The relationships of seasonality in prenatal diet and physical activity and postnatal micronutrient supplementation with growth in early life

Kimberley Bouckaert

Thesis submitted in fulfillment of the requirements for the degree of

Doctor (PhD) in Applied Biological Sciences

Promotors: Prof. Dr. Patrick Kolsteren and Prof. Dr. Ir. John Van Camp

Department of Food Safety and Food Quality

Faculty of Bioscience Engineering

Ghent University

Dean: Prof. Dr. Ir. Marc Van Meirvenne

Rector: Prof. Dr. Anne De Paepe

Citation: Bouckaert, K.P. (2016). *The relationships of seasonality in prenatal diet and physical activity and postnatal micronutrient supplementation with growth in early life*. Ghent University. Faculty of Bioscience Engineering, Ghent, Belgium.

Dutch title: De verbanden tussen seizoenaliteit in prenatale voeding en fysieke activiteit en postnatale micronutriënten supplementatie met groei in het vroege leven.

ISBN: 978-90-5989-920-9

Cover: Daniël Bouckaert, 2016

Printed by University press – Leegstraat 15 – 9060 Zelzate – Belgium

Thank you's

Here I am, at the top of the PhD mountain, taking in the view of my struggles and breakthroughs. What a journey! The experience will be unforgettable. There were many hands that pushed me forward and there were many words of encouragement on the trail. For that, I am very grateful.

A very big thank you to Patrick Kolsteren. You have opened the doors of maternal and child nutrition research for me. You picked up my motivation that day when I had an exploring appointment with you. Your advice led me back to the classroom, and eventually you welcomed me on board of the Unit. I am glad you pulled me in… I ended up where I needed to be. Thank you for believing in me. I want to thank John Van Camp for your encouragement, for helping me put ideas into practice and to find solutions to the many problems I faced. Thank you Bruno De Meulenaer for your dedication to the research in Ethiopia. I will not forget the time we spent experimenting with omega 3's and dried blood spots in the lab. I am thankful for the members of the Jury – Philippe Donnen, Inge Brouwer, Inge Huybrechts, Marijke D'Haese, Carl Lachat – and John and Patrick who've made time to read my work and improved its quality.

I have had the privilege to work with a wonderful team in the past seven years. I don't want to brag, but they were really the best. They've inspired me to go beyond what I believed I was capable of or what was possible, and have their share in who I am as a researcher today. Dominique, Carl, Lieven and Roos, I hope I made you proud. Thank you for all your bright ideas, critical comments, help, listening ears, and the many, many beers and good times we shared together. It was priceless…

My research led me to my first steps in Africa. Laeticia and Hermann, you have given me a second chance. I am forever grateful! Thanks for all of your support in helping me move forward. I have also learned a lot from our collaborations. Tefera, Mekitie, Alemayehu, you have introduced me to your beautiful country and culture. It has been one of the most life-changing experiences for me. I am happy we can say that we've accomplished a lot together! I am very grateful for all the fruitful collaborations during my stay in Jimma and Ghent. A big thanks to Kora, Teklu, Berhanu, Zeleke, Delenasew, Marita, Jan, Johan and Mulusew for your essential contributions to the project, and your support. Thank you also to the people at Jimma University who arranged practical matters and made the project possible.

A special word of appreciation to Anne Opsomer, Helke Baeyens, Mira Jashari, Mie Remaut, Marian Mareen, Godelieve Casier, Lieve De Greef and Monique Ceulemans who have gone through great lengths to organize shipments, calculate budgets, and/or other practicalities to achieve the ambitious goals. Thank you also for your encouragements and the good times

throughout my time at Ghent University and ITM. I would like to extend a warm thank you to my fellow colleagues and co-PhD-students at Ghent University who've spent a great deal of time extracting and analyzing the biological samples from Jimma, who have trained me in conducting lab experiments and sensory testing, who have taught me more about statistics and programming, who've helped in setting up a nutrition laboratory in Jimma, and who've helped operationalize a peanut butter dosing machine.

Thank you to all my friends and family for cheering me on by the side of the road and recharging my batteries. A special thanks to my mom and dad who taught me how to be resilient and preached that only calmness can save me. The last one in particular has been a tough one to live by!

Lieven, we were in this together. You have made sacrifices without which I would probably not be standing here. Thank you for your unconditional and never-ending support. It has been a tremendous source of strength for me.

Jury members

Prof. Dr. Patrick Kolsteren — Promotor
Department of Food Safety and Food Quality
Ghent University

Prof. Dr. Ir. John Van Camp — Co-promotor
Department of Food Safety and Food Quality
Ghent University

Prof. Dr. Ir. Peter Bossier — Chairman
Department of Animal Production
Ghent University

Prof. Dr. Ir. Marijke D'Haese — Secretary, Reading Committee
Department of Agricultural Economics
Ghent University

Prof. Dr. Carl Lachat — Reading Committee
Department of Food Safety and Food Quality
Ghent University

Associate Prof. Dr. Ir. Inge Brouwer — Reading Committee
Department of Agrotechnology and Food Sciences
Wageningen University

Prof. Dr. Philippe Donnen — Reading Committee
Centre d'Epidémiology, Biostatistiques et Recherche clinique
Université Libre de Bruxelles

Prof. Dr. Inge Huybrechts — Reading Committee
Section of Nutrition and Metabolism
International Agency for Research on Cancer

List of abbreviations

AGA	Appropriate-for-Gestational Age
BMI	Body Mass Index
CPM	Counts Per Minute
DHS	Demographic and Health Survey
E%	Energy percentage
EE	Energy Expenditure
EBF	Exclusive Breastfeeding
FAO	Food and Agriculture Organization
H/A	Height-for-Age
HAZ	Height-for-Age Z-score
Hb	Hemoglobin
HIC	High Income Country
HR	Hazard Ratio
IRR	Incidence Rate Ratio
IFA	Iron and Folic Acid
IUGR	Intra Uterine Growth Restriction
L/A	Length-for-Age
LAZ	Length-for-Age Z-score
LBW	Low Birth Weight
LMIC	Low and Middle Income Country
MET	Metabolic Equivalent of Task
MISAME	Micronutriments pour la Santé de la Mère et de l'Enfant
MMN	Multiple Micronutrients
MUAC	Mid-Upper Arm Circumference
NPNL	Non-Pregnant Non-Lactating
OR	Odds Ratio
P	Pregnant
PA	Physical Activity
PAEE	Physical Activity Energy Expenditure
PTB	Preterm Birth
RCT	Randomized Controlled Trial
RNI	Recommended Nutrient Intake
RR	Relative Risk
SD	Standard Deviation

SGA	Small-for-Gestational Age
TEE	Total Energy Expenditure
UNICEF	United Nations Children's Fund
UNIMMAP	UNICEF/WHO/UNU International Multiple Micronutrient Preparation
UN-MDG	United Nations Millennium Development Goal
UNU	United Nations University
W/A	Weight-for-Age
W/H	Weight-for-Height
W/L	Weight-for-Length
WAZ	Weight-for-Age Z-score
WHO	World Health Organization
WHZ	Weight-for-Height Z-score
WLZ	Weight-for-Length Z-score

Table of contents

Chapter 1

INTRODUCTION

1.1 Conceptual framework

1.1.1 Women's health in low and middle income countries

Women in the most vulnerable population groups in any low and middle income country (LMIC) endure a high burden of stress as a result of their social and economic roles, as well as environmental exposures (1). First, women are the driving force in the household and are in charge of caregiving, food preparation, cleaning, seeking health care and supervising their children. Second, they are often involved in agricultural activities, activities at the market or at home, and/or other work. Third, the lives of women and others in subsistence-farming populations in rural areas of LMICs are heavily influenced by the seasons. The prevailing climatologic conditions in LMICs allow for diseases to thrive while the unsafe water supply and inadequate sanitation and hygiene exacerbates the situation. Moreover, food stocks from the previous harvest typically run low in the annual rainy or hungry season putting the population at risk of inadequate food intake. Such challenges require strong health systems. Yet, it is mostly minimally trained and unsupported health workers at the lowest levels of the health system who provide care to the poor (1, 2). Adverse exposures and weak health systems may not only jeopardize the health and development of women but also that of their unborn children. Sustainable development will only be achieved once women are healthy, valued, enabled and empowered to reach their full potential.

1.1.2 The life cycle and the theory of programming

The life course theory postulates that health trajectories are built or diminished over the course of life by an integrated continuum of exposures to both protective and risk factors. Harmful exposures are thought to have their greatest impact at sensitive time periods in human development, such as in utero, early childhood and adolescence (3). As such, growth failure during these critical stages in life strongly implies that the environment deleteriously interacts with genetically endowed growth potential. Growth restriction is a widespread phenomenon in LMICs and poses a serious public health problem.

The life course theory also includes the concept of intergenerational programming which entails that environmental exposures may have long-term consequences as they program the health and development of the next generation. The most cited example comes from a community-based nutrition intervention trial in Guatemala in which, among others, young children were supplemented with either a high-energy and high-protein supplement (intervention) or a low-energy and low-protein (control) supplement. Many years later, a significant difference in the next generation's birth weight was found relating to the type of supplement their mothers were supplemented with at a young age (4). In addition, childhood growth restriction in mothers played a role as a 150 g difference in their offspring's birth weight was found depending on the severity of maternal stunting at 3 years of age (5). Another study in India demonstrated that the relative risk (RR) of under-5 child mortality and linear growth restriction is lower per cm increase in maternal height (respectively, RR: 0.978, 95%CI: 0.970-0.987; RR: 0.971, 95%CI: 0.968-0.973) (6).

The intergenerational nature of growth failure in a depriving environment is summarized in **Figure 1**. A small baby born with low birth weight (LBW) will continue to falter in growth during infancy, childhood and perhaps adolescence, and complete the vicious cycle by in turn giving birth to a small newborn. The figure also emphasizes the role of early pregnancy in adolescence as it may result in a small newborn as well as the restriction in maternal growth (7). The cycle illustrates that growth faltering is a complex phenomenon, and effectively breaking it requires both prenatal and postnatal approaches.

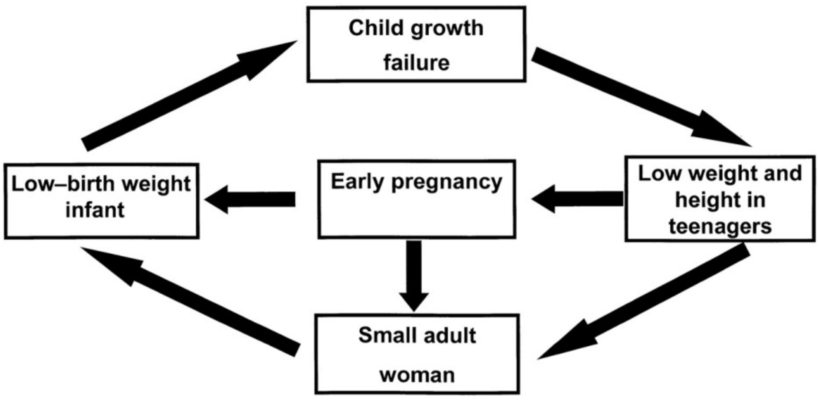


Figure 1. The conceptual framework of the intergenerational cycle of growth failure (ACC/SCN, 1992 (7)).

1.2 Growth restriction

1.2.1 Intra uterine growth restriction

1.2.1.1 Indicators and definitions

There is no single indicator for fetal or intra uterine growth restriction (IUGR), but until today it has been diagnosed in LMICs by using measurements at birth. IUGR has traditionally been assessed by the proxy indicator LBW, defined as a birth weight <2500 g (8). However, LBW has distinct causal pathways: preterm birth (PTB; defined as delivery <37 completed weeks of gestation (8)) and IUGR, or a combination of both. The classification into LBW alone does not differentiate between these causes, yet it has been shown that the determinants as well as the consequences of PTB and IUGR are different (9, 10). The main reasons as to why LBW is still being used today are that birth weight is measured routinely with acceptable validity and precision, and the lack of valid estimates of gestational age in LMICs. The latter is hampered by late and/or infrequent access to prenatal care and the unavailability of early ultrasound measurements, as well as inadequate recall of the last menstrual period (11). However, if valid estimates are available LBW can be further classified into term LBW (defined as birth weight <2500 g and delivery ≥37 completed weeks of gestation) and preterm LBW. The former is more likely to result from IUGR alone.

IUGR has also been used interchangeably with the indicator small-for-gestational age (SGA). SGA classifies newborns based on their size and gestational age at birth, but contrary to LBW, the measurements are compared to a reference population. An SGA newborn is defined as having a birth weight below that of a cutoff of a sex-specific reference population (12, 13). A more sensitive cut-off at the 10^{th} centile is preferred for operational reasons, so as not to exclude those infants at risk who might benefit from an intervention. Researchers have also used other cut-offs with a higher specificity at the 3^{rd} and 5^{th} centile. Just as with LBW, SGA can be broken down into term SGA and preterm SGA depending on the time of delivery.

The indicators LBW and SGA present some artefacts in the diagnoses of IUGR. First, neither indicator is able to classify a newborn as thin, short or both. However, it has been proposed that thinness and shortness follow different causal pathways and pose different health risks (12, 14). Thinness, also called asymmetrical growth restriction or wasting, is the disproportionate decrease in growth whereby linear growth continues normally and soft tissue growth is reduced. Shortness, also called symmetrical growth restriction or stunting, is the proportionate decrease in both skeletal and soft tissue growth. The distinction can be made by the Rohrer’s ponderal index which is calculated by dividing the newborn’s birth weight by the third power of her/his crown-to-heel length and multiplying by 100 (g/cm^3). There have been several hypothesis that relate the timing of the adverse exposure during pregnancy with the development of either of the two IUGR phenotypes (15-19). Yet, until today, the timing and exact etiology of growth restriction remains unclear. Second, there is the inherent problem of the arbitrary selection of a cut-off for classification. Some newborns who have experienced IUGR may be misclassified if their birth size is higher than the chosen cut-off, while others who did not experience IUGR but are constitutionally small and in the lower tail of the growth curve will be considered as having pathological IUGR. Third, until 2014 there was no universal recommendation on which reference population to choose and several, locally derived or national, references have therefore been in use. The lack has hampered straightforward comparisons of the outcomes between studies. However, recently, the INTERGROWTH-21st population-based project established universal multiethnic cross-sectional newborn growth charts using standard procedures from approximately 20,000 healthy babies coming from urban areas in eight countries: Brazil, China, India, Italy, Kenya, Oman, the UK and the USA (20). Moreover, as birth weight references can only identify suboptimal fetal growth retrospectively, another component of this project recently developed international standards for fetal growth based on longitudinal ultrasound measurements in 4,300 pregnant women (21). Both of these studies have demonstrated that fetal growth and newborn length were similar across these different settings, under the condition that the nutrition and health needs of the mother are met, antenatal care is available and environmental constraints are low (22). This finding demonstrates that a universal reference can be used to assess fetal and child growth restriction worldwide.

1.2.1.2 Epidemiology

It has been estimated that every year 20 million newborns are born around the world with LBW. This equates to 15.5% of all births, of which 95.6% are born in LMICs (23). Moreover, it was recently estimated that 43.3 million infants representing 36% livebirths were born too small (SGA) or too soon (PTB), or both, in LMICs in 2010 (24). The majority of these, 32.4 million newborns or 27% livebirths, were SGA births of whom 29.7 million were term SGA and 10.6 million were term LBW. In addition, it was estimated that 2.8 million infants were born preterm SGA.

Figure 2 shows the prevalence of preterm SGA, term SGA, preterm appropriate-for-gestational age (AGA; birth weight-for-gestational age above the 10th centile of the sex-specific reference population), and LBW in livebirths from the United Nations Millennium Development Goal (UN-MDG) regions for 2010 (24). The figure clearly demonstrates that there are substantial regional differences in SGA births. The highest prevalence of SGA births was registered in South Asia, followed by Sub-Saharan Africa and Southeast Asia, respectively 44.5%, 25.5% and 24.3%. Moreover, the analyses showed that the composition of LBW newborns varies across regions. Many LBW newborns (65%) are attributable to term SGA in South Asia, while just over half of LBW newborns from Sub-Saharan Africa are attributable to PTB. On the contrary, a substantial proportion of SGA newborns do not have a LBW and are not born preterm. It should be noted that these estimates were derived from community-based birth cohorts and facility-based surveys with imperfect data, i.e. missing or biased. However, only those studies meeting a priori data quality criteria were included in the analyses, missing birth weight data were imputed and bias adjustments were performed whenever possible. Sensitivity analyses showed that estimates were comparable between analyses including all birth cohorts and population representative studies.

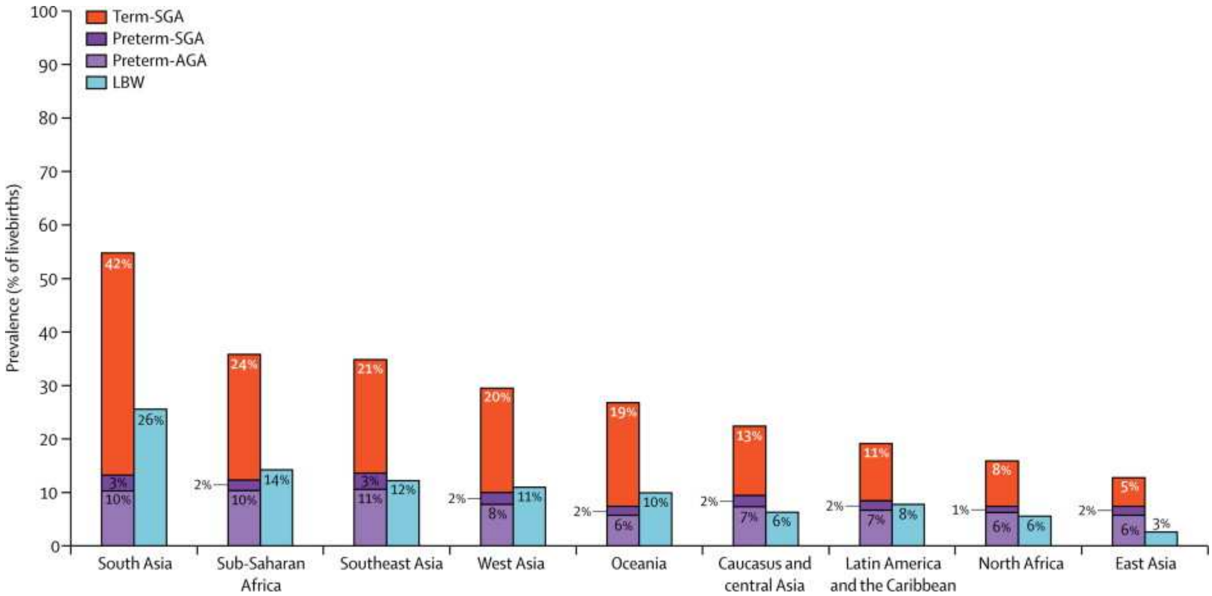


Figure 2. Prevalence of SGA births, PTBs and LBW births (% live births) by UN-MDG region in 2010 (Lee et al., 2013 (24)). AGA= Appropriate-for-Gestational Age; LBW= Low Birth Weight; PTB= Preterm Birth; SGA= Small-for-Gestational Age.

In general, the lack of birth weight and gestational age data as well as high quality data remains a barrier in the quantification of LBW, SGA and PTB in LMICs. First, birth weight data is usually collected as part of national household surveys and other routine registration programs. However, it has been estimated that more than half of newborns in LMICs don't have their weights recorded at birth, especially those born outside health facilities (23). Home delivery is often the only option for the most vulnerable women, who are more likely to be at higher risk. Moreover, it is also common that newborns who die soon after home delivery are not weighed. It is therefore expected that the prevalence of LBW, and especially preterm SGA with the highest mortality risks, is underestimated. Preterm SGA incidence may also be underestimated due to the use of birth weight compared to fetal growth charts. On the other hand, data from facility-based deliveries are more likely to be biased by the nature and amount of facilities in the area and the amount of deliveries at home. Second, gestational age is mostly estimated in LMICs by recalling the last menstrual period. This method is influenced by recall and variation in the duration of the menstrual cycle, and has an estimated error of 3 weeks standard deviation (SD) (25). As a comparison, an ultra sound performed in the first 20 weeks of gestation generates an estimate with an error of 7 days SD.

1.2.1.3 Consequences

Growth restriction, as measured by LBW and SGA, has been associated with numerous adverse health outcomes stretching across the life course. The World Health Organization (WHO) took a major leap forward in the 50's when they confirmed that LBW newborns were at an increased risk of morbidity and mortality (26). More recently, a pooled analysis of 20 cohorts from South and Southeast Asia, Sub-Saharan Africa and Latin America has shown differential mortality risks for PTB and/or SGA births, varying by severity, as well as across early life (9). The pooled RRs of neonatal mortality were higher in PTBs (RR: 6.82, 95%CI: 3.56 – 13.07) compared to SGA births (RR: 1.83, 95%CI: 1.34 – 2.50), but highest in those who were born both preterm and SGA (RR: 15.42, 95%CI: 9.11 – 26.12). Although the mortality risk attributable to SGA is lower compared to that associated with PTB, the risk is considered to be important in light of the fact that a very high number of newborns in LMICs are affected by SGA. The increased mortality risks of term SGA babies, compared to term AGA babies, did not vary over the neonatal period (**Figure 3**). However, the main risk of mortality associated with PTB was shown to occur in the early neonatal period, i.e. birth to 7 days. Moreover, statistically significant increased mortality risks persisted throughout the post-neonatal period or first year of life for PTBs and/or SGA newborns.

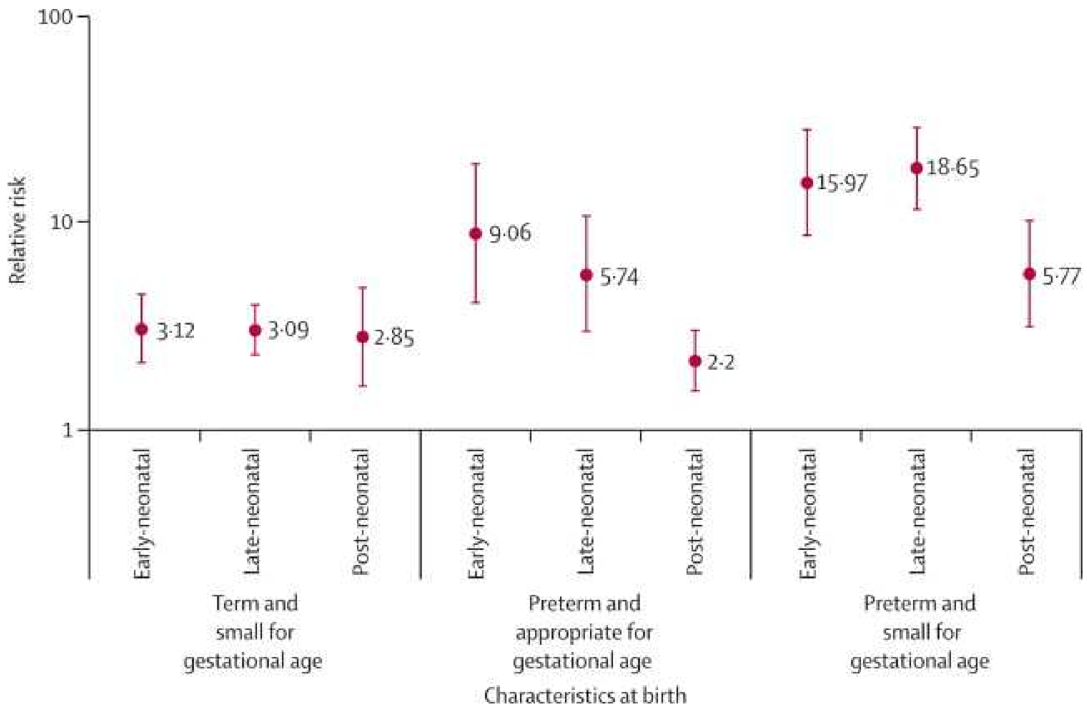


Figure 3. Relative risks of mortality associated with preterm and small-for-gestational age births in the early neonatal, late neonatal and post-neonatal periods (Katz et al., 2013 (9)).

Figure 4 displays the global causes of death of under-5 year old children, including neonates, in 2013 (27). It has been argued that IUGR is not a direct cause of death, but contributes indirectly to death due to intrapartum-related birth asphyxia and infections, such as sepsis, pneumonia and diarrhea, which account for more than half of all neonatal deaths (28). The relationship between growth faltering and infection is complex, both interacting synergistically and antagonistically, but increased mortality rates have been linked to a reduced immunological response (29, 30).

Furthermore, IUGR is associated with adverse conditions beyond the post-neonatal period. It has been shown to be an important contributor to undernutrition and growth restriction in early childhood. A meta-analyses of 19 birth cohorts in LMICs showed significantly increased odds ratios (ORs) for stunting (Length/Height-for-Age Z-score <-2) in young children 12-60 months of age of 2.43 (95%CI: 2.22 – 2.66), 1.93 (95%CI: 1.71 – 2.18) and 4.51 (95%CI: 3.42 – 5.93) for respectively term SGA births, PTBs, and preterm SGA births (31). Similar ORs were found for wasting in the same age category. In addition, the population attributable stunting risk related to SGA totaled 20% which further suggests that childhood stunting may have its origins in the fetal period. Intra uterine growth restriction, measured as term LBW, has also been associated with lower cognitive and psychomotor development in infants and younger children in LMICs (32, 33). Effect sizes were small to moderate compared to newborns with a birth weight ≥2500 g. However, evidence for longer-term effects was mixed.

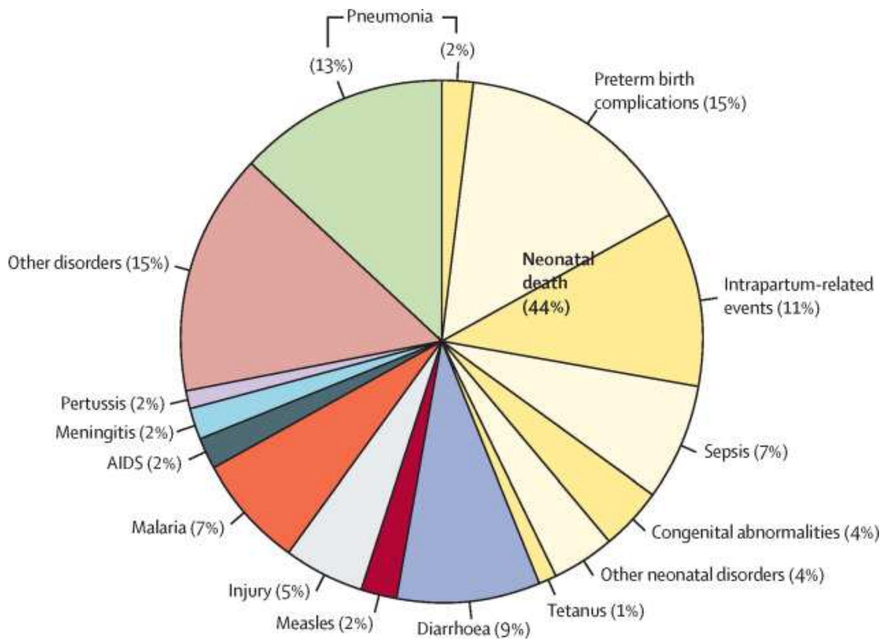


Figure 4. Global causes of death and their relative importance in under-5 children, including neonates, for 2013 (Liu et al., 2015 (27)).

In the late '80s, Barker and colleagues found an inverse association between ischemic heart disease mortality in English men and their weight at birth and infancy (34). They first coined the 'fetal origins of disease' theory which postulates that adult disease can be linked to early exposures in life. More analyses of cohort studies in high income countries (HICs) followed and supported the theory (35, 36). A first hypothesis about the mechanism of early nutrition programming is the concept of the thrifty phenotype. The hypothesis proposes that fetuses experiencing nutrient restriction in utero become metabolically attuned to nutritional deprivation, a state that remains imprinted during later life (37). A metabolic mismatch would then come into effect when children are exposed to a more abundant nutritional environment later in life. Recent evidence supports this hypothesis. A pooled analyses of 5 birth cohorts from LMICs showed that undernourished children who experience rapid weight gain after infancy are more prone to chronic disease (38, 39). These findings would be particularly relevant to adults currently living in urban areas of LMICs, as they are more likely to have been born at a time when undernutrition rates were higher but are now exposed to rapidly changing environments and diets. A second hypothesis is that early life nutrition exposure influences adult immunocompetence through effects on the developing immune system.

Researchers in The Gambia have found a relationship between being born in the hunger season, when SGA incidence peaks, and premature death from infectious disease (40). Adults born in this season were 11 times more likely ($P < 0.00009$) to die from infectious disease compared to those born in the harvest season.

1.2.2 Infant and young child growth restriction

1.2.2.1 Indices, indicators and definitions

There are three commonly used anthropometric indices for infants and children that combine recumbent length (age <2 years) or standing height (age ≥2 years), weight and age: Length/Height-for-Age (L/A or H/A), Weight-for-Age (W/A) and Weight-for-Length/Height (W/L or W/H). These indices are then compared to a sex-specific reference population. There are several growth charts available for under-5 children, the most recent being the international multiethnic 2006 WHO Child Growth Standards derived from 8440 healthy breastfed infants and young children in Brazil, Ghana, India, Norway, Oman and the USA (41). The most widely used method of reporting anthropometric indices in population-based studies are the Z-scores. The anthropometric value is expressed as a number of SDs, or Z-scores, above or below the reference mean or median. One then obtains the Length/Height-for-Age Z-score (LAZ or HAZ), Weight-for-Age Z-score (WAZ) and Weight-for-Length/Height Z-score (WLZ or WHZ). As a general rule, indices below -2 SD or Z-scores relative to the reference mean or median is indicative of “abnormal” anthropometry or growth restriction. Indices below -3 Z-scores are considered the result of severe growth restriction. It is important to note that the cutoff is different than that for SGA (10^{th} centile) when comparing fetal growth restriction with childhood growth restriction.

Similar to the fetal growth indicators, these three child growth indices and indicators reflect different etiologies and outcomes. Length/Height-for-Age is a measure that reflects cumulative linear growth of the child (42). Deficits in L/A or H/A, as compared to the reference population, may indicate long-term or frequent inadequacies in health or nutrition in the past. Small deficits are referred to as shortness which can result from normal variation or a pathological process. An LAZ or HAZ <-2 is defined as stunting and is considered pathological, as it implies that the child fails to reach her/his linear growth potential. Weight-for-Length/Height, on the other hand, is a measure of current nutritional status and only reflects short-term or recent exposure. A low W/L or W/H is referred to as thinness, and is not necessarily the result of a pathological process. However, an WLZ or WHZ <-2 is called wasting and describes recent and severe weight loss due to acute starvation and/or illness. The advantage of this index is that it doesn’t require the age of the child to be known, which is a common problem in poor communities of LMICs. Weight-for-Age is a composite index of the two indices mentioned above and is influenced by both the child's height and weight. The index combines the health and growth effects of short- and long-term exposures. A low W/A is defined as lightness, while WAZ <-2 is more pathological and called underweight.

There are a few other anthropometric indices available to describe a child's health. Mid-upper arm circumference (MUAC) is a measure of the diameter of the mid-upper arm and thus includes both fat and lean mass. It is easily measured in field settings and has been used as an alternative index for nutritional status. A MUAC <125 mm has been used as a proxy for wasting although MUAC and W/L correlate poorly (43). Moreover, the WHO has recommended the addition of MUAC <115 mm as an independent criterion to diagnose severe acute malnutrition in the community-based management of young children 6-59 months old (44, 45). MUAC has also been standardized for age (MUAC-for-Age) as MUAC significantly increases with age in infants and young children. Head circumference, or occipital-frontal circumference, is a measurement that's taken around the largest part of a child's head. It has been used to assess nutritional status but only in children up to 3 years of age, the time of rapid head growth. Deficits may also signal a developmental risk for the child. Unlike MUAC, head circumference has been rarely used in field settings.

1.2.2.2 Epidemiology

It has been estimated that there were 667 million under-five children around the world in 2014 (46). Of these, 159 million were affected by stunting and 50 million by wasting. **Table 1** shows the global and regional prevalence estimates of moderate (≤-2 Z-scores) or severe (≤-3 Z-scores) stunting, wasting and underweight in under-five children for the period 2009-2013. The figures show regional patterns with the highest prevalence estimates found in both South Asia and Sub-Saharan Africa. Moreover, Asia had more than half of all stunted under-five children while one third lived in Africa. Although a decreasing trend in stunting has been reported since 1990 in these regions, and especially Asia, the progress has been uneven among sub-regions. In addition, Africa has seen an increase in its population and has shown increased numbers of stunted children in the Eastern, Middle and Western sub-regions. It has also been reported that all wasted children lived in Asia and Africa, and not one sub-region in Africa has an acceptable level (46).

Table 1. Estimated prevalence of stunted, wasted and underweight under-five children in the UNICEF (sub)regions for the period 2009-2013 (adapted from UNICEF, 2014 (47)).

Region/Sub-region	Stunting (%)	Wasting (%)	Underweight (%)
Sub-Saharan Africa	37	9	21
Eastern and Southern	39	7	18
West and Central	36	11	23
Middle East and North Africa	18	8	7
South Asia	38	15	32
East Asia and Pacific	12	4	5
Latin America and Caribbean	11	1	3
Central Eastern Europe/CIS	11	1	2
Least developed countries	37	9	22
World	**25**	**8**	**15**

CIS= the Commonwealth of Independent States.

1.2.2.3 Consequences

Two recent meta-analyses of 10 prospective studies in Africa, Asia and South America, including approximately 54,000 under-five children, have confirmed that childhood growth restriction is associated with mortality (48, 49). Mortality risks increased with severity of growth restriction, while wasting was the strongest determinant of mortality. Hazard ratios (HRs) for moderate growth restriction (-3≤ Z-score <-2) were 2.3 (95%CI: 1.9 – 2.7) for stunting, 2.6 (95%CI: 2.2 – 3.1) for underweight, and 3.4 (95%CI: 2.9 – 4.0) for wasting, compared to those children with Z-scores ≥-1. HRs for severe growth restriction (Z-score <-3) were 5.5 (95%CI: 4.6 – 6.5) for stunting, 9.4 (95%CI: 8.0 – 11.0) for underweight, and 11.6 (95%CI: 9.8 – 13.8) for wasting (49). Moreover, mild to severe growth restriction consistently increased the hazards of death caused by respiratory tract infections and diarrhea. Furthermore, under-five children with multiple growth deficits, all defined by Z-scores <-2, were at a heightened risk of all-cause mortality compared to those with Z-scores ≥-2. HRs were 3.4 (95%CI: 2.6 – 4.3) in children who were stunted and underweight but not wasted; 4.7 (95%CI: 3.1 – 7.1) in children who were wasted and underweight but not stunted; and 12.3 (95%CI: 7.7 – 19.6) in children who were stunted, wasted and underweight (48).

Stunting and underweight have also been associated with poor child development and schooling. Data from four cohorts in settings with moderate to high stunting prevalence showed that a lower LAZ at 2 years of age was associated with a lower cognitive Z-score in children within the age range of 4 to 9 years (β coefficients 0.17 – 0.19 per unit change LAZ) (50). Another study in five LMICs showed that both WAZ and HAZ at 2 years of age were predictors of schooling (adjusted β coefficients respectively 0.52 and 0.50 years of additional schooling per unit change in Z-score) (39). In addition, data from 6 cohorts in LMICs showed that stunting between 1 and 3 years of age predicted poorer cognition and/or educational attainment in middle childhood. Effect sizes were moderate to large when moderate or severe stunted children were compared to non-stunted children (HAZ ≥-1) (51).

The evidence on the relationships between childhood undernutrition and the development of adult diseases was discussed in section 1.2.1.3, and will not be repeated here.

1.2.3 Determinants

It has been widely recognized that the determinants of LBW, IUGR and PTB are multifactorial. The many determinants of intra uterine growth and gestational duration can be grouped in broad categories relating to genetics and constitution, demographics, obstetrics, nutrition, morbidity and toxic exposure. The mechanisms underlying these biological relationships are covered in an extensive review by Kramer (52). Kramer has identified which factors have well-established direct causal links with IUGR and quantified their relative importance in two typical settings, a rural non-smoking pregnant population of a LMIC as well as a pregnant population of a HIC (**Figure 5**) (10). The figure shows marked differences between both settings. Toxic exposure to cigarettes is considered the most important determinant in the HIC. On the other hand, maternal nutritional factors such as low pre-pregnant weight, low caloric intake and low weight gain, short stature and maternal LBW are considered to make up 50% of the etiology of IUGR in the rural LMIC. It should be noted, however, that much more heterogeneity exists between these two typical settings. Moreover, the fractions are likely to be overestimated because not all determinants are known. The figures also do not include two important indirect factors, maternal age and socioeconomic status, whose impact goes through one or more direct factors.

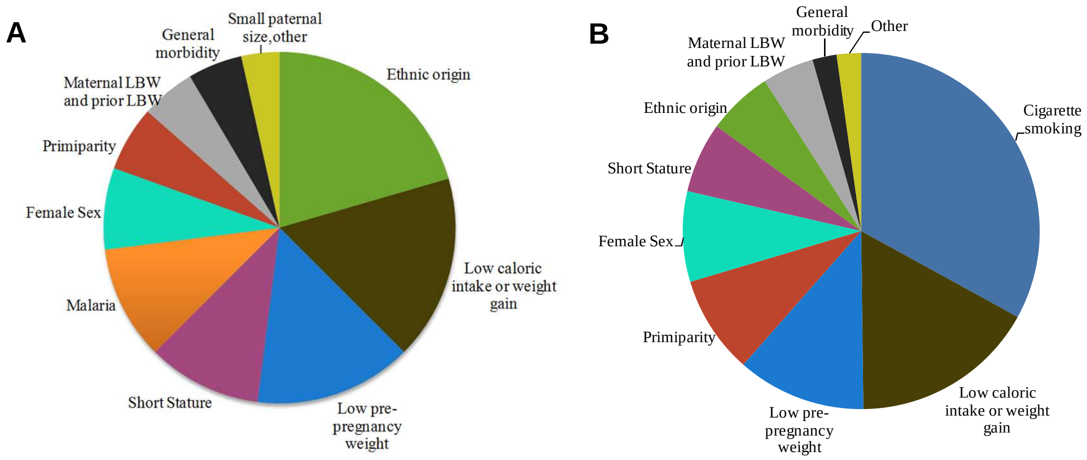


Figure 5. The relative importance of determinants with direct causal links on IUGR in a rural LMIC (A) and a HIC (B) (adapted from (10)). LBW= Low Birth Weight; LMIC= Low-Middle Income Country; HIC: High Income Country.

The determinants of infant and young child growth restriction are also multifactorial, and encompass intra uterine growth restriction, infectious disease, poor feeding practices, micronutrient deficiencies, socioeconomic status, and possibly environmental enteropathy. The determinants of intra uterine and infant and young child growth restriction are further elaborated below.

1.2.3.1 Genetic and constitutional factors

Female compared to male newborns have a slightly lower growth velocity at birth. As such, neonates of the female sex are well known to be smaller than their male counterparts (52). The difference in birth weight between both sexes is relatively smaller in LMICs than HICs, because the distribution is shifted more to the left. Moreover, the relative risk of IUGR in females compared to males is increased and has been estimated at 1.19 in LMICs. However, growth velocity in females levels off to that of males around the age of seven months, but is relatively faster afterwards until four years of age (53). A meta-analysis of data from 16 Demographic and Health Surveys in 10 Sub-Saharan countries has shown that male children under five years of age are more likely to become stunted than females, especially in the lowest socio-economic groups (54). The authors therefore suggested that males become more vulnerable in early childhood.

The health status of the mother before pregnancy influences birth outcomes. Short maternal stature and low pre-pregnancy weight have been identified as important independent direct factors in the causal pathway to IUGR, with an estimated population attributable risk of 25% in rural LMICs (10). Although attained height reflects the process of interaction between genetic and environmental factors, much of the variation in height in LMICs can be ascribed to environmental constraints on linear growth, especially in early life (55). A meta-analyses combining 25 datasets and 177,000 livebirths from LMICs showed that women with a stature <155 cm had increased risks of adverse birth outcomes compared to those ≥155 cm (56). Women <145 cm were considered at highest risk compared to women ≥155 cm and had relative risks of 2.03 (95%CI: 1.76 – 2.35) for term SGA, 1.45 (95%CI: 1.26 – 1.66) for preterm AGA and 2.13 (95%CI: 1.42 – 3.21) for preterm SGA. Pre-pregnancy maternal weight is a result of past growth performance and recent health status of the mother. It reflects the amount of nutritional reserves - lean and fat mass - available to the growing fetus. Since maternal weight and height are strongly correlated, maternal weight has also been adjusted for height to isolate its effects, e.g. the body mass index (BMI; weight/height^2). Underweight women with a pre-pregnancy BMI <18.5 kg/m^2 are considered thin and chronically energy deficient (57). A recent meta-analyses estimated that women from LMICs who are underweight before pregnancy are at an 1.52 increased risk (RR 95%CI: 1.25 – 1.85) of having a LBW newborn (58).

1.2.3.2 Obstetrics

Parity refers to the number of times a woman has been pregnant up to 20 weeks of gestation, resulting in either live or stillbirths. Although the exact mechanisms are unclear, birth size is typically more favorable with increasing parity (52). The relative risk of IUGR for primiparae has been estimated at 1.23. Maternal age is associated with parity, and it has therefore been questioned if age is an independent determinant of intra uterine growth or gestational duration. However, a large retrospective cohort study of nulliparous pregnant women in the USA showed that teenagers had an independently increased risk of adverse birth outcomes compared to women aged 20-24 years (59). These findings could possibly be explained by biological immaturity and/or a competition for nutrients between the growing mother and fetus.

1.2.3.3 Nutrition

The most sensitive indicator of acute nutritional stress during pregnancy is maternal or gestational weight gain. Weight gain can be divided over four components: deposition of fat stores, breast and uterine tissue growth, increased plasma volume, and growth of the fetus, placenta and amniotic fluid (52). However, only the first component actually contributes to fetal energy provision. Maternal energy stores prior to pregnancy are also a major source of nutrients, and pre-pregnancy BMI is a modifier of the effect of gestational weight gain on intra uterine growth. The association of gestational weight gain with birth size has been shown to be much stronger in underweight women (60). A path analysis provided support of the evidence in that the causal pathway maternal nutrition → weight gain → birth outcome is only sustained in the context of nutritional deprivation (61).

Maternal caloric intake is also a determinant, but more nutritionally focused, and more straightforward, compared to gestational weight gain. However, caloric intake should be considered along with energy expenditure because of the increased consumption of calories with increasing energy expenditure (52). Similar to gestational weight gain, the effect of maternal caloric intake on intra uterine growth has shown modification by pre-pregnancy BMI. The effects of acute caloric restriction during pregnancy on birth outcomes have been clearly demonstrated during a few historic ‘natural experiments’. One is the Dutch winter famine towards the end of World War II, in the period of October 1944 – May 1945, when a German embargo on rail transport was imposed and the western regions of The Netherlands experienced acute famine. Food rations had been stable during the war at ± 1800 kcal/d and suddenly dropped to levels <1500 kcal/d. Near the end of the famine, the average diet merely supplied ± 700 kcal/d in the western Netherlands. Several studies have consistently reported declines in birth weight during this period. Birth length and placental weight were also shown to be affected, although the decline in birth length was less pronounced (62). Birth anthropometric measures increased to approximate pre-famine values once the embargo was lifted.

Micronutrient deficiencies can have a direct impact on health, such as anemia, delayed motor development in infants and young children (iron), impaired neurological development (vitamin B_{12}), in utero neural tube defects and acute lower respiratory morbidity (folate), xerophthalmia (vitamin A), impaired child growth (zinc) and hypothyroid disorders (iodine) (32, 63-66). However, several micronutrients also play a key role in immune function and deficiencies may therefore feed into the interaction between infectious disease and malnutrition. It has been shown that growth becomes compromised when vitamin A or iodine deficiency is severe (65, 67). The prevalence of inadequate zinc intake has also been shown to be highly correlated with the prevalence of stunting in under-five children (66). A meta-analysis of zinc supplementation trials in LMICs has also shown significant protective effects against stunting (68).

1.2.3.4 Morbidity

Maternal malaria is well known to contribute to growth faltering. Prenatal malarial infection is positively associated with LBW (69, 70). In malaria endemic areas, the prevalence of malaria is higher in pregnant compared to non-pregnant women, especially primiparae (52). Moreover, there have been suggestions that malarial infection in the final trimester of pregnancy is particularly harmful (71). Prenatal malaria prophylaxis has shown to be effective in reducing LBW and IUGR (72, 73).

Infectious diseases, such as diarrhea, measles, pneumonia, meningitis and malaria, are an important determinant of childhood stunting (29). There are several nutritional pathways through which infectious disease can affect growth. Disease often results in decreased caloric intake as a result of appetite suppression. At the same time, nutrient absorption may be impaired and/or nutrient losses may occur while the metabolic stress of disease can increase nutrient requirements. Diarrheal disease has a relatively larger impact on growth faltering, possibly due to the combined effects of malabsorption as well as reduced appetite. It has been estimated that for every diarrheal episode in the first 24 months of life, the odds of stunting at 24 months increases by a factor 1.05 (OR: 1.05; 95%CI: 1.03 – 1.07) (28).

In this context, it is also important to mention environmental or tropical enteropathy, an acquired intestinal disorder associated with enteric infections and believed to be widely present in young children of LMICs (74). Its etiology is still unclear, but the symptoms can be compared to that of a ‘leaky gut’, such as reduced intestinal absorptive capacity, reduced barrier integrity, as well as mucosal inflammation. It has been hypothesized that the functional changes of the intestine may as such contribute to growth restriction (67).

1.2.4 Seasonality

Determinants of birth size, such as maternal nutritional status and anemia, in rural areas of LMICs show typical variations across seasons (75, 76). Women of reproductive age in subsistence-farming communities experience profound seasonal fluctuations in food security, dietary quantity and quality, and energy expenditure due to prevailing climatologic conditions (77-83). The rainy or hunger season is generally considered the most energetically challenging season. The season is thought to impose a negative energy balance due to the deterioration of nutritional status as food stocks from the previous harvest dwindle, infectious disease incidence increases, and manual agricultural labor on the field in preparation of the next harvest increases.

Birth size in rural environments of LMICs also shows such seasonal patterns. Monthly means of birth weight and birth length typically plummet during the rainy season (15, 75, 78, 84). Moreover, seasonality also influences the tail of the distribution. A retrospective cohort study in The Gambia investigated annual seasonal cycles of maternal weight, hours of work and malarial infections in an attempt to explain the differential etiologies of SGA births and PTBs (75). The authors concluded that maternal weight gain/loss cycles closely paralleled the seasonal patterns of SGA incidence. The nadir or minimum in SGA incidence occurred during those months when maternal weight was highest (early rainy season) and the peak or maximum in SGA births occurred when weight was lowest (end rainy season). Maximum maternal workload and malaria incidence respectively matched the first (early rainy season) and second (late rainy season) sharp peaks in PTB incidence during the year.

A longitudinal study in Malawi has shown that exposure to the energetically stressful rainy season during the third trimester of pregnancy influences both dimensions of fetal growth, birth weight and birth length, but not the ponderal index (15). As such, the study findings did not support the widely assumed 'timing hypothesis' that postulates that linear growth velocity peaks in the second trimester and soft tissue accretion (~ ponderal index) in the third trimester (85). These findings rather suggest that linear growth may respond sooner or may be more sensitive to prenatal seasonal stress compared to the ponderal index. A study of the Dutch winter famine also found larger effects of caloric restriction on birth weight and birth length in those pregnancies who were exposed to the famine, defined by <1500 kcal/d, in the third trimester of pregnancy. The effects were approximately halved in those exposed during the second trimester, and absent in those exposed during the first trimester (86). A comparison of birth weight-for-gestational age curves of newborns in the UK and rural The Gambia demonstrated that faltering in birth weight in The Gambia was most apparent >37 weeks of gestation (87). The difference in birth weight between countries jumped from ± 290 g at 37 weeks to 450 g at 39 weeks, 520 g at 40 weeks and 640 g at 41 weeks. The researchers later estimated that these differences were only minimally attributed to a shorter stature of women in The Gambia.

Another Malawian study, performed in the same research setting, showed that exposure to the rainy season acted independently from maternal weight gain in early/mid and late pregnancy which were all associated with birth weight and length (19). The researchers therefore suggested that the seasonal stress of the rainy season may operate through both direct and indirect effects by respectively the fetal substrate supply in late pregnancy and maternal weight gain at all stages of pregnancy.

However, other mechanisms have also been proposed. Recently, a study in The Gambia showed that seasonality in the dietary intake of micronutrients affects the epigenetic regulation of DNA methylation in pregnant women, and may as such influence embryogenic and fetal development (88). Another retrospective study in that same research setting showed that seasonality patterns going back to as early as the mother's time of birth affects birth weight and head circumference of the next generation (89).

1.2.5 The window of opportunity

Interventions to prevent child growth restriction are considered most efficacious when implemented during the so-called 'window of opportunity' which spans the 1,000 days between a woman's conception and her child's second birthday. This was demonstrated by a pooled data analysis from 54 countries in the WHO Global Database on Child Growth and Malnutrition. The analysis showed that infants from different continents are born with growth restriction and subsequent growth faltering occurred rapidly from three to 24 months of age (**Figure 6**) (90). Infants from Sub-Saharan Africa (orange plot - AFRO) were reported to have only slight catch-up growth beyond this period (0.005 z score increase/month), whereas infants from South Asia (green plot - SEARO) showed no sign of catch-up growth.

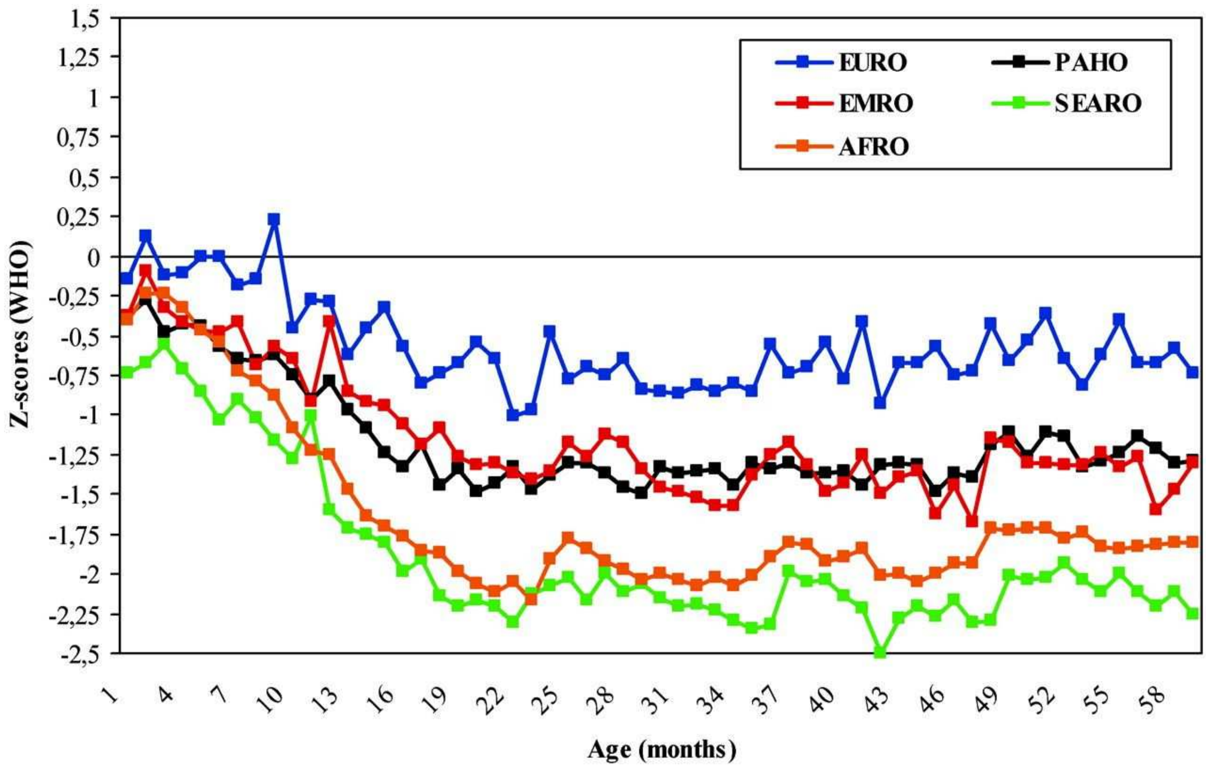


Figure 6. Mean Height-for-Age (Z-scores) in under-5 children, relative to the WHO Child Growth Standards, by age (months) and WHO region (Victora et al., 2010 (90)). EURO= Europe and Central Asia; PAHO= Latin America and the Caribbean; EMRO= North Africa and the Middle East; SEARO= South Asia; AFRO= Sub-Saharan Africa.

There is also an increasing amount of evidence in support of prenatal and early life interventions for the attainment of later human capital and health. Pooled data from five prospective cohort studies in Brazil, Guatemala, India, the Philippines and South Africa has consistently shown that small size at birth and, especially, smaller HAZ at two years of age are associated with reduced human capital, i.e. adult height, schooling, economic productivity and offspring birthweight (38, 39). The cohort data was also used to investigate adult chronic disease risk as the participants of these studies were born when undernutrition prevalence was high, but grew up in an environment that promoted the development of obesity and chronic disease risks. The data suggested that interventions aimed at increasing birth weight and linear growth in the first two years of life may offer some protection from adult chronic disease risk factors, such as high blood pressure and plasma glucose concentrations. In fact, associations between relative weight gain - independent of linear growth - and adult adiposity and cardiometabolic risk factors became more important after the age of two years (38).

1.3 Modifiable maternal factors

The determinants of intra uterine and childhood growth restriction are multifactorial. However, interventions that target growth restriction should primarily focus on those determinants that are both modifiable and quantitatively important in terms of effect size and prevalence (10). Among others, maternal nutrition has had a high priority on the research agenda of LMICs because of its potential substantial impact on the short term. Although not elaborated in the previous section on determinants, energy expenditure from strenuous maternal work (e.g. agricultural work during the rainy season) has also been given a high priority for future research. The hypothesis that increased strenuous work during pregnancy may result in adverse birth outcome has biological plausibility, but the evidence is still scarce and inconclusive.

The PhD research described in this manuscript covers both maternal nutrition and physical activity in rural areas of LMICs. More specifically, the research is focused on the seasonal impact of prenatal nutrition and physical activity as well as postnatal micronutrient supplementation strategies in lactating mothers on child growth restriction. The following sections provide a state-of-the-art of the evidence and explores some of the research gaps within these areas. The overview is not intended to be exhaustive, but highlights the topics within the scope of the PhD research.

1.3.1 Prenatal nutrition

1.3.1.1 Background

The nutrient demands of pregnancy are high in order to sustain fetal development as well as the growth of maternal tissues (91). The total energy cost of pregnancy has been estimated at 77,000 kcal (92). The energy demands increase over the course of pregnancy. While the energy needs in the first trimester are minimal, it has been estimated that an additional 350 and 500 kcal/d is required in the, respectively, second and third trimester for healthy women with a normal BMI (93). The basal metabolic rate increases at the onset of the second trimester in well-nourished populations. Around this time, growth rates of the uterus, placenta, fetus and mammary glands substantially increases, blood volume of the mother increases as well as her cardiovascular and respiratory work. Nonetheless, the additional energy needs are smaller compared to some nutrients. Hence, pregnant women have been advised to consume nutrient-dense foods. It is noteworthy that the metabolic efficiency for some nutrients, such as calcium and phosphorus, is adjusted in pregnant women by hormone-regulated intestinal absorption and urinary excretion (91). Requirements for these nutrients therefore do not increase during pregnancy.

Observational studies have shown that energy intake and gestational weight gain are positively associated with birth outcome (52, 94). The association is stronger in undernourished women with a low pre-pregnancy BMI. Although the prevalence of low BMI (<18.5 kg/m²) has been reduced since the ‘80s, it is still higher than 10% in Africa and Asia (28). Moreover, although data is scarce, studies have shown that women of reproductive age in both rural and urban areas of LMICs are at risk of inadequate intakes and deficiencies of micronutrients (95-99). These findings have been linked to the monotonous plant-based diet, low intakes of micronutrient-rich foods, higher infection rates, higher fertility rates, etc. that are common in LMICs. While some women start pregnancy with deficiencies, women with sufficient preconception stores often develop deficiencies during pregnancy (100).

Iron deficiency is a common nutritional deficiency. Iron deficiency anemia is an advanced stage of iron deficiency, when hemoglobin (Hb) production can no longer be supported and its concentration runs low. Although anemia is caused by several factors - such as genetic factors, infections, intestinal parasites, chronic diseases and/or poor nutrition - iron deficiency has been estimated to contribute to as much as 50% of the anemia cases worldwide in women of reproductive age (101). Anemia has therefore been used as a proxy for iron deficiency anemia. The global anemia prevalence in pregnant women was estimated at 32 million in 2011 (102). The burden is particularly high in Africa and South Asia. Other micronutrient deficiencies such as folate, vitamin A, vitamin B_2, vitamin B_6, vitamin B_{12}, vitamin C and selenium have also been associated with anemia in LMICs (103-107).

The global prevalence of folate and vitamin B_{12} deficiency has not been estimated, because data is scarce and often not nationally representative. However, a few national surveys suggest that deficiencies are common in both LMICs and HICs (108). There are also few nationally representative data from low income countries for zinc. However, it was estimated that 17.3% of the global population has inadequate zinc intakes based on country-specific national food supplies and dietary requirements (66). Sub-Saharan Africa and South Asia had the highest regional prevalence in the world, respectively 25.6% and 29.6%. It has also been estimated that 1.9 billion people have insufficient intakes of iodine (109). Around 40% and 32% of these people lived in Africa and South-East Asia, respectively. The prevalence of subclinical vitamin A deficiency, i.e. low serum retinol concentrations, has been estimated at 19.1 million or 15.3% of pregnant women (110).

1.3.1.2 Prenatal supplementation

1.3.1.2.1 Protein/energy supplementation

Maternal malnutrition and food insecurity in LMICs has been targeted by nutrition interventions ranging from nutrition education, balanced protein energy supplementation (protein < 25 energy% (E%)), high protein supplementation (protein ≥ 25 E%) and isocaloric balanced protein supplementation (protein < 25 E% without extra energy, i.e. protein replaces an equal amount of non-protein energy in the control group) (111). Among these different strategies, balanced protein energy supplementation has shown most promise in the prevention of adverse birth outcomes, including IUGR.

A recent Cochrane review of (cluster-) randomized controlled trials showed that balanced protein energy supplementation significantly increased mean birth weight (mean difference (MD): +40.96 g; 95%CI: 4.66 – 77.26; moderate quality evidence) and reduced the risk of SGA births by 21% (RR: 0.79; 95%CI: 0.69 – 0.90; moderate quality evidence) (112). The authors ascribed the modest increase in birth weight to non-compliance and dietary substitution, as a much larger effect on mean birth weight was seen in a trial in The Gambia that supplied a high energy supplement (± 1030 kcal/d) (113). However, it should be noted that some trials combined the protein energy supplement with multiple micronutrients (MMN) or used MMN supplementation as the control group. MMN supplementation has also been shown to have a beneficial effect on birth weight and low birth weight (see section 1.3.1.2.2) (114). The Cochrane review also analysed effect modification by pre-pregnancy nutritional status on birth weight but did not find any significant differences between groups ($\chi^2 = 2.35$; $P = 0.12$). However, the effect was higher in undernourished women (MD: +67.0 g; 95%CI: 13.1 – 120.8) compared to adequately nourished women (MD: +15.9; 95%CI: -20.8 – 52.7). This contradicts the findings of another more recent systematic review which found significant and more pronounced effects in malnourished women only (115, 116). It is important to mention that these findings warrant careful interpretation as maternal nutritional status is not always clearly defined by a criterion in the included studies, and pregnancy is most often not detected in the early stages. Balanced protein energy supplementation also showed an important reduction in the risk of stillbirth by 40% (RR: 0.60; 95%CI: 0.39 – 0.94; moderate quality evidence) but showed no effects on birth length, head circumference, weekly gestational weight gain, gestational duration and PTB. There are only a few trials that assessed postnatal growth after balanced energy protein supplementation. In these trials, infants 12 months of age did not show any differences in linear growth, head circumference, morbidity or mortality between mothers in the intervention and control groups (117-119).

Although there were only a few nutrition education interventions included in the Cochrane review, of which the quality of evidence was evaluated as low, nutrition education appeared effective in increasing protein intake in pregnant women and resulted in a significant increase in birth weight in undernourished women only (MD in undernourished women: +489.76 g, 95%CI: 427.93 – 551.59, vs, MD in adequately nourished women: +15.00 g, 95%CI: -76.30 – 106.30) (112). Nutrition education trials reported no beneficial effect on the incidence of SGA, but an important 54% reduction in PTB in adequately nourished women. High protein and isocaloric protein supplementation demonstrated a lack of benefit as well as evidence of potential harm, and future research was therefore not considered justified for these two supplementation strategies.

1.3.1.2.2 Micronutrient supplementation

The WHO has long targeted micronutrient deficiencies by recommending single-micronutrient interventions or programs, i.e. with one or two micronutrients. In an effort to tackle iron deficiency anemia and its adverse consequences, it has recommended daily supplementation of 30-60 mg iron (depending on anemia prevalence) + 400 µg folic acid for women throughout pregnancy, and at least three months after delivery for postpartum mothers of LMICs as part of the routine antenatal care (120, 121). However, it recently recommended intermittent weekly supplementation of 120 mg iron + 2800 µg folic acid in settings where anemia prevalence in pregnant women is <20% because of the reported fewer side effects and improved compliance rates (122). Due to safety concerns of iron supplementation in malaria-endemic areas, supplementation in such areas is advised to be accompanied by efforts to prevent, diagnose and treat malaria.

A recent meta-analysis concluded that daily oral iron supplementation during pregnancy significantly reduces the risk of maternal anemia, iron deficiency anemia and iron deficiency at term compared to no iron supplementation or placebo (123). On the other hand, infant outcomes were only marginally improved. The mean birth weight of newborns was 23.8 g higher (95%CI: -3.0 - 50.5; moderate quality evidence), and the risk of LBW newborns as well as PTBs were reduced by respectively 16% (RR: 0.84; 95%CI: 0.69 - 1.03; low quality evidence) and 7% (RR: 0.93; 95%CI: 0.84 - 1.03; moderate quality evidence). The few studies that assessed the effects of iron and folic acid (IFA) supplementation showed similar effects on maternal outcomes, but there was no clear evidence for infant outcomes.

Since the end of the ‘90s, there has been increasing interest in multiple micronutrient (MMN) supplementation. Firstly, there was accumulating evidence from observational studies that deficiencies in B-complex vitamins, vitamin A, iron, zinc, calcium, magnesium, copper and selenium are associated with LBW (124, 125). Secondly, studies have shown that micronutrient deficiencies in women from LMICs are multiple and do not always occur independently (80, 97, 98). Deficiency problems have in fact been shown to overlap and interact, e.g. vitamin B_6 deficiency usually occurs in combination with other B-complex vitamins (126). The benefit of single-micronutrient supplementation strategies was therefore questioned, and a joint call by UNICEF, WHO and the UN University was launched to test the benefits of prenatal MMN supplementation on pregnancy outcomes compared to the standard IFA supplementation. The recommended MMN supplement, called UNIMMAP, contains 15 micronutrients at a dose of one recommended daily allowance, based on American and Canadian standards, except for folic acid which is dosed at the WHO standard (127). The 15 micronutrients are those for which deficiencies are widespread and formulation into a tablet was feasible: thiamine, riboflavin, niacin, vitamin B_6, folic acid, vitamin B_{12}, vitamin C, vitamin A, vitamin D, vitamin E, zinc, iron, copper, selenium and iodine.

The most recent meta-analysis of the concerted effort on MMN supplementation in pregnant women compared to iron, with or without folic acid, showed a significant risk reduction for LBW (RR: 0.88; 95%CI: 0.85 – 0.91; high quality evidence) and SGA newborns (RR: 0.90; 95%CI: 0.83 – 0.97; moderate quality evidence), and a borderline risk reduction for PTBs (RR: 0.96; 95%CI: 0.89 – 1.03; high quality evidence) in LMICs (114). Moreover, MMN supplementation relatively reduced the rate of stillbirth (RR: 0.91; 95%CI: 0.85 – 0.98; high quality evidence). Subgroup analyses showed that the effect on the risk of SGA births was modified by maternal pre-pregnancy BMI and height. MMN supplementation resulted in significant reductions in SGA incidence in those mothers with a good nutritional status at baseline (BMI >20 kg/m², height >153.9 cm). On the other hand, subgroup analyses for PTBs showed that MMN supplementation was favorable only for those women with a low BMI (BMI ≤20 kg/m²).

Follow-up data of the UNIMMAP and other MMN trials is scarce and their results are contradictory. A longitudinal study in Burkina Faso showed that MMN compared to IFA supplementation resulted in a significantly increased LAZ of 0.13 (95%CI: 0.02 – 0.24; P = 0.02) and a 27% reduction in stunting (HR: 0.73; 95%CI: 0.60 – 0.24; P < 0.01) in the first year of life (128). However, the effects on linear growth disappeared and an increased WHZ of 0.20 (95%CI: 0.06 – 0.34; P = 0.04) was observed at an average age of 2.5 years. It should be noted that infant growth was poor, with wasting and stunting rates of respectively 35.1 and 29.2 per 1000 infant-months in the MMN group. A cross-sectional study in Nepal showed that 2.5 year old children of mothers who received prenatal MMN compared to IFA supplementation had an improved WAZ (+0.14; 95%CI: 0.001 – 0.27; P = 0.05), while HAZ and WHZ were marginally improved (resp. +0.08, 95%CI: -0.06 – 0.22, P = 0.05; +0.12, 95%CI: -0.02 – 0.26, P = 0.10) (129). However, another cross-sectional study from Nepal showed no differences between prenatal MMN compared to IFA supplementation at eight years of age (130). Although these findings suggest that prenatal supplementation may yield long-term effects, its clinical relevance is unclear. Although there is evidence that both IFA and MMN might improve perinatal survival, so far, there is no evidence that MMN compared to IFA supplementation improves child survival (128, 131, 132).

1.3.1.3 Problem statement

The evidence from maternal supplementation trials shows effect modifications by maternal pre-pregnancy BMI. Balanced protein energy supplementation appears to favor birth weight of newborns from mothers with a lower BMI, however, it is still unclear how the interaction affects other birth outcomes. MMN supplementation favors SGA and PTB outcome of newborns from mothers with a respectively higher and lower BMI. What is interesting is that maternal nutritional status fluctuates across seasons in rural areas of LMICs, and suggests that nutrition interventions may have differential effects across the year. It has therefore been hypothesized that seasonality modifies the effect of maternal nutrition supplementation on birth outcomes.

There have only been a few studies reporting the effects of balanced protein energy supplementation on birth outcomes by season, in an attempt to clarify the rather modest impact on birth outcome. Two studies in rural The Gambia, one cohort and one randomized controlled trial, demonstrated that balanced protein energy supplementation in pregnant women showed larger and significant effects on birth weight and SGA in the rainy season only (87, 113). Mean monthly birth weights of newborns in the control and supplemented villages in the randomized controlled trial are shown in **Figure 7** (113). Women in this trial had a mean BMI of 21.0 kg/m^2. Another cohort study of underweight pregnant women (BMI <18.5 kg/m^2) in rural Bangladesh showed that balanced protein energy supplementation only improved birth weight after a period of food insecurity, while noting that beneficial effects on newborns of the most undernourished women only appeared after mothers were supplemented for more than 4 months (133). Women in these studies were also supplemented with iron, with or without folic acid, at different doses as part of routine antenatal care, and intake of these supplements was not closely monitored. However, there are currently no studies assessing seasonal variations in the effects of balanced protein energy compared to MMN (UNIMMAP) supplementation on birth outcomes.

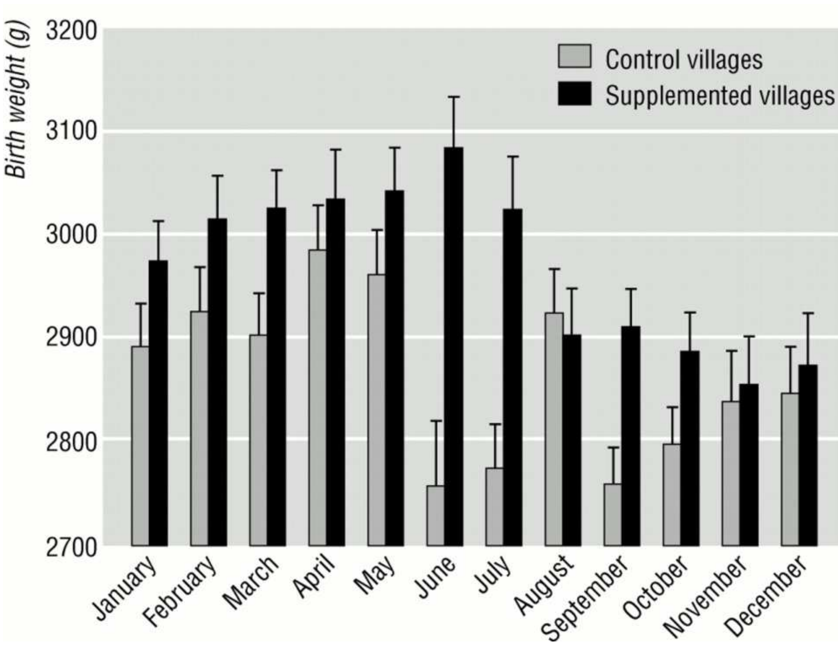


Figure 7. Monthly variations in birth weight (g) of newborns of mothers in the control and supplemented villages in the West Kiang region of The Gambia (Ceesay et al., 1997 (113)).

It is important to mention that the interaction between seasonality and nutrition interventions on birth outcomes has not been adequately investigated. Seasonality in birth size has usually been modelled by a simple linear regression model or regression models that take an annual or bimodal (six months) cycle into account (134-136). However, it has been shown that truncated Fourier series, which use birth dates transformed into cyclic radians, models seasonal cycles of birth size more naturally with pairs of sines and cosines (137). A more accurate analysis holds better promise to inform on the timing of beneficial effects of maternal supplementation and could lead to better targeted and cost-effective interventions to improve maternal and newborn outcomes.

1.3.2 Prenatal physical activity

1.3.2.1 Background

Subsistence-farming communities in rural areas of LMICs are dependent on agricultural food crop production. The first rains of the year are a sign to start preparing the field for the next harvest and women, including pregnant women, are an important part of the agricultural work force. However, the climatologic conditions are such that field preparation is condensed to only a few months per year which often results in long days of strenuous work depending on the agricultural demand. Activity levels of such communities have in fact shown sharp increases in the rainy season compared to the dry season (79, 81, 82, 138). Rural pregnant women in Nepal and The Gambia were reported to have a total energy expenditure (TEE) as high as 2390 kcal/d and 2589 kcal/d during the rainy season (81, 139). However, the increased energy needs of pregnant women can make them vulnerable to strenuous work, especially in the context of the rainy season when food stocks from the previous harvest run low and infectious diseases thrive. Data from LMICs consistently show that birth outcomes are poorest in the rainy season. Pregnant women in late pregnancy may be particularly at risk as their energy needs are highest.

There is evidence, mainly from The Gambia, that pregnant women are able to adapt to cues of energy imbalance by changing their metabolism in order to maintain a positive energy balance (140). This observed metabolic plasticity, although highly individual, consists of adaptations in metabolic rate as well as the amount of maternal fat stored during pregnancy. Longitudinal studies in England and The Gambia demonstrated that well-nourished pregnant women had an increasing basal metabolic rate until delivery, while undernourished women showed a suppressed metabolism in early pregnancy up until the last trimester of pregnancy (**Figure 8**) (140, 141). Undernourished women seemed as such to increase their metabolic efficiency and managed to save a net amount of energy over the full course of pregnancy. These women were estimated to have a total metabolic cost of pregnancy of only ± 34,000 kcal by 36 weeks of gestation. The researchers also reported that more metabolic energy was saved by women with low maternal weight gain, while Gambian women who were participating in a high energy supplementation trial spared less energy than women who were not supplemented (142). These findings suggest that current maternal nutritional status and/or energy supply may trigger these adaptations, although a plasticity threshold may exist at which women can no longer maintain a positive energy balance. The threshold level may well be determined by a woman’s body size, pre-pregnancy reserves and her level of physical activity (PA) (87). It should be noted that the efficiency of energy utilization for exercise/activity also increased, as the energy cost of activity did not increase in line with maternal weight gain. However, the energy savings were much lower than those obtained by the suppression in basal metabolism.

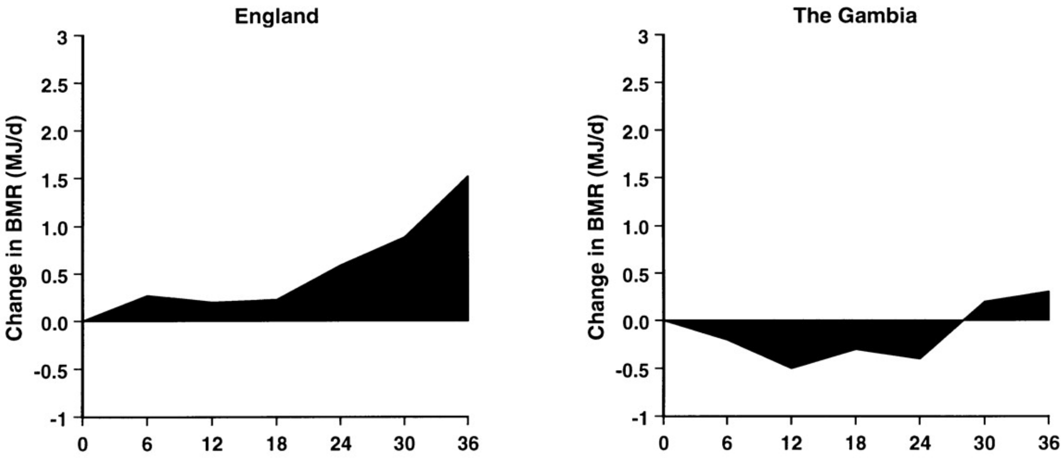


Figure 8. Mean changes in basal metabolic rate (MJ/d) over the course of pregnancy (weeks of gestation) in women from Cambridge (England) and Keneba (The Gambia) (Prentice and Goldberg, 2000 (140)).
BMR= Basal Metabolic Rate.

The average total weight gain of a well-nourished woman is 12.5 kg, without edema, and can be broken down into the following compartments: fetus - 3,400 g, placenta - 650 g, uterus - 970 g, mammary tissue - 405 g, amniotic fluid - 800 g, expansion of blood volume - 1,450 g, extracellular and extravascular water - 1,480 g, and maternal fat - 3,345 g (143). The former six are considered obligate needs and the latter, including maternal fat, are considered optional. Weight gain during pregnancy is typically low for many women in LMICs which may at least be partially explained by exposure to the rainy season. Prentice et al. have shown that pregnant women in The Gambia mobilize their fat stores during this season, while they gain adipose tissue during the dry season (76). Yet, the higher incidence of adverse birth outcomes in LMICs suggests that even obligate needs may not always be met. Data from 83 studies in HICs and LMICs showed that women from LMICs gained less weight relative to their pre-pregnancy weight and gave birth to newborns whose birth weights made up a greater proportion of total maternal weight gain (140). These findings further support the notion of compromised energy economics between mother and child.

1.3.2.2 Increased maternal physical activity

The evidence on the impact of increased maternal PA on birth outcomes is surprisingly scant. The most recent and updated Cochrane review on increased aerobic exercise, i.e. on at least two or three days/week, in pregnant women included 10 (quasi-) randomized trials from HICs (144). Sample sizes were small and gave rise to large confidence intervals. Increased exercise in previously sedentary women did not show any effects on mean birth weight (MD: +49.49 g; 95%CI: -27.72 – 126.73), while the risk for SGA births was not estimable. Increased exercise resulted in a, non-significant, increased risk of PTBs (risk ratio: 1.82; 95%CI: 0.35 – 9.57). The authors therefore concluded that the evidence was insufficient and warranted further research.

Three more recent meta-analyses of observational studies from both HICs and LMICs showed that long working hours - >40 h/week versus less - and physically demanding work are associated with small to moderate increased risks of PTB (long hours: OR: 1.25, 95%CI: 1.01 – 1.54; RR: 1.23, 95%CI: 1.13 – 1.34; demanding work: OR: 1.4, 95%CI: 1.19 – 1.66) (145-147). The risks for SGA births were smaller (RR: 1.04; 95%CI: 0.94 – 1.16), but the evidence was based on only a few studies of which the majority came from HICs where SGA incidence is lower. Studies investigating associations of occupational work, agricultural work, and total maternal activity with birth outcomes in LMICs have shown conflicting results. Moreover, comparisons are hampered due to methodological differences, such as the definition of exposure, population group, outcome assessments, etc. (138, 148-152).

There is also some evidence from HICs and LMICs that timing and intensity of PA influence birth outcomes, but results are somewhat contradictory (138, 145, 146, 153-155). For instance, two studies in the US in different ethnic groups showed that the highest vs lowest quartile of TEE in late pregnancy increased the odds of an SGA birth (OR: 3.0; 95%CI: 1.4 – 6.7; $P < 0.01$), and that the highest vs lowest quartile of total activity at the start of late pregnancy reduced the likelihood of an SGA birth (RR: 0.42; 95%CI: 0.21 – 0.81; $P_{trend} < 0.01$) (154, 155).

1.3.2.3 Problem statement

Pregnant women are an important contributor to agricultural work in the field, and have been shown to continue their routine household activities and child care, during the rainy season in rural parts of LMICs (79, 138). The rainy season usually dramatically increases PA levels and has been associated with poorer birth outcomes. In addition, a retrospective study in The Gambia showed that the typical long hours of work in the field early in the rainy season corresponded with the maximum annual peak in PTB incidence (75). It has therefore been hypothesized that increased maternal PA during the rainy season adversely affects birth outcomes.

Yet, so far, there have been no investigations into the impact of increased PA in the rainy season on birth outcomes. Moreover, it is not clear if women in late pregnancy with the highest energy needs are spared from such strenuous activities in the field. Furthermore, it is unclear to what extent the timing of prenatal exposure to increased PA in the rainy season, as well as prenatal exposure to different intensities of PA during that season, may influence birth outcome. Such data could provide further insights into the mechanisms by which seasonality influences birth outcomes.

1.3.3 Postnatal micronutrient intake and status

1.3.3.1 Background

Infancy, or the first year of life, is a period marked by rapid growth during which birth weight triples and birth length increases by more than 50% in a healthy term infant. Since 2001, the WHO has recommended exclusive breastfeeding (EBF) up to the age of six months based on a systematic review of the literature in term infants (156, 157). The evidence shows that term infants with EBF up to six months experience less morbidity from gastrointestinal infections and no deficits in weight or length gain in both HICs and LMICs. Nonetheless, the authors have warranted for more research as the evidence from LMICs draws upon a small amount of studies with small sample sizes. Moreover, infant micronutrient status is frequently suboptimal at the age of six months. Although micronutrient deficiencies have been proposed to be tackled across the continuum of pregnancy and lactation (158, 159), young infants and their lactating mothers have received relatively little attention, possibly because of concerns that breastfeeding might be discouraged (160).

Pregnancy and lactation are both anabolic phases that redirect micronutrients to the placenta or mammary glands for the development of the fetus or infant (161). As pregnant women in LMICs often become deficient during pregnancy, they are more likely to have suboptimal micronutrient stores at the onset of lactation. Moreover, micronutrient requirements in lactation are mostly higher than those of pregnancy in order to support the rapidly growing infant with relatively high requirements (**Table 2**). Already existing micronutrient deficiencies in the mother may thus be compounded by breastfeeding (161). The requirements for young infants, less than six months of age, listed in Table 2 can be considered as minimum requirements, as infections during childhood typically result in micronutrient losses and/or increased needs for catch-up growth.

Table 2. FAO/WHO recommended nutrient intakes for pregnant and lactating women and young infants (162).

	Women		Infants
	Pregnant	Lactating	< 6 months of age
	RNI[1]	RNI[2]	RNI
Thiamine, mg/d	1.4	1.5	0.2
Riboflavin, mg/d	1.4	1.6	0.3
Niacin, mg NE/d	18	17	2[3]
Vitamin B_6, mg/d	1.9	2.0	0.1
Vitamin B_5, mg/d	6.0	7.0	1.7
Vitamin B_7, µg/d	30	35	5
Folate, µg DFE/d	600	500	80
Vitamin B_{12}, µg/d	2.6	2.8	0.4
Vitamin C, mg/d	55	70	25
Vitamin A, µg RE/d[4]	800	850	375
Vitamin D, µg/d	5	5	5
Vitamin E, mg α-TE/d[5]			2.7
Vitamin K, µg/d	55	55	5
Calcium, mg/d	1,200	1,000	300[6]
Magnesium, mg/d	220	270	26[6]
Selenium, µg/d	30	35	6
Zinc, mg/d[7]	10.0	9.5	2.8
Iron, mg/d[8]	FN[9]	15	FN[10]
Iodine, µg/d	200	200	90

α-TE: α-tocopherol equivalents; DFE: Dietary Folate Equivalents; FAO: Food and Agriculture Organization; FN: Footnote; NE: Niacin Equivalents; RE: Retinol Equivalents; RNI: Recommended Nutrient Intake; WHO: World Health Organization.

[1] Third trimester of pregnancy.

[2] Lactating women in months 0-3.

[3] Preformed niacin.

[4] Values are recommended safe intakes, and not RNIs.

[5] Data were not strong enough to formulate recommendations. The value for infants represents the best estimate.

[6] Breastfed infants.

[7] Moderate bioavailability.

[8] 10% bioavailability.

[9] Iron supplements are recommended for pregnant women. The dose depends on the hemoglobin concentration of the woman. Non-anemic women should be given 100 mg ferrous sulfate during the second half of pregnancy, while anemic women should receive a higher dose.

[10] Neonatal iron stores are sufficient to meet the iron requirements in full term infants.

Some studies report very low breast milk concentrations of some micronutrients – most B vitamins, vitamin A, vitamin C, iodine and selenium – in lactating women from LMICs (63, 158, 160, 163-165). It has been estimated that breast milk from undernourished women provides only 60% of the EBF infant recommendation for thiamin, 53% for riboflavin, 80% for vitamin B_6, 25-55% for vitamin B_{12}, 56% for vitamin A, 50% for vitamin C, 6-23% for iodine and 52% for selenium (158). Folate deficiency occurs less in breastfed infants than their mothers, because their blood concentrations are kept at much higher levels (166). The risk of deficiency in lactating mothers thus increases with low folate intakes. On the other hand, EBF infants are particularly vulnerable to vitamin B_{12} deficiency. Vitamin B12 deficiency in the infant is the result of maternal malabsorption and/or vegetarianism, as the vitamin is exclusively found in animal source foods. Neonatal vitamin A deficiency is often the result of low concentrations of vitamin A in breast milk because newborns, even in well-nourished populations, are born with small vitamin A stores (158). Healthy suckling infants with deficient mothers have been shown to be at an increased risk of deficiency (167-169). In fact, there have been reports of concurrent micronutrient deficiencies in lactating women and their suckling infants (170). Moreover, LBW and preterm infants are at an increased risk of micronutrient deficiencies during infancy because of their lower micronutrient reserves at birth and may deplete their micronutrient stores sooner (171-174).

1.3.3.3 Micronutrient supplementation

The WHO has a few recommendations for single-micronutrient supplementation to lactating women and young infants, but is working on developing more specific guidelines for maternal nutrition interventions after birth. It has so far recommended daily supplementation of 60 mg iron and 400 µg folic acid to postpartum mothers in LMICs, as an extension of the recommendation for pregnant women, until three months post-delivery in order to prevent iron deficiency anemia (120). Surprisingly, the evidence on the effects of supplementation during lactation on infant outcomes is limited. Furthermore, the WHO recommends supplementation with 250 µg/d iodine or 400 mg/y iodized oil in pregnant and lactating women in countries in which less than 20% of households have access to iodized salt (175). As such, breastfeeding infants are thought to benefit from supplementation of their lactating mothers. Currently, the WHO neither recommends vitamin A supplementation in term neonates, nor breastfeeding mothers and their infants aged less than six months (164, 176). The evidence respectively shows a 14% reduction in the risk of mortality at six months of age by supplementation in neonates, but needs to be confirmed by ongoing studies, and no benefits on mortality or morbidity during infancy by supplementation in the lactation phase

(177, 178). Moreover, young infants are only recommended to be directly supplemented with zinc (10 mg) in the management of acute diarrhea, and not for prevention (179).

The WHO has developed separate guidelines for the optimal feeding of LBW infants during early infancy in LMICs. Recommendations for direct supplementation in young infants are targeted towards very low birth weight infants, weighing 1.0-1.5 kg, for whom the WHO recommends daily supplementation with iron (2-4 mg/kg/d) from two weeks to six months of age, vitamin D (400-1000 International Units (IU)/d) until six months of age, and calcium and phosphorus (respectively 120-140 mg/kg/d and 60-90 mg/kg/d) during the first months of life (180). So far, there is not enough supporting evidence to recommend direct supplementation of breastfed LBW infants with daily vitamin A or routine zinc supplementation.

1.3.3.3 Problem statement

It has been postulated that micronutrient deficiencies in early life may contribute to the vicious cycle of growth faltering in LMICs, but lactating women and their infants have only received little attention (67, 160, 164). The often suboptimal infant micronutrient status at six months of age and the co-existence of micronutrient deficiencies in vulnerable populations of LMICs suggest that lactating mothers and their suckling infants are at risk of multiple micronutrient deficiencies. Micronutrient deficiencies have also been shown to interact with other deficiencies. It has therefore been hypothesized that MMN supplementation compared to supplementation with IFA to lactating mothers improves infant growth. The reported beneficial effects of MMN compared to iron, with or without folic acid, supplementation in pregnant women on birth outcomes (see section 1.3.1.2.2) further supports this hypothesis.

However, the evidence on the impact of MMN supplementation in lactating women on maternal and infant outcomes is limited. A recent Cochrane review found no published studies testing the effects of MMN supplementation in lactating mothers compared to supplementation with two or less micronutrients on maternal morbidity and infant micronutrient deficiencies, mortality and morbidity (181). Moreover, we found no such studies investigating growth during infancy.

The advantages of supplementation in lactating women to improve maternal and infant outcomes could outweigh benefits from other supplementation strategies in the child. Firstly, supplementing lactating women with micronutrients has the potential to improve outcomes in both mother and child. Secondly, EBF has been associated with less gastrointestinal infections in the suckling infant through breast milk's supply of immune factors and the elimination of bacterial infections associated with handling foods. Thirdly, the infant is supplied with a safe and more bioavailable form of the micronutrient (182).

1.4 The MISAME project in rural Burkina Faso

The different research hypotheses raised in the previous section were tested in the context of the MISAME project which included two randomized controlled nutrition intervention trials in pregnant and lactating women from two rural villages in the Houndé health district of Burkina Faso from 2004 to 2008. The PhD research described in this manuscript presents the results of secondary analyses of these trials.

1.4.1 Research setting

Burkina Faso is a country in West Africa. The country was estimated to have a population of 17.4 million in 2014 with a fertility rate of 5.7 births per woman. The country has been ranked the sixth least developed country in the world and it has been estimated that 44.5% of its population earns less than 1.25$ per day (183).

Burkina Faso has a Sudano-Sahelian climate with two distinct seasons, i.e. a dry and rainy season. The rainy season runs from May to September/October (**Figure 9**). The main cereal crops, sorghum, maize and millet, are harvested from October to February. Burkina Faso is part of the meningitis belt of Sub-Saharan Africa from Senegal to Ethiopia. This area has annual outbreaks of meningococcal meningitis in the dry season. In 2010, a nationwide meningococcal A conjugate vaccine was introduced in the country (184). The most recent case estimates date back to 2012 and report a total of 6,957 people (185). Burkina Faso is also malaria holo-endemic and shows seasonal variations in its incidence. In 2008, a total of 36,514 malaria cases were reported (185).

The research setting is part of the Houndé health district area, Tuy province, in the mid-west Hauts-Bassins region of Burkina Faso. Houndé city is the capital of Tuy province and counted 39,800 citizens during the time of research. The health system of Houndé district includes 27 rural health centres and one district hospital which employs two physicians. The two MISAME randomized controlled trials were performed in two rural villages, Karaba and Koho, each belonging to a different health sector, respectively 1 and 3 (**Figure 10**). Karaba is a small village that approximately counts 2,400 citizens and is mostly inhabited by the indigenous Bwa people. Koho is a village that approximately counts 4,200 citizens and has a slight majority of Mossi people, who make up the largest ethnic group in Burkina Faso. Koho is also inhabited by a smaller group of Bwa people and a minority of Peul people.

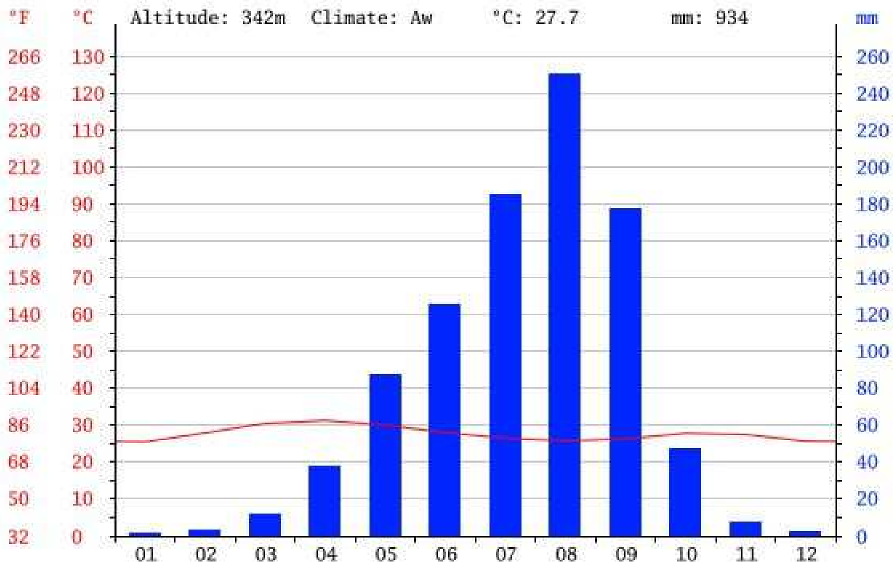


Figure 9. Mean rainfall and temperatures in Houndé, Burkina Faso, during the different months of the year (historical data from (186)).

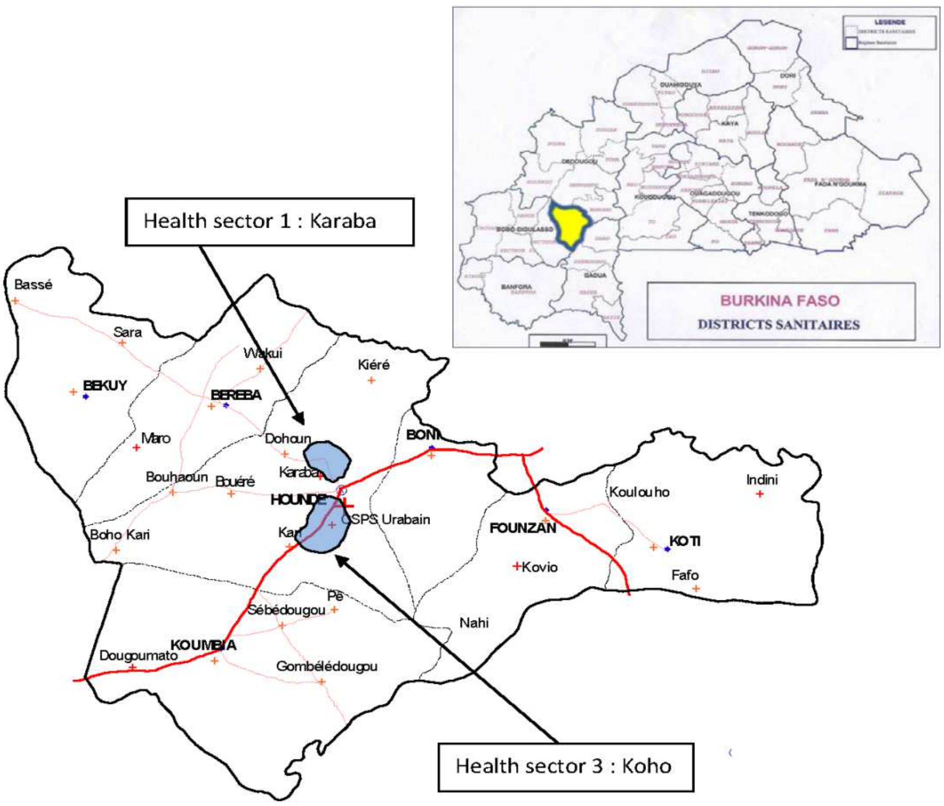


Figure 10. Location of the two research villages (blue) in the health district of Houndé (yellow), Tuy Province, Burkina Faso (Source: Health District of Houndé, 2006).

1.4.2 The MISAME trials

The PhD research described in this manuscript is embedded within the MISAME (MIcronutriments pour la SAnté de la Mère et de l'Enfant, or, micronutrients for maternal and child health) project which involved 2 randomized controlled trials in rural Burkina Faso from 2004 to 2008:

1) The MISAME1 trial investigated the effect of multiple micronutrient (MMN; intervention) supplementation versus iron and folic acid (IFA; control) supplementation in 1426 pregnant women on birth outcomes and, subsequently, the effect of MMN (intervention) versus IFA (control) supplementation in lactating women on infant growth (ClinicalTrials identifier: NCT00642408);

2) The MISAME2 trial investigated the effect of supplementation with a balanced protein energy lipid-based nutrient supplement fortified with MMN (LNS; intervention) versus only MMN (control) in 1296 pregnant women on birth outcomes (ClinicalTrials identifier: NCT00909974).

The MISAME1 trial was implemented in response to the call by UNICEF, WHO and the UN University to generate evidence on the efficacy of MMN - more specifically the UNICEF/WHO/UNU International Multiple Micronutrient Preparation or UNIMMAP – compared to the standard IFA supplementation on birth outcomes. Pregnant women were also re-randomized, at the time of enrolment in the MISAME1 trial, to either MMN (intervention) or IFA (control) supplementation post-delivery to test the efficacy of maternal MMN compared to IFA supplementation during lactation on infant growth. The MISAME1 trial was implemented from 2004 to 2006.

The MISAME2 trial was undertaken as a response to an interim analyses of MISAME1 trial data that suggested modest effects. It was hypothesized that maternal energy deficiency reduces the effect of MMN vs IFA supplementation. The intervention lipid-based nutrient supplement (LNS) therefore combined MMN (same composition as UNIMMAP) with balanced protein energy (14.7 g protein and 67 E% from fat supplying a modest 372 kcal/d to avoid dietary substitution). The LNS supplement was locally produced and was made ready-to-consume in daily packages of 72 g. The control group received the MMN (UNIMMAP) supplement. A dietary assessment study in December, the end of the harvest season, in the research area had shown that women consumed a monotonous cereal-based diet resulting in insufficient intakes of the B-complex vitamins, vitamin A, vitamin C, calcium, iron and zinc (99). Pregnant women did not increase their energy intake compared to non-pregnant women (2096 kcal/d vs 1994 kcal/d), although women in late pregnancy had a slightly, non-significantly, higher energy intake compared to those in other stages of pregnancy (2153 kcal/d vs 2050 kcal/d). The MISAME2 trial was implemented from 2006 to 2008.

Locally trained home visitors went to the homes of women of childbearing age every month. When women reported amenorrhea, they were referred to the nearby health center where pregnancy was confirmed by a urine test. After obtaining informed consent, women were randomly assigned to one of the two study groups until delivery. Nutrition supplement intake was monitored on a daily basis.

The MISAME1 and MISAME2 trials reported a respective LBW incidence of 15% and 13%, an SGA incidence of 40% and 34%, and a PTB incidence of 14% and 15%. The MISAME1 trial demonstrated a significant increase in birth weight (+52 g; 95%CI: 4 – 100; $P = 0.04$) and birth length (+3.6 mm; 95%CI: 0.8 – 6.3; $P = 0.01$) from MMN vs IFA supplementation (187). The MISAME2 trial showed a significant increase in birth length only (+4.6 mm; 95%CI: 1.8 – 7.3; $P = 0.001$) from LNS vs MMN supplementation (188). Birth weight non-significantly increased by +31 g (95%CI: -16 – 78; $P = 0.20$). Both studies reported significant effect modifications by baseline maternal nutritional status on birth size. The effect of MMN on birth weight was only apparent in women with a BMI ≥22 kg/m² (+119 g; 95%CI: 26 – 212; $P = 0.01$) in MISAME1. Conversely, the effect of LNS on birth length was only apparent in women with a BMI <18.5 kg/m² (+12.0 mm; 95%CI: 3.7 – 20.2; $P = 0.01$) in MISAME2.

1.5 Aims of the PhD research

The research of this PhD is situated within the framework of the prevention of growth restriction during the first 1,000 days window of opportunity. The specific aims are to investigate the impact of seasonality in prenatal nutrition and physical activity on fetal growth as well as the role of postnatal micronutrient nutrition on growth during infancy.

The different hypotheses of the research work have been mentioned throughout the introduction, and are detailed below:

- Seasonality modifies the effect of prenatal supplementation with a fortified lipid-based nutrient supplement compared to only multiple micronutrients on birth outcomes;
- Increased prenatal physical activity during the rainy season adversely affects birth outcomes;
- Postnatal multiple micronutrient compared to iron and folic acid supplementation to lactating mothers improves infant outcomes.

These research hypotheses will be tested within the context of the two MISAME trials: prenatal nutrition supplementation and physical activity during the rainy season (MISAME2) and postnatal micronutrient supplementation (MISAME1). The climatologic variations in the research area of the Houndé health district in rural Burkina Faso offers a unique opportunity to test the hypothesis relating to seasonality. Moreover, a dietary assessment study in the research area has shown that the intake of micronutrients was insufficient in comparison to the recommended daily allowances. As such, the research area is also a good candidate to test the hypothesis relating to micronutrient supplementation of lactating women.

1.6 Structure of the manuscript

The research relating to prenatal nutrition supplementation and physical activity during the rainy season are discussed in chapters 2 to 4 (MISAME2), whereas the research on the effect of postnatal micronutrient supplementation will be covered in chapter 5 (MISAME1) (**Figures 11 & 12**). Chapter 2 assesses the modification of the effect of maternal nutrition supplementation on birth size by seasonality. Chapter 3 describes the development and validation of a questionnaire to classify women into levels of physical activity during the rainy season. Chapter 4 firstly investigates the activity patterns of pregnant women in the rainy season, and compares them to non-pregnant and non-lactating women. Secondly, chapter 4 assesses the cross-sectional associations between higher compared to lower maternal physical activity during the rainy season with birth outcomes. Chapter 5 assesses the effect of multiple micronutrient compared to iron and folic acid supplementation in lactating women on infant outcomes. The general conclusions of the PhD research and recommendations for further research and policy are discussed in chapter 6.

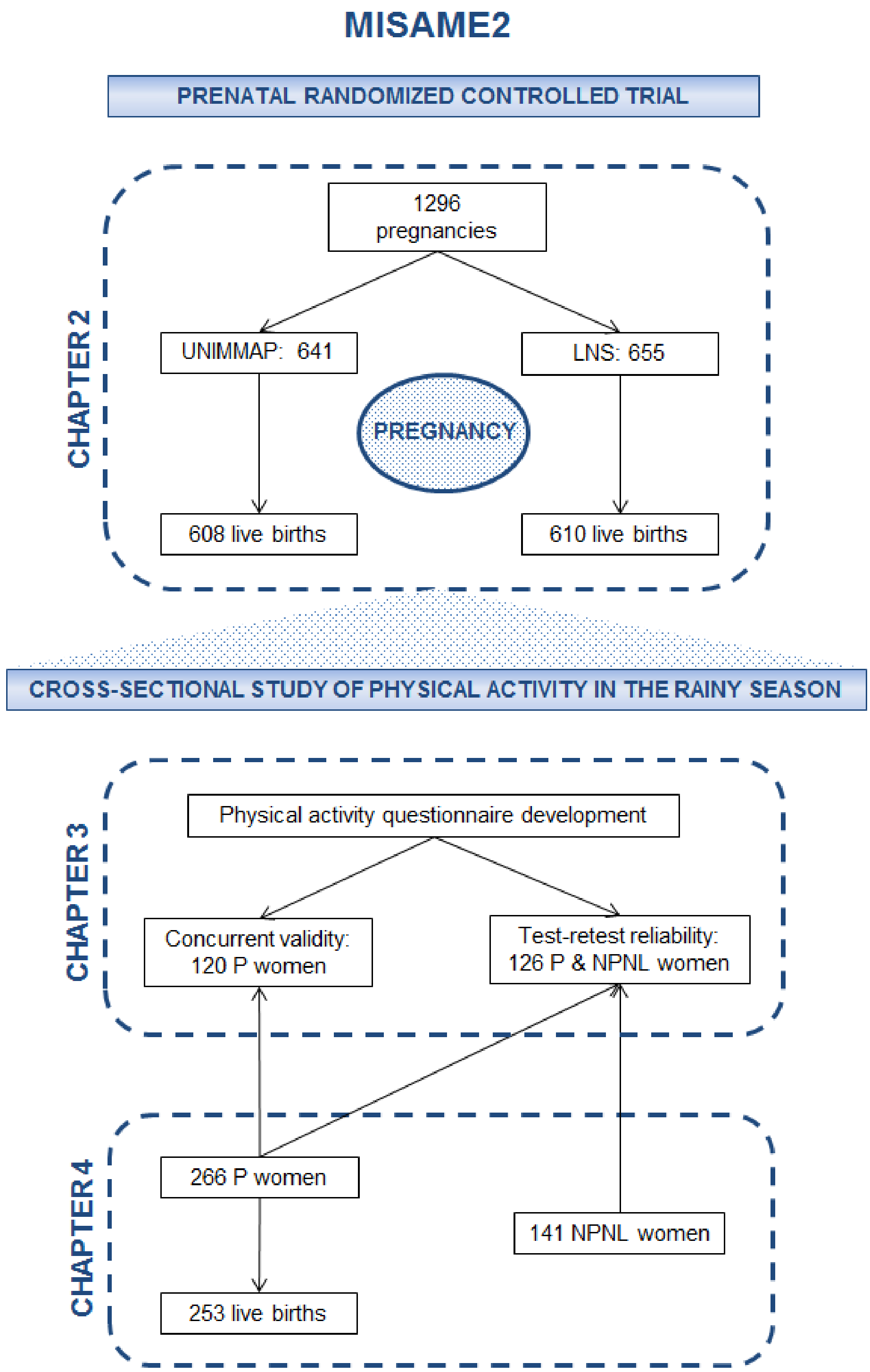


Figure 11. Situation of Chapters 2, 3 and 4 of the PhD research within the MISAME2 trial. LNS: Lipid-based Nutrient Supplement; NPNL: Non-Pregnant Non-Lactating; P: Pregnant; UNIMMAP: UNICEF/WHO/UNU Multiple Micronutrient Preparation.

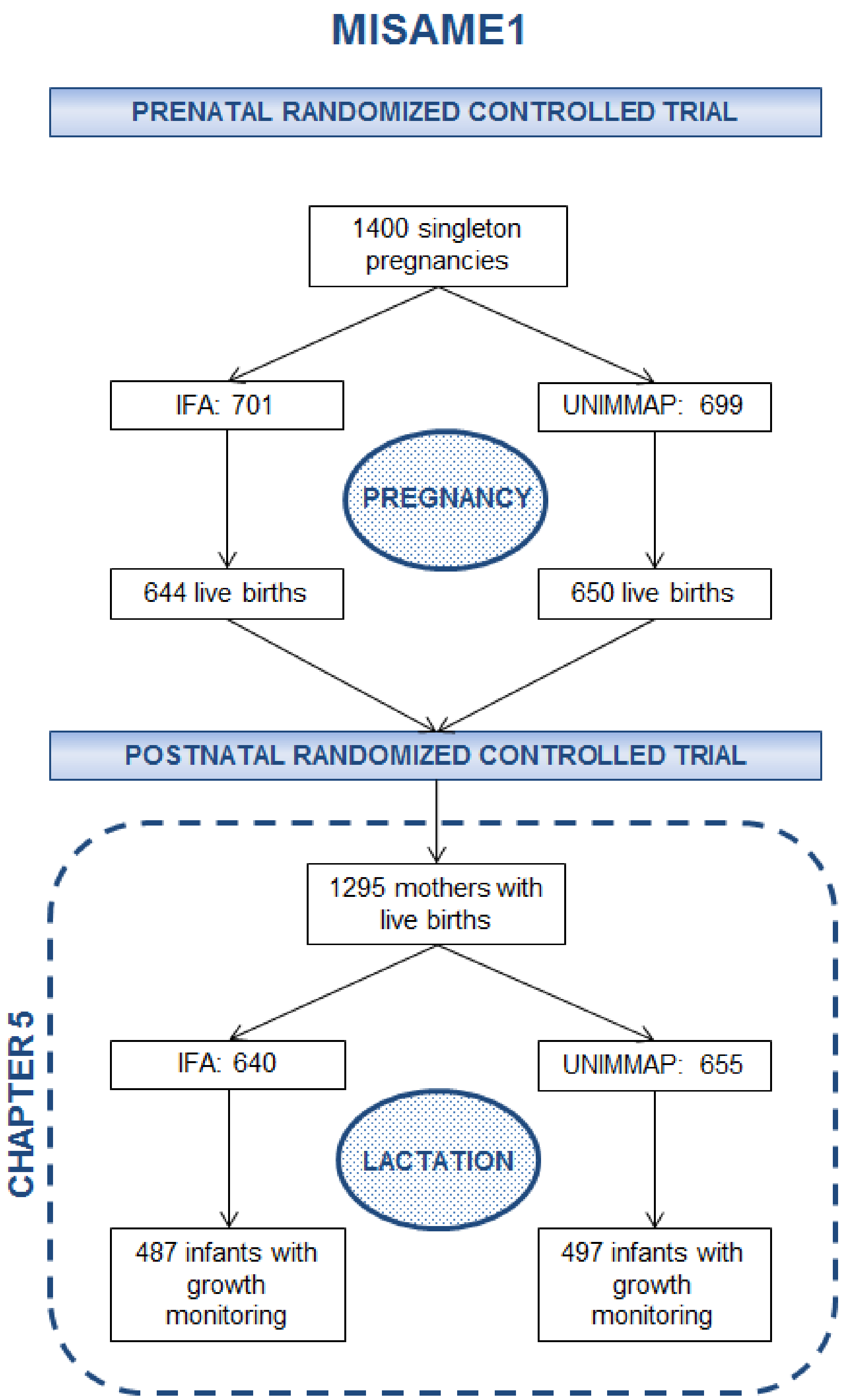


Figure 12. Situation of Chapter 5 of the PhD research within the MISAME1 trial. IFA: Iron and Folic Acid; UNIMMAP: UNICEF/WHO/UNU Multiple Micronutrient Preparation.

Chapter 2

SEASONALITY MODIFIES THE EFFECT OF A LIPID-BASED NUTRIENT SUPPLEMENT FOR PREGNANT RURAL WOMEN ON BIRTH LENGTH

Redrafted from: Bouckaert KP, Toe LC, De Beuf K, Roberfroid D, Meda N, Thas O, Van Camp J, Kolsteren PW, Huybregts LF. Seasonality Modifies the Effect of a Lipid-Based Nutrient Supplement for Pregnant Rural Women on Birth Length. J Nutr 2015; 145: 634-9.

2.1 Abstract

Background: Maternal nutritional status is a major determinant of low birth weight and fluctuates across seasons in rural areas of LMICs. Seasonality may influence the outcome of prenatal nutrition interventions that aim to enhance fetal growth.

Objective: This study investigated seasonal modifications of the efficacy of a randomized controlled prenatal nutrition intervention trial in pregnant women to improve fetal growth in rural Burkina Faso.

Methods: The second Micronutriments et Santé de la Mère et de l'Enfant study compared a lipid-based nutrient supplement (LNS) fortified with multiple micronutrients (MMNs) to an MMN tablet (UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation). Truncated Fourier series were used to characterize seasonality in birth outcomes. Models that included the Fourier series and newborn and maternal characteristics were used to assess seasonal effect modifications of prenatal supplementation on birth outcomes.

Results: Birth weight, birth length, small-for-gestational-age as a proxy for intrauterine growth retardation, and preterm birth were significantly related to date of birth and showed important seasonal variations. LNSs, which supply energy in addition to MMNs, resulted in a significant increase in birth length (+13.5 mm; 95% CI: 6.5 - 20.5) at the transition from rainy to dry season (September to November) compared to MMNs alone.

Conclusions: The climatologic and agricultural seasonal patterns in Burkina Faso affect the efficacy of prenatal LNSs on birth length. In this context, prenatal MMN supplementation programs should be complemented by energy supplementation during the annual rainy season to promote fetal growth. This trial was registered at clinicaltrials.gov as NCT00909974.

2.2 Introduction

Birth size is driven by growth processes in the fetus which can be hampered by intrauterine growth restriction (IUGR) and/or interrupted by preterm birth (PTB), both leading to low birth weight (LBW) (189, 190). Maternal nutritional status before and during pregnancy appears to be the most important determinant of LBW as maternal undernutrition accounts for >50% of LBW cases in many developing countries (191). Seasonal variations or seasonality in birth size are well known in developing countries (189). Seasonality is defined as intra-annual fluctuations occurring with regular as well as predictable patterns. The seasonal variations in birth size have been attributed to seasonal variations in maternal nutritional status, caused by periodical food shortages and agricultural labor (76, 87) that coincide with seasonal epidemics of infectious and parasitic diseases (192). Such variations could partly explain the modest and/or conflicting results achieved by various prenatal nutrition interventions to improve birth size (187, 188, 191). One study in The Gambia showed that protein-energy supplementation resulted in a more important increase in birth weight during the rainy season (June to October) compared to the dry season (November to May) (113). Yet, a sound investigation of seasonal variations in birth size could allow for more accurate analyses of nutrition interventions and could as such contribute to better-targeted nutrition interventions. The analysis of seasonality in birth size ranges from a simple linear regression (193, 194) to a time series summary of monthly means followed by the fit of a regression model that takes a 12-month periodicity (annual model) and/or a 6-month shift (bimodal model) (135, 195) into account. However, those methods can lead to over-parameterization or may introduce abrupt changes by the arbitrary choice of seasonal cut-offs. Fulford and Rayco-Solon et al. proposed using truncated Fourier series, which models seasonal variations more naturally (75, 137).

Burkina Faso is a low income country where multiple micronutrient deficiencies are common and with a 16.2% LBW incidence in 2006 (196). The country is characterized by two distinct seasons, a dry season and a rainy season that runs from May/June to September/October. We previously conducted a randomized controlled efficacy trial (Micronutriments et Santé de la Mère et de l'Enfant study 2 (MISAME2)) in which a lipid-based nutrient supplement (LNS) fortified with multiple micronutrients (MMNs) was compared to an MMN supplement (UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women) in rural Burkina Faso with the aim of improving birth size (188). The study found that prenatal daily LNS resulted in a significantly higher birth length (4.6 mm, $P = 0.001$).

This study aimed to investigate seasonal trends in birth weight, birth length, IUGR (approximated by small-for-gestational-age (SGA)) and PTB, and to investigate if the efficacy of the prenatal nutrition supplements on birth outcomes was modified by the seasonal patterns in Burkina Faso.

2.3 Methods

2.3.1 Study area and subjects

The data for this study was derived from the MISAME2 study that was conducted in the catchment area of 2 health centers in the Houndé health district of Burkina Faso, West Africa. The climate of the region is Sudano-Sahelian. The rainy season runs from May to September/October and the dry season from October to April. The region is malaria endemic. The diet is essentially cereal-based. Maize is the main staple food and is harvested in October/November. In 2004 and 2006, food consumption surveys, conducted by our research team using interactive repeated 24 hour recalls, estimated the average caloric intake during pregnancy to be 8.6 and 8.1 MJ/day during postharvest and preharvest seasons, respectively (77).

The MISAME2 trial was held from March 2006 to December 2007 (188). Trained community health workers home visited women of childbearing age in the study area monthly. In case of reported amenorrhea, a participating woman was referred to the local health center where a medical doctor confirmed the pregnancy by means of a urine test. After explaining the study aims and modalities in the local languages, written consent was sought and obtained from all participants. A consultant obstetrician assessed gestational age as soon as possible after study inclusion using transabdominal ultrasound fetal biometry. When the result of an ultrasound biometry was unavailable, gestational age was computed on the basis of recalled last menstrual period.

2.3.2 Study design

The study was organized as an open-label, randomized controlled efficacy trial. Individual randomization was done based on a computer-generated program in permuted blocks of 4. Randomization numbers were sealed in opaque envelopes and when an informed consent was obtained from an eligible participant, the study physician opened the next envelope and assigned the participant to a treatment group. Women allocated to the control group received one daily tablet of MMNs (UNIMMAP; Scanpharm, Copenhagen, Denmark), while women in the intervention group received a daily LNS (MMN premix obtained by Nutriset, Malauny, France) portion of 72 g (**Table 3**). Daily supplement intake was directly observed. Additionally, as part of a different study on malaria prevention during pregnancy, women were also randomly assigned to be either directly administered a double dose or a triple dose of sulfadoxine-pyrimethamine in their second and third trimesters. However, compliance to the intervention was low as only 23% of women received their triple dose (197). The full methodology of the study is described by Huybregts et al. (188). Briefly, maternal height and weight were measured and maternal characteristics, such as gravidity, were recorded at enrolment. Newborns' sex, weight and length were recorded at birth in the health centers. Only measurements taken within 24 h after birth were included for analysis. Length was measured to the nearest 1 mm with a SECA 207 scale. Weight was measured to the nearest 10 g with a SECA 725 scale. Gestational age at birth was computed by ultrasound measurements of fetal size at 10-12 weeks of gestation.

2.3.3 Data analysis

A total of 1296 pregnancies were randomly allocated to the two groups, i.e. 655 in the intervention group and 641 in the control group. Only singleton pregnancies were included in the analysis because birth anthropometric measures of multiple pregnancies are not primarily nutrition-related. The few data of stillbirths, miscarriages, maternal deaths and other mothers lost to follow-up (i.e. migration and unknown reasons) were also excluded for analysis. Intrauterine growth retardation was approximated by SGA, which is defined by a birth weight for gestational age below the 10th percentile of the reference population by Kramer et al. (198). Preterm birth was defined as being born at <37 wk of gestation. Data on birth weight, birth length and gestational age in the text are presented as means ± SDs, whereas incidence data of SGA and PTB are presented as mean percentages.

Table 3. Nutritional composition of a single dose of the UNICEF/WHO/UNU International Multiple Micronutrient Preparation (UNIMMAP), and the lipid-based nutrient supplement fortified with multiple micronutrients (LNS)[1].

Nutrient	UNIMMAP	LNS
Energy, *MJ*	-	1.56
Energy from protein, *%*	-	15.8
Energy from fat, *%*	-	67.0
Carbohydrates, *g*	-	15.9
Protein, *g*	-	14.7
Fat, *g*	-	27.6
SFA, *g*	-	8.1
MUFA, *g*	-	12.1
PUFA, *g*	-	7.3
ω3 Fatty acids, *g*	-	0.4
ω6 Fatty acids, *g*	-	7.0
Total dietary fiber, *g*	-	9.1
Vitamin A, *RE*	800	881
Vitamin D, *IU*	200	200
Vitamin E, *mg*	10	13
Thiamine, *mg*	1.4	1.6
Riboflavin, *mg*	1.4	1.6
Niacin, *mg*	18	21
Vitamin B6, *mg*	1.9	2.0
Folic acid, *µg*	400	461
Vitamin B12, *µg*	2.6	2.6
Vitamin C, *mg*	70	71
Zinc, *mg*	15	17
Iron, *mg*	30	35
Copper, *mg*	2.0	2.7
Selenium, *µg*	65	65
Iodine, *µg*	150	150
Calcium, *mg*	-	90

LNS, lipid-based nutrient supplement; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; RE, retinol equivalents; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement.

[1] The composition of the UNIMMAP tablet (control) was comparable to the multiple micronutrient premix embedded in the LNS supplement (intervention).

The seasonal trend of birth outcomes was modeled with truncated Fourier terms, as previously done by Fulford et al. (137). Dates of birth were transformed into cyclic data i.e. a continuous variable with a circular distribution, with the starting point set at 1 January. Each date of birth was represented by an angle $\theta_i = 2\pi\ (D_i \bmod 365.25)/365.25$ expressed in radians, so that the 2π radians covers an average year (365.25 d). D_i is the number of days between 1 January 1960 and the i^{th} child's birth. The first p pairs of terms of the Fourier series are included in the regression models as follows:

$$S(\theta_i, p) = \sum_{r=1}^{p} \beta_{r=1} \sin(r\theta_i) + \gamma_r \cos(r\theta_i)$$

where r is the order of the Fourier term. Seasonal effects acting at the time of delivery are modelled by adding $S(\theta_i, p)$ to the linear predictor so that β_i and γ_i become parameters in a regular multiple regression model. Pairs of Fourier terms (sine and cosine of the same order) were included in a regression model by increasing order, starting with the 1st order pair up to the 3rd order pair. The first order terms (sinθ and cosθ) model 6-mo cycles, the second order terms (sin2θ and cos2θ) 3-mo cycles and the third order (sin3θ and cos3θ) 1.5-mo cycles.

Fourier terms (sine and cosine) of the same order represent the same period and are orthogonal. Therefore, if one of these components is significantly associated with the outcome of interest, the order is also considered significantly related to this outcome. Regression coefficients are interpreted as in a simple regression equation, except for the coefficients of Fourier terms that have to be interpreted conjointly. The interpretation of these models can be best explained using an example. Assume that after convergence the model for birth weight over time of delivery would be: $\hat{y} = 2700 + 100\cos\theta - 200\sin\theta + e$. We calculate that on 1 July or at θ=π radians, the predicted average birth weight would be 2,700 + 100 x -1 + -200 x 0 = 2,600g, whereas on 1 April, or θ=π/2 radians, 2,700 + 100 x 0 + -200 x 1 results in a birth weight of 2,500 g. A convenient way to calculate the angle where a maximum or minimum of the outcome is situated is with use of the derivative of the model. A derivative or slope equal to zero would indicate local maxima/minima in the outcome of interest over time. If we consider the above equation, the derivative is $\hat{y}' = -100\sin\theta - 200\cos\theta$. Set to zero, that gives $\tan\theta = -2$. Solving this equation yields 2 angles, 2.03 and 4.25 radians, which represent 29 April and 5 September, respectively.

To determine which orders of Fourier terms fully captured the seasonality of birth outcomes, higher order models were compared to lower order models using a likelihood ratio test. Models that include up to 1st, 2nd or 3rd order Fourier terms are respectively named F1, F2, or F3 models.

Seasonal trends in continuous birth outcomes (birth weight and birth length) were analyzed using linear regression models, while those of binary outcomes (SGA -a proxy for IUGR- and PTB) were analyzed using logistic regression models. The models only included Fourier terms as predictors in order to model the crude trend of seasonality in birth outcomes.

Seasonal effect modifications of prenatal nutrition supplementation on birth weight, birth length, SGA and PTB were assessed by comparing a model that included interactions between intervention and Fourier terms (date of birth) and intervention and year of birth to a model without interactions, using a likelihood ratio test. The prior crude modeling of birth outcome over date of birth determined the number of pairs of Fourier terms in these models. All regression models were adjusted for season invariant covariates to gain statistical efficiency, i.e. health center, intervention, primigravidity, year of birth, group of malaria prophylaxis (3 vs. 2 doses of sulfadoxin-pyrimethamin), maternal height and infant sex.

Intervention effects were plotted over time with 95% CI bands, computed by non-parametric bootstrapping (n = 5000) with replacement to visualize the modulating effect of seasonality of prenatal nutrition supplementation on birth outcomes. Furthermore, baseline maternal BMI and mid-upper arm circumference (MUAC) as well as maternal malarial infection prevalence and blood parasite concentration were plotted over time to characterize the seasonal context of the study setting. All statistical tests were 2-sided and the significance level was set at 5%, except for interactions for which the 10% level was used. Analyses were performed in Stata 12.0 (199) and R 3.0.2 (200).

2.3.4 Ethical considerations

The trial was approved by the ethical committees of the Centre Muraz, Burkina Faso, and the Institute of Tropical Medicine, Belgium, and was registered as NCT00909974 on clinicaltrials.gov.

2.4 Results

Maternal characteristics at randomization and birth outcomes are presented in **Table 4**. Maternal characteristics were similar in both study groups, though maternal height was slightly lower in the LNS group (-0.62 cm, P = 0.06). Birth weight and birth length was 2937 ± 445 g and 478 ± 25 mm, respectively, while gestational length was 39.0 ± 2.8 weeks. Prematurity incidence was 15.0% and SGA incidence 34.0%.

The comparison of the goodness of fit of different seasonality models with increasing order is presented in **Table 5**. The data show no evidence that more than the first pair of Fourier terms, i.e. F1 model, and the first two pairs, i.e. F2 model, are necessary to explain the seasonality in birth weight and birth length, respectively. Both birth weight (P < 0.01) and birth length (P < 0.0001) were significantly related to date of birth. The seasonal variations in SGA (P < 0.01) and PTB (P < 0.01) were also found statistically significant and were modeled best by an F3 model.

Table 4. Maternal characteristics at randomization and birth outcomes of singleton pregnancies in the control (UNIMMAP) and intervention (LNS) groups of the MISAME2 trial[1].

	UNIMMAP		LNS	
	n	**Value**	**n**	**Value**
Maternal characteristics				
Age, *y*	641	24.5 ± 6.3	655	24.6 ± 6.2
Height, *cm*	638	162.9 ± 5.8	649	162.3 ± 6.1
BMI, *kg/m²*	632	21.0 ± 2.2	641	20.8 ± 2.2
Primiparity, *n (%)*	641	133 (20.8)	655	132 (20.2)
Trimester of inclusion, *n (%)*	641		655	
First		251 (39.2)		255 (38.9)
Second		356 (55.5)		346 (52.8)
Third		34 (5.3)		54 (8.2)
Birth outcomes				
Birth weight, *g*	497	2931 ± 433	523	2943 ± 456
Birth length, *mm*	497	476 ± 24	522	480 ± 26
Gestational age, *weeks*	580	39.1 ± 2.5	591	38.9 ± 3.0
Premature, *n (%)*	580	81 (14.0)	591	95 (16.1)
SGA, *n (%)*	494	177 (35.8)	518	167 (32.2)
Male, *n (%)*	510	243 (47.7)	529	271 (51.2)

[1] Data are presented as mean ± SD, unless indicated otherwise. LNS, lipid-based nutrient supplement; SGA, small-for-gestational-age; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement.

Table 5. Comparison of the goodness of fit of seasonality models for birth outcomes of singleton pregnancies in the MISAME2 trial[1].

	Birth weight		Birth length		SGA		PTB	
Model comparison[2]	**LR $\chi2$ (df)**	***P***	**LR $\chi2$ (df)**	***P***	**LR $\chi2$ (df)**	***P***	**LR $\chi2$ (df)**	***P***
F0 - F1	10.0 (2)	<0.01	28.9 (2)	<0.0001	8.1 (2)	0.02	14.0 (2)	<0.001
F1 - F2	5.8 (2)	0.06	11.4 (2)	<0.01	1.8 (2)	0.42	8.5 (2)	0.02
F2 - F3	2.0 (2)	0.37	3.1 (2)	0.21	11.1 (2)	<0.01	11.9 (2)	<0.01

[1] Values are the likelihood ratio chi-square test statistic (LR $\chi2$) and its corresponding degrees of freedom (df) of model comparisons for birth weight (n=1020), birth length (n=1019), SGA (n=1012) and PTB (n=1171). The dependent variables in the models are birth weight, birth length, SGA and PTB and the independent variables are Fourier terms.

[2] F0 is the intercept-only model. F1 is a model including only the 1st order Fourier pair, while F2 also includes the 2nd order pair, etc. PTB, preterm birth; SGA, small-for-gestational age.

From these fitted Fourier models it can be concluded that birth outcomes showed marked periodic variations throughout the year. Birth weights and birth lengths peaked at the end of the dry season, more precisely in April (2994 ± 2 g and 486 ± 0.3 mm, respectively) and May (2979 ± 7 g and 485 ± 1 mm, respectively), whereas their nadirs appeared in the rainy season, respectively in September (2879 ± 2 g) and August (470 ± 0.2 mm) (**Figure 13**). The rainy season was characterized by a dramatic rise in PTB cases (August, mean: 31.2%). SGA incidence showed several peaks throughout the year: 39.0%, 34.0% and 46.5% in February, June and October, respectively (**Figure 14**). Interestingly, the nadir of SGA incidence in the rainy season coincided with the peak in PTB and was followed by a distinct increase to the SGA maximum in October.

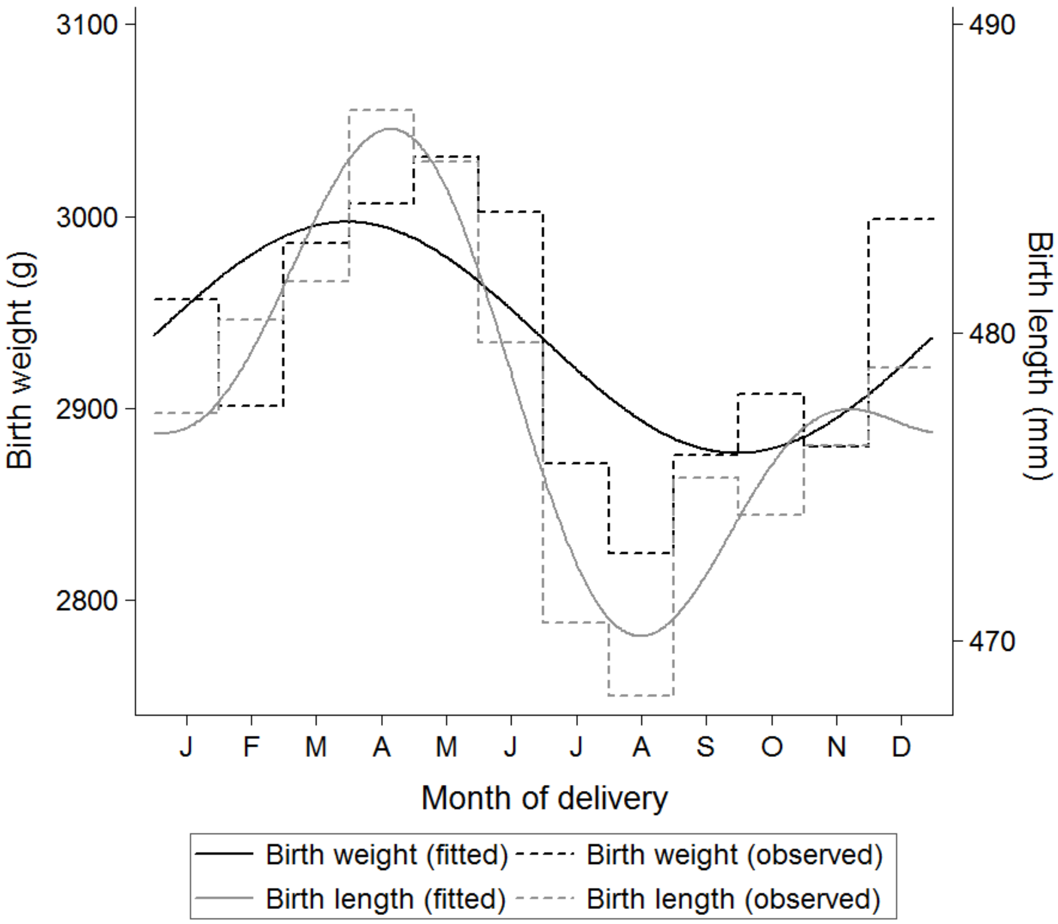


Figure 13. Monthly means and seasonal variations in birth weights (n=1020) and birth lengths (n=1019) of singleton newborns in the MISAME2 trial. The dotted lines represent the observed monthly means, the solid lines the modelled seasonal variations. The birth weight model was fitted to the 1st order Fourier pair. The birth length model was fitted to the 1st and 2nd order Fourier pairs.

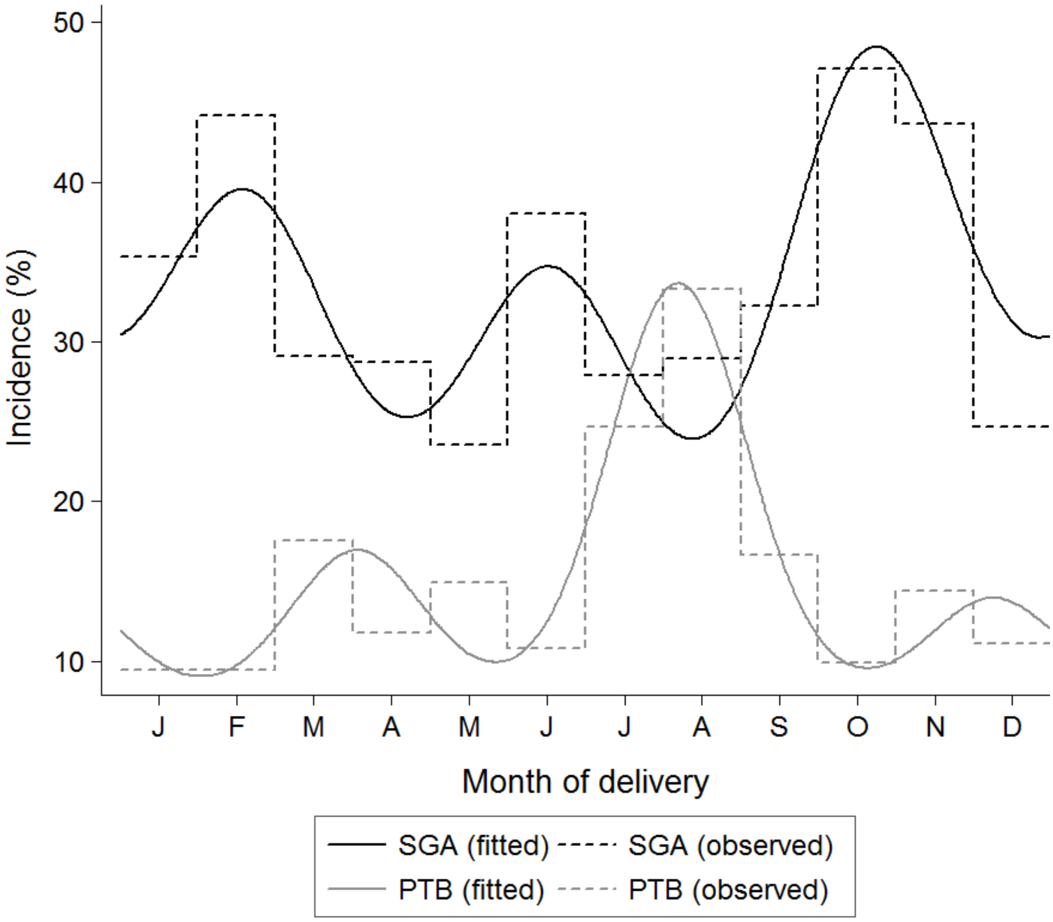


Figure 14. Monthly proportions and seasonal variations in incidence of SGA (n=1012) and PTB (n=1171) of singleton newborns in the MISAME2 trial. The dotted lines represent the monthly incidence, the solid lines the modelled seasonal variations. All models were fitted to the 1st, 2nd and 3rd order Fourier pairs. PTB, preterm birth; SGA, small-for-gestational-age.

Malaria prevalence increased sharply from August to September, i.e. its annual maximum, and the mean concentration of blood parasites was highest in August (**Supplemental Figures 1 and 2**). Baseline BMI and MUAC of pregnant women in their first trimester were plotted to describe maternal nutritional status across seasons (**Supplemental Figures 3 and 4**). Both BMI and MUAC revealed similar patterns, showing a peak in June/July followed by a steady decline toward the annual minimum in November.

Birth weight and birth length data for seasonal effect modification analyses were available for, respectively, 518 and 517 pregnancies in the intervention group and 493 pregnancies in the control group. Seasonality modified the effect of prenatal supplementation on birth length ($P < 0.1$) (**Supplemental Tables 1 and 2**). This observation reveals differential efficacy of the intervention group compared to the control group by date of birth. It is also of note that the interaction between prenatal supplementation and year of birth on birth length was statistically significant.

The intervention effect of an LNS on birth length was most pronounced from September to November at the transition from rainy to dry season (+13.5 mm; 95% CI: 6.5 - 20.5) compared to the UNIMMAP tablet (**Figure 15**). No significant effect of LNS on birth length was observed in other periods of the year. We did not observe any important effect modification on birth weight or SGA.

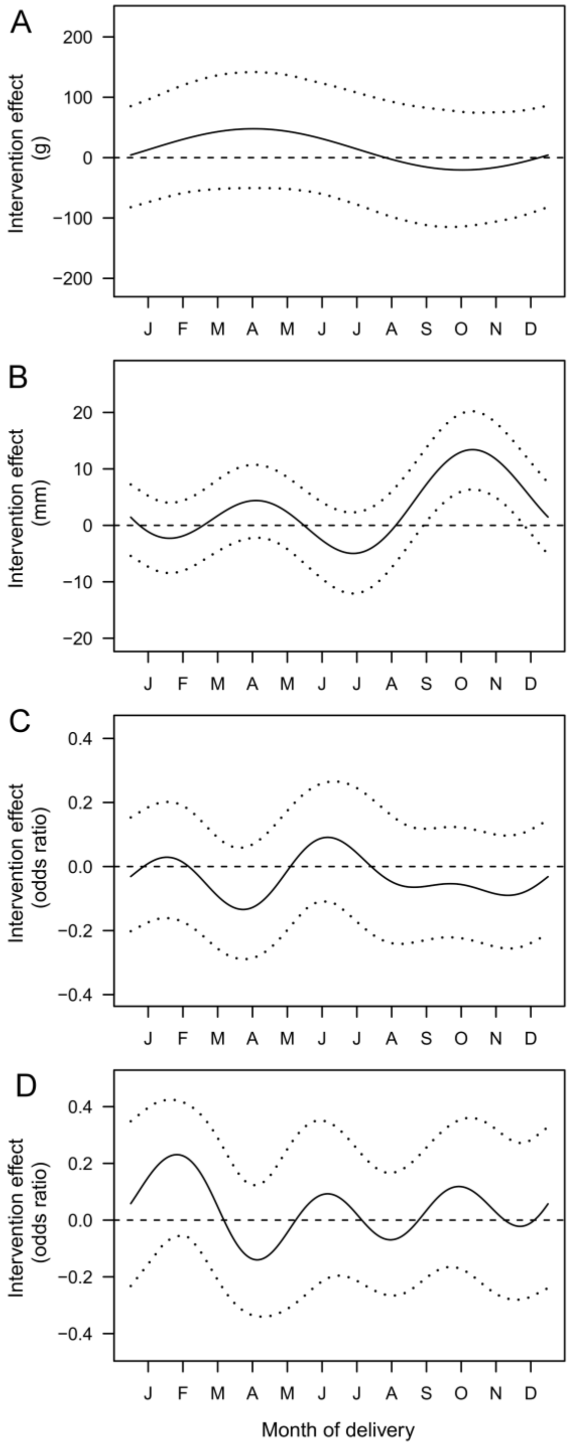


Figure 15. Seasonality in the intervention effect of LNS compared to UNIMMAP on (A) birth weight (n=1011), (B) birth length (n=1010), (C) SGA (n=1011) and (D) PTB (n=1038) of singleton newborns in the MISAME2 trial. Solid lines represent the intervention effect, dotted lines represent the 95% confidence intervals. Models included 1st order (birth weight), 1st and 2nd order (birth length) and 1st, 2nd and 3rd order (SGA and PTB) Fourier pairs and their interactions with supplementation group, and were adjusted for supplementation group, primiparity, birth year, group of malaria prevention (3 doses sulfadoxin-pyrimethamin vs 2 doses sulfadoxin-pyrimethamin), health centre, maternal height and infant sex, as well as the interaction of birth year with supplementation group. LNS, lipid-based nutrient supplement; PTB, preterm birth; SGA, small-for-gestational-age; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement.

2.5 Discussion

Fourier seasonality models showed that prenatal supplementation with LNS, compared to the UNIMMAP supplement, has important effect modifications on birth length by date of birth. To our knowledge, this is the first study to demonstrate these seasonal effect modifications for prenatal supplementation with an LNS on birth size.

The seasonality in birth weight and birth length showed distinct patterns with a peak at the end of the dry season and a nadir in the rainy season. A study in Malawi demonstrated that both prenatal linear growth and weight gain falter by exposure to the rainy season. However, the timing of exposure appeared essential in that the direct effect of the seasonal stress was only apparent in third trimester pregnancies (15). Similarly, a study in The Gambia reported a 250 g difference in birth weight between its maximum at the end of the dry season and minimum in the rainy season (113). In India, Rao et al. (78) found significant differences of 142 g birth weight and 15 mm birth length between the maximum in early postharvest and minimum at harvest, when intensive agricultural work takes place.

The incidence of SGA, a proxy for IUGR, and PTB demonstrated opposing fluctuations across seasons. The incidence of SGA showed a nadir in the rainy season that coincided with a sharp maximum peak in PTB incidence. Of notice, the nadir in SGA and peak in PTB were matched by the nadir in birth weight and birth length. Rayco-Solon et al. (75) found the same opposing trend between SGA and PTB in The Gambia. Furthermore, a recent pooled data analysis by Katz et al. (201) showed that the proportion of SGA babies in Asia and Africa is relatively lower prior to 37 weeks of gestation. These findings could suggest that IUGR in the rainy season takes greater hold in the last weeks of pregnancy. However, it may also be that SGA incidence was systematically underestimated for preterm babies due to the use of a birth weight reference instead of a fetal growth standard (201, 202). The maximum peak in PTB incidence and subsequent maximum in SGA incidence can be regarded as results of insults in the rainy season. Although the presence of intrauterine inflammation/infection, bacterial vaginosis, asymptomatic bacteriuria and high maternal plasma cortisol levels in early pregnancy can also lead to PTB (203-205), in our context, the peak in PTB incidence during the rainy season can predominantly be regarded as the result of acute insults such as acute infections and an increased workload. Conversely, SGA is considered to be the consequence of a chronic accumulation of insults. Therefore, when the rainy season sets in, preterm birth incidence rises sharply while SGA incidence rises gradually, the longer the exposure to the rainy season. In Burkina Faso, the rainy season (May to September/October) is characterized by a seasonal increase in agricultural labor and energy expenditure (206) as well as food scarcity due to diminishing food stocks. The arrival of the first rains in early June is the sign for many rural households to start sowing, whereas the period around October is dedicated to harvesting. A survey on physical activity patterns, of a convenience sample of ~250 pregnant women in the research area, showed that women in their third trimester of gestation were not spared from daily seeding, weeding and harvesting activities in bending or squatting postures (Huybregts L, observation, 2006). Such long working hours and an increased physical workload have been shown to be associated with a higher risk of preterm delivery (153, 207). Furthermore, the combination of a strenuous workload and limited food availability in the rainy season could result in a negative maternal energy balance (78, 87, 113), which probably triggered the descending trend in maternal BMI and MUAC (Supplemental Figures 3 and 4). Finally, additional data from MISAME2 and other reports (192) showed characteristic increases in malaria transmission rates in the rainy season. It is well documented that acute malarial infections at the end of pregnancy cause an induction of preterm delivery, whereas malaria-associated IUGR was suggested to be associated with placental insufficiency and parasitemia in the wider antenatal period (73, 208, 209). As a matter of fact, a malaria-infected placenta acts as a site for active immune response that may stimulate early labor, resulting in premature delivery, or hinder nutrient transport to the fetus, resulting in IUGR (70).

LNSs, which provided energy and MMNs compared to only an MMN supplement (UNIMMAP), triggered a distinct positive effect on linear growth at the transition from rainy to dry season (September-November). This observation brings up a few non-exclusive explanations. First, LNSs could lead to greater length gain the longer the exposure to the rainy season in the second and early third trimester of gestation, a time when linear growth velocity is maximal (85). Second, exposure to the full extent of seasonal stress of the rainy (lean) season in the second half of pregnancy requires the provision of additional energy that allows micronutrients to exert a functional effect on linear fetal growth. In this light, it is interesting to compare our results to a Gambian study (113) that showed a maximal effect on birth weight in the lean season and no significant effect on birth length. The pronounced effect on birth weight in The Gambia could be explained by the daily energy dose which was almost 3 times as high as our LNS (4.25 vs 1.56 MJ/d), and the fact that our control group received MMNs, which are also known to increase birth weight (187, 210-212). The lack of any effect on birth length is possibly related to the absence of additional MMNs in the Gambian supplement. Providing energy as “empty calories” in the presence of functional micronutrient deficiencies might primarily lead to an accumulation of adipose tissue (213) and, hence, higher birth weight without effects on linear growth. The results of the Gambian and our study therefore lead us to the hypothesis that the sole provision of MMNs, without additional energy, is not sufficient to support linear growth in pregnancies where the second half of pregnancy covers the rainy (lean) season, i.e. a time when energy needs are higher due to increased maternal energy expenditure and/or infections.

Our study has some limitations that warrant caution. First, the lack of additional longitudinal data on individual exposure, e.g. dietary intake and energy expenditure data as well as infectious diseases, hampers a straightforward interpretation of the results. As such, we cannot make causal inferences about which determinants of birth size trigger the seasonality in birth size. Second, we did not objectively assess rainfall and temperature in the research area throughout the intervention implementation. We can therefore not precisely pinpoint the onset and end of the rainy season. The references to the onset and end of the seasons in this work are therefore based on the generally accepted time period of the seasons. Third, the results should be interpreted carefully because of the duration of the intervention. Annual variation was demonstrated by a significant interaction between prenatal supplementation and year of birth. Yet, this variation is rather expected to be the result of harvest yield and market prices because no other interventions were implemented in the research area at the time. Moreover, our analyses showed that the annual variation did not contribute as much to the effect modification on birth length compared to seasonality within the year (+5.18 mm/year compared to +13 mm at the transition of the rainy to dry season).

In conclusion, we provide evidence that the previously reported effect of an LNS on birth length in rural Burkina Faso is mainly concentrated in pregnancies where the second half of pregnancy covers the rainy season. This result implies that MMN interventions during the annual rainy/lean season in Sub-Saharan African countries should be accompanied by additional energy supplementation to be more efficacious in supporting prenatal linear growth and, ultimately, preventing child stunting during the first 1,000 d from conception to 24 months of age (67).

Supplemental Table 1. Regression coefficient estimates of independent predictors of birth weight and birth length of singleton newborns[1].

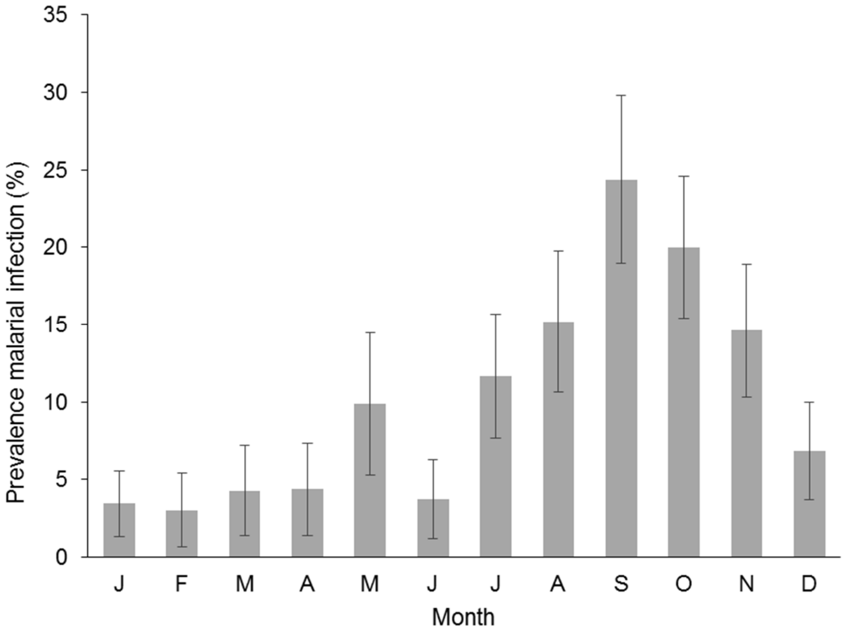
	Birth weight		Birth length	
	Estimate	***P***	**Estimate**	***P***
Health center	-57.63 (-114.70; -0.57)	0.05	4.54 (1.35; 7.73)	<0.01
Malaria prevention[2]	27.02 (-23.31; 77.36)	0.29	0.09 (-2.72; 2.91)	0.95
Maternal height	12.10 (7.66; 16.54)	<0.001	0.69 (0.44; 0.94)	<0.001
Sex[3]	165.64 (115.18; 216.09)	<0.001	9.62 (6.80; 12.44)	<0.001
Primigravidity[4]	-338.10 (-401.14; -275.06)	<0.001	-13.32 (-16.85; -9.79)	<0.001
Year of birth	15.43 (-50.74; 81.61)	0.65	2.10 (-1.66; 5.87)	0.27
Intervention[5]	13.99 (-36.34; 64.33)	0.59	2.84 (0.01; 5.67)	0.05
Sinθ[6]	23.90 (-35.65; 83.45)	0.43	4.71 (1.38; 8.04)	<0.01
Cosθ[6]	3.37 (-49.36; 56.09)	0.90	-1.33 (-4.27; 1.61)	0.38
Sin2θ[7]			-1.58 (-4.49; 1.33)	0.29
Cos2θ[7]			1.19 (-1.67; 4.05)	0.42
Sinθ x Intervention	32.05 (-50.55; 114.65)	0.45	-3.72 (-8.35; 0.90)	0.11
Cosθ x Intervention	-9.27 (-83.87; 65.33)	0.81	2.93 (-1.24; 7.10)	0.17
Sin2θ x Intervention			-4.26 (-8.29; -0.23)	0.04
Cos2θ x Intervention			-4.40 (-8.40; -0.40)	0.03
Year of birth x Intervention	18.56 (-73.29; 110.41)	0.69	5.18 (-0.03; 10.38)	0.05

[1] Values are regression coefficient estimates (95% confidence intervals) of linear regression models for birth weight (n=1011) and birth length (n=1010); [2] Malaria prevention by 3 doses of Sulfadoxin-pyrimethamin versus 2 doses of Sulfadoxin-pyrimethamin; [3] Male infants versus Female infants; [4] Primigravid versus multigravid women; [5] Lipid-based nutrient supplementation (LNS) versus supplementation with the UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women (UNIMMAP); [6] First order Fourier terms of seasonal predictors; [7] Second order Fourier terms of seasonal predictors (not included in birth weight model).

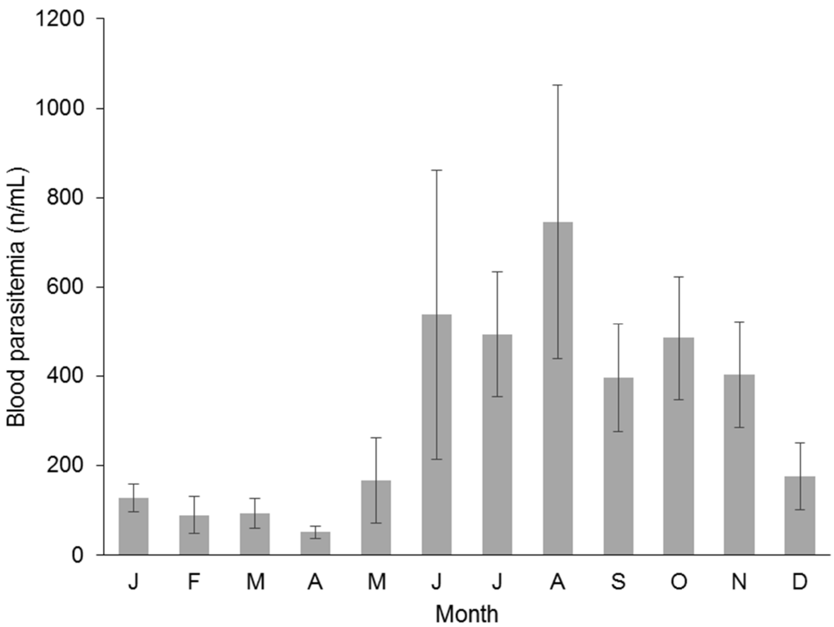
Supplemental Table 2. Odds ratios of independent predictors of small-for-gestational age and preterm birth of singleton newborns[1].

	SGA		PTB	
	Estimate	***P***	**Estimate**	***P***
Health center	1.18 (0.86; 1.60)	0.31	1.78 (1.10; 2.86)	0.02
Malaria prevention[2]	1.04 (0.79; 1.36)	0.79	0.77 (0.52; 1.13)	0.18
Maternal height	0.98 (0.95; 1.00)	0.04	0.97 (0.93; 1.00)	0.04
Sex[3]	1.29 (0.98; 1.69)	0.07	0.75 (0.51; 1.11)	0.15
Primigravidity[4]	2.10 (1.52; 2.92)	<0.001	1.82 (1.18; 2.81)	0.01
Year of birth	1.32 (0.92; 1.91)	0.13	0.70 (0.41; 1.19)	0.18
Intervention[5]	0.87 (0.66; 1.15)	0.33	1.17 (0.78; 1.76)	0.45
Sinθ[6]	0.75 (0.54; 1.03)	0.08	0.97 (0.61; 1.55)	0.90
Cosθ[6]	1.30 (0.97; 1.72)	0.08	0.65 (0.42; 1.01)	0.05
Sin2θ[7]	0.86 (0.65; 1.14)	0.29	1.37 (0.90; 2.08)	0.14
Cos2θ[7]	0.80 (0.61; 1.05)	0.10	0.90 (0.60; 1.36)	0.62
Sin3θ[8]	1.07 (0.81; 1.40)	0.65	0.53 (0.35; 0.81)	<0.01
Cos3θ[8]	0.80 (0.61; 1.06)	0.12	1.31 (0.87; 1.96)	0.19
Sinθ x Intervention	1.02 (0.65; 1.61)	0.92	1.00 (0.53; 1.88)	1.00
Cosθ x Intervention	0.88 (0.59; 1.33)	0.55	1.29 (0.71; 2.34)	0.40
Sin2θ x Intervention	1.07 (0.72; 1.58)	0.74	1.23 (0.70; 2.18)	0.47
Cos2θ x Intervention	1.28 (0.87; 1.88)	0.22	1.13 (0.65; 1.97)	0.66
Sin3θ x Intervention	1.20 (0.81; 1.77)	0.37	1.43 (0.81; 2.51)	0.22
Cos3θ x Intervention	0.90 (0.61; 1.32)	0.58	0.73 (0.43; 1.27)	0.27
Year of birth x Intervention	0.99 (0.60; 1.66)	0.98	0.76 (0.37; 1.57)	0.46

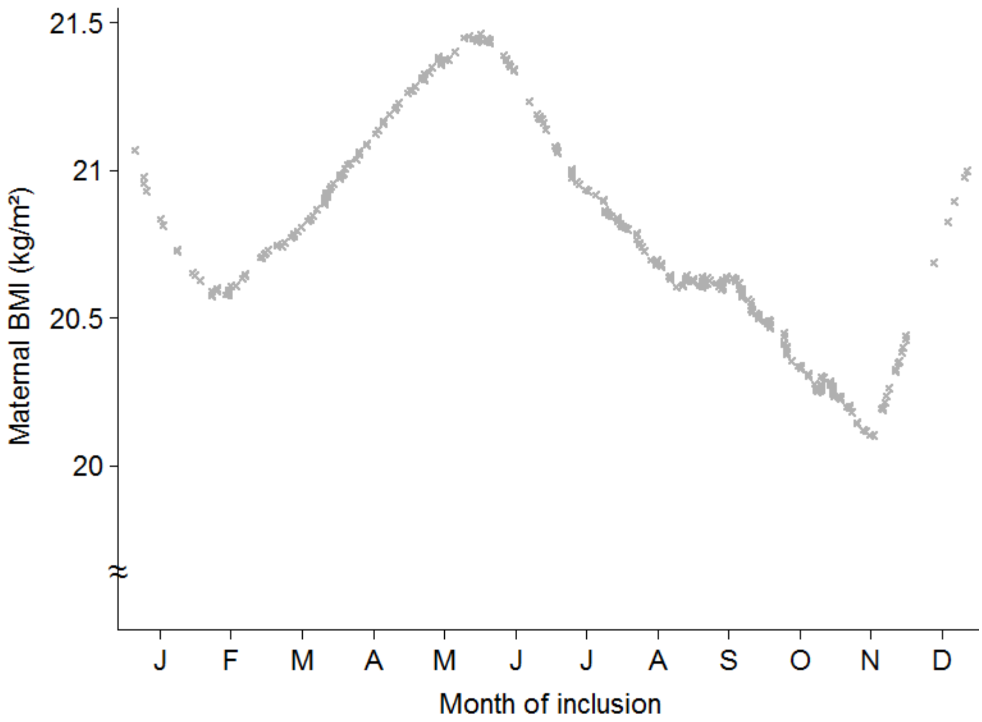
[1] Values are odds ratio estimates (95% confidence intervals) of logistic regression models for SGA (n=1011) and PTB (n=1038); [2] Malaria prevention by 3 doses of Sulfadoxin-pyrimethamin versus 2 doses of Sulfadoxin-pyrimethamin; [3] Male infants versus Female infants; [4] Primigravid versus multigravid women; [5] Lipid-based Nutrient Supplementation (LNS) versus supplementation with the UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women (UNIMMAP); [6] First order Fourier terms of seasonal predictors; [7] Second order Fourier terms of seasonal predictors; [8] Third order Fourier terms of seasonal predictors. OR, odds ratio; PTB, preterm birth; SGA, small-for-gestational-age.



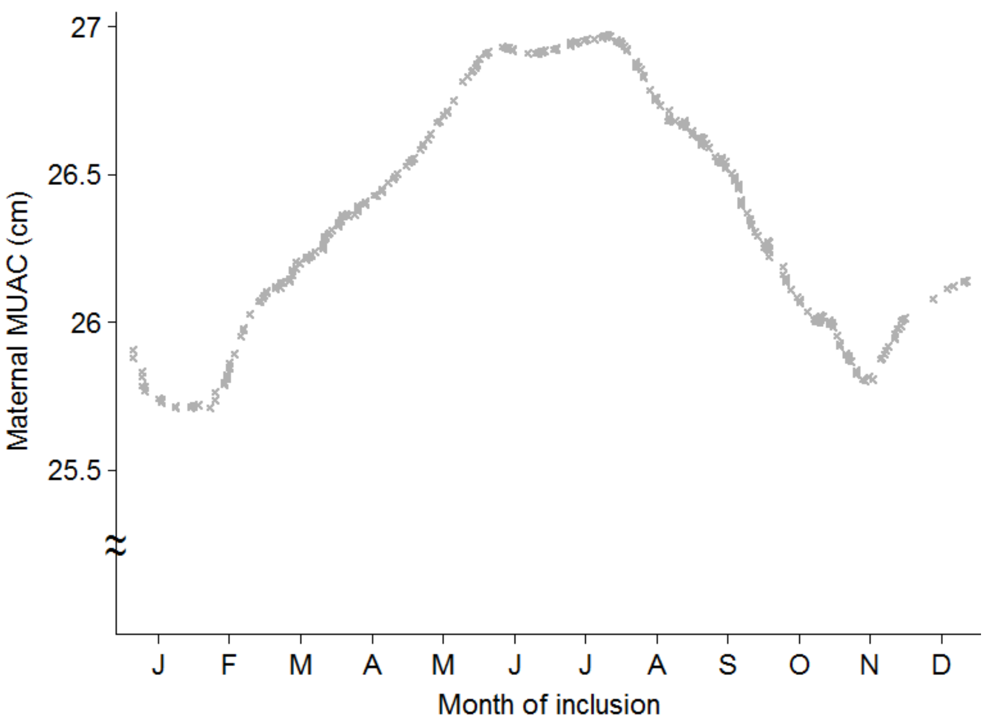
Supplemental Figure 1. Prevalence of malarial infection in pregnant women of the MISAME2 trial in rural Burkina Faso. Values are mean percentages ± 95% confidence intervals, n=10,668.



Supplemental Figure 2. Monthly blood levels of malaria parasites in pregnant women of the MISAME2 trial in rural Burkina Faso. Values are means ± SEM, n=10,668.



Supplemental Figure 3. BMI in 1st trimester pregnancies at baseline of the MISAME2 trial in rural Burkina Faso. Values are smoothened, n=508.



Supplemental Figure 4. Mid-upper arm circumference (MUAC) in 1st trimester pregnancies at baseline of the MISAME2 trial in rural Burkina Faso. Values are smoothened, n=506.

Chapter 3

DEVELOPMENT AND CONCURRENT VALIDATION OF A QUESTIONNAIRE FOR THE ASSESSMENT OF PHYSICAL ACTIVITY DURING THE RAINY SEASON IN PREGNANT WOMEN OF RURAL BURKINA FASO

Redrafted from: Bouckaert KP, Vandevijvere S, Hagströmer M, Lachat C, Verstraeten R, Kolsteren P, Van Camp J, Huybregts L. Rainy Season Activity in Pregnant Women and Birth Outcome: A Cross-Sectional Study in Rural Burkina Faso. PLOS ONE, under review.

3.1 Abstract

Background: The rainy season in rural areas of low and middle income countries (LMICs) introduces strenuous agricultural work to the daily activities. At the same time, there is evidence that the rainy season shows a peak in preterm births and small-for-gestational age births. It is unclear to what extent increased physical activity (PA) during the rainy season influences birth outcomes. However, there is no valid or reliable questionnaire available to assess PA levels in pregnant (P) women in rural areas of LMICs. The objectives are to develop and validate a questionnaire to assess PA levels of P women in rural Burkina Faso.

Methods: Three heterogeneous focus groups were assembled, comprising a total of 24 P rural Burkinabé women, during which a comprehensive list of common activities of the rainy season and quantifiable components were established. A structured, interviewer-administered, questionnaire was developed that encompassed four domains of activity (agricultural work, domestic activities including child care, transportation and leisure) to assess usual PA in the last week. Women were selected from the research area by convenience sampling to assess reliability and validity. Test-retest reliability was measured by administering two interviews by the same interviewer over a two-week time interval. Concurrent validity was assessed by comparing the data with data collected by an ActiGraph accelerometer during the recall period of the questionnaire.

Results: A total of 111 and 105 women were included in the analysis of respectively test-retest reliability and concurrent validity. Intra-class correlations of test-retest reliability ranged from 0.50 to 0.67, while classification agreement was evaluated as fair to moderate (Cohen's Kappa: 0.39 – 0.46). The 95% prediction interval of total accelerometer counts from physical activity energy expenditure (PAEE) showed a wide average (± SD) width of 4,037,935 ± 19,436 counts/week. The 95% limits of agreement for light and moderate intensity PA were also wide and showed systematic bias. Time spent in light intensity PA was consistently underestimated by the PA questionnaire, partly due to the exclusion of leisure time. Rank correlation and classification agreement of PAEE and moderate intensity PA with accelerometer data was respectively evaluated as moderate (Spearman rank correlations: 0.42 – 0.47) and fair (Cohen's Kappa: 0.24 – 0.31).

Conclusions: PA assessments in the research community were hampered by a lack of an awareness of time. The PA questionnaire shows limited absolute measurement agreement with accelerometer data for individual assessments, yet shows acceptable classification agreement for both concurrent validity and test-retest reliability. The questionnaire can be used to classify P women into PA levels at the population level.

3.2 Introduction

Studies have shown that activity patterns of rural populations in low and middle-income countries (LMICs) are subject to seasonal changes (81, 82). The start of the rainy season introduces agricultural labor to the daily activities which implies long and laborious work for several months on end. Pregnant (P) women in LMICs are typically not spared from working on the field. Interestingly, the rainy season also shows an increased incidence of preterm and a rise in small-for-gestational age births (214, 215). Yet, it remains unclear to what extent physical activity (PA) in the rainy season influences birth outcomes in LMICs.

PA is traditionally assessed by means of a diary or questionnaire in epidemiologic research. In illiterate populations, interviewer-administered questionnaires are often the method of choice. The main purpose of such questionnaires is to rank and discriminate individuals according to their levels of PA in a population. There are a few, non-exclusive, considerations pertaining to the measurement of PA in the rainy season of P women that are key for the evaluation of its associations with birth outcomes. First, it has been argued that questionnaires for P women require greater sensitivity to smaller differences as their range of PA may be narrower than that of non-pregnant women (216), though this should be confirmed for P women in LMICs. It has therefore been postulated that questionnaires should encompass different dimensions of PA, such as frequency, duration, intensity and type, to increase accuracy and precision in detecting associations between PA and birth outcomes (217). Second, a study in India and our own observations in Burkina Faso have demonstrated that routine domestic activities and child care remain an important contributor to usual PA in P rural women during the labor-intensive rainy season (138). These activities should therefore not be excluded from the questionnaire. Third, the assessment of PA in the rainy season requires the recall period to be more than a few days to reduce intra-individual variability, yet, short enough to avoid the inherent seasonal shifts in activity patterns. Lastly, there is no valid and reliable questionnaire available for the assessment of PA in the rainy season of rural Sub-Saharan African women. The International Physical Activity Questionnaire (IPAQ), the most widely used standardized questionnaire, has previously shown poor validity in P women and poor reliability in populations from rural areas of LMICs (218, 219).

The aim of this study was therefore to develop an interviewer-administered structured questionnaire to rank women in rural Burkina Faso into levels of PA in the rainy season, and to assess its test-retest reliability and concurrent validity.

3.3 Materials and Methods

3.3.1 Study setting and participants

The development of the PA questionnaire is part of a cross-sectional study that investigated associations between maternal PA in the rainy season and birth outcome in rural Burkina Faso. The study was conducted within the framework of the second MISAME (Micronutriments et Santé de la Mère et de l'Enfant) randomized controlled trial that compared the efficacy of a prenatal multiple micronutrient supplement (MMN; comparison) to a lipid-based nutrient supplement (LNS; experimental) in 1296 P women. The trial was implemented from March 2006 to July 2008 in the catchment area of 2 health centers, Karaba and Koho, in the Houndé health district of Tuy Province in Burkina Faso, West Africa. These rural communities are dependent upon subsistence farming. The region has a Sudano-Sahelian climate with one important rainy season that runs from May through September/October.

The reliability and concurrent validity assessment was conducted in the rainy season of respectively 2008 (July) and 2006 (August/September). Both P women and non-pregnant non-lactating (NPNL) women, whose youngest child was at least 1 year old, in the Karaba and Koho communities were eligible for participation. NPNL women were also included because the second objective of the main study was to compare the PA patterns of P women with those of NPNL women. In total, 126 P and NPNL women and 120 P women were selected by convenience sampling from the research area for respectively reliability and concurrent validity testing. Each MISAME 2 project staff member randomly identified 4 women in their designated area. The validity study entailed wearing an accelerometer and was solely performed in P women in order to increase compliance, as these women were visited daily by MISAME 2 project staff. Socio-demographic data were available for these women only.

The MISAME 2 trial was approved by the ethics committees of the Centre Muraz in Bobo-Dioulasso, Burkina Faso, and the Institute of Tropical Medicine in Antwerp, Belgium. The aims and procedures of the study were explained in the local language and written informed consent was obtained. Women were also explained about the aims and procedures of the observational validation study after which oral consent was obtained.

3.3.2 PA questionnaire

3.3.2.1 Development

An interviewer-administered PA questionnaire was developed to categorize rural women into higher and lower PA levels in the rainy season. Focus group discussions were used to establish a comprehensive list of common activities in the rainy season and to identify quantifiable components, such as duration, weekly frequency, mode of transport, distance, production units of activity (e.g. washing <10, 10-30 and >30 dishes), as well as whether or not women carried an infant or load, and/or received help from other people. Three focus groups, heterogeneous with regard to age, ethnicity and religion, were assembled in which a total of 24 P women voluntarily participated.

The structured questionnaire recalls 23 selected activities from the past week within 4 domains (agricultural labor, transportation, domestic activities including child care, and leisure), and has an open-ended section for women to report other and/or exceptional activities (**Addendum**). Furthermore, the questionnaire probes about the amount of people and children in the household. The time frame of 1 week was chosen such that it would capture usual activity, including agricultural labor in the field which could discriminate women. The questionnaire was pre-tested and minor adaptations were incorporated. Interviewers were trained and collected data within a time span of 4 weeks. Two study supervisors randomly back-checked 10% of the conducted interviews during the assessments.

3.3.2.2 PA energy expenditure and time

The pre-test showed that respondents had serious difficulty estimating duration and distance with standard units. As such, we decided to estimate duration from production units and recorded duration and production units of activities in 10-15 nonparticipating women. For instance, washing one dish took an average of 0.5 min. If a woman then reported having washed <10 dishes, an average of five dishes was multiplied with the average of 0.5 min/dish. Duration estimates of activities without production units, such as cooking and child care, were taken from the average local recordings or data from Burkinabe female farmers (79). Duration estimates of leisure (e.g. eating, chatting, watching TV, small activities, …) and sleep were not used as our observations showed that these were very difficult to estimate. A similar categorizing approach was used to estimate distance. We asked respondents to categorize distance based on distances between well-known local landmarks. Duration estimates of transportation were then derived by multiplying average distance with the average speed of the mode of transport. A 10 min break was assumed for every reported hour of agricultural labor.

Energy expenditure (EE) scores (kcal/kg) were calculated for each activity by multiplying the duration estimate (min) with energy cost (kcal/kg/min) as obtained from two indirect calorimetry studies in African women (79, 220). Data from Lawrence et al. (220) was given priority as its data was more exhaustive and internally comparable. If the activity was not listed in either source, energy costs were taken from the Compendium of Physical Activities (221) by converting 1 Metabolic Equivalent of Task (MET) to 1 kcal/kg/h and dividing by 60 minutes. The energy cost of agricultural labor was considered to be equal to bending and digging with a short-handled hoe, the main agricultural activity for the period August-September in rural Burkina Faso. Scores of women reporting to walk were corrected by a factor x1.05 if the woman reported carrying an infant or a load up to 10kg, or by a factor x1.15 for heavier loads (adapted from (220)). Scores and durations were corrected for helping hands by dividing by the total amount of people involved in the activity.

Overall PAEE (kcal/kg/week) and PA time (min/week) were derived by respectively multiplying each EE score and duration estimate with the reported weekly frequency of the activity, and adding up the scores and durations for all activities. Activities were also categorized by type (EE in agricultural labor, transportation and domestic activities) and intensity of PA (time spent in light and moderate intensity PA, respectively 1.6-2.9 MET and 3.0-5.9 MET (221)). Light intensity PA included the activities cooking, washing dishes and child care. Most activities, including agricultural labor, were classified as moderate intensity PA. Women did not report vigorous intensity PA (≥6.0 MET). In addition, all sedentary intensity PA (≤1.5 MET) in the questionnaire was represented by leisure time and thus removed from the analyses.

3.3.3 Test-retest reliability and Concurrent validity

3.3.3.1 Test-retest reliability

Test-retest reliability was assessed by completing a second PA questionnaire at a time interval of 11.0 ± 1.6 days in the rainy season by the same interviewer. Women with only one PA questionnaire (n = 14) or missing dates (n = 1) were excluded from the reliability analyses.

3.3.3.2 Concurrent validity

Concurrent validity of the questionnaire was assessed by comparing the data of the questionnaire, which recalls activities of the past 7 days, with accelerometer data registered during the same time period in the rainy season. ActiGraph accelerometers have been shown to correlate reasonably well with energy expenditure derived from the doubly-labeled water technique in non-pregnant populations (222). We used the uniaxial ActiGraph models 71256 and 7164 (Manufacturing Technology Inc., Fort Walton Beach, USA) which were set at 1 minute-epochs. Women were asked to wear the accelerometers around the waist for 8 full days except when sleeping or showering after which they were instructed to put them back on. Accelerometers started recording in the morning of day 2. Women returned the accelerometers on the morning of day 9 when the PA questionnaire was completed. Exceptional days of activity, such as Fridays and Sundays, were equally represented.

Women with no or extreme accelerometer data (n = 8), who had an abortion or gave birth (n = 2) or who reported more than the week limit of 7140 min/week (24h/d – 7h/d sleep) in the PA questionnaire (n = 2) were removed from the validity analyses. A lot of women wore the accelerometer at night, and a day-time filter of 5:00AM–10:00PM was applied to standardize the window of accelerometer counts. Non-wear time of the accelerometer was defined as 60 min of consecutive zero counts, allowing for a 2 min peak of <100 counts, within day-time. Weartime was estimated by subtracting non-wear time from the total observation time per day. A day was considered valid if weartime ≥10 h/d. Cut-points of intensity were defined as <100 counts per minute (cpm) for sedentary (223), 101-759 cpm for light and ≥760 cpm for total moderate intensity PA (760-2019 cpm for moderate non-ambulatory (224) and ≥2020 cpm for moderate ambulatory-to-vigorous intensity PA (225)). We did not use a separate cut-point for vigorous intensity PA because only a negligible amount of women spent very little time at vigorous intensity (≥5999 cpm). Similar to the PA questionnaire, sedentary intensity PA was not included in further analyses. The Actigraph accelerometer has been shown to be a valid and reliable instrument for assessing free-living PA in adults using 3 up to 5 measurement days (226). All women with ≥3 valid days were included in the validity analyses. Sensitivity analyses showed that accelerometer measures were not significantly different between women with 7 (n = 77) and ≥3 (n = 105) valid days. Mean daily measures of those women with less than 7 valid days were adjusted to weekly measures by multiplying by 7 days.

3.3.4 Data analysis

Socio-demographic data were available for women in the validity test only. Women were identified as being involved in a specific activity if they reported performing the activity over the past week, regardless of any missing data in the dimensions of that activity or being an over-reporter (reported time >7140 min/week). Descriptives are presented for those women included in the analysis. It should be noted that the activity 'chopping wood' was not included in the questionnaire for 22% of the participants in the validity test due to a technical problem. The different PAEE and PA time measures of the questionnaire did not follow a normal distribution and are reported as medians (25^{th}-75^{th} percentile). Accelerometer data of all women were standardized to the limit of 7140 min/week. Overall PA measured by the accelerometer was therefore expressed in counts/week, whereas time spent in different intensities of PA were expressed in min/week. Accelerometer measures followed a normal distribution and are reported as means (± SD). Differences between time captured by repeated measures of the PA questionnaire, and the accelerometer and PA questionnaire were tested by paired ttests.

Absolute agreement and classification agreement between repeated measures of the PA questionnaire were evaluated by respectively intra-class correlations (ICC) and Cohen's Kappa tests. Agreement between total accelerometer counts and overall PAEE was evaluated by the 95% prediction interval around the line of best fit as proposed by Bland and Altman (227). Agreement between both methods for time spent in light and moderate intensity PA was evaluated by the 95% limits of agreement (LOA) (228). Ranking and classification agreement, using the median cut-off, between both methods was assessed by respectively Spearman rank correlations and Cohen's Kappa tests. Strength of agreement for Kappa statistics was evaluated using the standards proposed by Landis and Koch (229).

All analyses were performed in Stata 12.0 (StataCorp, College Station, TX, USA). The significance level was set at P <0.05 and all tests were two-sided.

3.4 Results

Socio-demographic characteristics of P women enrolled in the concurrent validity study are presented in **Table 6**. The majority of women participating in the validity study and the two measurements of the reliability study - respectively 81.5%, 77.0% and 84.8% - reported to have worked on the field in the past week (**Table 7**). The reliability and validity test were performed in the rainy season of different years. Women in the validity test reported higher involvement levels in some activities, mostly of moderate intensity PA, compared to women in the reliability test.

Table 6. Characteristics of pregnant women enrolled in the concurrent validity study[1].

Maternal characteristics	N	Value
Age, y	107	24.9 ± 6.7
Height, cm	107	163.9 ± 6.2
Health center, n(%)	107	
Karaba		37 (34.6)
Koho		70 (65.4)
Household help, n(%)	110	44 (40.0)
Number of children, n(%)	110	
0		30 (27.3)
≥ 1		80 (72.7)
Education, n(%)	109	
Illiterate		81 (74.3)
Primary school		15 (13.8)
Secondary school		5 (4.6)
Undetermined		8 (7.3)
Job, n(%)	107	
Housewife		103 (96.3)
Student		1 (0.9)
Other		3 (2.8)
Ethnicity, n(%)	109	
Bwa		48 (44.0)
Mossi		50 (45.9)
Peuhl		6 (5.5)
Other		5 (4.6)

[1] Values are means ± SD, unless indicated otherwise.

Table 7. Activity involvement (%) of rural Burkinabe women as reported by the PA interviews in the test-retest reliability and concurrent validity test.

	Test-retest reliability		Concurrent validity (n = 119)
	occasion 1 (n = 126)	occasion 2 (n = 112)	
Fieldwork	77.0	84.8	81.5
Transportation			
Market	23.0	17.0	37.8
Mill	65.1	74.1	72.3
Fetching water	92.9	92.9	88.2
Fetching wood	34.9	33.9	42.9
Field	74.6	84.8	81.5
Domestic activities			
Grinding	29.4	29.5	48.7
Preparing shea butter	6.3	9.8	30.3
Preparing tô	94.4	95.5	91.6
Cooking	94.4	96.4	90.8
Sweeping floor	95.2	94.6	98.3
Chopping wood [a]	26.2	24.1	53.8
Washing dishes	86.5	90.2	86.6
Washing clothes	77.0	79.5	82.4
Child care	60.3	58.9	74.8
Weeding	11.1	17.0	29.4
Animal care	48.4	56.3	40.3
Milking cows	0.8	1.8	1.7

[a] Data was available for 93 (78%) P women in the validity test.

A total of 111 women (68 NPNL and 43 P women) and 105 P women had complete data and were analyzed in the test-retest reliability study and concurrent validity study. The PA questionnaire measured similar levels of overall PA time at repeated interviews in the reliability test, respectively 3,960 and 4,045 min/week. Moreover, PAEE and time spent in light and moderate intensity PA were not significantly different between repeated interviews. The time captured by the PA questionnaire in the validity test was significantly lower than the standardized weartime of the accelerometer (2,412 vs 7,140 min/week, P <0.001) (**Table 8**). The accelerometer estimated significantly more time spent in light intensity PA compared to the PA questionnaire (2,182 vs 540 min/week, P <0.001). However, the estimated time spent in moderate intensity PA was similar between both methods.

Absolute agreement between repeated measures of the PA questionnaire ranged from moderate (overall PAEE and time spent in moderate intensity PA) to substantial (time spent in light intensity PA) (**Table 9**). Classification agreement was evaluated as fair (overall PAEE) to moderate (time spent in light and moderate intensity PA).

Table 8. Physical activity measures of rural Burkinabe women in the concurrent validity test and at repeated PA interviews in the reliability test.

	Concurrent validity (n = 105)					Test-retest reliability (n = 111)				
	Accelerometer [a]		PA questionnaire		*P*	PA questionnaire 1		PA questionnaire 2		*P*
	mean	SD	median	IQR		median	IQR	median	IQR	
Overall										
Counts/week	3,818,076	1,111,827	-	-		-	-	-	-	
PAEE, kcal/kg/week	-	-	134	(75 – 195)		241	(155 – 310)	248	(180 – 309)	0.48
PA time, min/week	7,140	-	2,412	(1,526 – 3,173)	<0.001	3,960	(2,888 – 4,830)	4,045	(3,136 – 5,173)	0.53
Intensity, min/week										
Light [b]	2,182	513	540	(383 – 571)	<0.001	635	(439 – 1,022)	674	(452 – 1,056)	0.50
Moderate [b]	1,950	658	1,943	(990 – 2,657)	0.76	3,302	(2,074 – 4,125)	3,434	(2,553 – 4,064)	0.62

[a] Data are standardized against the maximum week limit of 7140 min/week.

[b] Light intensity PA range: 1.6-2.9 MET; Moderate intensity PA range: ≥3.0 MET.

Table 9. Correlations and classification agreement in test-retest reliability (N = 111) and concurrent validity (N = 105) of the PA questionnaire [a].

		Correlation [b]		% Agreement	Kappa	*P*
		Estimate	95% CI			
Test-retest reliability						
Overall PAEE		0.50	(0.37 – 0.64)	69.4	0.39	<0.0001
Intensity [c]						
	Light	0.67	(0.56 – 0.77)	73.0	0.46	<0.0001
	Moderate	0.51	(0.37 – 0.65)	73.0	0.46	<0.0001
Concurrent validity						
Overall PA [d]		0.42	(0.25 – 0.57)	65.7	0.31	<0.001
Intensity [c]						
	Light	-0.73	(-0.81 – -0.63)	48.6	-0.03	0.62
	Moderate	0.47	(0.30 – 0.60)	61.9	0.24	0.01

[a] Test-retest reliability of the PA questionnaire at a 11.0 ± 1.6 day time interval and concurrent validity of the PA questionnaire with objective accelerometer data.

[b] Absolute agreement intra-class correlation for test-retest reliability, and Spearman rank correlation for concurrent validity.

[c] Light intensity PA range: 1.6-2.9 MET; Moderate intensity PA range: ≥3.0 MET.

[d] Overall PA as measured by the PA questionnaire (expressed as kcal/kg/week) and by the accelerometer (expressed as counts/week).

The 95% prediction interval of total accelerometer counts from overall PAEE showed a wide scatter of individual observations (**Figure 16**). The average (± SD) width was 4,037,935 ± 19,436 counts/week. The PA questionnaire systematically estimated less time spent in light intensity PA compared to the accelerometer and bias increased with increasing average (A) (**Figure 17**). Moreover, women tended to both under- and over-report moderate intensity PA at respectively lower and higher average values (**Figure 18**). The regression-based lower and upper 95% LOA were wide for light intensity PA (-664.3 – 1.3A and 790.4 – 1.3A min/week) and moderate intensity PA (-2336.0 + 0.6A and -192.5 + 0.6A min/week). However, ranking and classification agreement between both methods were respectively evaluated as moderate and fair for both overall PAEE and time spent in moderate intensity PA (Table 9). The PA questionnaire showed good opposite ranking for time spent in light intensity PA and poor classification agreement with the accelerometer data.

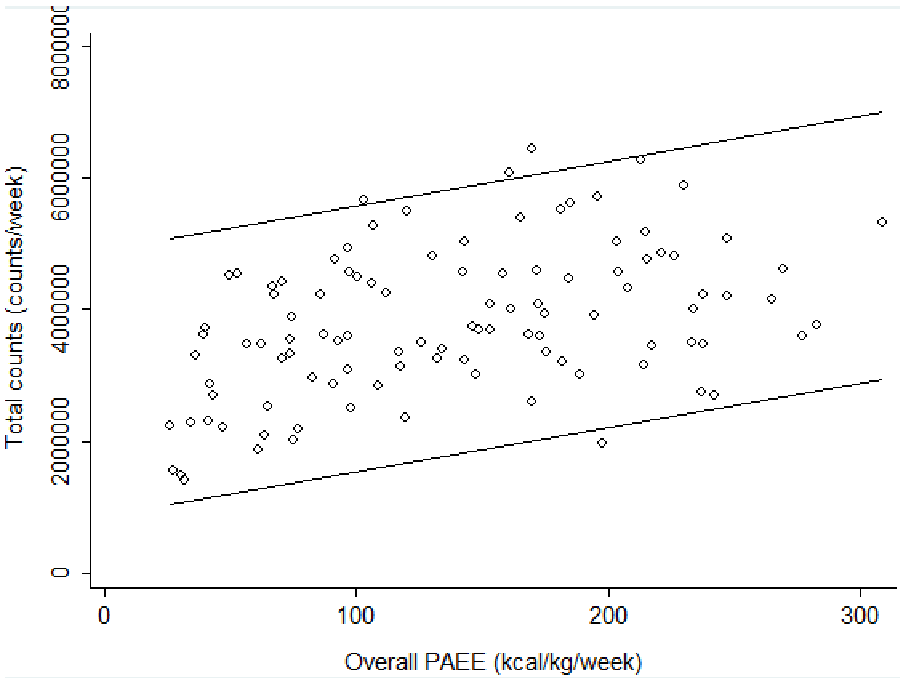


Figure 16. Prediction of total accelerometer counts by overall PAEE. Black lines represent the 95% prediction interval.

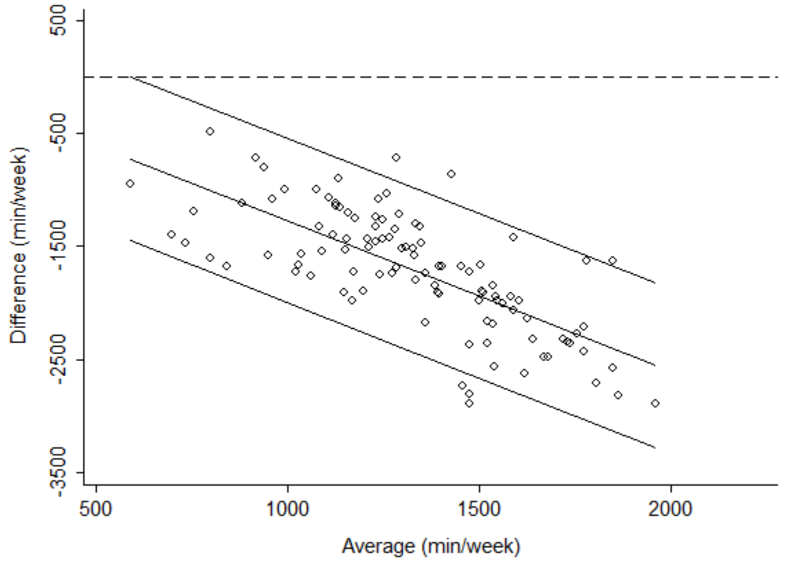


Figure 17. Bland-Altman plot for light intensity PA. The difference of time captured in light intensity PA by the questionnaire and the accelerometer against the mean of both methods. Outer black lines are the upper and lower 95% limits of agreement; the middle line represents the line of best fit. Dashed line is the no difference-line.

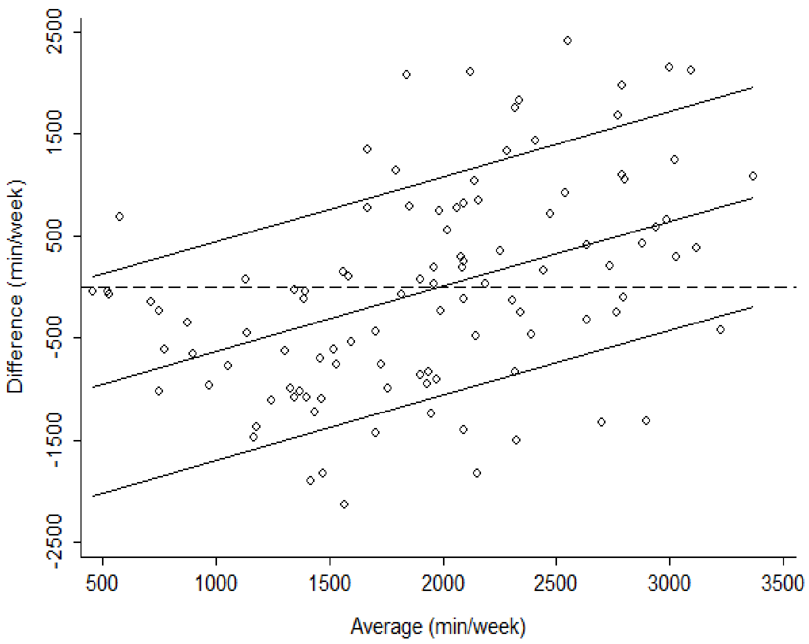


Figure 18. Bland-Altman plot for moderate intensity PA. The difference of time captured in moderate intensity PA by the questionnaire and the accelerometer against the mean of both methods. Outer black lines are the upper and lower 95% limits of agreement; the middle line represents the line of best fit. Dashed line is the no difference-line.

3.5 Discussion

We developed a structured interviewer-administered questionnaire encompassing 3 domains of activity - transportation, domestic activities including child care, and agricultural labor - and different dimensions of activity - duration, frequency, type - in order to rank women into levels of PA in the rainy season of rural Burkina Faso. Test-retest reliability of the questionnaire was satisfactory with moderate-to-substantial correlation agreement and fair-to-moderate classification agreement. Although the questionnaire had poor absolute measurement agreement with objective accelerometer data, the questionnaire showed moderate ranking and fair classification agreement for overall PAEE and time spent in moderate intensity PA at the population level.

Similar to other questionnaires for P women in high income countries (217), our questionnaire performed better in test-retest reliability than concurrent validity. The questionnaire proved to be robust in the assessment of test-retest reliability. Classification agreement was fair-to-moderate for overall PAEE and moderate for time spent in light and moderate PA. Although intra-class correlations were satisfactory, they were lower compared to other questionnaires for P women in high income countries (230-232). A multi-country validity and reliability study of the IPAQ has also demonstrated relatively lower reliability of the questionnaire in rural areas of LMICs compared to high income countries as well as urban areas of LMICs (218). Errors in the reliability estimates may be related to the population's illiteracy and/or greater variability in PA. It should be noted that the repeated assessments were conducted at an average 11.0 ± 1.6 day time interval, and it is therefore unlikely that PA patterns substantially changed during this time frame. The reported activity involvement levels (Table 7) support this assumption, although slightly more women reported fieldwork at the second visit.

The PA questionnaire performed satisfactory in ranking agreement with objective data from the ActiGraph accelerometer for overall PAEE and time spent in moderate intensity PA. Ranking agreement by our questionnaire was better than or comparable to questionnaires for P women in high income countries that were validated against the ActiGraph accelerometer (219, 230-233). Classification agreement for overall PAEE and time spent in moderate intensity PA was rated as fair.

The PA questionnaire performed inferior in absolute measurement agreement with the accelerometer data. The wide 95% prediction interval of total accelerometer count as well as the wide 95% LOA for light and moderate intensity PA indicated low precision of the questionnaire. Moreover, the Bland-Altman plots showed systematic bias at both levels of intensity. Time spent in light intensity PA was consistently underestimated by the PA questionnaire. This may at least be partly explained by the fact that we did not include leisure time, captured by sedentary (eating, watching TV and chatting) and light intensity activities (praying, braiding hair and dehulling peanuts), in the analyses. The pre-test results demonstrated that women were unable to provide reliable duration estimates, while leisure time can only be estimated by time and is quite variable among women. In addition, the plot showed higher systematic bias at higher average values. The plot for moderate intensity PA also showed a trend in bias, yet here, time was under- and overestimated by the PA questionnaire at respectively lower and higher average values. The average estimated time spent in moderate intensity PA was not different between both methods. Our findings therefore suggest that the validity of the PA questionnaire is limited for individual PA assessments. Similar trends in bias for light and moderate intensity PA, in MET min^{-1}, were seen for the IPAQ validity study in Australian P women (219). It has been reported that good measures of light and moderate intensity PA, such as domestic activities and child care, are difficult to obtain by questionnaires (234, 235).

This study has several strengths and some limitations. First, the PA questionnaire has been developed for use in P women in rural Burkina Faso by using several focus group discussions in a culturally representative group. Second, the PA questionnaire includes a broad spectrum of activities that are relevant to women in rural Burkina Faso. Yet, an important limitation in this community was the absence of an awareness of time which seriously hampered the assessment of PA. Duration of activities that could be expressed in terms of production, such as amounts of washed clothes, were estimated by the amounts produced. However, leisure time estimates which are solely defined by time were considered unreliable and were not included in the analyses. Third, activity scores were expressed in kcal/kg/min. The indirect calorimetry study in The Gambia indicated that weight-adjusted energy expenditure was relatively lower in the overall second/third trimesters of pregnancy for certain types of activities which may reflect an increased energy cost efficiency (220). However, we chose not to systematically correct the activity scores in our study because of the uncertainty to which activities to apply a correction factor as well as the uncertainty of the extent of energy cost efficiency in each trimester of pregnancy separately. Fourth, the validity of the PA questionnaire was established against an objective measure with which PA was registered over the same length (i.e. 7 days) and period of time (i.e. no time lag in measurement). Nonetheless, choosing the accelerometer for P women also comes with disadvantages. Accelerometer cutpoints of PA intensity have not yet been developed or validated for pregnant women, as this is a relatively new research domain. The ActiGraph accelerometer has also been shown to be inaccurate in measuring activities involving upper-body movement as well as standing, static activity and pushing or carrying a load (236). Moreover, wearing the accelerometer introduces a burden to the participant which may affect behavior. Finally, accelerometer estimates in P women may also be affected by the placement site and the tilt of the monitor due to their higher abdominal circumference (237). Though it is less likely that the latter issue had a substantial effect. As such, the accelerometer was considered a criterion measure for concurrent validity.

In conclusion, women in the research community had difficulty estimating time and leisure time activities were therefore not included in the analyses. The questionnaire showed poor absolute measurement agreement with the accelerometer. However, the questionnaire showed acceptable classification agreement with the accelerometer and between repeated measures. The questionnaire may therefore be considered a satisfactory method to classify P women into PA levels at the population level. As such, this questionnaire can be used to investigate associations of PA in the rainy season with birth outcomes in rural Burkina Faso.

Chapter 4

RAINY SEASON ACTIVITY IN PREGNANT WOMEN AND BIRTH OUTCOME: A CROSS-SECTIONAL STUDY IN RURAL BURKINA FASO

Redrafted from: Bouckaert KP, Vandevijvere S, Hagströmer M, Lachat C, Verstraeten R, Kolsteren P, Van Camp J, Huybregts L. Rainy Season Activity in Pregnant Women and Birth Outcome: A Cross-Sectional Study in Rural Burkina Faso. PLOS ONE, under review.

4.1 Abstract

Background: The rainy season in rural parts of low- and middle-income countries typically comes with an increased incidence of preterm and small-for-gestational age newborns, along with intensified agricultural labor. It is hypothesized that women in late pregnancy are not spared from strenuous work and that higher physical activity (PA) may influence birth outcomes. The objectives of this study are to characterize PA patterns of pregnant women in the rainy season, and to test associations of higher PA energy expenditure (PAEE) and more time spent in light and moderate intensity PA with birth outcomes.

Methods: A cross-sectional study of PA in 266 pregnant women in rural Burkina Faso was conducted in the rainy season. A validated interviewer-administered PA questionnaire recalled agricultural labor, transportation and domestic activity over the past seven days. PA patterns of pregnant women were compared across stages of pregnancy as well as with those of non-pregnant and non-lactating women. Regression models assessed associations of maternal physical activity with gestational age, prematurity, birth weight, small-for-gestational age and placental weight at delivery.

Results: The majority of pregnant women, including those close to term, were involved in agricultural labor (75.9%). Overall PAEE was high, but relatively lower than that of non-pregnant non-lactating women (P <0.001). Activity patterns were similar across pregnancy stages. Women in early and mid-pregnancy with higher PAEE and more time spent in moderate intensity physical activity respectively showed a significant 48% reduced risk (RR = 0.52; 95% CI: 0.28,0.98; P = 0.04) and a borderline significant 70% reduced risk (RR = 0.30; 95% CI: 0.09, 1.01; P = 0.05) of a small-for-gestational age newborn versus women with lower PAEE and less time.

Conclusions: Increased agricultural labor in the rainy season imposes participation of women in late pregnancy. Higher compared to lower overall PAEE in early and mid-pregnancy was found to be protective against fetal growth restriction.

4.2 Introduction

Newborns in low- and middle-income countries (LMIC) who are born preterm (PTB) and/or small-for-gestational-age (SGA, a proxy for intra-uterine growth restriction (IUGR)) are at a higher risk of neonatal and post-neonatal mortality, have greater odds of developing undernutrition during childhood and may be predisposed to chronic diseases later in life (9, 31, 238). Previously, our group and Rayco-Solon *et al.* have shown that the incidence of PTB and SGA in rural communities of respectively Burkina Faso and The Gambia is subject to important seasonal variations (214, 215). In both settings, the rainy season showed a maximum in PTB incidence followed by a rise in SGA incidence. The rainy season is generally associated with a higher disease load, intensive agricultural labor in preparation of the harvest, declining food stocks and a negative maternal energy balance as a result (79, 81, 82, 139). The etiologies of PTB and IUGR have been argued to differ (214). Exposure to a single or combination of these factors could acutely precipitate PTB and/or could lead to more pronounced IUGR the longer the extent and higher the intensity of the exposure.

Seasonal shifts in disease load and agricultural labor in LMIC have profound impacts. The positive association between malarial infection during pregnancy and low birth weight is well documented (69, 70). It has also been hypothesized that strenuous work, such as manual agricultural labor, could be associated with birth outcome (214). Evidence on physical activity (PA) and birth outcomes mainly comes from high-income countries (HIC) and has a primary focus on maternal exercise and occupational activities. A review of 10 (quasi-) randomized trials concluded that the available data on increased aerobic exercise in healthy pregnant (P) women is insufficient (144). Nevertheless, a non-significant increase in PTB risk (RR= 1.82; 95% CI: 0.35, 9.57) warranted further study. Moreover, three recent meta-analyses of observational studies showed that long working hours and physically demanding work are associated with small but significantly higher risks of PTB (146, 147, 207). The available evidence for SGA was less extensive and pointed to smaller increased risks, though the bulk of the data came from HIC where its incidence is lower. Timing and intensity of PA during pregnancy have also been shown to influence birth outcome (138, 146, 153-155, 207, 239), but the available data is scarce. The few studies performed in LMIC used different methodologies in diverse study populations and raised contradictory findings (138, 148-152). None focused on the characteristic increase in agricultural labor during the rainy season. Furthermore, we did not find any study that documented to what extent women in late pregnancy are spared from agricultural labor in the rainy season.

In this study, we assess if women in late pregnancy are spared from strenuous work on the field in the rainy season by comparing PA patterns across the stages of pregnancy as well as with those of non-pregnant and non-lactating women. In addition, we assess if exposure to higher compared to lower PA energy expenditure (PAEE) and more compared to less time in different PA intensity levels of P women in the rainy season affects birth outcome. Finally, we explore the influence of exposure at different stages of pregnancy as well as interactions of maternal PA with parity and pre-pregnancy nutritional status on birth outcome.

4.3 Methods

4.3.1 Study setting and design

This study was conducted within the framework of the second MISAME (Micronutriments et Santé de la Mère et de l'Enfant) randomized controlled trial that compared the efficacy of a prenatal multiple micronutrient supplement (MMN; comparison) to a lipid-based nutrient supplement (LNS; experimental) in 1296 P women. P women were re-randomized to receive either a double (comparison) or triple dose (experimental) of sulfadoxine-pyriméthamine malaria prophylaxis once in their second and third trimester. The full methodology of the trial has been described elsewhere (188). The trial was implemented from March 2006 to July 2008 in the catchment area of 2 health centers, Karaba and Koho, in the Houndé health district of Tuy Province in Burkina Faso, West Africa. These rural communities are dependent upon subsistence farming. The region has a Sudano-Sahelian climate with one important rainy season that runs from May through September/October. Maize is the main staple food and is harvested in October/November. The region is malaria holoendemic with higher transmission during the rainy season (240). The malarial infection incidence rate in the P women of this study was 72.5 per 1000 women-months (95%CI: 56.4, 88.6). SGA and PTB incidence in the study area was estimated at respectively 36.8% and 14.4% (187, 188).

The present study is a cross-sectional community-based assessment of PA in P women and was conducted in the rainy season, August and September, of 2006. All P women in the Karaba and Koho communities, and participating in the MISAME 2 trial (MMN and LNS groups), were eligible and were included in the study. A comparison group of non-pregnant non-lactating (NPNL) women, whose youngest child was at least 1 year old, was selected by convience sampling from the same communities. NPNL women were included until a sample size was reached that equaled the MMN and LNS groups. In total, 266 P and 141 NPNL women were included in the study. The MISAME 2 trial was approved by the Institutional Ethics Committee of the Centre Muraz in Bobo-Dioulasso, Burkina Faso, and the Institutional Review Board of the Institute of Tropical Medicine in Antwerp, Belgium (CME/HV/dvm/953).

The aims and procedures of the study were explained in local language and written informed consent was obtained. The MISAME 2 trial was registered at clinicaltrials.gov as NCT00909974.

4.3.2 Measurements

PA was self-reported by means of a structured interviewer-administered 7d-recall questionnaire. The questionnaire recalls 18 selected activities within 3 categories (agricultural labor, transportation, domestic activities), and has an open-ended section for women to report other and/or exceptional activities. Energy expenditure (EE) scores (kcal/kg) were calculated for each activity by multiplying the duration estimate (min) with energy cost (kcal/kg/min) as obtained from two indirect calorimetry studies in African women (79, 220) or the Compendium of Physical Activities (221). The main agricultural activity for the period August-September in rural Burkina Faso was bending and digging with a short-handled hoe. Scores for walking were corrected by a factor x1.05 if the woman reported carrying an infant or a load up to 10kg, or by a factor x1.15 for heavier loads (adapted from (220)). Scores and durations were corrected for helping hands by dividing by the total amount of people involved in the activity. Overall PAEE (kcal/kg/week) and PA time (min/week) were derived by respectively multiplying each score and duration estimate with the reported weekly frequency of the activity, and adding up the scores and durations for all activities. Activities were also categorized by type (score in agricultural labor, transportation and domestic activities) and intensity of PA (time spent in light and moderate intensity PA, respectively 1.6-2.9 MET and 3.0-5.9 MET (221)). Women did not report vigorous intensity PA (≥6.0 MET).

Maternal characteristics such as socio-demographic data, anthropometry, parity, etc., were recorded at MISAME 2 trial enrolment. Socio-demographic data and anthropometry of P and NPNL women were also measured at the time of interview. P women had their body temperature recorded daily. Malaria was diagnosed by thick and thin film analysis in case of fever or reported fever. Newborns' sex, weight, gestational age and placental weight were recorded at birth by a study nurse in the health center. Prematurity was defined as a gestational age at birth <37 completed weeks. IUGR was approximated by SGA and was defined as having a birth weight <10th centile of a sex-specific birth weight for gestational age reference. The full methodology of the MISAME 2 trial is described elsewhere (188).

4.3.3 Data analysis

The group of women in their 1st trimester of pregnancy was small (N = 34) and had a mean (± SD) gestational age of 10.1 (± 2.3) weeks, 4 weeks before the 2nd trimester cut-off. The activity pattern of 1st trimester women was mostly similar to that of 2nd trimester women. Therefore, both groups were pooled to gain statistical power (early and mid-pregnancy, ≤25 completed weeks of gestation). Women with >25 completed weeks of gestation were considered as late pregnancy.

Differences in maternal characteristics and birth outcomes were assessed by X^2 tests for categorical variables, and anova tests with post-hoc Tukey-Kramer tests (3 groups) and two-sample t-tests (2 groups) for continuous variables. Women were identified as being involved in a specific activity if they reported performing the activity over the past week, regardless of any missing data in the dimensions of the activity or being an overreporter. In total, seven women were identified to overreport (> week limit of 7140 min/week), i.e. three NPNL women, two P women in their 2nd trimester and two in their 3rd trimester. These women were excluded from calculations and analyses. The different PAEE and PA time measures did not follow a normal distribution and are reported as medians (25th-75th percentile). Differences between NPNL women and P women at different stages of pregnancy were tested by multiple linear regression analysis adjusted for confounding factors (interviewer, ethnicity, husband's job, presence of children in the household, number of household members, and reporting exceptional days of activity) with post-hoc Tukey-Kramer tests. Residual histograms of regressions were symmetric. A sample size of 120 women in each group allows for a detection of an effect size of 0.16 with a power of 80.0% and a type I error of 5%.

P women were categorized into higher or lower PAEE (kcal/kg/week) and more or less time spent in light (min/week) and moderate intensity PA (min/week) by using the median cut-off. Associations of higher compared to lower PAEE with birth outcomes were assessed by linear (birth weight, gestational age and placental weight) and robust poisson (PTB and SGA) regression models adjusted for confounding factors (maternal age at interview, maternal height, primiparity, infant sex, prior or current malarial infection, presence of children in the household and number of household members) and clustering at the woman-level. Models for time spent in light and moderate intensity PA were also adjusted for PAEE and thus reflect intensity independent of their contribution to the total volume of PA (241). Analyses were performed in all P women, further adjusted for trimester of pregnancy, and in P women at different stages of pregnancy. A sample size of 252 P women allows testing for an effect size of 0.35 in continuous outcomes and a difference of 15 percentage points in SGA and PTB with a respective power of 79.3% and 84.8% and a type I error of 5%.

Pre-pregnancy nutritional status and parity were explored as effect modifiers of PAEE by inserting interaction terms in the models. Women who start their pregnancy underweight (BMI <18.5 kg/m²) and primiparous women have a higher risk of low birth weight offspring (242). Significant interactions were subsequently explored in subgroup analyses.

All analyses were performed in Stata 12.0 (StataCorp, College Station, TX, USA). The significance level was set at *P* <0.05 for testing main effects and *P* <0.10 for interactions. All tests were two-sided.

4.4 Results

More NPNL women reported to have children in the household (*P* <0.0001) and to receive help with domestic activities (*P* = 0.06) (**Table 10**). There were also slightly more Bwa (P = 0.08) and less Mossi (P = 0.07) in the NPNL group. Prematurity and SGA incidence was 9.5% and 32.8%, respectively. There were less premature (7.8% vs 11.6%) and more SGA (38.4% vs 26.5%) newborns in women interviewed in late pregnancy compared to women in early and mid-pregnancy. Moreover, gestational age was significantly higher (*P* = 0.02) and placental weight was significantly lower (*P* <0.01) in women interviewed in late pregnancy compared to women in early and mid-pregnancy. Primiparous and underweight women respectively represented 17.3% and 14.5% of P women.

Table 10. Maternal characteristics of NPNL and P rural Burkinabe women and birth outcomes.

	NPNL women (N = 141)		P women: Early and mid-pregnancy (N = 121)		P women: Late pregnancy (N = 145)		*P* [2]
	n	**Value** [1]	***n***	**Value** [1]	***n***	**Value** [1]	
Maternal characteristics							
Age, years	*99*	26.0 ± 6.3	*121*	24.3 ± 5.9	*143*	24.7 ± 6.8	0.14
Height, cm	*135*	162.5 ± 5.9	*118*	162.9 ± 6.1	*139*	163.1 ± 6.0	0.70
BMI [3], kg/m²	*135*	21.5 ± 2.8	*120*	20.8 ± 2.1	*142*	21.1 ± 2.2	0.08
<18.5 kg/m², n (%)	*132*	16 (12.1)	*120*	16 (13.3)	*142*	22 (15.5)	0.71
MUAC [3], mm	*136*	27.3 ± 2.7	*119*	26.5 ± 2.1	*141*	26.2 ± 2.3	<0.001[a,b,b]
Hb, g/dL		-	*118*	11.6 ± 1.6	*143*	11.6 ± 1.7	0.80
<11 g/dL, n (%)			*118*	39 (33.1)	*143*	47 (32.9)	0.98
Primiparous, n (%)		-	*121*	23 (19.0)	*145*	23 (15.9)	0.50
Health center							
Koho, n (%)	*139*	93 (66.9)	*121*	72 (59.5)	*145*	95 (65.5)	0.42
Household help, n (%)	*141*	78 (55.3)	*121*	50 (41.3)	*145*	65 (44.8)	0.06
Children, n (%)	*141*	133 (94.3)	*121*	90 (74.4)	*145*	105 (72.4)	<0.0001[a,b,b]
School attendance, n (%)	*141*	23 (16.3)	*121*	14 (11.6)	*145*	22 (15.2)	0.53
Job husband							
Farmer, n (%)	*103*	90 (87.4)	*121*	114 (94.2)	*145*	135 (93.1)	0.14
Ethnicity							
Mossi, n (%)	*139*	56 (40.3)	*121*	63 (52.1)	*145*	76 (52.4)	0.07
Bwa, n (%)	*139*	72 (51.8)	*121*	50 (41.3)	*145*	57 (39.3)	0.08
Peuhl, n (%)	*139*	5 (3.6)	*121*	4 (3.3)	*145*	7 (4.8)	0.79
Other, n (%)	*139*	6 (4.3)	*121*	4 (3.3)	*145*	5 (3.4)	0.89
Birth outcomes							
Weight, g		-	*113*	2,948 ± 475	*125*	2,959 ± 424	0.86
Gestational age, wk		-	*121*	38.7 ± 3.1	*142*	39.4 ± 1.9	0.02
PTB, %		-	*121*	14 (11.6)	*142*	11 (7.8)	0.29
SGA, %		-	*113*	30 (26.5)	*125*	48 (38.4)	0.05
Placental weight, g		-	*110*	604 ± 134	*99*	548 ± 119	<0.01
Male, %		-	*113*	64 (56.6)	*134*	66 (49.3)	0.25

BMI, Body Mass Index; MUAC, Mid-Upper Arm Circumference; NPNL, Non-Pregnant Non-Lactating women; P, Pregnant women; PTB, Preterm Birth; SGA, Small-for-Gestational Age.

[1] Values are mean ± SD unless indicated otherwise.

[2] Letters refer to NPNL women, women in early and mid-pregnancy and women in late pregnancy in order of appearance. Different letters indicate significant differences between the respective groups of women.

[3] BMI/MUAC at interview for NPNL women and pre-pregnancy BMI/MUAC at MISAME 2 trial enrollment for P women.

The majority of NPNL and P women were engaged in agricultural labor in the rainy season (NPNL = 82.3%; P = 75.9%) (**Table 11**). Moreover, 64.3% of women close to term (>34 weeks gestational age) reported to be working in the field. In total, 39.0% NPNL women, 20.7% women in early and mid-pregnancy and 24.1% women in late pregnancy worked full days in the field on ≥6 days/week. Among the types of PA, agricultural labor had the highest median EE and thus contributed most to the overall PAEE of NPNL women and women in late pregnancy. The median EE in agricultural labor of women in late pregnancy (55 kcal/kg/wk; IQR = 0–138) was lower compared to NPNL women (83 kcal/kg/wk; IQR = 21–165) and higher compared to early and mid-pregnancy (28 kcal/kg/wk; IQR = 0–110) (**Table 12**). EE in agricultural labor was significantly different between groups ($P < 0.01$) with EE of women in early and mid-pregnancy being significantly smaller than that of NPNL women. EE in transportation and domestic activities were also different between groups ($P = 0.01$ and $P = 0.04$, respectively). Post-hoc tests showed that P women had significantly lower EE in transportation than NPNL women, and women in late pregnancy had significantly lower EE in domestic activities than NPNL women. Moreover, groups were significantly different for overall PAEE ($P < 0.001$) and PA time ($P < 0.001$) and time spent in moderate intensity PA ($P < 0.001$), with PAEE, PA time and time in moderate intensity PA of P women being smaller than those of NPNL women. Time spent in light intensity activity was similar across groups.

Table 11. Involvement (%) of rural Burkinabe NPNL and P women in agricultural labor, transportation and domestic activities.

		NPNL women (N = 141)	P women	
			Early and mid-pregnancy (N = 121)	Late pregnancy (N = 145)
Agricultural labor		82.3	76.9	75.5
Transportation				
	Market	29.1	34.7	26.2
	Mill	78.7	75.2	66.9
	Fetching water	94.3	95.0	93.1
	Fetching firewood	34.8	37.2	44.1
	Field	83.7	76.9	77.9
Domestic activities				
	Grinding	33.3	35.5	29.7
	Preparing shea butter	24.1	19.8	19.3
	Preparing tô	97.2	96.7	93.1
	Cooking	95.0	89.3	87.6
	Sweeping floor	98.6	95.0	95.2
	Washing dishes	87.9	86.0	82.8
	Washing clothes	80.1	70.2	77.9
	Child care	87.9	69.4	62.8
	Chopping wood [1]	62.7	42.0	47.4
	Weeding	27.0	19.8	22.8
	Animal care	43.3	30.6	32.4
	Milking cows	1.4	0.0	1.4

NPNL, Non-Pregnant Non-Lactating women; P, Pregnant women.

[1] Data for 102 (72.3%) NPNL women, 88 (72.7%) women in early to mid-pregnancy and 76 (52.4%) women in late pregnancy.

Table 12. Physical activity of NPNL and P rural Burkinabe women.

		NPNL women (N = 138)		P women: Early and mid-pregnancy (N = 119)		P women: Late pregnancy (N = 143)		P^1
		Median	(25^{th} – 75^{th} pctl)	Median	(25^{th} – 75^{th} pctl)	Median	(25^{th} – 75^{th} pctl)	
PAEE, kcal/kg/week		187	(101 – 252)	113	(60 – 214)	143	(63 – 229)	<0.001[a,b,b]
PA time, min/week		3,350	(1,876 – 4,016)	2,011	(1,190 – 3,287)	2,289	(1,288 – 3,697)	<0.001[a,b,b]
Type, kcal/kg/week								
	Agricultural labor	83	(21 – 165)	28	(0 – 110)	55	(0 – 138)	<0.01[a,b,ab]
	Transportation	32	(16 – 46)	21	(10 – 42)	26	(11 – 43)	0.01[a,b,b]
	Domestic activities	45	(38 – 61)	41	(30 – 58)	39	(28 – 52)	0.04[a,ab,b]
Intensity, min/week								
	Light [2]	468	(244 – 613)	445	(244 – 571)	452	(210 – 558)	0.80
	Moderate [2]	2,608	(1,360 – 3,600)	1,535	(811 – 2,880)	1,948	(848 – 3,167)	<0.001[a,b,b]

NPNL, Non-Pregnant Non-Lactating women; P, Pregnant women; PA, Physical Activity; PAEE, Physical Activity Energy Expenditure.

[1] Multiple linear regression adjusted for interviewer, ethnicity, husband's job, presence of children in the household, number of household members, and having exceptional days of activity. Different superscript letters indicate significant differences between the groups of women in the post-hoc Tukey-Kramer tests. The first superscript letter refers to NPNL women, the second letter to women in early and mid-pregnancy and the third letter to women in late pregnancy.

[2] Light intensity PA range: 1.6-2.9 MET; Moderate intensity PA range: ≥3.0 MET.

Data for the analysis of associations between maternal PA in the rainy season and birth outcomes were available for a total of 253 P women, and excluded miscarriages (n = 1), stillbirths (n = 3), and MISAME 2 trial dropouts with no birth outcome data (n = 6). Higher compared to lower PAEE in the rainy season was not significantly associated with birth outcomes in P women as a whole (**Table 13**). However, women in early and mid-pregnancy with higher PAEE had a significant risk reduction for an SGA birth by 48% (risk ratio (RR) = 0.52; 95% CI: 0.28, 0.98; *P* = 0.04) compared to those with lower PAEE. Higher compared to lower levels of moderate intensity PA also reduced the risk of an SGA newborn by 70%, albeit borderline significant (RR = 0.30; 95% CI: 0.09, 1.01; *P* = 0.05), in these women. Risk ratio's of PTB were mostly not-significantly higher but showed wider confidence intervals, especially for moderate intensity PA. There were 13 PTB cases in each subgroup of P women.

Parity modified the association of higher compared to lower PAEE with SGA and gestational age in, respectively, women in early and mid-pregnancy and late pregnancy ($P_{interaction}$ = 0.10 and 0.01, respectively) (**Supplemental Table 3**). Multiparous women with higher PAEE in early and mid-pregnancy had a significant 76% reduced risk of having an SGA newborn (RR = 0.24; 95% CI: 0.09, 0.62; *P* <0.01) compared to those with lower PAEE. Moreover, primiparous women with higher PAEE in late pregnancy had a shorter gestational age, albeit not significant (β = -2.9 weeks; 95% CI: -5.9, 0.1; *P* = 0.06), compared to those with lower PAEE. In addition, pre-pregnancy maternal nutritional status modified the association between PAEE and placental weight in both P women as a whole and women in early and mid-pregnancy ($P_{interaction}$ = 0.05 and <0.01, respectively). Higher compared to lower PAEE was associated with significantly higher placental weights in P women (β = 178.7 g; 95% CI: 59.2, 298.2; *P* <0.01), and women in early and mid-pregnancy in particular (β = 312.3 g; 95% CI: 69.9, 554.6; *P* = 0.02), who started their pregnancy underweight (BMI <18.5 kg/m^2). Interaction coefficients of pre-pregnancy nutritional status and parity for PTB were large. The few cases of PTB in the subgroups of underweight and primiparous women yielded insufficient power to examine interactions.

Table 13. Associations of higher compared to lower overall PAEE and time spent in light and moderate intensity PA in the rainy season at pregnancy with birth outcomes in rural Burkinabe women.

			Overall PAEE		**Light intensity PA**		**Moderate intensity PA**	
		n	**Estimate [1] (95% CI)**	***P***	**Estimate [1] (95% CI)**	***P***	**Estimate [1] (95% CI)**	***P***
P women [2]								
	Gestational age	240	-0.13 (-0.71 – 0.46)	0.67	0.15 (-0.38 – 0.68)	0.58	-0.18 (-1.25 – 0.89)	0.74
	PTB	240	1.40 (0.54 – 3.64)	0.49	1.28 (0.49 – 3.31)	0.61	1.73 (0.36 – 8.29)	0.49
	Birth weight	233	-13.37 (-144.72 – 117.96)	0.84	34.27 (-90.44 – 158.98)	0.59	-79.43 (-330.10 – 171.23)	0.53
	SGA	233	0.76 (0.52 – 1.10)	0.14	1.00 (0.68 – 1.48)	0.99	0.69 (0.34 – 1.40)	0.30
	Placental weight	205	8.20 (-31.42 – 47.82)	0.68	8.67 (-29.95 – 47.29)	0.66	-11.74 (-88.71 – 65.22)	0.76
Early and mid-pregnancy [2]								
	Gestational age	112	-0.40 (-1.39 – 0.59)	0.43	0.11 (-0.84 – 1.06)	0.82	-0.38 (-2.17 – 1.41)	0.68
	PTB	112	1.92 (0.45 – 8.12)	0.38	1.41 (0.44 – 4.54)	0.56	1.23 (0.08 – 18.22)	0.88
	Birth weight	112	0.35 (-214.79 – 215.50)	1.00	-63.35 (-276.63 – 149.93)	0.56	4.36 (-378.12 – 386.83)	0.98
	SGA	112	0.52 (0.28 – 0.98)	0.04	1.51 (0.79 – 2.89)	0.21	0.30 (0.09 – 1.01)	0.05
	Placental weight	108	30.33 (-27.66 – 88.32)	0.30	47.89 (-12.35 – 108.14)	0.12	38.66 (-77.05 – 154.38)	0.51
Late pregnancy [2]								
	Gestational age	128	0.01 (-0.73 – 0.71)	0.97	0.05 (-0.57 – 0.66)	0.88	-0.55 (-1.71 – 0.60)	0.34
	PTB	128	0.88 (0.22 – 3.43)	0.85	1.53 (0.39 – 5.96)	0.54	2.85 (0.29 – 28.25)	0.37
	Birth weight	121	-48.40 (-205.46 – 108.66)	0.54	128.22 (-28.20 – 284.64)	0.11	-222.24 (-505.27 – 60.79)	0.12
	SGA	121	0.91 (0.59 – 1.40)	0.66	0.70 (0.43 – 1.15)	0.16	1.51 (0.63 – 3.62)	0.35
	Placental weight	97	-38.72 (-84.14 – 6.69)	0.09	-8.49 (-56.48 – 39.50)	0.73	-41.60 (-138.98 – 55.78)	0.40

PA, Physical Activity; PAEE, Physical Activity Energy Expenditure; PTB, Preterm Birth; SGA, Small-for-Gestational Age.

[1] Estimates are β coefficients (continuous variables) or risk ratios (PTB and SGA) of a higher vs lower level of PAEE, time spent in light intensity PA and time spent in moderate intensity PA. Models are adjusted for maternal age at interview, maternal height, primiparity, infant sex, prior or current malarial infection, presence of children in the household and number of household members.

[2] Medians of lower and higher PA levels: PAEE – P women = Lower (62 kcal/kg/wk), Higher (219 kcal/kg/wk); Early and mid-pregnancy = Lower (60 kcal/kg/wk), Higher (197 kcal/kg/wk); Late pregnancy = Lower (62 kcal/kg/wk), Higher (227 kcal/kg/wk). Light intensity PA - Pregnant = Lower (239 min/wk), Higher (558 min/wk); Early and mid-pregnancy = Lower (253 min/wk), Higher (571 min/wk); Late pregnancy = Lower (185 min/wk), Higher (558 min/wk). Moderate intensity PA - Pregnant = Lower (829 min/wk), Higher (3014 min/wk); Early and mid-pregnancy = Lower (814 min/wk), Higher (2733 min/wk); Late pregnancy = Lower (836 min/wk), Higher (3172 min/wk).

4.5 Discussion

This study shows that the majority of P women in rural Burkina Faso, including women close to term, are engaged in fieldwork in response to the increased agricultural demands of the rainy season. Overall PAEE was high in P women, though relatively lower compared to NPNL women. Of all types of PA, agricultural labor contributed most to the overall PAEE of women in late pregnancy. Interestingly, our findings revealed that women in early and mid-pregnancy, multiparous women in particular, with a higher PAEE had a significantly reduced risk for an SGA newborn. The risk reduction was also apparent in women in early and mid-pregnancy with higher moderate intensity PA, but was borderline significant. We did not find any associations between higher maternal PA and birth outcomes in P women as a whole. This could indicate that exposure to higher levels of PA in the rainy season during early and mid-pregnancy may potentially protect against IUGR.

Two studies have demonstrated high levels of daily total EE in rural P women at the time of peak agricultural activity in the rainy season (81, 139). Converting to weekly total EE, women expended 304.8 kcal/kg/week in mid- and late pregnancy in The Gambia while women in Nepal expended 326.2 kcal/kg/week in late pregnancy. The P women in our study had an overall median PAEE of 127 kcal/kg/week (IQR: 62-222) which excluded sleep and leisure time. If we assume that women sleep 7 h/d in the rainy season and spend their unaccounted time sitting and standing (50%/50%), the estimated total EE would amount to 294 kcal/kg/week. This would suggest that the P women in rural Burkina Faso, who were mostly in mid- and late pregnancy, attained equally high levels in the rainy season.

P women in our study reported lower overall PAEE than NPNL women. P women spent significantly less time in moderate intensity PA compared to NPNL women and reported less involvement in activities with a relatively higher energy cost, i.e. agricultural labor, chopping wood and animal care. Nonetheless, P women's involvement in agricultural labor was high (75.9%) with more than half of women beyond 34 weeks gestational age (64.3%) reporting to be working in the field. In addition, EE in agricultural labor of women in late pregnancy was not significantly lower - their weekly duration of fieldwork being only slightly lower - than that of NPNL women. P women in The Gambia, including those in their final month of pregnancy, also spent as much time on the field as NPNL women though they reduced the amount of heavy farming jobs (82). These findings suggest that the increased work demands in the rainy season imposes the involvement of women in late pregnancy in agricultural work, including those women who are close to term. Furthermore, activity patterns of women in early and mid-pregnancy and late pregnancy were generally comparable in the rainy season, contrary to HIC where PA levels tend to fall during pregnancy (243-245). Moreover, the relatively lower overall PAEE of women in late pregnancy compared to NPNL women does not indicate that the additional energy requirement of 500 kcal/d in late pregnancy is sufficiently compensated for (93). Rao and colleagues also reported activity patterns to be comparable between women in mid-pregnancy and late pregnancy in a subsistence-farming community in India, though analyses were independent of season and the assessment included resting (138).

We found that fetal growth was most benefitted when women were more physically active in early and mid-pregnancy. Some biologically plausible mechanisms could explain these results. Increased activity in early and mid-pregnancy has been shown to stimulate placental growth and functionality (246) and mid-pregnancy placental volume is a positive independent predictor of birth weight (247, 248). The fetus may therefore likely be at an advantage when entering the final trimester of gestation, in this context, the more nutritionally abundant and less challenging dry season. However, the lack of an association with birth weight in our study may point out that the benefit only extends to those newborns in the lower extremes of birth weight. Moreover, we did not find important relationships of higher PA in the first two trimesters with placental weight at delivery, except for mothers who were underweight in early pregnancy. Underweight women and those who are experiencing caloric restriction in early pregnancy have been reported to have smaller, yet, more efficient placentas (249, 250). These findings may indicate that placental growth-promoting activity in early and mid-pregnancy has a lasting effect on the placenta of vulnerable women in whom feto-protective mechanisms are already at work. Our findings are in contrast to those of Rao et al. (138) who found that rural Indian women with a pre-pregnant weight of <45 kg and medium-to-high activity levels at mid-pregnancy had significantly lower placental weights at delivery compared to those with low activity levels. The influence of higher PAEE in early and mid-pregnancy on SGA incidence was more important in multiparous women. We did not find any study documenting an interaction between parity and PA on SGA incidence. Primigravidous gestations typically result in smaller newborns for reasons that are not fully understood, yet the most popular hypothesis puts forward the uterine constraints in the first pregnancy. It may be that these constraints also interfere in the influence of PA on fetal growth.

We explored differential associations of PA intensity levels on birth outcomes and found that more time spent in moderate intensity PA induced similar protective effects against IUGR. The models controlled for PAEE to offset the inherent differences in energy cost between intensity levels, making the measures independent of their contribution to total EE. If confirmed by other studies, this may indicate that both a higher PAEE and spending more time in moderate intensity PA in early and mid-pregnancy may spur feto-protective mechanisms.

We did not find associations of maternal PA levels in the rainy season with PTB, gestational age and birth weight. The current evidence base has been inconsistent which may be partly ascribed to methodological differences in the timing of assessment. A study in 1327 British women showed that trunk bending for >1 h/d at 34 weeks of gestation was associated with a threefold risk of PTB (153). In our study, 64.3% of women beyond 34 weeks of gestation spent long working hours in the field typically bending forward to use a short-handled hoe for seeding and weeding.

This community-based study has a few strengths and limitations. Other studies have shown that P women who are working in the field continue their routine domestic activities (138). As such, our questionnaire probed for several types of activities ranging from agricultural labor and transportation to domestic activities (including child care). The sum of scores can be considered as a proxy of PAEE and was used to rank women by level of PA. Furthermore, a longitudinal study design would have been preferred to investigate associations over the different stages of pregnancy. However, the specific purpose of this study was to investigate PA in the rainy season which limited the design of the study. Although data of 253 P women were available for analyses there were too few cases of PTB which restricted analyses. Moreover, maternal dietary intake was not included in the analysis but has been suggested to have a moderating effect on the relationship between maternal PA and birth outcome (246). The women in the study were also receiving nutritional supplements as part of the MISAME2 trial. However, the allocation proportions to the LNS and MMN groups were not significantly different between PA levels. Finally, our analyses adjusted for malarial infection, but not other infections such as urinary tract infections, intrauterine inflammation, etc. Such infections have also been shown to be associated with adverse birth outcomes (203).

In conclusion, we observed that P women have different activity patterns in the rainy season compared to NPNL women although the majority remained involved in agricultural labor, the main contributor to overall PAEE in late pregnancy. Overall PAEE was high and our data suggest that the additional energy requirement in late pregnancy is not fully compensated for by a reduction in PA. Finally, we found that higher PAEE levels and more time spent in moderate intensity PA in the rainy season during early and mid-pregnancy may protect against IUGR, particularly in multiparous women.

Supplemental Table 3. Interactions of higher overall PAEE levels in the rain season with pre-pregnancy nutritional status and parity on birth outcomes in rural Burkinabe women.

	Pregnant		Early and mid-pregnancy		Late pregnancy	
	n	*P*[1]	n	*P*[1]	n	*P*[1]
Gestational age, weeks						
Nutritional status	239	0.18	112	0.20	127	0.95
Parity	240	0.20	112	0.57	128	0.01
Birth weight, g						
Nutritional status	232	0.99	112	0.65	120	0.37
Parity	233	0.35	112	0.77	121	0.23
SGA, n(%)						
Nutritional status	232	0.52	112	0.64	120	0.28
Parity	233	0.94	112	0.10	121	0.16
Placental weight, g						
Nutritional status	204	0.05	108	<0.01	96	0.88
Parity	205	0.81	108	0.94	97	0.89

SGA: Small-for-Gestational Age

[1] Models are adjusted for materal age at interview, maternal height, infant sex, prior or current malarial infection, presence of children in the household, number of household members and primiparity. The latter only for models of maternal nutritional status.

Chapter 5

EFFECT OF MULTIPLE MICRONUTRIENT SUPPLEMENTATION IN LACTATING WOMEN ON INFANT GROWTH AND MORBIDITY: A DOUBLE-BLIND RANDOMIZED CONTROLLED TRIAL IN RURAL BURKINA FASO

5.1 Abstract

Background: Micronutrient deficiencies contribute to the vicious cycle of growth restriction in low and middle-income countries (LMICs). Lactating women and their breastfeeding infants are at risk of inadequate intakes and deficiencies in multiple micronutrients due to the imbalance between their relatively high micronutrient requirements and the typical low intake of micronutrient-dense foods. Infants born small-for-gestational age (SGA) or preterm (PTB) may be particularly vulnerable. However, there is currently no evidence on the effect of multiple micronutrient supplementation in lactating women on infant growth and morbidity.

Methods: In a randomized controlled trial in rural Burkina Faso, 1426 pregnancies were allocated to daily supplementation with either multiple micronutrients (UNICEF/WHO/UNU Multiple Micronutrient Preparation or UNIMMAP; intervention) or iron and folic acid (IFA; control) for three months after delivery. In total, 1295 mothers with live births were enrolled in the study. Daily tablet intake was directly observed by trained community workers. Anthropometry, morbidity and hemoglobin concentration of infants were measured monthly until the first birthday. Linear mixed-effects models, accounting for repeated measurements, were used to assess effects on monthly growth and hemoglobin concentration rates. Stunting and anemia prevalence at the last follow-up visit were compared between groups by logistic regression. Effects on cumulative incidences of diarrhea, cough, fever, wasting and anemia were assessed by generalized linear latent and mixed models. We tested the intervention’s impact on infants who were born SGA and PTB in subgroup analyses.

Results: Linear growth and relative weight gain during infancy remained suboptimal despite supplementation of lactating mothers. We noted a borderline increased linear growth rate of infants in the UNIMMAP group (β = 0.0119 length-for-age Z-score/month; 95% CI: -0.0026 – 0.0263; $P_{UNIMMAPxage}$ = 0.107) compared to IFA. Infants who were born SGA had significantly higher linear growth rates (β = 0.0229 length-for-age Z-score/month; 95% CI: -0.0006 – 0.0465; $P_{UNIMMAPxage}$ = 0.057), but did not have significant reductions in stunting. Infants born PTB had increased hemoglobin concentration rates (β = 0.1558 g/dL/month; 95% CI: -0.0182 – 0.3299; $P_{UNIMMAPxage}$ = 0.079). There was a borderline tendency of UNIMMAP to reduce relative infant weight gain rate (β = -0.0159; 95% CI: -0.0349 – 0.0031 weight-for-age Z-score/month; 95% CI: -0.0006 – 0.0465; $P_{UNIMMAPxage}$ = 0.101).

Conclusions: Supplementing UNIMMAP instead of IFA during the first three months of lactation leads to better linear growth in infants born SGA and improves the hemoglobin concentration of infants born PTB.

5.2 Introduction

Women of reproductive age in low and middle income countries (LMICs) are at risk of inadequate intakes and deficiencies of multiple micronutrients (97, 98). The burden has mainly been ascribed to poor quality diets and low intakes of micronutrient rich foods, along with increased infection rates due to poor hygiene and infectious diseases. The high global prevalence of anemia and the evidence linking maternal anemia to adverse pregnancy outcomes has spurred research and policy recommendations for pregnant women. Far less is known about the effects of low maternal intakes and deficiencies during lactation, when nutrients are redirected to breast milk, on infant health and growth. Lactation is known to considerably increase the requirements for most micronutrients, even more than pregnancy, as growth rates soar during infancy (161). The risk of deficiencies in lactating women is thus expected to be higher, particularly in those who were nutritionally depleted before or during pregnancy. Maternal deficiencies in some micronutrients have been associated with a decline in breast milk concentrations and concurrent deficiencies in their infants (160, 251). It has therefore been hypothesized that micronutrient deficiencies in lactating women are a potential contributor to growth faltering during infancy (67, 160, 164).

The World Health Organization has proposed single nutrient (two or less micronutrients) supplementation strategies, such as iron and folic acid (IFA) to prevent or treat anemia during pregnancy and improve pregnancy outcomes (121). Postpartum mothers too have been recommended to be supplemented with IFA for three months post-delivery to prevent anemia (120). Yet, there is accumulating evidence that deficiencies are not limited to one micronutrient in populations from LMICs (80, 252-255). Moreover, several micronutrients may be involved in the absorption or metabolism of one micronutrient, e.g. the case of iron and its complex interactions in haematopoiesis (256). Both of these matters could explain the varying findings of single nutrient interventions across different settings. Several trials in pregnant women from different settings were therefore launched in a concerted effort to test the efficacy of multiple micronutrient supplementation (UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation) compared to the routine IFA supplementation. These trials have shown significantly reduced risks of adverse birth outcomes, i.e. stillbirth, low birth weight, small-for-gestational age (SGA; birth weight for gestational age $<10^{th}$ centile of a sex-specific reference population) and preterm birth (PTB; gestational age <37 completed weeks) (114).

The findings of these trials raise the question whether UNIMMAP supplementation might equally benefit lactating women and their suckling infants. However, the evidence on the effects of supplementation of lactating women on infant health and growth is surprisingly limited. A systematic search concluded that there are currently no published trials testing the efficacy of multiple micronutrients compared to IFA supplementation in lactating mothers on infant micronutrient deficiencies, mortality or morbidity (181). Furthermore, we did not find any such study investigating effects on growth during infancy. It is also unclear if SGA and preterm newborns, who are more likely to have undernourished mothers and who have been reported to have less micronutrient stores at birth, are especially benefited by UNIMMAP compared to IFA supplementation in their lactating mothers during early infancy (171-174).

The objectives of this study are therefore to: 1) assess the efficacy of UNIMMAP compared to IFA supplementation in early lactation on infant nutritional status, growth and morbidity, and 2) to test its efficacy in subgroups of vulnerable infants born SGA or PTB.

5.3 Methods

5.3.1 Study setting and design

From March 2004 to February 2006, a randomized controlled trial was conducted in 1426 pregnant women who attended two health centers in the district of Houndé in rural Burkina Faso. The study was designed to examine the effects of prenatal UNIMMAP supplementation compared to IFA supplementation on birth outcomes. Details of the trial have been described elsewhere (187). Briefly, women with confirmed pregnancies were randomly assigned to receive either a daily dose of UNIMMAP (intervention) or IFA (control) from enrolment until delivery. Pregnant women were also randomized at enrolment in the MISAME1 trial to receive either a daily dose of UNIMMAP (intervention) or IFA (control) during early lactation, i.e. three months post-delivery. Women with live births were enrolled in the postnatal supplementation trial at delivery. The micronutrient composition of both supplements is presented in **Table 14**. The intervention and control micronutrient tablets appeared identical. The tablets were produced by Scanpharm (Copenhagen, Denmark) who sealed them in containers labeled with a letter code corresponding to the study group. The letter codes were kept secret from the research staff and participants until data analysis was completed. As part of a different study, women were also randomized at enrolment in the MISAME1 trial to a weekly dose of Chloroquine (intervention; 300mg) or no Chloroquine (control) post-delivery. Of the women in the postnatal IFA group, 51% received prenatal IFA and 49% received prenatal UNIMMAP. Moreover, 51% received postnatal chloroquine whereas 49% received no malaria prophylaxis. The respective proportions for the women in the postnatal UNIMMAP group were all 50%. Trained community workers visited the homes of the women in the study and directly observed intake of the tablets. The study was approved by the ethics committees of the Center Muraz (Burkina Faso) and the Institute of Tropical Medicine (Belgium). The trial was registered at clinicaltrials.gov (identifier NCT00642408).

Table 14. Micronutrient composition of the IFA and UNIMMAP tablets.

Micronutrient	IFA	UNIMMAP
Vitamin A, µg	-	800
Thiamine, mg	-	1.4
Riboflavin, mg	-	1.4
Niacin, mg	-	18
Vitamin B6; mg	-	1.9
Folic acid, µg	400	400
Vitamin B12, µg	-	2.6
Vitamin C, mg	-	70
Vitamin D, IU	-	200
Vitamin E, mg	-	10
Zinc, mg	-	15
Iron, mg	60	30
Copper, mg	-	2
Selenium, µg	-	65
Iodine, µg	-	150

IFA, Iron and Folic Acid; IU, International Units;
UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation.

5.3.2 Subjects and measurements

Mothers were invited to attend monthly visits at the nearest health center to assess their child's growth and morbidity during the first year of life, i.e. from 1 to 12 months of age. Mothers who missed an appointment were visited at home and encouraged to attend the visits. Infant length, weight, mid-upper arm circumference (MUAC) and head circumference were measured at each visit. Infant length was measured to the nearest 1mm with a SECA 207 measuring rod (SECA Gmbh & Co, Hamburg, Germany), whereas weight was measured to the nearest 10g with a SECA 725 scale (SECA Gmbh & Co, Hamburg, Germany). The weights of infants aged >6 months were measured to the nearest 100g with an electronic UNIscale (UNICEF, Copenhagen, Denmark). MUAC and head circumference were measured to the nearest 1 mm with a SECA girth measuring tape or a SECA 212

(SECA Gmbh & Co, Hamburg, Germany). The circumference of the arm was measured halfway the tip of the olecranon and the acromion. The circumference of the head was taken at the maximum occipito-frontal measurement. The accuracy and precision of the measurements were established through monthly standardization sessions. Anthropometry was measured in duplicate by study nurses, and then averaged. Data were screened for consistency by a supervisor. If there was a large difference between duplicate measurements (200g for weight or 5mm for other measurements), the valid measure was confirmed by the supervisor. At the monthly visits, the study nurses also collected data on diarrhea (defined as ≥3 loose stools in the past 24 hours), fever, and cough episodes that had occurred in the two weeks prior to the visit, and mortality cases. In addition, finger blood samples were collected at intermittent visits for Hb determination with a HemoCue device (HemoCue Ltd, Dronfield, United Kingdom). Cord blood was also collected at birth. The HemoCue device was calibrated daily at the time of the measurements with a HemoCue Control Cuvette. Recommendations for exclusive breastfeeding and optimal complementary feeding were provided to all participants. As per national recommendations, women received 200,000 IU of vitamin A after delivery, while infants received 100,000 IU and 200,000 IU at respectively 6 and 12 months of age. Infants were also vaccinated as per the national schedule. Infants who were sick and/or had lost weight since the last visit were referred for treatment.

5.3.3 Data analysis

Live newborns of randomized mothers with at least 1 set of anthropometric measurements after delivery were included in the intention-to-treat analyses. Infants were considered lost to follow-up when they didn't attend their last monthly visit at the age of 12 months. Data from infants who were lost to follow-up were included in the analyses up to the last visit.

The primary outcomes of this study are length-for-age Z–score (LAZ) and hemoglobin concentration. Secondary outcomes included weight-for-length Z–score (WLZ), weight-for-age Z–score (WAZ), MUAC, head circumference, stunting (LAZ <-2), wasting (WLZ <-2), anemia and morbidity. Anthropometric Z-scores were calculated based on the 2006 World Health Organization child growth standards reference (257). Anemia was defined as a Hb concentration of respectively <9.0 g/dL and <10.5 g/dL in finger blood of infants aged 1 to 3 months and those aged 4 to 12 months (258). Infant growth outcomes were analysed with linear mixed-effects models. Fixed effects included postnatal intervention (UNIMMAP versus IFA), maternal height, primiparity, child gender, child age, and prenatal intervention (UNIMMAP versus IFA) and health center to account for the study design. An additional quadratic term for child age, reflecting the typical non-linear pattern of growth trajectories, improved the fit of the models (likelihood ratio test, *P* <0.0001). The models also included a random intercept for child, to account for the repeated measurements, and a random slope for child age to account for the variations in individual growth trajectories. Fitting a quadratic term for child age in the random slope improved the fit of the models (restricted estimated likelihood ratio test, *P* <0.0001). All models included an interaction term between postnatal intervention and child age to assess the intervention effect over time. The covariance matrices of the models were unstructured. The effect of the postnatal intervention on the prevalence of stunting at endpoint, i.e. the last visit of the child, was analyzed with logistic regression. The cumulative incidence of wasting was compared between study groups using generalized linear latent and mixed models, which allowed the fitting of multilevel mixed-effects Poisson regression models. We opted to use Poisson regression to present an adjusted incidence rate ratio rather than an odds ratio. A robust estimation of the variance was adopted to relax the assumption of a Poisson distribution. A child identifier was added to the models as a random effect to account for clustering.

Infant hemoglobin (Hb) concentrations were modelled with linear mixed-effects models. Fixed terms included postnatal intervention (UNIMMAP compared to IFA), Hb in cord blood at delivery, child age, prenatal intervention (UNIMMAP compared to IFA) and health center. A quadratic term for child age improved the fit of the models (likelihood ratio test, *P* <0.0001). The models included a random intercept for child. A random slope for child age did not improve the fit of the model (likelihood ratio test, *P* = 1.0). The hemoglobin models also included an interaction term between postnatal intervention and child age to assess the effect of the intervention over time. The effect on anemia prevalence at the last visit was assessed with logistic regression models. The cumulative incidence of anemia, diarrhea, cough and fever was compared between both study groups using generalized linear latent and mixed models, as described above.

In the second study objective, we assessed the efficacy of postnatal UNIMMAP compared to IFA supplementation on these outcomes in infants who were born preterm (gestational age <37 completed weeks) and those born SGA (birth weight for gestational age <10th centile of the sex-specific Kramer reference population (198)). Analyses were not adjusted for postnatal malaria prophylaxis supplementation, because only very low amounts of chloroquine are transferred into breast milk and do not protect the infant (259-261). All statistical analyses were two-sided. A *P*-value <0.05 was considered statistically significant in all analyses, except for the appreciation of interaction terms for which we used *P* <0.10 (262). Data management and analyses were carried out with Stata 13.0 (StataCorp, College Station, TX). Values are means ± SD or mean percentages, unless indicated otherwise.

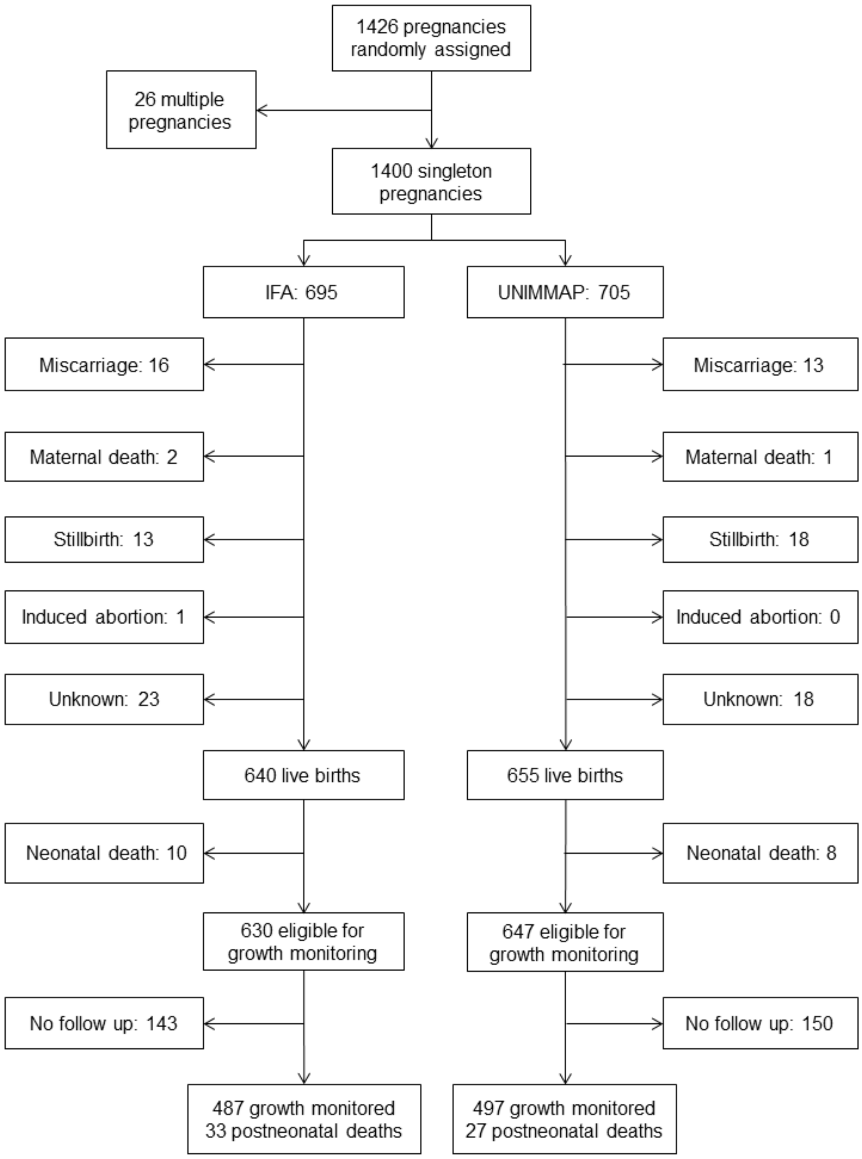


Figure 19. Trial profile of the postnatal study groups. IFA,: Iron and Folic Acid; UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation.

5.4 Results

During the course of two years, a total of 1426 pregnancies were identified and randomly assigned to prenatal and postnatal supplementation with either UNIMMAP or IFA. Women were mostly young (age= 24.4 ± 6.3 y) at the start of the prenatal trial and 19.8% were primiparous. There was no difference in the proportion of cases lost to follow-up during pregnancy between the postnatal study groups (data not shown) (**Figure 19**). Maternal characteristics and the birth characteristics of their 1295 singleton live newborns were balanced between study groups (**Table 15**). Eighteen infants died during the neonatal period (IFA = 10; UNIMMAP = 8; P = 0.60), leaving 1277 infants eligible for growth monitoring.

Table 15. Characteristics of mothers and their singleton live newborns[1].

	IFA (n=640)	UNIMMAP (n=655)
Maternal characteristics[2]		
Maternal age, y	24.3 ± 6.3	24.2 ± 6.1
Maternal height, cm	162.3 ± 6.0	162.0 ± 5.8
BMI, kg/m²	21.0 ± 2.1	20.8 ± 2.1
BMI <18.5 kg/m², n (%)	56 (9.1)	78 (12.5)
MUAC, mm	25.9 ± 2.2	25.7 ± 2.1
Primiparity, n (%)	132 (20.6)	131 (20.0)
Newborn characteristics		
Birth length, mm	481 ± 26	482 ± 23
Birth weight, g	2,902 ± 448	2,889 ± 432.3
LBW, n (%)	72 (13.9)	87 (16.4)
SGA, n (%)	204 (40.2)	201 (38.7)
LGA, n (%)	23 (4.5)	21 (4.1)
Gestational age, wk	39.2 ± 3.0	39.2 ± 3.1
Premature, n (%)	84 (13.8)	86 (13.7)
Hb in cord blood, g/dL	15.4 ± 2.7	15.5 ± 2.7
Female, n (%)	323 (51.9)	313 (48.5)

BMI, Body Mass Index; Hb, Hemoglobin; IFA, Iron and Folic Acid; LBW, Low Birth Weight; LGA, Large-for-Gestational Age; MUAC, Mid-Upper Arm Circumference; UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation; SGA, Small-for-Gestational Age.

[1] Values are means ± SD, unless indicated otherwise.

[2] At enrolment in the prenatal intervention trial.

Mean compliance to the daily intake of tablets was estimated at 73.8% and was not significantly different between groups (IFA = 73.6%; UNIMMAP = 73.9%; P = 0.79). The total duration of follow-up was 7,210 infant-months with infants attending an average of 5.6 ± 4.1 monthly visits. A total of 293 infants had no follow-up data and 865 infants were lost to follow-up before reaching their first birthday, mainly because of migration out of the study area. There was no difference in the proportion of lost to follow-up infants between the study groups. Moreover, there were no differences between birth characteristics of infants lost to follow-up and who were growth monitored (data not shown). Sixty infants died during the follow-up period (IFA = 33; UNIMMAP = 27; P = 0.37).

Linear growth and relative weight gain of infants, measured by respectively LAZ and WLZ, faltered during the course of the first 12 months of life in the overall study sample (**Figure 20**). Infants whose mothers received UNIMMAP had a slightly lower LAZ at 1 month of age (mean ± SE; IFA = -0.73 ± 0.05; UNIMMAP = -0.85 ± 0.6; P = 0.11) and showed an increase in mean LAZ until 3-4 months of age. Linear growth was similar in both study groups after. Growth patterns in relative weight gain, MUAC and head circumference were parallel in both study groups, although infant growth appeared to be marginally better in the UNIMMAP group until the final months of infancy. Infants whose mothers received UNIMMAP had a tendency for an increased linear growth rate (β = 0.0119 LAZ/month; 95% CI: -0.0026 – 0.0263; $P_{UNIMMAPxage}$ = 0.107) and a tendency for a decreased WLZ rate (β = -0.0159 WLZ/month; 95% CI: -0.0349 – 0.0031; $P_{UNIMMAPxage}$ = 0.101) compared to infants whose mothers received IFA (**Table 16**).

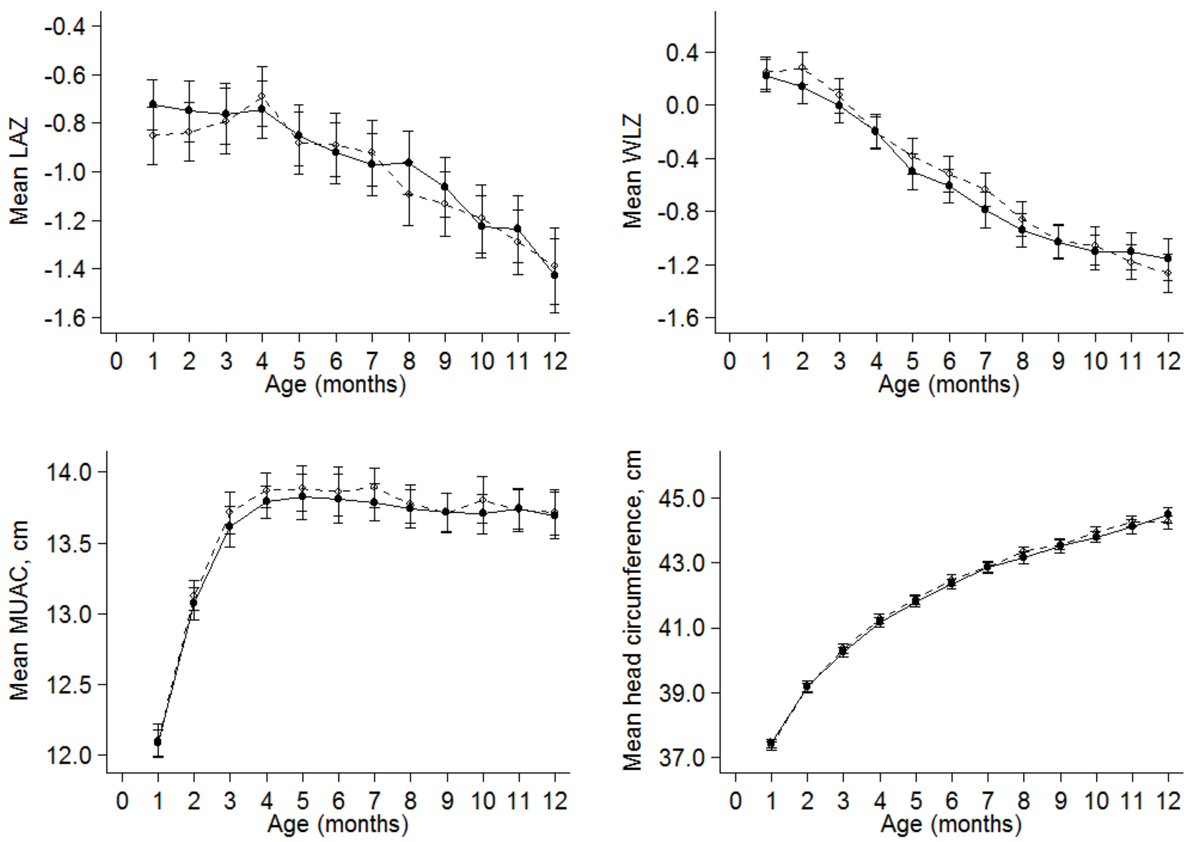


Figure 20. Mean monthly length-for-age Z-scores (LAZ), weight-for-length Z-scores (WLZ), mid-upper arm circumference (MUAC) and head circumference during infancy. Dashed lines represent the intervention group (UNIMMAP), and solid lines the control group (IFA). Bars are the upper and lower confidence intervals of the monthly means. IFA, Iron and Folic Acid; UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation.

Table 16. Effect of postnatal supplementation with UNIMMAP compared to IFA on infant growth and hemoglobin concentration.

	IFA		UNIMMAP		Crude model		Adjusted model	
Outcome	N[1]	Mean ± SD	N[1]	Mean ± SD	β (95%CI)[2]	*P*	β (95%CI)[3]	*P*
Length-for-Age, Z-score	3596	-0.93 ± 1.12	3604	-0.97 ± 1.18	0.0101 (-0.0035 – 0.0236)	0.144	0.0119 (-0.0026 – 0.0263)	0.107
Hemoglobin, g/dL	923	9.3 ± 1.9	907	9.3 ± 1.9	-0.0003 (-0.0456 – 0.0450)	0.990	0.0051 (-0.0436 – 0.0538)	0.837
Weight-for-Length, Z-score	3596	-0.50 ± 1.27	3603	-0.46 ± 1.27	-0.0105 (-0.0283 – 0.0074)	0.251	-0.0159 (-0.0349 – 0.0031)	0.101
Weight-for-Age, Z-score	3596	-1.00 ± 1.16	3605	-1.00 ± 1.19	0.0015 (-0.0127 – 0.0157)	0.836	-0.0011 (-0.0159 – 0.0138)	0.887
MUAC, cm	3604	13.5 ± 1.3	3603	13.5 ± 1.4	-0.0024 (-0.0191 – 0.0143)	0.775	-0.0052 (-0.0230 – 0.1264)	0.568
Head circumference, cm	3604	41.6 ± 2.7	3603	41.7 ± 2.6	0.0095 (-0.0071 – 0.0262)	0.261	0.0090 (-0.0088 – 0.0267)	0.324

IFA, Iron and Folic Acid; MUAC, Mid-Upper Arm Circumference; UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation for pregnant and lactating women.

[1] Total number of monthly measurements during the follow up period.

[2] Interaction coefficient between postnatal intervention allocation (UNIMMAP compared to IFA) and child age, estimated from quadratic mixed models and expressed as monthly rate. Fixed effects for growth models included postnatal intervention, child age, child age squared, and health center. Random effects for growth models included a child identifier, child age and child age squared. Fixed effects of the hemoglobin model included postnatal intervention, child age, child age squared, and health center. A child identifier was included as a random effect in the hemoglobin model.

[3] Interaction coefficient between postnatal intervention allocation (UNIMMAP compared to IFA) and child age, estimated from quadratic mixed models and expressed as monthly rate. Growth models were further adjusted for prenatal intervention allocation (UNIMMAP compared to IFA), maternal height, primiparity, child gender, and premature and small-for-gestational age birth, as fixed effects. Hemoglobin models were further adjusted for prenatal intervention, hemoglobin concentration in cord blood, and premature and small-for-gestational age birth, as fixed effects.

Linear growth rates were higher in infants born SGA (β = 0.0229 LAZ/month; 95% CI: -0.0006 – 0.0465; $P_{UNIMMAPxage}$ = 0.057), while there were no effects on those who weren't SGA at birth (β = 0.0047 LAZ/month; 95% CI: -0.0136 – 0.0230; $P_{UNIMMAPxage}$ = 0.613) (**Table 17**). SGA newborns of lactating mothers receiving UNIMMAP showed increased growth during the time period of supplementation (**Figure 21**). While these infants showed a trend towards reduced odds of stunting at their last anthropometry visit, the reduction was not significant (**Table 18**). On the other hand, UNIMMAP compared to IFA supplementation of lactating mothers of PTB newborns improved hemoglobin concentration rates during infancy (β = 0.1558 g/dL/month; 95% CI: -0.0182 – 0.3299; $P_{UNIMMAPxage}$ = 0.079) (Table 17). The interaction coefficient remained significant when the model was further adjusted for SGA birth (β = 0.1532 g/dL/month; 95% CI: -0.0209 – 0.3273; $P_{UNIMMAPxage}$ = 0.085). Finally, no differences in morbidity episodes were observed between the study groups (**Table 19**).

Table 17. Effect of postnatal supplementation with UNIMMAP compared to IFA on growth and hemoglobin concentration of infants born SGA and PTB.

	SGA			PTB		
	N[1]	**β (95%CI)[2]**	***P***	**N[1]**	**β (95%CI)[2]**	***P***
Length-for-Age, Z-score/mo	2464	0.0229 (-0.0006 – 0.0465)	0.057	667	0.0151 (-0.0314 – 0.0617)	0.524
Hemoglobin, g/dL/mo	570	0.0182 (-0.0543 – 0.0908)	0.623	146	0.1558 (-0.0182 – 0.3299)	0.079
Weight-for-Length, Z-score/mo	2464	-0.0185 (-0.0481 – 0.0112)	0.222	667	-0.0149 (-0.0749 – 0.0450)	0.258
Weight-for-Age, Z-score/mo	2465	0.0034 (-0.0202 – 0.0270)	0.777	667	0.0088 (-0.0438 – 0.0614)	0.743
MUAC, cm/mo	2465	0.0016 (-0.0257 – 0.0289)	0.909	667	0.0087 (-0.0425 – 0.0600)	0.738
Head circumference, cm/mo	2465	0.0096 (-0.0223 – 0.0416)	0.556	667	0.0159 (-0.0495 – 0.0813)	0.633

IFA, Iron and Folic Acid; MUAC, Mid-Upper Arm Circumference; PTB, Preterm Birth; SGA, Small-for-Gestational Age; UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation for pregnant and lactating women.

[1] Number of monthly measurements during the follow-up period.

[2] Interaction coefficient between postnatal intervention allocation (UNIMMAP compared to IFA) and child age, estimated from quadratic mixed models and expressed as monthly rate. Fixed effects for growth models included postnatal intervention, maternal height, primiparity, child gender, child age, child age squared, prenatal intervention (UNIMMAP compared to IFA) and health center. Random effects for growth models included a child identifier, child age and child age squared. Fixed effects of the hemoglobin model included postnatal intervention, hemoglobin concentration in cord blood, child age, child age squared, prenatal intervention and health center. A child identifier was included as a random effect in the hemoglobin model.

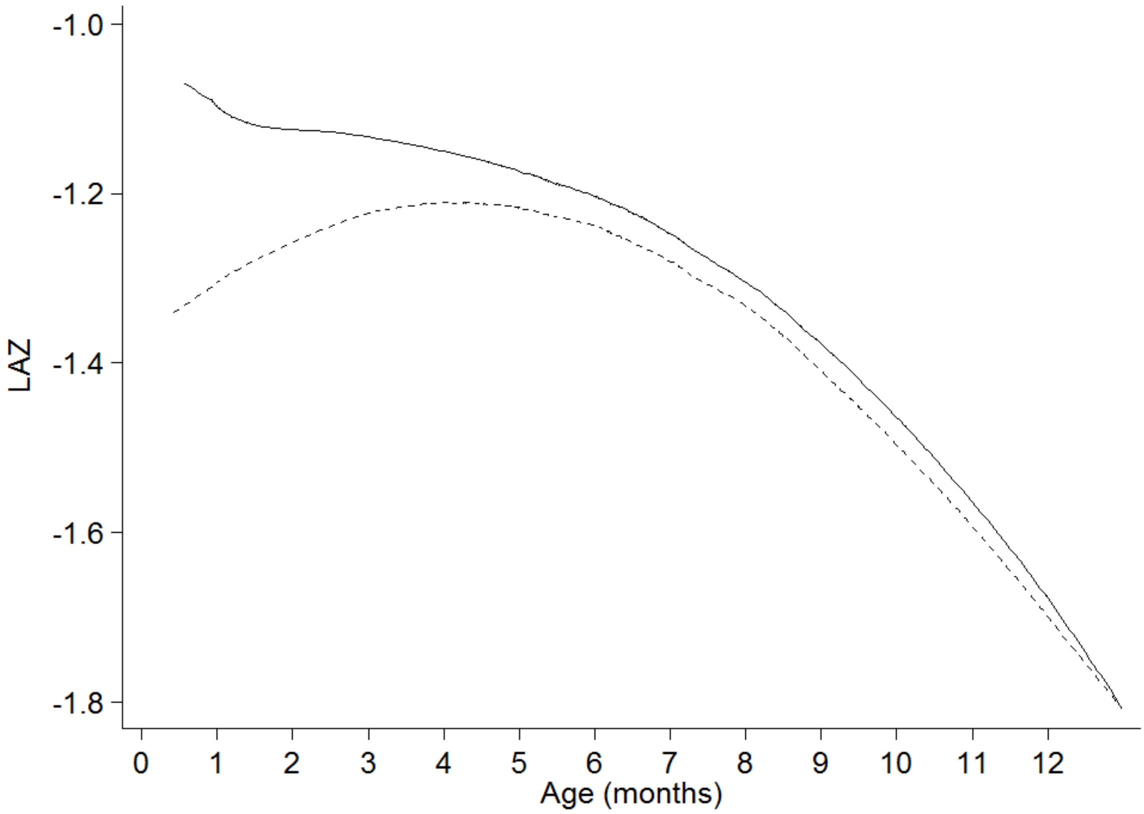


Figure 21. Length-for-age Z-scores (LAZ) of small-for-gestational age newborns of lactating mothers in the UNIMMAP and the IFA group during infancy. The dashed line represents a locally weighted regression on the fitted values of the growth model for the intervention group (UNIMMAP), and the solid line that of the control group (IFA). IFA, Iron and Folic Acid; UNIMMAP, UNICEF/WHO/UNU Multiple Micronutrient Preparation.

Table 18. Effect of postnatal supplementation with UNIMMAP compared to IFA on the prevalence of stunting and anemia at the last visit.

	All			SGA			PTB		
	N	**OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***
Stunting[1]	950	1.00 (0.74 – 1.36)	0.992	325	0.77 (0.48 – 1.25)	0.292	112	1.12 (0.49 – 2.58)	0.783
Anemia[2]	864	0.94 (0.67 – 1.32)	0.720	287	1.02 (0.55 – 1.86)	0.958	101	0.72 (0.25 – 2.10)	0.545

IFA, Iron and Folic Acid; UNIMMAP; UNICEF/WHO/UNU Multiple Micronutrient Preparation; OR, Odds Ratio; PTB, Preterm Birth; SGA, Small-for-Gestational Age.

[1] ORs estimated by logistic regression with adjustment for maternal height, primiparity, child gender, child age at last visit, prenatal intervention (UNIMMAP compared to IFA), and health center.

[2] ORs estimated by logistic regression with adjustment for primiparity, child gender, child age at last visit, prenatal intervention (UNIMMAP compared to IFA), and health center.

Table 19. Effect of postnatal supplementation with UNIMMAP compared to IFA on the cumulative incidence of diarrhea, cough, fever, wasting and anemia.

	All			SGA			PTB		
	Infant-months[1]	**IRR (95%CI)[2]**	***P***	**Infant-months[1]**	**IRR (95%CI)[2]**	***P***	**Infant-months[1]**	**IRR (95%CI)[2]**	***P***
Diarrhea	3524.5	1.02 (0.82 – 1.28)	0.846	1233.5	0.98 (0.65 – 1.46)	0.911	333.0	1.10 (0.53 – 2.27)	0.794
Cough	3524.0	0.92 (0.78 – 1.08)	0.301	1233.5	1.02 (0.79 – 1.33)	0.855	333.0	0.78 (0.48 – 1.29)	0.337
Fever	3523.5	0.99 (0.86 – 1.14)	0.879	1233.5	1.15 (0.90 – 1.47)	0.278	333.0	0.97 (0.65 – 1.46)	0.895
Wasting	7077.0	0.98 (0.75 – 1.29)	0.904	2475.0	0.86 (0.56 – 1.32)	0.486	673.0	0.88 (0.48 – 1.61)	0.675
Anemia	1796.0	0.99 (0.93 – 1.05)	0.768	614.0	0.98 (0.88 – 1.10)	0.755	191.0	0.98 (0.82 – 1.17)	0.829

IRR, Incidence Rate Ratio; IFA, Iron and Folic Acid; UNIMMAP; UNICEF/WHO/UNU Multiple Micronutrient Preparation for pregnant and lactating women; PTB, Preterm Birth; SGA, Small-for-Gestational Age.

[1] Infant-months for diarrhea, cough and fever were adjusted for total morbidity recall period.

[2] IRRs estimated by general linear latent and mixed models with fixed effects primiparity, child gender, prenatal intervention (UNIMMAP compared to IFA) and health center, and child identifier as random effect.

5.5 Discussion

To our knowledge, this is the first study to report the effects of UNIMMAP compared to IFA supplementation in lactating women on infant nutritional status, growth and morbidity. There was no effect on infant anthropometry, hemoglobin concentration and morbidity in the overall group. Interestingly, the intervention showed selective yet differential effects in subgroups of infants born SGA and PTB. On one hand, the intervention increased linear growth rates of SGA born infants. Linear growth was sustained until three months of age but levelled off and faltered afterwards, leaving no apparent advantage compared to those whose mothers were supplemented with IFA during early lactation. On the other hand, the intervention increased hemoglobin concentration rates in PTB born infants only.

Both linear growth and relative weight gain faltered during infancy. Yet, infants whose mothers were supplemented with UNIMMAP had a trend towards higher linear growth rates compared to their peers whose mothers received daily IFA. There are a few biologically plausible mechanisms which could explain these findings. First, lactating mothers in the research area had inadequate intakes of multiple micronutrients and/or low micronutrient status. A previous dietary intake study in the study area has shown that pregnant and non-pregnant and non-lactating women did not meet recommended intakes for several micronutrients, even in the post-harvest season (99). Moreover, a recent study in an adjacent province in Burkina Faso showed that women of reproductive age had concurrent deficiencies in iron, zinc and vitamin A along with a high anemia prevalence (263). Lactating mothers may have been at an even higher risk of inadequate intakes and/or status as the requirements of most micronutrients, except iron and folate, are highest during lactation. Second, the UNIMMAP supplement may not have provided a sufficiently high dose of multiple micronutrients. The supplement was designed to supply one recommended daily allowance for all of its micronutrients, except folate, for healthy pregnant women in the US and Canada (127). Such an approach will reduce the chances of harm to women, but may also be inadequate for women who are at greater risk of inadequate intakes and/or status. Third, breast milk quality was likely only improved in those mothers receiving UNIMMAP. Breast milk concentrations and thus infant intake has been shown to be most affected by maternal intake of the water-soluble vitamins thiamin, riboflavin, vitamin B_6 and vitamin B_{12}, vitamin A, as well as iodine and selenium (160, 251, 264-266). Breast milk concentrations of iron, folate, zinc, copper and vitamin D are less susceptible to maternal dietary intake as they are more regulated by the mammary gland and protect the lactating mother from depletion (267-270). It should be noted that all women in our study received a single high-dose of vitamin A after delivery regardless of study group in order to prevent vitamin A deficiency. Women in both groups may therefore have had improved breast milk concentrations of vitamin A, and other micronutrients in the UNIMMAP supplement may have accounted for the apparent trend in improved linear growth rates. Fourth, low dietary intakes and/or status of thiamin, vitamin B_6 and vitamin B_{12} have been associated with poor infant growth (160). It may be that these micronutrients were limited in breast milk and restricted growth of infants in the study area. Fifth, the trend in reduced relative weight gain rates in the UNIMMAP group could suggest that the increased linear growth of infants concomitantly increased their energy requirement which was however not met by their dietary intake.

We found beneficial intervention effects in infants who are most at risk of childhood undernutrition and stunting (31). This is important as recent estimates show that respectively 27.0% and 11.3% of live newborns in LMICs are SGA and PTB (24). Mothers of infants born SGA and/or PTB are more likely to have inadequate micronutrient intakes and/or status of multiple micronutrients during pregnancy, and as such may also be at an increased risk during lactation. There is evidence that SGA and PTB newborns are born with less reserves of several micronutrients and may deplete their stores earlier in infancy (171-174, 271-273). However, both types of newborns responded differently to the postnatal intervention with UNIMMAP. This finding points towards the existence of prioritized pathways depending on the health status of the newborn.

In our study, linear growth rates during infancy were higher and reached statistical significance in SGA newborns which seems to suggest that the intervention was more effective in this subgroup. Despite the typical decrease in LAZ during the first two years of life in LMICs, one could speculate that the interruption of supplementation contributed to the observed growth faltering at 3-4 months (90). An extended supplementation period supplying UNIMMAP may prevent further growth restriction of SGA newborns on the condition that breastfeeding levels are sufficient. Nevertheless, prolonging the intervention will not have an impact on breast milk concentrations of iron and zinc which may need to be directly administered later in infancy. It is important to mention that accelerated growth leads to the expansion of hemoglobin and myoglobin mass and increases the demand for iron. As such, higher growth velocity during infancy has been shown to increase the risk of iron deficiency (274, 275). However, we did not find an effect on hemoglobin concentration rates nor anemia prevalence in infants born SGA. Although iron in breast milk is highly bioavailable, its concentration is rather low. Infants younger than six months of age therefore draw from their own iron stores at birth and the iron that is released from fetal hemoglobin during the first two weeks of life (171). Moreover, it is also important to consider whether the effect on linear growth is related with improved health. The findings of our study showed that UNIMMAP supplementation did not reduce the incidence of diarrhea, cough, and fever. However, we cannot exclude that the intervention reduced the severity of such episodes. The interpretation of these findings is hampered by a lack of biomarker data. In fact, dried blood spots of the study infants were collected but could not be analysed because of methodological problems with blood elution.

Supplementing mothers of PTB born infants with UNIMMAP increased infants' hemoglobin concentration rates without any effect on linear growth. These findings may be explained by differences in iron metabolism between PTB and SGA infants. A study in Chilean infants with low birth weights showed that PTB, appropriate-for-gestational age, infants had significantly lower hemoglobin levels compared to term SGA infants (10.3 g/dL vs 11.3 g/dL; P <0.05) at four months of age (276). Moreover, a study in Finland showed that PTB newborns have a more profound initial drop in hemoglobin concentration compared to term infants (277). When supplemented with iron, PTB infants showed increased hemoglobin concentrations once erythropoiesis became more active and gradually caught up with hemoglobin concentrations of term infants by the age of five months. Several vitamins are needed in the process of erythropoiesis, such as vitamin A, folate, vitamin B12, riboflavin and vitamin B6 (105, 107, 256). These vitamins were all supplied by the UNIMMAP tablet and may have led to an increased red blood cell production.

Our study has a number of strengths and limitations. First, community workers directly administered tablets to lactating women during daily home visits. Compliance to daily tablet intake was somewhat lower than anticipated. However, tablet compliance and the amount of visits for anthropometry measurements was not different between study groups. Second, study nurses and health center staff provided mothers with recommendations on optimal breastfeeding practices. However, we did not estimate breast milk intake in study infants. Such quantitative measurements are difficult to collect and we did not expect UNIMMAP to exert a differential effect on breastfeeding practices as compared to IFA supplementation. A previous study in neighboring southwest Burkina Faso estimated that only 35% of infants aged 3 months were exclusively breastfed in 2011 (278). The most recent Demographic Health Survey reported that 99.0% of infants 2-3 months are breastfed, the majority of which are also being fed water, juice, broth, and other liquids (279). It is therefore difficult to assess if the infants received a sufficient amount of breast milk to be able to benefit from the maternal intervention. Third, we did not collect any breast milk and venous blood samples at baseline, end of supplementation and endline. Data on breast milk micronutrient concentrations and micronutrient status in both mother and infant would have improved the interpretation of our findings.

In conclusion, our findings raise the possibility that supplementation of lactating mothers with UNIMMAP compared to IFA during three months post-delivery may increase linear growth rates of infants, and more so in infants born SGA, in a region with high growth faltering rates. Moreover, PTB newborns had improved hemoglobin concentration rates during infancy. Our findings therefore suggest that supplementation may be particularly effective for infants who are most at risk of childhood undernutrition. It is not clear if the study findings are externally valid as there are no similar studies to which we can compare our results. Nevertheless, we expect that these findings can be replicated in other settings provided that infants have sufficient access to breast milk, i.e. a high prevalence of exclusive breastfeeding up to six months of age and continued breastfeeding beyond.

Chapter 6

GENERAL DISCUSSION

As many as 36% of live births in LMICs are newborns who are either born too small and/or too soon (24). The burden of being small-for-gestational age is especially high in South Asia and Sub-Saharan Africa. Growth restriction in early life is an important determinant of infant mortality, childhood undernutrition and chronic disease in later life. Moreover, the condition is typically passed on from one generation to the next entrapping many in a vicious cycle. The health of women of reproductive age is therefore crucial and calls for attention in any attempt to break this pattern. The first 1,000 days of life, spanning the time period of conception to a child's second birthday, are considered a window of opportunity to prevent growth restriction (90). In this PhD work, we have investigated the role of modifiable prenatal and postnatal maternal factors on intrauterine and infant growth in rural Burkina Faso. More specifically, the research focused on the relationships of seasonality in prenatal nutrition and physical activity as well as postnatal micronutrient supplementation on growth restriction in early life.

6.1 Seasonality and the rainy season

Burkina Faso, as other landlocked countries in West Africa, has one main rainy season which coincides with food shortages due to dwindling supplies from the previous harvest. The rainy season is therefore also called the hunger or lean season. Subsistence-farming communities in rural areas are particularly affected. Studies in Benin, Senegal and The Gambia have shown that women have a reduced energy and protein intake during this rainy season (76, 280-282). Whereas the rainy season shows a decline in energy intake, agricultural activity in the field is typically increased and could as such lead to energy imbalance. Non-pregnant women in northeast Burkina Faso showed an average weight loss of -1.9 kg between the start and end of the rainy season (283). This change in body weight was mainly attributed to a decrease in body fat (from 23.1% to 20.3%; $P < 0.001$), which reflects the mobilization of fat stores to cover periods of food shortage. The mean BMI after the rainy season fell under 21 kg/m^2 and the prevalence of undernutrition increased from 11.1% to 17.1% during the rainy season. Furthermore, another study demonstrated that women appear to lower their basal metabolic rate in response to cues of maternal energy status (140). Energy imbalance and such adaptive responses during the rainy season have been demonstrated in non-pregnant women in central Mali, non-pregnant women in southwest Benin, and pregnant women in The Gambia (281, 284, 285).

Birth outcomes in rural areas of LMICs show distinct seasonal fluctuations. Newborns born during the rainy season are on average smaller in both length and weight. Moreover, a study in The Gambia showed that maternal weight cycles paralleled those of SGA incidence which peaked at the end of the rainy season (75). A few studies have suggested that the relation between exposure to caloric restriction or the rainy season and birth size is most apparent during late pregnancy (15, 86). Healthy women in late pregnancy need an additional 500 kcal/d compared to non-pregnant women and may thus be most vulnerable to reduced food availability (93). However, smaller associations have also been reported between caloric restriction during mid-pregnancy and birth size (86).

A dietary intake study in the study area of the rural Houndé health district in Burkina Faso showed that women had a respective estimated median energy intake of 2,019 kcal/d (IQR: 1,602 – 2,319 kcal/d) in early/mid-pregnancy and 2,060 kcal/d (IQR: 1,662 – 2,328 kcal/d) in late pregnancy at the end of the rainy season (286). The PA study in this PhD work was conducted at the same time and we estimated a respective median PA energy expenditure of 283 kcal/kg/wk and 306 kcal/kg/wk in early/mid and late pregnancy, after accounting for sleep and leisure time activity. From the MISAME 2 trial data, we calculated that women had a respective mean weight of 56 kg and 61 kg in early/mid and late pregnancy. As such, total energy expenditure was estimated at 2,261 kcal/d and 2,667 kcal/d. However, it should be noted that these are rough estimates as the PA questionnaire showed not be a valid instrument for absolute measurements. Furthermore, we did not record individual weights of pregnant women at the time of PA assessment, and women in late pregnancy may have perceived their PA as more strenuous which could increase over-reporting. Nonetheless, these findings suggest that women in late pregnancy may have been more at risk of energy imbalance during the rainy season.

6.1.1 Energy supplementation with multiple micronutrients during pregnancy

The study presented in Chapter 2 showed that women who were exposed to the rainy season during the second half of pregnancy benefited from energy supplementation in the presence of MMNs (MISAME 2 trial). The intervention, supplying 372 kcal/d through a LNS supplement, showed a clinically important effect on birth length of +13.5 mm (95%CI: 6.5 – 20.5) compared to UNIMMAP at the transition from the rainy to dry season. A recent randomized controlled trial (RCT) in Ghana reported that a lower dose of LNS (20 g; 118 kcal/d) compared to MMN supplementation improved birth length (mean difference: +6.7 mm; 95%CI: 0.6 – 12.7; P = 0.03) and birth weight (mean difference: +139 g; 95%CI: 5.8 – 272.0; P = 0.04) only in primiparous gestations. However, this study was implemented in a better nourished peri-urban population with a mean BMI of 24.8 kg/m^2 and 2.4% underweight women at study inclusion (287). A similar recent trial conducted by the same research consortium using the same doses of LNS in Malawi was unable to demonstrate any effect on birth size (288).

The MISAME 2 trial was unable to demonstrate a positive LNS effect on birth weight and, more importantly, SGA incidence remained very high (32%). There are two work hypotheses that could explain this lack of effect. First, it is possible that the energy content of the LNS supplement (372 kcal/d) was insufficient to induce a response in birth weight during the rainy season. Fetal weight gain predominantly occurs in late pregnancy, during which an additional energy intake of 500 kcal/d is recommended (93). A quantitative dietary intake study at the end of the rainy season demonstrated that women from the LNS group had a significantly higher median energy intake compared to their peers from the UNIMMAP group. More specifically, women given LNS during their early/mid-pregnancy consumed 371 kcal (P <0.001) more energy per day, whereas women in late pregnancy consumed 201 kcal (P <0.01) more compared to the comparison group (286). The smaller increase in total energy intake in late pregnancy was explained by a higher energy intake by the UNIMMAP group, whereas LNS mothers in late pregnancy had a slightly lower energy intake compared to their peers in early/mid-pregnancy. In contrast to our findings, a meta-analysis of RCTs showed that supplementation with balanced protein-energy improves birth weight (mean difference: + 41.0 g; 95%CI: 4.7 – 77.3) and reduces the risk of SGA birth (RR: 0.79; 95%CI: 0.69 – 0.90) (112). Moreover, an RCT in rural Gambian pregnant women showed that supplementation with high-energy biscuits (1,016 kcal/d; without added MMNs) compared to a control group that was given no supplements resulted in a significant increase in birth weight (mean ± SE) of 201 ± 35 g (P <0.001) in the rainy season (113). Moreover, an observational cohort study in rural Bangladesh reported that an IFA-fortified mixed flour (608 kcal/d) increased birth weight by ±183 g when mean birth weight reached its yearly minimum (133). However, the researchers did not measure birth length. We could therefore speculate that, in our study, the increase in fetal linear growth may have outpaced fetal weight growth because the LNS supplement did not provide sufficient energy for women in late pregnancy during the rainy season.

A second possible explanation of why prenatal LNS did not result in increased weight growth is the complex pathway by which the rainy season impacts fetal weight growth. It is possible that nutrition supplementation is an insufficient intervention during the rainy season, because of the presence of other contextual factors like malaria and maternal workload which also peak in this season (79, 289). A meta-analysis of clinical trials on birth outcomes has shown that the risk of LBW can be reduced by up to 43% through successful malaria prevention (69). However, the MISAME 2 trial systematically provided either two or three doses of sulfadoxine-pyrimethamine as malaria prophylaxis. The evidence on the influence of maternal workload in LMICs is less substantiated, but data from The Gambia and this PhD research in rural Burkina Faso suggest that strenuous agricultural work in the rainy season in late pregnancy could be linked to adverse birth outcomes (75). A review of observational studies, in mostly high income countries, showed that long working hours and physically demanding work have been associated with adverse birth outcomes (145-147).

In the study area in Burkina Faso, it was previously reported that LNS supplementation was most effective in mothers with a low BMI (<18.5 kg/m²) at study inclusion (188). Women in the LNS group who were underweight gave birth to taller newborns (birth length: +12.0 mm (95%CI: 3.7 – 20.2; *P* <0.01)). This effect modification has also been confirmed by a meta-analysis of balanced protein/energy supplementation studies that showed impacts on birth weight in undernourished women only (mean difference: +67.0 g; 95%CI: 13.1 – 120.8; *P* = 0.02) (112). On the other hand, the results of Chapter 2 show that LNS resulted in a significant increase in birth length (+13.5 mm; 95%CI: 6.5 - 20.5) at the transition from the rainy to dry season (September to November) compared to prenatal MMN. The conception period of these pregnancies spans the months of January to March, a period that coincided with the annual nadir of maternal BMI measured at study inclusion of the MISAME 2 trial (**Supplemental Figure 3**). The reported effect modification of LNS on birth length by season may therefore be, at least partially, confounded by a higher prevalence of underweight women at study inclusion. However, both groups responded differently in birth weight outcome. LNS showed a tendency to increase birth weight by +111 g (95%CI: -34 – 256; *P* = 0.13) in women who were underweight at study inclusion (N = 118), whereas this tendency was not observed at all in deliveries taking place between September and November. This discrepancy suggests that LNS is effective in increasing birth length both in mothers with a relative negative energy balance at early pregnancy, i.e. underweight, and during late pregnancy when exposed to the rainy season. However, the energy dose of LNS was most likely insufficient to improve birth weight substantially during the rainy season. Such a hypothesis warrants further research in larger groups of underweight women that guarantee sufficient statistical power. In addition, it is recommended to carefully assess the seasonality of maternal nutritional status, and energy intake and expenditure throughout the year. Such data, especially between the start and end of the rainy season, could have provided more insight into the extent of energy deficiency.

Finally, the results should be interpreted in light of the double burden of disease in LMICs. It is unknown if an LNS supplement for pregnant women in rural Burkina Faso will lead to unhealthy weight gain in these women and their offspring. However, we consider it unlikely that the intake of the supplement will lead to overweight or other risk factors of cardiovascular disease. Firstly, the MISAME2 trial was performed in rural Burkina Faso where undernutrition is a significant concern. Secondly, cohort data from 5 LMICs show that interventions aimed at increasing birth size in the first two years of life may offer some protection from adult chronic disease risk factors in offspring, such as high blood pressure and plasma glucose concentrations (38, 39). In fact, these studies showed associations between relative weight gain after the age of two years and adult adiposity. Moreover, birth weight was only non-significantly increased in the main trial (+ 31 g; 95%CI: -16 – 78; P = 0.20) and the increase in birth length at the transition of the rainy to dry season was not accompanied by a concurrent significant increase in birth weight. LNS supplements can also be seen as safe. Such products have a low water activity which prevents the growth of bacteria, yeast and fungi and can reduce non-enzymatic browning and lipid oxidation. Nonetheless, products with a high fat content and micronutrients with oxidative capacity such as iron are vulnerable to oxidation and may produce rancid flavors. However, these can be overcome by recent technological innovations such as encapsulation. Moreover, LNS products are usually also tested for oil and nutrient stability under field conditions.

In conclusion, the results from this PhD research suggest that an increased prenatal energy intake in the presence of MMNs has clinically important effects on fetal linear growth at the transition of the rainy to dry season in a rural setting of a LMIC. There are relatively few studies that have tested the effect of balanced energy protein across seasons in LMICs, and all have consistently shown improvements in birth size around the period of food insecurity. However, ours is the first to show an effect on linear growth which exhibits a different, and often more complex, physiologic regulation compared to gains in body mass. The hypothesis that the inclusion of MMNs into an energy-dense supplement increases linear growth should be replicated in other settings. Currently, Manary and colleagues are conducting an RCT in Malawi with three study arms in moderately undernourished pregnant women (cfr. ClinicalTrials.gov; Identifier: NCT02120599). The study groups receive equicaloric supplements as follows: a corn-soy flour supplement fortified with IFA (comparator), a corn-soy supplement fortified with UNIMMAP (first experimental arm) and LNS with 200% of the daily recommended dietary allowance for most micronutrients (second experimental arm). Such a study is interesting as on average more underweight women will participate, and the study setting allows to test many of the hypotheses that were generated in this discussion. It is expected that birth weight will increase in all study groups. However, a dose-response effect of MMNs would show evidence of more important effects on birth length in function of the MMN doses, when sufficient energy supplementation of 750 kcal/d is provided. A limitation of this study is that different food matrices are being used in the different study arms which may affect study compliance and acceptability, which may lead to a more difficult interpretation of study results.

6.1.2 Physical activity in the rainy season during pregnancy

Chapter 2 of this PhD work showed a strong peak (33%) in PTB incidence during the rainy season, whereas SGA incidence peaked at 47% at the end of the rainy season. One of the hypothesis was that a higher maternal workload and its related energy expenditure is, at least partly, responsible for these observations. Chapter 4 of this PhD work discussed the results of a physical activity study that was set up to test this hypothesis.

PA patterns of pregnant women were characterized with the use of a structured interviewer-administered questionnaire in a community-based cross-sectional study at the end of the rainy season. Pregnant women were classified into higher or lower PA levels and associations with birth outcomes were investigated. Our analyses suggested that the agricultural demands at the end of the rainy season imposed the participation of women in late pregnancy. Agricultural labor contributed most to the estimated PA energy expenditure in these women. The findings also showed that more than half of pregnant women ≥34 weeks of gestation (64.3%) reported to be working in the field.

As shown by the calculations made earlier in this chapter, we assumed that many women in late pregnancy had a negative energy balance, which probably caused the annual minimum in birth weight at the end of the rainy season (September to November; **Figure 13**). Yet, we found no evidence that higher compared to lower PA energy expenditure (PAEE) during the rainy season in late pregnancy was associated with smaller birth size.

There is a paucity of well-designed research on the effects of PA on birth outcomes in LMICs, and none have investigated the typical increase in PA in the rainy season. A longitudinal study in a subsistence-farming community in India showed that term newborns of mothers in the highest PA tercile had lower birth weights (2626 vs. 2695 g; P = 0.02) compared to those of mothers in the lowest tercile (138). The negative association appeared to be independent from seasonality (78). A retrospective study in Brazil showed that performing strenuous agricultural work during the last three months of pregnancy, but not less, was associated with a marginal reduction in birth weight (mean difference: -117 g; P = 0.05) compared to mothers performing household work (151). Moreover, a longitudinal study of term pregnancies in an adequately nourished population in the US showed that women in the highest compared to lowest quartile of total energy expenditure during late pregnancy gave birth to newborns with a lower birth weight (mean difference: -97.0 g; P = 0.04; P_{trend} = 0.10) and an increased risk of SGA (OR: 3.0; 95%CI: 1.4 – 6.7; P <0.01; P_{trend} = 0.07) (155). The reduction in birth weight seemed to be more related to the loss of adipose tissue, as air displacement plethysmography showed a significant reduction in fat mass (mean difference:

-41.1 g; P = 0.03; P_{trend} = 0.04). These findings therefore warrant careful considerations of the role of higher PA in late pregnancy.

On a different note, we found that newborns of women in early/mid-pregnancy with higher compared to lower PA levels were at a 48% reduced risk (95%CI: 0.28 – 0.98; P = 0.04) of being SGA. This finding leads us to hypothesize high PA levels in early/mid-pregnancy protect against intra uterine growth restriction. There is some evidence towards the biological plausibility of this finding. Placental growth and functionality have been shown to be stimulated by increased activity in early/mid-pregnancy, and mid-term placental weight is a positive independent predictor of birth weight (246, 248). However, we found no association between higher PA and birth weight in our study and only women who started pregnancy underweight showed a higher placental weight at delivery (β = 312.3 g; 95%CI: 69.9 – 554.6; P = 0.02). The improvement in the lower tail of the distribution may be partly attributed to the reported adaptive metabolic response in women who are at risk of energy imbalance during the rainy season. Pregnant women have been shown to adapt to an energy-constraining environment by suppressing their basal metabolic rate until the energy requirements of late pregnancy no longer support such an adaptation (140). The adaptation allows women to save energy during early/mid-pregnancy. Interestingly, the extent of this energy-sparing mechanism seems to depend on maternal energy status. One study in rural The Gambia showed a reduction in the adaptive response of women who were supplemented with energy (285). There is currently insufficient evidence whether this coping strategy is harmful to the mother.

Our study had a number of methodological limitations that need to be considered. The study was limited by the number of women who were pregnant and participating in the MISAME 2 trial during the rainy season. As a result, the effect size was set at 0.35 for continuous outcomes which corresponds to a least significant difference in birth weight outcome of ~140g. The study was therefore only powered to detect larger differences in birth outcomes. Moreover, the duration of several leisure activities could not be estimated by study participants. Hence, we were obliged to remove these activities from the analysis. However, the few questionnaires designed to assess PA in pregnant women in high income countries have shown that concurrent validity compared to objective measures is generally low and relatively lower compared to test-retest reliability (217). Chapter 3 of this PhD work showed that the PA questionnaire in our study showed similar results and showed acceptable classification agreement into higher and lower PAEE levels (test-retest reliability: ICC: 0.50, Cohen's Kappa: 0.39; concurrent validity: Spearman rank correlation: 0.42, Cohen's Kappa: 0.31). In addition, our questionnaire probed for the total duration spent working in the field, and therefore did not capture any potential changes in their agricultural tasks nor the intensity with which women perform their work. Rural Gambian pregnant women have been reported to spend as many hours on the field as their non-pregnant peers but reduced the amount of heavy work (82). Furthermore, the above mentioned calculations of total energy expenditure showed that women in late pregnancy had a higher estimated energy expenditure compared to those in early/mid-pregnancy. Our results showed that women in late pregnancy reported more time spent in PA, but this may possibly have been the result of over-reporting. Women in late pregnancy experience more fatigue which could bias their self-reported durations of PA. Probing women about their specific tasks on the field and perceived work intensity would have improved the interpretation of our findings.

In conclusion, we found that higher levels of PA during early/mid-pregnancy reduced the risk of an SGA newborn in a rural setting of a LMIC. Furthermore, we were unable to demonstrate that higher PA levels were associated with large changes in birth size. This is the first study to assess associations between PA levels in the rainy season during pregnancy and birth size in a rural setting of a LMIC.

6.2 Multiple micronutrient supplementation during lactation

A dietary intake study in the study area of the Houndé health district as well as a recent study in the nearby Sanguié province have shown that the majority of women of reproductive age in rural Burkina Faso do not meet recommended intakes of several micronutrients such as riboflavin, niacin, vitamin B6, folate, vitamin A, vitamin C and iron in the post-harvest season (263, 286). It was hypothesized that poor dietary micronutrient intake of lactating women may contribute to growth faltering during infancy, as most micronutrient requirements are highest at this stage (162). Chapter 5 presented the results of an RCT that tested the efficacy of UNIMMAP supplementation supplying 15 micronutrients (intervention) compared to only IFA supplementation (control) for three months post-delivery on infant growth and morbidity.

Women who were previously enrolled in the prenatal MISAME 1 trial and gave birth to a live newborn were included in the postnatal supplementation trial. Infants generally failed to thrive during infancy as demonstrated by decreasing mean LAZ scores in both study groups. UNIMMAP supplementation did not result in improved growth in the overall group of infants. Nonetheless, the intervention showed a borderline tendency towards a slightly improved linear growth rate (β = 0.0119 LAZ/month; 95%CI: -0.0026 – 0.0263; $P_{\text{UNIMMAPxage}}$ = 0.107), but also a decrease in relative weight gain rate (β = -0.0159 WLZ/month; 95%CI: -0.0349 – 0.0031; $P_{\text{UNIMMAPxage}}$ = 0.101) compared to infants in the IFA group during the first 12 months of life. The intervention effect on postnatal linear growth rate was stronger and statistically significant in infants born SGA.

We calculated, from the mean growth rates, that UNIMMAP would result in a 0.14 higher LAZ (95%CI: -0.03 – 0.32) and 0.19 lower WLZ (95%CI: -0.42 – 0.04) compared to IFA over the course of 12 months. A pooled data analysis of the WHO Global Database on Child Growth and Malnutrition shows that infants in the Sub-Saharan African region falter in linear growth by an estimated average of ±1 LAZ over the same period of time (90). The observed increase in linear growth rate in our study could therefore result in an approximate 14% decline in infant linear growth faltering. The increase in linear growth rate of SGA infants was higher and estimated to result in an approximate 27% decline in linear growth faltering between study groups. These estimates suggest that the effect of UNIMMAP compared to IFA supplementation on infant growth is relatively small, yet possibly important, especially in the group of newborns who experienced intra uterine growth restriction. These infants also showed a, non-significant, negative trend in stunting prevalence at their last visit (OR: 0.77; 95%CI: 0.48 – 1.25; P = 0.29). However, it remains unclear what triggered the relatively higher growth rates in this group. A possible explanation might be that SGA newborns, in general, show some catch-up growth in the first months of postnatal life due to a delay in normal growth plate senescence (290). It could thus be speculated that UNIMMAP provides more essential micronutrients to support the relative growth spurt.

A concomitant negative trend in the rate of relative weight gain in the overall group suggests that energy intake may have been insufficient to keep up with the relatively higher linear growth rates. Although we provided lactating women with recommendations on optimal breastfeeding, the rate of exclusive breastfeeding in our population was estimated to be low. The most recent DHS in Burkina Faso showed a 25.3% prevalence of exclusive breastfeeding of infants at 2 to 3 months of age (279). Almost all these infants were reported to be breastfed but most were fed additional liquids such as water, juices, broths, etc. As such, UNIMMAP supplementation might have benefited from a more intensive concurrent breastfeeding promotion program. A peer counselling intervention in southwest Burkina Faso demonstrated to be effective in sustaining exclusive breastfeeding throughout the first six months of life (278). Respectively 79% and 73% of women were breastfeeding exclusively in the intervention group at three and six months post-delivery, compared to 35% and 22% in the control group.

It could be argued that a combined supplementation of protein energy and UNIMMAP during lactation might have resulted in a stronger impact on infant growth. The caloric content of term breast milk in populations with an adequate nutritional status has been estimated to be

60 kcal/dL (± 2SD: 44 – 77) at one week of lactation (291). Starting from that time, breast milk fat content increases from 2.2 g/dL to 3.4 g/dL at three months of age. However, fat concentrations have been shown to decline with infant age when maternal nutritional status is inadequate. The fat content of breast milk of marginally nourished Bangladeshi mothers was reported to be only 2.8 g/dL at three months of age (292). It was therefore hypothesized that combined supplementation of both energy and MMNs to undernourished lactating women may have potential to improve infant growth beyond the promotion of exclusive breastfeeding. There are a few RCTs, in Malawi, Guatemala and South Africa, that examined the effects of maternal supplementation with energy and MMNs during lactation only on infant growth (293-295). However, none was able to demonstrate an effect on infant growth. Moreover, the evidence of such interventions starting from pregnancy is inconclusive. Even a recent RCT in rural Malawi that started supplementing women with LNS (20 g/d) during pregnancy until six months post-partum and subsequently their infants from 6 to 18 months of age was unable to demonstrate significant effects on infant growth (296).

The study presented in Chapter 5 has a number of limitations that hamper a straightforward interpretation of the findings. First, we did not assess breast milk intake of infants, nor micronutrient concentrations in breast milk and blood. Therefore, it cannot be excluded that suboptimal breastfeeding practices limited the potential impact of UNIMMAP. Second, we did not collect data on micronutrient status of the infants. Such data would have been rather useful as an independent coverage indicator, as well as an essential intermediate indicator on the mechanistic pathway to improved infant growth.

Finally, it must be emphasized that the supplements used in this study were considered to be safe and therefore beneficial to lactating women in their breastfeeding infants. The choice of which micronutrients and doses of the micronutrients in IFA and UNIMMAP have been subject to a critical appraisal of the available evidence on existing deficiencies in LMICs, the beneficial impact of supplementation on the mother and child, the supply by the diet, the tolerable upper intake level at which no adverse effects are observed, possible side effects of supplementation… Firstly, anemia is a serious problem in rural settings of LMIC and IFA has shown beneficial effects in pregnant women. IFA is used in lactating women for the same reasons to support recovery from pregnancy. Increased iron doses have been reported to be able to cause side effects, especially in women who are most vulnerable. However, it was reasoned that the advantages of providing IFA outweigh any possible risks in such populations. Secondly, the micronutrient composition of the UNIMMAP supplement was designed for healthy pregnant women in the US and Canada and most micronutrients were dosed at 1 RDA. This dose is considered safe, because the micronutrient intake of populations of LMICs is low and their requirements are considered to be much higher. Moreover, lactating women generally have higher requirements. Some micronutrients dosed at RDA levels may even be too low to correct for deficiencies. It is also noteworthy that such supplements are given in HICs without posing any health problems. Similarly, withholding such a supplement from malnourished women was considered inappropriate from a public health point of view. Lastly, the children in the lactation study received breast milk from their mothers and were not directly exposed to the (synthetic) tablet with MMNs. Because lactating women and infants have relatively higher requirements, and seeing the general lack of side effects in pregnant women, these children were hypothesized to benefit from the intervention. The intervention study in the study area also did not show any differential effects on morbidity in the two study groups.

In conclusion, UNIMMAP compared to IFA supplementation in lactating mothers may improve infant linear growth, especially in those who experienced intra uterine growth restriction, in a rural setting of a LMIC with low compliance to exclusive breastfeeding in the first six months of life. This is the first study to report the effects of UNIMMAP compared to IFA supplementation in lactating women on infant growth and morbidity. We expect that our results can be replicated in other settings if exclusive breastfeeding levels are sufficiently high during the first six months of infancy.

6.3 Recommendations for policy

Previous trials, including the MISAME2 trial, have demonstrated the efficacy of prenatal balanced protein energy supplementation on birth size, especially in undernourished pregnant women. The findings of the seasonality analyses of the MISAME2 trial in Chapter 2 suggest that pregnant populations in rural subsistence-farming communities could also benefit from blanket supplementation during the rainy season. The MISAME2 trial was a randomized controlled efficacy trial which was implemented under optimally controlled conditions, i.e. a specifically formulated food supplement that didn't substitute the usual diet, daily intake of the food supplement under direct observation, etc. As a next step, evidence from pragmatic studies performed under real-life conditions is required to inform and guide policy and health decision-makers on feasible, culturally acceptable and cost-effective programs.

It has increasingly been recognized that true progress requires a multi-sectoral approach and not just the up-scaling of nutrition-specific interventions, such as maternal dietary and micronutrient supplementation, which address the direct determinants of fetal and child nutrition (297). In fact, it has been recommended to integrate such interventions with nutrition-sensitive programs which address the underlying determinants of undernutrition such as food security, caregiving resources, access to health services, etc (**Figure 22**). On the one hand, such programs can act as delivery platforms of nutrition-specific interventions and are therefore likely to increase coverage and effectiveness of these interventions. On the other hand, nutrition-sensitive programs such as agricultural and food security programs as well as social safety nets have so far shown little or no impact on child nutritional status and could benefit from increasing their 'nutrition' sensitivity (297). The lack of impact may be due to poor timing and implementation, the duration of exposure to the program, and the lack of nutrition objectives. Nonetheless, such programs have shown beneficial effects on food security, reduced poverty, increased use of health services and women empowerment. These improvements could lead to spillover effects on nutrition over the longer term, and may reduce vulnerability to shocks, if only the direct determinants of nutrition are sufficiently changed. In this regard, it is of note that the rainy season not only exacerbates nutritional status but also the underlying causes of undernutrition. The integration of nutrition-specific and nutrition-sensitive programs is therefore likely to synergistically or additively benefit the outcome on maternal and child nutritional status. A few suggestions for such multi-sectoral approaches on the short and longer term are discussed below.

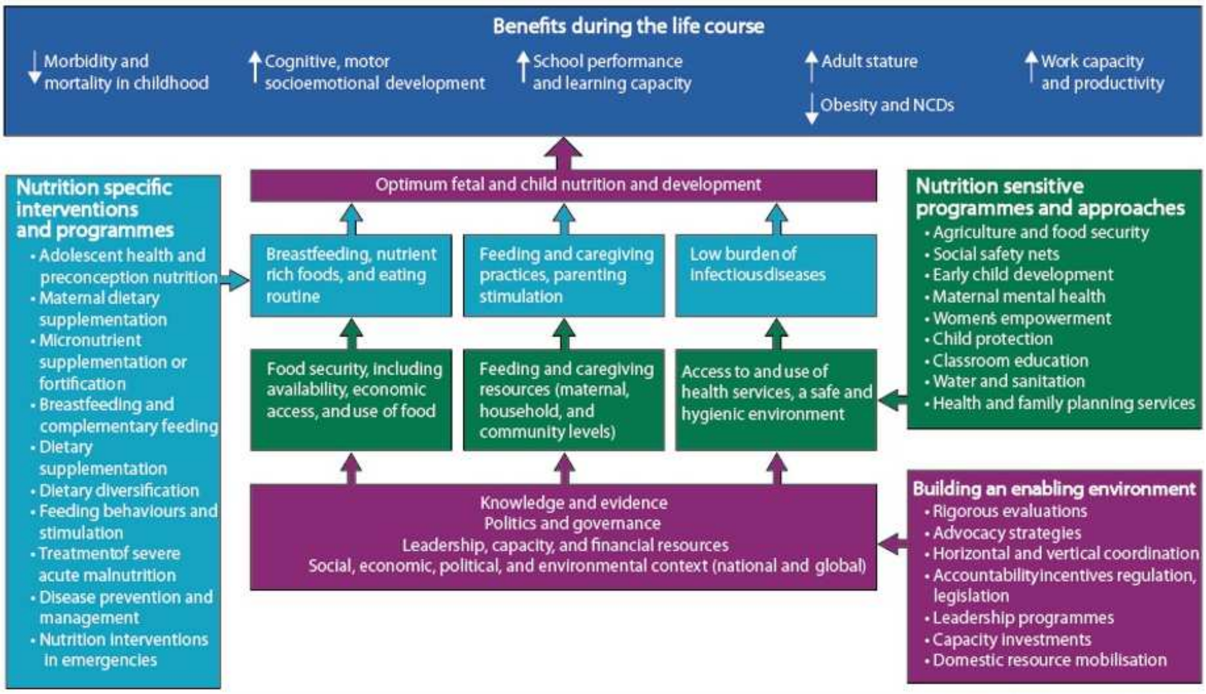


Figure 22. Action framework for optimal fetal and child nutrition and development (Black et al., 2013 (298)). NCDs, Noncommunicable Diseases.

Social safety nets are programs that provide cash and/or food transfers to vulnerable low-income households to augment income, investments in human capital and resilience to sudden shocks. Cash transfers improve the underlying determinants of nutrition. The cash may be used to create more sustainable household assets that can have an impact on nutrition, and it may lead to more available time for child care (299). Cash transfers leave households to decide how to meet their needs. However, transfers are sometimes combined with conditions or other interventions that enhance nutrition-sensitivity. For instance, the preventive distribution of a fortified flour or LNS supplement combined with cash transfers in the rainy season halved the incidence of child acute malnutrition compared to those who received cash or supplementary food only in Niger (300). Moreover, conditional cash transfers have been used to overcome the financial barrier in seeking health care (301). Recent evidence from Burkina Faso shows that antenatal care visits of pregnant women can be increased by providing cash on the condition that families attend preventive health care services (302).

Antenatal services present a unique opportunity to integrate nutrition-specific and other nutrition-sensitive interventions in pregnant women in rural Burkina Faso. Firstly, optimal antenatal care is positively associated with having a baby with a normal birth weight (OR: 1.62; 95%CI: 1.11 – 2.38) and could as such have spillover effects (303). Secondly, antenatal care services can be used as delivery platforms of preventive food distribution programs during the rainy season. Dietary patterns are unlikely to change over a short period of time and the distribution of energy- and nutrient-dense food supplements may be required on the short term. Alternative recipes of LNS supplements based on locally produced foods can lower the cost and increase cultural acceptability. Other programs have successfully used ingredients such as whey, soy protein, sesame, cashew and chickpea paste, and flavoring such as tomato and onion. A recent publication has already shown that these cheaper products are acceptable and not inferior for the treatment of severe acute malnutrition in under-five children (304). Moreover, engaging local women in the production of these food supplements will increase their skills and stimulate their livelihoods and empowerment. Thirdly, health centers are able to screen pregnant women for stage of pregnancy. The MISAME2 trial showed that only those women in the second half of pregnancy benefited from a food supplement. Programs are more (cost-)effective when interventions are targeted to the most vulnerable population group. Fourthly, antenatal care can be effectively used as a platform for nutrition education. A recent, unpublished, randomized controlled trial in the same study area in Burkina Faso has shown that patient-centered communication to promote good feeding practices for pregnant and lactating women and young children is associated with increased birth weight (Nikièma L., unpublished). Knowledge can potentially also increase compliance to food supplementation. Finally, as pregnant women are more likely to have under-five children, antenatal services could link up with water, sanitation and health (WASH) interventions to promote hygiene and reduce infectious diseases. A meta-analyses showed that WASH programs result in a small benefit on linear growth in under-five children when implemented for 9-12 months (305).

It should be noted that the beneficial synergies of such programs will be improved by good quality and accessibility of care, skilled staff and attendance at birth, childbirth at the health center, good infrastructure, and sufficient coverage of the programs (306, 307). Moreover, beneficial effects on birth outcomes are only expected if women attend a sufficient amount of consultations. The WHO recommends at least four antenatal care visits to pregnant women. Compliance to these conditions and coverage should therefore be monitored by the program. It is also of note that blanket food supplementation is expected to have a larger impact on birth size if implemented throughout the full duration of the rainy season, as our findings have shown. The onset and end of the rainy season have been shown to differ by as much as respectively two months and one month in Gaoua, approximately 100 km south of Houndé (308). Although monitoring local climatological conditions can pinpoint the exact timing of the rainy season, a timely and organized response in the distribution of nutritious foods would require a more pragmatic approach of selecting a fixed time period, such as from 1st May to the 1st October in our study area.

It is important to remark that more sustainable alternatives to supplementation with fortified foods will require more time. The population in the study area in rural Burkina Faso knows low employment levels, high poverty levels, and relies on agricultural production in small farms. Our results show that pregnant women in rural settings would benefit from more energy-dense and nutrient-dense foods. Agricultural programs could therefore invest more in cultivating energy-dense crops, such as peanuts and shea nuts which could be processed into oil/fat supplements for pregnant women, a diversification of the agricultural production such as bio-fortified crops, underutilized foods as well as raising small-scale livestock, and the production of more nutrient-dense foods. These programs are likely to improve nutrition outcomes over the long term by targeting women and linking these activities to women empowerment, e.g. nutrition education, acquiring skills, and promotion of decision-making power over gained income. Moreover, such programs could be combined with increasing domestic and global market access of these small farmers.

A few recommendations can also be put forward for the current national policy of Burkina Faso which prescribes food supplementation to undernourished pregnant and lactating mothers, i.e. MUAC <23 cm (309). In practice, the World Food Program (WFP) delivers fortified corn-soy blends (DSM, The Netherlands) to health centers in those areas with a high prevalence of undernutrition. The rations are intended to supplement the usual diet and are distributed every two weeks until these women attain the discharge criteria of the program. Firstly, to our knowledge, there have been no effectiveness studies on this program nor are there any currently available estimates of coverage. Screening for undernutrition in pregnancy is mostly performed during antenatal consultations at the health center. A minimum of four visits is recommended by the WHO. However, the latest DHS estimated that only 31% of mothers in rural areas of Burkina Faso complied with the recommended number of antenatal consultations (279). Therefore, the implementation of this policy should be better monitored. Secondly, the currently used food supplement has not been optimized for pregnant and lactating women. The policy advises the fortified blend to be mixed with a small quantity of oil at a dose of 900 - 1,100 kcal/d. Yet, the micronutrient composition of the corn-soy blend is not tailored to the nutritional needs of pregnancy and lactation. It is rather considered as an all-round supplement for women and children with lower nutrient requirements. Thirdly, it is recommended that supplements for underweight pregnant and lactating women are produced as much as possible with locally available foods of which the government should take ownership of in terms of availability, quality control and distribution. Currently, the WFP is still fulfilling this role.

The nature of the PA study of this PhD research does not lend itself to formulating policy recommendations. The exploratory study had a cross-sectional design and was nested in the MISAME2 trial. Moreover, this is the first study of the associations between prenatal PA during the rainy season and birth outcome in a rural setting of an LMIC. This research area would benefit from well-designed longitudinal and low-risk experimental trials in different settings.

Lastly, the findings of this PhD research on postnatal supplementation of lactating women with MMNs, the first study of its kind, do not present strong evidence that the current national policy of Burkina Faso which recommends IFA supplementation to lactating mothers should be changed to UNIMMAP supplementation. Further research on UNIMMAP supplementation during lactation should focus on integrated interventions that pay equal attention to breastfeeding practices as well as to supplementation itself.

6.4 Recommendations for further research

The findings of this PhD research raise a few points for further research into strategies to improve both prenatal and postnatal child growth and development.

Our findings in Chapter 2 showed that LNS compared to UNIMMAP supplementation had a maximal effect on birth length at the transition between the rainy and dry season. We recommend that future studies of nutrition supplementation in LMICs add the analysis of effect modifications by season in their evaluation design to gain more insight into possible mechanisms.

The differential effect of LNS on birth length and birth weight calls for more research on the optimal dose of energy, especially in late pregnancy. Furthermore, the MMN composition is subject for debate. We used the UNIMMAP formulation to deliver the MMNs as proposed by an expert committee. However, dose-response studies of LNS with different quantities of MMNs are warranted in order to assess the optimal doses to sustain fetal linear growth.

It remains unclear if the effect modification of LNS on birth length by seasonality is caused by a negative energy balance at the start of pregnancy or throughout pregnancy. Some authors have suggested that maternal nutrition in early pregnancy affects partitioning of energy and nutrients between the maternal and child compartment throughout pregnancy (310, 311). Therefore, we recommend measuring maternal nutritional status, energy intake and expenditure, throughout pregnancy and seasons to clarify the role of energy deficiency and possible differential effects of supplementation on infant and maternal outcomes.

It is important to consider whether combined energy and MMNs supplementation can be offered by other, preferably locally produced, food matrices instead of LNS. The choice of the matrix in which to embed MMNs is subject to several factors which influence the absorption and metabolism of MMNs, such as bulkiness, anti-nutrient content, interactions among micronutrients and with the food matrix. In our study, a small energy-dense LNS supplement was preferred to avoid any substitution effects in the home diet and to increase convenience for community workers who monitored the consumption of such a small quantity. Nevertheless, additional research should investigate alternative formulations that are potentially more cost-effective. Our recommendation for Burkina Faso would be to first compare the LNS used in the MISAME 2 trial with the SuperCereal® that is currently being distributed to undernourished pregnant women in Burkina Faso as part of the national policy.

There is a lack of research into the development of instruments to assess PA in rural populations of LMICs. Validated questionnaires such as the International Physical Activity Questionnaire perform poorly in such settings (218). Our study showed that the population had serious difficulty estimating durations of activity due to a lack of awareness of time. The combination of questionnaires and accelerometers with heart rate monitoring allows to assess different dimensions of PA and may as such improve accuracy and precision of individual estimates of PA of pregnant women (217, 312-315). Moreover, more research is needed on the energetic cost of activities at different stages of pregnancy and the selection of appropriate accelerometer cut-points for pregnant women in LMICs.

The cross-sectional study to assess the association between PA in the rainy season and birth outcome had a rather simple design to test such a complex association. Longitudinal studies in women in whom either the second or third trimester falls into the rainy season need be setup to confirm the current estimates. Moreover, an intervention study in which one group of pregnant women is assigned to performing no agricultural labor, the main contributor to PA energy expenditure, could provide more evidence on the effects of agricultural labor in the rainy season. Such an intervention will however reduce available resources and should be combined with sufficient support of livelihoods.

The postnatal trial in lactating women was the first study to report the effect of UNIMMAP compared to IFA supplementation. As such, the study needs to be replicated in different settings. Similar to the prenatal trials, the effect of combined energy and MMNs supplementation and the dose-response effect of different doses of MMNs should be investigated and supported by measurements of breast milk intake and micronutrient concentrations in breast milk and blood. Future intervention studies should also ensure that breastfeeding levels are sufficiently high during micronutrient supplementation. Furthermore, the hypothesis that supplementation of mothers of the subgroup of SGA born infants could to some extent decrease linear growth restriction should be replicated by intervention studies including larger samples of SGA newborns to guarantee sufficient statistical power.

References

1. Langer A, Meleis A, Knaul FM, Atun R, Aran M, Arreola-Ornelas H, et al. Women and health: The key for sustainable development. Lancet. 2015;386(9999):1165-210.

2. George A. Human resources for health: a gender analysis. Paper commissioned by the Women and Gender Equity Knowledge Network. Geneva: World Health Organization, 2007.

3. Fine A, Kotelchuck M. Rethinking MCH: The life course model as an organizing framework. Washington, DC: US Department of Health and Human Services, 2010.

4. Martorell R, Rivera JA, Schroeder DG, Ramakrishnan U, Pollitt E, Ruel M. Consecuencias a largo plazo del retardo en crecimiento en la ninez. Arch Latinoam Nutr. 1995;45:109-13S.

5. Martorell R, Ramakrishnan U, Rivera J, Melgar P. Stunting at 3 years of age in Guatemalan girls and birth weight of their children. FASEB J. 1996;10(3):1091.

6. Subramanian SV, Ackerson LK, Smith GD, John NA. Association of maternal height with child mortality, anthropometric failure, and anemia in India. J Am Med Assoc. 2009;301(16):1691-701.

7. ACC/SCN. 2nd Report on the World Nutrition Situation. Geneva: United Nations Standing Committee on Nutrition, 1992.

8. WHO. International statistical classification of diseases and related health problems - 10th revision. Geneva: World Health Organization, 2011.

9. Katz J, Lee ACC, Kozuki N, Lawn JE, Cousens S, Blencowe H, et al. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. Lancet. 2013;382(9890):417-25.

10. Kramer MS. Intrauterine growth and gestational duration determinants. Pediatrics. 1987;80(4):502-11.

11. Kramer MS. The epidemiology of adverse pregnancy outcomes: An overview. J Nutr. 2003;133(5):S1592-6.

12. Maulik D. Fetal growth compromise: Definitions, standards, and classification. Clin Obstet Gynecol. 2006;49(2):214-8.

13. WHO. Physical status: the use of and interpretation of anthropometry. Report of a WHO expert committee. Geneva: World Health Organization, 1995.

14. Godfrey KM. Maternal regulation of fetal development and health in adult life. Eur J Obstet Gynecol Reprod Biol. 1998;78(2):141-50.

15. Neufeld L, Pelletier DL, Haas JD. The timing hypothesis and body proportionality of the intra-uterine growth retarded infant. Am J Hum Biol. 1999;11(5):638-46.

16. Rasmussen S, Kiserud T, Albrechtsen S. Foetal size and body proportion at 17-19 weeks of gestation and neonatal size, proportion, and outcome. Early Hum Dev. 2006;82(10):683-90.
17. Tanner JM. Foetus into man: physical growth from conception to maturity. Cambridge, MA: Harvard University Press; 1978. 250 p.
18. Vik T, Vatten L, Jacobsen G, Bakketeig LS. Prenatal growth in symmetric and asymmetric small-for-gestational-age infants. Early Hum Dev. 1997;48(1-2):167-76.
19. Neufeld L, Pelletier DL, Haas JD. The timing of maternal weight gain during pregnancy and fetal growth. Am J Hum Biol. 1999;11(5):627-37.
20. Villar J, Ismail LC, Victora CG, Ohuma EO, Bertino E, Altman DG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. Lancet. 2014;384(9946):857-68.
21. Papageorghiou AT, Ohuma EO, Altman DG, Todros T, Ismail LC, Lambert A, et al. International standards for fetal growth based on serial ultrasound measurements: the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project. Lancet. 2014;384(9946):869-79.
22. Villar J, Papageorghiou AT, Pang R, Ohuma EO, Ismail LC, Barros FC, et al. The likeness of fetal growth and newborn size across non-isolated populations in the INTERGROWTH-21st Project: the Fetal Growth Longitudinal Study and Newborn Cross-Sectional Study. Lancet Diabetes Endocrinol. 2014;2(10):781-92.
23. UNICEF, WHO. Low birthweight: country, regional and global estimates. New York: United Nations Children's Fund, 2004.
24. Lee ACC, Katz J, Blencowe H, Cousens S, Kozuki N, Vogel JP, et al. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. Lancet Glob Health. 2013;1(1):E26-36.
25. Kramer MS, McLean FH, Boyd ME, Usher RH. The validity of gestational age estimation by menstrual dating in term, preterm, and postterm gestations. J Am Med Assoc. 1988;260(22):3306-8.
26. WHO. Expert group on prematurity. Final Report. Geneva: World Health Organization, 1950.
27. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40.
28. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition 1 - Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008;371(9608):243-60.

29. Scrimshaw NS. Historical concepts of interactions, synergism and antagonism between nutrition and infection. J Nutr. 2003;133(1):316S-21S.
30. Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection, and immunity: An overview. Am J Clin Nutr. 1997;66(2):464-77.
31. Christian P, Lee SE, Angel MD, Adair LS, Arifeen SE, Ashorn P, et al. Risk of childhood undernutrition related to small-for-gestational age and preterm birth in low- and middle-income countries. Int J Epidemiol. 2013;42(5):1340-55.
32. Walker SP, Wachs TD, Gardner JM, Lozoff B, Wasserman GA, Pollitt E, et al. Child development in developing countries 2 - Child development: risk factors for adverse outcomes in developing countries. Lancet. 2007;369(9556):145-57.
33. Walker SP, Wachs TD, Grantham-McGregor S, Black MM, Nelson CA, Huffman SL, et al. Child Development 1 Inequality in early childhood: risk and protective factors for early child development. Lancet. 2011;378(9799):1325-38.
34. Barker DJP, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischemic heart-disease. Lancet. 1989;2(8663):577-80.
35. Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD. Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet. 1996;348(9040):1478-80.
36. Leon DA, Lithell HO, Vagero D, Koupilova I, Mohsen R, Berglund L, et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. Br Med J. 1998;317(7153):241-5.
37. Hales CN, Barker DJP. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992;35(7):595-601.
38. Adair LS, Fall CHD, Osmond C, Stein AD, Martorell R, Ramirez-Zea M, et al. Associations of linear growth and relative weight gain during early life with adult health and human capital in countries of low and middle income: findings from five birth cohort studies. Lancet. 2013;382(9891):525-34.
39. Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, et al. Maternal and child undernutrition 2 - Maternal and child undernutrition: consequences for adult health and human capital. Lancet. 2008;371(9609):340-57.
40. Moore SE, Cole TJ, Poskitt EME, Sonko BJ, Whitehead RG, McGregor IA, et al. Season of birth predicts mortality in rural Gambia. Nature. 1997;388(6641):434.
41. de Onis M, Martorell R, Garza C, Lartey A, WHO Multicentre Growth Reference. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr. 2006;95:76-85.
42. WHO. Physical status: The use and interpretation of anthropometry: report of a WHO Expert Committee. Review. Geneva: World Health Organisation, 1995, 0512-3054.

43. Roberfroid D, Huybregts L, Lachat C, Vrijens F, Kolsteren P, Guesdon B. Inconsistent diagnosis of acute malnutrition by weight-for-height and mid-upper arm circumference: contributors in 16 cross-sectional surveys from South Sudan, the Philippines, Chad, and Bangladesh. Nutr J. 2015;14:8.
44. Briend A, Maire B, Fontaine O, Garenne M. Mid-upper arm circumference and weight-for-height to identify high-risk malnourished under-five children. Matern Child Nutr. 2012;8(1):130-3.
45. WHO. Guideline: Updates on the management of severe acute malnutrition in infants and children. Geneva: World Health Organization, 2013.
46. UNICEF, WHO, The World Bank. UNICEF-WHO-World Bank Joint Child Malnutrition Estimates. New York, Geneva, Washington DC: United Nations Children's Fund, World Health Organization, The World Bank, 2015.
47. UNICEF. The State of the World's Children 2015. New York: United Nations Children's Fund, 2014.
48. McDonald CM, Olofin I, Flaxman S, Fawzi WW, Spiegelman D, Caulfield LE, et al. The effect of multiple anthropometric deficits on child mortality: meta-analysis of individual data in 10 prospective studies from developing countries. Am J Clin Nutr. 2013;97(4):896-901.
49. Olofin I, McDonald CM, Ezzati M, Flaxman S, Black RE, Fawzi WW, et al. Associations of suboptimal growth with all-cause and cause-specific mortality in children under five years: A pooled analysis of ten prospective studies. PLoS One. 2013;8(5):e64636.
50. Walker CLF, Lamberti L, Adair L, Guerrant RL, Lescano AG, Martorell R, et al. Does childhood diarrhea influence cognition beyond the diarrhea-stunting pathway? PLoS One. 2012;7(10):e47908.
51. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B, et al. Child development in developing countries 1 - Developmental potential in the first 5 years for children in developing countries. Lancet. 2007;369(9555):60-70.
52. Kramer MS. Determinants of low birth weight - Methodological assessment and meta-analysis. Bull World Health Organ. 1987;65(5):663-737.
53. Rogol AD, Clark PA, Roemmich JN. Growth and pubertal development in children and adolescents: effects of diet and physical activity. Am J Clin Nutr. 2000;72(2):S521-8.
54. Wamani H, Åstrøm AN, Peterson S, Tumwine JK, Tylleskär T. Boys are more stunted than girls in Sub-Saharan Africa: a meta-analysis of 16 demographic and health surveys. BMC Pediatr. 2007;7(1):1-10.
55. Stein AD, Wang M, Martorell R, Norris SA, Adair LS, Bas I, et al. Growth patterns in early childhood and final attained stature: Data from five birth cohorts from low- and middle-income countries. Am J Hum Biol. 2010;22(3):353-9.

56. Kozuki N, Katz J, Lee ACC, Vogel JP, Silveira MF, Sania A, et al. Short maternal stature increases risk of small-for-gestational-age and preterm births in low-and middle-income countries: Individual participant data meta-analysis and population attributable fraction. J Nutr. 2015;145(11):2542-50.
57. James WPT, Ferroluzzi A, Waterlow JC. Definition of chronic energy deficiency in adults. Report of a working party of the International Dietary Energy Consultative Group. Discussion. 1988 Dec. Report No.: 0954-3007 Contract No.: 12.
58. Han Z, Mulla S, Beyene J, Liao G, McDonald SD, Knowledge Synth G. Maternal underweight and the risk of preterm birth and low birth weight: a systematic review and meta-analyses. Int J Epidemiol. 2011;40(1):65-101.
59. Chen XK, Wen SW, Fleming N, Demissie K, Rhoads GG, Walker M. Teenage pregnancy and adverse birth outcomes: a large population based retrospective cohort study. Int J Epidemiol. 2007;36(2):368-73.
60. Institute of Medicine, National Research Council. Weight gain during pregnancy: reexamining the guidelines. Washington, DC: The National Academies Press; 2009.
61. Susser M. Maternal weight gain, infant birth weight, and diet - causal sequences. Am J Clin Nutr. 1991;53(6):1384-96.
62. Stein Z, Susser M. The Dutch Famine, 1944-1945, and the reproductive process. I. Effects on six indices at birth. Pediatr Res. 1975;9(2):70-6.
63. Dror DK, Allen LH. Effect of vitamin B-12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev. 2008;66(5):250-5.
64. Strand TA, Taneja S, Bhandari N, Refsum H, Ueland PM, Gjessing HK, et al. Folate, but not vitamin B-12 status, predicts respiratory morbidity in north Indian children. Am J Clin Nutr. 2007;86(1):139-44.
65. Sherwin JC, Reacher MH, Dean WH, Ngondi J. Epidemiology of vitamin A deficiency and xerophthalmia in at-risk populations. Trans R Soc Trop Med Hyg. 2012;106(4):205-14.
66. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. PLoS One. 2012;7(11):11.
67. Black RE, Alderman H, Bhutta ZA, Gillespie S, Haddad L, Horton S, et al. Maternal and child nutrition: building momentum for impact. Lancet. 2013;382(9890):372-5.
68. Imdad A, Bhutta ZA. Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries: a meta-analysis of studies for input to the lives saved tool. BMC Public Health. 2011;11:14.
69. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7(2):93-104.

70. Guyatt HL, Snow RW. Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. Clin Microbiol Rev. 2004;17(4):760-9.
71. Cottrell G, Mary JY, Barro D, Cot M. The importance of the period of malarial infection during pregnancy on birth weight in Tropical Africa. Am J Trop Med Hyg. 2007;76(5):849-54.
72. Landis SH, Lokomba V, Ananth CV, Atibu J, Ryder RW, Hartmann KE, et al. Impact of maternal malaria and under-nutrition on intrauterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo. Epidemiol Infect. 2009;137(2):294-304.
73. Steketee RW, Wirima JJ, Hightower AW, Slutsker L, Heymann DL, Breman JG. The effect of malaria and malaria prevention in pregnancy on offspring birthweight, prematurity, and intrauterine growth retardation in rural Malawi. Am J Trop Med Hyg. 1996;55(Suppl 1):33-41.
74. Guerrant RL, Oria RB, Moore SR, Oria MOB, Lima AAM. Malnutrition as an enteric infectious disease with long-term effects on child development. Nutr Rev. 2008;66(9):487-505.
75. Rayco-Solon P, Fulford AJ, Prentice AM. Differential effects of seasonality on preterm birth and intrauterine growth restriction in rural Africans. Am J Clin Nutr. 2005;81(1):134-9.
76. Prentice AM, Whitehead RG, Roberts SB, Paul AA. Long-term energy balance in child-bearing Gambian women. Am J Clin Nutr. 1981;34(12):2790-9.
77. Mumburi JM. Assessment of seasonal variation on food and nutrient intake of pregnant women in rural Burkina Faso. Ghent: Ghent University; 2007.
78. Rao S, Kanade AN, Yajnik CS, Fall CH. Seasonality in maternal intake and activity influence offspring's birth size among rural Indian mothers--Pune Maternal Nutrition Study. Int J Epidemiol. 2009;38(4):1094-103.
79. Bleiberg FM, Brun TA, Goihman S. Duration of activities and energy expenditure of female farmers in dry and rainy season in Upper-Volta. Br J Nutr. 1980;43(1):71-82.
80. Jiang TN, Christian P, Khatry SK, Wu L, West KP. Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural nepali pregnant women. J Nutr. 2005;135(5):1106-12.
81. Panterbrick C. Seasonality of energy expenditure during pregnancy and lactation for rural Nepali women. Am J Clin Nutr. 1993;57(5):620-8.
82. Roberts SB, Paul AA, Cole TJ, Whitehead RG. Seasonal changes in activity, birth weight and lactational performance in rural Gambian women. Trans R Soc Trop Med Hyg. 1982;76(5):668-78.
83. Rao S, Yajnik CS, Kanade A, Fall CHD, Margetts BM, Jackson AA, et al. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune maternal nutrition study. J Nutr. 2001;131(4):1217-24.

84. Hughes MM, Katz J, Mullany LC, Khatry SK, LeClerq SC, Darmstadt GL, et al. Seasonality of birth outcomes in rural Sarlahi District, Nepal: a population-based prospective cohort. BMC Pregnancy Childbirth. 2014;14:310.
85. Scammon RE, Calkins LA. The development and growth of the external dimensions of the human body in the fetal period. Minneapolis: The University of Minnesota Press; 1929.
86. Stein AD, Zybert PA, van de Bor M, Lumey LH. Intrauterine famine exposure and body proportions at birth: the Dutch Hunger Winter. Int J Epidemiol. 2004;33(4):831-6.
87. Prentice AM, Cole TJ, Foord FA, Lamb WH, Whitehead RG. Increased birthweight after prenatal dietary supplementation of rural African women. Am J Clin Nutr. 1987;46(6):912-25.
88. Dominguez-Salas P, Moore SE, Cole D, da Costa KA, Cox SE, Dyer RA, et al. DNA methylation potential: dietary intake and blood concentrations of one-carbon metabolites and cofactors in rural African women. Am J Clin Nutr. 2013;97(6):1217-27.
89. Rickard IJ, Courtiol A, Prentice AM, Fulford AJC, Clutton-Brock TH, Lummaa V. Intergenerational effects of maternal birth season on offspring size in rural Gambia. Proc R Soc B-Biol Sci. 2012;279(1745):4253-62.
90. Victora CG, de Onis M, Hallal PC, Blossner M, Shrimpton R. Worldwide timing of growth faltering: revisiting implications for interventions. Pediatrics. 2010;125(3):E473-80.
91. Richte L, King J. Nutrient recommendations and dietary guidelines for pregnant women. In: Lammi-Keefe CJ, Couch SC, Philipson EH, editors. Nutrition and health: Handbook of nutrition and pregnancy. Totowa: Humana Press; 2008. p. 3-25.
92. WHO, FAO, UNU. Human energy requirements: Report of a joint FAO/WHO/UNU expert consultation, Rome, Italy, 17-24 October 2001. Rome: Food and Agriculture Organization, 2004.
93. Butte NF, Wong WW, Treuth MS, Ellis KJ, Smith EO. Energy requirements during pregnancy based on total energy expenditure and energy deposition. Am J Clin Nutr. 2004;79(6):1078-87.
94. Institute of Medicine. Nutrition during pregnancy: Part I: Weight gain, Part II: Nutrient supplements. Washington, DC: The National Academies Press; 1990.
95. Becquey E, Martin-Prevel Y. Micronutrient adequacy of women's diet in urban Burkina Faso is low. J Nutr. 2010;140(11):S2079-85.
96. Lee SE, Talegawkar SA, Merialdi M, Caulfield LE. Dietary intakes of women during pregnancy in low- and middle-income countries. Public Health Nutr. 2013;16(8):1340-53.
97. Ramakrishnan U. Prevalence of micronutrient malnutrition worldwide. Nutr Rev. 2002;60(5):S46-52.
98. Torheim LE, Ferguson EL, Penrose K, Arimond M. Women in resource-poor settings are at risk of inadequate intakes of multiple micronutrients. J Nutr. 2010;140(11):S2051-8.

99. Huybregts LF, Roberfroid DA, Kolsteren PW, Van Camp JH. Dietary behaviour, food and nutrient intake of pregnant women in a rural community in Burkina Faso. Matern Child Nutr. 2009;5(3):211-22.

100. Puolakka J, Jänne O, Pakarinen PA, Vihko R. Serum ferritin as a measure of iron stores during and after normal pregnancy with and without iron supplements. Acta Obstet Gynecol Scand. 1980;95(S95):43-51.

101. Stoltzfus RJ, Mullany LC, Black RE. Iron deficiency anaemia. In: Ezzati M, Lopez AD, Rodgers A, Murray CJL, editors. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors. Geneva: World Health Organization; 2004. p. 163-210.

102. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. Lancet Global Health. 2013;1(1):E16-E25.

103. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. Lancet. 2011;378(9809):2123-35.

104. Bates CJ, Powers HJ, Thurnham DI. Vitamins, iron, and physical work. Lancet. 1989;2(8658):313-4.

105. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B-12, and iron. Annu Rev Nutr. 2004;24:105-31.

106. Van Nhien N, Khan NC, Ninh NX, Van Huan P, Hop LT, Lam NT, et al. Micronutrient deficiencies and anemia among preschool children in rural Vietnam. Asia Pac J Clin Nutr. 2008;17(1):48-55.

107. Zimmermann MB, Biebinger R, Rohner F, Dib A, Zeder C, Hurrell RF, et al. Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron. Am J Clin Nutr. 2006;84(3):580-6.

108. McLean FH, De Benoist B, Allen LH. Review of the magnitude of folate and vitamin B12 deficiencies worldwide. Food Nutr Bull. 2008;29:S38-51.

109. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. J Nutr. 2012;142(4):744-50.

110. WHO. Global prevalence of vitamin A deficiency in populations at risk 1995-2005. WHO Global Database on Vitamin A Deficiency. Geneva: World Health Organization, 2009.

111. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet. 2013;382(9890):452-77.

112. Ota E, Hori H, Mori TA, Tobe-Gai R, Farrar D. Antenatal dietary education and supplementation to increase energy and protein intake. Cochrane Database Syst Rev. 2015(6).

113. Ceesay SM, Prentice AM, Cole TJ, Foord F, Weaver LT, Poskitt EM, et al. Effects on birth weight and perinatal mortality of maternal dietary supplements in rural Gambia: 5 year randomised controlled trial. BMJ. 1997;315(7111):786-90.

114. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. Cochrane Database Syst Rev. 2015(11):130.

115. Imdad A, Bhutta ZA. Maternal nutrition and birth outcomes: Effect of balanced protein-energy supplementation. Paediatr Perinat Epidemiol. 2012;26:178-90.

116. Kramer MSK, R., Kakuma A. Energy and protein intake in pregnancy. Cochrane Database Syst Rev. 2003.

117. Blackwel.Rq, Chow BF, Chinn KSK, Blackwel.Bn, Hsu SC. Prospective maternal nutrition study in Taiwan: rationale, study design, feasibility and preliminary findings. Nutrition Reports International. 1973;7(5):517-32.

118. Lanou H, Huybregts L, Roberfroid D, Nikiema L, Kouanda S, Van Camp J, et al. Prenatal nutrient supplementation and postnatal growth in a developing nation: An RCT. Pediatrics. 2014;133(4):E1001-E8.

119. Rush D, Stein Z, Susser M. A randomized controlled trial of prenatal nutritional supplementation in New York City. Pediatrics. 1980;65(4):683-97.

120. World Health Organization, United Nations Development Programme, United Nations Children's Fund, The World Bank. Pregnancy, childbirth, postpartum and newborn care: a guide for essential practice. Geneva: World Health Organization, 2015.

121. World Health Organization. Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization, 2012.

122. WHO. Guideline: Intermittent iron and folic acid supplementation in non-anaemic pregnant women. Geneva: World Health Organization, 2012.

123. Pena-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. Cochrane Database Syst Rev. 2015(7).

124. Ramakrishnan U, Manjrekar R, Rivera J, Gonzales-Cossio T, Martorell R. Micronutrients and pregnancy outcome: A review of the literature. Nutr Res. 1999;19(1):103-59.

125. Scholl TO, Hediger ML, Bendich A, Schall JI, Smith WK, Krueger PM. Use of multivitamin/mineral prenatal supplements: Influence on the outcome of pregnancy. Am J Epidemiol. 1997;146(2):134-41.

126. McCormick DB. Thiamine. In: Shills ME, Young VR, editors. Modern nutrition in health and disease. 6th ed. Philadelphia: Lea and Febiger; 1988. p. 376-82.

127. United Nations Children's Fund, World Health Organization, United Nations University. Composition of a multi-micronutrient supplement to be used in pilot programmes among pregnant women in developing countries: report of a United Nations Children's Fund (UNICEF), World Health Organization (WHO), United Nations University (UNU) workshop held at UNICEF Headquarters, New York, July 9, 1999. New York: United Nations Children's Fund, 2000.
128. Roberfroid D, Huybregts L, Lanou H, Ouedraogo L, Henry MC, Meda N, et al. Impact of prenatal multiple micronutrients on survival and growth during infancy: a randomized controlled trial. Am J Clin Nutr. 2012;95(4):916-24.
129. Vaidya A, Saville N, Shrestha BP, Costello AMD, Manandhar DS, Osrin D. Effects of antenatal multiple micronutrient supplementation on children's weight and size at 2 years of age in Nepal: follow-up of a double-blind randomised controlled trial. Lancet. 2008;371(9611):492-9.
130. Stewart CP, Christian P, LeClerq SC, West KP, Khatry SK. Antenatal supplementation with folic acid plus iron plus zinc improves linear growth and reduces peripheral adiposity in school-age children in rural Nepal. Am J Clin Nutr. 2009;90(1):132-40.
131. West KP, Shamim AA, Mehra S, Labrique AB, Ali H, Shaikh S, et al. Effect of maternal multiple micronutrient vs iron-folic acid supplementation on infant mortality and adverse birth outcomes in rural Bangladesh: The JiVitA-3 randomized trial. JAMA. 2014;312(24):2649-58.
132. Ronsmans C, Fisher DJ, Osmond C, Margetts BM, Fall CHD, Mmssg. Multiple micronutrient supplementation during pregnancy in low-income countries: A meta-analysis of effects on stillbirths and on early and late neonatal mortality. Food Nutr Bull. 2009;30(4):S547-S55.
133. Shaheen R, de Francisco A, El Arifeen S, Ekstrom EC, Persson LA. Effect of prenatal food supplementation on birth weight: an observational study from Bangladesh. Am J Clin Nutr. 2006;83(6):1355-61.
134. Chodick G, Shalev V, Goren I, Inskip PD. Seasonality in birth weight in Israel: new evidence suggests several global patterns and different etiologies. Ann Epidemiol. 2007;17(6):440-6.
135. McGrath JJ, Barnett AG, Eyles DW. The association between birth weight, season of birth and latitude. Ann Hum Biol. 2005;32(5):547-59.
136. Murray LJ, O'Reilly J, Betts N, Patterson CC, Smith GD, Evans AE. Season and outdoor ambient temperature: Effects on birth weight. Obstet Gynecol. 2000;96(5):689-95.
137. Fulford AJ, Rayco-Solon P, Prentice AM. Statistical modelling of the seasonality of preterm delivery and intrauterine growth restriction in rural Gambia. Paediatr Perinat Epidemiol. 2006;20(3):251-9.

138. Rao S, Kanade A, Margetts BM, Yajnik CS, Lubree H, Rege S, et al. Maternal activity in relation to birth size in rural India. The Pune Maternal Nutrition Study. Eur J Clin Nutr. 2003;57(4):531-42.
139. Singh J, Prentice AM, Diaz E, Coward WA, Ashford J, Sawyer M, et al. Energy expenditure of Gambian women during peak agricultural activity measured by the doubly-labeled water method. Br J Nutr. 1989;62(2):315-29.
140. Prentice AM, Goldberg GR. Energy adaptations in human pregnancy: limits and long-term consequences. Am J Clin Nutr. 2000;71(5):S1226-32.
141. Poppitt SD, Prentice AM, Jequier E, Schutz Y, Whitehead RG. Evidence of energy sparing in Gambian women during pregnancy - a longitudinal study using whole body calorimetry. Am J Clin Nutr. 1993;57(3):353-64.
142. Lawrence M, Lamb WH, Lawrence F, Whitehead RG. Maintenance energy cost of pregnancy in rural Gambian women and influence of dietary status. Lancet. 1984;2(8399):363-5.
143. Hytten FE. Weight gain in pregnancy. Clinical physiology in obstetrics. Oxford: Blackwell Scientific Publications; 1980. p. 193 - 233.
144. Kramer MS, McDonald SW. Aerobic exercise for women during pregnancy. Cochrane Database Syst Rev. 2006(3):34.
145. Palmer KT, Bonzini M, Harris EC, Linaker C, Bonde JP. Work activities and risk of prematurity, low birth weight and pre-eclampsia: an updated review with meta-analysis. Occup Environ Med. 2013;70(4):213-22.
146. van Beukering MDM, van Melick M, Mol BW, Frings-Dresen MHW, Hulshof CTJ. Physically demanding work and preterm delivery: a systematic review and meta-analysis. Int Arch Occup Environ Health. 2014;87(8):809-34.
147. van Melick M, van Beukering MDM, Mol BW, Frings-Dresen MHW, Hulshof CTJ. Shift work, long working hours and preterm birth: a systematic review and meta-analysis. Int Arch Occup Environ Health. 2014;87(8):835-49.
148. Barnes DL, Adair LS, Popkin BM. Women's physical activity and pregnancy outcome: a longitudinal analysis from the Philippines. Int J Epidemiol. 1991;20(1):162-72.
149. Ha E, Cho SI, Park H, Chen DF, Chen CZ, Wang LH, et al. Does standing at work during pregnancy result in reduced infant birth weight? J Occup Environ Med. 2002;44(9):815-21.
150. Launer LJ, Villar J, Kestler E, de Onis M. The effect of maternal work on fetal growth and duration of pregnancy: a prospective study. Br J Obstet Gynaecol. 1990;97(1):62-70.
151. Lima M, Ismail S, Ashworth A, Morris SS. Influence of heavy agricultural work during pregnancy on birthweight in Northeast Brazil. Int J Epidemiol. 1999;28(3):469-74.

152. Tuntiseranee P, Geater A, Chongsuvivatwong V, Kor-anantakul O. The effect of heavy maternal workload on fetal growth retardation and preterm delivery - A study among southern Thai women. J Occup Environ Med. 1998;40(11):1013-21.
153. Bonzini M, Coggon D, Godfrey K, Inskip H, Crozier S, Palmer KT. Occupational physical activities, working hours and outcome of pregnancy: findings from the Southampton Women's Survey. Occup Environ Med. 2009;66(10):685-90.
154. Gollenberg AL, Pekow P, Bertone-Johnson ER, Freedson PS, Markenson G, Chasan-Taber L. Physical activity and risk of small-for-gestational-age birth among predominantly Puerto Rican women. Matern Child Health J. 2011;15(1):49-59.
155. Harrod CS, Chasan-Taber L, Reynolds RM, Fingerlin TE, Glueck DH, Brinton JT, et al. Physical activity in pregnancy and neonatal body composition: The Healthy Start Study. Obstet Gynecol. 2014;124(2):257-64.
156. WHO. The optimal duration of exclusive breastfeeding. Report of an Expert Consultation. Geneva: World Health Organisation, 2001.
157. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev. 2012(8):132.
158. Allen LH. Multiple micronutrients in pregnancy and lactation: An overview. Am J Clin Nutr. 2005;81(5):S1206-12.
159. Salam RA, Das JK, Bhutta ZA. Multiple micronutrient supplementation during pregnancy and lactation in low-to-middle-income developing country settings: Impact on pregnancy outcomes. Ann Nutr Metab. 2014;65(1):4-12.
160. Allen LH. B vitamins in breast milk: relative importance of maternal status and intake, and effects on infant status and function. Adv Nutr. 2012;3(3):362-9.
161. Picciano MF. Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements. J Nutr. 2003;133(6):S1997-2002.
162. FAO/WHO. Vitamin and Mineral Requirements In Human Nutrition. Rome: World Health Organization, Food and Agriculture Organization, 2004.
163. Bates CJ, Prentice A. Breast milk as a source of vitamins, essential minerals and trace elemants. Pharmacol Ther. 1994;62(1-2):193-220.
164. Butte NF, Lopez-Alarcon MG, Garza C. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. Geneva: World Health Organization, 2002.
165. Williams AM, Chantry CJ, Young SL, Achando BS, Allen LH, Arnold BF, et al. Vitamin B-12 concentrations in breast milk are low and are not associated with reported household hunger, recent animal-source food, or vitamin B-12 intake in women in rural Kenya. J Nutr. 2016:epub ahead of print.

166. Molloy AM, Kirke PN, Brody LC, Scott JM, Mills JL. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. Food Nutr Bull. 2008;29(2):S101-11.
167. de Pee S, Bloem MW, Sari M, Kiess L, Yip R, Kosen S. The high prevalence of low hemoglobin concentration among Indonesian infants aged 3-5 months is related to maternal anemia. J Nutr. 2002;132(8):2215-21.
168. Meinzen-Derr JK, Guerrero ML, Altaye M, Otega-Gallegos H, Ruiz-Palacios GM, Morrow AL. Risk of infant anemia is associated with exclusive breast-feeding and maternal anemia in a Mexican cohort. J Nutr. 2006;136(2):452-8.
169. Colomer J, Colomer C, Gutierrez D, Jubert A, Nolasco A, Donat J, Fernandez-Delgado R, Donat F, et al. Anaemia during pregnancy as a risk factor for infant iron deficiency: report from the Valencia Infant Anaemia Cohort (VIAC) study. Paediatr Perinat Epidemiol. 1990;4(2):196-204.
170. Dijkhuizen MA, Wieringa FT, West CE, Muherdiyantiningsih, Muhilal. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. Am J Clin Nutr. 2001;73(4):786-91.
171. Dallman PR, Siimes MA, Stekel A. Iron-deficiency in infancy and childhood. Am J Clin Nutr. 1980;33(1):86-118.
172. Shah RS, Rajalakshmi R. Vitamin A status of the newborn in relation to gestational age, body weight, and maternal nutritional status. Am J Clin Nutr. 1984;40(4):794-800.
173. Terrin G, Canani RB, Di Chiara M, Pietravalle A, Aleandri V, Conte F, et al. Zinc in early life: a key element in the fetus and preterm neonate. Nutrients. 2015;7(12):10427-46.
174. Elizabeth KE, Krishnan V, Vijayakumar T. Umbilical cord blood nutrients in low birth weight babies in relation to birth weight & gestational age. Indian J Med Res. 2008;128(2):128-33.
175. WHO, UNICEF. Reaching optimal iodine nutrition in pregnant and lactating women and young children: a joint statement by WHO and UNICEF. Geneva: World Health Organization, 2007.
176. WHO. Guideline: Neonatal vitamin A supplementation. Geneva: World Health Organization, 2011.
177. Gogia S, Sachdev HS. Vitamin A supplementation for the prevention of morbidity and mortality in infants six months of age or less. Cochrane Database Syst Rev. 2011(10):97.
178. Haider BA, Bhutta ZA. Neonatal vitamin A supplementation for the prevention of mortality and morbidity in term neonates in developing countries. Cochrane Database Syst Rev. 2011(10):54.

179. WHO, UNICEF. WHO/UNICEF Joint Statement: Clinical management of acute diarrhoea. Geneva, New York: World Health Organization, United Nations Children's Fund, 2004.

180. WHO. Guidelines on optimal feeding of low birth-weight infants in low- and middle-income countries. Geneva: World Health Organization, 2011.

181. Abe SK, Balogun OO, Ota E, Takahashi K, Mori R. Supplementation with multiple micronutrients for breastfeeding women for improving outcomes for the mother and baby. Cochrane Database Syst Rev. 2016(2).

182. Allen LH. SCN News, Number 11 - Maternal and child nutrition. Maternal micronutrient malnutrition: Effects on breast milk and infant nutrition, and priorities for intervention. 11 ed: ACC/SCN; 1994.

183. UNDP. Human Development Report 2015 - Work for human development. New York: United Nations Development Programme, 2015.

184. WHO. Meningococcal meningitis - Fact Sheet N°141. Geneva: World Health Organization. 2016 [updated November 2015]. Available from: http://www.who.int/mediacentre/factsheets/fs141/en/.

185. Global Health Observatory data [Internet]. World Health Organization. 2016 [cited April 5]. Available from: http://apps.who.int/gho/data/node.country.country-BFA.

186. CLIMATE-DATA.ORG. Climate: Houndé [Internet]. 2016 [September 1]. Available from: http://en.climate-data.org/location/53486/.

187. Roberfroid D, Huybregts L, Lanou H, Henry M-C, Meda N, Menten J, et al. Effects of maternal multiple micronutrient supplementation on fetal growth: a double-blind randomized controlled trial in rural Burkina Faso. Am J Clin Nutr. 2008;88(5):1330-40.

188. Huybregts L, Roberfroid D, Lanou H, Menten J, Meda N, Van Camp J, et al. Prenatal food supplementation fortified with multiple micronutrients increases birth length: a randomized controlled trial in rural Burkina Faso. Am J Clin Nutr. 2009;90(6):1593-600.

189. de Onis M, Blossner M, Villar J. Levels and patterns of intrauterine growth retardation in developing countries. Eur J Clin Nutr. 1998;52(Suppl 1):S5-15.

190. Regnault TRH, Limesand SW, Hay WW. Factors influencing fetal growth. NeoReviews. 2001;2(6):119e-28.

191. Kramer MS, Kakuma R. Energy and protein intake in pregnancy. Cochrane Database Syst Rev. 2003;4(4):CD000032.

192. Ouedraogo A, Tiono AB, Diarra A, Sanon S, Yaro JB, Ouedraogo E, et al. Malaria morbidity in high and seasonal malaria transmission area of Burkina Faso. PLoS One. 2013;8(1):e50036.

193. Chodick G, Shalev V, Goren I, Inskip PD. Seasonality in birth weight in Israel: New evidence suggests several global patterns and different etiologies. Ann Epidemiol. 2007;17(6):440-6.
194. Murray LJ, O'Reilly J, Betts N, Patterson CC, Smith GD, Evans AE. Season and outdoor ambient temperature: Effects on birth weight. Obstet Gynecol. 2000;96(5):689-95.
195. McGrath JJ, Keeping D, Saha S, Chant DC, Lieberman DE, O'Callaghan MJ. Seasonal fluctuations in birth weight and neonatal limb length; does prenatal vitamin D influence neonatal size and shape? Early Hum Dev. 2005;81(7):609-18.
196. UNSSCN. Sixth report on the world nutrition situation: Progress in nutrition. Geneva: United Nations System Standing Committee on Nutrition, 2010.
197. Valea I, Tinto H, Drabo MK, Huybregts L, Henry MC, Roberfroid D, et al. Intermittent preventive treatment of malaria with sulphadoxine-pyrimethamine during pregnancy in Burkina Faso: effect of adding a third dose to the standard two-dose regimen on low birth weight, anaemia and pregnancy outcomes. Malar J. 2010;9:9.
198. Kramer MS, Platt RW, Wen SW, Joseph KS, Allen A, Abrahamowicz M, et al. A new and improved population-based Canadian reference for birth weight for gestational age. Pediatrics. 2001;108(2):e35.
199. StataCorp. Stata Statistical Software: Release 12. College Station, TX, USA: StataCorp LP; 2011.
200. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.
201. Katz J, Lee AC, Kozuki N, Lawn JE, Cousens S, Blencowe H, et al. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. Lancet. 2013;382(9890):417-25.
202. Ott WJ. Intrauterine growth retardation and preterm delivery. Am J Obstet Gynecol. 1993;168(6 Pt 1):1710-5.
203. Goldenberg RL, Culhane JF, Iams JD, Romero R. Preterm birth 1 - Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.
204. Hobel CJ, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CP. Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. Am J Obstet Gynecol. 1999;180(1):S257-S63.
205. Sandman CA, Glynn L, Schetter CD, Wadhwa P, Garite T, Chicz-DeMet A, et al. Elevated maternal cortisol early in pregnancy predicts third trimester levels of placental corticotropin releasing hormone (CRH): Priming the placental clock. Peptides. 2006;27(6):1457-63.
206. Bleiberg FM, Brun TA, Goihman S. Duration of activities and energy-expenditure of female farmers in dry and rainy seasons in Upper-Volta. Br J Nutr. 1980;43(1):71-82.

207. Palmer KT, Bonzini M, Harris EC, Linaker C, Bonde JP. Work activities and risk of prematurity, low birth weight and pre-eclampsia: an updated review with meta-analysis. Occup Environ Med. 2013;70(4):213-22.

208. WHO. World Malaria Report. Geneva: World Health Organization, 2012.

209. Sullivan AD, Nyirenda T, Cullinan T, Taylor T, Harlow SD, James SA, et al. Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. J Infect Dis. 1999;179(6):1580-3.

210. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. Cochrane Database Syst Rev. 2006(4):CD004905.

211. Margetts BM, Fall CH, Ronsmans C, Allen LH, Fisher DJ, The Maternal Micronutrient Supplementation Study Group. Multiple micronutrient supplementation during pregnancy in low-income countries: review of methods and characteristics of studies included in the meta-analyses. Food Nutr Bull. 2009;30(Suppl 4):S517-26.

212. Shah PS, Ohlsson A, Knowledge Synthesis Group on Determinants of Low Birth Weight and Preterm Births. Effects of prenatal multimicronutrient supplementation on pregnancy outcomes: a meta-analysis. CMAJ. 2009;180(12):e99-108.

213. Golden MH. Proposed recommended nutrient densities for moderately malnourished children. Food Nutr Bull. 2009;30(Suppl 3):S267-342.

214. Rayco-Solon P, Fulford AJ, Prentice AM. Differential effects of seasonality on preterm birth and intrauterine growth restriction in rural Africans. Am J Clin Nutr. 2005;81(1):134-9.

215. Toe LC, Bouckaert KP, De Beuf K, Roberfroid D, Meda N, Thas O, et al. Seasonality modifies the effect of a lipid-based nutrient supplement for pregnant rural women on birth length. J Nutr. 2015;145(3):634-9.

216. Chasan-Taber L, Evenson KR, Sternfeld B, Kengeri S. Assessment of recreational physical activity during pregnancy in epidemiologic studies of birthweight and length of gestation: Methodologic aspects. Women Health. 2007;45(4):85-107.

217. Evenson KR, Chasan-Taber L, Downs DS, Pearce EE. Review of self-reported physical activity assessments for pregnancy: Summary of the evidence for validity and reliability. Paediatr Perinat Epidemiol. 2012;26(5):479-94.

218. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.

219. Harrison CL, Thompson RG, Teede HJ, Lombard CB. Measuring physical activity during pregnancy. Int J Behav Nutr Phys Act. 2011;8:8.

220. Lawrence M, Singh J, Lawrence F, Whitehead RG. The energy cost of common daily activities in African women: increased expenditure in pregnancy? Am J Clin Nutr. 1985;42(5):753-63.
221. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, et al. 2011 Compendium of Physical Activities: A Second Update of Codes and MET Values. Med Sci Sports Exerc. 2011;43(8):1575-81.
222. Plasqui G, Westerterp KR. Physical activity assessment with accelerometers: An evaluation against doubly labeled water. Obesity. 2007;15(10):2371-9.
223. Matthews CE, Chen KY, Freedson PS, Buchowski MS, Beech BM, Pate RR, et al. Amount of time spent in sedentary behaviors in the united states, 2003-2004. Am J Epidemiol. 2008;167(7):875-81.
224. Matthews CE. Calibration of accelerometer output for adults. Med Sci Sports Exerc. 2005;37(11):S512-S22.
225. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc. 2008;40(1):181-8.
226. Trost SG, McIver KL, Pate RR. Conducting accelerometer-based activity assessments in field-based research. Med Sci Sports Exerc. 2005;37(11):S531-S43.
227. Bland JM, Altman DG. Applying the right statistics: analyses of measurement studies. Ultrasound Obstet Gynecol. 2003;22(1):85-93.
228. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8(2):135-60.
229. Landis JR, Koch GG. Measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-74.
230. Chasan-Taber L, Schmidt MD, Roberts DE, Hosmer D, Markenson G, Freedson PS. Development and validation of a pregnancy physical activity questionnaire. Med Sci Sports Exerc. 2004;36(10):1750-60.
231. Evenson KR, Wen F. Measuring physical activity among pregnant women using a structured one-week recall questionnaire: evidence for validity and reliability. Int J Behav Nutr Phys Act. 2010;7:12.
232. Schmidt MD, Freedson PS, Pekow P, Roberts D, Sternfeld B, Chasan-Taber L. Validation of the Kaiser physical activity survey in pregnant women. Med Sci Sports Exerc. 2006;38(1):42-50.
233. Poudevigne MS, O'Connor PJ. Physical activity during pregnancy: Comparing an accelerometer to a 7-day recall and diary. Med Sci Sports Exerc. 2005;37:S112-S.
234. Washburn RA, Heath GW, Jackson AW. Reliability and validity issues concerning large-scale surveillance of physical activity. Res Q Exerc Sport. 2000;71(2):S104-S13.

235. Richardson MT, Leon AS, Jacobs DR, Ainsworth BE, Serfass R. Comprehensive evaluation of the Minnesota Leisure Time Physical Activity Questionnaire. J Clin Epidemiol. 1994;47(3):271-81.
236. Bassett DR. Validity and reliability issues in objective monitoring of physical activity. Res Q Exerc Sport. 2000;71(2):S30-S6.
237. DiNallo JM, Downs DS, Le Masurier G. Objectively assessing treadmill walking during the second and third pregnancy trimesters. J Phys Act Health. 2012;9(1):21-8.
238. Osmond C, Barker DJP. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. Environ Health Perspect. 2000;108:545-53.
239. Jukic AMZ, Evenson KR, Daniels JL, Herring AH, Wilcox AJ, Hartmann KE. A prospective study of the association between vigorous physical activity during pregnancy and length of gestation and birthweight. Matern Child Health J. 2012;16(5):1031-44.
240. Coulibaly SO, Gies S, D'Alessandro U. Malaria burden among pregnant women living in the rural district of Boromo, Burkina Faso. Am J Trop Med Hyg. 2007;77(6):56-60.
241. I-Min L. Epidemiologic methods in physical activity studies. New York: Oxford University Press; 2009. 340 p.
242. Frederick IO, Williams MA, Sales AE, Martin DP, Killien M. Pre-pregnancy body mass index, gestational weight gain, and other maternal characteristics in relation to infant birth weight. Matern Child Health J. 2008;12(5):557-67.
243. Poudevigne MS, O'Connor PJ. A review of physical activity patterns in pregnant women and their relationship to psychological health. Sports Med. 2006;36(1):19-38.
244. Borodulin KM, Evenson KR, Wen F, Herring AH, Benson AM. Physical activity patterns during pregnancy. Med Sci Sports Exerc. 2008;40(11):1901-8.
245. Evenson KR, Wen F. Prevalence and correlates of objectively measured physical activity and sedentary behavior among US pregnant women. Prev Med. 2011;53(1-2):39-43.
246. Clapp JF. Influence of endurance exercise and diet on human placental development and fetal growth. Placenta. 2006;27(6-7):527-34.
247. Kinare AS, Natekar AS, Chinchwadkar MC, Yajnik CS, Coyaji KJ, Fall CHD, et al. Low midpregnancy placental volume in rural Indian women: A cause for low birth weight? Am J Obstet Gynecol. 2000;182(2):443-8.
248. Clapp JF, Rizk KH, Applebywineberg SK, Crass JR. Second-trimester placental volumes predict birth weight at term. J Soc Gynecol Investig. 1995;2(1):19-22.
249. Roseboom TJ, Painter RC, de Rooij SR, van Abeelen AFM, Veenendaal MVE, Osmond C, et al. Effects of famine on placental size and efficiency. Placenta. 2011;32(5):395-9.

250. Wallace JM, Horgan GW, Bhattacharya S. Placental weight and efficiency in relation to maternal body mass index and the risk of pregnancy complications in women delivering singleton babies. Placenta. 2012;33(8):611-8.
251. World Health Organization. Complementary feeding of young children in developing countries: a review of current scientific knowledge. Geneva: World Health Organization, 1998.
252. Ahmed F, Khan MR, Banu CP, Qazi MR, Akhtaruzzaman M. The coexistence of other micronutrient deficiencies in anaemic adolescent schoolgirls in rural Bangladesh. Eur J Clin Nutr. 2008;62(3):365-72.
253. Bushra M, Elhassan EM, Ali NI, Osman E, Bakheit KH, Adam, II. Anaemia, zinc and copper deficiencies among pregnant women in central Sudan. Biol Trace Elem Res. 2010;137(3):255-61.
254. Ma AG, Schouten EG, Wang Y, Xu RX, Zheng MC, Li Y, et al. Micronutrient status in anemic and non-anemic Chinese women in the third trimester of pregnancy. Asia Pac J Clin Nutr. 2009;18(1):41-7.
255. Hettiarachchi M, Liyanage C. Coexisting micronutrient deficiencies among Sri Lankan pre-school children: a community-based study. Matern Child Nutr. 2012;8(2):259-66.
256. Fishman SM, Christian P, West KP. The role of vitamins in the prevention and control of anaemia. Public Health Nutr. 2000;3(2):125-50.
257. Mei ZG, Grummer-Strawn LM. Standard deviation of anthropometric Z-scores as a data quality assessment tool using the 2006 WHO growth standards: a cross country analysis. Bull World Health Organ. 2007;85(6):441-8.
258. Domellof M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, et al. Iron requirements of infants and toddlers. J Pediatr Gastroenterol Nutr. 2014;58(1):119-29.
259. Akintonwa A, Gbajumo SA, Mabadeje AFB. Placental and milk transfer of chloroquine in humans. Ther Drug Monit. 1988;10(2):147-9.
260. Ogunbona FA, Onyeji CO, Bolaji OO, Torimiro SEA. Excretion of chloroquine and desethylchloroquine in human milk. Br J Clin Pharmacol. 1987;23(4):473-6.
261. Edstein MD, Veenendaal JR, Newman K, Hyslop R. Excretion of chloroquine, dapsone and pyrimethamine in human milk. Br J Clin Pharmacol. 1986;22(6):733-5.
262. Fleiss JL. Analysis of data from multiclinic trials. Control Clin Trials. 1986;7(4):267-75.
263. Martin-Prevel Y, Allemand P, Nikiema L, Ayassou KA, Ouedraogo HG, Moursi M, et al. Biological status and dietary intakes of iron, zinc and vitamin A among women and preschool children in rural Burkina Faso. PLoS One. 2016;11(3):17.
264. Hampel D, Shahab-Ferdows S, Adair LS, Bentley ME, Flax VL, Jamieson DJ, et al. Thiamin and riboflavin in human milk: effects of lipid-based nutrient supplementation and

stage of lactation on vitamer secretion and contributions to total vitamin content. PLoS One. 2016;11(2):14.
265. Semba RD, Delange F. Iodine in human milk: perspectives for infant health. Nutr Rev. 2001;59(8):269-78.
266. Kumpulainen J, Salmenpera L, Siimes MA, Koivistoinen P, Perheentupa J. Selenium status of exclusively breastfed infants as influenced by maternal organic or inorganic selenium supplementation. Am J Clin Nutr. 1985;42(5):829-35.
267. Domellof M, Lonnerdal B, Dewey KG, Cohen RJ, Hernell O. Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. Am J Clin Nutr. 2004;79(1):111-5.
268. Lonnerdal B. Regulation of mineral and trace elements in human milk: exogenous and endogenous factors. Nutr Rev. 2000;58(8):223-9.
269. Lonnerdal B. Effects of maternal dietary intake on human milk composition. J Nutr. 1986;116(4):499-513.
270. Khambalia A, Latulippe ME, Campos C, Merlos C, Villalpando S, Picciano MF, et al. Milk folate secretion is not impaired during iron deficiency in humans. J Nutr. 2006;136(10):2617-24.
271. Berglund S, Westrup B, Domellof M. Iron supplements reduce the risk of iron deficiency anemia in marginally low birth weight infants. Pediatrics. 2010;126(4):E874-83.
272. Agarwal R, Virmani D, Jaipal M, Gupta S, Sankar MJ, Bhatia S, et al. Iron stores in low and normal birth weight infants at birth and in early infancy. Indian J Pediatr. 2014;81(3):279-82.
273. Powers HJ. Micronutrient deficiencies in the preterm neonate. Proc Nutr Soc. 1993;52(2):285-91.
274. Michaelsen KF, Milman N, Samuelson G. A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors. Acta Paediatr. 1995;84(9):1035-44.
275. Yang ZY, Lonnerdal B, Adu-Afarwuah S, Brown KH, Chaparro CM, Cohen RJ, et al. Prevalence and predictors of iron deficiency in fully breastfed infants at 6 mo of age: comparison of data from 6 studies. Am J Clin Nutr. 2009;89(5):1433-40.
276. Olivares M, Llaguno S, Marin V, Hertrampf E, Mena P, Milad M. Iron status in low birth weight infants, small and appropriate for gestational age: a follow-up study. Acta Paediatr. 1992;81(10):824-8.
277. Lundstrom U, Siimes MA. Red blood cell values in low birth weight infants: ages at which values become equivalent to those of term infants. J Pediatr. 1980;96(6):1040-2.

278. Tylleskar T, Jackson D, Meda N, Engebretsen IMS, Chopra M, Diallo AH, et al. Exclusive breastfeeding promotion by peer counsellors in sub-Saharan Africa (PROMISE-EBF): a cluster-randomised trial. Lancet. 2011;378(9789):420-7.

279. INSD, ICF International. Enquête Démographique et de Santé et à Indicateurs Multiples du Burkina Faso 2010. Calverton, MD: Institut National de la Statistique et de la Démographie, ICF International, 2012.

280. van Liere MJ, Ategbo E-AD, Den Hartog AP, Hautvast JGAJ. The consequence of seasonal food insecurity for individual food-consumption patters in north-western Benin. Food Nutr Bull. 1995;16:147-54.

281. Schultink JW, Vanraaij JMA, Hautvast J. Seasonal weight loss and metabolic adaptation in rural Beninese women: the relationship with body mass index. Br J Nutr. 1993;70(3):689-700.

282. Rosetta L. Sex differences in seasaonal variations of the nutritional status of Serere adults in Senegal. Ecol Food Nutr. 1986;18(3):231-44.

283. Savy M, Martin-Prevel Y, Traissac P, Eymard-Duvernay S, Delpeuch F. Dietary diversity scores and nutritional status of women change during the seasonal food shortage in rural Burkina Faso. J Nutr. 2006;136(10):2625-32.

284. Adams AM. Seasonal variations in energy-balance among agriculturalists in central Mali: compromise or adaptation. Eur J Clin Nutr. 1995;49(11):809-23.

285. Lawrence M, Coward WA, Lawrence F, Cole TJ, Whitehead RG. Energy requirements of pregnancy in The Gambia. Lancet. 1987;2(8567):1072-6.

286. Huybregts LF. Prevention of intrauterine growth retardation by food supplementation in rural Burkina Faso. Ghent, Belgium: Ghent University, Faculty of Bioscience Engineering; 2010.

287. Adu-Afarwuah S, Lartey A, Okronipa H, Ashorn P, Zeilani M, Peerson JM, et al. Lipid-based nutrient supplement increases the birth size of infants of primiparous women in Ghana. Am J Clin Nutr. 2015;101(4):835-46.

288. Ashorn P, Alho L, Ashorn U, Cheung YB, Dewey KG, Harjunmaa U, et al. The impact of lipid-based nutrient supplement provision to pregnant women on newborn size in rural Malawi: a randomized controlled trial. Am J Clin Nutr. 2015;101(2):387-97.

289. Brabin BJ, Romagosa C, Abdelgalil S, Menendez C, Verhoeff FH, McGready R, et al. The sick placenta - The role of malaria. Placenta. 2004;25(5):359-78.

290. Saenger P, Czernichow P, Hughes I, Reiter EO. Small for gestational age: Short stature and beyond. Endocr Rev. 2007;28(2):219-51.

291. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. BMC Pediatr. 2014;14:14.

292. Brown KH, Akhtar NA, Robertson AD, Ahmed MG. Lactational capacity of marginally nourished mothers: relationships between maternal nutritional status and quantity and proximate composition of milk. Pediatrics. 1986;78(5):909-19.
293. Gonzalez-Cossio T, Habicht JP, Rasmussen KM, Delgado HL. Impact of food supplementation during lactation on infant breast-milk intake and on the proportion of infants exclusively breast-fed. J Nutr. 1998;128(10):1692-702.
294. Kindra G, Coutsoudis A, Esposito F. Effect of nutritional supplementation of breastfeeding HIV positive mothers on maternal and child health: findings from a randomized controlled clinical trial. BMC Public Health. 2011;11:12.
295. Flax VL, Bentley ME, Chasela CS, Kayira D, Hudgens MG, Knight RJ, et al. Use of lipid-based nutrient supplements by HIV-infected Malawian women during lactation has no effect on infant growth from 0 to 24 weeks. J Nutr. 2012;142(7):1350-6.
296. Ashorn P, Alho L, Ashorn U, Cheung YB, Dewey KG, Gondwe A, et al. Supplementation of maternal diets during pregnancy and for 6 months postpartum and infant diets thereafter with small-quantity lipid-based nutrient supplements does not promote child growth by 18 months of age in rural Malawi: A randomized controlled trial. J Nutr. 2015;145(6):1345-53.
297. Ruel MT, Alderman H, Maternal Child Nutr Study Group. Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition? Lancet. 2013;382(9891):536-51.
298. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet. 2013;382(9890):427-51.
299. de Pee S, Grais R, Fenn B, Brown R, Briend A, Frize J, et al. Prevention of acute malnutrition: Distribution of special nutritious foods and cash, and addressing underlying causes-what to recommend when, where, for whom, and how. Food Nutr Bull. 2015;36:S24-S9.
300. Langendorf C, Roederer T, de Pee S, Brown D, Doyon S, Mamaty AA, et al. Preventing acute malnutrition among young children in crises: a prospective intervention study in Niger. PLoS Med. 2014;11(9):15.
301. Glassman A, Duran D, Fleisher L, Singer D, Sturke R, Angeles G, et al. Impact of conditional cash transfers on maternal and newborn health. J Heatlh Popul Nutr. 2013;31(4):48-66.
302. Akresh R, de Walque D, Kazianga H. Alternative cash transfer delivery mechanisms. Washington, DC: The World Bank, 2012. Contract No.: Policy research working paper 5958.
303. Mbuagbaw LCE, Gofin R. A new measurement for optimal antenatal care: determinants and outcomes in Cameroon. Matern Child Health J. 2011;15(8):1427-34.

304. Bahwere P, Balaluka B, Wells JCK, Mbiribindi CN, Sadler K, Akomo P, et al. Cereals and pulse-based ready-to-use therapeutic food as an alternative to the standard milk- and peanut paste-based formulation for treating severe acute malnutrition: a noninferiority, individually randomized controlled efficacy clinical trial. Am J Clin Nutr. 2016;103(4):1145-61.
305. Dangour AD, Watson L, Cumming O, Boisson S, Che Y, Velleman Y, et al. Interventions to improve water quality and supply, sanitation and hygiene practices, and their effects on the nutritional status of children. Cochrane Database Syst Rev. 2013(8):102.
306. Duysburgh E, Zhang WH, Ye M, Williams A, Massawe S, Sie A, et al. Quality of antenatal and childbirth care in selected rural health facilities in Burkina Faso, Ghana and Tanzania: similar finding. Trop Med Int Health. 2013;18(5):534-47.
307. Nikiema L, Kameli Y, Capon G, Sondo B, Martin-Prevel Y. Quality of antenatal care and obstetrical coverage in rural Burkina Faso. J Heatlh Popul Nutr. 2010;28(1):67-75.
308. Boubacar I. Caractérisation des saisons de pluies au Burkina Faso dans un contexte de changement climatique et évaluation des impacts hydrologiques sur le bassin du Nakanbé [Doctoral thesis]. Paris: Université Pierre et Marie Curie - Paris VI; 2012.
309. Ministère de la Santé du Burkina Faso. Protocole nationale: prise en charge integree de la malnutrition aiguë (pcima). Ouagadougou, Burkina Faso. 2014.
310. King JC. The risk of maternal nutritional depletion and poor outcomes increases in early or closely spaced pregnancies. J Nutr. 2003;133(5):1732S-6S.
311. Winkvist A, Habicht JP, Rasmussen KM. Linking maternal and infant benefits of a nutritional supplement during pregnancy and lactation. Am J Clin Nutr. 1998;68(3):656-61.
312. Haakstad LAH, Gundersen I, Bo K. Self-reporting compared to motion monitor in the measurement of physical activity during pregnancy. Acta Obstet Gynecol Scand. 2010;89(6):749-56.
313. Smith KM, Foster RC, Campbell CG. Accuracy of physical activity assessment during pregnancy: an observational study. BMC Pregnancy Childbirth. 2011;11:9.
314. Assah FK, Ekelund U, Brage S, Wright A, Mbanya JC, Wareham NJ. Accuracy and validity of a combined heart rate and motion sensor for the measurement of free-living physical activity energy expenditure in adults in Cameroon. Int J Epidemiol. 2011;40(1):112-20.
315. Brage S, Westgate K, Franks PW, Stegle O, Wright A, Ekelund U, et al. Estimation of free-living energy expenditure by heart rate and movement sensing: A doubly-labelled water study. PLoS One. 2015;10(9).

Addendum: Physical activity questionnaire

Nom : |__|__|__|__|__|__|__|__|__|__|__|__|__|__| Prénom : |__|__|__|__|__|__|__|__|__|__|__|

Date: .... /… /2006 ENQUÊTRICE n° : |__|
VISITE n° : |__|

ENCEINTE ? OUI / NON
ALLAITANTE ? OUI / NON
DATE NAISSANCE DERNIER ENFANT :…/…/….
APPAREIL : OUI / NON n°: |__|

NOMBRE DE PERSONNES DANS LA MAISON DE LA FEMME ENQUÊTÉE:…
NOMBRE DE PERSONNES QUI AIDENT A LA MAISON : …
NOMBRE D'ENFANTS MOINS DE 7 ANS : …
NOMBRE D'ENFANTS PLUS DE 7 ANS : …
LA FEMME TRAVAILLE AU CHAMPS : OUI / NON

Demander s' il y avait des jours spéciales pendant la semaine, quand elle a fait des activités différentes : OUI / NON
Si OUI, quand et raison ……………………………………….…. (1=jour de fête ; 2= maladie ; 3=autre)

Estimer la distance :

Karaba :
A=distance entre entrée Karaba et CPSS
B=distance entre entrée Karaba et Houndé
C=distance entre Houndé et Boni

Koho :
A=distance entre les barrages et l'école primaire de Koho
B=distance entre école primaire et Houndé centre
C=distance entre Houndé et Boni

Possibilités : <A , A , A-B , B , B-C , C , >C

Remarques :
1. Durée par session d’activité
2. Fréquence par semaine

---

1. ACTIVITE DEPLACEMENT

<u>Aller au marché à Houndé</u>	*MOYEN*	*ENFANT*	DISTANCE	*(KILO)METRES*	DUREE	FREQUENCE
	à pied	oui				
	à vélo	non				
	à moto					
	à la charette					

2. ACTIVITE A LA MAISON

** Préparer les ingrédients*

<u>Piler</u>	*QUANTITE*	ENFANT	AIDE	DUREE	FREQUENCE
	< 5 boites	oui	oui		
	5-10 boites	non	non		
	> 10 boites		… personnes		

<u>Faire beurre de karité</u>	QUANTITE*	ENFANT	AIDE	DUREE	FREQUENCE
* quantité de noix ramassés	< 3 boites	oui	oui		
	3-5 boites	non	non		
	> 5 boites		… personnes		

<u>Battre le tô</u>	QUANTITE	ENFANT	AIDE	DUREE**	FREQUENCE
** temps pour battre	< 2 boites	oui	oui		
	2-5 boites	non	non		
	> 5 boites		… personnes		

* *Faire la cuisine*		ENFANT	AIDE		DUREE	FREQUENCE
		oui	oui			
examples: Faire le petit déjeuner (=tô avec une sauce ou bouillie) faire dolo faire couscous, gnonkon faire du feu		non	non			
			…personnes			

* *Balayer*		ENFANT	AIDE		DUREE	FREQUENCE
		oui	oui			
		non	non			
			… personnes			

* *Aller au moulin*	*MOYEN*	ENFANT	CHARGE	DISTANCE	DUREE	FREQUENCE
	à pied	oui	< 2 tines			
	à vélo	non	2-5 tines			
	à moto		> 5 tines	(KILO)METRES		
	à la charette					

* *Aller chercher de l'eau*	*MOYEN*	ENFANT	*COMMENT*	DISTANCE	DUREE	FREQUENCE
	à pied	oui	pompe à main			
	à vélo	non	pompe à pied			
	à moto		à la rivière	(KILOMETRES)		
	à la charette		au puits			

** Aller chercher du bois*	*MOYEN*	ENFANT		DISTANCE	DUREE	FREQUENCE
	à pied	oui				
	à vélo	non				
	à moto			(KILO)METRES		
	à la charette					
** Hacher du bois*	QUANTITE				DUREE	FREQUENCE
** Laver les plats*	QUANTITE		AIDE		DUREE	FREQUENCE
	< 10 plats		oui			
	10-30 plats		non			
	> 30 plats		….personnes			
** Laver le linge*	QUANTITE	ENFANT	AIDE		DUREE	FREQUENCE
	< 10 habits	oui	oui			
	10-30 habits	non	non			
	> 30 habits		…personnes			
** Prendre soin des enfants*			AIDE		DUREE	FREQUENCE
			oui			
			non			
			…personnes			

examples:
laver les enfants
allaiter les enfants
donner à manger aux enfants

** Autour de la maison*

Desherber le jardin		ENFANT	AIDE		DUREE	FREQUENCE
		oui	oui			
		non	non			
			…personnes			

Prendre soin des animaux:		ENFANT	AIDE	DISTANCE	DUREE	FREQUENCE
		oui	oui			
examples : leur donner à boire leur donner à manger les déplacer		non	non			
			…personnes			

Traire des vaches	QUANTITE	ENFANT	AIDE		DUREE	FREQUENCE
	< 3 vaches	oui	oui			
	3-10 vaches	non	non			
	> 10 vaches		…personnes			

3. ACTIVITES AU CHAMP

Aller travailler au champ	*MOYEN*	ENFANT	CHARGE	DISTANCE	DUREE	FREQUENCE
	à pied	oui				
	à vélo	non				
	à moto			(KILO)METRES		
	à la charette					

Travailler au champs

	DUREE	FREQUENCE
1 demi journée 2 toute la journée		

4. ACTIVITE SE REPOSER

	FREQUENCE/jour	DUREE
** Manger*		
** Prier*		
** Tresser*		
** Activités petites comme arranger légumes, décortiquer arachides, tirer les feuilles*		
** Regarder télévision, bavarder avec amis ou famille*		

	DUREE
** Combien de temps est-ce que vous êtes assise au total par jour?*	

5. AUTRES ACTIVITES

Est-ce que vous avez fait d'autres activités ? OUI / NON
Lesquelles, combien de fois et combien de temps ?

____
____
____
____

6. ACTIVITE DORMIR

<u>Dormir</u> combien d'heures est-ce que vous dormez en général pendant la nuit? [   ] heures

Summary

The PhD research detailed in this manuscript centered on the topic of growth restriction in rural areas of LMICs during the first 1,000 days of life. More specifically, we investigated the role of seasonality in prenatal nutrition and physical activity on fetal growth and the effect of postnatal micronutrient supplementation in lactating women on infant growth.

The work built upon the results of the MISAME project which consisted of two randomized controlled nutrition intervention trials in pregnant women from subsistence-farming communities in the rural health district of Houndé, Burkina Faso. The MISAME1 trial showed that prenatal supplementation with multiple micronutrients (MMN, consisting of 15 micronutrients including iron and folic acid) compared to iron and folic acid (IFA) resulted in a modest increase in birth weight (+52 g; P = 0.04) and birth length (+3.6 mm; P = 0.01). The MISAME2 trial demonstrated that prenatal supplementation with a lipid-based nutrient supplement (LNS) including MMN compared to MMN resulted in a modest increase in birth length only (+4.6 mm; P = 0.001).

Both trials showed effect modifications by maternal nutritional status at baseline, with MMN compared to IFA favoring mothers with a higher BMI and LNS compared to MMN favoring underweight mothers. The PhD research further explored these findings as they imply that maternal nutrition interventions can be better targeted. Fluctuations in maternal nutritional status, linked to seasonal variations in nutrient intake and energy expenditure, are a common phenomenon in subsistence-farming communities of LMICs. Maternal nutritional status typically worsens during the rainy season. We therefore hypothesized that seasonality modifies the effect of maternal nutrition interventions on birth outcomes. A Fourier-based seasonality analysis of the effect of LNS compared to MMN revealed an important maximum increase in birth length (+13.5 mm; 95% CI: 6.5 – 20.5) at the transition from the rainy to dry season. We therefore concluded that prenatal micronutrient supplementation programs should be complemented with energy supplementation during the annual rainy season in order to promote fetal growth.

The analyses also demonstrated significant seasonal variations in birth weight, birth length, SGA and PTB incidence. The rainy season coincided with the yearly nadir in birth weight and birth length. PTB incidence rose sharply early in the season and was trailed by an upsurge in SGA birth incidence which peaked later, at the end of the season. The rainy season is not only nutritionally depriving, it also leads to sudden substantial increases in maternal work load on the field when the rains set in. It was therefore hypothesized that increased maternal physical activity (PA) during the rainy season negatively influences birth

outcomes in LMICs. In the first phase, we developed and validated an interviewer-administered structured questionnaire assessing agricultural, domestic, transportation and leisure time activity in the past week with the aim to classify pregnant rural women into levels of PA in the rainy season. However, women in the study area had much difficulty estimating time. Durations of reported activities were therefore deducted from production units of activity and distances between well-known landmarks in the area. Yet, leisure time activity could not be estimated as such and was excluded from the analyses. The questionnaire showed poor absolute measurement agreement with accelerometer data, used as an objective comparison, which suggests limited validity for individual assessments. However, the questionnaire achieved acceptable correlation and classification agreement in both test-retest reliability (absolute intra-class correlation: 0.50 – 0.67; Cohen's Kappa: 0.39 – 0.46) and concurrent validity (Spearman rank correlation: 0.42 – 0.47; Cohen's Kappa: 0.24 – 0.31) for overall PA and moderate intensity PA. These results were similar to other PA questionnaires for pregnant women. We therefore concluded that the developed questionnaire could be used to classify pregnant women into levels of PA at the population level. In the second phase of the research, we investigated if PA patterns of pregnant women in the rainy season and time spent in moderate and light intensity PA was associated with different birth outcomes. The majority of pregnant women, including those close to term, reported to be working in the field in the past week and the estimated overall PA Energy Expenditure (PAEE) was high. We found an approximate 50% risk reduction for an SGA birth in mothers with a higher PAEE in the rainy season during early and mid-pregnancy (RR: 0.52; 95% CI: 0.28 – 0.98; $P = 0.04$). Subgroup analyses showed that the association was only apparent in multiparous women in whom a reduction up to 76% was found (RR: 0.24; 95% CI: 0.09 – 0.62; $P < 0.01$). A large, albeit marginally significant, 70% SGA birth risk reduction (RR: 0.30; 95% CI: 0.09 – 1.01; $P = 0.05$) was observed in mothers who spent more time in moderate intensity PA. We concluded that the increased agricultural demands of the rainy season imposes the involvement of women in late pregnancy. Higher compared to lower PAEE was found to be protective of fetal growth restriction in women who were exposed in early and mid-pregnancy.

In the last stage of this PhD research, we looked at postnatal maternal nutritional factors that could influence child growth during infancy. Women of reproductive age have been shown to have inadequate intakes of multiple micronutrients. Yet, of all populations, lactating mothers have the highest requirements for most micronutrients and are therefore most at risk of inadequate intakes and deficiencies. Moreover, SGA and PTB newborns have been reported to have smaller micronutrient stores at birth which could become depleted earlier in infancy. It was therefore hypothesized that inadequate intakes of multiple micronutrients during lactation contribute to infant growth faltering in LMICs. In addition, it was hypothesized that infants born SGA or PTB are especially vulnerable to an inadequate supply of multiple micronutrients. A double-blind randomized controlled trial was implemented to assess the effect of MMN compared to the routine IFA supplementation in lactating mothers on infant growth and morbidity during the first 12 months of life. Although infant growth was suboptimal, infants whose lactating mothers were supplemented with MMN showed a borderline trend of increased linear growth rates (β = 0.0119 length-for-age Z-score/month; 95% CI: -0.0026 – 0.0263; $P_{MMNxage}$ = 0.107) compared to those whose mothers received IFA. Our results showed differential effects of the intervention in SGA and PTB newborns which suggest prioritized pathways. Subgroup analyses showed that the effect on linear growth was higher and statistically significant in infants who were born SGA (β = 0.0229 length-for-age Z-score/month; 95% CI: -0.0006 – 0.0465; $P_{MMNxage}$ = 0.057). Conversely, PTB infants had increased hemoglobin concentration rates (β = 0.1558 g/dL/month; 95% CI: -0.0182 – 0.3299; $P_{MMNxage}$ = 0.079). We concluded that supplementation of lactating women with MMN, compared to the routine IFA, for three months post-delivery may improve linear growth, especially of SGA born infants, and hemoglobin concentration rates in PTB infants.

Samenvatting

Het hierin beschreven doctoraatsonderzoek richtte zich op het thema van groeiachterstand gedurende de eerste 1,000 levensdagen in rurale gebieden van ontwikkelingslanden. Meer bepaald onderzochten we de rol van seizoenaliteit in prenatale voeding en fysieke activiteit op de foetale groei alsook het effect van postnatale supplementatie met micronutriënten in lacterende moeders op de groei van het jonge kind.

Dit werk bouwt verder op de resultaten van het MISAME project dat bestond uit 2 gerandomiseerde en gecontroleerde interventiestudies met zwangere vrouwen in het rurale gezondheidsdistrict van Houndé in Burkina Faso. De MISAME1 studie toonde aan dat prenatale supplementatie met meerdere micronutriënten (MMN; 15 micronutriënten inclusief ijzer en foliumzuur) ten opzichte van enkel ijzer en foliumzuur (IFA) leidde tot een hoger geboortegewicht (+52 g; P = 0.04) en hogere geboortelengte (+3.6 mm; P = 0.01). De MISAME2 studie ontdekte verder dat een vetrijk voedingssupplement (LNS) aangerijkt met MMN ten opzichte van MMN alleen (met dezelfde 15 micronutriënten) leidde tot een hogere geboortelengte (+4.6 mm; P = 0.001).

Beide studies toonden aan dat de grootte van het effect van de interventie bepaald werd door de voedingstoestand van de moeder aan het begin van de zwangerschap. Enerzijds was het effect van MMN, ten opzichte van IFA, sterker bij moeders die bij het begin van de zwangerschap een hogere BMI hadden. Anderzijds was het effect van LNS, ten opzichte van MMN, op geboortelengte enkel significant bij moeders met een lage BMI. Het doctoraatsonderzoek in dit werk verrichte een meer diepgaande analyse omdat deze resultaten aangeven dat voedingsinterventie studies beter toegewezen kunnen worden. De voedingstoestand van de moeder varieert immers nogal in rurale gemeenschappen uit ontwikkelingslanden vanwege de seizoenale fluctuaties in nutriënten inname en energieverbruik. De voedingstoestand van de moeder verslechtert doorgaans gedurende het jaarlijkse regenseizoen. Om die reden stelden we de hypothese voorop dat zulke fluctuaties het effect van een prenatale voedings-supplementatie in belangrijke mate kunnen beïnvloeden. Een Fourier-analyse toonde aan dat het eerder vermelde effect van LNS op geboortelengte, ten opzichte van MMN, maximaal was (+13.5 mm; 95% betrouwbaarheidsinterval (BI): 6.5 – 20.5) bij de transitie van het regenseizoen naar het droog seizoen. We besloten daarom dat prenatale supplementatie met micronutriënten best wordt aangevuld met een dosis energie om tot een beter foetale groei te komen in die zwangerschappen die het regenseizoen overbruggen.

De analyses toonden verder ook belangrijke variaties in geboortegewicht, geboortelengte, en de prevalentie aan te kleine pasgeborenen (SGA, een maat voor foetale groeiachterstand) en premature geboortes. Het jaarlijkse regenseizoen bleek samen te vallen met het jaarlijkse minimum in geboortegewicht en -lengte. Verder vertoonde de prevalentie aan premature geboortes een scherpe piek in dit seizoen. De piek werd gevold door een gradueel stijgend aantal SGA pasgeborenen dat piekte tegen het eind van het regenseizoen. Het jaarlijkse regenseizoen is niet enkel gekenmerkt door een suboptimale voedselbeschikbaarheid en een daarmee gepaard gaande suboptimale voedingsinname, het seizoen wordt ook gekenmerkt door een hogere werklast op het veld. Om die reden stelden we de hypothese voorop dat een hogere fysieke actieve activiteit tijdens het regenseizoen de geboortekenmerken van het kind negatief zou kunnen beïnvloeden in ontwikkelingslanden. In een eerste onderzoeksfase ontwikkelde en valideerde het studieteam een vragenlijst die activiteiten zoals landbouw, huishouden, vervoer en vrije tijd in vrouwen op een betrouwbare manier kon bevragen. Het doel van dit instrument was om zwangere vrouwen uit rurale gemeenschappen te klasseren volgens het niveau van fysieke activiteit in het regenseizoen. Vrouwelijke deelnemers hadden echter veel moeilijkheden bij het inschatten van de duurtijd van activiteiten en afstanden. Daarom werd een strategie geïntroduceerd om de tijdsduur af te leiden van de hoeveelheid arbeid die werd verricht (in productie eenheden) of om de afstand die werd afgelegd af te leiden uit afstanden tussen gekende oriëntatiepunten. De inschatting van de vrije tijd bleek te moeilijk om correct in te schatten en werd daarom niet meegenomen in de analyse. De vragenlijst vertoonde een redelijk slechte overeenkomst met accelerometer data wat suggereert dat het bewegingsprofiel niet accuraat werd ingeschat. De vragenlijst toonde echter een aanvaardbare correlatie en classificatie overeenkomst bij zowel de herhaalbaarheid (resp. absolute intra-klasse correlatie: 0.50 – 0.67; Cohen’s kappa: 0.39 – 0.64) als de concurrente validiteit met accelerometer data (resp. Spearman correlatie: 0.42 – 0.47; Cohen’s kappa: 0.24 – 0.31) voor de totale fysieke activiteitscore (PAEE) en de tijd besteed in fysieke activiteit van matige intensiteit. Deze resultaten waren vergelijkbaar met andere bestaande vragenlijsten voor zwangere vrouwen. Op basis van dit onderzoek besloten we dat de vragenlijst op voldoende wijze als instrument kan dienen om zwangere vrouwen te rangschikken volgens hun fysiek activiteitsniveau. In de tweede onderzoeksfase onderzochten we de fysieke activiteitspatronen van zwangere vrouwen tijdens het regenseizoen en associaties tussen een hogere totale fysieke activiteitscore en meer tijd besteed in activiteiten van lichte en matige intensiteit met de geboortekenmerken van de pasgeborenen. De meerderheid van de zwangere vrouwen, inclusief deze aan het eind van de zwangerschap, rapporteerde op het veld te werken tijdens de afgelopen week en het ingeschatte energieverbruik (PAEE) was relatief hoog. De studieresultaten toonden aan dat moeders met een hogere PAEE tijdens het regenseizoen in hun eerste en tweede trimester 50% minder risico liepen op een SGA pasgeborene (relatief risico (RR): 0.52; 95%BI: 0.28 – 0.98; *P* =0.04). Een subgroep analyse toonde verder aan dat dit verband sterker was in multipara zwangerschappen (RR: 0.24; 95%BI: 0.09 – 0.62; *P* <0.01). Daarnaast vonden we ook een grote vermindering van het risico op een SGA pasgeborene bij vrouwen die meer tijd spendeerden in matig intense fysieke activiteit (RR: 0.30; 95% BI: 0.09 – 1.01; *P* = 0.05), maar deze associatie was echter nauwelijks statistisch significant. Uit deze studie besloten we dat zwangere vrouwen niet gespaard worden van zware landbouw activiteiten, zelfs niet op het einde van hun zwangerschap. Een hogere versus lagere fysieke activiteitscore tijdens het eerste en tweede trimester van de zwangerschap werd gezien als een beschermende factor tegen foetale groeiachterstand.

In het laatste deel van het doctoraatsonderzoek onderzochten we de invloed van postnatale nutritionele factoren van de moeder op de groei van het jonge kind. Over het algemeen vertonen vrouwen van vruchtbare leeftijd in ontwikkelingslanden een onvoldoende inname van meerdere micronutriënten. Echter, de aanbevolen inname van de meeste micronutriënten is het hoogst tijdens de lactatiefase wat leidt tot een hoger risico op een onvoldoende inname en tekorten. Daarnaast hebben SGA en prematuur pasgeborenen kleinere reserves aan micronutriënten bij de geboorte die sneller dreigen uitgeput te geraken tijdens de groei. Uit deze vaststellingen werd de hypothese voorop opgesteld dat de suboptimale inname van meerdere micronutriënten door lacterende moeders bijdraagt tot de groeiachterstand van hun jonge, zogende, kinderen in ontwikkelingslanden. Bovendien werd vooropgesteld dat kinderen die SGA en prematuur geboren werden kwetsbaarder zijn voor een onvoldoende inname van meerdere micronutriënten. Een dubbel geblindeerde, gerandomiseerde en gecontroleerde interventiestudie werd geïmplementeerd om het effect na te gaan van postnatale supplementatie met MMN, ten opzichte van IFA, in lacterende moeders tot 3 maanden na de bevalling op de groei en morbiditeit van kinderen in het eerste levensjaar. Hoewel de groeiachterstand toenam in de ganse groep, werd een licht stijgende trend in de groeisnelheid van kinderen uit de interventiegroep waargenomen (β = 0.0119 lengte-voor-leeftijd Z-score/maand; 95% BI: -0.0026 – 0.0263; $P_{MMNxleeftijd}$ = 0.107) in vergelijking met kinderen in de controlegroep. Er werd een verschillend effect van MMN waargenomen in de subgroep van kinderen die SGA en prematuur geboren werden. Enerzijds nam de lineaire groei van SGA kinderen van moeders in de interventiegroep minder af (β = 0.0229 lengte-voor-leeftijd Z-score/maand; 95% BI: -0.0006 – 0.0465; $P_{MMNxleeftijd}$ = 0.057). Anderzijds leidde de interventie tot een iets hogere hemoglobine concentratie bij prematuur geboren kinderen (β = 0.1558 g/dL/maand; 95% BI: -0.0182 – 0.3299; $P_{MMNxleeftijd}$ = 0.079). We besloten daarom dat MMN supplementatie van lacterende vrouwen, ten opzichte van IFA, tot drie maanden na de bevalling een mogelijks positief effect kan hebben op de lineaire groei van kinderen die SGA geboren werden en op de hemoglobine concentraties van kinderen die prematuur geboren werden.

Curriculum vitae of the author

Kimberley Bouckaert was born on December 4th 1982 in Kortrijk, Belgium. She finished her secondary education in Mathematics and Science at Spes Nostra Kuurne in 2000. She graduated from Ghent University with a MSc degree in Bioscience Engineering in 2005 and obtained a second degree in Food Science and Nutrition in 2007. She briefly worked in the environmental and food industry business in Belgium after graduating. In 2008, she worked as a trainee student at the International Agency for Research on Cancer in Lyon in collaboration with the Institute of Food Research in Norwich on the standardization of folate databases for international nutrition studies. She also participated in several EU-projects as a scientific collaborator at the Department of Public Health and the Department of Food Safety and Food Quality of Ghent University. She started her research on maternal and child nutrition in low and middle income countries in 2009 at the Department of Food Safety and Food Quality of Ghent University in collaboration with the Nutrition and Child Health Unit at the Institute of Tropical Medicine in Antwerp.

Peer-review publications with scientific citation index (a1)

Papers published

- Wondafrash M, Huybregts L, Lachat C, Bouckaert K and Kolsteren P (2016). Dietary diversity predicts dietary quality regardless of season in 6-12 months old infants in south-west Ethiopia. Public Health Nutr, published ahead of print. doi: 10.1017/S1368980016000525.

- Bouckaert K*, Toe L*, De Beuf K, Roberfroid D, Meda N, Thas O, Van Camp J, Kolsteren P, and Huybregts L (2015). Seasonality modifies the effect of a lipid-based nutrient supplement for pregnant rural women on birth length. J Nutr, 145(3), 634-639. **shared first authorship*

- Kulwa K, Verstraeten R, Bouckaert K, Mamiro PS, Kolsteren P and Lachat C (2014). Effectiveness of a nutrition education package in improving feeding practices, dietary adequacy and growth of infants and young children in rural Tanzania: rationale, design and methods of a cluster randomized trial. BMI Public Health, 14, article number: 1077.

- Nikièma L, Huybregts L, Kolsteren P, Lanou H, Tiendrebeogo S, Bouckaert K, Kouanda S, Sondo B and Roberfroid D (2014). Treating Moderate Acute Malnutrition in first-line health

services: an effectiveness cluster-randomized trial in Burkina Faso. Am J Clin Nutr, 100(1), 241-249.

- Bouckaert K, Slimani N, Nicolas G, Vignat J, Wright A, Roe M, Witthöft C and Finglas P (2011). Critical evaluation of folate data in European and international databases: Recommendations for standardization in international nutritional studies. Mol Nutr Food Res, 55, 166-180.

- Jenab M, Salvini S, van Gils CH, Brustad M, Shakya-Shrestha S, Buijsse B, Verhagen H, Touvier M, Biessy C, Wallström P, Bouckaert K, et al. (2009). Dietary intakes of retinol, beta-carotene, vitamin D and vitamin E in the European Prospective Investigation into Cancer and Nutrition cohort. Eur J Clin Nutr, 63, S150-78.

- Slimani N, Deharveng G, Southgate DAT, Biessy C, Chajes V, van Bakel MME, Boutron-Ruault MC, McTaggart A, Grioni S, Verkaik-Kloosterman J, Huybrechts I, Amiano P, Jenab M, Vignat J, Bouckaert K, et al. (2009). Contribution of highly industrially processed foods to the nutrient intakes and patterns of middle-aged populations in the European Prospective Investigation into Cancer and Nutrition study. Eur J Clin Nutr, 63, S206-25.

Papers under review

- Argaw A, Wondafrash M, Bouckaert K, Kolsteren P, Belachew T and Huybregts L. N-3 LC-PUFA supplementation of lactating mothers improves development of their breastfed children in Ethiopia: a randomized controlled trial. Submitted to Am J Clin Nutr, under review.

- Wondafrash M, Huybregts L, Lachat C, Bouckaert K and Kolsteren P. Assessment of child feeding practices and nutritional status among infants and young children: based on data collected in two seasons in rural southwestern Ethiopia. Submitted to BMC Nutr, under review.

- Bouckaert K, Vandevijvere S, Hagströmer M, Lachat C, Verstraeten R, Kolsteren P, Van Camp J and Huybregts L. Rain season activity in pregnant women and birth outcome: a cross-sectional study in rural Burkina Faso. Submitted to PLOS One, under review.

Abstracts (c3)

- Bouckaert K, Kibebew M, Van Camp J, Kolsteren P. ω3 LCPUFA for healthy growth and development of young children in Ethiopia. 5th Annual symposium of the Ghent Africa Platform (GAPSYM-5): (r)Urban Africa: multidisciplinary approaches to the African city, 2nd December 2011, Ghent, Belgium. Poster abstract.

- Bouckaert K, Van Camp J, Kolsteren P. ω3 and ω6 fatty acids for growth, development and gut integrity of infants in developing countries. 2nd Annual meeting of the Belgian Nutrition Society: Microorganisms in human nutrition – exploring new pathways for health, 29th April 2011, Brussels, Belgium. p14 – oral communication abstract.

- Critical evaluation of folate data in European and international databases. Bouckaert KP, Slimani N, Deharveng G, Witthöft CM, Vignat J, Wright AJA, Finglas PM. 3rd International EuroFIR congress, 8th-10th September 2009, Vienna, Austria. p46-47 – short communication abstract 3_46, and p154 – poster abstract 3_46.

Training

- 2015: Estimating average treatment effects using Stata, Washington DC, USA

- 2014: European nutrition leadership programme, Luxemburg city, Luxemburg

- 2014: Creativity in research, Institute of Tropical Medicine, Antwerp, Belgium

- 2013: Speed reading, Ghent University, Belgium

- 2012: Clinical studies, Faculty of Medicine and Health Sciences, Ghent University, Belgium

- 2009 – 2010: Statistics: Analysis of Variance, Institute of Continuing Education in Science, Ghent, Belgium

Prizes

- Young investigator award for outstanding oral research communication: ω3 and ω6 fatty acids for young children in developing countries: an intervention study in Ethiopia. 2nd Belgian Nutrition Society congress, 29th April 2011, Brussels, Belgium.

Scholarships

- 2014 laureate of the Dubois-Brigué scholarship for the final year of a doctorate. Awarded by the Royal Academy of Medicine of Belgium.

- 2009 laureate of a VLADOC PhD scholarship for research on a development oriented subject. Awarded by the Flemish Interuniversity Council.