early life development support its use as the main outcome of interest for the purposes of this work. For consistency, we chose to use the historically defined dichotomous cut point of less than 2,500 grams. In addition to dichotomizing births at the 2,500 gram cutpoint, we also assessed mean birth weight as a continuous outcome (3,81).

Since we did have gestational age based on ultrasound in nine out of the thirteen studies, we conducted a sub-analyses looking at small-for-gestational age as an additional outcome. We assessed SGA among a sub-group of the study population with reliable gestational age at delivery as a proxy for IUGR. We used the new INTERGROWTH-21st birth weight reference (25).

Exposure assessment

Malaria infection

Malaria infection at enrollment

Upon enrollment into each study, malaria infection was diagnosed by examination of peripheral blood (82). Parasitemia was assessed using one or more of the three following approaches: light microscopic (LM) examination of a Giemsa-stained blood smear, polymerase chain reaction (PCR), and rapid diagnostic tests (RDTs) (Table 3.2). In all but two studies, malaria at enrollment was diagnosed by LM. In the FSP/MISAME study in Burkina Faso, LM was only performed if a woman presented with symptoms consistent of malaria, i.e. fever or history of fever (72). In the iLiNS Ghana study, malaria was diagnosed using an RDT (60).

For LM, peripheral blood collected from the study participant was applied as a blood smear, stained, and examined using a microscope with a 100X oil immersion objective (83). Microscopy is a relatively inexpensive diagnostic tool, used to visually detect, differentiate, and quantify parasites (82). The quality control for microscopy readings varied by study. Three studies had two separate microscopists read the slides with discordant readings adjudicated by a third reader (70,74,75), two studies sent a 10% sample of slides to be assessed by a independent technician (8,72), two studies did not report whether or not quality control measures were conducted (37,38), and six studies performed no quality control measures (60,71,73,76–78). RDTs check for malaria antigen in serum and are increasingly used in malaria-endemic areas when microscopy might not be readily or feasibly available (82,84). While RDTS are quick and easy, the antigenic variation may cause false negative results (28). Sensitivity of PCR, a DNA-based detection technique, is much higher than LM and RDTs, but the role of submicroscopic

infections detected by PCR alone in causing adverse outcomes has not been clearly determined (85).

While there is some heterogeneity in diagnostics used for malaria infection at enrollment, the main source of variability comes from the timing of malaria infection. As reported in Table 3.1, there was a wide range in gestational age of women at enrollment, both within the individual studies and between studies. While most studies enrolled women in the second trimester, there were women enrolled as early as four weeks gestation and as late as forty weeks gestation. Using one definition of 'malaria infection at enrollment' obscures the variability in timing of these measurements during pregnancy. Despite sparse data on the importance of timing of malaria infection during pregnancies, studies show that infections during all time points of pregnancy impair fetal growth, however the time period of most importance is uncertain (72,86–88). The evidence that malaria appears to be detrimental regardless of gestational age of infection gives support to our use of a composite exposure of malaria infection at enrollment.

Malaria infection at delivery

A majority of studies included in the pooled cohort tested for malaria infection at delivery. Some studies used the same peripheral blood diagnostics at delivery that were used at enrollment and described above, but some studies also sought to determine placental malaria infection at delivery (Table 3.3). Microscopic examination of a Giemsa-stained peripheral blood smear is the standard for malaria diagnosis among non-pregnant women (82), but may not capture placental infection. Specific adhesion of infected red blood cells to chondroitin sulfate A in the intervillous space of the placenta leads to sequestration of parasites in the placenta that may be absent or undetectable in peripheral blood (89).

Presence of malaria parasitemia in the placenta was assessed using LM of a placental blood smear, PCR, and/or placental histology (90). The diagnosis of malaria using placental histology is defined on the basis of presence of three features: infected erythrocytes, hemozoin in monocytes/macrophages, and hemozoin in fibrin deposits (91). Active placental infection diagnosed using histology is defined as presence of infected red blood cells, chronic placental infection is defined as detection of parasites in combination with hemozoin, and past infection is defined as placentas with hemozoin but no parasites (91).

Placental histology is considered the reference standard for placental infection, but is often not feasible in low-resource, malaria-endemic settings. A meta-analysis reported a summary estimate of sensitivity for peripheral LM and placental LM compared to placental histology was only 44% and 54%, respectively (28). Both PCR and RDTs were reported to have higher sensitivities for placental malaria (28). However, while histology is typically considered the most accurate reference standard, one recent study suggested the use of PCR as a more valid reference (89). This study among Mozambican women reported that microscopy, placental histology, and RDT combined did not identify 60% of malaria infections detected by PCR (89).

Definitions of malaria infection for analyses

The heterogeneity in diagnostic tests used across the pooled studies and the timing of malaria infection measurements complicates the definition of malaria exposure. We considered two separate main definitions of malaria infection: 1) malaria infection at enrollment and 2) malaria infection at delivery.

Because the FSP/MISAME study in Burkina Faso only performed LM at study enrollment if a woman presented with symptoms consistent with malaria, we excluded this study

from all analyses on malaria infection at enrollment (72). This study was only included when we were assessing malaria infection at delivery (which was routinely assessed) or malnutrition. All remaining twelve studies in M3 measured malaria infection at enrollment, using either LM, PCR, and/or RDT. At study enrollment, we defined malaria based on light microscopic (LM) examination of a Giemsa-stained peripheral blood smear or a rapid diagnostic test (RDT) for malaria antigen.(28) Given the uncertain impact of sub-microscopic infections on LBW and the variation in availability of polymerase chain reaction (PCR) diagnostics across studies, we excluded PCR results.(92) In sensitivity analyses, we explored definitions of malaria including PCR results, and 'any malaria', defined as a positive LM, RDT, or PCR at enrollment, delivery, or during pregnancy (in five studies with relevant data).

For delivery measurements, we also considered multiple classifications of malaria infection. Unlike malaria diagnostics at enrollment, the availability of malaria diagnostics at delivery is more limited and differential by study (Table 3.4). First, we defined malaria infection at delivery as a positive result for peripheral LM, placental LM, and placental histology (both active, chronic, and past). We conducted a complete case analysis for the exposure of malaria infection at delivery, excluding observations with missing malaria diagnostics, since malaria diagnostics were not systematically obtained at delivery. In sensitivity analyses, we explored including PCR results in the definition of malaria infection at delivery (92).

Maternal malnutrition

There are various methods for defining individual malnutrition, but in low-resource settings the most common anthropometric measures are mid-upper arm circumference (MUAC) or BMI. Included studies were required to have a measurement of either MUAC and/or BMI (height and weight) captured at a pregnant woman's first antenatal care visit or study enrollment.

MUAC

Mid-upper arm circumference is measured as the circumference of the left upper arm, assessed at the mid-point between the elbow and the tip of the shoulder (93). MUAC is used as an indicator of maternal protein reserves, fat stores, and malnutrition more broadly. There is no global consensus on a threshold to define malnutrition (94), but a conservative cut-point of MUAC <23 cm has been recommended for pregnant women in African and Asian contexts, regardless of gestational age (93). MUAC is only associated with modest changes throughout pregnancy (49), making it a useful measure of malnutrition when accurate gestational ages are unknown. We used MUAC at enrollment as our primary measure of maternal malnutrition, categorizing women as malnourished if MUAC< 23 cm and as well-nourished otherwise. However, it should be noted that loss of fat stores in the third trimester of pregnancy and a subsequent decrease in MUAC represents the mobilization of maternal stores to support fetal growth. In late pregnancy, when enrollment occurred for some women, MUAC might be less indicative of maternal fat stores (95). Thus, we can not discount our dichotomous measure of MUAC<23cm misclassifying some women as malnourished that might actually be having appropriate mobilization of fat stores. MUAC was measured in ten of the thirteen studies, which included 61% of our total study population (Table 3.5). For our main analysis, we multiply

imputed the missing MUAC for the two studies that were missing less than 20% of MUAC observations and we exclude the three studies that did not measure MUAC (96).

BMI

Weight and height measurements, which constitute BMI, were measured in addition to MUAC in nine of the main thirteen studies, were the sole measures of malnutrition in three studies, and one study did not collect height information (Table 3.5). In addition to defining malnutrition using MUAC, we also explored using BMI as the primary exposure measurement of malnutrition. There is not an exact one-to-one correlation between BMI and MUAC, thus cut-points using either of these exposures classify different women as well-nourished versus malnourished (94). However, according to the WHO, a pre-pregnancy BMI of <18.5 is a worldwide cut-point for malnutrition, and has been predictive of adverse birth outcomes (97). Low BMI is representative of wasting of lean and fat tissue. Thus, we were interested in looking at BMI as a measurement of malnutrition in addition to MUAC. Both are imprecise proxies for maternal malnutrition, but it is valuable to know which measure is of more importance when assessing the interaction between malaria infection and malnutrition.

BMI during pregnancy encompasses gestational weight gain, thus is a poor measure of maternal malnutrition after the first trimester (48). Despite the caution that there is insufficient evidence for a cut-point for BMI by gestational age in developing countries (93), previous work on this topic has used cut-points for low BMI (8,9). In a study by Landis and colleagues in the Democratic Republic of the Congo on the interaction between malaria and malnutrition (low BMI or low MUAC) on LBW, low BMI was defined at enrollment (≤22 gestational weeks) as <19.8 kg/m^2 (8). In a separate study by McClure and colleagues in Kenya on the interaction

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between malaria and malnutrition (low BMI) on birth weight, low BMI was defined as below the 10th percentile for gestational age at antenatal care visits (9). For this study population, the cut-point for the 10th percentile of BMI ranged from 19.8 to 20.7 at the beginning of the second trimester and third trimester, respectively. However, given that we are pooling multiple studies with heterogeneous distributions of BMI, defining low BMI using a percentile cut point would obscure comparability between studies.

While the WHO recommends a cut point of <18.5 for defining low BMI for prepregnancy BMI, there are no standard cut points for defining low BMI during the course of pregnancy. In most of the studies BMI was not recorded until time of study enrollment, which varied by study (Table 3.1). This complicates creating a precise cut point for BMI due to the changes in maternal physiology and additional weight of the fetus during pregnancy. We defined a low BMI as an imputed pre-pregnancy BMI <18.5 kg/m^2. To impute pre-pregnancy BMI, we first regressed BMI on age, age squared, and age cubed and saved both the predicted value of BMI and the residual (actual BMI value minus predicted value). The mean BMI of the first trimester (proxy pre-pregnancy BMI) was then determined. Finally, the imputed pre-pregnancy BMI values for each woman were calculated as the mean BMI of the first trimester plus the individual residual value determined in the first step. This approach was taken by the iLiNS-Dyad study team, whose statistical analysis plan is publically available (http://ilins.org/ilins-project-research/data-analysis).

In conclusion, we assessed two classifications of malnutrition: 1) MUAC <23 cm, regardless of gestational age; 2) imputed pre-pregnancy BMI <18.5 kg/m^2. While no measurement is perfect, MUAC and BMI are easily measured in low resource settings and represent the best available data for assessing malnutrition in our pooled dataset.

Covariate assessment/definitions

Additional covariates that are likely to be important in the current analysis and were assessed in at least some of the studies include maternal age, gravidity, gestational age at enrollment, gestational age at delivery, sex of infant, hemoglobin and/or hematocrit at enrollment, area of residence, implemented malaria prevention interventions for each study, and nutritional interventions for each study. Availability of each covariate by study is reported in Tables 3.2 and 3.3.

Maternal age

Maternal age was ascertained through self-report for all women in all included studies. We used Akaike information criterion (AIC) to determine the best fit for control of maternal age using restricted cubic splines, linear splines, or quadratic, cubic, or linear transformations of maternal age (98).

Gravidity

Gravidity was ascertained through self-report for all women in all included studies. We categorized gravidity as primigravid (gravidity=1), secundigravid (gravidity=2), and multigravida (gravidity ≥3).

Gestational age at enrollment

Gestational age at enrollment was ascertained using either US or SFH. Only 0.4% of the study population was missing gestational age by one of these measures. US measurements were

available for nine of the thirteen studies (Table 3.2). In the first two trimesters US is the gold standard for gestational age estimation, with waning accuracy in the third trimester (99). SFH, plus or minus one to three centimeters, estimates gestational age beginning in the second trimester, although this does not take into account IUGR (100). When available, we used US measures for gestational age, otherwise we used SFH.

Delivery gestational age

Reliable gestational age at delivery using US were available for nine of the thirteen studies (Table 3.3).

Sex of infant

Information on the sex of the infant was ascertained for all women in the study population (Table 3.3). While sex of the infant is not a confounder, the sex of the infant was important for definition of the secondary outcome, SGA, which requires use of a gestational age and sex specific birth weight reference (25).

Timing of birth weight

Six of the twelve studies restricted their study population to infants that were weighed within 24 hours of delivery, and the remaining seven allowed for weighing within one week. In some of the studies the majority of women delivered at home, thus obtaining birth weight within 24 hours was challenging. Allowing for inclusion of data on infants measured within one week increases the amount of available data. To account for typical infant weight loss within that first

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week, we estimated birth weight using a cubic regression model, which was determined to be the best fit model based on AIC (101).

Hemoglobin and hematocrit at enrollment

Low hemoglobin and hematocrit are indicators for anemia (102). 98.2% of the study population had either a hemoglobin or hematocrit measure at enrollment (Table 3.2). Hemoglobin is an iron-rich protein in red blood cells that is responsible for delivery of oxygen to tissues and hematocrit is a measure of how much space red blood cells take up in the blood (102). We defined anemia as a hemoglobin < 11 g/dL of venous blood, if available, or hematocrit <33 g/dL, in the first and third trimesters, and less than 10.5 and 32, respectively for the second trimester (103). Hemoglobin and hematocrit were measured at enrollment, thus the trimester of measurement varied by study (Table 3.1).

Area of residence

Whether women lived in a rural versus urban setting was recorded for >99% of the study population (Table 3.2). Five studies described their study population as all living in rural settings (71–73,78,88), two studies were from urban settings (8,60), and six studies differentiated between those living in urban and rural residencies (37,38,70,74,76,77). However, the distinction between rural and urban was not systematically assessed, thus while this variable is available for all studies it may have slightly variable meanings in each context.

Malaria Prevention and Nutritional Supplementation during pregnancy

Details of antimalarials and nutritional supplementation in these studies can be found in Table 3.1. Variables to indicate the malaria prevention policy for the region at the time for observational studies and what the malaria intervention arm was for RCTs were included in the M3 dataset. Information on how many doses of prophylaxis women actually received was available for six of the thirteen studies, and for the IPTp arms of the ISTp-Malawi and STOPMIP-Kenya studies. Self-reported bed net ownership at enrollment was also ascertained in eight out of the thirteen studies; 62% reported bed net ownership, which likely overestimates the usage of bed nets.

Table 3.1. Characteristics of the 13 individual studies included in the Maternal Malnutrition and Malaria (M3) Initiative.

Countries	Study Name	Design	Period	Median GA (IQR)*	Malaria prevention†	Nutritional intervention	$N^{\ddagger}$	$n^{\S}$
Benin	STOPPAM I	Cohort	2008-2010	17 (14-20)	IPT-SP	None	1037	791
BF	FSP/MISAME	RCT	2006-2008	16 (11-21)	IPT-SP(2), IPT-SP(3)	MMS, FFS	1296	1020
DRC	ECHO	Cohort	2005-2006	19 (17-21)	IPT-SP	None	182	164
Ghana	iLiNS-DYAD	RCT	2009-2012	17 (15-20)	IPT-SP	LNS, MMN, IFA	1320	1068
Kenya	EMEP & IPTpMon	Cohort #	2011-2013	23 (16-29)	IPTp-SP	None	1453	471#
Kenya	ITN	RCT	1996-1999	24 (20-30)	IPT-SP started during study	None	911	711
Kenya	Kisumu cohort	Cohort	1996-2001	36 (34-37)	IPT-SP started during study	None	3155	3388**
Kenya	STOPMIP	RCT	2012-2015	23 (20-26)	IPT-SP, IPT-DHA-PQ, IST-DHA-PQ	None	1546	1203
Malawi	ISTp	RCT	2011-2013	21 (19-23)	IPT-SP, IST-DP	None	1873	1601
Malawi	LAIS	RCT	2003-2006	20 (18-23)	IPT-SP(2), IPT-SP(4) IPT-SPAZ	None	1320	1190
PNG	IPTp study	RCT	2009-2013	22 (19-25)	IPT-SPAZ; Single dose SP and	None	2793	1943
PNG	Sek cohort	Cohort	2005-2007	25 (22-28)	Single dose SP and weekly CQ	None	470	293
Tanzania	STOPPAM II	Cohort	2008-2010	19 (15-21)	IPT-SP	None	995	789

BF=Burkina Faso. CQ= chloroquine. DHA-PQ=Dihydroartemisinin-piperaquine. DRC=Democratic Republic of the Congo. FFS=fortified food supplementation. IFA=iron and folic acid supplementation. IPT (doses)=intermittent preventive treatment in pregnancy. IST=intermittent screening for malaria infection. LNS=lipid-based nutrient supplementation. MMS=multiple micronutrients supplementation. PNG=Papua New Guinea. RCT=randomised controlled trial. SP=sulphadoxine-pyrimethamine. SPAZ= SP and azithromycin.

* Median (IQR): Gestational age at enrolment assessed by fetal biometry, or symphysis-pubis fundal height when ultrasound unavailable

†If RCT, describes the intervention, if cohort, describes the national policy during the study period

‡N=Enrolled in parent study.

§ *n*=live birth pregnancies that met inclusion criteria for M3.

The EMEP study was a prospective cohort study with some overlapping enrolment with the cross-sectional study IPTp-MON. 111 pregnancies were enrolled in both EMEP and IPTp- MON; information on malaria infection at delivery was obtained for the subset of women in IPTp-MON.

**Includes additional women from a sub-study not included in the parent study which otherwise met inclusion criteria for the pooled data.

Figure 3.1. Geographical locations where the thirteen parent studies were conducted: Benin, Burkina Faso, Democratic Republic of the Congo, Ghana, Kenya, Malawi, Papua New Guinea, and Tanzania.

Table 3.2. Key measures at enrolment across the 13 studies included in the Maternal Malnutrition and Malaria (M3) Initiative.

Variable	STOPPAM - Benin	FSP/ MISAME - BF	ECHO - DRC	iLiNS-DYAD-Ghana	EMEP& IPTpMon-Kenya	ITN-Kenya	Kisumu - Kenya	STOPMIP -Kenya	ISTp-Malawi	LAIS-Malawi	IPTp-PNG	Sek-PNG	STOPPAM -Tanzania
Essential variables													
Malaria													
LM	X	X*	X		X	X	X	X	X	X	X	X	X
PCR								X	X		X	X	
RDT	X†			X‡				X§	X§				X†
Anthropometrics													
MUAC	X*	X	X	X		X*		X#		X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X		X
Gravidity	X	X	X	X	X	X	X	X	X	X	X	X	X
Anaemia††													
Haemoglobin	X	X		X	X	X	X	X	X	X	X	X	X
Haematocrit			X										
Optional variables													
Gestational age													
Ultrasound	X	X	X	X	X*				X	X	X*		X
Fundal height	X	X*	X	X	X*	X	X	X	X	X	X	X	X
Age	X	X	X	X	X	X	X	X	X	X	X	X	X
Smoking (Y/N)	X		X		X				X	X	X	X	X
HIV	X		X	X	X	X*	X	X	X	X			X
Rural/urban area	X		X	X	X	X	X	X	X	X	X	X	X
Bed net ownership	X		X			X		X	X	X	X	X	X*

Available information indicated with X, missing data >10% indicated by asterisk, and systematically missing data indicated with grey highlighting. BF=Burkina Faso. DRC=Democratic Republic of the Congo. HIV=human immunodeficiency virus. IPTp=intermittent preventive treatment in pregnancy. ISTp=intermittent screening for malaria infection in pregnancy. ITN=insecticide-treated bed nets. LM=light microscopy. MUAC=mid-upper arm circumference. PNG=Papua New Guinea. PRC=polymerase chain reaction. RDT=rapid diagnostic test. † The STOPPAM Benin and Tanzania studies primarily used the Parascreen RDT (Zephyr Biomedical Systems, Goa, India), which detects *P. falciparum* histidine protein-2 (HRP-2) and *Plasmodium* lactate dehydrogenase (pLDH). ‡ The iLiNS-DYAD-Ghana study used the Clearview Malaria Combo RDT (British Biocell International Ltd., Dundee, United Kingdom), which detects HRP-2 and plasmodium aldolase § Women randomized to the ISTp arms of the STOPMIP-Kenya and ISTp-Malawi studies were tested with a First Response Malaria Ag. (pLDH/HRP2) Combo RDT (Premier Medical Corporation, India) # Maternal MUAC measured at delivery †† Nine studies measured haemoglobin using a HemoCue haemoglobinometer (Hemocue, Angelholm, Sweden),[20–22,26–31] one study relied on haemoglobin measures from a local laboratory (unknown make),[23] one study used Sysmex hematological analyzer (Kobe, Japan),[32] and one study collected capillary blood (finger stick) specimens for determination of haematocrit or haemoglobin levels using a standardised chart.[10] In the ITN-Kenya study, haemoglobin was measured using a HemoCue haemoglobinometer (Hemocue, Angelholm, Sweden) before 1997, and from 1997 onwards capillary blood (finger stick) specimens were collected for determination of haematocrit levels, which were divided by a factor of three and presented as haemoglobin values for consistency with the 1996 data.

Table 3.3. Key measures at delivery across the 13 studies included in the Maternal Malnutrition and Malaria (M3) Initiative.

Variable	STOPPAM - Benin	FSP/ MISAME - BF	ECHO - DRC	iLiNS- DYAD- Ghana	EMEP& IPTpMon- Kenya	ITN- Kenya	Kisumu - Kenya	STOPMIP -Kenya	ISTp- Malawi	LAIS- Malawi	IPTp- PNG	Sek- PNG	STOPPAM -Tanzania
Essential variables													
Malaria- peripheral													
LM	X*	X*	X		X*†	X	X	X	X	X*	X	X	X
PCR										X*	X	X	
Malaria-placental													
LM	X*	X*			X*†	X	X	X	X		X*	X*	X*
PCR								X	X		X*	X*	X*
Histology			X					X	X		X*	X*	
IPTp doses	X	X*	X		X			X	X*	X	X		X
Birthweight	X	X	X	X	X	X	X	X	X	X	X	X	X
Infant sex	X	X	X	X	X	X	X	X	X	X	X	X	X
Optional variables													
Gestational age													
By ultrasound	X	X	X	X	X*				X	X	X*		X
Neonatal measurements													
Abdomen circumference	X*		X								X		X
Chest circumference		X								X			
Head circumference	X	X	X	X	X			X	X	X	X	X	X
Crown heel length		X	X	X	X	X		X			X	X	
Timing of measurements	X		X	X	X	X	X	X	X	X	X	X	X

Available information indicated with X, missing data >10% indicated by asterisk, and systematically missing data indicated with grey highlighting.

BF=Burkina Faso. DRC=Democratic Republic of the Congo. IPTp=intermittent preventive treatment in pregnancy. ISTp=intermittent screening for malaria infection in pregnancy. ITN=insecticide-treated bed nets. LM=light microscopy. PNG=Papua New Guinea. PRC=polymerase chain reaction

† For the EMEP/IPTpMON study, only women co-enrolled in the IPTpMON study (n=111) had malaria diagnostics at delivery.

Table 3.4. Percentage of study population for which various diagnostics of malaria at **delivery** were measured. .

Study Name	Countries	N	Peripheral		Placental			Missing any malaria measurement at delivery
			LM	PCR	LM	PCR	Histology	
Sek cohort	PNG	293	100 %	94 %	78 %	74 %	76 %	0 %
IPTp study	PNG	1943	98 %	97 %	81 %	65 %	71 %	1 %
iLiNS	Ghana	1068	-	-	-	-	-	100 %
ISTp	Malawi	1602	96 %	-	97 %	90 %	93 %	2 %
STOPMIP	Kenya	1546	99%	-	97 %	95%	97%	1%
STOPPAM I	Benin	791	75 %	-	70 %	-		20 %
STOPPAM II	Tanzania	789	97 %	-	46 %	-	-	2 %
LAIS	Malawi	1190	38 %	38%	-	-	-	62 %
Kisumu cohort	Kenya	3388	90 %	-	98 %	-	-	1 %
Asembo Bay, ITN	Kenya	711	92 %	-	90 %	-	-	8 %
EMEP& IPTpMon	Kenya	471	24%	-	23%	-	-	76%*
FSP/MISAME	Burkina Faso	1020	70%	-	-	-	-	30%
ECHO	DRC	164	100 %	-	-	-	100 %	0 %

PNG=Papua New Guinea. DRC=Democratic Republic of the Congo. BF=Burkina Faso. IPTp=intermittent preventive treatment in pregnancy. ISTp=intermittent screening for malaria infection in pregnancy.* The EMEP study was a prospective cohort study with some overlapping enrolment with the cross-sectional study IPTp-MON. 111 pregnancies were enrolled in both EMEP and IPTp- MON; information on malaria infection at delivery was obtained for the subset of women in IPTp-MON

Table 3.5. Percent of observations with <u>measured</u> mid-upper arm circumference (MUAC) and body mass index (BMI). Also reported is the range in gestational age of anthropometric measurement at enrollment.

Study Name	N	MUAC	BMI	GA (wks)*
Sek study, PNG	293	100 %	0 %	8-35
IPTp study, PNG	1943	100 %	100 %	6-33
iLiNS, Ghana	1068	100 %	100 %	7-39
ISTp, Malawi	1590	0%	100 %	14-30
STOPMIP Kenya	1203	100% (delivery)	100%	8-36
STOPPAM I, Benin	791	88%	100 %	4-29
STOPPAM II, Tanzania	789	100 %	100 %	6-24
LAIS, Malawi	1190	100 %	100 %	14-26
Kisumu, Kenya	3388	0 %	100 %	30-40
ITNs study, Kenya	711	87%	100 %	4-40
EMEP&IPTpMON, Kenya	471	0%	100%	4-41
ECHO study, DRC	164	100 %	100 %	10-22
FSP/MISAME, BF	1034	100 %	100 %	4-38

*Range in gestational age at enrollment as measured by symphysis-pubis fundal height or ultrasound

CHAPTER IV: ANALYTIC METHODS

Analytic approach for aim 1

Aim 1: To investigate the joint effects of malaria infection and maternal malnutrition during pregnancy on the risk of LBW.

Hypothesis: We hypothesized that there would be a synergistic interaction between malaria infection and malnutrition in causing LBW, such that the observed joint effect of being both infected with malaria and malnourished would be greater than expected if considering each exposure independently.

Pooled analysis

Since we pooled individual-level data from thirteen separate studies, we needed to account for variation in the estimate effects across studies due to differences in patient populations, study procedures, study location and calendar time (104,105). There are two commonly employed approaches for handling individual pooled data, a one-step approach and a two-step approach, although there is no consensus as to which approach is preferable (104,106,107). The one-stage approach analyzes all of the data simultaneously but accounting for clustering among patients in the same study using separate intercepts for each study (fixed effect) or random effects for intercepts and slopes. A two-stage approach analyzes each study separately and then combines summary estimates using standard meta-analytic techniques (104,107). Comparisons of one and two-stage approaches based on examples, theory, and

simulation studies indicate similar results between the two approaches (104–108). However, two-stage approaches are generally considered more easily interpretable, and allow the investigator to visually present forest plots and quantify statistical heterogeneity (106). Thus, we employed a two-stage approach, analyzing each study separately and then pooling summary estimates using standard meta-analytic techniques. As a secondary analysis, we also examined the consistency of results with a one-stage approach, fitting a generalized mixed model with random intercept and slope effects.

For stage one of the two-stage approach, study-specific effect estimates were calculated using linear and log binomial regression models controlling for a minimally sufficient set of confounders identified through the evaluation of a directed acyclic graphic (DAG, See Section 4.1.2) through inverse probability of treatment weighting (IPTW, See Section 4.1.2). In the second stage, effect estimates and interaction statistics (Section 4.1.3) were pooled using random-effects models (108). For the random effects model we used the restricted maximum likelihood method of DerSimonian and Laird to obtain mean and variance (τ^2) of random effects distributions (109). This random effects model assumes that the true effects among the different studies are drawn from a normal distribution (109). The estimated mean is a weighted average of the estimates from each individual study with weights inversely proportional to the total variance of each estimate (109). When study variance (τ^2) was greater than zero we calculated 95% population effects intervals (PEI) (110,111). The 95% PEI is the range within which 95% of the different study populations' effects are estimated to lie within. We assessed the influence of study characteristics on the heterogeneity of effect estimates using meta-regression. We decided *a priori* to evaluate modification of the results by time period (before versus after 2008) due to changes in antimalarial recommendations, study type (trial/cohort), location (Africa/Western

Pacific), and malaria infection prevalence (based on M3 data). The separation between studies done before 2008 is to distinguish between studies conducted before and after the change in IPTp recommendations.

While these approaches follow the methodology of individual patient-level meta-analyses (IPD-MA), this project did *not* involve the systematic ascertainment of studies from a systematic review, thus we refrained from using the terminology "meta-analysis" (108). While we have no reason to believe that the obtained studies in M3 are different from studies not included, we cannot discount the possibility that these studies may not be a random sample of all available data and could potentially result in selection bias.

Directed acyclic graph (DAG)

Confounders were identified using the directed acyclic graph (DAG) in Figure 4.1. Measured confounders common for all analyses include maternal age, gravidity, area of residence, and HIV infection. When assessing malaria at delivery instead of malaria at enrollment, additional confounders include the number of doses of IPTp received during pregnancy and anemia at enrollment. Potential unmeasured confounders include helminth infections, and other micronutrient deficiencies, particularly iron deficiency.

Notably, when considering the exposure of malaria infection at enrollment, anemia at enrollment is a mediator, thus should not be included in the multivariable model. Malaria infection contributes to anemia through both increased removal of red blood cells by the host immune system and decreased production of red blood cells in the bone marrow (112). When the exposure is malaria infection at delivery, anemia at enrollment is a confounder and should be included in the model. However, when considering the interaction between malaria at enrollment

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and malnutrition at enrollment, anemia is a mediator between malaria and LBW but a confounder between malnutrition and LBW (Figure 4.1).

We cannot control for confounding through traditional multivariable regression due to the relationship between anemia at enrollment, malaria at enrollment, malnutrition at enrollment, and LBW. However, by using inverse-probability of treatment weighted (IPTW) marginal structural models (MSM), we can circumvent this problem (113). IPTW-MSM control for confounding by modeling the relationship between the exposures of interest and the confounding variables. We created separate treatment weights for malaria and malnutrition, which enabled the inclusion of anemia in the weights for malnutrition but not for malaria at enrollment.

Assessment of interaction

We estimated associations between malaria infection and malnutrition and the outcome of LBW using log binomial and linear regressions. Using log binomial regression we estimated risk ratios and using the linear regression model we estimated differences in mean birthweight separately for each study. Specifically, we modeled the probability of LBW or mean birthweight with respect to the two exposures of interest, malaria infection and malnutrition, the interaction between these two variables (coded as a product term between these two variables in the model), and used the inverse-probability of treatment weights to control for confounding. The inclusion of the interaction term in the model allows for the estimates for malaria infection versus no malaria infection to be heterogeneous across strata of malnutrition, and vice versa for the effect of malnourished versus well-nourished across strata of malaria infection. There are four possible combinations of the two exposures: malaria-uninfected/well-nourished, malaria-uninfected/malnourished, malaria-infected/well-nourished, and malaria-infected/malnourished.

This approach allows us to consider malnutrition as a co-primary exposure, along with malaria infection, instead of just as a modifier of the malaria and LBW relationship. Epidemiologists often use effect measure modification and interaction synonymously, but there is a nuanced distinction between these two concepts (114). In this work, we were specifically interested in the interaction between malaria infection and malnutrition, since both exposures are modifiable and we have confounders measured for both exposures relative to the risk of LBW. Specifically, we were not only interested in how malnutrition modifies the relationship between malaria infection and LBW as suggested by prior studies, but were more precisely interested in the joint effects of both malaria and malnutrition on LBW. Interaction is valuable in addition to looking at effect measure modification because we could potentially intervene on both malaria and malnutrition.

We assessed interaction on both the multiplicative and additive scales, however, it has been argued that the additive scale is more applicable for public health (115). We calculated an estimate of the interaction contrast (IC), which is a measure of the difference between the observed risk for women jointly exposed to being both infected with malaria and malnourished and the expected risk for women jointly exposed under the assumption of additive risks (no interaction). An IC greater than zero indicates a net increase in the positive, harmful association of malaria with joint exposure to malnutrition. We calculated IC statistics and multiplicative product terms separately for each study and then pooled across studies to obtain an overall IC and overall multiplicative product term (See Section 4.1.1).

Analytic approach for aim 2

Aim 2. To explore the impact of hypothetical targeted antimalarial and nutrition interventions on population-level pregnancy outcomes.

Implementation of the g-formula

There are the four steps to estimating interventional estimates.(14,66)

1)   First, we modeled the association between the exposure(s) (malaria, malnutrition, ITN ownership, IPTp dosage) we hope to modify and the outcome, LBW. We used logistic regression models, controlling for known and measured confounders, and generalized estimating equations to account for within-study correlation. Confounders for the relationship between malaria infection at delivery and LBW were determined from a causal directed acyclic graph (DAG) (116) based on prior literature on the relationship between covariates, and included study site, maternal age, gravidity, rural versus urban residence, HIV infection, anemia at enrollment, and the number of IPTp doses. Confounders for the malnutrition-LBW relationship were obtained from a separate DAG and included study site, maternal age, gravidity, rural versus urban residence, anemia at enrollment, and HIV infection. Study site was the only measured confounder for the bed net coverage-LBW relationship and study site and HIV infection were the only measured confounders for the IPTp-LBW relationship.

2)   Second, we "set" the exposure (malaria, malnutrition, ITN ownership, IPTp use) to the level specified for the particular hypothesized intervention (See Sections 4.2.2-4.2.4). With this exposure "set", we imputed the probability of having a LBW infant for each mother.

3)   Third, we averaged the imputed probabilities for LBW across the population, for each "set" level of exposure. Comparison of the average outcomes for the different "set" exposures provides us with population-wide predicted effect of changing exposure on the prevalence of LBW in the whole population, the population-standardized risk difference. We also estimated the

number needed to treat (NNT) for each contrast of "set" exposures. The NNT is calculated as the reciprocal of the absolute value of the risk difference.

4)   We calculated confidence intervals using non-parametric bootstrapping techniques (200 samples with replacement from original dataset) (117).

Interventional Estimates: Overview

In epidemiology, we typically assess the etiologic causal question regarding the effect of one or more exposures on a particular outcome. To assess this question, we use analytic tools (regression analyses or g-methods) to ask what the risk of the outcome is if the population had all been exposed versus none of the population had been exposed, known as the population average causal effect (118). Assuming the circles in Table 4.1 illustrate the factual or counterfactual study population, where the black portion is representative of the exposure prevalence, then scenario 1 illustrates a contrast between the risk when the whole population is exposed and the risk when no one is exposed; we refer to this as the average causal effect. Note, the circles in Table 4.1 do not illustrate the expected risk of LBW, rather they exclusively focus on the exposure distribution among the study population.

Aim 1 of this work focuses on the etiologic synergistic effects of maternal malnutrition and malaria infection on LBW. Questions regarding the etiologic effect of malaria and malnutrition would be: 1) what if everyone in the population had malaria compared to none, 2) what if everyone in the population were malnourished compared to none, and 3) what if everyone in the population had both malaria and malnutrition, compared to neither (and compared to just malaria or malnutrition independently).

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However, understanding the implications of implementing realistic interventions on malaria and malnutrition for population-level birth outcomes is equally, if not more, informative for public health policy decisions. We used an imputation-based causal inference method called the parametric g-formula(15,67) to stochastically simulate seven different intervention scenarios (13–15). **The different intervention scenarios were modeled are as follows:**

Malaria interventions

The malaria interventions to be assessed intervene on the following four exposures: malaria infection at delivery, ITN ownership at enrollment, and receipt of intermittent preventive therapy during pregnancy (IPTp).

Interventions specific to malaria infection at delivery

For malaria infection at delivery, we calculated population attributable contrasts, which compares the observed distribution of malaria to the population had no women been infected with malaria (Table 4.1, scenario 2). However, this intervention contrast only assesses a hypothetical intervention with non-specific mechanisms of prevention. While this contrast does not guide policies for direct interventions during pregnancy, it does highlight the potential impact of increased ITN coverage in women of reproductive age.

Bed net ownership and IPTp use during pregnancy

To better guide policies for specific evidence-based interventions, we also assessed the impact of scaling up existing malaria prevention efforts implemented in the pooled studies, specifically bed net ownership at enrollment and IPTp prophylaxis during pregnancy. Unlike the prior contrasts where we were looking at removal of malaria, now we are specifically looking at scale-up of malaria prevention strategies. In our pooled data, among women with available self-

reported information on bed net ownership, 62% reported owning a bed net at enrollment. Even though this variable is likely an overestimation of actual bed net use (40), we can use this information for an intention to treat (ITT) analysis of the ownership of bed nets under typical usage. Again, we calculated a population attributable contrast, comparing the observed distribution of bed net ownership at enrollment to the population had all women owned bed nets enrollment (Table 4.1, scenario 3).

Another existing malaria prevention strategy is IPTp prophylaxis during pregnancy. We had information on what antimalarial interventions were the national public health policy, which drugs were assigned (if an RCT), and how many doses of prophylaxis were received before delivery. Of the 14,633 women in M3, 1,148 (8%) lack data on how many doses of IPT they received and were excluded for this interventional estimate. Of the remaining 13,485 women, 39% received no IPTp doses, 15% received only one dose of IPT, 18% received only two doses (the recommended schedule prior to 2007), and 28% received three or more doses (similar to the monthly schedule recommended since 2007) (Figure 9f).

First, we only intervened among those who received less than two doses of IPTp and 'set' (simulate) their exposure to be 2 doses. This allowed us to estimate a generalized intervention contrast, comparing the observed distribution of IPTp receipt to the population where everyone has two or three doses of IPTp (Table 4.1, scenario 4a). Second, we simulate everyone receiving three+ doses (similar to the monthly schedule recommended since 2007) (Table 4.1, scenario 4b). Given that the national target for many of the malaria-endemic countries represented by our study population is 80% coverage, we also assessed increasing the dosage to at least three doses of IPTp in 80% of the study population (40).These interventional estimates informed on the

population level impact that scaling up IPTp could have in malaria endemic settings. However, this approach does not take into account drug type differences or regional drug resistance.

Malnutrition interventions

Next, we considered a hypothetical intervention on maternal malnutrition . We took a similar approach to this exposure as we did to malaria, first comparing the risk of LBW if the population of women all had MUAC below 23 cm (all-shaded circle) versus if all of the women had MUAC at or above 23 cm (unshaded circle), and then comparing the *observed* distribution of MUAC (the partially-shaded circle) to the hypothetical population in which women had MUAC at or above 23 cm (unshaded circle) (Table 4.1, scenarios 5-6). Women with MUAC counterfactual to these scenarios were set either below or above the cut point of 23 cm depending on the contrast. For example, in the scenario where all women were simulated to have normal MUAC, a woman with factual MUAC of 20 cm would have their simulated MUAC set to 23 cm, improving their nutritional status to a normal measurement of MUAC. Additionally, it is unrealistic to expect that an intervention could achieve complete removal of maternal malnutrition; therefore we also compared the observed distribution of malnutrition prevalence at enrollment to the population in which only 5% of each separate study population is malnourished at enrollment (the lowest prevalence of malnutrition from any of our studies). However, again this contrast requires the assumption of a hypothetical interventions with non-specific mechanisms to improve nutrition. Also, since we only have malnutrition assessments at enrollment, this assumes an intervention could be implemented that would target malnutrition prior to pregnancy.
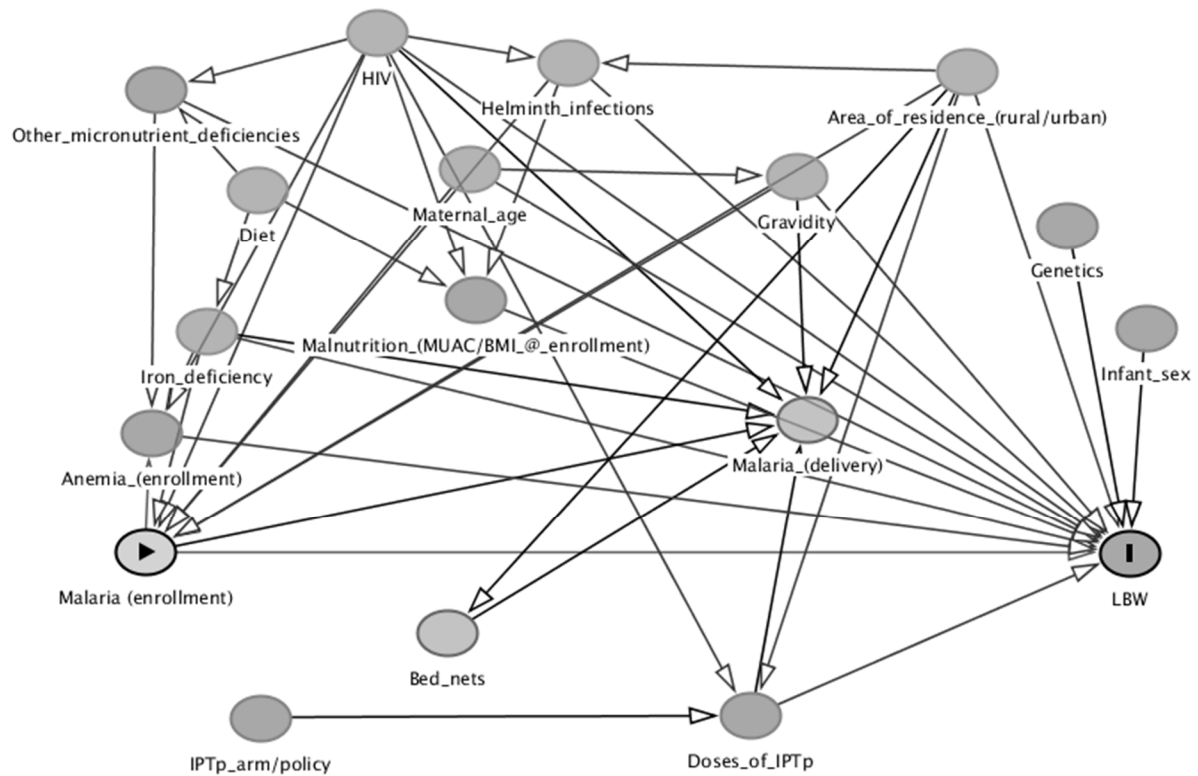
Figure 4.1. Directed acyclic graph (DAG) created for the relationship between malaria at enrollment, malaria at delivery, malnutrition, and LBW.
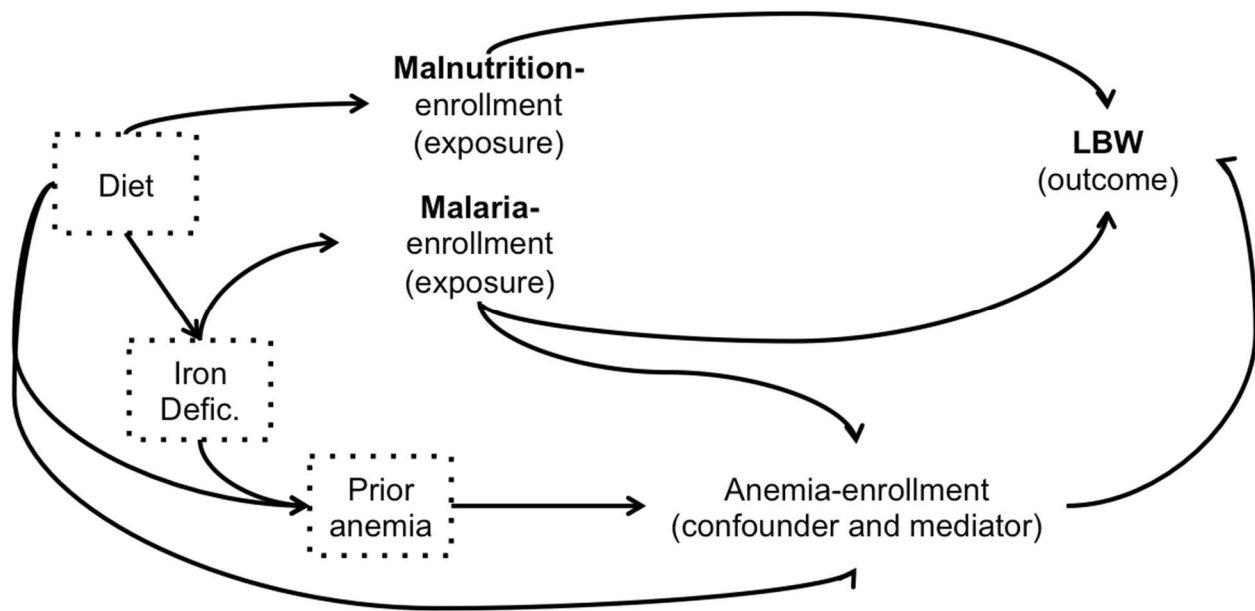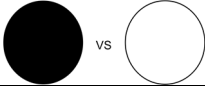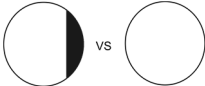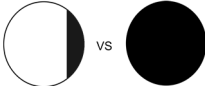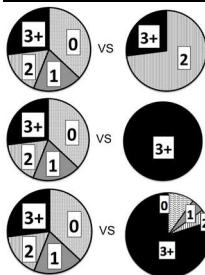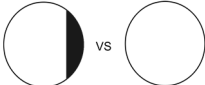
Figure 4.2. Simplified directed acyclic graph (DAG) illustrating the relationship between malaria, anemia, malnutrition, and LBW. The dotted boxes reflect relevant variables that are unknown in the M3 dataset. The figure illustrates that anemia is a mediator of the relationship between malaria and LBW but is a confounder of the relationship between malnutrition and LBW.

Table 4.1. Population average causal effects, population attributable effects, and generalized intervention effects for the risk of low birthweight (LBW) associated with malaria infection and malnutrition during pregnancy. For example, the second scenario depicts the population attributable effect for malaria infection at delivery, comparing the risk of LBW under the observed distribution of malaria infection at delivery in the study population to the risk under a counterfactual setting in which there is hypothetical complete and instant eradication of malaria.

	Exposure	Contrasts	Intervention
	Malaria interventions		
1	Malaria infection at delivery	**Population average causal effect:** The population with everyone exposed to malaria infection at delivery compared to the population of women with no infection.	N/A
2	Malaria infection at delivery	**Population attributable effect:** The observed distribution of malaria infection at delivery compared to the population of women with no infection.	Hypothetical complete and instant eradication of malaria infection at delivery.
3	Bed net ownership at enrollment	**Population attributable effect:** The observed distribution of bed net ownership compared to the population of women where everyone owns a bed net.	Scaling up of existing bed net intervention.
4	Number of IPTp doses	**Generalized intervention effect**: The observed distribution of intermittent preventive therapy during pregnancy (IPTp) doses compared to the population where (i) everyone has at least two doses of IPTp, (ii) everyone has at least three doses of IPTp, or (iii) at least 80% of the study population has at least three doses of IPTp.	Scaling up of existing IPTp interventions.
	Malnutrition interventions		
5	Malnutrition at enrollment	**Population average causal effect:** The population with everyone malnourished at enrollment compared to the population of women with no malnutrition.	N/A
6	Malnutrition at enrollment	**Population attributable effect:** The observed distribution of malnutrition (MUAC<23 cm) compared to the population of women with no malnutrition.	Hypothetical complete and instant eradication of malnutrition prior to pregnancy or in early pregnancy.
7	Malnutrition at enrollment	**Generalized intervention effect**: The observed distribution of malnutrition (MUAC<23 cm) compared to the population where only 5% of each separate study population is malnourished.	Hypothetical intervention with a non-specific mechanism that would reduce malnutrition prevalence in each study in M3 to 5%.

CHAPTER V: COHORT PROFILE: THE MATERNAL MALARIA AND MALNUTRITION (M3) INITIATIVE, A POOLED BIRTH COHORT OF THIRTEEN PREGNANCY STUDIES IN AFRICA AND THE WESTERN PACIFIC.[1]

Overview

Purpose:

The Maternal Malaria and Malnutrition (M3) initiative has pooled together thirteen studies with the hope of improving understanding of malaria-nutrition interactions during pregnancy and to foster collaboration between nutritionists and malariologists.

Participants:

Data was pooled on 14,633 singleton, live birth pregnancies from women who had participated in one of 13 pregnancy studies. The thirteen studies cover eight countries in Africa and Papua New Guinea in the Western Pacific conducted from 1996 to 2015.

Findings to date:

Data is available at the time of antenatal enrolment of women into their respective parent study and at delivery. The dataset comprises essential data such as malaria infection status, anthropometric assessments of maternal nutritional status, presence of anaemia, and birthweight, as well as additional variables such gestational age at delivery for a subset of women.

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[1] This chapter previously appeared as an article in the Journal BMJ Open. The original citation is as follows: Unger, Holger W., Jordan E. Cates, Julie Gutman, Valerie Briand, Nadine Fievet, Innocent Valea, Halidou Tinto, et al. "Maternal Malaria and Malnutrition (M3) Initiative, a Pooled Birth Cohort of 13 Pregnancy Studies in Africa and the Western Pacific." *BMJ Open* 6, no. 12 (December 21, 2016).

Participating studies are described in detail with regards to setting and primary outcome measures, and summarised data is available from each contributing cohort.

Future plans:

This pooled birth cohort is the largest pregnancy data set to date to permit a more definite evaluation of the impact of plausible interactions between poor nutritional status and malaria infection in pregnant women on fetal growth and gestational length. Given the current comparative lack of large pregnancy cohorts in malaria-endemic settings, compilation of suitable pregnancy cohorts is likely to provide adequate statistical power to assess malaria-nutrition interactions, and could point towards settings where such interactions are most relevant. The M3 cohort may thus help to identify pregnant women at high risk of adverse outcomes who may benefit from tailored intensive antenatal care including nutritional supplements and alternative or intensified malaria prevention regimens, and the settings in which these interventions would be most effective.

Introduction

It is estimated that each year over 125 million pregnant women residing in low- and middle-income countries (LMICs) are at risk of infection with *Plasmodium falciparum* and *P. vivax* (4). Malaria contributes to the high burden of maternal morbidity and mortality in these settings and may affect placental development and fetal growth (2). Sequestration of *P. falciparum* parasites in the placenta has been associated with fetal growth restriction (FGR) and low birthweight (LBW, <2,500 grams), thus contributing to infant mortality and possibly long-term health problems (119,120). *P. vivax* has also been associated with adverse pregnancy outcomes (121). Malaria during pregnancy may cause maternal anaemia, which itself can have

deleterious effects on fetal development (2). Maternal undernutrition is also common amongst pregnant women in these settings (11,122,123), and undernourished women are more likely to have growth-restricted fetuses and babies with reduced birthweight (17). To date it remains unclear whether the susceptibility to and the impact of malarial infection are affected by maternal nutritional status. A small number of studies suggest that macronutrient nutritional status modifies the effects of malaria in pregnancy, specifically the impact of *P. falciparum* parasitaemia on fetal growth and birthweight (8–10). Although largely overlooked, this may not be surprising given that both macro- and micronutrient nutritional status affect immune function more broadly (124,125). As such, there may be scope to design interventions that prevent adverse pregnancy outcomes by protecting women and their offspring from the deleterious consequences of both malaria and undernutrition. This aligns with the 2015 Global Strategy for Women's, Children's and Adolescents' Health recommendations to reduce the risk of LBW and maternal anaemia through the prevention and treatment of malaria and through adequate nutrition during pregnancy (126,127).

The Malaria in Pregnancy Consortium (MiPc), which receives funding from the Bill & Melinda Gates Foundation, brings together scientists whose aim is to reduce the burden and impact of malaria in pregnancy in LMICs (128,129). At the 2014 MiPc meeting in New Orleans, Louisiana USA, Consortium members identified the need to study the relationship between macronutrient nutritional status and malaria in pregnancy, given the high prevalence of undernutrition in malaria-endemic countries. The MiPc and other malaria researchers have conducted a number of malaria in pregnancy studies (observational studies and randomised controlled trials [RCTs] of malaria prevention strategies) and many had collected maternal anthropometrics and data on anaemia. Similarly, some recent trials of nutritional

supplementation during pregnancy collected malariometric indices. Drawing on these data, there is a unique opportunity to study the interaction between undernutrition and malaria, together with other risk factors such as maternal anaemia.

The Maternal Malaria and Malnutrition (M3) initiative has pooled data from thirteen studies with the hope of improving the understanding of malaria-nutrition interactions and to foster collaboration between nutritionists and malariologists. Reduction of LBW and anaemia were two of the six global nutrition targets for 2025 agreed upon at the 2012 World Health Assembly (126). As of 2014, the Global Nutrition Report found that there was little progress towards this target (21).[19] Risk factors for, and interventions to prevent, LBW are all too commonly studied in isolation and without cross-discipline collaboration. Topics of interest for M3 include an evaluation of whether macronutrient undernutrition modifies the impact of malaria infection during pregnancy; the investigation of potential interventions that address both malaria risk and nutritional status during pregnancy; and the study of the role of anaemia in relation to adverse pregnancy outcomes.

Pooled cohort description

Study populations

The M3 initiative comprises thirteen studies (seven RCTs and six cohort studies) conducted amongst pregnant women in malaria-endemic countries from 1996 to 2015 (8,37,60,70–74,76–80,88). Studies were selected based on the availability of a predetermined set of essential variables for each woman, adequate ethical clearance, and willingness of collaborators to share data. Essential variables included the assessment of malariometric indices (light microscopy [LM], quantitative polymerase chain reaction (qPCR), and/or rapid diagnostic tests [RDT]) at enrolment/first antenatal care visit (ANC), assessment of anthropometric

indicators at enrolment (mid-upper arm circumference [MUAC] and/or body mass index [BMI]), gravidity, type of malaria prevention used, and maternal age. Essential delivery outcomes included birthweight and newborn sex. With birthweight being the primary focus, we restricted inclusion to women who delivered live singleton infants without congenital abnormalities, as previously recommended (69).

We included 14,633 singleton, live birth pregnancies from women who had participated in one of 13 pregnancy studies (Table 5.1) conducted in seven countries in Africa and one in the Western Pacific (Figure 5.1, Table 5.1). Of the seven RCTs, four assessed the effectiveness of different pharmacological malaria prevention regimens (70,71,79,80), one additionally evaluated nutritional supplementation (72), one was designed solely to assess the effectiveness of nutritional supplementation (60), and one measured the impact of insecticide-treated bed nets (ITNs) on birthweight (73). The six prospective cohort studies were all designed to assess risk factors and consequences of malaria infection and/or antimalarial treatment during pregnancy in different locations and among different study populations (8,74,76–78,88).

For ten of the thirteen studies, pregnant women were identified and recruited at one of their self-initiated ANC, usually the booking visit but sometimes at a subsequent visit (77). The EMEP study enrolled women early in pregnancy, but a subset of these same women were also enrolled in a cross-sectional study at delivery, the IPTp-MON study (78,79). The Asembo Bay, ITN cohort (Kenya) identified pregnant women through monthly community census, and the FSP/MISAME (Burkina Faso) study recruited through a community-based network of home visitors (72,73). Three studies excluded HIV-infected pregnant women (37,60,80), three studies did not assess HIV status (70,72,74), and the remaining seven studies included both HIV-infected and HIV-negative pregnant women.

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The predominant malaria species observed in all studies was *P. falciparum*. The two studies in Papua New Guinea (PNG) also reported *P. vivax* infections (70,74). Some studies detected *P. ovale* and *P. malariae* infections but prevalence was very low (<1%). Studies not differentiating between species (n=4) were conducted in areas where *P. falciparum* is the principal malaria species, and *P. vivax* is thought to be largely absent (60,72,73,88).[20–22,25] Eleven studies reported high and perennial malaria transmission, while two studies reported medium to high perennial malaria transmission (60,70).

Measurements

Women in all studies had at least two study visits, at enrolment and delivery, but the frequency and timing of follow-up visits during pregnancy varied by study. Given logistical and computational intensity required in pooling longitudinal measures and substantial heterogeneity in frequency and timing of measurements, only data at enrolment and at delivery were pooled from every study. Table 5.2 and Table 5.3 show available enrolment and delivery measurements for each included cohort. Measurements included both those variables deemed essential for the main analyses on malaria, anaemia, and malnutrition, and those variables deemed optional but informative. A few measurements, specifically including malaria infection diagnostics, malnutrition anthropometrics, and anaemia diagnostics warrant further discussion.

Malaria infection at enrolment: At enrolment malaria infection was diagnosed by examination of peripheral blood (82). Parasitaemia was assessed using one or more of the three following approaches: light microscopic (LM) examination of a Giemsa-stained blood smear, qPCR, and rapid diagnostic tests (RDTs) (Table 5.2). In all but two studies, malaria at enrolment was routinely diagnosed by LM. In the FSP/MISAME study in Burkina Faso, LM was only performed if a woman presented with symptoms consistent with malaria, i.e. fever or history of

fever (72). In the iLiNS-DYAD Ghana study, malaria was only diagnosed using a RDT (60). RDTs detect circulating target malaria antigens and are increasingly used in malaria-endemic areas when microscopy is not readily available (82,84). However, persistence of circulating target antigens after parasite clearance can lead to false positive test results (82). Sensitivity of qPCR is much higher than LM and RDTs, but the role of submicroscopic infections detected by qPCR alone in causing adverse outcomes has not been clearly determined (85,92).

Malaria infection at delivery: In addition to peripheral blood diagnostics at delivery, some studies also assessed placental malaria infection at delivery (Table 5.3). Placental malaria was diagnosed by LM of a placental blood smear, qPCR, and/or placental histology (90). Three criteria are used to classify placental malaria by histology: presence of infected erythrocytes; haemozoin in monocytes/macrophages; and haemozoin in fibrin deposits (91). Placental histology is considered the diagnostic reference standard for placental infection.

Mid-upper arm circumference (MUAC) and body mass index (BMI): Maternal macronutrient nutritional status was assessed by measurement of women's MUAC and/or BMI at enrolment. MUAC, the circumference of the upper arm, assessed at the mid-point between the elbow and the tip of the shoulder, is frequently used as a broad indicator of maternal protein reserves, fat stores, and macronutrient nutritional status (94). MUAC was measured in ten of the thirteen studies, which included 61% of our total study population (Table 5.2). Weight and height (to derive BMI) were additionally measured in nine of these studies as well as in the three studies where MUAC was unavailable (Table 5.2). While neither measurement is perfect, MUAC and BMI are easy and low-cost nutritional assessments that can be undertaken in low-resource settings, and are clinically relevant (93).

Anaemia at enrolment: Most women had haemoglobin measurements done at first ANC, and most received routine iron/folate supplements, although information on their use was not routinely collected. The dosage of folate supplementation (0.4 to 5 mg) varied by study site.

Findings to date

Findings from each individual study emphasise how pooling this data has the potential to generate important information on malaria-nutrition interactions in pregnancy.

The ECHO study in the DRC was the first study to report an interaction between malaria and malnutrition on the risk of FGR (8). In this study, the risk of FGR was two to eight times higher among women with evidence of malnutrition. In this study, the magnitude of the difference in effect sizes depended on the measurement for malnutrition (BMI vs MUAC vs short stature vs weight gain) and the measurement for malaria (cross-sectional versus cumulative); however, with all measurements of malnutrition and malaria the pattern and directionality of the modification was consistent. This study, together with a small number of follow-up studies, provided the necessary preliminary data for the M3 initiative (9,11).

Two participating studies provided evidence regarding nutritional interventions during pregnancy. The FSP/MISAME in Burkina Faso study concluded that multiple micronutrient-fortified food supplementation (FFS) increased mean birthweight, and that this effect was most pronounced amongst women who were anaemic or undernourished early in their pregnancy (130). The iLiNS-DYAD trial in Ghana found modest overall increases in birthweight with the provision of lipid-based nutrient supplements compared to iron and folic acid supplementation, but showed that the intervention may be most effective when given to primiparous women (60). While nutritional assessment was not the primary aim of the remaining studies, the IPTp RCT in PNG and the STOPPAM cohort study in Benin reported associations between low maternal

anthropometrics and birthweight (88,131).

Other studies largely focused on malaria prevention. In the late 90s, the Kisumu cohort study found that implementation of two doses of IPT-SP halved the prevalence of placental malaria infection. In another study conducted concurrently in Kenya (Asembo Bay study) use of ITNs was associated with a marked reduction in placental malaria and LBW, but (as expected) with no apparent impact on nutritional status (73). The LAIS study, conducted in Malawi from 2003-2006, found that adding azithromycin to two courses of monthly SP (SPAZ) was better at reducing malaria at delivery, LBW, and preterm birth than monthly SP or two doses of SP (71,132,133). SPAZ was also superior when compared to the standard malaria prevention regimen used in PNG (SP plus chloroquine),(70) where placental infection is an important risk factor for LBW (74). The EMEP cohort study found that use of artemisinin combination treatments during the first trimester was not associated with increased risk of miscarriage (78). The IPTp-MON study found that with increasing resistance, the efficacy of IPTp-SP in clearing existing infections or preventing new ones is compromised, however, where the sextuple mutant is rare, it remains associated with improvements in birthweight and maternal haemoglobin (79).

The STOPPAM cohort studies conducted in Benin and Tanzania provided observational data on the effects and potential limitations of two-dose IPTp-SP. Specifically, the STOPPAM-Benin study found that a substantial proportion of women acquired malaria infections late in pregnancy, some time after the second IPTp dose had been given (88). The STOPPAM-Tanzania study also found that malaria infections in the first or second trimester were associated with altered fetal growth that might not be detectable until the 3rd trimester (76). Trial data from the FSP/MISAME study further corroborated these observational studies; the FSP/MISAME study found that malaria infection during the first trimester was associated with higher risk of LBW

and that additional doses of IPTp might be more effective (72,134). These studies, along with others, have highlighted the importance of early and frequent malaria prevention to improve birth outcomes, and IPT-SP is now routinely given monthly until delivery (34,135).

The ISTp Malawi study assessed another malaria prevention strategy by comparing IPTp-SP to IPTp with intermittent screening for malaria infection (ISTp) at ANC visits using RDTs and treating infected women with dihydroartemisinin-piperaquine (DHA-PQ) (38). The STOPMIP Kenya study also assessed ISTp with DHA-PQ, comparing it to IPTp with DHA-PQ as well as IPTp-SP. Analyses from both studies suggest that ISTp-DHA-PQ is not superior to the existing strategy of IPTp-SP, although IPTp-DHA-PQ was more effective than both ISTp-DP and IPTp-SP (37).

Future plans

We plan to assess whether maternal macronutrient nutritional status, as assessed by MUAC, BMI and maternal height, alters the risk of reduced birthweight that is associated with malaria infection during pregnancy. Among women who underwent a dating ultrasound in early pregnancy, analyses will be performed using preterm birth and small-for-gestational age as endpoints instead of LBW. To inform policy decisions, we further plan to use advanced epidemiologic methods to stochastically model the impact that plausible targeted antimalarial and nutritional interventions during pregnancy could have on the number of babies born LBW in malaria endemic areas (13–15). Additional future analyses include, but are not limited to, the following: the impact of anaemia on LBW; the interaction between anaemia and malaria with regards to increasing the risk of LBW; the mediation of the effect of malaria through anaemia on LBW; and the usefulness and agreement of MUAC versus BMI to predict adverse birth outcomes. Short maternal stature, potentially indicative of stunting and reduced adolescent catch-

up growth (chronic undernutrition), has also been associated with increased risks of adverse birth outcomes and will be explored using this pooled dataset (136). Finally, we will assess aforementioned relationships and effects amongst pregnant women with HIV, a patient cohort known to be at increased risk of adverse outcomes due to malaria and malnutrition (63).

Strengths and limitations

This pooled birth cohort holds promise as the largest pregnancy dataset to date to address questions regarding malaria and nutrition that are of great importance to maternal and infant health. Prior research on this topic has been limited by insufficient statistical power to examine the interaction between malaria and malnutrition. The pooled M3 provides offers the opportunity to overcome this limitation by increasing statistical power and precision, which is particularly valuable for conducting subgroup analyses and investigating interactions. The substantial size of the pooled dataset also facilitates the application of rigorous methodological approaches for systematically missing data, such as multiple imputation by chained equations, standardisation, latent variable methods, or transformation of measurements to create commensurate measures (137,138). Availability of individual-level data, rather than aggregate data from each study, allows definitions of exposures and outcomes to be harmonised. Individual-level information on malaria and malnutrition further enables implementation of complex modelling to assess targeted antimalarial and nutritional interventions during pregnancy. The diversity of study populations included in the M3 cohort, e.g. with respect to location, ethnicity, malaria transmission intensity, malaria and nutrition interventions, health infrastructure, and HIV prevalence, could provide important data with regards to generalisability of our findings. Specifically, this level of population diversity, coupled with availability of data on multiple risk factors for LBW and greater statistical power to assess subgroup effects, may allow the identification of high risk

women who could benefit most from more intensive antenatal care, nutritional supplements, and alternative malaria prevention regimens, and of the settings in which these interventions would be most effective.

The main limitations of the pooled dataset are related to missing data and heterogeneity of collected data. First, information that was collected by all studies varied, leading to systematically missing data on a number of important variables (Tables 5.2 and 5.3). The limited availability of accurately measured gestational age hinders the ability to conduct preterm birth and small-for-gestational age analyses for the entire dataset (Table 5.3), but should allow for meaningful subanalyses. Second, exclusion of miscarriages and stillbirths may cause selection bias (left truncation) for some analyses, given the possibility of differential rates of early pregnancy losses in relation to infection and nutritional status (139). We will assess this potential bias using data on miscarriages and stillbirths extracted from a subset of studies (37,38,72,78). Third, potential confounders such as concomitant helminth infection and micronutrient deficiencies were not measured in participant studies (140,141). Fourth, while MUAC only modestly changes throughout pregnancy, BMI tends to increase with gestational age: gestational age-adjusted BMI may be one approach to address this problem. Fifth, while we have information on HIV status for a majority of studies, information on use of antiretroviral therapy (ART) was not extracted, and secular changes over time regarding use and efficacy of ARTs could influence the comparability of results across studies that enrolled HIV-infected women. Finally, since the intention was to concentrate on areas of moderate to high *P. falciparum* transmission, we did not include any studies representing other malaria-affected regions such as Central/South America and India.

Table 5.1. Characteristics of the 13 individual studies included in the Maternal Malnutrition and Malaria (M3) Initiative.

Countries	Study Name	Design	Period	Median GA (IQR)*	Malaria prevention†	Nutritional intervention	N‡	n§
Benin	STOPPAM I	Cohort	2008-2010	17 (14-20)	IPT-SP	None	1037	791
BF	FSP/MISAME	RCT	2006-2008	16 (11-21)	IPT-SP(2), IPT-SP(3)	MMS, FFS	1296	1020
DRC	ECHO	Cohort	2005-2006	19 (17-21)	IPT-SP	None	182	164
Ghana	iLiNS-DYAD	RCT	2009-2012	17 (15-20)	IPT-SP	LNS, MMN, IFA	1320	1068
Kenya	EMEP & IPTpMon	Cohort #	2011-2013	23 (16-30)	IPTp-SP	None	1453	471#
Kenya	ITN	RCT	1996-1999	24 (20-30)	IPT-SP started during study	None	911	711
Kenya	Kisumu cohort	Cohort	1996-2001	36 (34-37)	IPT-SP started during study	None	3155	3388**
Kenya	STOPMIP	RCT	2012-2015	23 (20-26)	IPT-SP, IPT-DHA-PQ, IST-DHA-PQ	None	1546	1203
Malawi	ISTp	RCT	2011-2013	21 (19-23)	IPT-SP, IST-DP	None	1873	1602
Malawi	LAIS	RCT	2003-2006	20 (18-23)	IPT-SP(2), IPT-SP(4) IPT-SPAZ	None	1320	1190
PNG	IPTp study	RCT	2009-2013	22 (19-25)	IPT-SPAZ; Single dose SP and weekly CQ	None	2793	1943
PNG	Sek cohort	Cohort	2005-2007	25 (22-28)	Single dose SP and weekly CQ	None	470	293
Tanzania	STOPPAM II	Cohort	2008-2010	19 (15-21)	IPT-SP	None	995	789

BF=Burkina Faso. CQ= chloroquine. DHA-PQ=Dihydroartemisinin-piperaquine. DRC=Democratic Republic of the Congo. FFS=fortified food supplementation. IFA=iron and folic acid supplementation. IPT (doses)=intermittent preventive treatment in pregnancy. IST=intermittent screening for malaria infection. LNS=lipid-based nutrient supplementation. MMS=multiple micronutrients supplementation. PNG=Papua New Guinea. RCT=randomised controlled trial. SP=sulphadoxine-pyrimethamine. SPAZ= SP and azithromycin.
* Median (IQR): Gestational age at enrolment assessed by fetal biometry, or symphysis-pubis fundal height when ultrasound unavailable.
†If RCT, describes the intervention, if cohort, describes the national policy during the study period
‡N=Enrolled in parent study.
§ *n*=live birth pregnancies that met inclusion criteria for M3.
# The EMEP study was a prospective cohort study with some overlapping enrolment with the cross-sectional study IPTp-MON. 111 pregnancies were enrolled in both EMEP and IPTp- MON; information on malaria infection at delivery was obtained for the subset of women in IPTp-MON.
**Includes additional women from a sub-study not included in the parent study which otherwise met inclusion criteria for the pooled data.

Table 5.2. Key measures at enrolment across the 13 studies included in the Maternal Malnutrition and Malaria (M3) Initiative.

Variable	STOPPAM - Benin	FSP/ MISAME - BF	ECHO - DRC	iLiNS-DYAD-Ghana	EMEP& IPTpMon-Kenya	ITN-Kenya	Kisumu - Kenya	STOPMIP -Kenya	ISTp-Malawi	LAIS-Malawi	IPTp-PNG	Sek-PNG	STOPPAM -Tanzania
Essential													
Malaria													
LM	X	X*	X		X	X	X	X	X	X	X	X	X
PCR								X	X		X	X	
RDT	X†			X‡				X§	X§				X†
Anthropometrics													
MUAC	X*	X	X	X		X*		X#		X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X		X
Gravidity	X	X	X	X	X	X	X	X	X	X	X	X	X
Anaemia††													
Haemoglobin	X	X		X	X	X	X	X	X	X	X	X	X
Haematocrit			X										
Optional													
Gestational age													
Ultrasound	X	X	X	X	X*				X	X	X*		X
Fundal height	X	X*	X	X	X*	X	X	X	X	X	X	X	X
Age	X	X	X	X	X	X	X	X	X	X	X	X	X
Smoking (Y/N)	X		X		X					X	X	X	X
HIV	X		X	X	X	X*	X	X	X	X			X
Rural/urban area	X		X	X	X	X	X	X	X	X	X	X	X
Bed net	X		X		X	X		X	X	X	X	X	X*

Available information indicated with X, missing data >10% indicated by asterisk, and systematically missing data indicated with grey highlighting.

BF=Burkina Faso. DRC=Democratic Republic of the Congo. HIV=human immunodeficiency virus. IPTp=intermittent preventive treatment in pregnancy. ISTp=intermittent screening for malaria infection in pregnancy. ITN=insecticide-treated bed nets. LM=light microscopy. MUAC=mid-upper arm circumference. PNG=Papua New Guinea. PRC=polymerase chain reaction. RDT=rapid diagnostic test.

† The STOPPAM Benin and Tanzania studies primarily used the Parascreen RDT (Zephyr Biomedical Systems, Goa, India), which detects *P. falciparum* histidine protein-2 (HRP-2) and *Plasmodium* lactate dehydrogenase (pLDH).

‡ The iLiNS-DYAD-Ghana study used the Clearview Malaria Combo RDT (British Biocell International Ltd., Dundee, United Kingdom), which detects HRP-2 and plasmodium aldolase.

§ Women randomized to the ISTp arms of the STOPMIP-Kenya and ISTp-Malawi studies were tested with a First Response Malaria Ag. (pLDH/HRP2) Combo RDT (Premier Medical Corporation, India).

Maternal MUAC measured at delivery.

†† Nine studies measured haemoglobin using a HemoCue haemoglobinometer (Hemocue, Angelholm, Sweden),[20–22,26–31] one study relied on haemoglobin measures from a local laboratory (unknown make),[23] one study used Sysmex hematological analyzer (Kobe, Japan),[32] and one study collected capillary blood (finger stick) specimens for determination of haematocrit or haemoglobin levels using a standardised chart.[10] In the ITN-Kenya study, haemoglobin was measured using a HemoCue haemoglobinometer (Hemocue, Angelholm, Sweden) before 1997, and from 1997 onwards capillary blood (finger stick) specimens were collected for determination of haematocrit levels, which were divided by a factor of three and presented as haemoglobin values for consistency with the 1996 data.

Table 5.3. Key measures at delivery across the 13 studies included in the Maternal Malnutrition and Malaria (M3) Initiative.

Variable	STOPPAM - Benin	FSP/ MISAME - BF	ECHO - DRC	iLiNS-DYAD- Ghana	EMEP& IPTpMon- Kenya	ITN- Kenya	Kisumu - Kenya	STOPMIP -Kenya	ISTp- Malawi	LAIS- Malawi	IPTp- PNG	Sek- PNG	STOPPAM -Tanzania
Essential variables													
Malaria- peripheral													
LM	X*	X*	X		X*†	X	X	X	X	X*	X	X	X
PCR									X*		X	X	
Malaria-placental													
LM	X*	X*			X*†	X	X	X	X		X*	X*	X*
PCR								X	X		X*	X*	X*
Histology			X					X	X		X*	X*	
IPTp doses	X	X*	X		X			X	X*	X	X		X
Birthweight	X	X	X	X	X	X	X	X	X	X	X	X	X
Infant sex	X	X	X	X	X	X	X	X	X	X	X	X	X
Optional variables													
Gestational age													
By ultrasound	X	X	X	X	X*				X	X	X*		X
Neonatal anthropometrics													
Abdomen circumference	X*		X								X		X
Chest circumference		X								X			
Head circumference	X	X	X	X	X		X	X	X	X	X	X	X
Crown heel length		X	X	X	X	X		X			X	X	
Timing of anthropometrics	X		X	X	X	X	X	X	X	X	X	X	X

Available information indicated with X, missing data >10% indicated by asterisk, and systematically missing data indicated with grey highlighting.

BF=Burkina Faso. DRC=Democratic Republic of the Congo. IPTp=intermittent preventive treatment in pregnancy. ISTp=intermittent screening for malaria infection in pregnancy. ITN=insecticide-treated bed nets. LM=light microscopy. PNG=Papua New Guinea. PRC=polymerase chain reaction

† For the EMEP/IPTpMON study, only women co-enrolled in the IPTpMON study (n=111) had malaria diagnostics at delivery.

CHAPTER VI: MALARIA, MALNUTRITION, AND BIRTH WEIGHT:
A POOLED ANALYSIS OF 14,633 PREGNANCIES

Introduction

Annually, over 20 million infants are born low birthweight (LBW; <2500 g),

predominantly in low- and middle-income countries (LMICs) (16). LBW can have negative

impacts on neonatal mortality and childhood neurological, metabolic, and physical development

(2). The World Health Organization (WHO) has set a Global Nutrition Target of 30% reduction

in LBW by 2025 (16).

One preventable cause of LBW in LMICs is maternal malaria infection (1,2). Its

prevalence remains high, despite targeted malaria prevention programs (2). Annually, 125

million pregnant women are at risk for malaria (4). The predominant species, *Plasmodium*

falciparum, sequesters in the placenta, causing LBW through fetal growth restriction (FGR) and

preterm delivery (2). Prior estimates from Africa suggest that malaria infection doubles the risk

of LBW (2,4). Prevention of malaria in pregnancy remains a public health priority.

Another modifiable risk factor for impaired fetal growth is maternal malnutrition,

specifically undernutrition (17). Up to 20% of African women of reproductive age are

undernourished (17,46,93). Maternal protein-energy-fat (macronutrient) and micronutrient

reserves and dietary consumption influence fetal growth. Micronutrient deficiencies are difficult

and costly to assess and therefore anthropometrics are commonly used as sensitive but non-

specific indicators of protein reserves, fat stores, and malnutrition more broadly (93).

Recent evidence indicates that the relationship between malaria infection and LBW may depend upon the mother's nutritional status (51). Studies in Papua New Guinea (PNG) and Benin found inconsistent evidence of modification of the malaria infection -LBW relationship by maternal anthropometric status, but studies from Kenya and the Democratic Republic of the Congo (DRC) reported significant modification (8,9,11,12). Notably, in DRC the risk of FGR associated with malaria infection was two to eight times higher among malnourished women (8). Malaria infection and malnutrition may act along similar physiological pathways by affecting placental development and nutrient transfer (2,4,17).

To date, work on this potential interaction has been limited to 4 studies with only 1,318 pregnant women from Africa and 1,369 pregnant women from PNG. Not only were these studies somewhat inconsistent in their findings, but their interpretation is hindered by relatively small sample sizes, potentially limiting their generalizability to other malaria -endemic countries. The objective of this study was to investigate the putative interaction between maternal malaria infection and malnutrition in relation to LBW using a large pooled dataset of thirteen studies conducted in multiple LMICs. We hypothesized that there would be a synergistic interaction, such that the observed joint effect of being both infected with malaria and malnourished would be greater than expected if considering each exposure independently.

Methods

Study population

We used data from 14,633 singleton live birth pregnancies from women participating in thirteen studies in eight African countries and the Western Pacific (PNG) as part of the Maternal Malaria and Malnutrition (M3) initiative (8,11,37,60,71–75,77–80,142).

Outcomes and exposures

The main outcome was birth weight (BW), analyzed continuously and dichotomized at

2,500 grams (LBW) (16). Weights measured after 24 hours (13% of weights) were adjusted

using a cubic regression model to account for weight changes in the first week of life (143).

Among nine studies with ultrasound-dated gestational age we used small-for-gestational age

(SGA) as a secondary outcome, defined as a BW less than the 10th percentile of the

INTERGROWTH-21st reference (25).

Diagnostics for malaria were collected at study enrollment and at delivery. At study

enrollment, we defined malaria based on light microscopic (LM) examination of a Giemsa-

stained peripheral blood smear or a rapid diagnostic test (RDT) for malaria antigen (28). At

delivery, we defined malaria based on peripheral or placental LM or placental histology (active

or past infection). Given the uncertain impact of sub-microscopic infections on LBW and the

variation in availability of polymerase chain reaction (PCR) diagnostics across studies, we

excluded PCR results (92). In sensitivity analyses, we explored definitions of malaria including

PCR results, and 'any malaria', defined as a positive LM, RDT, or PCR at enrollment, delivery,

or during pregnancy (in five studies with relevant data).

The primary measure of maternal malnutrition was low mid-upper arm circumference

(MUAC) at enrollment, dichotomized at 23 cm (93). MUAC changes little over pregnancy,

making it a useful measure of malnutrition (93). Since some studies did not measure MUAC, we

used body mass index (BMI) as a secondary measure of malnutrition. According to the WHO, a

pre-pregnancy BMI <18.5 kg/m^2 is predictive of adverse birth outcomes (48). BMI at enrollment

was used to estimate pre-pregnancy BMI by adjusting maternal weight measured in the

second/third trimesters using a cubic regression model to account for gestational weight gain (48). Low adjusted-BMI was defined as values under 18.5 kg/m^2. As the correlation between BMI and MUAC is not perfect, indicators were analyzed separately (93).

Statistical analysis

We analyzed maternal malaria infection and malnutrition as co-primary exposures and assessed malnutrition as a modifier of the malaria -LBW relationship. While effect measure modification (EMM) assesses how the effect of one exposure varies across strata of another variable, interaction analyses assess the joint effects of two exposures (114). We performed both interaction and EMM analyses, however in the context of this work, interaction is preferable to EMM because interventions for both malaria infection and malnutrition might prevent LBW.

We employed a two-stage approach for pooling individual-level data from each study. We examined the consistency of results with a one-stage approach, fitting a generalized mixed model with random intercepts and slopes. Study-specific risk ratios (RRs) and mean BW differences were calculated using linear and log binomial regression models controlling for confounding using inverse probability of treatment weights (IPTW) truncated at the 1st and 99th percentiles. A minimally sufficient set of confounders was identified using a directed acyclic graph based upon background knowledge of covariate relationships (116). We identified confounders for both malaria infection and malnutrition relative to LBW since we were analyzing them as co-primary exposures. Confounders for the relationship between malaria infection at enrollment and LBW included maternal age, gravidity, rural versus urban residence, malnutrition (MUAC when available, otherwise BMI), and HIV infection. Because malaria infection is a cause of anemia, the latter was considered a mediator, and not a confounder. We

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explored modification of the effect of malaria infection at enrollment on LBW by maternal gravidity and doses of intermittent preventive therapy (IPTp) received. When assessing malaria infection at delivery, anemia at enrollment and the number of IPTp doses were considered confounders. Confounders for the malnutrition-LBW relationship included maternal age, gravidity, rural versus urban residence, anemia at enrollment, and HIV infection. Partially missing data were imputed using multivariate normal multiple imputation (144). We calculated interaction estimates using a product term in the multiplicative and additive model for LBW and the additive model for mean BW (114). These estimates reflect whether the effect of exposure to both malaria infection and malnutrition exceeds the product (or sum) of the effects of each exposure considered separately, and quantifies this difference. A product term greater than one on the multiplicative scale or greater than zero on the additive scale is indicative of interaction between malaria infection and malnutrition.

Study-specific estimates were pooled using DerSimonian and Laird restricted maximum likelihood method random effects models (109). When τ^2, the estimated variance of the random effects distribution, was greater than zero, we calculated 95% population effects intervals (PEI), which incorporate the estimated variance between studies (109). If τ^2 equaled zero, the random effects model was interpreted as a fixed effects model. We decided *a priori* to evaluate modification of the results by time period (before versus after 2008) due to changes in antimalarial recommendations, study type (trial/cohort), location (Africa/Western Pacific), and malaria infection prevalence (based on M3 data), using meta-regression.

Results

Study population characteristics

The trimester at enrollment, anemia prevalence, gravidity distribution, area of residence, and HIV prevalence varied across studies (Table 6.1). The prevalence of malaria infection at enrollment, low MUAC, and joint malaria infection and low MUAC also varied by study (Figure 6.1). Among 8,152 women with both measurements, only 2% had both low MUAC and malaria infection at enrollment. The prevalence of malaria infection among women with low MUAC was 16%, compared to 12% among well-nourished women (p=0.0005). The prevalence of low BMI varied across studies, and was different from, although correlated with, the prevalence of low MUAC (χ^2 p<.0001; Supplemental Figure 6.1). The joint prevalence of malaria infection at enrollment and low BMI was also 2%. Of all 14,633 women, 35% were infected with malaria at either enrollment or delivery or had low MUAC or BMI. The prevalence of LBW was 9% (range 5% to 15%).

Independent effects of malaria infection and malnutrition

The pooled IPTW-adjusted risk ratio (aRR) for the effect of malaria infection at enrollment on LBW was 1.14 (95% CI: 0.91, 1.42; 95% PEI: 0.72, 1.80), and the mean BW difference was -55 g (95% CI: -79, -30) (Figure 6.2a). The effect of malaria infection at delivery was more pronounced: aRR, 1.32 (95% CI: 1.08, 1.62; 95% PEI: 0.91, 1.91) (Figure 6.2b). When considering SGA as the outcome, results were the same for malaria infection at enrollment and slightly attenuated for malaria infection at delivery. The effect of malaria infection at enrollment was attenuated among those with more than one IPTp dose versus one or zero doses (aRR 0.98 vs 1.22) and was stronger among primi/secundigravid versus multigravida women (aRR 1.19 vs

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1.14). A slightly stronger effect of malaria infection was seen among women enrolled in studies conducted prior to 2008, in Africa, or with malaria infection prevalence at or above the median (Supplemental Figure 6.2).

The aRR for the effect of low MUAC on LBW was 1.60 (95% CI: 1.34, 1.87); the mean BW difference was -142g (95% CI: -171, -113) (Figure 6.3a). Results were similar for low BMI: aRR, 1.49 (95% CI: 1.26, 1.76); mean BW difference -133g (95% CI: -158, -108) (Figure 6.3b). There was no modification by study characteristics on the malnutrition-LBW relationship (Supplemental Figure 6.3). Similar trends were observed when SGA was used as the outcome among the studies with ultrasound data.

Interaction and EMM

The joint aRR for both malaria infection at enrollment and low MUAC was 2.13 (95% CI: 1.21, 3.73; 95% PEI: 0.80, 5.67) and the mean BW difference was -163 g (95% CI: -253, -75; 95% PEI: -328, 0). The multiplicative interaction term for LBW was 1.30 (95% CI: 0.62, 2.72; 95% PEI: 0.39, 4.31), the additive interaction term for LBW was -0.01 (95% CI: -0.09, 0.08; 95% PEI: -0.13, 0.11), and the additive interaction term for mean BW difference was 38 g (95% CI: -90, 166; 95% PEI: -219, 295). Sensitivity analyses that varied the definitions of malaria, malnutrition, outcome, and analytic approach did not qualitatively alter the results (Supplemental Table 6.1). Meta-regression indicated apparent multiplicative interaction and slight additive interaction between MUAC and malaria infection at enrollment among studies conducted in Africa (multiplicative interaction term, 2.47 (95% CI: 1.12, 5.42); additive interaction contrast, 0.06 (95% CI: -0.05, 0.17) Supplemental 6.4), but this interaction was not seen when assessing malaria infection at delivery or BMI or when accounting for multiple

comparisons with a Bonferroni correction (99% CI: 0.88, 6.95). In EMM analyses, the aRR for

the effect of malaria infection at enrollment on LBW among low MUAC women was 1.32 (95%

CI: 0.66, 2.63; 95% PEI: 0.36, 4.79), compared to 0.98 (95% CI: 0.74, 1.29) among well-

nourished women.

Discussion

Using the large M3 dataset, we found that pregnant women who were both infected with

malaria and malnourished were at greater risk of LBW and reduced mean BW compared to their

uninfected, well-nourished counterparts. The relative risk of LBW associated with joint malaria

infection at enrollment and low MUAC was 2.13 (95% CI: 1.21, 3.73; 95% PEI: 0.80, 5.67).

While this relative risk was greater than the individual effects of malaria infection and

malnutrition separately, there was overall no convincing evidence of synergism, i.e. excess risk

due to interaction. This suggests that malaria infection and malnutrition largely act independently

to influence fetal growth.

A 2004 review estimated that women infected with placental malaria were twice as likely

to have a LBW infant (19). Our findings are broadly consistent with this review, although with

weaker effects on LBW (overall aRR for malaria infection at delivery: 1.32 [95% CI: 1.08, 1.62],

aRR restricted to African studies: 1.55 [95% CI: 1.29, 1.85]), possibly reflecting increased access

to preventive strategies and fewer chronic infections (1,4). In support of this, the effect of

malaria infection on LBW appears lower in women who received more doses of IPTp. The

effects of malaria infection at enrollment on LBW were weaker than at delivery, contradicting

the theory that malaria infection earlier in pregnancy is more disruptive to placental function (2).

This weaker effect at enrollment could either suggest that antimalarial treatment, provided in

most studies, cleared infection and allowed catch-up growth or that infection at delivery represents more severe infections that were not cleared despite medications.

Our data are consistent with a 2011 meta-analysis, which estimated that underweight women had increased risks of LBW (aRR: 1.64 [95% CI: 1.38, 1.94]), although studies included in that meta-analysis used different definitions for underweight (52). In our study, using consistent cut points of malnutrition across studies, both low MUAC (aRR 1.60 [95% CI: 1.36, 1.87]) and low BMI (aRR 1.49 [95% CI: 1.26, 1.76]) increased the risk of LBW. This information is consistent with other evidence that adequate maternal nutrition is integral for fetal growth (17).

Prior literature on the interaction between malaria infection and malnutrition is sparse. Two studies in the DRC and Kenya showed that the association between malaria infection and reduced fetal growth was greatest among malnourished women (8,9). In a third study in Benin, the effect of malaria infection on fetal growth velocity was greatest among women with low anthropometric status, but there was no modification of the effect of malaria infection on birthweight z-scores. A fourth study in PNG found that the effect of histology-defined placental malaria infection on LBW was higher among women with a low BMI, but found malnutrition did not modify the association between peripheral blood malaria infection parasitemia and SGA (11). The Benin, Congo, and PNG studies were included in the present analysis, but our analytic approach differed from the original publications in the assessment of both interaction and modification. Unlike these prior studies, our pooled results suggest that there is a negligible impact of maternal anthropometry on the relationship between malaria infection and LBW and further indicate that there is no evidence of excess risk of LBW due to interaction (i.e. synergism). We note that in an *a priori* sensitivity analysis restricted to African studies, there

was apparent interaction between malaria infection at enrollment and MUAC, which is consistent with the prior publications. Regional differences could be due to genetics, low MUAC or anemia prevalence; however, these sub-region effects were not statistically significant when properly accounting for multiple comparisons, and were absent when using other definitions of malaria (i.e. at delivery) or malnutrition (i.e. BMI). Notably, only 183 women (2%) were jointly infected and malnourished (low MUAC). Thus, even if there is a multiplicative interaction between malaria infection and MUAC among African women, the proportion of women implicated is small, and does not indicate a large public health burden. However, even in the absence of strong interaction between malaria infection and malnutrition on LBW, we emphasize that interventions on both malaria infection and malnutrition are warranted given their independent effects.

This work had several strengths and limitations. We substantially increased the number of women in whom the hypothesized interaction between malaria infection, malnutrition, and LBW was investigated; notably, the number of pregnant women from Africa was almost ten times more than all prior studies. Analyzed studies were performed in a variety of settings, increasing the generalizability of these results. Furthermore, availability of individual-level data enabled us to harmonize definitions and minimize heterogeneity. On the other hand, we were obliged to pool malaria diagnostics of varying sensitivity and specificity, and we were limited to two cross-sectional assessments of malaria infection. Nevertheless, sensitivity analyses that evaluated alternative definitions of malaria, or incorporated repeat diagnostics during pregnancy, were consistent with the main results. Additionally, there may be selection bias due to excluding pregnancy losses. There were only 116 (3%) pregnancy losses in four studies (N=4,571) in M3 that collected these data, but this is almost certainly an underestimate, since many studies

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enrolled women after the first trimester. We were limited to extrapolating a pre-pregnancy BMI using gestational age and BMI at enrollment. Additionally, the M3 was a convenience sample of studies with available malaria and nutrition measurements, rather than a random sample of extant data. Furthermore, women enrolled in studies were likely healthier and received better antenatal care than the general population; the effects of malaria and malnutrition in reality might well be greater than observed within these research settings. Finally, we cannot discount possible unmeasured confounding, particularly by helminth infections, sexually transmitted infections, environmental pollutants, or micronutrient deficiencies; however, it is important to note that because neither malnutrition nor malaria could be randomized, large-scale, multi-site cohort analyses such as this one are necessarily the gold standard for addressing these scientific questions. Future studies may wish to explore joint effects of malaria and other nutritional indicators (e.g., height, obesity, anemia) on preterm birth as well as SGA and LBW.

In summary, our findings suggest that women who are both infected with malaria and malnourished are at greater risk of LBW than their uninfected, well-nourished counterparts, but that there is no conclusive evidence of synergistic interaction between the two. Rather, we propose that malaria infection and malnutrition act independently to disrupt fetal growth, and that malnutrition in particular has a strong effect on LBW. Of all 14,633 pregnancies, 35% were affected by malaria infection and/or malnutrition, illustrating the high burden of at-risk pregnancies in LMICs. Malaria infection and malnutrition represent two established and modifiable causes of LBW that should both be addressed to optimize pregnancy outcomes in LMIC.

Table 6.1. Characteristics of women participating in thirteen studies included in the Maternal Malaria and Malnutrition (M3) initiative

	Kisumu- Kenya	IPTp-PNG	ISTp-Malawi	STOPMIP-Kenya	LAIS-Malawi	iLiNS-Ghana
	(N=3388)	(N=1943)	(N=1602)	(N=1203)	(N=1190)	(N=1068)
Study enrollment (years)	1996-2001	2009-2013	2011-2013	2012-2015	2003-2006	2009-2012
Maternal age	20 (18-24)	24 (20-28)	21 (18-26)	22 (19-27)	24 (20-29)	26 (22-30)
Gravidity						
1 (Primi-)	1656 (49)	966 (50)	542 (34)	403 (34)	267 (22)	349 (33)
2 (Secundi-)	748 (22)	494 (21)	448 (28)	237 (20)	213 (18)	351 (33)
3+ (Multi-)	984 (29)	573 (29)	612 (38)	563 (47)	710 (60)	368 (34)
Trimester*						
1	0 (0)	72 (4)	0 (0)	21 (2)	0 (0)	103 (10)
2	0 (0)	1780 (92)	1585 (99)	991 (82)	1190 (100)	881 (82)
3	3388 (100)	91 (5)	17 (1)	191 (16)	0 (0)	81 (8)
Missing GA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0)
Anemic†						
Yes	2548 (75)	1348 (69)	533 (33)	591 (49)	459 (39)	305 (29)
No	808 (24)	512 (26)	1069 (67)	612 (51)	731 (61)	763 (71)
Missing	32 (1)	83 (4)	0 (0)	0 (0)	0 (0)	0 (0)
HIV						
Yes	810 (24)	-	0 (0)	0 (0)	144 (12)	0 (0)
No	2560 (76)	-	1602 (100)	1203 (100)	931 (78)	1059 (99)
Missing	18 (1)	1943 (100)	0 (0)	0 (0)	115 (10)	9 (1)
Area of Residence						
Rural	722 (21)	1185 (61)	1590 (99)	1027 (85)	1190 (100)	0 (0)
Urban	2666 (77)	758 (39)	10 (1)	169 (14)	0 (0)	1068 (100)
Missing	0 (0)	0 (0)	2 (0)	7 (1)	0 (0)	0 (0)
Bed net ownership						
Yes	-	1798 (93)	327 (20)	681 (57)	877 (74)	-
No	-	145 (7)	1275 (80)	522 (43)	313 (26)	-
Missing	3388 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1068 (100)

Table 6.1 Continued.

	FSP/ MISAME- BF (N=1020)	STOPPAM- Benin (N=791)	STOPPAM- Tanzania (N=789)	ITN- Kenya (N=711)	EMEP/IPTp MON-Kenya (N=471)	Sek-PNG (N=293)	ECHO- DRC (N=164)
Study enrollment (years)	2006-2008	2008-2010	2008-2010	1996-1999	2011-2013	2005-2007	2005-2006
Maternal age	23 (19.5-28)	25 (22-30)	26 (22-31)	24 (20-30)	24 (20-30)	24 (21-28)	27 (23.5-31)
Gravidity							
1 (Primi-)	205 (20)	147 (19)	162 (21)	127 (18)	94 (20)	115 (39)	43 (26)
2 (Secundi-)	216 (21)	173 (22)	201 (25)	118 (17)	77 (16)	54 (18)	22 (13)
3+ (Multi-)	599 (59)	471 (59)	426 (54)	466 (66)	300 (64)	124 (42)	99 (60)
Trimester*							
1	385 (38)	174 (22)	88 (11)	3 (0)	67 (14)	0 (0)	6 (4)
2	595 (58)	616 (78)	701 (89)	376 (53)	247 (52)	214 (73)	158 (96)
3	40 (4)	1 (0)	0 (0)	292 (41)	140 (30)	75 (26)	0 (0)
Missing GA	0 (0)	0 (0)	0 (0)	40 (6)	17 (4)	4 (1)	0 (0)
Anemic†							
Yes	372 (36)	354 (45)	289 (37)	416 (59)	169 (36)	272 (93)	43 (26)
No	630 (62)	433 (55)	497 (63)	293 (41)	297 (63)	21 (7)	107 (65)
Missing	18 (2)	4 (1)	3 (0)	2 (0)	5 (1)	0 (0)	14 (9)
HIV							
Yes	-	13 (2)	39 (4)	51 (7)	0 (0)	-	4 (2)
No	-	699 (88)	693 (88)	234 (33)	468 (99)	-	160 (98)
Missing	1020 (100)	79 (10)	57 (7)	426 (60)	3 (1)	293 (100)	0 (0)
Area of Residence							
Rural	1020 (100)	791 (100)	430 (55)	711 (100)	471 (100)	282 (96)	0 (0)
Urban	0 (0)	0 (0)	354 (45)	0 (0)	0 (0)	8 (3)	164 (100)
Missing	0 (0)	0 (0)	5 (1)	0 (0)	0 (0)	3 (1)	0 (0)
Bed net ownership							
Yes	-	254 (32)	571 (72)	348 (49)	-	240 (82)	164 (100)
No	-	537 (68)	52 (7)	363 (51)	-	49 (17)	0 (0)
Missing	1020 (100)	0 (0)	166 (21)	0 (0)	471 (100)	4 (1)	0 (0)

Categorical variables are expressed as N (%) and continuous variables are expressed as median (IQR). Dash indicates information on particular factor was not assessed in parent study. BF=Burkina Faso. DRC=Democratic Republic of the Congo. GA=gestational age. HIV=human immunodeficiency virus. PNG=Papua New Guinea. * Based on ultrasound if measured, otherwise based on Ballard's score or symphysis-pubis fundal height (SFH). When using SFH, to adjust for misclassification in the first trimester, a fundal height< 7 cm was defined as first trimester, while SFH<28 cm was defined as second trimester, and SFH≥28cm defined as third trimester. † Anemic= hemoglobin <11 g/dL of venous blood, if available, or hematocrit <33%, in the first and third trimesters, and less than 10.5 and 32, respectively for the second trimester.

Figure 6.1. Prevalence of malaria infection at enrollment, malnutrition (MUAC<23cm or BMI < 18.5 kg/m2), and joint malaria infection and malnutrition among 14,633 live birth pregnancies from women participating in studies (years 1996-2015) included in the Maternal Malaria and Malnutrition (M3) initiative.
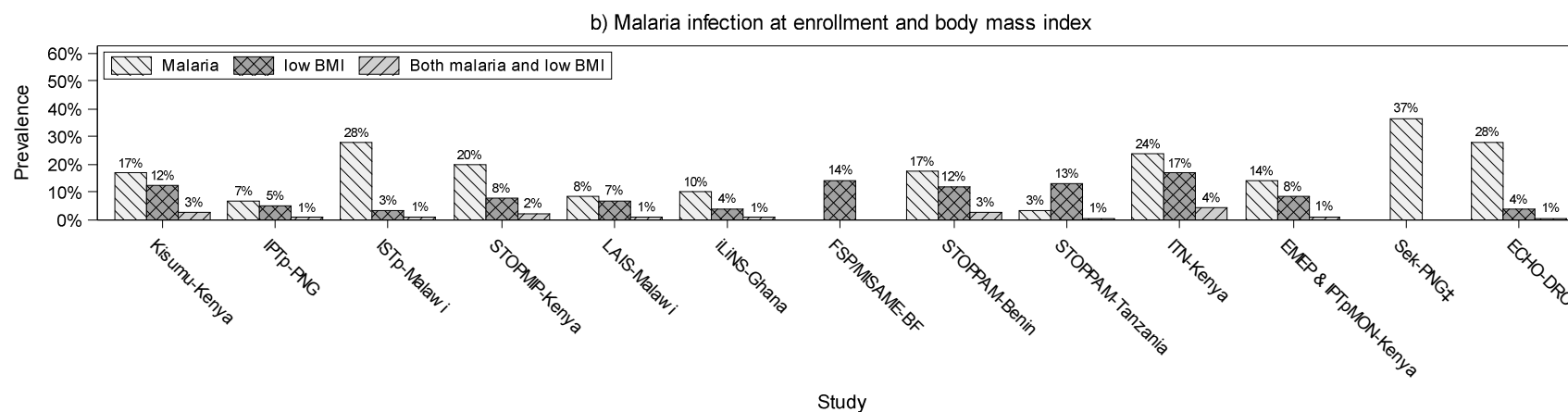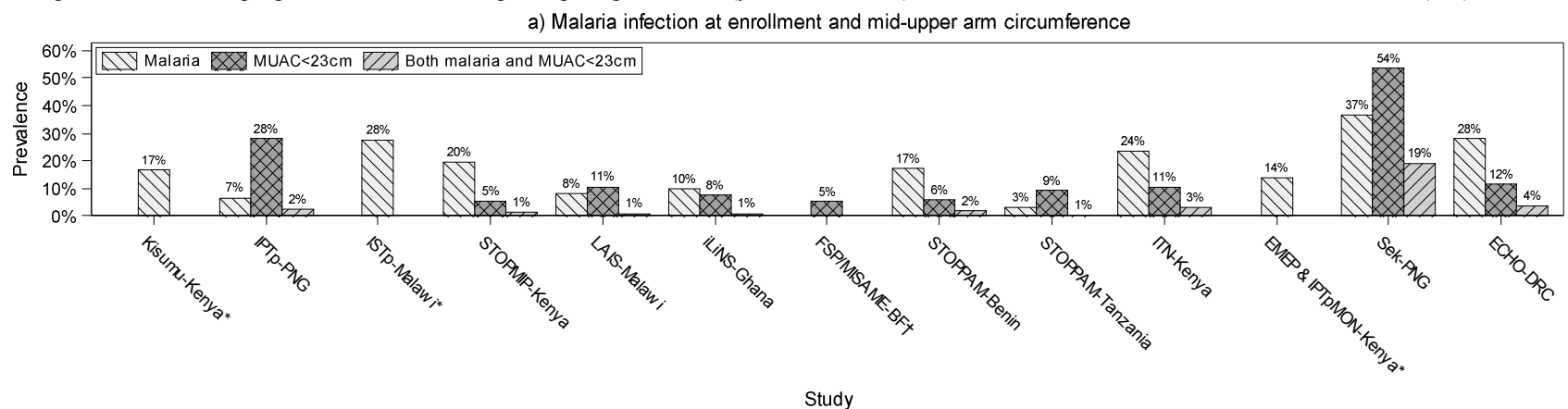
a) Malaria infection at enrollment and mid-upper arm circumference

b) Malaria infection at enrollment and body mass index

BF=Burkina Faso. DRC=Democratic Republic of the Congo. MUAC=mid-upper arm circumference. PNG=Papua New Guinea.

* Did not record mid-upper arm circumference

† Did not systematically diagnose malaria infection at study enrollment

‡ Did not record maternal height

Figure 6.2. The independent effects of (a) malaria infection at enrollment and (b) malaria infection at delivery on risk of low birthweight and mean birthweight among women enrolled in one of thirteen studies from the Maternal Malaria and Malnutrition (M3) initiative. Inverse probability of treatment weighted (IPTW) estimates controlled for confounding between malaria at enrollment and LBW by maternal age, gravidity, area of residence, mid-upper arm circumference at enrollment, and HIV infection (where available). IPTW estimates controlled for confounding between malaria at delivery and LBW additionally controlled for anemia and number of doses of antimalarial intermittent preventive therapy received during pregnancy. aRR= adjusted risk ratio. BF=Burkina Faso. BW=Birthweight. CI= Confidence interval. DRC=Democratic Republic of the Congo. LBW=Low birthweight. N/A=not available. N/C= no model convergence. PNG=Papua New Guinea.

(a) Effect of Malaria Infection at Enrollment on Low Birthweight (LBW) and mean Birthweight (BW)

Study	LBW aRR [95% CI]	Study	Mean BW Difference (g) [95% CI]
Kisumu-Kenya	1.51 [1.06 , 2.15]	Kisumu-Kenya	-74 [-116 , -31]
IPTp-PNG	0.65 [0.39 , 1.09]	IPTp-PNG	26 [-57 , 108]
ISTp-Malawi	1.68 [1.23 , 2.30]	ISTp-Malawi	-73 [-125 , -21]
STOPMIP-Kenya	0.86 [0.41 , 1.81]	STOPMIP-Kenya	-14 [-92 , 64]
LAIS-Malawi	1.28 [0.68 , 2.41]	LAIS-Malawi	-98 [-212 , 17]
iLiNS-Ghana	0.79 [0.39 , 1.58]	iLiNS-Ghana	-20 [-101 , 61]
FSP/MISAME-BF	N/A	FSP/MISAME-BF	N/A
STOPPAM-Benin	1.18 [0.68 , 2.05]	STOPPAM-Benin	-97 [-198 , 4]
STOPPAM-Tanzania	0.84 [0.20 , 3.42]	STOPPAM-Tanzania	-54 [-217 , 108]
ITN-Kenya	1.36 [0.75 , 2.46]	ITN-Kenya	-48 [-134 , 37]
EMEP & IPTpMon-Kenya	0.47 [0.14 , 1.58]	EMEP & IPTpMon-Kenya	107 [-51 , 264]
Sek-PNG	1.22 [0.70 , 2.12]	Sek-PNG	-110 [-219 , -2]
ECHO-DRC	0.90 [0.25 , 3.23]	ECHO-DRC	-124 [-257 , 10]
	1.14 [0.91 , 1.42]		-55 [-79 , -30]

(b) Effect of Malaria Infection at Delivery on Low Birthweight (LBW) and mean Birthweight (BW)

Study	LBW aRR [95% CI]	Study	Mean BW Difference (g) [95% CI]
Kisumu-Kenya	1.66 [1.19 , 2.31]	Kisumu-Kenya	-80 [-122 , -39]
IPTp-PNG	0.97 [0.71 , 1.33]	IPTp-PNG	-32 [-92 , 29]
ISTp-Malawi	1.50 [1.07 , 2.10]	ISTp-Malawi	-42 [-92 , 8]
STOPMIP-Kenya	1.17 [0.66 , 2.07]	STOPMIP-Kenya	-69 [-128 , -11]
LAIS-Malawi	0.40 [0.06 , 2.85]	LAIS-Malawi	37 [-162 , 237]
iLiNS-Ghana	N/A	iLiNS-Ghana	N/A
FSP/MISAME-BF	1.71 [0.94 , 3.12]	FSP/MISAME-BF	-68 [-218 , 83]
STOPPAM-Benin	1.51 [0.87 , 2.62]	STOPPAM-Benin	-72 [-186 , 42]
STOPPAM-Tanzania	N/C	STOPPAM-Tanzania	33 [-495 , 560]
ITN-Kenya	1.87 [1.00 , 3.47]	ITN-Kenya	-106 [-201 , -11]
EMEP & IPTpMon-Kenya	N/C	EMEP & IPTpMon-Kenya	-144 [-318 , 31]
Sek-PNG	0.77 [0.45 , 1.32]	Sek-PNG	20 [-82 , 123]
ECHO-DRC	1.99 [0.55 , 7.17]	ECHO-DRC	5 [-231 , 240]
	1.32 [1.08 , 1.62]		-59 [-81 , -36]

Figure 6.3. The independent effect of malnutrition at enrollment, (a) MUAC<23cm and (b) BMI*<18.5 kg/m^2), on risk of low birthweight and mean birthweight among 14,633 women enrolled in one of thirteen studies from the Maternal Malaria and Malnutrition (M3) initiative from 1996 to 2015. BMI adjusted for gestational age to reflect estimated first trimester weight. Inverse probability of treatment weighted (IPTW) estimates controlling for confounding between malnutrition (MUAC or BMI) at enrollment and LBW by maternal age, gravidity, area of residence, anemia, and HIV infection (where available). BMI=Body mass index. BW=Birthweight. CI= Confidence interval. DRC=Democratic Republic of the Congo. MUAC=mid-upper arm circumference. N/A=not available. N/C= no model convergence. PNG=Papua New Guinea.

(a) Effect of MUAC<23cm at Study Enrollment on Low Birthweight (LBW) and Mean Birthweight (BW)

Study	LBW aRR [95% CI]	Study	Mean BW Difference (g) [95% CI]
Kisumu-Kenya	N/A	Kisumu-Kenya	N/A
IPTp-PNG	1.59 [1.27 , 1.99]	IPTp-PNG	-145 [-192 , -98]
ISTp-Malawi	N/A	ISTp-Malawi	N/A
STOPMIP-Kenya	1.92 [0.76 , 4.88]	STOPMIP-Kenya	-177 [-307 , -47]
LAIS-Malawi	1.25 [0.74 , 2.13]	LAIS-Malawi	-108 [-198 , -18]
iLiNS-DYAD-Ghana	1.70 [0.86 , 3.38]	iLiNS-DYAD-Ghana	-124 [-216 , -33]
FSP/MISAME-Burkina Faso	1.98 [1.20 , 3.28]	FSP/MISAME-Burkina Faso	-227 [-336 , -119]
STOPPAM-Benin	1.81 [0.92 , 3.55]	STOPPAM-Benin	-175 [-313 , -36]
STOPPAM-Tanzania	0.95 [0.38 , 2.42]	STOPPAM-Tanzania	-131 [-230 , -31]
Asembo Bay, ITN-Kenya	1.97 [0.96 , 4.06]	Asembo Bay, ITN-Kenya	-143 [-272 , -13]
EMEP & IPTpMon-Kenya	N/A	EMEP & IPTpMon-Kenya	N/A
Sek-PNG	1.36 [0.78 , 2.38]	Sek-PNG	-84 [-186 , 19]
ECHO-DRC	2.21 [0.45 , 10.78]	ECHO-DRC	-167 [-337 , 2]
	1.60 [1.36 , 1.87]		-142 [-171 , -113]

0.25 0.50 1.00 2.00 4.00   -300 -150 0 150 300

(b) Effect of BMI<18.5 kg/m^2 at Study Enrollment on Low Birthweight (LBW) and Mean Birthweight (BW)

Study	LBW aRR [95% CI]	Study	Mean BW Difference (g) [95% CI]
Kisumu-Kenya	1.35 [0.90 , 2.05]	Kisumu-Kenya	-146 [-190 , -103]
IPTp-PNG	1.53 [1.02 , 2.29]	IPTp-PNG	-159 [-263 , -55]
ISTp-Malawi	1.80 [0.99 , 3.27]	ISTp-Malawi	-140 [-257 , -23]
STOPMIP-Kenya	1.38 [0.59 , 3.20]	STOPMIP-Kenya	-154 [-244 , -64]
LAIS-Malawi	1.64 [0.89 , 3.02]	LAIS-Malawi	-145 [-258 , -32]
iLiNS-DYAD-Ghana	1.75 [0.75 , 4.10]	iLiNS-DYAD-Ghana	-124 [-235 , -13]
FSP/MISAME-Burkina Faso	1.49 [1.01 , 2.19]	FSP/MISAME-Burkina Faso	-97 [-173 , -20]
STOPPAM-Benin	1.89 [1.18 , 3.02]	STOPPAM-Benin	-147 [-248 , -47]
STOPPAM-Tanzania	0.98 [0.44 , 2.16]	STOPPAM-Tanzania	-103 [-196 , -9]
Asembo Bay, ITN-Kenya	0.89 [0.40 , 1.95]	Asembo Bay, ITN-Kenya	-124 [-210 , -39]
EMEP & IPTpMon-Kenya	1.36 [0.41 , 4.52]	EMEP & IPTpMon-Kenya	-112 [-230 , 6]
Sek-PNG	N/A	Sek-PNG	N/A
ECHO-DRC	N/C	ECHO-DRC	-49 [-303 , 206]
	1.49 [1.26 , 1.76]		-133 [-158 , -108]

0.25 0.50 1.00 2.00 4.00   -300 -150 0 150 300

Supplemental Figure 6.1. Prevalence of low mid-upper arm circumference (MUAC<23cm) compared to prevalence of low body-mass index (BMI< 18.5 kg/m^2) among the thirteen studies in the Maternal Malaria and Malnutrition (M3) initiative.
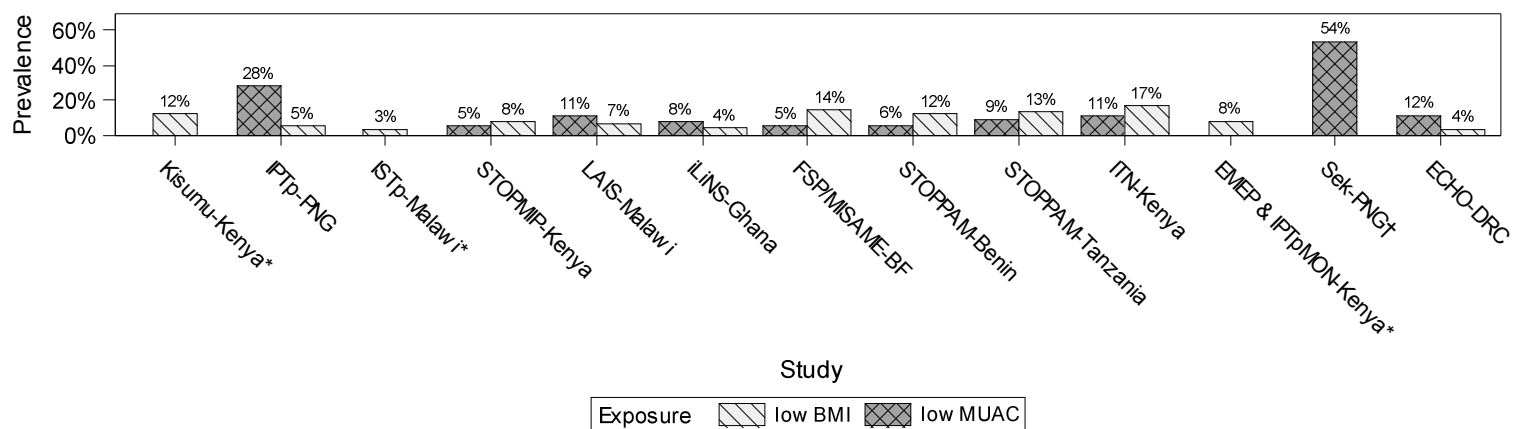
BF=Burkina Faso. BMI=body mass index. DRC=Democratic Republic of the Congo. MUAC=mid-upper arm circumference. PNG=Papua New Guinea. BMI adjusted for gestational age to reflect extrapolated pre-pregnancy/first trimester weight.

* Did not measure mid-upper arm circumference (MUAC).

† Did not measure maternal height.

Supplemental Figure 6.2. Meta-regression results for the effects of malaria infection at enrollment and delivery on risk o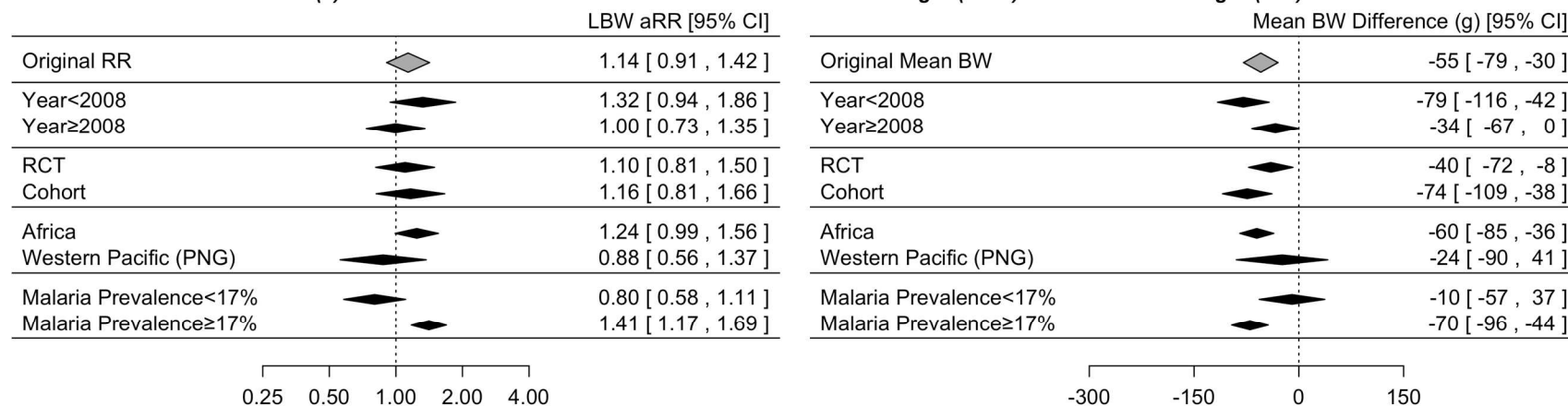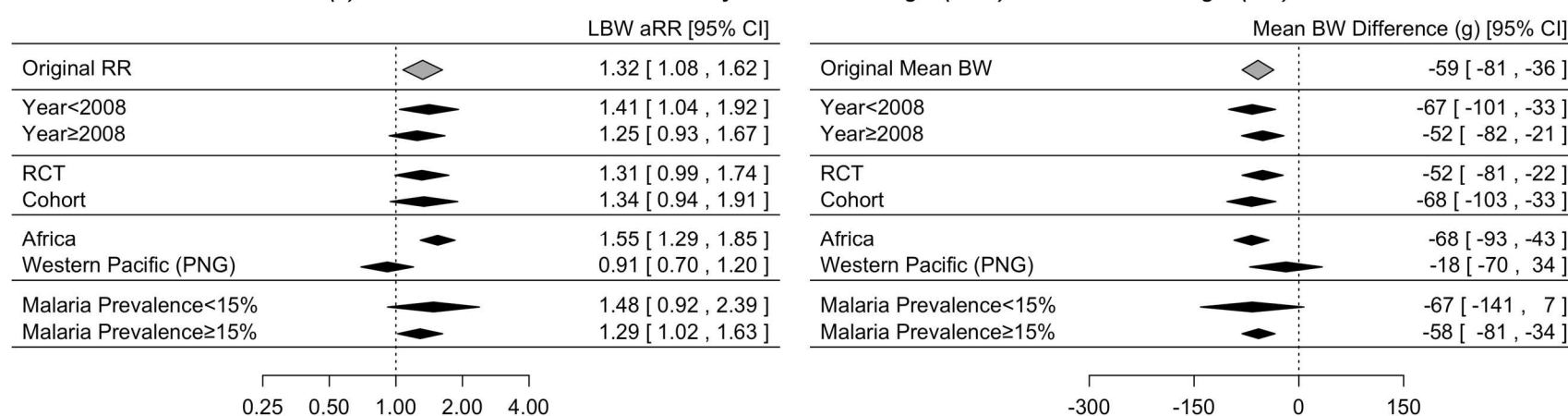f low birthweight (LBW) and mean birthweight (BW) by time period, study type, location, and malaria infection prevalence. Median malaria infection prevalence across studies was 17% at enrollment and 15% at delivery. RCT=randomized control trial.

(a) Effect of Malaria Infection at Enrollment on Low Birthweight (LBW) and mean Birthweight (BW)

	LBW aRR [95% CI]		Mean BW Difference (g) [95% CI]
Original RR	1.14 [0.91 , 1.42]	Original Mean BW	-55 [-79 , -30]
Year<2008	1.32 [0.94 , 1.86]	Year<2008	-79 [-116 , -42]
Year≥2008	1.00 [0.73 , 1.35]	Year≥2008	-34 [-67 , 0]
RCT	1.10 [0.81 , 1.50]	RCT	-40 [-72 , -8]
Cohort	1.16 [0.81 , 1.66]	Cohort	-74 [-109 , -38]
Africa	1.24 [0.99 , 1.56]	Africa	-60 [-85 , -36]
Western Pacific (PNG)	0.88 [0.56 , 1.37]	Western Pacific (PNG)	-24 [-90 , 41]
Malaria Prevalence<17%	0.80 [0.58 , 1.11]	Malaria Prevalence<17%	-10 [-57 , 37]
Malaria Prevalence≥17%	1.41 [1.17 , 1.69]	Malaria Prevalence≥17%	-70 [-96 , -44]

(b) Effect of Malaria Infection at Delivery on Low Birthweight (LBW) and mean Birthweight (BW)

	LBW aRR [95% CI]		Mean BW Difference (g) [95% CI]
Original RR	1.32 [1.08 , 1.62]	Original Mean BW	-59 [-81 , -36]
Year<2008	1.41 [1.04 , 1.92]	Year<2008	-67 [-101 , -33]
Year≥2008	1.25 [0.93 , 1.67]	Year≥2008	-52 [-82 , -21]
RCT	1.31 [0.99 , 1.74]	RCT	-52 [-81 , -22]
Cohort	1.34 [0.94 , 1.91]	Cohort	-68 [-103 , -33]
Africa	1.55 [1.29 , 1.85]	Africa	-68 [-93 , -43]
Western Pacific (PNG)	0.91 [0.70 , 1.20]	Western Pacific (PNG)	-18 [-70 , 34]
Malaria Prevalence<15%	1.48 [0.92 , 2.39]	Malaria Prevalence<15%	-67 [-141 , 7]
Malaria Prevalence≥15%	1.29 [1.02 , 1.63]	Malaria Prevalence≥15%	-58 [-81 , -34]

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Supplemental Figure 6.3. Meta-regression results for the effects of malnutrition at enrollment, (a) low mid-upper arm circumference (MUAC<23cm) and (b) low BMI (BMI<18.5 kg/m2), on risk of low birthweight (LBW) and mean birthweight (BW) by time period, study type, location, and malaria infection prevalence. Median malaria infection prevalence across studies was 17% at enrollment and 15% at delivery. RCT=randomized control trial.

(a) Effect of MUAC<23cm at Study Enrollment on Low Birthweight (LBW) and Mean Birthweight (BW)
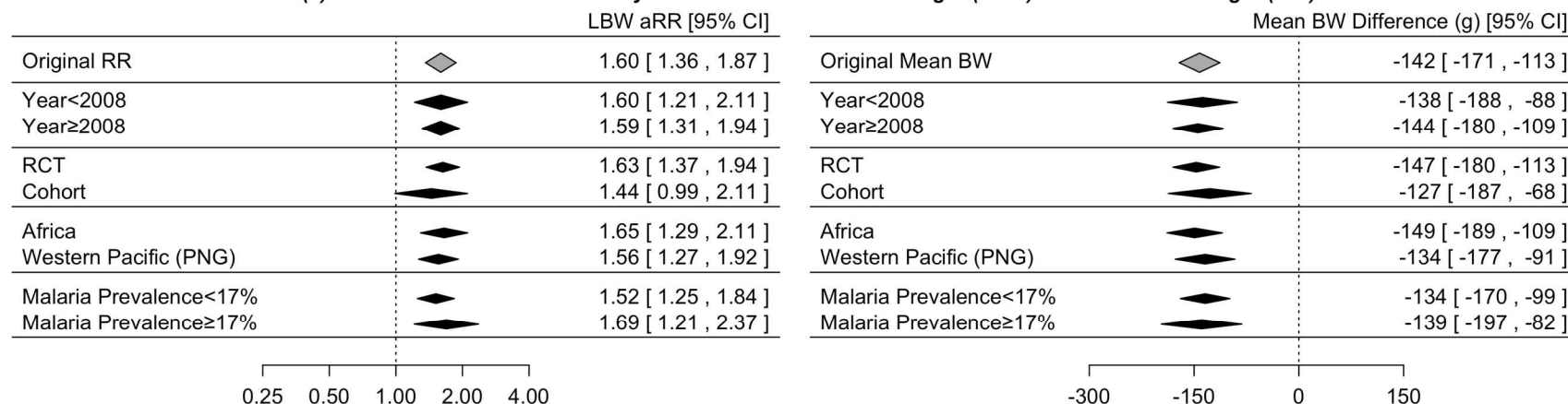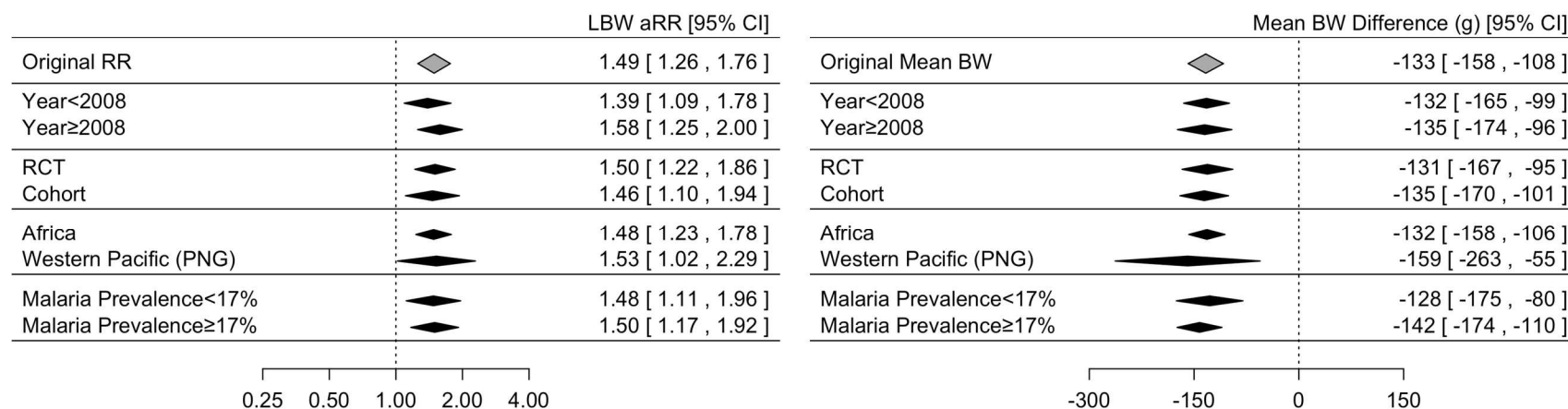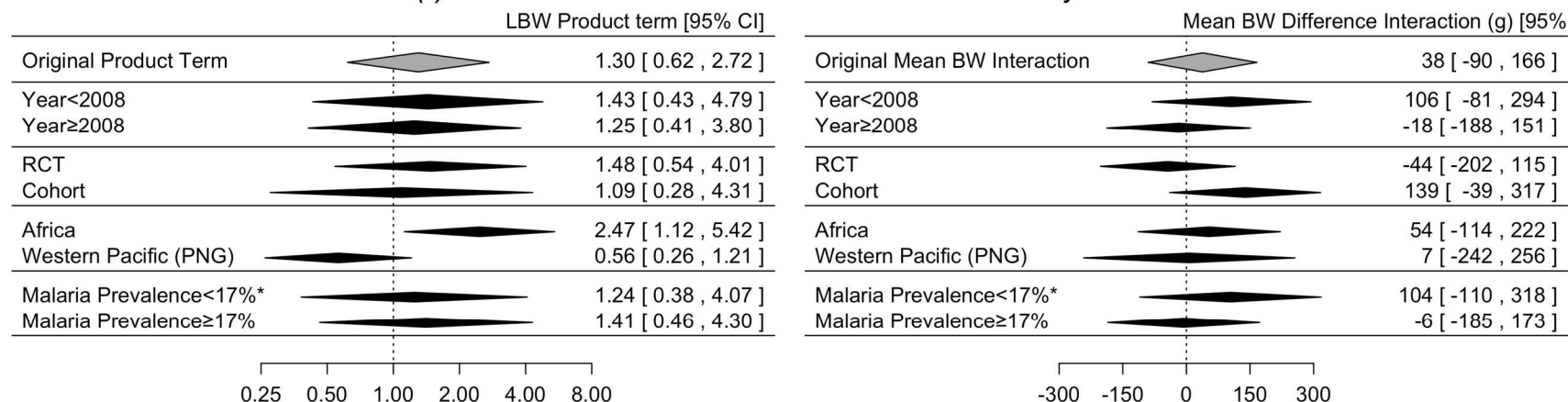
	LBW aRR [95% CI]		Mean BW Difference (g) [95% CI]
Original RR	1.60 [1.36 , 1.87]	Original Mean BW	-142 [-171 , -113]
Year<2008	1.60 [1.21 , 2.11]	Year<2008	-138 [-188 , -88]
Year≥2008	1.59 [1.31 , 1.94]	Year≥2008	-144 [-180 , -109]
RCT	1.63 [1.37 , 1.94]	RCT	-147 [-180 , -113]
Cohort	1.44 [0.99 , 2.11]	Cohort	-127 [-187 , -68]
Africa	1.65 [1.29 , 2.11]	Africa	-149 [-189 , -109]
Western Pacific (PNG)	1.56 [1.27 , 1.92]	Western Pacific (PNG)	-134 [-177 , -91]
Malaria Prevalence<17%	1.52 [1.25 , 1.84]	Malaria Prevalence<17%	-134 [-170 , -99]
Malaria Prevalence≥17%	1.69 [1.21 , 2.37]	Malaria Prevalence≥17%	-139 [-197 , -82]

LBW axis: 0.25 0.50 1.00 2.00 4.00
Mean BW axis: -300 -150 0 150

(b) Effect of BMI<18.5 kg/m² at Study Enrollment on Low Birthweight (LBW) and Mean Birthweight (BW)

	LBW aRR [95% CI]		Mean BW Difference (g) [95% CI]
Original RR	1.49 [1.26 , 1.76]	Original Mean BW	-133 [-158 , -108]
Year<2008	1.39 [1.09 , 1.78]	Year<2008	-132 [-165 , -99]
Year≥2008	1.58 [1.25 , 2.00]	Year≥2008	-135 [-174 , -96]
RCT	1.50 [1.22 , 1.86]	RCT	-131 [-167 , -95]
Cohort	1.46 [1.10 , 1.94]	Cohort	-135 [-170 , -101]
Africa	1.48 [1.23 , 1.78]	Africa	-132 [-158 , -106]
Western Pacific (PNG)	1.53 [1.02 , 2.29]	Western Pacific (PNG)	-159 [-263 , -55]
Malaria Prevalence<17%	1.48 [1.11 , 1.96]	Malaria Prevalence<17%	-128 [-175 , -80]
Malaria Prevalence≥17%	1.50 [1.17 , 1.92]	Malaria Prevalence≥17%	-142 [-174 , -110]

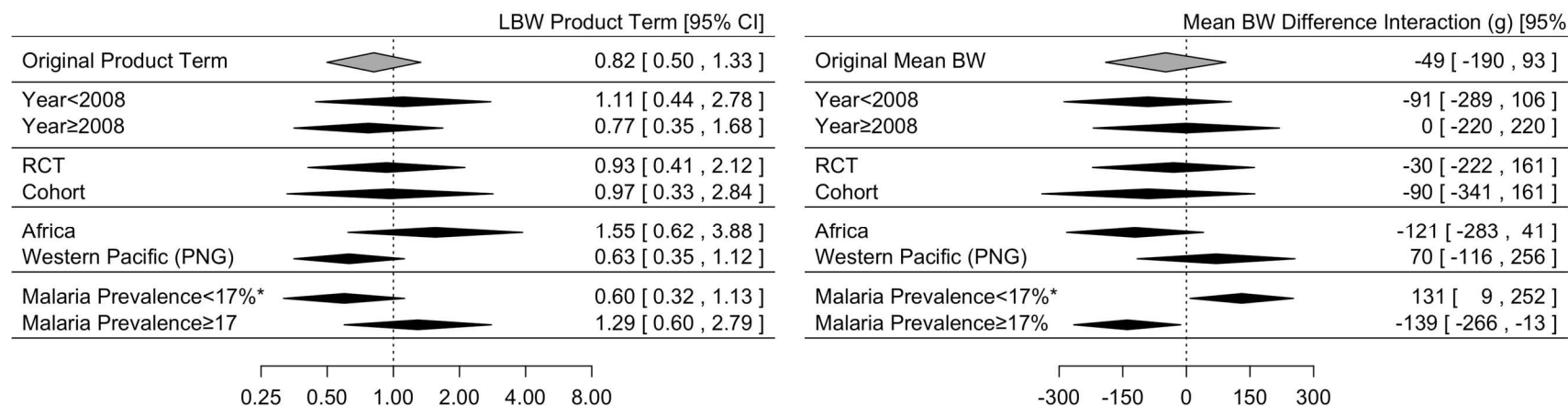LBW axis: 0.25 0.50 1.00 2.00 4.00
Mean BW axis: -300 -150 0 150

Supplemental Figure 6.4. Meta-regression results for the multiplicative and additive interaction effects for malaria infection at enrollment or delivery and low mid-upper arm circumference (MUAC<23cm) on risk of low birthweight (LBW) and mean birthweight (BW) by time period, study type, location, and malaria infection prevalence. Median malaria infection prevalence across studies was 17% at enrollment and 15% at delivery.

(a) Interaction Estimates between low MUAC and Malaria at Study Enrollment

	LBW Product term [95% CI]		Mean BW Difference Interaction (g) [95%
Original Product Term	1.30 [0.62 , 2.72]	Original Mean BW Interaction	38 [-90 , 166]
Year<2008	1.43 [0.43 , 4.79]	Year<2008	106 [-81 , 294]
Year≥2008	1.25 [0.41 , 3.80]	Year≥2008	-18 [-188 , 151]
RCT	1.48 [0.54 , 4.01]	RCT	-44 [-202 , 115]
Cohort	1.09 [0.28 , 4.31]	Cohort	139 [-39 , 317]
Africa	2.47 [1.12 , 5.42]	Africa	54 [-114 , 222]
Western Pacific (PNG)	0.56 [0.26 , 1.21]	Western Pacific (PNG)	7 [-242 , 256]
Malaria Prevalence<17%*	1.24 [0.38 , 4.07]	Malaria Prevalence<17%*	104 [-110 , 318]
Malaria Prevalence≥17%	1.41 [0.46 , 4.30]	Malaria Prevalence≥17%	-6 [-185 , 173]

0.25 0.50 1.00 2.00 4.00 8.00   -300 -150 0 150 300

(b) Interaction Estimates between low MUAC and Malaria at Delivery

	LBW Product Term [95% CI]		Mean BW Difference Interaction (g) [95%
Original Product Term	0.82 [0.50 , 1.33]	Original Mean BW	-49 [-190 , 93]
Year<2008	1.11 [0.44 , 2.78]	Year<2008	-91 [-289 , 106]
Year≥2008	0.77 [0.35 , 1.68]	Year≥2008	0 [-220 , 220]
RCT	0.93 [0.41 , 2.12]	RCT	-30 [-222 , 161]
Cohort	0.97 [0.33 , 2.84]	Cohort	-90 [-341 , 161]
Africa	1.55 [0.62 , 3.88]	Africa	-121 [-283 , 41]
Western Pacific (PNG)	0.63 [0.35 , 1.12]	Western Pacific (PNG)	70 [-116 , 256]
Malaria Prevalence<17%*	0.60 [0.32 , 1.13]	Malaria Prevalence<17%*	131 [9 , 252]
Malaria Prevalence≥17	1.29 [0.60 , 2.79]	Malaria Prevalence≥17%	-139 [-266 , -13]

0.25 0.50 1.00 2.00 4.00 8.00   -300 -150 0 150 300

Supplemental Figure 6.5. Prevalence malaria infection at delivery among the thirteen studies in the Maternal Malaria and Malnutrition (M3) initiative.
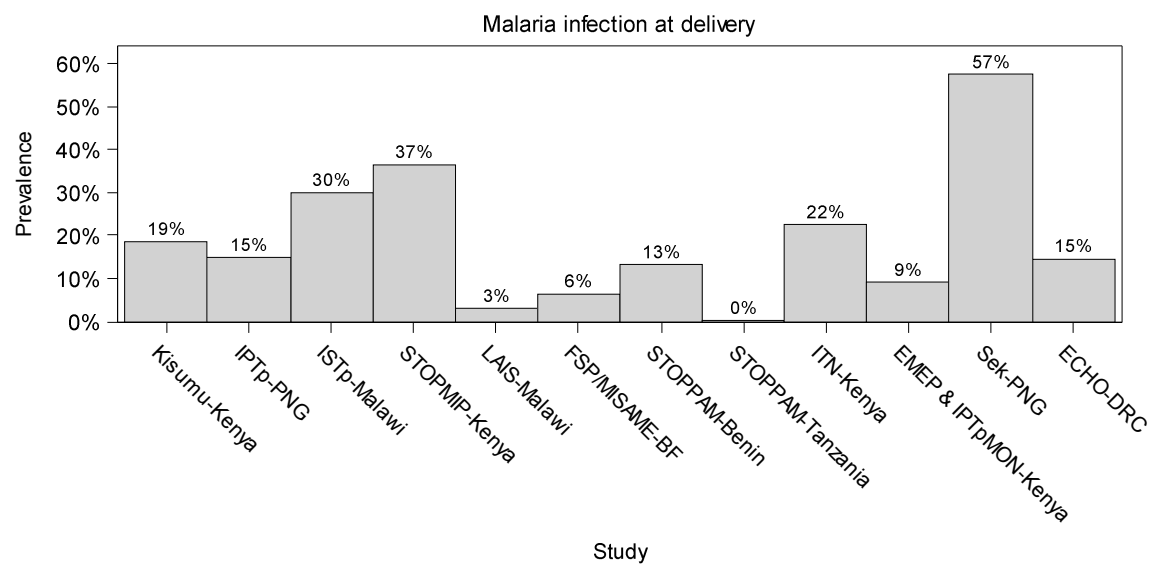
Malaria infection at delivery

BF=Burkina Faso. DRC=Democratic Republic of the Congo. MUAC=mid-upper arm circumference. PNG=Papua New Guinea.

iLiNS-Dyad-Ghana study did not assess malaria infection at delivery.

Supplemental Table 6.1. Selected sensitivity analyses results for the multiplicative interaction effects for malaria and malnutrition on risk of adverse birth outcomes among the thirteen studies in the Maternal Malaria and Malnutrition (M3) initiative. Sensitivity analyses varied the definitions of malaria, malnutrition, and the approach taken in pooling study results, and are stratified by outcome definition.

Malaria Definition	Malnutrition Definition	Method	Product term* (95% CI)
LBW			
Enrollment	MUAC<23 cm	Two-stage	1.30 (0.62, 2.72)
Enrollment	MUAC<23 cm	Two-stage[†]	1.44 (0.64, 3.25)
Enrollment	BMI<18.5 kg/m^2	Two-stage	1.26 (0.77, 2.05)
Enrollment	MUAC<23 cm	One-stage	1.12 (0.71, 1.78)
Delivery	MUAC<23 cm	Two-stage	0.82 (0.50, 1.33)
Delivery	BMI<18.5 kg/m^2	Two-stage	0.90 (0.56, 1.45)
Enrollment- with PCR	MUAC<23 cm	Two-stage	1.57 (0.81, 3.05)
Delivery- with PCR	MUAC<23 cm	Two-stage	0.80 (0.41, 1.56)
Any malaria[‡]	MUAC<23 cm	Two-stage	1.29 (0.70, 2.38)
Any malaria[‡]	BMI<18.5 kg/m^2	Two-stage	0.82 (0.54, 1.24)
SGA			
Enrollment	MUAC<23 cm	Two-stage	0.94 (0.47, 1.85)
Enrollment	BMI<18.5 kg/m^2	Two-stage	1.01 (0.46, 2.21)
Delivery	MUAC<23 cm	Two-stage	1.72 (0.85, 3.48)
Delivery	BMI<18.5 kg/m^2	Two-stage	0.82 (0.17, 3.85)

BMI=body mass index. BW=birth weight. CI= confidence interval LBW=low birthweight. MUAC=mid-upper arm circumference. PCR=polymerase-chain reaction. SGA=small-for-gestational age. * Estimate of the departure from multiplicative interaction. † Analysis included only birth weights measured within 24 hours of delivery. ‡Any malaria infection defined as a positive LM, RDT, or PCR at enrollment, delivery, or during pregnancy (among studies that were able to retrospectively share repeat diagnostics during pregnancy).

CHAPTER VII: INTERVENTIONS AGAINST MATERNAL MALARIA AND
MALNUTRITION: POTENTIAL IMPACT ON PREGNANCY OUTCOMES

Introduction

Low birthweight (LBW) remains a significant global health concern, affecting over 25
million infants annually (16,17). Defined as birth weight less than 2,500 grams, LBW is
associated with a marked increase in infant mortality and contributes to long-term morbidity (5).
In 2012, the WHO endorsed a target to reduce the incidence of LBW by 30% by 2025. As of
2014, the Global Nutrition Report found that there was little progress globally towards this goal
(21). Interventions in low- and middle-income countries (LMICs) to improve fetal growth have
the potential to produce substantial public health effects, improving a wide array of factors
ranging from cognitive development to enhanced neonatal survival.

Two important risk factors for LBW in many LMICs are maternal malnutrition and
malaria infection during pregnancy (1,2,44,51). In malaria-endemic countries, up to one in four
pregnant women are infected with malaria during pregnancy, and estimates of malnutrition
prevalence also reach as high as 20% among women of reproductive age in LMICs (1,17,46).
While these two factors are highly prevalent in many LMICs, they are often studied and
intervened upon independently. Our group, the Maternal Malaria and Malnutrition (M3)
Initiative, has endeavored to better understand this co-burden of malaria infection and
malnutrition during pregnancy. Specifically, in a recent publication using data pooled from
thirteen studies across sub-Saharan Africa and the Western Pacific, we found that women who

were both infected with malaria and malnourished were at greater risk of delivering a LBW infant than their uninfected, well-nourished counterparts, and that malnutrition in particular has a strong effect on LBW (REF Paper 1 "Malaria, Malnutrition, and Birth Weight: A Pooled Analysis of 14,633 Pregnancies").

While results from this study were informative for furthering our understanding of the biologic mechanisms that affect fetal growth and development, they do not address the public health impact of intervening on malaria infection and malnutrition during pregnancy. Policy makers may wish to know how many LBW infants could be prevented by intervening to prevent malaria infection during pregnancy or maternal malnutrition. The current WHO Policy Recommendations for malaria prevention during pregnancy involve three separate evidence-based strategies: insecticide treated bed nets (ITN), intermittent preventive therapy during pregnancy (IPTp), and effective case management (32). The current strategy for IPTp involves monthly doses of sulfadoxine/pryimethamine (SP) starting in the second trimester (32). Despite an increase in the proportion of women receiving IPTp, the level of coverage remains inadequate (39–41). Additionally, interventions to address maternal malnutrition during pregnancy have not been programmatically integrated into antenatal care (51,145).

The objective of this study was two-fold. First, we aimed to estimate the impact of implementing hypothetical targeted antimalarial and nutritional interventions on population-level estimates of LBW. State-of-the-art epidemiologic methods allow us to use complex, real-world data to estimate the effects of plausible public health interventions (13,65,146,147). Second, we aimed to see if the introduction of any combination of these hypothetical targeted antimalarial and nutritional interventions might meet the WHO goal of a 30% reduction in LBW. These results have the potential to improve guidelines for clinical practice among this population and

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provide motivation for development or improvement of interventions to address the dual burden of malaria and malnutrition on pregnancy outcomes.

Methods

Study population

The study population comprised 14,633 live birth pregnancies pooled together in the Maternal Malaria and Malnutrition (M3) initiative, although the sample sizes for each specific analysis depended on the availability of relevant data. The M3 initiative has been described in detail previously (68). Briefly, the study population consisted of pregnant women enrolled in one of thirteen studies conducted from 1996 to 2015 in Sub-Saharan Africa (seven countries) and the Western Pacific (one country). Parent studies were initially undertaken with a range of objectives related to investigating the etiology of malaria during pregnancy, evaluating antimalarial interventions during pregnancy, such as IPTp or ITN, or assessing the effectiveness of nutritional supplementation during pregnancy.

Outcome and exposures

The main outcome of interest for this proposed work was LBW (3). Every study measured birth weight (BW) within one week of delivery, and BW measured after 24 hours (13% of weights) was adjusted using a regression model to account for subsequent changes in weight during the first week of life, and weights measured after 24 hours were excluded in a sensitivity analysis (143). Presence of malaria parasitemia in peripheral blood or in the placenta at delivery was assessed using light microscopic (LM) examination of a Giemsa-stained peripheral or placental smear and/or placental histology (active or past infection) (90). Given the variation in availability of polymerase chain reaction (PCR) diagnostics across studies and the uncertain impact of sub-microscopic infections on adverse birth outcomes, we excluded PCR

results from our definition of malaria infection (92). Most studies had some strategy for malaria prevention during pregnancy, either as part of a randomized control trial or as the national policy during the study period. Information on how many doses of IPTp women received was available for 92% of the study population, and self-reported bed net ownership at study enrollment was also ascertained in nine out of the thirteen studies. However, data on bed net ownership did not distinguish between untreated and insecticide-treated bed nets (ITN), although most recent studies will have been ITN. Currently, the WHO recommends use of SP for IPTp, which is what a majority (64%) of the women received, but some women did receive SP with azithromycin (16%), SP plus chloroquine (15%), or dihydroartemisinin-piperaquine (5%) (32). We used mid-upper-arm circumference (MUAC) at study enrollment as our primary measure of maternal malnutrition, categorizing women as malnourished if their MUAC was less than 23 cm and as well-nourished otherwise (93). MUAC is an indicator of maternal protein reserves, fat stores, and malnutrition more broadly (93). We used gestational-age-adjusted body-mass index less than 18.5 kg/m^2 as a secondary anthropometric indicator of malnutrition.

Population Intervention Effects

Table 7.1 describes a series of contrasts between observed and counterfactual population distributions of malaria infection, IPTp dosage, bed net ownership, and maternal MUAC. First in Table 7.1, we show the comparison of the risk of LBW if the population of women had all been infected with malaria at delivery (all-shaded circle) versus if none of the population had been infected (unshaded circle). While this estimate informs on the etiologic effect of malaria infection at delivery, it is a contrast of two counterfactual exposure distributions and tells us little about the real-world impacts of reducing malaria infection at delivery. To further understand the implications of implementing interventions to reduce malaria infection at delivery, we compared

the *observed* distribution of malaria infection at delivery (the partially-shaded circle) to the

hypothetical population in which no women were infected with malaria (the unshaded circle;

Table 7.1, scenario 2). (REF Westreich et al, in press at *Epidemiology*, "From patients to policy:

population intervention effects in epidemiology"). Although hypothetical and not based on a

specific antimalarial intervention, this contrast provides our best estimate about what would

happen if malaria infection at delivery were completely eliminated.

To better guide policies for specific evidence-based interventions, we also assessed the

impact of scaling up *existing* malaria prevention efforts implemented in the pooled studies,

specifically (i) bed net ownership at enrollment and (ii) IPTp prophylaxis during pregnancy.

Unlike the prior etiologic and intervention effects where we were considering complete

elimination of malaria, now we are specifically estimating the impact of the *scale-up of existing*

malaria prevention strategies. For bed net ownership at enrollment, we compared the *observed*

distribution of bed net ownership (approximately 62% of participants with available information

on bed net ownership) to a counterfactual scenario in which everyone owns a bed net (Table 7.1,

scenario 3). To inform the population-level impact of scaling up IPTp in malaria endemic

settings, we compared the *observed* distribution of IPTp doses to the hypothetical population in

which everyone has i) at least two doses of IPTp or ii) at least three doses of IPTp (similar to the

monthly schedule recommended by the WHO since 2007) (Table 7.1, scenario 4) (32). Given

that the national target for many of the malaria-endemic countries represented by our study

population is 80% coverage, we also assessed increasing the dosage to at least three doses of

IPTp in 80% of the study population (Table 7.1, scenario 4) (40).

Table 7.1 also details population-level contrasts between observed or counterfactual

distributions of maternal malnutrition in early pregnancy, as defined by low MUAC. We took a

similar approach to this exposure as we did to malaria, first comparing the risk of LBW if the population of women all had MUAC below 23 cm (all-shaded circle) versus if all of the women had MUAC at or above 23 cm (unshaded circle), and then comparing the *observed* distribution of MUAC (the partially-shaded circle) to the hypothetical population in which women had MUAC at or above 23 cm (unshaded circle) (Table 7.1, scenarios 5-6). Women with MUAC counterfactual to these scenarios were set either below or above the cut point of 23 cm depending on the contrast. For example, in the scenario where all women were simulated to have normal MUAC, a woman with factual MUAC of 20 cm would have their simulated MUAC set to 23 cm, improving their nutritional status to a normal measurement of MUAC. Additionally, it is unrealistic to expect that an intervention could achieve complete removal of maternal malnutrition; therefore we also compared the observed distribution of malnutrition prevalence at enrollment to the population in which only 5% of each separate study population is malnourished at enrollment (the lowest prevalence of malnutrition from any of our studies).

Statistical Analysis

We used the parametric g-formula to estimate the aforementioned population intervention effects detailed in Table 7.1. The parametric g-formula has been described in detail previously (13,14,148). Briefly: first, we modeled the effect of each exposure (malaria, malnutrition, bed net ownership, or IPTp dosage) on LBW. We used logistic regression models, controlling for known and measured confounders, and generalized estimating equations to account for within-study correlation. Confounders for the relationship between malaria infection at delivery and LBW were determined from a causal directed acyclic graph (DAG) (116) based on prior literature on the relationship between covariates, and included study site, maternal age, gravidity, rural versus urban residence, HIV infection, anemia at enrollment, and the number of IPTp doses.

Confounders for the malnutrition-LBW relationship were obtained from a separate DAG and included study site, maternal age, gravidity, rural versus urban residence, anemia at enrollment, and HIV infection. Study site was the only measured confounder for the bed net coverage-LBW relationship and study site and HIV infection were the only measured confounders for the IPTp-LBW relationship.

Second, we "set" the exposure (malaria, malnutrition, IPTp use, or bed net coverage) to the level specified for the particular hypothesized intervention (see Table 7.1). With this exposure "set" (sometimes counter to the fact of the observed exposure), we predicted the probability of the outcome (having a LBW infant) for each individual.

Third, we averaged the imputed probabilities for having a LBW infant for each individual across the population, for each "set" level of exposure. Comparison of the average outcomes for the different "set" exposure provides us with population-wide predicted effect of changing exposure on the prevalence of LBW in the whole population, a population-standardized risk difference for the specified exposure contrast. We also estimated the number needed to treat (NNT) for each contrast of "set" exposures (except for the unrealistic contrasts of all exposed versus non-exposed), as the reciprocal of the absolute value of the risk difference. NNTs for hypothesized interventions that are only targeted towards the exposed were calculated as the prevalence of the exposure times the reciprocal of the absolute value of the risk difference. Confidence intervals were calculated using a non-parametric bootstrap (200 samples with replacement from original dataset) (117).

Results

Study Population

Characteristics of the 14,633 women participating in the thirteen M3 studies have been reported previously, and are included in Supplemental Table 7.1. While a majority of studies enrolled between 20% to 30% primigravida women, some studies, such as the Kisumu, Kenya, study and the IPTp-PNG study, enrolled roughly 50% primigravida women. The variation in prevalence of anemia was also noticeable, with the lowest prevalence of 26% in ECHO-DRC and the highest prevalence of 93% in the Sek PNG study. The *observed* overall prevalences of malaria infection at delivery, IPTp doses, bed net, and distribution of MUAC at study enrollment are depicted in Figure 1. Specifically, 2,312 (20%) of the 11,832 women with malaria diagnostics were infected with malaria at delivery and 1,224 (14%) of the 8,963 women with MUAC measured were malnourished at enrollment (low MUAC) (Figure 1). However, these prevalences varied greatly by study site, with the prevalence of malaria at delivery ranging from <1% (0.3%) to 57% and the prevalence of malnutrition ranging from 5% to 54% (Supplemental Table 7.1). The median number of IPTp doses among the 13,485 women with IPTp dosage information was 1 (interquartile range: 0, 3) and 5,260 (62%) of the 8,516 women reported owning a bed net (Figure 7.1). The prevalence of LBW was 9% (range 5% to 15%).

Intervention Estimates

The population-standardized risk differences relating to malaria infection at delivery or antimalarial interventions are displayed in Table 7.2. The risk difference for LBW when comparing the population had everyone been infected with malaria at delivery versus if none of the population had been infected was 2.6% (confidence interval [CI]: 1.1%, 3.8%). The population attributable risk difference was smaller (RD: 0.6% [95% CI: 0.2%, 0.8%]) because it

compared the *observed* prevalence of malaria (where only 20% of women overall were infected) to complete elimination of malaria infection.

We then estimated the effects for scaling up existing interventions of bed net ownership and IPTp. The risk difference for the comparison of observed bed net ownership to the population had everyone owned a bed net was 0.4% (95 CI: -0.2%, 1.0%). The risk difference for at least two doses of IPTp compared to the observed distribution was modest (0.6% [95% CI: -0.3%, 1.4%]), but the effect for increasing the dosage to three doses for all women was markedly stronger (RD: 2.9% [95% CI: 2.2%, 3.6%]; NNT: 36 [95% CI: 28, 46]). Increasing dosage to 3+ for at least 80% of the study population also produced substantial impacts on LBW (RD: 2.1% [95% CI: 1.6%, 2.6%]), albeit weaker than complete IPTp 3+ coverage. Results were consistent when excluding women with non-SP exclusive regimens (i.e. SP with azithromycin, SP plus chloroquine, or dihydroartemisinin-piperaquine) and when excluding infants with birth weight measured after 24 hours.

The population-standardized risk differences relating to maternal malnutrition at enrollment are shown in Table 7.3. The risk difference for LBW when comparing the population had everyone had low MUAC at study enrollment versus if all of the population had MUAC at or above 23 cm was 4.0% (95% CI: 3.0%, 5.1%). The risk differences for LBW were smaller when comparing the observed distribution of MUAC to the population had there been no malnutrition (RD: 0.3%, 95% CI: [0.2%, 0.4%]) or to the population where only 5% of each study was malnourished (RD: 0.2%, 95% CI: [0.2%, 0.3%]). The population-standardized risk differences when using BMI as an anthropometric indicator of maternal malnutrition were weaker (Supplemental Table 1).

Discussion

In this work we calculated intervention estimates for the potential impact of reductions in malaria infection at delivery, scale-up of bed net ownership and intermittent preventive therapy (IPTp) during pregnancy, and reductions in maternal malnutrition during early pregnancy on the risk of delivering a low birthweight (LBW) infant using data from thirteen studies in malaria-endemic countries in sub-Saharan Africa and the Western Pacific. Calculation of interventional effects is rarely done in epidemiologic studies, despite the utility of such results from a policy and public health perspective (13,65,146,147). Most epidemiologic studies estimate the effects of specific exposures, contrasting hypotheticals in which everyone is exposed, compared to everyone who is unexposed (REF Westreich et al, in press at *Epidemiology*, "From patients to policy: population intervention effects in epidemiology", (149)). However, these exposure effect estimates may be less valuable for policy decision-makers when considering interventions or changes in clinical practice, protocols, or guidelines (13,14,65,149,150). Overall, these results showed that increasing uptake of IPTp has the potential to have a marked impact on the numbers of infants born LBW in malaria endemic countries.

The absolute difference in the risk of LBW of 2.6% when comparing the extreme contrast in which everyone is infected with malaria at delivery, compared to everyone who is uninfected, illustrates the harmful impact of maternal parasitemia on the risk of LBW. This etiologic effect is consistent with the other etiologic effect measures for malaria infection reported in prior work by the M3 initiative and by other observational studies related to malaria infection during pregnancy (REF Paper 1 "Malaria, Malnutrition, and Birth Weight: A Pooled Analysis of 14,633 Pregnancies", (19)). We previously reported that women infected with malaria at delivery had an average risk of LBW that was 1.3 times greater than uninfected women (adjusted risk ratio: 1.32

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(95% CI: 1.08, 1.62) (REF Paper 1 "Malaria, Malnutrition, and Birth Weight: A Pooled Analysis of 14,633 Pregnancies"). Our risk difference reported here is consistent with that relative effect, and is perhaps the more relevant measure for assessing public health impact (151). In addition to estimating the etiologic effects of malaria infection at delivery on LBW, we also provided estimates for the effect of interventions that would hypothetically eliminate existing malaria infection at delivery. Complete elimination of malaria infection at delivery in these settings seems possible, given that at least one of the studies had a prevalence of malaria infection at delivery that was only 0.3%, and given the current global drive to malaria elimination. However, our population attributable effect illustrates that even if we were to completely remove malaria infection within these settings, there would only be a 0.6% absolute reduction in the prevalence of LBW.

The population attributable effect for bet net ownership was also notably weak: we estimated that a complete uptake of bed net ownership would only result in a 0.4% absolute reduction in the prevalence of LBW compared to current levels over all studies in this cohort. However, in our pooled data set, 62% of women with available information reported owning a bed net at enrollment. This is likely an overestimate: as of 2010 ITN coverage in sub-Saharan Africa was only 41% (5,40,152). If bed net ownership is overestimated, the actual population intervention estimate is likely greater than we estimated. Furthermore, M3 only captured information on bed net ownership; actual usage of the bed nets may be even lower. Additionally, while we did control for study site in our model, we did not have more detailed information on malaria transmission, regional insecticide residence, or most importantly whether or not the bed nets were untreated or insecticide-treated, which may influence the validity of our results. A 2009 Cochrane Review summarizing information from five randomized controlled trials found

that insecticide-treated bed nets were effective at reducing the risk of LBW (RR 0.77, 95% CI: 0.61, 0.98) among women (153). Given the limitations surrounding our measurement of bed net usage, we urge caution in the interpretation of the bed net results, emphasizing that bed nets, in particular insecticide-treated bed nets, remain a valuable tool for preventing malaria and improving fetal development in malaria-endemic countries (152,153).

Conversely, our results suggest that scaling up existing coverage of IPTp to three or more doses could have a marked impact on the absolute number of infants born LBW. We estimated that, had all individuals under study had at least three doses of IPTp during pregnancy, the incidence of LBW could have been reduced from 8.5% to 5.6%, a 33% (95% CI: 25%, 43%) relative reduction. This reduction in LBW exceeds the WHO 2025 Global Nutrition Target of a 30% reduction in LBW, and corresponds to an NNT of 36. Even 80% coverage of 3+ was estimated to reduce LBW by a 24% (95% CI: 19%, 29%) relative reduction. However, the effects for IPTp are stronger than the estimated effect of complete elimination of malaria infection at delivery . This discrepancy could be related to the fact that increased IPTp coverage indicates treatment and prevention of malaria throughout pregnancy and not just at delivery, which was not something we captured in our malaria analysis. However, it could also suggests that the apparent effect of IPTp may be attributable to other mechanisms than the prevention of malaria infection at delivery. In regards to other mechanisms, SP, the primary drug used for IPTp, is an effective broad-spectrum antibiotic in addition to an antimalarial and may improve fetal growth through clearance of other pathogens, anti-inflammatory action, and/or alteration of gut microbiome composition (154,155). However, we also cannot discount potential unmeasured confounding and selection bias influencing the validity of these results. First, the intervention estimates for IPTp do not account for regional drug resistance. However, a recent multi-country

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study found that even in areas of high-resistance to SP, IPTp-SP remains associated with reductions in the risk of LBW (79). Second, women who receive three or more doses of IPTp are likely receiving earlier and more frequent antenatal care. Women who have the resources and ability to access frequent antenatal care are potentially healthier due to associated health-promoting behaviors and related socio-economic status (156,157). Our inability to control for these potential unmeasured confounders and selection biases indicates that our intervention effects may be biased and that the impact of IPTp may not be as great as we have estimated. Future studies are warranted that attempt to elucidate the various mechanisms of action for SP and to untangle to effects of increased dosage with the potential selection bias related to more frequent antenatal care. Despite these limitations, these findings are consistent with prior evidence on the importance of multiple doses of IPTp during pregnancy (34). Recent mathematical modeling conducted by Walker and colleagues also indicated substantial reductions in LBW through increasing IPTp coverage (158). While there has been an increase in the proportion of women receiving IPTp over the past decade, the level of coverage remains inadequate (39–41). In 2013, an estimated 43% of the 35 million eligible pregnant women did not receive even a single dose of IPTp (41). Our findings provide further incentive for increasing the uptake of IPTp coverage.

In addition to the intervention effects for malaria and antimalarial exposures, we also reported intervention effects for maternal malnutrition. The absolute difference in the risk of LBW when comparing the extreme contrast of everyone being malnourished at study enrollment (MUAC<23 cm), compared to everyone being well-nourished (RD: 4.0% [95% CI: 3.0%, 5.1%]), is consistent with prior publications that highlight significant associations between maternal anthropometrics and fetal development (REF Paper 1 "Malaria, Malnutrition, and Birth Weight:

A Pooled Analysis of 14,633 Pregnancies", (48,52)). However, we estimated that even if we were to improve the nutritional status of all women to a normal MUAC by a novel, hypothetical intervention, there would only be a 0.3% absolute reduction in the incidence of LBW, and that a more realistic reduction of low MUAC prevalence to 5% by a novel, hypothetical intervention would still only result in a 0.2% absolute reduction in the incidence of LBW. Thus, while low MUAC is a strong predictor of LBW, our intervention effects illustrate that complete or partial removal of this risk factor from this population would have only faint population-level impacts on LBW. However, despite the minimal population-level impact, our 'number needed to treat (NNT)' results suggest that a hypothetical nutritional intervention targeted towards only malnourished women could be an efficient approach to addressing the burden of LBW. Specifically, if this hypothetical intervention was provided to only women that were malnourished, then the number of women who would need to receive the hypothetical nutrition intervention to see one less infant born LBW is 43 (95% CI: 34, 63). Results were similar, although weaker, when using body mass index as an anthropometric indicator (Supplemental Table 7.2).

Unlike the malaria interventions, where we were able to assess real interventions (bed nets and IPTp), we were not able to assess a real nutrition intervention, but rather had to rely on assuming a hypothetical intervention on maternal anthropometrics. We only had malnutrition assessments at antenatal enrollment, thus our nutrition models require the assumption that an intervention could be implemented that would target malnutrition prior to pregnancy or in early pregnancy. For example, an aggressive community-based anti-malnutrition campaign targeted at women of reproductive age could potentially produce the changes in maternal malnutrition that we model. There has been a push by nutritional researchers to move beyond prenatal nutritional

interventions, and to expand the focus to maternal nutrition to the periconceptional period as well
(44). This is further supported by animal studies showing that protein deficiencies during the
peri-implantation and rapid placental development stages significantly affect fetal growth (44).
Alternatively, there are prenatal dietary supplementation interventions that could be given to
women who present undernourished to antenatal care early in pregnancy, such as lipid-based
nutrient supplements or balanced protein energy supplementation, but these interventions are
unlikely to have a marked impact on maternal MUAC in such a short time period. Future studies
may wish to consider using measurement of gestational weight gain to estimate impacts of
hypothetical nutritional interventions that would improve gestational weight throughout
pregnancy.

In additional to the aforementioned limitations, the extent of unmeasured confounding
biasing results is unknown. For example, potential unmeasured confounders include helminth
infection, sexually transmitted infections, environmental pollutants, or micronutrient deficiencies.
Additionally, all of the intervention effects reported are a function of background prevalences of
the exposure of interest in each analysis, thus not generalizable to settings with dramatically
different baseline prevalences. Supplemental Figure 1 illustrates the distribution of risk
differences for increasing IPTp uptake for each of the studies, with varying levels of exposure
and covariate prevalences.

Public Health Implications

In conclusion, our intervention effects provide valuable information to policy makers
regarding the scale-up of malaria prevention, specifically IPTp, during pregnancy. Provision of
interventional effects in addition to exposure effects provides results that are more immediately
useful to policy-makers. For example, outputs from this work could be directly used in cost-

effectiveness models created by health policy professionals for understanding the impact of scaling up IPTp. Additionally, while the IPTp estimates were notably strong, hypothetical interventions to partially or even completely reduce malaria or improve MUAC do not appear to have a strong impact on population-level incidence of LBW. Although we cannot discount potential unmeasured confounding and selection bias of the IPTp estimates, if we assume that they are valid then this suggests that IPTp-SP improves fetal growth through mechanisms independent of both malaria and malnutrition.

Overall, our findings highlight the impact that scaling up of this existing antenatal intervention could have on the absolute numbers of infants born LBW. Of interventions evaluated in this work, early antenatal initiation and frequent antenatal follow-up to ensure adequate dosage of IPTp throughout pregnancy appears to be a key tool for achieving the WHO's Global Nutrition Target of a 30% reduction in LBW by 2025.

Table 7.1. Population average causal effects, population attributable effects, and generalized intervention effects for the risk of low birthweight (LBW) associated with malaria infection and malnutrition during pregnancy. For example, the second scenario depicts the population attributable effect for malaria infection at delivery, comparing the risk of LBW under the observed distribution of malaria infection at delivery in the study population to the risk under a counterfactual setting in which there is hypothetical complete and instant eradication of malaria.
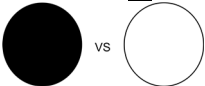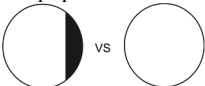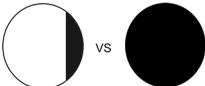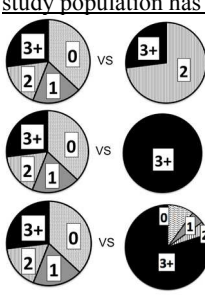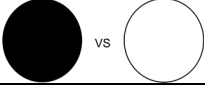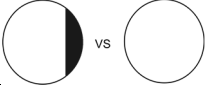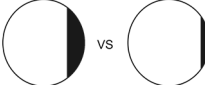
	Exposure (s)	Contrasts	Intervention
	Malaria interventions		
1	Malaria infection at delivery	**Population average causal effect:** The population with everyone exposed to malaria infection at delivery compared to the population of women with no infection. 	N/A
2	Malaria infection at delivery	**Population attributable effect:** The observed distribution of malaria infection at delivery compared to the population of women with no infection. 	Hypothetical complete and instant eradication of malaria infection at delivery.
3	Bed net ownership at enrollment	**Population attributable effect:** The observed distribution of bed net ownership compared to the population of women where everyone owns a bed net. 	Scaling up of existing bed net intervention.
4	Number of IPTp doses	**Generalized intervention effect**: The observed distribution of intermittent preventive therapy during pregnancy (IPTp) doses compared to the population where (i) everyone has at least two doses of IPTp, (ii) everyone has at least three doses of IPTp, or (iii) at least 80% of the study population has at least three doses of IPTp. 	Scaling up of existing IPTp interventions.
	Malnutrition interventions		
5	Malnutrition at enrollment	**Population average causal effect:** The population with everyone malnourished at enrollment compared to the population of women with no malnutrition. 	N/A
6	Malnutrition at enrollment	**Population attributable effect:** The observed distribution of malnutrition (MUAC<23 cm) compared to the population of women with no malnutrition. 	Hypothetical complete and instant eradication of malnutrition prior to pregnancy or in early pregnancy.
7	Malnutrition at enrollment	**Generalized intervention effect**: The observed distribution of malnutrition (MUAC<23 cm) compared to the population where only 5% of each separate study population is malnourished. 	Hypothetical intervention with a non-specific mechanism that would reduce malnutrition prevalence in each study in M3 to 5%

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Figure 7.1. Distribution of malaria infection at delivery, number of doses of intermittent preventive therapy during pregnancy (IPTp), bed net ownership at study enrollment, and maternal mid-upper arm circumference of women enrolled in studies participating in Maternal Malaria and Malnutrition (M3) cohort initiative from 1996 to 2015.
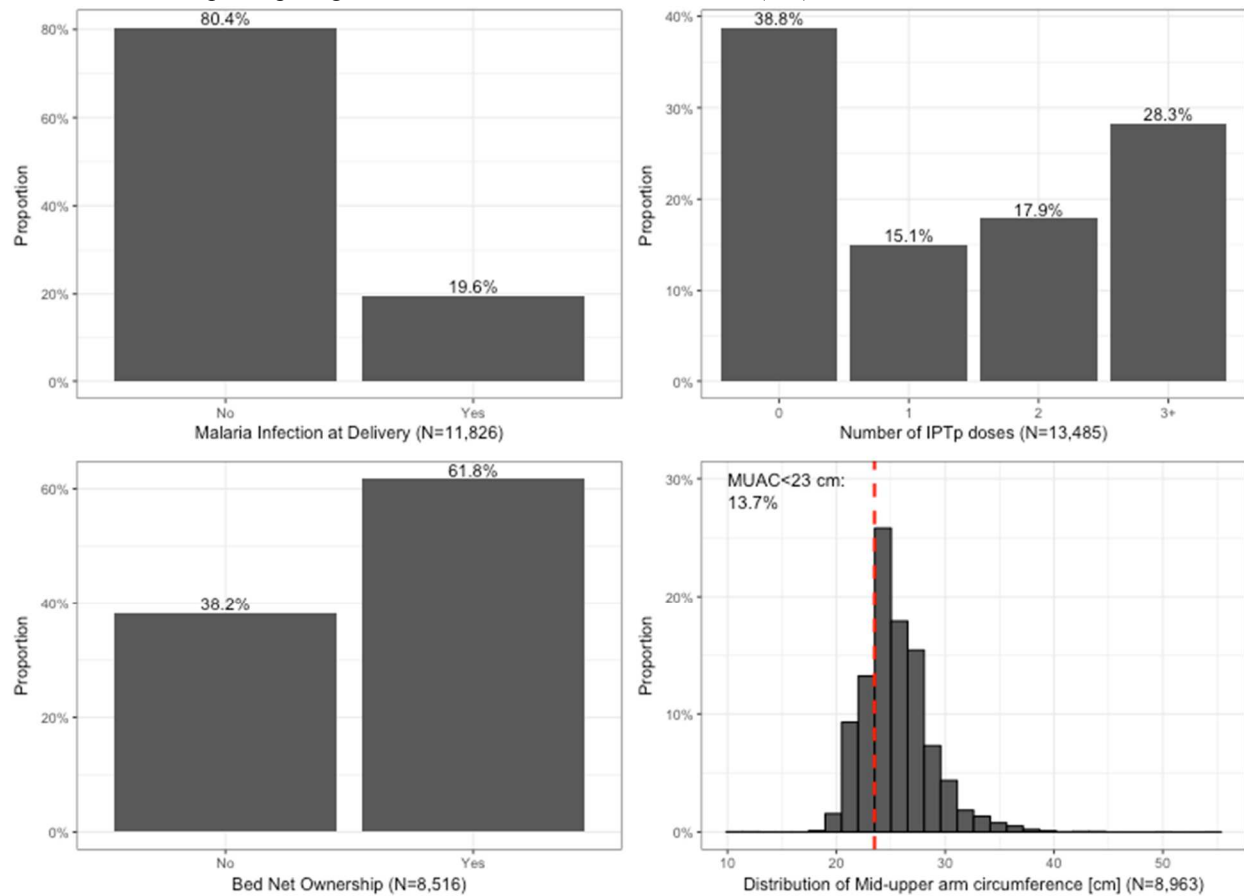
Table 7.2. Estimated population-level impact of malaria infection during pregnancy and potential interventions to reduce malaria infection on low birthweight among women enrolled in studies participating in Maternal Malaria and Malnutrition (M3) cohort initiative from 1996 to 2015.

	Contrasts	Risk of LBW	Risk difference (95% CI)	NNT
Malaria infection at delivery				
N=11,826 (2,312 infected)				
Population average causal effect				
All exposed		10.5%	2.6% (1.1%, 3.8%)	
None exposed		7.9%	0.	
Population attributable effect				
Observed		8.5%	0.6% (0.2%, 0.8%)	181 (120, 405)
None exposed		7.9%	0.	
Bed net ownership				
N=8,516 (5,260 owned bed net)				
Population attributable effect				
Observed		9.7%	0.4% (-0.2%, 1.0%)	253 (-620, 101)
All exposed		9.3%		
IPTp dosage				
N=13,483 (Avg 1.5 doses)				
Population attributable effect				
Observed (3623 [27%] with 3+)		8.5%		
All at least 2 doses		7.9%	0.6% (-0.3%, 1.4%)	423 (-320 73)
All 3+ doses		5.6%	2.9% (2.2%, 3.6%)	36 (28, 46)
80% of each study 3+ doses		6.4%	2.1% (1.6%, 2.6%)	51 (39, 64)

Table 7.3. Estimated population-level impact of maternal malnutrition (low mid-upper arm circumference [MUAC]), and potential interventions to reduce malnutrition on low birthweight among women enrolled in studies participating in Maternal Malaria and Malnutrition (M3) cohort initiative from 1996 to 2015.

		Risk of LBW	Risk difference (95% CI)	NNT
Maternal Malnutrition				
N=8,963 (1,224 with low MUAC)				
Population average causal effect				
All exposed		13.6%	4.0% (3.0%, 5.1%)	
None exposed		9.6%	0.	
Population attributable effect				
Observed		9.9%	0.3% (0.2%, 0.4%)	312 (243, 424) [†]
None exposed		9.6%	0.	43 (34, 63) [‡]
Generalized intervention effect				
Observed		9.9%	0.2% (0.2%, 0.3%)	439 (336, 579) [†]
Exposure reduced to realistic lower bound (5%)*		9.7%		61 (47, 90) [‡]

* The lowest prevalence of malnutrition (MUAC<23cm) within the pooled M3 dataset was observed in the STOPMIP-Kenya cohort (5%).

† Number needed to treat, assuming an intervention that is administered to all women to improve their nutrition, regardless of their baseline nutrition level.

‡ Number needed to treat, assuming an intervention that is only administered to women that were malnourished at baseline.

Supplemental Table 7.1. Characteristics of women participating in thirteen studies included in the Maternal Malaria and Malnutrition (M3) initiative.

	Kisumu-Kenya (N=3388)	IPTp-PNG (N=1943)	ISTp-Malawi (N=1602)	STOPMIP-Kenya (N=1203)	LAIS-Malawi (N=1190)	iLiNS-Ghana (N=1068)
Study enrollment (years)	1996-2001	2009-2013	2011-2013	2012-2015	2003-2006	2009-2012
Maternal age	20 (18-24)	24 (20-28)	21 (18-26)	22 (19-27)	24 (20-29)	26 (22-30)
Gravidity						
1 (Primi-)	1656 (49)	966 (50)	542 (34)	403 (34)	267 (22)	349 (33)
2 (Secundi-)	748 (22)	494 (21)	448 (28)	237 (20)	213 (18)	351 (33)
3+ (Multi-)	984 (29)	573 (29)	612 (38)	563 (47)	710 (60)	368 (34)
Anemic[†]						
Yes	2548 (75)	1348 (69)	533 (33)	591 (49)	459 (39)	305 (29)
No	808 (24)	512 (26)	1069 (67)	612 (51)	731 (61)	763 (71)
Missing	32 (1)	83 (4)	0 (0)	0 (0)	0 (0)	0 (0)
HIV						
Yes	810 (24)	-	0 (0)	0 (0)	144 (12)	0 (0)
No	2560 (76)	-	1602 (100)	1203 (100)	931 (78)	1059 (99)
Missing	18 (1)	1943 (100)	0 (0)	0 (0)	115 (10)	9 (1)
Malaria (LM or histology)	624 (19)	288 (15)	470 (30)	436 (37)	14 (3)	NA
IPTp doses	0 (0-0)	1 (1-3)	0.5 (0-4)	1 (0-2)	4 (2-4)	-
Bed net ownership						
Yes	-	1798 (93)	327 (20)	681 (57)	877 (74)	-
No	-	145 (7)	1275 (80)	522 (43)	313 (26)	-
Missing	3388 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1068 (100)
Low MUAC (<23 cm)	NA	541 (28)	NA	65 (5)	NA	82 (8)

Supplemental Table 7.1 Continued.

	FSP/ MISAME- BF (N=1020)	STOPPAM - Benin (N=791)	STOPPAM -Tanzania (N=789)	ITN- Kenya (N=711)	EMEP/IPTp MON-Kenya (N=471)	EMEP/IPTp MON-Kenya (N=473)	Sek-PNG (N=293)	ECHO- DRC (N=164)
Study enrollment (years)	2006-2008	2008-2010	2008-2010	1996-1999	2011-2013	2011-2013	2005-2007	2005-2006
Maternal age	23 (19.5-28)	25 (22-30)	26 (22-31)	24 (20-30)	24 (20-30)	24 (20-30)	24 (21-28)	27 (23.5-31)
Gravidity								
1 (Primi-)	205 (20)	147 (19)	162 (21)	127 (18)	94 (20)	94 (20)	115 (39)	43 (26)
2 (Secundi-)	216 (21)	173 (22)	201 (25)	118 (17)	77 (16)	77 (16)	54 (18)	22 (13)
3+ (Multi-)	599 (59)	471 (59)	426 (54)	466 (66)	300 (64)	302 (64)	124 (42)	99 (60)
Anemic†								
Yes	372 (36)	354 (45)	289 (37)	416 (59)	169 (36)	170 (35)	272 (93)	43 (26)
No	630 (62)	433 (55)	497 (63)	293 (41)	297 (63)	298 (63)	21 (7)	107 (65)
Missing	18 (2)	4 (1)	3 (0)	2 (0)	5 (1)	5 (1)	0 (0)	14 (9)
HIV								
Yes	-	13 (2)	39 (4)	51 (7)	0 (0)	0 (0)	-	4 (2)
No	-	699 (88)	693 (88)	234 (33)	468 (99)	486 (99)	-	160 (98)
Missing	1020 (100)	79 (10)	57 (7)	426 (60)	3 (1)	3 (1)	293 (100)	0 (0)
Malaria (LM or histology)	46 (6)	83 (13)	2 (0.3)	147 (22)	10 (9)	10 (9)	168 (57)	24 (15)
IPTp dosage	2 (1-2)	2 (2-2)	3 (3-3)	0 (0-0)	3 (2-3)	3 (2-3)	-	2 (2-2)
Bed net ownership								
Yes	-	254 (32)	571 (72)	348 (49)	-	-	240 (82)	164 (100)
No	-	537 (68)	52 (7)	363 (51)	-	-	49 (17)	0 (0)
Missing	1020 (100)	0 (0)	166 (21)	0 (0)	471 (100)	473 (100)	4 (1)	0 (0)
Low MUAC (<23 cm)	53 (5)	40 (6)	73 (9)	66 (11)	NA	NA	157 (54)	19 (12)

Categorical variables are expressed as N (%) and continuous variables are expressed as median (IQR). Dash indicates information on particular factor was not assessed in parent study. BF=Burkina Faso. DRC=Democratic Republic of the Congo. GA=gestational age. HIV=human immunodeficiency virus. IPTp=intermittent preventive treatment in pregnancy. LM=light microscopy. MUAC=mid-upper arm circumference. PNG=Papua New Guinea. * Based on ultrasound if measured, otherwise based on Ballard's score or symphysis-pubis fundal height (SFH). When using SFH, to adjust for misclassification in the first trimester, a fundal height< 7 cm was defined as first trimester, while SFH<28 cm was defined as second trimester, and SFH≥28cm defined as third trimester. † Anemic= hemoglobin <11 g/dL of venous blood, if available, or hematocrit <33%, in the first and third trimesters, and less than 10.5 and 32, respectively for the second trimester.

Supplemental Table 7.2. Estimated population-level impact of maternal low BMI, and potential interventions to reduce malnutrition on low birthweight among 14,633 women enrolled in Maternal Malaria and Malnutrition (M3) cohort initiative from 1996 to 2015.

		Risk of LBW	Risk difference (95% CI)	NNT
Maternal Malnutrition				
Low BMI				
N=14,322 (1,287 with low BMI) Population average causal risk difference				
All exposed		11.1%	2.5%	
None exposed		8.5%	(1.9%, 3.3%)	
Population attributable contrast				
Observed		8.6%	0.08%	1217 (907, 1805) †
None exposed		8.5%	(0.06%, 0.11%)	109 (82, 162) ‡
Generalized intervention contrast				
Observed		8.6%	0.06%	1837(1342, 2692) †
Exposure reduced to realistic lower bound (3%)*		8.6%	(0.04%, 0.08%)	165 (121, 242) ‡

* The lowest prevalence of low BMI within the pooled M3 dataset was observed in the ISTp-Malawi dataset (3%).

† Number needed to treat, assuming an intervention that is administered to all women to improve their nutrition, regardless of their baseline nutrition level.

‡ Number needed to treat, assuming an intervention that is only administered to women that were malnourished at baseline.

Supplemental Figure 7.1. The population attributable effect of increases IPTp dosage to at least three doses for all pregnancies, stratified by the parent studies. This figure does not incorporate the variance of each estimate. The overall prevalence of 3 or more doses of IPTp=27%, corresponding to an overall population attributable risk difference of 2.9% (2.2%, 3.6%).
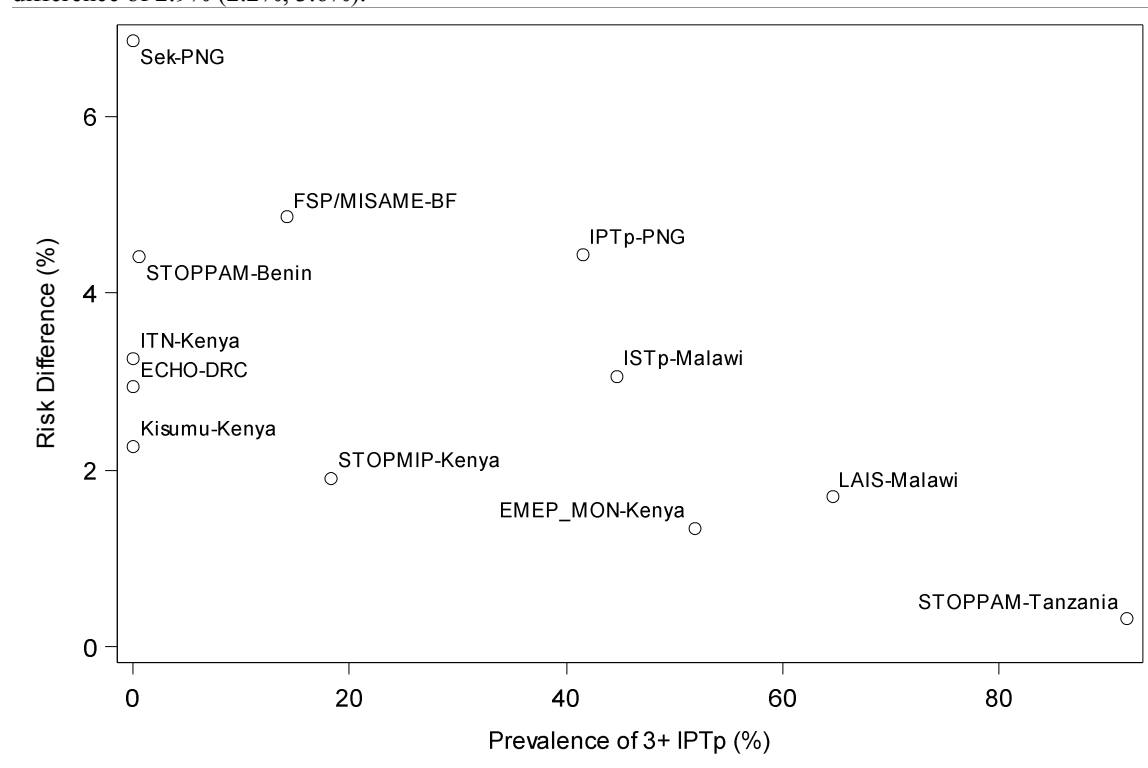
CHAPTER VIII: DISCUSSION

Overview

Despite widespread, well-supported public health campaigns over the past two decades to scale up malaria prevention programs for pregnant women, the prevalence of malaria infection during pregnancy remains around 25% in highly-endemic countries, a considerable public health burden. Malaria infection during pregnancy can have detrimental effects on the newborn, including low birthweight (LBW, <2,500g), which itself is associated with increased infant morbidity and mortality and adulthood health problems.

Evidence from four prior studies indicated that the relationship between malaria infection and LBW may be influenced by the mother's nutritional status. Two studies found a significant association between malaria infection and measures of fetal growth among malnourished pregnant women (defined using low mid-upper arm circumference [MUAC] or low body mass index [BMI]), but little or no association among well-nourished women. Two additional studies had inconsistent results. The first aim of this dissertation was to investigate the potential interaction between malaria infection and maternal undernutrition (low MUAC or low BMI) with regard to LBW, by pooling data from 14,633 pregnancies from malaria-endemic countries. We hypothesized that there would be a synergistic interaction between malaria infection and malnutrition in causing LBW, such that the observed joint effect of being both infected with malaria and malnourished would be greater than expected if considering each exposure independently. Our second aim was to translate our etiologic estimates related to the effects of malaria and malnutrition during pregnancy on LBW into intervention estimates that would

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inform on the absolute reduction in LBW that could be seen in malaria-endemic countries with hypothetical antimalarial and nutritional interventions.

Summary of findings

The M3 dataset comprised eleven studies from across sub-Saharan Africa and two studies from Papua New Guinea, representing a diverse study population of 14,633 pregnant women from multiple settings. While the prevalence of malaria infection at enrolment and delivery was overall fairly common (16% of women were infected at study enrolment and 20% of women were infected at delivery), the prevalence varied across studies. Similarly, while the prevalence of low MUAC and low BMI was 14% and 9%, respectively, these prevalences also varied across studies. When considering the dual burden of malaria infection and malnutrition at the population level, among 8,152 women with both measurements, only 2% had both low MUAC and malaria infection at enrollment; proportions were similar when low BMI instead of MUAC.

Aim 1

The results of Aim 1 contribute to the sparse body of literature that had previously addressed the interaction between malaria infection and malnutrition with regard to LBW. We found that women who were both infected with malaria and malnourished during pregnancy had a greater risk of delivering a baby with LBW and reduced mean birthweight compared to their uninfected, well-nourished counterparts. While these risks were greater than the individual risks of malaria infection and malnutrition separately, there was no evidence of excess risk due to interaction on either the multiplicative or additive scale. There was apparent multiplicative interaction between malaria infection at enrollment and low MUAC with regards to LBW among an *a priori* analysis restricted to African studies, but this was not statistically significant when properly accounting for multiple comparisons, and was absent when using other definitions of

malaria (i.e. at delivery) or malnutrition (i.e. BMI). And notably, the additive interaction, which has been argued at the more relevant measure for public health impact (114), was only slightly elevated among the African studies only and was not strong or precise enough to warrant public health action. Also of public health relevance, only 183 women (2%) were jointly infected and malnourished (low MUAC). Thus, even if there were a multiplicative interaction between malaria infection and MUAC among African women, the proportion of women implicated is small, and does not indicate a large public health burden. Overall, while not all analyses are consistent with a completely null finding, there was overall no convincing evidence of synergism, i.e. excess risk due to interaction, that would warrant public health significance.

We also provided updated evidence on the independent consequences of maternal malaria infection and malnutrition in a large and diverse population. Even in the absence of strong interaction between malaria infection and malnutrition on LBW, our findings emphasize that malaria infection and malnutrition represent two established and modifiable causes of LBW that should be considered as targets of combined interventions to optimize pregnancy outcomes in low- and middle-income countries.

Aim 2

The findings of Aim 2 further elucidate the implications of targeted interventions related to malaria and malnutrition on the population-level burden of low birthweight. Of the hypothetical antimalarial and nutritional interventions evaluated in this work, the most notable interventional estimate was associated with scaling up IPTp dosage to three or more doses during pregnancy. This is aligned with the 2007 WHO recommendations for monthly IPTp-SP during pregnancy starting in the second trimester. While the IPTp estimates were notably strong, hypothetical interventions to partially or even completely reduce malaria or improve MUAC do

not appear to have much impact on population-level incidence of LBW. Although we cannot discount potential unmeasured confounding and selection bias of the IPTp estimates, if we assume they are valid then this suggests that IPTp-SP improves fetal growth through mechanisms independent of both malaria and malnutrition.

Strengths

This work rigorously addressed the previously unclear relationship between malaria during pregnancy, maternal malnutrition, and LBW. Prior pregnancy studies that assessed malaria infection-undernutrition interactions have had small sample sizes, and more subjects from more study sites were needed to determine the magnitude and generalizability of the combined effect, particularly within Africa. Prior work also exclusively focused on how malnutrition did or did not modify the relationship between malaria and LBW, rather than the interaction between the two risk factors. Our work expanded upon this framework by evaluating maternal malaria infection and malnutrition as co-primary exposures with putative synergistic pathways of interaction. Interaction is preferable to EMM because interventions for both malaria infection and malnutrition might prevent LBW.

Our work harmonized data from thirteen different parent studies into a large, robust dataset, coined the Maternal Malaria and Malnutrition (M3) Initiative dataset, providing malaria epidemiologists with an unprecedented and highly valuable data resource for other potentially high impact research questions. The M3 dataset includes combined data from 12,395 pregnancies from eight African countries and 2,236 pregnancies from Papua New Guinea, for a total of 14,633 pregnancies in malaria-endemic areas. To our knowledge, this is the largest study to date to assess interactions between malaria infection, malnutrition, and LBW, enlarging the study population within Africa by almost ten-fold compared to prior research. Compilation of these

studies also provided us with a large, diverse study population with increased generalizability. The study population draws from a diverse setting of rural and urban areas, representing a wide range of malaria transmission and malnutrition prevalences, as well as diversity in modifying factors, such as gravidity, HIV infection, and maternal anemia.

Availability of individual data, rather than just aggregate data from each study, further enabled us to synchronize our definitions of exposures and outcomes where possible and minimize measurement heterogeneity. We used modern and appropriate statistical analyses to account for between-study variation, confounding, and missing data. Data pooled from different patient populations is sometimes incorrectly analyzed as one large dataset, however this ignores variation in the effect estimates across studies due to differences in patient populations, study procedures, study location, and calendar time. We strengthened our analysis by conducting a two-step approach to analyzing our pooled data, as well as confirming our findings using a one-stage generalized mixed model with random effects. Furthermore, in our two-stage analysis, we also calculated 95% population effects intervals (PEI). PEI are rarely reported in meta-analyses, despite their importance in incorporating the estimated variance between studies (111). To handle confounding, we appropriately used inverse probability of treatment weights to separately adjust for exposure-specific confounders. This was necessary since anemia at enrollment was a mediator of the malaria infection at enrollment-LBW relationship and a confounder of the malnutrition-LBW relationship.

Our work is further strengthened by the emphasize on providing results that are more readily translatable to policy decisions in Aim 2. To our knowledge, no one has calculated and reported interventional effect estimates for the impact of hypothetical targeted antimalarial and malnutrition interventions on pregnancy outcomes, specifically LBW. Provision of interventional

effects in addition to exposure effects provides results directly useful to policy-makers. For example, output from this work could be directly applied to cost-effectiveness models used by health policy professionals for understanding the impact of implementing and/or scaling up interventions on malaria and malnutrition.

Limitations

The main limitations of the pooled M3 dataset are related to missing data and heterogeneity of collected data. Information that was collected by all studies varied, leading to systematically missing data on a number of important variables. Notably, not all studies collected data on maternal mid-upper arm circumference, the preferred anthropometric for defining maternal macronutrient malnutrition. While we did have BMI measurements on a majority of women, BMI changes throughout pregnancy due to maternal and fetal weight gain, thus we were limited to extrapolating a pre-pregnancy BMI using gestational age and BMI at enrollment. The availability of consistent and frequent malaria diagnostics was also a limiting factor. We were obliged to pool malaria diagnostics of varying sensitivity and specificity, and we were limited to two cross-sectional assessments of malaria infection. Nevertheless, sensitivity analyses that evaluated alternative definitions of malaria, or incorporated repeat diagnostics during pregnancy, were consistent with the main results.

Additionally, there may be selection bias in both aims due to conditioning on fetal survival, also called left truncation or competing risk bias. Left truncation occurs when a study includes participants who enter the study after they have already been at risk for the outcome of research interest. This occurs in pregnancy studies where women are often not enrolled until their first antenatal care visit or later, due to the difficulty of following women from conception, especially in resource poor settings. Typically, this left truncation is assumed to be non-

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informative, and analyses are restricted to live singleton pregnancies as a result. Thus, pregnancies that ended in miscarriage or stillbirth may be missing from the study due to enrollment restrictions or missing from the analysis due to exclusion criteria. However, when there are differential rates of early pregnancy losses among those who are exposed and unexposed (singly or jointly exposed), this could cause a selection bias potentially distorting the true causal associations (139). This has also been described as bias resulting from survival selection by conditioning on a competing risk (159).

As illustrated in Figure 8.1, restricting analyses to only live births could potentially be restricting on a collider if there is an unmeasured factor that influences both pregnancy loss and fetal growth and if both malaria and malnutrition affect pregnancy loss. This unmeasured factor (U) is a realistic problem, given that there are likely genetic or environmental factors that affect both successful live birth and appropriate fetal growth. Additionally, studies of malaria infection during pregnancy suggest an increased risk of miscarriage and stillbirth due to malaria infection (28,160). To address this potential bias, four of the individual studies, the STOPMIP-Kenya, EMEP-Kenya, FSP/MISAME-Burkina Faso, and ISTp-Malawi, agreed to share all pregnancy outcomes, including miscarriages and stillbirths, which was not part of the original data request for the M3 cohort initiative. However, there were only 116 (3%) pregnancy losses in four studies (N=4,571) in M3 that collected these data, limiting our ability to further address this selection bias issue. However this is almost certainly an underestimate, since many studies enrolled women after the first trimester. Additional instances of selection bias might also have resulted from the sample of studies available and the selection of women into these studies. The M3 was a convenience sample of studies with available malaria and nutrition measurements, rather than a random sample of extant data, and thus may not be fully representative. Furthermore, women

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enrolled in studies were likely healthier and received better antenatal care than the general population; the effects of malaria and malnutrition in reality might well be greater than observed within these research settings.

As mentioned previously, LBW is limited as an outcome because it is a result of intrauterine growth restriction, preterm birth, or both. Additionally, LBW does not distinguish between infants who were constitutionally small versus those that truly experienced pathological intrauterine growth restriction. The distribution of constitutionally small babies may differ across different ethnicities, and we did not account for this difference across the various studies included in our analysis.

Finally, inherent in most observational data is the extent of unmeasured confounding biasing results. As identified through our posited DAG in Figure 4.1, potential unmeasured confounders include helminth infections, sexually transmitted infections, environmental pollutants, or micronutrient deficiencies; however, it is important to note that because neither malnutrition nor malaria could be randomized, large-scale, multi-site cohort analyses such as this one are necessarily the gold standard for addressing these scientific questions. Additional limitations specific to the second aim included selection bias for the IPTp analysis, mismeasurement of bed net usage, and limited availability of more appropriate measures of maternal nutrition during pregnancy (such as gestational weight gain) or real nutritional interventions (e.g. supplementation).

Most of the aforementioned limitations are related to the causality assumptions required to identify and consistently estimate effect estimates. Specifically, these assumptions are conditional exchangeability, treatment-version irrelevance, positivity, no measurement bias, no interference, and no model misspecification. Most of these assumptions are still likely required

for estimating intervention effects. For example, positivity, no interference, no measurement bias, and no model misspecification all still need to be assumed for g-formula estimation of intervention effects. As for conditional exchangeability, the assumption is slightly less restrictive. In the population average causal effect, where you are coming the study population if everyone was exposed versus if no one was exposed, your are assuming that those who are unexposed can 'stand in' (i.e. are exchangeable) for those who are exposed, if they have been exposed, and vice versa. However, intervention effects, where one contrast is the observed exposure, you only have one-sided conditional exchangeability, such that the exposed need to be exchangeable with the unexposed, but you do not need the unexposed to be exchangeable with the exposed. For treatment-version irrelevance, you likely are less restrictive again because you are forced to be more specific when specifying the intervention that is being simulated in the intervention contrast.

Future directions

As previously discussed, our work was limited to primarily analyzing LBW and mean BW. In sensitivity analyses conducted in aim 1, we did evaluate SGA as a secondary outcome, but future work may seek to repeat the analyses of aim 2 using SGA as an additional outcome. Further, while we did attempt to asses SGA, we didn't assess preterm birth. Preterm birth could be assessed using this data among a sub-group of the study population with reliable gestational age (ultrasound measurements). Although SGA and preterm birth share common risk factors, they are two distinct phenomena with differing etiology, and LBW does not differentiate between the two (3). One recent study estimated that approximately half of LBW babies in low-to middle-income countries are preterm (23), higher than previously assumed (24).

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Future studies may also wish to explore joint effects of malaria with other nutritional indicators, such as height, obesity, and anemia. Maternal height is typically interpreted as a measure of stunting, chronic/childhood nutritional status and a proxy for low SES, whereas BMI and MUAC are more reflective of current nutritional status and represent the reserves available to nourish the developing fetus. Mechanisms of putative interaction between height and malaria infection could be due to chronic undernutrition affecting endocrinological and immune function responses to malaria infection. The potential interaction between anemia and malaria would be harder to disentangle, given the cyclical relationship between the two. The second aim of our work was focused on hypothetical antimalarial and nutrition interventions during pregnancy. Future work would ideally expand upon this work, to evaluate interventional effects for other risk factors for impaired fetal growth, such as sexually transmitted infections.

One area for future exploration is the complex relationship between malaria, malnutrition, and iron deficiency. Studies to date focusing on macronutrient malnutrition, malaria, and LBW, including this one, are potentially biased by inadequate control for confounding by maternal iron deficiency. Iron deficiency and macronutrient health are highly correlated, since both factors are influenced by a common cause, insufficient dietary consumption. Iron deficiency is associated with low birthweight (161), but unlike macronutrient health, has a very intricate relationship with malaria risk (162,163). There is evidence showing that iron deficiency is protective against malaria (162), and that iron supplementation could possibly increase the risk of malaria infection and severity (161,163). Undoubtedly, iron deficiency is tightly interconnected with the relationship between malaria, macronutrient malnutrition, and LBW. Confounding by iron deficiency potentially masks the interaction between malaria and macronutrient malnutrition in causing LBW. To adequately control for confounding by iron deficiency in the assessment of

interaction between malaria, macronutrient malnutrition, and LBW, one would need to conduct a longitudinal study where blood samples are collected and test not just for malaria but for ferritin and transferrin co-receptors as biomarker measurements of maternal iron status. The study would need to be longitudinal in nature to capture the temporality of malaria infection and iron deficiency, due to the cyclical nature of these two factors (Figure 8.2).

Conclusions

In conclusion, our work contributes to the body of evidence supporting a harmful impact of malaria infection during pregnancy and maternal malnutrition on low birthweight of the baby, and the potential impact that scaling up IPTp could have on the absolute numbers of infants born LBW. In the largest study to do so, we assessed potential interactions between malaria infection and malnutrition with regards to LBW, and overall found no convincing evidence of synergistic interaction that would warrant public health action. However, one public health action that is strongly supported by our work is the use of multiple doses of IPTp during pregnancy in malaria-endemic countries, given the impact this intervention can have on the overall burden of LBW.
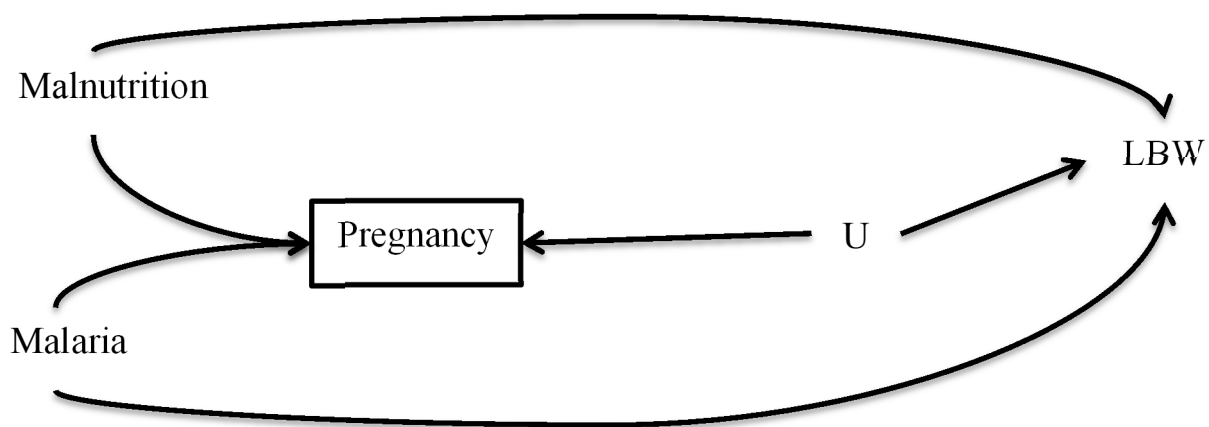
Figure 8.1. Simplified directed acyclic graph (DAG) illustrating potential selection bias due to restriction to live births.
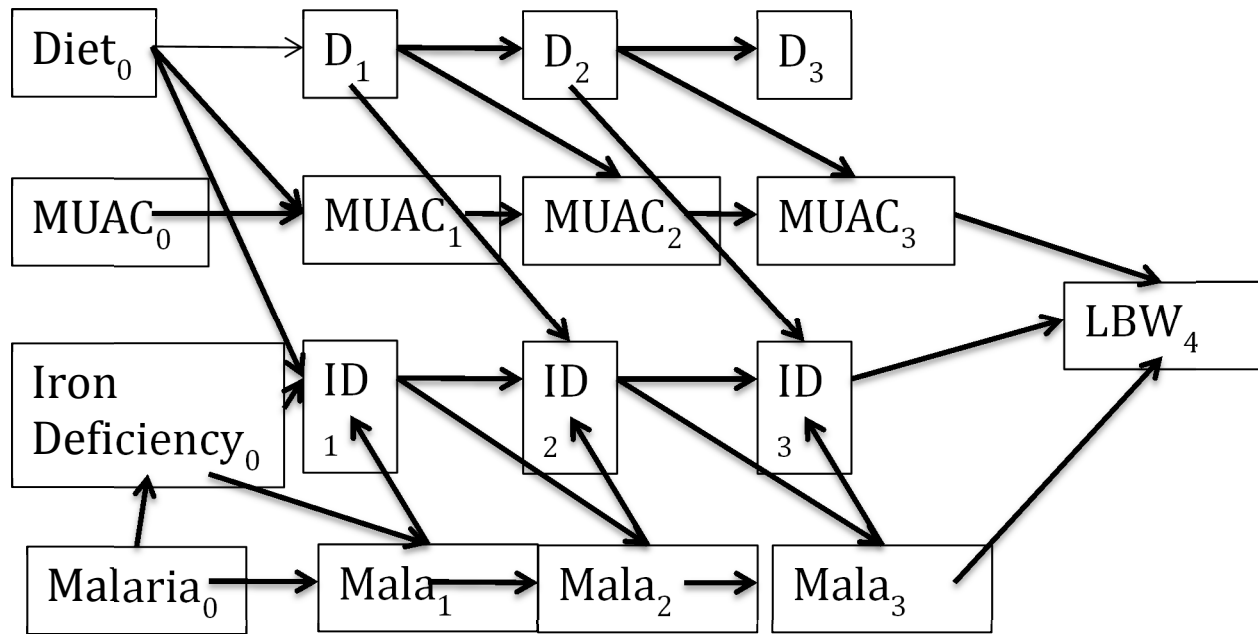
Figure 8.2. Simplified directed acyclic graph (DAG) illustrating the temporality between mid-upper arm circumference (MUAC), iron deficiency (ID), malaria (mala), and low birthweight (LBW).

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