The efficacy of fortified balanced energy-protein supplementation on diet of pregnant women and birth outcomes in rural Burkina Faso

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Abbreviations

24HR	24-Hour recall
AA	Arachidonic acid
AGA	Appropriate-for-gestational age
AI	Adequate intake
ANC	Antenatal care
BEP	Balanced energy-protein
BMGF	Bill and Melinda Gates Foundation
BMI	Body mass index
BOND	Biomarkers of Nutrition for Development
CI	Confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CSB	Corn-soy blend
CSPro	Census and Survey Processing System
CSPS	Centre de Santé et Promotion Sociale
DFE	Dietary folate equivalents
DHA	Docosahexaenoic acid
DIAAS	Digestible indispensable amino acid score
DSMB	Data and Safety Monitoring Board
EAR	Estimated average requirement
EPA	Eicosapentaenoic acid
FA	Femme accompagnante
FANTA	Food and Nutrition Technical Assistance
FAO	Food and Agricultural Organization of the United Nations
FBF	Fortified blended food
FCT	Food composition table
FGD	Focus groups discussion
GI	Glycemic index
GL	Glycemic load
Hb	Hemoglobin
IFA	Iron-folic acid
INSPIRE	Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence
INTERGROWTH	International Fetal and Newborn Growth Consortium
IQR	Interquartile range

ITT	Intention-to-treat
IUGR	Intrauterine growth retardation
LAZ	Length-for-age z-score
LBW	Low birth weight
LGA	Large-for-gestational age
LMICs	Low- and middle-income countries
LMP	Last menstrual period
LNS	Lipid-based nutrient supplement
LQ-LNS	Large quantity lipid-based nutrient supplement
MDD-W	Minimum Dietary Diversity for Women
MINT	Maternal and Infant Nutrition Trial
MISAME	Micronutriments pour la Santé de la Mère et de l'Enfant
MMN	Multiple micronutrients
MQ-LNS	Medium quantity lipid-based nutrient supplement
MUAC	Mid-upper arm circumference
NAM	National Academy of Medicine; *formerly* the Institute of Medicine (IoM)
NAR	Nutrient adequacy ratio
PAL	Physical activity level
PP	Percentage points
RAE	Retinol activity equivalent
RCT	Randomized controlled trial
RE	Retinol equivalent
RUSF	Ready-to-use supplementary food
SAE	Serious adverse event
SD	Standard deviation
SELEVER	Soutenir l'Exploitation familiale pour Lancer l'Élevage des Volailles et Valoriser l'Économie Rurale
SGA	Small-for-gestational age
SQ-LNS	Small quantity lipid-based nutrient supplement
UN	United Nations
UNICEF	United Nations Children's Fund
UNIMMAP	UNICEF/WHO/UNU international multiple micronutrient preparation
WASH	Water, sanitation and hygiene
WAZ	Weight-for-age z-score
WFP	World Food Programme
WHO	World Health Organization

Summary

Globally, malnutrition in all its forms remains a major challenge with serious consequences for health and wellbeing. It affects millions of people around the world and has a devastating developmental, economic and social impact on communities. Especially in low- and middle-income countries, unacceptable levels of undernutrition persist. Climate change, ongoing conflicts and the COVID-19 pandemic have triggered a global food crisis, putting even more women, infants and young children at risk of malnutrition.

Poor maternal nutritional status not only affects a woman's health, but also influences the health of her child (elaborated on in **Chapter 1**). Deficiencies in macro- and micronutrients during pregnancy are linked to poor fetal growth and development, which can lead to stillbirth, preterm delivery, small size at birth, and increased mortality and morbidity – during early childhood lasting into adulthood. Pregnancy is therefore viewed as a window to future health. To meet maternal needs and support fetal development, nutrition interventions that deliver additional energy and nutrients have the potential to improve pregnancy and birth outcomes.

There is limited evidence that balanced energy-protein (BEP) supplementation (with less than 25% of energy from protein) during pregnancy improves birth weight, and reduces the risk of stillbirth and born small-for-gestational age (SGA). These conclusions on the impact of BEP supplementation should however be interpreted with caution due to the large heterogeneity in supplements, study populations and quality of the study designs. To address the critical need for high-quality studies, the 'Micronutrients for Maternal and Child Health-III' (in French: 'Micronutriments pour la Santé de la Mère et de l'Enfant', MISAME-III) research project in Burkina Faso was designed.

In MISAME-III, an individually randomized controlled efficacy trial design was used to test the hypothesis that BEP supplementation during pregnancy results in a lower prevalence of SGA. In addition, the effect on large-for-gestational age, low birth weight (LBW; less than 2500 g), preterm birth (before 37 weeks of pregnancy), gestational duration, birth weight, birth length, Rohrer's ponderal index, head circumference, thoracic circumference, arm circumference, fetal loss and stillbirth was evaluated. The study protocol of MISAME-III is described in detail in **Chapter 2** of this PhD thesis.

The effectiveness of BEP supplementation as a nutrition intervention largely depends on women's adherence to daily supplementation. Therefore, a formative study was designed to assess acceptability and utilization of BEP supplements. The first part of this formative research, presented in **Chapter 3**, included an evaluation of 12 product formulations by 40 pregnant women in the local community. A mixed methods approach revealed that products with a sweet flavor and resemblance to familiar foods were preferred. The perceived health benefits of BEP supplements were identified as a promoting factor and an unpleasant odor of a product as a limiting factor for daily consumption. Sharing the BEP supplement with children or other family members was raised as a possible concern for the impact of the intervention.

In the second part of this formative research, presented in **Chapter 4**, the two highest-ranked products – a lipid-based peanut paste and vanilla biscuit – were evaluated during a 10-week home feeding trial by 80 Burkinabe pregnant women to assess medium-term acceptability and adherence. The results showed that both BEP supplements were well accepted. For implementation in the main trial, the lipid-based peanut paste fortified with multiple-micronutrients was selected. Structured and easy-to-understand communication by health care professionals on the use and benefits of prenatal food supplements, and engaging community leaders and family members were identified as important promoting factors for adherence. These context-specific findings that can be used to promote optimal supplement use, demonstrate the value of conducting preliminary research prior to the actual implementation.

In **Chapter 5**, a cross-sectional dietary intake study is presented that investigates another important attenuating factor for the efficacy of supplementation, namely the possible displacement of nutrient-dense foods by BEP supplements. Using a 24-h dietary recall, energy and nutrient intakes from pregnant women in the intervention and control group were compared. Our results showed that supplementation significantly increased dietary energy and macro- and micronutrients intakes, with a difference in median energy intake equivalent to a daily dose of the BEP supplement. Nutrient adequacy was low for all micronutrients in both groups when excluding the BEP supplement and increased to the estimated average requirement for pregnant women when including the BEP supplement.

In conclusion, this study showed that BEP supplementation can fill nutrient gaps without displacing food intake.

Findings of the main MISAME-III randomized controlled trial, conducted between October 2019 and August 2021, are presented and discussed in **Chapter 6**. In total, 1,788 pregnant women from 6 health center catchment areas in rural Burkina Faso were enrolled in the study and met the inclusion criteria. The women were randomized to receive either daily fortified BEP supplements and iron-folic acid (IFA) tablets (intervention) or IFA (control), consumed under supervision of trained village-based project workers during home visits. Mean gestational age at enrolment was 11 weeks, and adherence rates for daily observed intake were 83% for BEP, and 89% and 91% for IFA in the control and intervention group, respectively. Birth anthropometry was measured of 1,708 pregnancies at latest 12 hours after delivery by trained study midwives. All statistical analysis were conducted following the intention-to-treat principle.

The results showed that BEP supplementation led to a mean 3.1 percentage points (pp) reduction in SGA with a 95% confidence interval (CI) of −7.39 to 1.16 (P = 0.151), indicating a wide range of plausible true treatment efficacy. The intervention significantly improved the duration of gestation (+0.20 weeks, 95% CI 0.05 to 0.36, P = 0.010), birth weight (50.1 g, 8.11 to 92.0, P = 0.019), birth length (0.20 cm, 0.01 to 0.40, P = 0.044), thoracic circumference (0.20 cm, 0.04 to 0.37, P = 0.016), arm circumference (0.86 mm, 0.11 to 1.62, P = 0.025), and decreased LBW prevalence (−3.95 pp, −6.83 to −1.06, P = 0.007) as secondary outcomes measures. No differences in serious adverse events (fetal loss and stillbirth) between the study groups were found. The subgroup analyses revealed that the intervention was efficacious in reducing SGA prevalence among mothers with a more adequate baseline nutritional status.

Overall, the findings of the MISAME-III trial did not support our primary study hypothesis that fortified BEP supplementation during pregnancy decreased SGA in a rural setting in Burkina Faso. However, the intervention was efficacious in increasing gestational duration, birth weight, birth length, thoracic and arm circumference, and decreasing the prevalence of LBW babies. To estimate the clinical relevance of BEP supplementation, future research in MISAME-III will assess additional maternal and child biochemical parameters to provide further insights.

In conclusion, this PhD research provides robust evidence on the effect of prenatal BEP supplementation on birth outcomes in a rural sub-Saharan African setting. In **Chapter 7**, the implications of the study findings are discussed and recommendations for future research and policy are provided. As many risk factors play a role in maternal nutrition and fetal growth, future research should invest in reliable biomarkers for a mechanistic understanding of the impact of BEP supplementation. In combination with other well-designed trials, the MISAME-III results can guide future policy recommendations. Nonetheless, prenatal BEP supplementation is not an alternative to healthy diets but can be used as a complementary solution. A multi-sectoral approach that addresses underlying and contextual determinants of malnutrition and engages the target population is required to tackle the complex problem of poor birth outcomes.

Samenvatting

Ondervoeding blijft één van de grootste wereldwijde uitdagingen met ernstige gevolgen voor de gezondheid en welzijn. Miljoenen mensen lopen het risico op ondervoeding met verstrekkende sociale en economische gevolgen. Vooral in lage- en middeninkomenslanden eist ondervoeding een hoge tol. Door klimaatverandering, conflicten en de COVID-19 pandemie is er een wereldwijde voedselcrisis ontstaan die nog meer vrouwen en kinderen in gevaar brengt.

Gezonde en evenwichtige voeding tijdens de zwangerschap is essentieel. Een slechte voedingsstatus van de moeder heeft namelijk niet alleen een negatief effect op haar eigen gezondheid, maar beïnvloed ook de groei en ontwikkeling van haar kind (**Hoofdstuk 1**). Een tekort aan macro- en micronutriënten tijdens de zwangerschap is gelinkt aan een verminderde foetale groei en ontwikkeling, wat kan leiden tot doodgeboorte, vroeggeboorte, een te klein geboren kind, en een verhoogde kans op mortaliteit en morbiditeit in de eerste levensjaren en op volwassen leeftijd. De zwangerschap wordt daarom beschouwd als een belangrijke periode om de toekomstige gezondheid te beïnvloeden. Gedurende de zwangerschap zijn de voedingsbehoeften verhoogd om zowel de moeder, als de ontwikkeling van de baby te ondersteunen. Om in deze verhoogde behoefte te voorzien, kunnen voedingsinterventies die extra energie en voedingsstoffen voorzien mogelijk een positieve bijdrage leveren aan de zwangerschap en geboorte uitkomsten.

Er is beperkt bewijs dat energie-proteïne supplementen, met minder dan 25% energie uit eiwit, (Engels: 'balanced energy-protein supplement', BEP) tijdens de zwangerschap een positief effect hebben op het geboortegewicht en de kans verlagen op doodgeboorte en een te klein geboren baby. De studies voor dit wetenschappelijke bewijs verschillen echter enorm in kwaliteit. Het is daarnaast niet eenvoudig om de studieresultaten te vergelijken door grote verschillen in het type voedingssupplement en deelnemers, waardoor het belangrijk is om voorzichtig te zijn met definitieve conclusies. Om aanvullend bewijs te leveren is het grootschalige onderzoek 'Micronutriënten voor de Gezondheid van Moeder en Kind-III' (Frans: 'Micronutriments pour la Santé de la Mère et de l'Enfant', MISAME-III) in Burkina Faso opgezet.

MISAME-III is een individueel gerandomiseerd onderzoek met een controlegroep om het effect van een verrijkt BEP supplement tijdens de zwangerschap te testen. De belangrijkste hypothese die werd onderzocht, is of een dagelijkse inname van het voedingssupplement leidt tot minder baby's die te klein worden geboren voor de zwangerschapsduur (Engels: 'small-for-gestational age', SGA). Ook werd gekeken naar het effect op te groot geboren baby's, laag geboortegewicht (minder dan 2500 gram), vroeggeboorte (voor 37 weken zwangerschap), de duur van de zwangerschap, ponderal index (gewicht gedeeld door de lengte tot de derde macht), hoofd-, arm-, en borstomtrek, en het aantal gevallen van een miskraam en doodgeboorte. Het studieprotocol van MISAME-III is in detail beschreven in **Hoofdstuk 2** van deze PhD thesis.

Het effect van de voedingssupplementen hangt voor een groot deel af van de mate waarin vrouwen de supplementen dagelijks innemen. Om deze reden is een verkennende studie opgezet om de acceptatie en het gebruik van BEP supplementen te onderzoeken. Het eerste deel van dit verkennende onderzoek komt aan bod in **Hoofdstuk 3** en omvat een evaluatie van 12 producten door 40 zwangere vrouwen in de lokale gemeenschap. Met behulp van kwalitatieve en kwantitatieve onderzoeksmethoden kwam naar voren dat vrouwen in onze setting een voorkeur hebben voor producten met een zoete smaak en producten die herkenbaar zijn. De gezondheidsvoordelen van de supplementen werd aangeduid als een belangrijke stimulans om de producten dagelijks te consumeren, terwijl een sterke geur als hinderlijk werd ervaren. Het delen van de voedingssupplementen met kinderen of andere familieleden werd aangemerkt als mogelijk risico voor een lagere impact van de voedingsinterventie.

In het tweede deel van de verkennende studie, beschreven in **Hoofdstuk 4**, werden de twee producten die het best scoorden, een verrijkte pinda-pasta en vanille biscuit, in de thuisomgeving getest door 80 zwangere vrouwen gedurende 10 weken. Deze studieopzet diende om inzicht te geven in de mate van acceptatie en bereidheid om het supplement dagelijks te consumeren gedurende een langere tijd. De resultaten laten zien dat beide voedingssupplementen hier positief op scoren. Voor de interventiestudie is uiteindelijk de verrijkte pinda-pasta geselecteerd.

Om ervoor te zorgen dat zwangere vrouwen zich aan het advies van één portie per dag houden, blijkt informatie over het gebruik en de positieve effecten voor de moeder en het (ongeboren) kind een belangrijke rol te spelen. Evenals het informeren en betrekken van belangrijke personen binnen de gemeenschap en familieleden om draagvlak te creëren. Gezondheidsprofessionals worden gezien als meeste betrouwbare bron om deze informatie op een gestructureerde en begrijpelijke wijze over te brengen. Deze context-specifieke bevindingen kunnen worden gebruikt om de introductie van supplementen zo optimaal mogelijk te maken en benadrukken de meerwaarde van een verkennende studie voorafgaand aan een klinische studie of supplementatieprogramma met een groot bereik.

In **Hoofdstuk 5** wordt een cross-sectionele voedselconsumptiestudie beschreven die een andere mogelijke impact factor onderzoekt, namelijk een verandering van het dagelijkse eetpatroon door de consumptie van het voedingssupplement. Als nutriëntrijke voeding wordt vervangen door de verrijkte pinda-pasta, zou de impact van de interventie namelijk lager uit kunnen vallen. Met een interactieve methode om de exacte voedingsinname van de afgelopen 24-uur te meten, is een vergelijking gemaakt tussen de zwangere vrouwen in de interventie- en controlegroep. De resultaten van de studie laten zien dat de supplementen de inname van energie en macro- en micronutriënten significant verhogen, met een verschil dat gelijk is aan één dosis van het voedingssupplement. De inname van vitaminen en mineralen bleek (ver) onder de grens van 'adequate inname' tijdens de zwangerschap voor beide groepen en werd bereikt met de toevoeging van het supplement aan het dieet van de vrouwen. De studie levert dus bewijs dat het BEP supplement een werkelijke toevoeging is aan het dieet en nutritionele tekorten kan aanvullen.

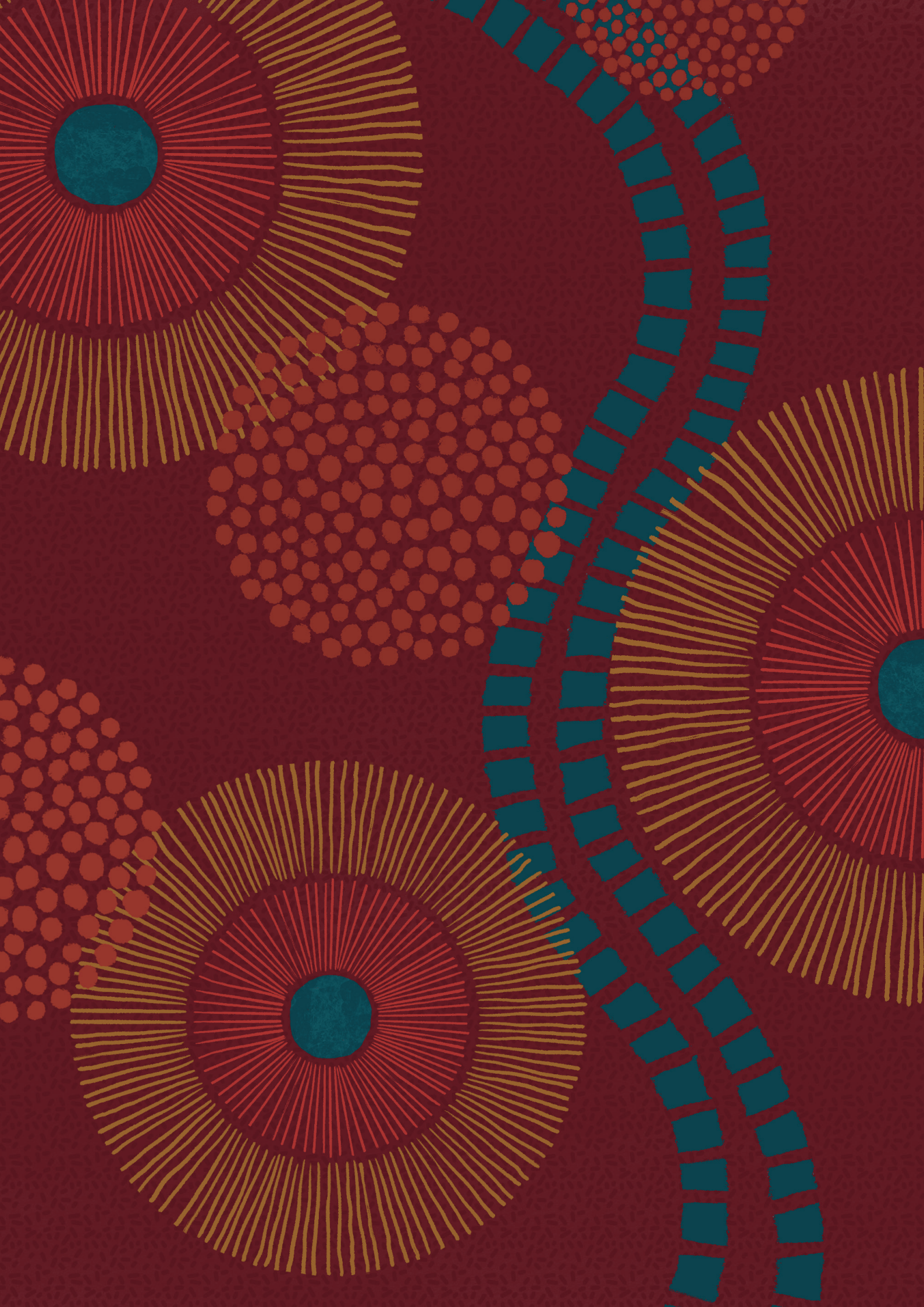
De resultaten van de MISAME-III gerandomiseerde interventiestudie worden voorgesteld en besproken in **Hoofdstuk 6**. In totaal namen 1788 zwangere vrouwen deel aan de studie binnen het bereik van 6 gezondheidscentra rondom Houndé in ruraal Burkina Faso. De vrouwen werden gerandomiseerd om dagelijks het verrijkte BEP supplement en een ijzer- en foliumzuurtablet (interventiegroep) of enkel een ijzer en foliumzuurtablet (controlegroep) te ontvangen. Beide supplementen werden dagelijks huis-aan-huis gebracht door projectmedewerkers uit de dorpen en geconsumeerd onder supervisie.

De gemiddelde zwangerschapsduur was 11 weken bij deelname aan de studie. In de interventiegroep namen 83% van de vrouwen het BEP supplement dagelijks onder supervisie, en in de controlegroep was het aandeel 89% voor het innemen van de ijzer- en foliumzuurtabletten. Antropometrie bij de geboorte werd gemeten van 1708 zwangerschappen, ten laatste 12 uur na de bevalling, door verloskundigen van het MISAME-III project. Alle statistische analysen volgden het principe van 'intention-to-treat'.

De resultaten toonden aan dat BEP supplementatie tot een gemiddeld lagere prevalentie van het aantal te klein geboren (SGA) baby's leidde, met een verlaging van 3.1 percentage punt (pp) en een 95% betrouwbaarheidsinterval (Engels: confidence interval, CI) van −7.39 tot 1.16 (P = 0.151), wat een brede range van een aannemelijk effect weergeeft. De interventie zorgde daarnaast voor een significant langere zwangerschapsduur (+0.20 weken, 95% CI 0.05 tot 0.36, P = 0.010), een hoger geboortegewicht (50.1 g, 8.11 tot 92.0, P = 0.019), geboortelengte (0.20 cm, 0.01 tot 0.40, P = 0.044), borstomtrek (0.20 cm, 0.04 tot 0.37, P = 0.016), armomtrek (0.86 mm, 0.11 tot 1.62, P = 0.025), en een vermindering in het aantal baby's met een laag geboortegewicht (−3.95 pp, −6.83 tot −1.06, P = 0.007). Er werden geen verschillen gevonden in het aantal ernstige ongewenste voorvallen (miskraam en doodgeboorte) tussen de studiegroepen. Subgroep analyse toonde aan dat de BEP supplementatie een positiever effect had bij vrouwen met een betere voedingsstatus aan het begin van de zwangerschap.

De resultaten van de MISAME-III studie leverden geen bewijs dat verrijkte BEP supplementen tijdens de zwangerschap het aantal te klein geboren baby's verlaagd in een rurale setting in Burkina Faso. Wel leverde de studie het bewijs dat het geven van verrijkte BEP voedingssupplementen een effectieve interventie is om de zwangerschapsduur te verlengen, geboortegewicht- en lengte, en borst- en armomtrek te verhogen, en de kans op een laag geboortegewicht te verkleinen. Om de klinische relevantie van deze uitkomsten te bepalen, is het vervolgonderzoek binnen het MISAME-III onderzoeksproject van belang. Extra biochemische parameters van moeder en kind leveren mogelijk nieuwe inzichten op over het effect van de interventie.

Dit PhD onderzoek heeft bijgedragen aan het leveren van robuust bewijs over het effect van verrijkte BEP supplementen tijdens de zwangerschap op geboorte uitkomsten in een rurale setting in sub-Sahara Afrika. In **Hoofdstuk 7**, worden de implicaties van de onderzoeksresultaten besproken en aanbevelingen gedaan voor verder onderzoek en beleid. Gezien de vele factoren die een rol spelen in de voedingstoestand van de moeder en groei van de foetus, is het belangrijk om te investeren in betrouwbare biologische markers die verder inzicht kunnen geven in de mechanistische werking en de impact van het BEP supplement. Door de resultaten van MISAME-III samen te brengen met andere interventiestudies van hoge kwaliteit, kunnen de bevindingen een basis vormen voor toekomstig beleid. Het is belangrijk om te benadrukken dat voedingssupplementen tijdens de zwangerschap geen alternatief zijn voor een gezond dieet, maar het kan zeker onderdeel zijn van een strategie voor de aanpak van ondervoeding. Een multi-sectoren benadering die ook de onderliggende en contextuele factoren van ondervoeding aanpakt, in samenspraak met de lokale gemeenschap, is gewenst om het complexe probleem van maternale ondervoeding en verminderde groei en ontwikkeling van baby's aan te pakken.



I

General introduction

1.1 Malnutrition: a global challenge

Globally, malnutrition in all its forms remains a major challenge. The term malnutrition refers to deficiencies, excesses or imbalances in a person's energy or nutrient intake and addresses three broad group of conditions: (1) undernutrition; (2) micronutrient-related malnutrition; (3) overweight, obesity and diet-related noncommunicable diseases [1]. Poor nutrition has serious consequences for health, including impaired physical and cognitive development, reduced immune function and an increased risk of developing noncommunicable diseases, such as diabetes, heart disease and cancer [2]. It limits people's ability to reach their full developmental potential and restricts economic productivity of individuals and societies. Childhood stunting (low height-for-age) is estimated to lead to an average of 5-7% loss of per capita income [3]. In addition, anemia (reduction in the oxygen carrying capacity of blood due to decreases in hemoglobin [Hb] concentration and red blood cell volume) is associated with adverse birth outcomes, poor cognitive and motor development in children, and work capacity in adults [4].

1.1.1 Global prevalence of malnutrition

Every country worldwide is affected by one or more forms of malnutrition. Overweight and obesity are an increasing concern: an estimated 2.2 billion (40%) adults and 38.9 million (5.7%) children are overweight or obese [5]. On the other hand, unacceptable levels of undernutrition persist, mainly in low- and middle-income countries (LMICs). In 2020, 20.5 million newborns (14.6% of all live births) had a low weight at birth, 149.2 million (22%) children under 5 years of age were stunted and 45.4 million (6.7%) were wasted (low weight-for-height) [5]. It was estimated that around 3.2 million children under 5 die every year due to undernutrition, which is 45% of all child deaths [2].

1.1.2 Sustainable Development Goals and Decade of Action on Nutrition

The alarmingly high number of people that suffer from hunger and undernutrition requires swift action. The United Nations (UN) Decade of Action on Nutrition (2016-2025) is a commitment by UN Member States to undertake 10 years of sustained and coherent implementation of policies, programs and increased investment to eliminate malnutrition

in all its forms, in all countries over the world [6]. The Nutrition Decade focuses on the Sustainable Development Goals 2: Zero Hunger and 3: Good Health and Wellbeing [7].

Even before the COVID-19 pandemic, the world was sadly not on track to achieve the targets for any of the nutrition indicators by 2030. The pandemic has challenged health systems, increased household food insecurity, reversed economic growth and set back nutritional improvements across LMICs [8]. The consequences will exacerbate maternal and child undernutrition and child mortality. It has been estimated that over 10 million additional children will be malnourished, and that the crisis will lead to 168,000 additional child deaths, 2.1 million maternal anemia cases and 2.1 million children born to women with a low BMI by 2022 if no additional investments are made. This will lead to a disadvantaged start for many children and a loss of productivity of $79m due to anemia during pregnancy in a moderate impact scenario [9].

While a substantial shift in the global food and agriculture system is needed to recover from the devastating impact of the COVID-19 pandemic and reach both Sustainable Development Goals 2 and 3, it has been recognized that the use of micronutrient supplements for vulnerable groups can be an appropriate interim measure to improve nutritional status and subsequent health, social and economic consequences [10].

1.2 The impact of maternal malnutrition

Millions of women around the world suffer from malnutrition in all its forms, including underweight, overweight, short stature, anemia and micronutrient deficiencies (**Figure 1.1**). According to the UNICEF Global Nutrition Database, approximately 170 million women (9.1 %) are underweight, defined as body mass index (BMI) less than 18.5 kg/m^2, while 610 million women (32.5%) are overweight (BMI ≥25 kg/m^2) [11]. The burden of low BMI is especially high in west, east and central Africa and Asia, and remains elevated. According to the 2017 World Health Organization (WHO) African region data, nine countries in Africa had a prevalence rate above 15% [12]. Short stature (height <150 cm) is another indicator of intergenerational and chronic undernutrition [13]. In LMICs, an estimated 7% of women aged 20-49 years are affected by short stature [14]. In addition, over half a billion (29.9%) women of reproductive age were anemic in 2019, of which 29.6% prevalence in non-pregnant women and 36.5% in pregnant women [15]. Up-to-date global estimates of micronutrient deficiencies and diet quality among women are more difficult to obtain, due to limited data availability.

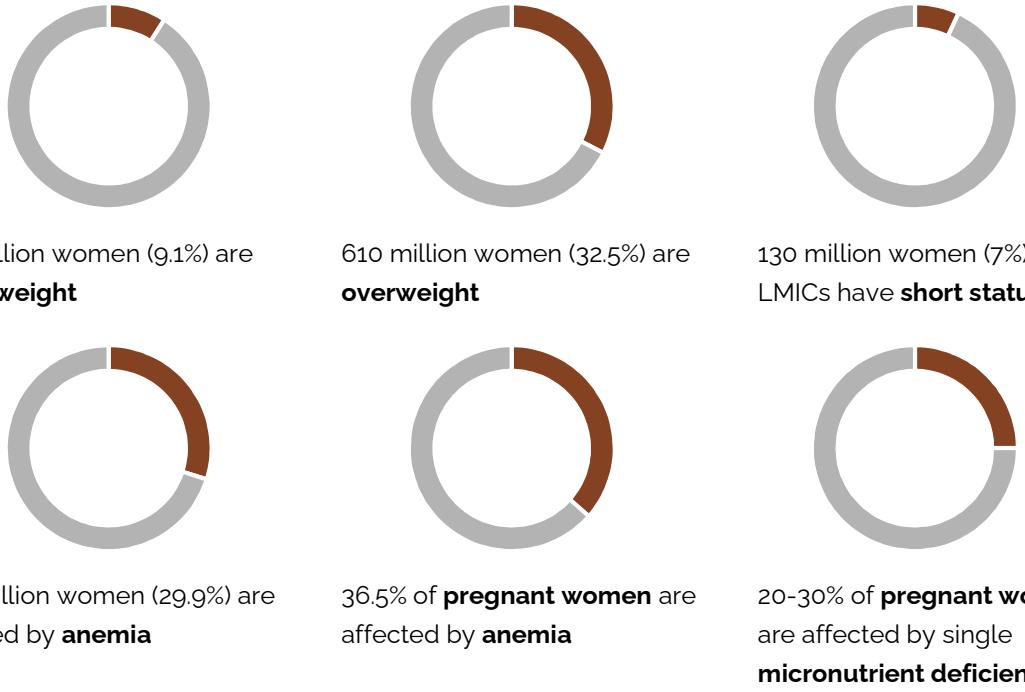


Figure 1.1 Global prevalence of malnutrition in (pregnant) women.

Adapted from: Mousa et al. 2019 [16]; UNICEF 2021 [11]; Victora et al. 2021 [14]; WHO 2021 [15].

Overall, in many LMICs, and in particular in food-insecure settings, the prevalence of micronutrient deficiencies among women, irrespective of their weight, is presumed to be high [17]. Single micronutrient deficiency estimates among pregnant women range from 20% to 30% worldwide [16] and up to 40% in Africa [18].

1.2.1 Consequences for maternal and child health

Since the early 1980s, many observational studies linked poor maternal nutrition with birth weight and survival. This formed the basis of the 'Barker hypothesis', which posits that harmful intrauterine exposure, such as suboptimal malnutrition, followed by plentiful food in adulthood programs the fetus to develop chronic disease later in life. An assessment of the Dutch Hunger Winter, the tragic postwar period in which pregnant women were consuming only 400-800 kcal/day, showed that food restriction in utero had an effect on subsequent metabolism and cardiovascular adult health and cognitive functions. Undernutrition can thus stress the developing fetus and adversely reprogram its evolving phenotype [19].

In addition, evidence from animal, tissue and molecular studies support the importance of maternal nutrition before and during different stages of pregnancy for the growth and (embryonic) development of the fetus [20]. Maternal malnutrition influences the balance between maternal and fetal needs and results in a biological competition with an impact on [20,21]:

- The process of embryogenesis and implantation (the first 3 days after conception);
- Placental development (within ~3 weeks of conception) and function (e.g. nutrient transfer capacity) by which the interchange of nutrients, hormones and immune factors between mother and fetus can be affected. A reduced maternal nutrient supply to the fetus may subsequently restrict growth;
- Tissue cell growth and proliferation (crucial for organ size, structure and functioning);
- Methylation status of genes and their subsequent expression [19,22];
- Other physiological mechanisms as hormonal axes regulating growth and appetite and microbiome maturation [23].

Maternal malnutrition therefore directly impacts pregnancy outcomes and fetal growth. Women who are undernourished are more susceptible to disease, have an increased risk of miscarriage and are more likely to give birth to babies whose survival and long-term health is at risk [24].

Short maternal stature increases the risk of caesarean delivery and death of the mother at delivery [13]. In combination with iron deficiency anemia, short stature accounts for at least 20% of maternal mortality [24]. Furthermore, short stature in women is associated with poor birth outcomes (SGA and preterm birth) and is strongly associated with stunting in children [25].

Lastly, maternal malnutrition in the form of micronutrient deficiencies not only affects a woman's health and survival, but also of her offspring. Deficiencies in essential vitamin and minerals are linked to miscarriage, stillbirths, birth defects, LBW, infant mortality, impaired cognitive development and metabolic disease risks later in life [26].

For a better understanding of the effects on fetal growth, each consequence will be described in more detail in the subsequent paragraphs.

Intrauterine growth restriction

A restriction in fetal growth is called intrauterine growth retardation (IUGR) and is a condition where the fetus fails to achieve its appropriate genetic growth potential [27]. Fetal growth is a complex process influenced by many physiological and environmental factors of the fetus, placenta and mother. Restriction in fetal growth during pregnancy confers an increased risk of morbidity and mortality in the first 28 days of life and beyond [28]. A fetus that is IUGR is programmed to lower its metabolic rate and use all energy efficiently and catch-up growth may therefore lead to increased adiposity, insulin resistance and the metabolic syndrome [29].

Small-for-gestational age

Small-for-gestational age is defined as a birth weight below the 10^{th} percentile of a standard optimal reference population for a given gestational age and sex [30]. SGA is often caused by growth restriction in the womb and is therefore a common proxy for IUGR. SGA has been associated with neonatal mortality and an increased risk of growth faltering

in the first 2 years of life [2]. It is even estimated that 20% of stunting might be attributable to fetal growth restriction [2]. In addition, SGA has been linked to an increased risk of morbidity later in life, especially noncommunicable diseases [31].

IUGR and SGA are used interchangeable in the medical literature, although the concepts are different. Most IUGR infants are SGA, but not all SGA infants are considered growth retarded [27]. These infants normally grew in utero, but are naturally small and fall below the threshold used to define SGA. Similarly, some infants are considered IUGR because their fetal growth sufficiently deviated from their expect growth but are appropriate-for-gestational age (AGA) as their birth weight is above the set threshold [28].

Low birth weight

Low birth weight is defined as an infant's weight less than 2500 g at birth, regardless of gestational age. Babies that are born LBW, either did not grow properly (IUGR), were born too soon (preterm birth), or both [32]. As birthweight depends directly on gestational age and sex of the newborn, SGA is commonly used to indicate adverse fetal growth [33].

LBW contributes to a range of poor health outcomes. This includes fetal and neonatal mortality and morbidity, and it is estimated that infants born LBW are about 20 times more likely to die than heavier infants [32]. In addition, it may lead to inhibited growth and cognitive development and noncommunicable diseases later in life, specifically metabolic syndrome, diabetes type 2 and hypertension [30,31].

In many LMICs ultrasound is unavailable to determine gestational age. To illustrate, in rural sub-Saharan Africa less than 7% of pregnant women have access to ultrasound [34]. As gestational age estimation by last menstrual period (LMP) is often unreliable and poorly correlates with the estimation by ultrasound [35], LBW if often used to as an indicator of a multifaceted public health problem, including maternal malnutrition, in low-income settings [32].

Preterm birth

Preterm birth refers to infants that are born before 37 completed weeks of gestation. The mortality risk associated with preterm birth is higher compared to SGA. In combination with SGA, preterm birth leads to mortality risks that continue beyond the neonatal period, i.e.

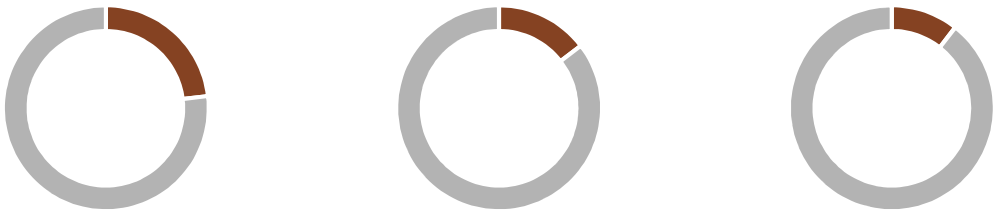
the first 28 days of life [30]. Preterm birth was positively associated with neurocognitive impairment (e.g. learning difficulties, developmental delay, cerebral palsy, hearing and visual impairment) [36] and chronic diseases (e.g. systemic arterial hypertension) in adult life [31].

Stillbirth

Stillbirth refers to fetal death (born without sign of life) occurring ≥28 weeks of gestation, before or during birth. Early stillbirth is also defined as fetal loss, which can be further categorized in: i) <22 weeks of gestation; ii) between ≥22 weeks and <28 weeks of gestation; and iii) <28 weeks of gestation.

1.2.2 Global prevalence of poor birth outcomes

Poor fetal growth is rarely a direct cause of death, but it is important to recognize that indirectly it can contribute to many neonatal deaths due to birth asphyxia (lack of oxygen and blood flow to the brain) and infections (sepsis, pneumonia and diarrhea), which together account for about 60% of neonatal deaths [24]. Annually, 32.4 million infants (23.1%) are born SGA [37], 20.5 million (14.6%) are born with LBW [38], 14.9 million (10.6%) are born preterm [39], with the highest prevalence in LMICs (**Figure 1.2**).



32.4 million (23.1%) infants are born **small-for-gestational age**

20.5 million (14.6%) infants are born with **low birth weight**

14.9 million (10.6%) infants are born **preterm**

Figure 1.2 Global annual prevalence of children born small-for-gestational age, low birth weight and preterm.

Adapted from: Black 2015 [37]; Blencowe et al. 2012 [39], 2019 [38].

Of the 2.9 million annual neonatal deaths worldwide, 2.4 million (80%) are attributable to SGA and/or preterm birth [40], an estimated 606,500 (21.9%) neonatal deaths are attributable to SGA [41], 1 million (34%) neonatal deaths are directly caused by complications due to preterm birth, and an additional 1.47 million neonatal deaths is due to indirect effects (e.g. infections) of preterm birth [40]. The number of stillborn infants is estimated to be 2.6 million per year, of which 98% occur in LMICs [42].

The 2008, 2013 and 2021 *Lancet* Series on maternal and child undernutrition and its progress, highlight this enormous global health burden and call for well-designed research and strengthening of intervention programs to effectively address this challenge [43]. Briefly, the *Lancet* series identified 10 core interventions (e.g. vitamin A supplementation, promotion of breastfeeding, therapeutic zinc supplementation for diarrhea management) effective in reducing maternal and child undernutrition. Many countries and agencies have scaled up these interventions following up on this. Despite these global efforts however, malnutrition persists at unacceptably high levels. In addition, the number of good quality and effectiveness studies has grown, yet evidence gaps remain.

1.3 Predictors of adverse birth outcomes

Understanding the risk factors for adverse birth outcomes may help to identify the most effective interventions to prevent newborns from being born too small and/or too soon and reduce the high mortality and morbidity rates. Suboptimal fetal development is a complex phenomenon caused by a variety of pathways including fetal, placental, environmental and maternal risk factors [44].

1.3.1 Fetal risk factors

Fetal risk factors are less prevalent and include birth defects caused by genetic abnormalities (e.g. Down syndrome) or single gene defects (e.g. cystic fibrosis); viral, parasitic or bacterial infections; multiple gestations; and placental/cord abnormalities [45–47]. Birth defects only account for a small fraction (~7%) of stillbirths [42].

1.3.2 Placental risk factors

The placenta is the key organ between the mother and fetus and defects in its metabolic and transport function may reduce the supply of nutrients and oxygen to the fetus and disturb fetal growth [48] or even result in stillbirth [42]. Placental insufficiency accounts for many cases of IUGR, but affects only a small number (~3%) of pregnancies. In addition to placental function, a decrease in placental mass, immunological disturbance at the maternal-fetal interface and other placental malformations can impair fetal growth [49].

1.3.3 Environmental risk factors

Exposure to harmful substances, either lifestyle-associated (e.g. tobacco, alcohol or drugs) or environmental pollutants (e.g. biomass cooking fuels, pesticides) is known to reduce fetal growth by the increased risk of chronic fetal hypoxia (deprived oxygen supply) or birth defects [50].

Poor access to healthcare services due to long distance or financial constraints is also documented to increase risk of undiagnosed/untreated infections and obstetric complications, which may particularly increase stillbirth rates in LMICs [50].

1.3.4 Maternal risk factors

Maternal conditions are major determinants for SGA, LBW and preterm birth including: (1) genetic predisposition; (2) reproductive factors; (3) morbidities; and (4) diet and nutritional status.

Genetic predisposition

Several maternal gene polymorphisms encoding for proteins involved in cell growth and differentiation have been associated with SGA in both mice and human studies [51,52]. Although there is growing evidence that disturbances on the growth hormone-insulin-like growth factor axis are linked to fetal growth retardation, a better understanding of (environmental) interaction factors and precise physiological implications is still required. In addition, not all maternal and fetal DNA variants linked to birth size have been identified to date, requiring further research to fully understand and describe this risk factor [23].

Reproductive factors

Reproductive factors such as age, parity and birth interval have been associated with IUGR. Maternal age at pregnancy, either young (<18 yrs) or advanced (>35 yrs) age, is a risk factor for IUGR [49], LBW [49], preterm birth [53] and stillbirth [42]. In addition, parity plays a role in fetal development, in which first pregnancies tend to be more evident of fetal growth restriction [52] and preterm birth [53], while the risk of gestational diabetes – a condition associated with stillbirth – increases with grand multiparty (>4 pregnancies) [50]. A short pregnancy interval is also associated with SGA [54,55] and preterm birth [55]. The optimal interval identified to prevent adverse birth outcomes is 18-23 months [54].

Maternal morbidities and infections

Maternal morbidities including gestational diabetes, (pre-)eclampsia and infectious diseases increase the risk for IUGR fetuses and stillbirths [42]. Common infections include viral (e.g. rubella, CMV, herpes, HIV), parasitic (e.g. toxoplasmosis, syphilis, malaria) and bacterial infections (e.g. chlamydia, listeria, tuberculosis) and can result in fetal growth restriction, LBW and preterm delivery due to direct cell damage or placental passage of the infection [49]. In LMICs, especially those in sub-Saharan Africa, malaria infection during pregnancy may result in fetal loss, IUGR, LBW and preterm delivery as a result of poor

oxygen and nutrient transfer due to the destruction of red blood cells and placental malaria [49,56]. Also urinary tract infections are highly prevalent (affecting 9-80% of pregnancies in sub-Saharan Africa) and are associated with a twofold elevated risk of preterm delivery, as well as helminthic infections which are associated with systemic inflammation, LBW and preterm birth and genital tract infections with increased risk of preterm birth [57].

Maternal diet and nutritional status

Women's nutrition before and during pregnancy is crucial for the development, growth and health of their child – in the womb and throughout early childhood. Nutritional vulnerability and requirements are increased during pregnancy, and undernutrition (including chronic energy and micronutrient deficiencies) during this critical life phase has been strongly associated with SGA, LWB and to a lesser extent with preterm birth [58] and stillbirth [42]. More specifically, short maternal stature is associated with a greater risk of SGA and preterm birth [25]; low BMI in early pregnancy with SGA [2] and to a less certain extent with preterm birth [59]; and low weight gain during pregnancy with SGA [60], preterm birth and LBW [61]. The crucial role of maternal nutrition for early childhood has been demonstrated by the association of short maternal height and low BMI with lower height-for-age and weight-for-height at 2 years of age, which is probably mediated by small size at birth [14].

During pregnancy, increased requirement and poor dietary availability of iron results in a high prevalence of anemia in women. A recent systematic review and meta-analysis of Young and colleagues [62] showed that anemia is associated with SGA, LBW, preterm birth and stillbirth. It is estimated that 50-60% of all anemia cases is attributable to iron deficiency, which demonstrates the link between maternal malnutrition and adverse birth outcomes [14].

Three different mechanisms have been identified by which maternal nutrition and metabolism affect newborn health: (1) at the start, the embryo or placentation could be affected by early exposures; (2) later in pregnancy, during organogenesis (i.e. embryotic development), nutritional supply might alter the number and function of cell types in the

heart or pancreas which predisposes the child to disease; and (3) after fetal organs have been formed, maternal nutrition could impact its function [29].

Interaction between maternal nutrition and infections

Not only maternal malnutrition as a risk factor on itself, also the interaction with infections leads to poor birth outcomes. Maternal malnutrition makes women vulnerable for infections and vice versa, leading to a vicious cycle leading to fetal growth restriction. Chronic protein energy malnutrition impairs immune function, increasing the risk for infection, while chronic infections may lead to undernutrition due to reduced dietary intake, malabsorption, increased nutrient losses and/or increased requirements [57].

Socioeconomic, demographic and contextual factors

Underlying the maternal risk factors, socioeconomic factors as maternal education, early marriage, gender inequality in intra-household food distribution and household income play a significant role. Research has shown that women with a lower socioeconomic position are more likely to live in poor and unsanitary areas and have higher odds of maternal mortality [63]. More frequent exposure to pathogens could also be linked with adverse birth outcomes. Overall, differences in the burden of poor maternal nutrition clearly demonstrate the gap between poor and wealthy families within LMICs and between countries [14].

1.4 Nutritional requirements during pregnancy

Nutritional requirements increase during pregnancy to maintain maternal metabolism, increase body tissue reserves, promote development of the placenta, and support fetal growth and development [64]. Compared with pre-pregnancy, energy requirements increase by an average of 300 kcal/day during pregnancy [65]. In addition, requirements for many, but not all, micronutrients increase and depend on the pre-pregnancy nutritional status of the woman, weight gain and infectious diseases during pregnancy [66].

Inadequate micronutrient intakes are relatively common in LMICs among pregnant women [18]. It is often a result of inadequate food intake, poor quality diets and poor bioavailability. As an example, in situations with food insecurity, women often have primarily access to plant-based diets and staple foods which are often not a good source of high-quality protein and micronutrients. In addition, not meeting the nutritional requirements is strongly linked to inadequate health systems with poor capacity, poverty and socio-cultural factors as early marriage and pregnancies, repeated and short-interval pregnancies, and traditional dietary practices [17,66].

The consequences of maternal deficiencies can be devastating for mother and child. It may even affect future generations, as there is increasing evidence that the effect of maternal undernutrition may persist well into adulthood, with possible intergenerational effects [67]. The function and status of micronutrients during pregnancy are however less often studied and more difficult to assess e.g. due to increased plasma volumes and poor biomarkers [66], **Table 1.1.** provides an overview and the following paragraphs tend to describe the role, requirement and risk association for each macro- and micronutrient in more detail.

1.4.1 Energy and macronutrients

Additional energy during pregnancy is required for the growth of new tissue (fetus, placenta and amniotic fluid) and existing tissue (uterus, breast and maternal adipose tissue), with the greatest increase between 10-30 weeks of gestation. Energy needs vary depending on physical activity levels, pre-pregnancy BMI and metabolic rate, while on average pregnancy is estimated to costs a total of 77,000 kcal [68].

Requirements for carbohydrates during pregnancy are not increased [68]. Preliminary research in high-income countries however indicates that food with a low glycemic index (GI: glycemic response induced by carbohydrates), low glycemic load (GL: taking into account both the quality (GI) and quantity of carbohydrates) and/or high fiber might be beneficial in pregnancy [16].

Protein has an important biological role involved in structural (e.g., keratin, collagen) and functional (e.g. enzymes, protein transport, hormones) mechanisms necessary for growth, repair and maintenance. Within the first weeks of pregnancy, adjustments in protein metabolism occur to maintain maternal homeostasis while supporting fetal growth and preparing for lactation. In the second and third trimester, a substantial increase in protein synthesis occurs [16] and a 50% higher intake of protein (within 25% of total energy) is therefore advised [69].

Essential fatty acids, omega-3 (α-linolenic acid) and omega-6 (linoleic acid), as well as their long-chain derivatives (arachidonic acid [AA], eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]), are structural components of cell membranes and are essential for tissue formation. DHA plays an important role in brain and retina development in the fetus and EPA may reduce the risk of pre-eclampsia and timing of childbirth [16]. Adequate intake (AI) slightly increases during pregnancy and deficiency in omega-3 fatty acids has been related to (early) preterm birth, perinatal death and LBW infants [70].

1.4.2 Vitamins

Water-soluble vitamins (B-complex, C)

B-complex vitamins act as coenzymes in several metabolic pathways for the production and release of energy in cells; the metabolism of protein, fat and carbohydrates; and the formation of blood cells [71]. Pregnant women have a heightened requirement for B-vitamins due to increased energy and protein needs, particularly during the third trimester. Adaptive responses during pregnancy reduce urinary excretion of some B-vitamins to help meet increasing demands [16]. For some B-vitamins (B1, B2, B6, folate, B12) body stores are limited and regular dietary supply is required to maintain optimal levels, while for other B-vitamins (B3, B5, B8) there is no direct evidence for a (large) change in requirement during pregnancy. Deficiency can impact brain development and fetal growth and has also been

associated with pre-eclampsia [16,72]. Especially the link between preconception folate status and the risk of neural tube and other birth defects is well-known [73].

Both vitamin B12 and folate play a role in energy metabolism and are strongly linked. Vitamin B12 coenzyme regenerates a folic acid derivative to its active form and is thus necessary for the formation of new cells. Deficiency in vitamin B12 can result in impaired activity of this vitamin B12 dependent reaction.

Vitamin C is a powerful antioxidant to combat oxidative stress, a key feature in the development of complications in pregnancy [72]. It is actively transported across the placenta which leads to reduced maternal plasma levels and increased requirements during pregnancy and lactation. Low intake could be associated with pre-eclampsia, anemia and IUGR [16,74].

Fat-soluble vitamins (A, D, E, K)

Additional vitamin A is essential to support organ and skeletal growth, development of vision and tissue maintenance in the fetus, provide fetal liver reserves, and aid in maternal metabolism and immune system [16,75]. Pregnant women are susceptible to vitamin A deficiency throughout gestation, but deficiency is most common in the third trimester due to accelerated fetal development and the increase in blood volume during this period. Seasonal food availability and periods when infectious disease rates are high can also increase susceptibility to vitamin A deficiency [76]. Currently, there are no reliable estimates for the increased requirements to support these processes. Studies in Nepal and India showed a correlation between maternal blindness and an increased risk of LBW and infant mortality due to infectious diseases such as diarrhea, measles and respiratory infections [2,77,78].

Vitamin D is important in skeletal growth and immune function [72]. During pregnancy, the fetus relies completely on maternal vitamin D stores for its development and requirements are difficult to meet with diet alone [79]. Vitamin D deficiency during pregnancy and early infancy has been associated with several adverse pregnancy outcomes as poor maternal weight gain, gestational diabetes, pre-eclampsia, and poor birth outcomes as preterm birth and SGA infants [80].

Vitamin E is a powerful antioxidant and combats oxidative stress, synergistically with vitamin C [16,72]. Placental transfer of vitamin E is not very efficient and losses of vitamin E to the fetus are thought to be minimal. Since maternal losses are less expected, no additional intake of alpha-tocopherol is therefore recommended during pregnancy [16,81]. Oxidative stress is presumably a key mechanism underlying the pathophysiology of several pregnancy complications including preeclampsia [72], preterm birth, IUGR and prelabor rupture of membranes [16].

Vitamin K is important for the synthesis of blood-clotting proteins and bone proteins [72]. As vitamin K cannot cross the placental barriers efficiently to reach the fetus, no additional requirements are known during pregnancy [81] Newborn infants are vulnerable to vitamin K deficiency and may suffer from hemorrhagic disease as fetal reserves of vitamin K in the liver are low, the intestines are sterile at birth, and human milk is a poor source of vitamin K [82].

1.4.3 Minerals

Calcium is important for the mineralization of bones and teeth, and provide rigidity and structure [71]. Furthermore, it is involved in muscle contraction and relaxation, nerve functioning, blood clotting, blood pressure and immune defense [72]. For the general population, no additional calcium requirements are set during pregnancy [81]. However, in areas where dietary calcium intake is low, calcium supplementation during pregnancy is recommended by WHO for the prevention of pre-eclampsia [83]. Furthermore, calcium deficiency has been associated with LBW infants and may induce IUGR [72].

Magnesium is an enzyme cofactor and activator involved in bone mineralization, building of protein, normal muscle contraction, nerve impulse transmission, maintenance of teeth and functioning of immune system [71,72]. A deficiency in magnesium interferes with fetal growth and may lead to hyperparathyroidism (an increase in parathyroid hormone levels in the blood) [72].

Phosphorus is part of every cell and a major element for many biological processes as DNA synthesis, ATP synthesis (energy transfer), membrane synthesis and protein phosphorylation. It is also involved in the mineralization of bones and teeth [71,72]. No additional requirements are set for phosphorus intake during pregnancy.

Potassium facilitates many reactions: maintains normal fluid and electrolyte balance, supports cell integrity, and assists in nerve impulse transmission and muscle contractions [71,72]. A slight increase for AI has been set for potassium intake during pregnancy, despite the fact that effects on birth outcomes due to hypokalemia (maternal deficiency in potassium) are unknown [84].

1.4.4 Trace minerals

Copper is part of several enzymes, helps to form hemoglobin, and is responsible for vessel integrity. A slight increase in intake is advised for pregnant women. A deficiency can lead to brain malformation and vascular hemorrhage [72].

Iodine is a key component of thyroid hormone that helps to regulate growth, brain development and metabolic rate [72]. As it is essential for healthy fetal development, a sharp increase in iodine requirement is set as a result of increased thyroidal activity and fetal transfer [85]. Deficiency results in hypothyroidism or, if severe, in cretinism, leading to irreversible brain damage [72].

Iron is important in hemoglobin synthesis and production of red blood cells carrying oxygen to the brain, but is also involved in DNA synthesis and electron transport [72]. Deficiency has been associated with the risk of preterm birth, LBW or SGA infants, impaired maternal immune system, and cognitive and behavior development issues persisting into adulthood despite treatment [16,86].

Manganese is a component of enzymes involved in bone formation and various metabolic processes [71]. A slightly higher intake is recommended during pregnancy to reach adequacy. Deficiency may lead to poor fetal growth and an interference with normal skeletal development [84].

Selenium is an essential trace element that plays a role in the immune system and antioxidant defense. A slightly higher dietary intake is advised during pregnancy. A deficiency in selenium has been associated with miscarriage, pre-eclampsia and IUGR [72].

Zinc has a functional and structural role in many enzymes and is important for gene expression, cell growth and division, neurotransmission, and reproductive and immune functions [84]. Deficiency has been linked to poor fetal growth and if severe, it might lead

to teratogenesis (prenatal toxicity characterized by structural or functional defects in the developing embryo or fetus) [72].

Beyond the role of single micronutrients, it is desirable to maintain an optimal balance between micronutrients for fetal development as interactions between micronutrient-dependent physiological and biological actions can be both positive (e.g. zinc and vitamin A) and negative (e.g. zinc/copper and iron) [66].

Table 1.1 Function, requirements and deficiency risks of macro- and micronutrients during pregnancy.

Macronutrients	**Function during pregnancy**	**Requirements during pregnancy**	**Deficiency and risk for fetal development**
Carbohydrate	Energy supply; Maintenance of maternal and fetal brain function; Healthy digestive system	EAR increases from 100 g/d pre-pregnancy to 135 g/d during pregnancy[a]	Impaired fetal growth
Protein	Part of structural and functional mechanisms for growth, repair and maintenance	EAR increases from 0.66 g/kg/d pre-pregnancy to 0.88 g/kg/d during pregnancy[a]	Impaired fetal growth
Omega-3 fatty acids	Development of brain and retina; Reducing the risk of pre-eclampsia and timing of childbirth	AI increases from 1.1 g/d pre-pregnancy to 1.4 g/d during pregnancy[b]	(early) Preterm birth; Perinatal death; LBW
Omega-6 fatty acids	Key structural component of cell membranes and vital for tissue formation; Positively affects cholesterol (moderate intake)	AI increases from 12 g/d pre-pregnancy to 13 g/d during pregnancy[b]	LBW (both low and high intake)

Table 1.1 (continued)

Vitamins	Function during pregnancy	Requirements during pregnancy	Deficiency and risk for fetal development
Vitamin A	Essential to support organ and skeletal growth; Development of vision and tissue maintenance; Provide fetal reserves; Aid in maternal metabolism and immune system	Slight increase from 500 µg/d pre-pregnancy to 550 µg/d during pregnancy[a,c]	LBW; Infant mortality (due to suppressed immune system and higher susceptibility to infectious diseases); Maternal blindness
Thiamin (B1)	Co-enzyme important in lipid and nucleotide synthesis enzymes, especially in developing the brain	Increase from 0.9 mg/d pre-pregnancy to 1.2 mg/d during pregnancy[a]	Impairs brain development; Impairs fetal growth
Riboflavin (B2)	Part of 2 flavin coenzymes used in energy metabolism	Increase from 0.9 mg/d pre-pregnancy to 1.2 mg/d during pregnancy[a]	Preeclampsia; Congenital heart defects; LBW
Niacin (B3)	Part of coenzymes involved in energy production	Increase from 11 mg/d pre-pregnancy to 14 mg/d during pregnancy[a,d]	Preeclampsia; Congenital heart defects; LBW
Vitamin B5	Part of coenzymes involved in energy production	AI increases from 5 mg/d pre-pregnancy to 6 mg/d during pregnancy[b]	Deficiencies are rare and there is little information on the effects
Vitamin B6	Vital role in numerous metabolic processes including nervous system development and functioning	Increase from 1.1 mg/d pre-pregnancy to 1.6mg/d during pregnancy[a]	Preeclampsia; Gastrointestinal carbohydrate intolerance; Neurological deficits in infants
Folate	Co-enzyme particularly important during embryonic and fetal stages of pregnancy where there is rapid cell division and tissue growth	Folate[e]: sharp increase from 320 µg/d pre-pregnancy to 520 µg/d during pregnancy[a]	Neural tube and other birth defects; Pre-eclampsia

Table 1.1 (continued)

Vitamins	Function during pregnancy	Requirements during pregnancy	Deficiency and risk for fetal development
Vitamin B12	Essential for the methylation of DNA, RNA and proteins; Critical for placental development and fetal growth; Involved in the development of red blood cells	Increase from 2.0 µg/d pre-pregnancy to 2.2 during pregnancy[a]	Placental abruption; Stillbirth; LBW; Preterm delivery; Neural tube defects
Vitamin C	Powerful antioxidant to combat oxidative stress and avoid complications in pregnancy	Increase from 60 mg/d pre-pregnancy to 70 mg/d during pregnancy[a]	Pre-eclampsia; IUGR; Anemia
Vitamin D	Important in skeletal growth and immune function	Similar to pre-pregnancy[a]	Poor maternal weight gain; Gestational diabetes; Pre-eclampsia; Preterm birth; SGA
Vitamin E	Powerful antioxidant to combat oxidative stress	Similar to pre-pregnancy[a,f]	Pre-eclampsia Preterm birth IUGR Prelabor rupture of membranes
Vitamin K	Synthesis of blood-clotting proteins; Important for bone health	Similar to pre-pregnancy[b]	Newborns are vulnerable to vitamin K deficiency (leading to hemorrhagic disease) as vitamin K cannot cross the placental barriers efficiently to reach the fetus

Table 1.1 (continued)

Minerals	Function during pregnancy	Requirements during pregnancy	Deficiency and risk for fetal development
Calcium	Development of skeleton; Involved in muscle and nerve functioning, blood clotting, blood pressure and immune defenses	Similar to pre-pregnancy[a]	Preeclampsia; LBW; IUGR
Copper	Vessel integrity; Part of several enzymes, e.g. helps to form hemoglobin	Slight increase from 700 µg/d pre-pregnancy to 800 µg/d during pregnancy[a]	Brain malformation; Damaged blood vessels
Iodine	Key component of thyroid hormone that helps to regulate growth, (brain) development and metabolic rate	Sharp increase in requirements from 95 µg/d pre-pregnancy to 160 µg/d in pregnancy[a]	Irreversible brain damage
Iron	Participates in many metabolic processes, including oxygen transport (as part of the protein hemoglobin), DNA synthesis and electron transport	Sharp increase from 8.1 mg/d pre-pregnancy to 22 mg/d during pregnancy[a]	Poor fetal growth; Cognitive and behavior problems persisting into adulthood despite treatment
Manganese	Manganese-dependent enzymes are involved in bone formation and various metabolic processes	AI increases from 1.8 mg/d pre-pregnancy to 2.0 mg/d during pregnancy[b]	Poor fetal growth; Interference with normal skeletal development
Magnesium	Involved in bone mineralization, building of protein, muscle contraction, nerve impulse transmission, and functioning of immune system	Slight increase from 255/265 mg/d pre-pregnancy to 290/300 mg/d during pregnancy[a]	Interference with fetal growth; Hyperparathyroidism (increase in parathyroid hormone levels in the blood)
Phosphorus	Development of skeleton; Major element essential for many biological processes as DNA synthesis	Similar to pre-pregnancy[a]	Muscular and bone weakness

Table 1.1 (continued)

Minerals	Function during pregnancy	Requirements during pregnancy	Deficiency and risk for fetal development
Potassium	Facilitates many reactions: maintains normal fluid and electrolyte balance, supports cell integrity, assists in nerve impulse transmission and muscle contractions	AI increases slightly from 2600 mg/d pre-pregnancy to 2900 mg/d during pregnancy[b]	Effects on birth outcomes due to hypokalemia (maternal deficiency in potassium) are unknown
Selenium	Antioxidant defense; Supports immune system	Slight increase from 45 µg/d pre-pregnancy to 49 µg/d during pregnancy[a]	Miscarriage; Pre-eclampsia; IUGR
Zinc	Part of many enzymes; Important for embryogenesis and fetal growth; Supports immune system	Increase from 6.8 mg/d pre-pregnancy to 9.5 mg/d during pregnancy[a]	Poor fetal growth; If severe: teratogenesis (prenatal toxicity characterized by structural or functional defects in the developing embryo or fetus)

[a] Estimated average requirements (EAR): the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group.

[b] Adequate intake (AI): covers the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[c] As retinol activity equivalent (RAE).

[d] As niacin equivalent.

[e] As dietary folate equivalent (DFE). 1 DFE = 1 µg food folate = 0.6 µg of folic acid from fortified food or as a supplement consumed with food.

[f] As α-tocopherol.

IUGR, intra-uterine growth restriction; LBW, low birth weight; SGA, small-for-gestational age.

Adapted from: IOM 2006 [69]; Middleton et al. 2018 [70]; Mousa et al. 2019 [16]; NAM 2019 [87]; de Pee et al. 2017 [72]; Whitney and Rolfes 2015 [71].

1.4.5 Global prevalence of micronutrient deficiencies

Overall, there is limited national representative data available to assess prevalence of vitamin and mineral deficiencies, especially during pregnancy [17]. In addition, physiological changes during pregnancy make it difficult to quantify the true prevalence of deficiencies in pregnancy. However, several studies have shown that certain diets (e.g. low in meat, fish, legumes; or high in polished rice, maize, fermented fish, tea leaves and betel nut), infections (e.g. malaria and hookworm) or emergency situations (e.g. conflict and drought) can increase the risk of maternal deficiency [82]. A recent analysis of individual-level biomarker data for micronutrient status from nationally representative, population-based surveys, estimated that two-thirds of non-pregnant women of reproductive age worldwide have micronutrient deficiencies. Iron, zinc and folate deficiency appears to be highly prevalent among this population group in most countries, and especially in sub-Saharan Africa in which 80% (70-89) is estimated to be deficient in at least one of these three micronutrients. The authors specifically highlight the lack of data that hindered the inclusion of pregnant women in their analysis [88].

Earlier studies report that deficiencies in thiamine (B1) [89,90], riboflavin (B2) [17], niacin (B3) [91], vitamin B6 [91], folate [17], vitamin B12 [16] and vitamin C [92] could be of global concern affecting millions of women around the world. For vitamin B12 specifically, it is estimated that deficiency affects 25% of pregnancies worldwide [16].

For vitamin A deficiency, more specific data is available. Night blindness affects up to 7.8% of pregnant women (9.8 million), and 15.3% (19.1 million) have deficient serum retinol concentrations [2], by which it is considered a public health issue at population level [93]. For vitamin D deficiency during pregnancy, global estimates range from 40-98%, with 15-84% severely deficient [94]. The number of studies on vitamin D status in Africa is relatively small. Risk factors including very low calcium intake and a high burden of infectious disease, whereby utilization and turnover of vitamin D is increased, can influence the number of vitamin D deficient women in African countries [95]. Deficiencies for pantothenic acid (B5) on a global scale are rare and for vitamin E and K, there is not sufficient information available.

Iron deficiency is thought to be the most common nutrient deficiency among pregnant women, and the global prevalence of anemia among pregnant women was estimated to

be 36.5% in 2019 [4,15]. It is estimated that 3.5 billion people are at risk of calcium deficiency. In certain regions, for example West Africa, calcium intakes are even lower and it is challenging to reach recommended calcium intake levels from local foods [96]. Despite that the most severe consequence of iodine deficiency, cretinism, has been eradicated by iodine supplementation strategies, it is assumed that iodine deficiency remains a global health problem [97]. The same holds for zinc deficiency, which in severe form is considered rare, but mild to moderate deficiency may be relatively common in many countries [98]. For the other minerals copper, manganese, magnesium, phosphorus, potassium and selenium it is difficult to present global prevalence rates.

1.5 Nutrition interventions to improve birth outcomes

Deficiencies that often occur simultaneously during pregnancy can cause adverse outcomes in mother and child. Nutrition interventions could mitigate these adverse health effects. There are several interventions for increasing maternal intake of macro- and micronutrients, which can be nutrition-specific (addressing the immediate determinants of fetal and child nutrition and development) or nutrition-sensitive (addressing the underlying determinants of fetal and child nutrition and development) (**Table 1.2**). The strategy could aim at (1) improving dietary quality by increasing the consumption of animal source foods, fruits and vegetables – a sustainable, but challenging and long-term approach [99]. Only a few high-quality intervention studies have investigated this approach using micronutrient-rich foods or nutrition education and reported improved pregnancy outcomes (e.g. decreased risk of preterm and LBW babies) [99,100]. Another strategy (2) is to provide supplementation in the form of tablets, powders, fortified foods: staple foods (e.g. wheat, flour, rice, salt, sugar, oil and milk) or supplements (e.g. ready-to-use nutrient supplement and biscuits) – a more targeted approach with more immediate results [66,99].

Table 1.2 Examples of nutrition-specific and nutrition-sensitive interventions to improve maternal nutritional status.

Nutrition-specific interventions	Nutrition-sensitive interventions
Dietary diversification	Agriculture and food security
Food fortification; micronutrient powders	Health and family planning services
IFA, MMN and protein–energy supplementation	Water and sanitation
Disease prevention and management	Education
	Women's empowerment
	Social safety nets

IFA, iron-folic acid; MMN, multiple micronutrients.

Adapted from: Christian et al. 2015 [101].

1.5.1 Single micronutrient supplements

Providing single micronutrient supplementation during pregnancy has only resulted in improvements in a few outcomes: (pre-)eclampsia (calcium, vitamin D) [79,102]; maternal anemia (iron) [4]; preterm birth (omega-3 fatty acids) [70]; and gestational diabetes, LBW and severe postpartum hemorrhage (vitamin D) [79].

For vitamin A [103], riboflavin (B2), niacin (B3) [16], vitamin B6 [104], vitamin C [105], vitamin E [74], iodine [106], magnesium [107] and zinc [98] supplementation alone, there is sparse or no evidence of improvements in maternal or neonatal outcomes.

1.5.2 Iron-folic acid supplementation

WHO currently recommends daily oral IFA supplementation with 30-60 mg elemental iron and 400 µg folic acid during pregnancy [108]. This recommendation is supported by a recent meta-analysis that showed that IFA supplementation reduced anemia with 48% (95% CI, 34-59%) and LBW prevalence with 12% (95% CI, 1-22%) [109]. Folic acid supplementation alone can reduce the chance of neural tube defects if taken before conception, while evidence on supplementation during pregnancy is inconclusive [110].

1.5.3 Multiple micronutrient supplements

In 2020, WHO updated its antenatal care (ANC) guideline 'WHO recommendations on antenatal care for a positive pregnancy experience' on the use of antenatal multiple micronutrient (MMN) supplements and concluded that MMN is recommended in the context of rigorous research (i.e. implementation research) [46]. This update was based on two recent (Cochrane) reviews and meta-analysis [111,112] that assessed trials comparing MMN with IFA conducted in LMICs. Compared to IFA, MMN reduced LBW by 12% (95% CI: 9-15%), SGA by 8% (3-12%), stillbirth by 5% (14% reduction, 4% increase) and preterm birth by 5% (10% reduction, 1% increase) [111]. The effect on LBW appeared to be greater when the dose of iron was less than 60 mg, and using the UNICEF/WHO/UNU International Multiple Micronutrient Preparation (UNIMMAP) formulation including 15 micronutrients, compared to supplements with 60 mg iron or formulations that included only three to four micronutrients [109]. In addition, the effect on birth outcomes was reported to be larger in women with anemia, compared to non-anemic women [112]. No negative effects were

observed for maternal or neonatal mortality [111]. The debate to switch from IFA to MMN as standard of care is ongoing, as benefits – which highly depend on accurate assessment of gestational age by ultrasound (i.e. SGA, preterm birth) – may be limited and costs are substantially higher. Furthermore, the effect of providing 30 mg iron (UNIMMAP) versus 60 mg iron (IFA) in settings where the prevalence of anemia is high was unclear [46]. A recent meta-analysis by Gomes and colleagues (2022) [113] showed that there are no differences between MMN (30 mg iron) and IFA (60 mg iron) on hemoglobin concentration, anemia or iron deficiency in the third trimester. Keeping in mind that the data on iron deficiency anemia was limited, the authors do suggest that policymakers in LMICs proceed with the transition from IFA to MMN.

1.5.4 Food supplements with micronutrients

There are various food supplements for pregnant women on the market, which can be roughly categorized into Fortified Blended Foods (FBFs) and Ready-to-Use Supplementary Foods (RUSFs).

Fortified Blended Foods

Fortified Blended Foods are blends of partially pre-cooked and milled cereals, soya, beans and pulses fortified with micronutrients, with or without vegetable oil or milk powder. Corn-soy blend (CSB) is the most commonly blended food for children distributed by the World Food Programme (WFP) but can also be provided to pregnant women. The blend can be consumed as a porridge or gruel, mixed with an appropriate portion of flour and clean water, and thus requires preparation and cooking [114]. There is very limited evidence on the use of FBFs and micronutrient powders for home fortification by pregnant women and the potential benefits for maternal and infant health outcomes [115].

Ready-to-Use Supplementary Foods

Ready-to-Use Supplementary Foods do not require preparation and include a range of products that vary in lipid and protein content. The various types are discussed in more detail in the following paragraphs.

Lipid-based Nutrient Supplements

Lipid-based nutrient supplements (LNS) in various quantities – large quantity (LQ: ~70 g/d), medium quantity (MQ: ~50 g), or small quantity (LQ: ~20 g/d) – contain micronutrients embedded in a food matrix providing energy, fats and protein. Generally it is an individually packed paste/spread made with heat treated oil seeds/pulses/cereals, sugar, milk powder and vegetables oils [114].

Several intervention trials have studied the effect of LNS on birth outcomes, mainly in Africa and a few in Asia and South America. These trials have provided mixed results. A multi-country (Democratic Republic of the Congo, Guatemala, India and Pakistan) randomized controlled trial (RCT), the 'Women First Trial', found a positive effect on mean birth size (length-for-age z-score, LAZ), SGA and newborn stunting when women consumed a 20 g LNS supplement (and an additional 55 g LNS for women with a BMI <20) for at least 3 months before conception and the duration of their pregnancy [116]. A large-scale cluster-randomized efficacy trial in Bangladesh found higher birth weights, weight-for-age z-scores (WAZ), head circumference-for-age z-scores, BMI z-scores, and in the adjusted models increased birth length and LAZ. The SQ-LNS reduced the risk of newborn stunting and small head size, especially in infants with higher risk of fetal growth restriction [117]. In addition, a study in Ghana with SQ-LNS for women starting supplementation before 20 weeks pregnancy, did not find an effect on SGA, but found positive effects on mean birth weight, WAZ, BMI-for-age z-score, and a lower risk of LBW in the intervention group compared to the IFA group [118]. In Malawi, researchers found only a statistically significant difference for newborn mid-upper arm circumference (MUAC) in the intervention group with SQ-LNS [119]. Few years earlier, researchers showed that LQ-LNS compared to MMN led to higher birth length in an individually RCT among pregnant women in Burkina Faso. The latter study, MISAME-II will be discussed below in more detail.

In studies in Malawi [120], The Gambia [121] and Niger [122], no effects of LNS for pregnant women were found on birth anthropometry or SGA prevalence.

A Cochrane review in 2018 included the four trials in Bangladesh, Ghana, Malawi and Burkina Faso. It concluded that the beneficial findings of LNS should be interpreted with caution, as the evidence comes from a small number of trials and effect sizes are too small to propose any concrete recommendation for practice [123].

Isocaloric protein supplements

Isocaloric protein supplementations refers to supplementation where protein replaces an equal amount of non-protein energy. Two trials from the 1980s with very low sample sizes, evaluated as low quality evidence, did not find any effect of this intervention on birth weight or gestational weight gain [100].

High protein supplements

Supplementation with ≥25% energy from protein is considered 'high protein' supplementation. A trial from the 1980s in poor neighborhoods in New York showed that the risk of SGA increased significantly, with no additional effects of gestational weight gain or other neonatal outcomes [100]. Hence, no additional intervention studies were done with high protein supplementation.

Balanced energy-protein supplements

In BEP supplements, less than 25% of energy comes from protein. These supplements can be fortified with MMN or not. The WHO recommendation for antenatal care for a positive pregnancy experience include context-specific recommendations on balanced energy-protein supplementation for pregnant women in undernourished populations to reduce the risk of stillbirths and SGA neonates. This recommendation is based on evidence from a Cochrane review in 2015 by Ota and colleagues [100]. This specific review on BEP supplementation included 12 randomized trials (n = 6,705) from 1973 to 2009 and concluded based on 5 trials (moderate-quality evidence) that the risk of stillbirth was significantly reduced with a 95% CI of 6-61%, mean birth weight was significantly increased with 5-77 g (11 trials, moderate-quality evidence), and the risk for SGA was significantly reduced with 10-31% (7 trials, moderate-quality evidence). No significant effect was observed for preterm birth, neonatal death or gestational weight gain [100].

It is however key to look at the individual study details to put the authors' conclusion in this Cochrane review [100] in perspective of current standards of good research practices. First, the conclusion on stillbirth was based on five trials of which MISAME-II is the most recent (which did not find any effect on stillbirth), while the other studies (with a possible effect) date from more than 25 years ago. Also, for SGA, the conclusion was based on the results

of seven studies, of which six were conducted between 1973 and 1997 and one (MISAME-II) in 2009 with no observed effect of BEP (i.e. LQ-LNS) on SGA. Second, in a 5-year cluster-randomized trial (n = 2,047) in The Gambia, gestational age during pregnancy was estimated "as judged by clinical examination" and gestational age at birth was estimated using the Parkin method, which is a simplified Dubowitz procedure, easier to apply for fieldworkers. It is based on skin color, skin texture, ear firmness and breast development and has a 95% CI of 15 days, which can make a huge difference for classifying infants SGA [124]. Also, for the other trials published 38-49 years ago, it is difficult to find out whether gestational age was estimated using the golden standard of ultrasound examination. Third, the sample size of some studies was very low: the study in India contained only 20 women [125], the 'Bacon Chow' study in Taiwan 225 women [126], and the study in Colombia 456 women [127]. Fourth, it is not always clear whether the BEP supplements under study were fortified with MMN or not. In some studies the intervention is clearly described as "balanced energy/protein 16-oz beverage supplement containing 322 kcal energy, 6 g protein, and vitamins/minerals ('complement')", while for example pregnant women in the 'Barry Caerphilly study' in Wales received milk tokens "which entitled them to additional free milk delivered by their milkman" [128].

Two other systematic reviews emphasized that the effect of BEP supplementation was larger among undernourished women. In 2013, a meta-analysis on (quasi-) RCTs evaluating the impact of BEP supplementation in pregnancy, including 11 trials, indicated that BEP supplementation resulted in a significant reduction of SGA by 31% (95% CI 15–44%) and mean birth weight increased by 60 g (33-87 g) with a more pronounced effect in malnourished women [129]. Stevens and colleagues performed another systematic review in 2015 [130] and concluded, based on seven trials between 1970 and 2015 – including MISAME-II labeled as 'strong quality of evidence', that BEP supplementation significantly improved birth weight in undernourished women. No significant benefits were found on birth length or head circumference. The authors conclude the review with the need for new evidence to identify the impact on longer-term infant growth.

1.6 The MISAME-III research project

To address the current knowledge gap and provide robust scientific evidence concerning the efficacy of fortified BEP supplementation during pregnancy and lactation on birth outcomes and child growth, the MISAME-III research project was designed.

MISAME-III is an individually randomized controlled efficacy trial design that aims to test the efficacy of fortified BEP supplementation for pregnant and lactating women in rural Burkina Faso. The study protocol of MISAME-III is described in detail in **Chapter 2** of this PhD thesis.

1.6.1 Project history

The present MISAME study is the third in a row to elucidate the relationship between maternal nutrition and birth outcome, infant growth and morbidity.

The first trial, MISAME-I (NCT00642408), started in 2002 in Houndé, the district capital of the province of Tuy in the mid-west area of Burkina Faso. It was an individual RCT comparing the effect of a multivitamin-mineral supplement (UNIMMAP formulation) to IFA, the regular supplementation provided through government programs, during pregnancy. The villages were spread around the health centers of Koho and Karaba. Overall, birth weight (52 g, P = 0.035) and birth length (3.6 mm, P = 0.012) increased in the intervention group, however, the difference with the control group was small [131]. This modest result led to the hypothesis that maternal energy deficiency could be the limiting factor to reach the desired effect of the provided micronutrients.

A second trial was therefore designed to test this hypothesis. MISAME-II (NCT00909974), took place in the same area and in the same health centers. The individual RCT compared the effect of the multi-vitamin-mineral supplement (UNIMMAP formulation) to a lipid-based food supplement enriched with the same dose of minerals and vitamins. The results again showed an improvement in birth length (4.6 mm, P = 0.001) and birth weight (31 g, P = 0.197), but again the effects were small [132].

Since MISAME-II used the UNIMMAP as a control, a complete assessment of the impact of the fortified lipid-based food supplement was not possible. Therefore, in this third trial MISAME-III, we supplement pregnant and lactating women with a BEP food supplement,

including a range of minerals and vitamins in the same study area and compare the results to IFA supplementation, the current standard of care. Furthermore, we extend the supplementation postnatally to investigate the net contribution of prenatal and postnatal BEP supplementation on child linear growth up to 6 and 12 months of age.

1.6.2 Study area

MISAME-III was conducted in the district of Houndé in Burkina Faso, a landlocked country situated in West Africa. The climate of the country is Sudano-Sahelian, with a dry season from October to March/April and a rainy season from May until September/October. The diet is essentially cereal based with maize as the main staple food.

Burkina Faso has an infant mortality rate of 53 per 1.000 live births [133], with an estimated low birth weight (LBW) prevalence at 14% in 2013 [134]. The prevalence of SGA has been estimated to be between 32.2% and 41.6% in the district of Houndé [131]. The Demographic and Health Survey of 2010 reported that 16% of women had a BMI below 18.5 kg/m^2, which indicates the presence of chronic energy deficiencies in the zone [135]. The highest prevalence can be found in the eastern region, where 31% of women have a BMI lower than 18.5 kg/m^2, that is, low BMI [135]. Moreover, in particular, adolescent Burkinabe girls between the age of 15 and 19 years have a low BMI, with an estimated prevalence of 23% [136]. Micronutrient deficiencies also remain a major problem in both infants and women of reproductive age in the country [136,137].

In the rural areas, pregnant women typically continue working on the fields throughout pregnancy. Unpublished results of the MISAME-II trial, using an accelerometer and physical activity questionnaire, showed that the physical activity level (PAL = total energy expenditure / resting metabolic rate) among pregnant women (1,79) was lower compared to non-pregnant women (2,05), but no adaptations during the second or third trimester were measured compared to the first trimester. Overall their physical activity level can be considered high (MSc Thesis Stefanie Vandevijvere, Ghent University).

The incidence of food allergies in the region is undocumented and is expected to be much lower compared to European/North-American countries. The few studies that are available from African countries reflect sensitization to food or self-reported symptoms,

rather than diagnostic testing to identify food allergies and indicate that the incidence is rare, but could however be an emerging problem [138].

For the MISAME-III study, six health center catchment areas were included based on their accessibility (also during the rainy season), agricultural model (distance to fields), languages and birth figures: Boni, Dohoun, Dougoumato II, Karaba, Kari and Koumbia (**Figure 1.3**).

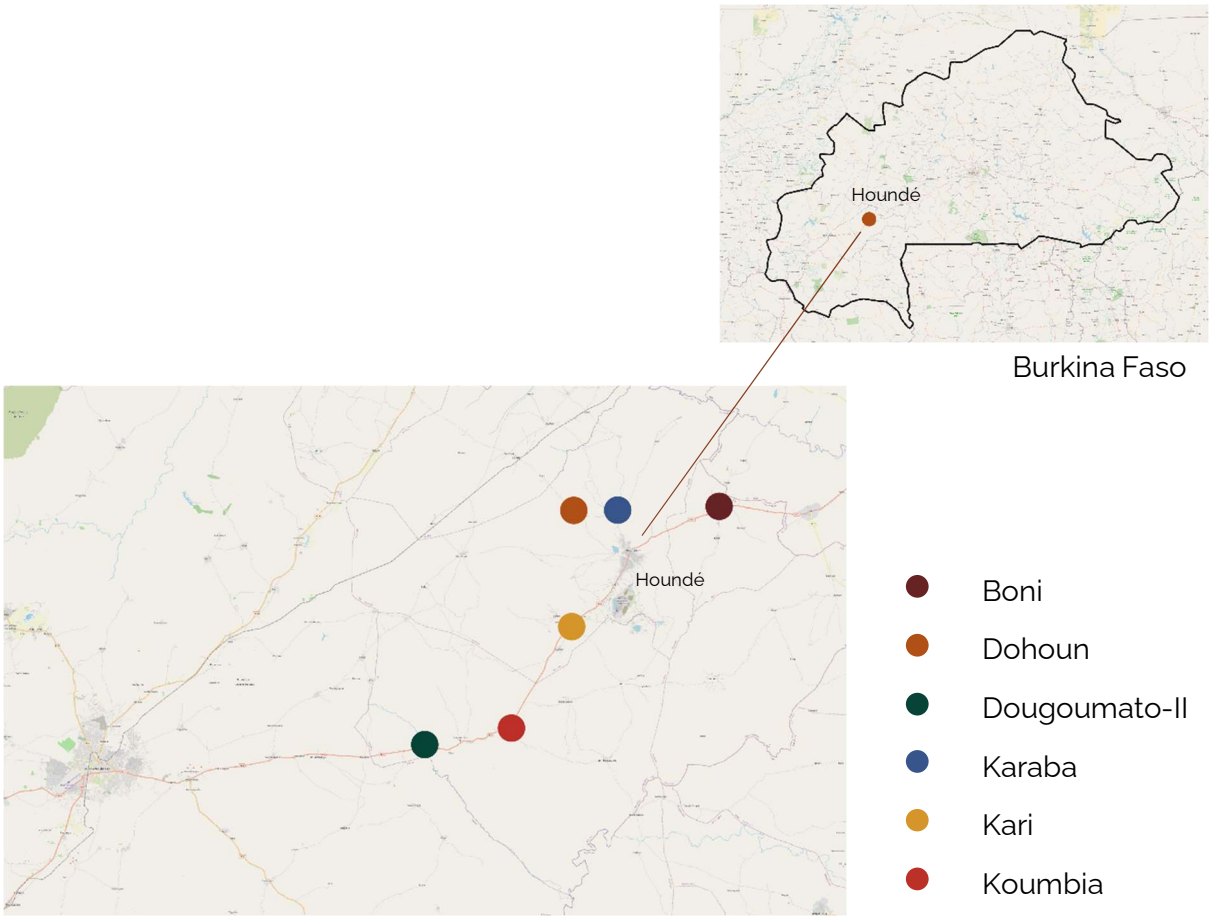


Figure 1.3 Map of the MISAME-III study area with the six health center catchment areas.

1.7 Structure of the PhD thesis

This PhD thesis includes seven chapters. After an introduction on the topic of maternal malnutrition during pregnancy in **Chapter 1**, Chapters 2-6 include published manuscripts of the MISAME-III research project. Although slight modifications have been adopted for the purpose of this PhD thesis, some overlap may exist.

The detailed study protocol of the MISAME-III research project is presented in **Chapter 2**. The published protocol provides a comprehensive overview of the study objectives, design, setting and population of both the pre-and postnatal intervention. It includes information on all study outcomes, data collection, management and analysis procedures, ethical aspects and the dissemination plan.

The study proposal was designed in 2017 by Prof. em. dr. Patrick Kolsteren and colleagues to receive funding and ethical approval for the research project. In the first year of my PhD, I focused on developing the Standard Operating Procedures for the trial, including an elaboration on the (secondary) outcome parameters, data collection methods and time points. In collaboration with colleagues and research partners, I had a primary role in designing the data collection tools and data management plan, procurement of study material and training of the field data collectors. Finally, I co-authored the published manuscript.

The following two chapters present the preliminary study, which consisted of two phases, to identify the most suitable BEP supplement for implementation in the RCT. **Chapter 3** includes the first phase, in which 12 BEP supplement types were evaluated using a single-meal rapid assessment to understand product preferences and contextual factors that might influence product acceptability and use. Ghent University and research partners Harvard T.H. Chan School of Public Health, Anthrologica and Applying Evidence for Nutrition had already developed a study protocol for this first phase of the formative research. My contributions included: training of enumerators, coordinating data collection, analyzing the quantitative data, interpretation of study findings and reviewing the manuscript.

Chapter 4 includes in the second phase of the formative study, in which the two most preferred products of phase 1, a lipid-based peanut paste and vanilla biscuit, were evaluated using a mixed methods acceptability study in the home setting. In collaboration with our research partners, we refined the research questions and study design. My main responsibilities involved: designing and analyzing the quantitative research tools, coordinating data collection, interpretation of study findings and writing the manuscript.

Chapter 5 describes a cross-sectional study of MISAME-III evaluating differences in food intake between the intervention and control group using a 24h-recall method. For this sub-study, I developed the study protocol, procedures and maternal, including the questionnaires and analysis plan. Together with my colleagues, I trained and coordinated the data collection. Finally, I was responsible for the management, cleaning and analysis of the data, and writing the manuscript.

Chapter 6 presents the main findings of the effect of prenatal fortified BEP supplementation on birth outcomes. The large-scale clinical trial MISAME-III involved a great collaborative effort among researchers, the field team and community members. I had a key role in the project implementation and coordination throughout the whole study duration, including communication with our research partners and field team. In addition, I was responsible for data management, cleaning and analysis, interpretation of study findings, writing the manuscript and dissemination of the results.

Finally, a general discussion of the implications of this PhD research and future research and policy perspectives are provided in **Chapter 7**.

2

Effect of fortified balanced energy-protein supplementation during pregnancy and lactation on birth outcomes and infant growth in rural Burkina Faso: Study protocol for a randomized controlled trial

Redrafted from: Vanslambrouck, K., de Kok, B., Toe, L. C., De Cock, N., Ouédraogo, M., Dailey-Chwalibóg, T., Hanley-Cook, G., Ganaba, R., Lachat, C., Huybregts, L., & Kolsteren, P. (2021). Effect of balanced energy-protein supplementation during pregnancy and lactation on birth outcomes and infant growth in rural Burkina Faso: Study protocol for a randomized controlled trial. *BMJ open*, *11*(3), e038393.

Effect of fortified balanced energy-protein supplementation during pregnancy and lactation on birth outcomes and infant growth in rural Burkina Faso: Study protocol for a randomized controlled trial

Abstract

Background: Adequate nutrition during pregnancy is crucial to both mother and child. Maternal malnutrition can be the cause of stillbirth or lead to poor birth outcomes such as preterm delivery and small-for-gestational-age newborns. There is a probable positive effect of providing pregnant women a balanced energy-protein (BEP) food supplement, but more evidence is needed. The Micronutriments pour la Santé de la Mère et de l'Enfant (MISAME)-III project aims to improve birth outcomes and infant growth by testing a BEP supplement during pregnancy and lactation in rural Burkina Faso. This paper describes the study protocol.

Methods: MISAME-III is a four-arm individually randomized efficacy trial implemented in six rural health center catchments areas in the district of Houndé. Eligible pregnant women, aged between 15 and 40 years old and living in the study areas, will be enrolled. Women will be randomly assigned to one of the four study groups: (1) prenatal intervention only, (2) postnatal intervention only, (3) prenatal and postnatal intervention or (4) no prenatal or postnatal intervention. The intervention group will receive the BEP supplement and iron/folic acid (IFA) tablets, while the control group will only receive the IFA tablets following the national health protocol. Consumption will be supervised by trained village-based project workers on a daily basis by means of home visits. The primary outcomes are small-for-gestational age at birth and length-for-age z-score at 6 months of age. Secondary outcomes will be measured at birth and during the first 6 months of the infants' life. Women will be enrolled from October 2019 until the total sample size is reached.

Ethics and dissemination: MISAME-III has been reviewed and approved by the University Hospital of Ghent and the ethics committee of Centre Muraz, Burkina Faso. Informed consent will be obtained. Results will be published in relevant journals and shared with other researchers and public health institutions.

Key messages

- This trial will help to fill the evidence gap on the effect of balanced energy-protein (BEP) supplements in pregnant and lactating women on birth outcomes and infant growth.
- Formative research to select the most suitable BEP supplement will ensure that the selected BEP is well accepted by the study population.
- Strengths of the study include the monthly home visits in the study area to encourage early enrollment, daily observed BEP intake, and (early) measurement of birth outcomes and child growth by trained midwives.

Introduction

Pregnancy is a challenging period in the life of many women in LMICs. Maternal mortality remains high, and many neonates suffer from premature delivery and/or IUGR, both in length and in weight accumulation [139]. An indicator to measure neonatal growth is SGA, defined as a birth weight below the 10^{th} percentile of a standard optimal reference population for a given gestational age and sex [30]. SGA is often caused by growth restriction in the womb and has been associated with neonatal and post-neonatal mortality [30]. It has also been linked to an increased risk of morbidity later in life, especially noncommunicable diseases [140]. SGA affected 23.3 million term children in LMICs in 2012 [41]. Adequate nutrition during pregnancy is crucial for optimal maternal and newborn health [108,130], and maternal malnutrition has been associated with fetal growth restriction [141]. An adequate dietary balance is necessary to ensure sufficient energy intake for adequate growth of the fetus [16]. Unfortunately, maternal undernutrition remains a public health challenge in regions across sub-Saharan Africa and Asia [142,143].

Several types of food supplements have been developed and evaluated over the past years. A positive effect of MMN supplements during pregnancy on birth outcomes has been found in previous studies [111]. Keats and colleagues [111] concluded in their review that MMN during pregnancy gave a probable reduction in SGA and preterm births and can thus be used for future guidance. According to a multi-country RCT done in LMICs, a positive effect of LNS on fetal growth-related birth outcomes can be seen when starting

supplementation before conception or during the first trimester [116]. Moreover, the latest evidence indicates a possible positive effect of providing pregnant women a BEP food supplement [100,130,144,145]. In line with that evidence, the 2016 WHO's antenatal care guidelines state that pregnant women in undernourished populations should receive, depending on the context, BEP supplements to reduce the risk of stillbirth and SGA [108]. Researchers, however, still highlight the limited amount of evidence and a need to evaluate the effect of balanced supplements on birth outcomes, such as SGA [100,130]. Experimental trials of high-quality and large sample sizes, especially in undernourished pregnant women, are thus needed [100]. Following this recommendation, compositional guidance for a ready-to-use food supplement for pregnant women was developed by the Bill and Melinda Gates Foundation (BMGF) in 2016 [146].

In summary, the MISAME-III study hypothesizes that providing women with a BEP supplement during pregnancy will decrease the incidence of SGA compared with the control group; and (2) providing them with a BEP supplement during the postnatal period will increase children's length by the age of 6 months compared with the control group.

Methods

This protocol has been developed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials guidelines (**Annex 1.1**).

MISAME-III study design

The MISAME-III project is an individually randomized 2×2 factorial efficacy trial aiming to improve birth outcomes and infant growth in rural Burkina Faso by testing a BEP supplement during pregnancy and lactation (**Figure 2.1**). At inclusion, pregnant women will be individually and randomly allocated to a prenatal intervention or control group and a postnatal intervention or control group. The intervention group will receive a daily BEP supplement to be consumed under supervision for the duration of pregnancy/lactation. Both intervention and control groups will receive the standard IFA tablet through the national antenatal care. In addition to the main trial, we propose a number of sub-studies to test specific hypotheses in a subsample of pregnant/lactating women and children.

MISAME-III began with a formative study to identify the preferred product type for the provision of a fortified BEP supplement during the RCT.

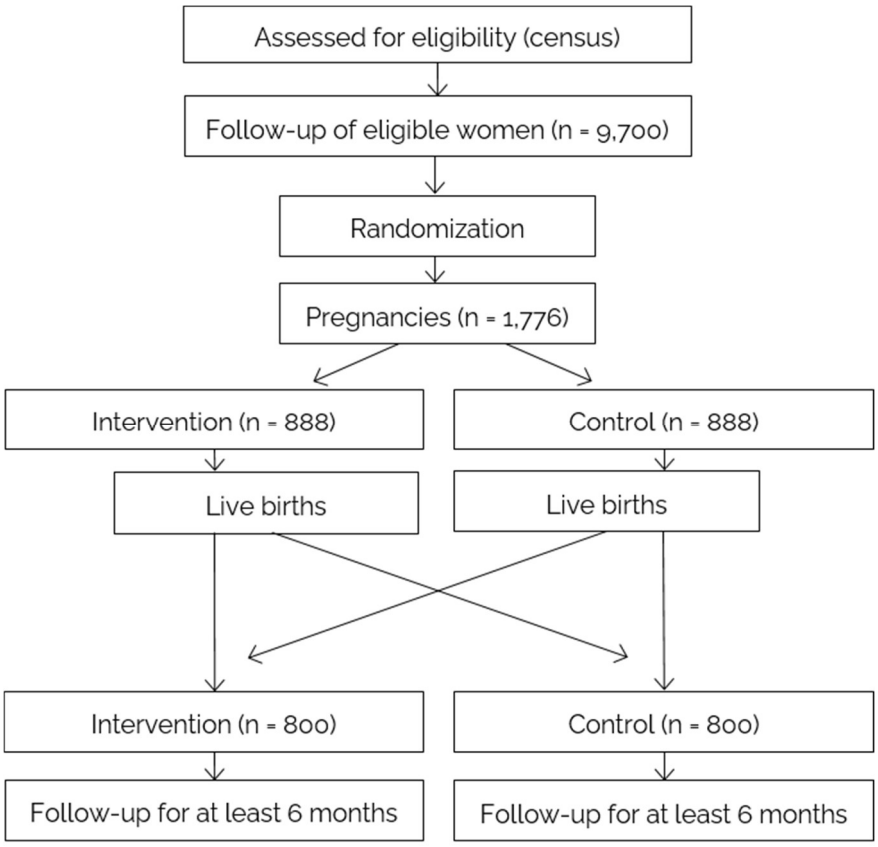


Figure 2.1 Study design of the randomized controlled trial.

Study setting

The study will be conducted in the district of Houndé in Burkina Faso, a landlocked country situated in West Africa; similar to the two previous MISAME studies. The study villages are concentrated around six health centers within a reasonable distance from the district hospital. These six health centers were selected based on their accessibility during the rainy season, agricultural model (i.e. villages in which women would not reside in the fields during the entire harvest season), language (i.e. Mooré, Dioula or Bwamu) and an average monthly birth rate of at least 25 to reach the planned recruitment rate within the project timeframe.

Study population and recruitment

Women living in the study villages and aged between 15 and 40 years will be identified through a census. Trained village-based project workers (femme accompagnante [FA]), selected in collaboration with the community leaders, will visit the households every 5 weeks to ask about women's menstruation (n = 9,700 women in **Figure 2.1**). In case of amenorrhea, women will be sent to the nearest health center for a pregnancy test and a first antenatal consultation by our project midwife when tested positive (n = 1,776 pregnancies in **Figure 2.1**). An ultrasound examination will be completed soon after inclusion by the project medical doctor to assess gestational age. A baseline interview will also be done by the project interviewers to assess the household members' characteristics, household properties, water sanitation and hygiene (WASH) and household food security.

Inclusion criteria:

- Women between 15 and 40 years old at study inclusion.
- Confirmed pregnancy by a pregnancy test and ultrasound.
- Women who signed the informed consent form.

Exclusion criteria:

- Pregnancies >20 weeks of gestational age.
- Women planning on leaving the area during their pregnancy.
- Women planning on delivering outside the study area.
- Women who are allergic to peanuts.
- Women with multifetal gestations (exclusion from analysis).

FAs will be informed by the project midwife when a participant has been included. FAs will visit pregnant women on a daily basis to distribute the BEP supplement and/or IFA tablet and to supervise consumption. During the postnatal period, FAs will distribute the supplements and IFA tablets to the intervention group on a daily basis until 6 weeks after birth. From that moment onwards, they will receive a week's worth of BEP supplements. The postnatal control group will receive the IFA tablets on a daily basis during the first 6 weeks after birth, and participants will thereafter be visited once a week (without any supplementation) to minimize the effect of home visits. The FA will inform women on the

supplement's function, the importance of antenatal visits during pregnancy, maintaining a healthy diversified diet, the importance of delivering at a health facility, the importance of exclusive breast feeding and the introduction of complementary foods at the age of 6 months. Throughout the study, the FAs will be supervised by project interviewers. Supervision visits will be conducted using Lot Quality Assurance Sampling schemes and empty sachet counts to ensure that study participant are visited according to the project protocol.

Manufacturing of 12 fortified BEP supplements and the formative study

Twelve fortified BEP supplements were pretested before the start of the RCT during a formative research phase. Several food manufacturing companies were invited to produce ready-to-use BEP supplements following the compositional guidelines proposed during an expert meeting hosted by the BMGF [146] in September 2016 (**Table 2.1**). The BEP supplements had to be: (1) ready to consume, (2) not need a cold chain and (3) microbiologically stable.

Seven out of 12 supplements were characterized as sweet and five as savory. Products were produced in different forms, including a biscuit, pillow, wafer, bar, paste, instant drink and soup.

In a first screening step of the formative study, the two most preferred BEP supplements were identified by using a combined evaluation approach consisting of a single meal test, sensory evaluation and focus group discussions, in a convenience sample of 40 pregnant women. In a next step, we compared the acceptability of the two preselected BEP supplements using a 10-week home feeding study, with 80 pregnant women, to select the most preferred product for the RCT. We refer to both papers (Chapter 3 and 4) for detailed information [147,148].

Table 2.1 The compositional guidelines for macronutrients and micronutrients.[a]

Nutrition component	Target per serving
Total energy	250–500 kcal per serving
Fat content	10%–60% of energy intake
Protein content	16 g (14–18 g) with a Digestible Indispensable Amino Acid Score of ≥0.9[b]
Carbohydrates	Between 45 g and 32 g per 100 g (added sucrose between 20 g and 10 g per 100 g)
Trans fats	<1% of energy intake
Fatty acid	(Optional): min of 1.3 g of n-3 or 300 mg docosahexaenoic acid (DHA) + eicosapentaenoic acid (of which 200 mg DHA) to achieve a healthy n-6: ratio of the supplement of 5:1
Micronutrients	Vitamin A, D, E, K, B1 (thiamin), B2 (riboflavin), B3 (niacin), B6 (pyridoxine), B9 (folate), B12 and C; minerals: iron, zinc, iodine, calcium, phosphorous, copper and selenium
Optionally	Pantothenic acid, manganese, potassium, biotin and choline will be included

[a] The final composition of the product will be determined by the selected product as the manufacturing process will influence the macronutrient composition.

[b] The Digestible Indispensable Amino Acid Score (DIAAS) is a protein quality method proposed by the FAO in 2013 [149]. It is a more accurate measure of the amounts of amino acids absorbed by the body, specifically at the end of the small intestine, and the protein's contribution to human amino acid and nitrogen requirements. A score of ≥0.9 indicates higher quality protein.

Allocation/randomisation

We will apply a stratified permuted block randomisation schedule to allocate women to the prenatal intervention or control group and in a next step to allocate women to a postnatal intervention and control group. Per health center (i.e., stratum), women will be individually randomly in permuted blocks of 8 so that, per block, equal numbers are obtained in the prenatal intervention (n = 4) and control (n = 4) group and equal numbers are also obtained in the postnatal intervention (n = 4) and control (n = 4) group. The double random sequence will be generated before the start of the study using Stata (v. 15.1, StataCorp, USA) by an external research analyst. The allocation group will be coded with two letters (A or B for the prenatal and Y or Z for the postnatal study group) and placed in a sequentially numbered sealed opaque envelopes by project employees, not in direct contact with participants. At study enrolment, the project midwife will draw the next sealed envelope and allocate the participant to the study group defined by the letter code in the envelope.

Blinding of participants and community-based project workers will not be possible since the supplements are identifiable. Field staff responsible for measuring primary and secondary study outcomes are not directly involved in the daily supplementation of the study participants and can therefore be considered to be partially blinded.

Outcomes

Primary outcomes of the RCT

The trial has two primary study outcomes that will be used to assess the impact of the prenatal and the postnatal intervention, respectively:

- Incidence of SGA, defined as birth weight <10th centile of the INTERGROWTH-21st reference [150].
- LAZ calculated using the WHO 2006 growth reference at 6 months of age [151].

Secondary outcomes of the RCT

A list of the trial's secondary outcomes can be found in **Table 2.2**.

Birth weight measurements will be defined using the INTERGROWTH-21st reference [150], and child anthropometry will be defined using the Child Growth Standards developed by the WHO [151].

Outcomes of the sub-studies

Sub-study 1: impact of the intervention on dietary intake. A dietary assessment study will be conducted, using a 24-hour dietary recall in a subsample of women. This sub-study will enable us to assess possible substitution of the prenatal diet by the BEP supplement.

Sub-study 2 (outside the scope of this PhD thesis): impact of the intervention on neonatal and maternal body composition 2-3 weeks after delivery. Body composition will be determined in mother-child dyads by deuterium dilution and analysis of saliva by a Fourier Transform Infrared reader (Agilent FTIR 4500 series). The sub-study will also assess if early gestation maternal BMI (defined as body weight in kilograms divided by the square of height in meters) modifies the intervention's effect on neonatal body composition.

Sub-study 3 (outside the scope of this PhD thesis): impact of the intervention on breastmilk. Breastmilk samples will be taken in the four study groups to compare the composition and to analyze the interaction between the supplementation periods.

Table 2.2 Secondary outcomes of the RCT on maternal, newborn and child level.

Maternal outcomes	**Newborn**	**Child**
Total and trimester-specific prenatal weight gain and gestational weight change	Birth weight (measured within 72 hours after birth)	Weight-for-age z-score at 6 months of age (WAZ) (and 9 and 12 months on a subsample)
Probable and possible maternal postnatal depression at 2 and 6 months of child age	Birth length (measured within 72 hours after birth)	Weight-for-length z-score at 6 months of age (WLZ) (and 9 and 12 months on a subsample)
Maternal anemia at the third antenatal consultation	Ponderal or Rohrer's index at birth	Stunting at 6 months of age
Women's mean and minimum dietary diversity score (measured twice weekly)	Gestational age at delivery	Wasting at 6 months of age
	Large-for-gestational age	Underweight at 6 months of age
	Chest circumference (measured within 72 hours after birth)	Duration of exclusive breastfeeding during the first 6 months of age
	Head circumference (measured within 72 hours after birth)	Incidence of child wasting over first 6 months of life
	Arm circumference (measured within 72 hours after birth	Weight gain over first 6 months of life
	Incidence of preterm birth	Child mortality (between birth and 6 months of age)
	Fetal loss	Monthly change in length-for-age z-score (LAZ) over first 6 months of life
	Stillbirths	Monthly change in WLZ over first 6 months of life
		Monthly change in WAZ over first 6 months of life
		Monthly change in head circumference over first 6 months of life
		Child morbidity symptoms over first 6 months of life
		Anemia at 6 months of age
		Hemoglobin concentration at 6 months of age

LAZ, length-for-age z-score; RCT, randomized controlled trial; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score.

Sample size

With an SGA prevalence of 32% and an anticipated decrease of 7%, a sample of 652 subjects per prenatal arm is required with $\alpha = 0.05$ and $\beta = 0.20$ [152]. To accommodate for possible losses, the number of subjects per arm was increased to 888 (total subjects: 1,776). Possible losses are based on previous MISAME studies where the prevalence was ~26% due to a combination of miscarriage, stillbirths, multifetal pregnancies, out-migrants, maternal deaths and incomplete data [132]. For the analysis of an effect of the postnatal intervention on LAZ at 6 months of age, the minimally detectable effect depends on the presence or absence of an interaction effect between the prenatal and postnatal intervention. In the absence of a statistically significant interaction between prenatal and postnatal intervention, at sample size of 588 children per postnatal study arm would allow us to detect a difference of 0.18 z-score (SD = 1.1) based on a cross-sectional survey conducted in the Gourcy health district in Burkina Faso [153], between study arms with $\alpha = 0.05$ and $\beta = 0.20$. This implies that if ~1,400 singleton live births are available, we allow for a maximum loss to follow-up of 16%. In the presence of a statistically significant interaction between prenatal and postnatal intervention, a total sample size of 1,176 represents 294 children per factorial combination of the prenatal and postnatal study group (four groups in total). A subgroup size of 294 would allow us to detect a difference in LAZ at 6 months of age of 0.28 assuming an SD of 1.1, $\alpha = 0.025$ (Bonferroni correction for two primary endpoints analyses) and $\beta = 0.20$.

Data collection

Anthropometric and clinical procedures

At enrolment, anthropometric measurements from all women will be taken. Gestational age will be determined during an ultrasound consultation by measuring crown-rump length (7-13 weeks) or by calculating the mean of three to four measurements: biparietal diameter, head circumference, abdominal circumference and femur length (12–26 weeks) [154]. During pregnancy, clinical follow-up will consist of antenatal visits following the national guidelines.

At birth, anthropometric measurements of all neonates will be assessed in duplicate within the first 72 hours of life (in practice, the aim is to measure within the 24 hours of life). After birth, mother and child will visit the healthcare centers monthly for a follow-up on clinical, anthropometric and child morbidity measures (signs including fever, vomiting, diarrhea, cough, difficulty breathing and running nose). A subsample will be measured at the healthcare centers or at home by the project interviewers to collect postnatal data at months 9 and 12.

Hemoglobin concentrations will be measured in women at enrolment and during the third ANC visit. This will be conducted at 6 months of age among children.

Baseline questionnaires

Prenatal and postnatal maternal depression will be assessed using the standardized Edinburgh Postnatal Depression Scale questionnaire consisting of 10 questions [155]. Project midwives will be trained for this, and the questionnaire will be asked at inclusion and at months 2 and 6 after birth. Socioeconomic and demographic information from all participants will be collected once included. Trained project interviewers will ask questions on household members' characteristics, household properties, WASH environment and household food security. The women's dietary diversity score will be measured in all participating women by the FA during the home visits. This will be enumerated twice a week per participant using the Women's Dietary Diversity Score with 11 food groups [156].

Table 2.3 shows the overview of the time schedule and measurements of the trial.

Table 2.3 Participant timeline schedule of enrolment, interventions, assessment and visits.

	Enrolment		**Pregnancy and birth**					**After birth**							
	Start	5-wk visits	ANC 1	Household visit	Ultra-sound	ANC 2, 3 and 4	Birth	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12
Enrolment															
Census	X														
Pregnancy identification		X													
Pregnancy confirmatory test			X												
Informed consent and allocation to study group			X												
Study groups															
Prenatal BEP + IFA			X	—	—	—	X								
Prenatal IFA			X	—	—	—	X								
Postnatal BEP + IFA[a]							X	—	—	—	—	—	X		
Postnatal IFA[a]							X	—	X						
Assessments - mothers															
Baseline questionnaire				X											
Gestational age determination					X										
Skinfold measurements					X										
Weight (kg) and arm circumference (mm)[b,c]			X			X	X	X	X	X	X	X	X	X	X
Height (cm)			X												
Hemoglobin (g/dL)						X (ANC3)		X							
Women's dietary diversity score (biweekly)				X											
Maternal depression			X						X				X		

Table 2.3 (continued)

	Enrolment		Pregnancy and birth					After birth							
	Start	5-wk visits	ANC 1	House hold visit	Ultra-sound	ANC 2, 3 and 4	Birth	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12
Infants															
Birth weight (kg)							X								
Birth length (cm)							X								
Head circumference (mm)							X	X	X	X	X	X	X		
Chest circumference (mm)							X								
Arm circumference (mm)								X		X			X		X
Morbidity								X	X	X	X	X	X		
Mortality							X	X	X	X	X	X	X		
Weight (kg) and height (cm)								X	X	X	X	X	X	X	X
Breastfeeding practices								X	X	X	X	X	X		
Hemoglobin (g/dL)													X		

[a] The IFA tablets will be given during the first 6 weeks after birth in the postnatal intervention group, according to the national health protocol.

[b] Only maternal weight will be taken at birth.

[c] In subsample during month 9 and 12.

ANC, antenatal care; BEP, balanced-energy protein; IFA, iron-folic acid.

Quality of all study data will be insured by a thorough training of all field staff. Procedures to handle data collection tools (questionnaires, anthropometric and clinical measurement material, and laboratory procedures) will be pretested in the field during a dry-run of ±3 months. Anthropometric measurement standardizations of the field staff will be repeated bimonthly throughout the trial. Anthropometric measurements will be taken in duplicate. Newborns will be measured within 72 hours after birth (preferably within the 24 hours), and all weighing scales and HemoCue 201+ devices will undergo weekly quality control. A WhatsApp group will be set up where problems can be communicated and solved quickly.

All data collection forms of the trial can be found on: https://www.misame3.ugent.be/.

Women will be designated as lost to follow-up if they move from the study area or withdraw their participation. Reasons for discontinuation will be recorded.

Women will be enrolled in the study from October 2019 until the total sample size has been reached.

Data management and analysis

FAs will use smartphones with computer-assisted person interviewing programmed in Census and Survey Processing System (CSPro; v. 7.3.1, Census Bureau, USA) to collect data during household visits. The study data collected by the project medical doctor, project midwives and interviewers will be done by Survey Solutions data entry software (v. 19.12.6, World Bank Group, USA) on tablets. This data will be uploaded to a central server on a weekly basis. All questionnaires were programmed and have been tested on the Survey Solutions Designer website and include validation codes to promote the quality of the data entry in the field. Assignments will be sent once a week to the tablets of the field team, and preloaded data collected at an earlier contact moment will be used to lower the amount of incorrect data. Paper forms will also be available on the field as a backup.

Further data quality checks will be conducted in Stata (v. 14.2, StataCorp, USA). Missing or inconsistent data outliers will be sent back to the field for revision.

Statistical analysis

We refer to the Statistical Analysis Plan of the trial 'Statistical Analysis Plan: Impact of a prenatal and postnatal BEP supplement on birth size and postnatal child growth in Burkina Faso' published on: https://www.misame3.ugent.be/.

Data monitoring

Data monitoring and auditing

The Data and Safety Monitoring Board (DSMB) is an independent multidisciplinary group whose members are not involved in the trial. The board consists of a Belgian endocrinologist, a Belgian pediatrician, a Burkinabe pediatrician, a Belgian gynecologist and a Belgian ethicist.

Serious adverse events

FAs will be trained to recognize health issues and will actively refer those participants to see the project midwife in the primary health facilities or Centre de Santé et Promotion Sociale (CSPS) in the event they occur. All serious adverse events (SAEs) will be recorded on a case-by-case basis, and verbal autopsies will be conducted for maternal, neonatal and infant deaths by the field medical doctor.

Ethics and dissemination

Ethics approval and consent to participate

MISAME-III has been reviewed and approved by the University Hospital of Ghent University (B670201734334) and the Burkinabe ethics (N°2018–22/MS/SG/CM/CEI) committee. Important protocol changes will be noted on ClinicalTrials.gov. When eligible women meet the inclusion criteria, project midwives will explain the background and procedure of the complete trial. Written informed consent or assent will be asked from the participating women. In case of illiteracy, a thumb print will be asked and witnessed by the recruiting investigator and one extra witness. Participants will be told that all data collected during the trial is confidential and that they are allowed to withdraw at any time. A copy of the informed consent and assent can be found on misame3.ugent.be and as supplementary file (**Annex 1.2**).

Patient and public involvement

MISAME-III has been well accepted by the community, because of the previous positive experiences they had with the MISAME-I and II studies. Through the formative study, women were involved in the choice of BEP supplement. Workshops will be planned at the end of the study in order to communicate the study results to the community.

Ancillary care

The MISAME-III project will pay for ancillary care when participants have health issues and in case the costs are not covered by the national healthcare program. Participants suffering harm due to their trial participation will be covered.

Confidentiality

A data management plan has been put in place to address concerns regarding the General Data Protection Regulation rules. During the trial, the data files containing personal identifying information will be stored on the Survey Solutions server. Only the principal investigators and the project coordinators will be able to access those files.

Dissemination plan

On completion of the trial, all anonymized study data will be available on request. Final results will be communicated to the participants, the Burkinabe Ministry of health, the field staff, the BMGF, Ghent University researchers and students, AFRICSanté, healthcare professionals and other relevant international public institutions. Papers on the study results will be published in peer-reviewed journals and will be available on the project website. All investigators contributing to the realization of the project and publication of results will be included as an author. Other contributors such as the participants, FA and field staff members will be mentioned in the acknowledgements.

MISAME-III has been well accepted by the community, because of the previous positive experiences they had with the MISAME-I and II studies. Through the formative study, women were involved in the choice of BEP supplement. Workshops will be planned at the end of the study in order to communicate the study results to the community.

Discussion

In this paper, the protocol of an individually randomized four-arm efficacy trial in rural Burkina Faso has been described in which pregnant and lactating women in the intervention group will receive a BEP supplement together with IFA tablets. The control group will only receive the standard IFA treatment.

The key features of the present trial are, first, the inclusion of a formative study for a better understanding of which type of supplement is preferred, what taste is most acceptable and which factors affect adherence in the study population. Second, the supplementation will be given during pregnancy and during the first 6 months after birth. This will give us the opportunity to assess the specific value of postnatal supplementation on several outcomes. Third, the observed daily intake of intervention and control supplements is a key feature to ensure adherence and to avoid sharing of the supplements with other household members. Fourth, MISAME-III has the advantage of being the third trial of its kind in the study area. This presents an opportunity to anticipate the issues that arose in previous trials. For instance, women in specific villages tended to leave their homes for a longer period to go work on the fields outside the village. This posed problems in the continuation of the supplementation in the past and will be taken into consideration during MISAME-III. Fifth, four sub-studies are nested in the main trial that will provide insight into the mechanism by which prenatal BEP supplementation affects birth and infant outcomes. And last, similar studies are being conducted in other countries, allowing for comparison between results from different contexts.

The MISAME-III study will provide evidence on the impact of BEP supplements on birth and infant size using a rigorous study design. The study results will further strengthen and refine WHO's recommendation on the use of context-specific BEP supplementation during pregnancy and lactation.

3

Acceptability of twelve fortified balanced energy-protein supplements - Insights from Burkina Faso

Redrafted from:

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Acceptability of 12 fortified balanced energy protein supplements - Insights from Burkina Faso

Abstract

Background: Poor maternal nutrition contributes to poor birth outcomes, including low birth weight and small-for-gestational age births. Fortified balanced energy protein (BEP) supplements may be beneficial, although evidence is limited.

Methods: This mixed method study, conducted among pregnant women in Burkina Faso, is part of a larger clinical trial that seeks to understand the impact of fortified BEP supplements on pregnancy outcomes and child growth. The formative research reported here, a single-meal rapid assessment of 12 product formulations, sought to understand product preferences for provision of BEP supplements and contextual factors that might affect product acceptability and use.

Results: Our data indicate a preference for products perceived as sweet rather than salty/savory and for products perceived as familiar, as well as a sensitivity to product odors. Women expressed a willingness and intention to use the products even if they did not like them, because of the health benefits for their babies. The findings also indicate that household food sharing practices may impact supplement use, although most women denied any intention to share the products. Sharing behavior should therefore be monitored, and strategies to avoid sharing should be developed during the succeeding parts of the research.

Conclusion: The lipid-based peanut paste and vanilla biscuit received the highest overall ranking for appreciation and acceptability during pregnancy.

Key messages

- Current evidence indicates that balanced energy-protein supplements may be effective in improving birth outcomes, but more needs to be known about preferred types of supplements and potential facilitators and barriers to product use. To inform decision making, 12 BEP supplements were tested by pregnant women in Burkina Faso.
- Although women in this study preferred products with familiar flavors that are perceived as sweet rather than savory/salty, they expressed a willingness to eat any product they can tolerate because of the potential health benefits for their baby.
- Future studies and programs should pay attention to strategies that support women to avoid sharing nutritious food supplements with children or other household members.

Introduction

Low birth weight is a significant risk factor for infant mortality, estimated to account directly or indirectly for 60-80% of neonatal deaths worldwide [157]. Poor maternal nutrition status, including energy and micronutrient deficiencies, contributes to poor birth outcomes [26,158,159]. Women who enter pregnancy with low BMI or short stature are at increased risk of adverse health outcomes [160] as well as SGA births [58]. Although Burkina Faso has seen significant improvements in maternal and infant health over the last 25 years, the infant mortality rate has remained high, at 53 per 1000 live births in 2016 [161]. Of those babies with a reported birth weight (63.6% of all births), 13.9% were LBW [135]; a previous study by members of this research team estimated that the number of SGA births is between 32.2% and 41.6% in the Houndé district [131,132]. The overall prevalence of underweight in women of reproductive age in Burkina Faso is 15.7%, although prevalence is nearly twice that (31.1%) in some regions [135].

Evidence indicates that providing pregnant women with a BEP food supplement may have a positive effect on birth outcomes [100,129], particularly among undernourished women. WHO antenatal care guidelines recommend provision of BEP supplements in populations where the prevalence of undernourished women (low BMI) is greater than 20% [108].

In addition, recent reviews support the conclusion that MMN supplements are beneficial in countries with a high prevalence of multiple micronutrient deficiencies [18,112,162]. Transitioning from IFA supplements to MMN has also been found to be cost-effective [163,164].

Guidance from the BMGF therefore recommends development of a multi micronutrient fortified BEP supplement as a ready-to-consume product [146]. However, more research is needed to quantify the impact of specific fortified BEP supplements on birth outcomes, as well as to understand how factors such as product preferences and community influences on product use may affect acceptability and uptake of specific supplements in a given context.

Currently there is limited evidence on the acceptability of food supplements for pregnant women in LMICs settings. Two studies were conducted in Asia (Bangladesh [165] and Cambodia [166]), one in Central-America (Mexico [167]), three in East-Africa (Ghana [168,169], Malawi [168] and Uganda [170]) and three in West-Africa (Niger [171,172] and Mali [173]). The only studies conducted in Burkina Faso evaluate the acceptability of supplementary foods among young children [174–177]. Overall, the acceptability studies among pregnant and lactating women indicate that the supplements (i.e. MMN, LNS or CSB) are well accepted. Promoting factors for the high adherence include the expected health benefits for the mother and baby [165,166,168,171,173,178], favorable sensory attributes [168–170,172], and simplicity of use and/or preparation [167,170]. Barriers include the bad smell or taste (e.g. overly sweet, too oily, bitter aftertaste) of supplements [165–167,169,179], feeling nausea or vomiting [165,167–169,172], rumors on childbirth complications [171] and fears of weight gain during pregnancy [166]. In addition, studies report sharing of food supplements with small children, family members or friends as a potential risk factor [168,171]. Overall, the studies indicate that in light of potential widespread use of nutritious food supplements, future studies, including test-meals and home-feeding trials over longer time periods (e.g. across different seasons), are warranted to better understand acceptability in different participants and regions of the world and support optimal use.

This paper focuses on the first part of phase 1 of the MISAME-III study, a two-phase study seeking to evaluate the preferred product type for fortified BEP supplements (phase 1 -

Chapters 3 & 4) and their impact on pregnancy outcomes and child growth in Burkina Faso (phase 2 - Chapter 6). Data collection activities during this part of phase 1 sought to (1) assess the hedonic properties of 12 formulations of fortified BEP supplements, (2) identify preferred product type(s) for fortified BEP supplements (for further testing in the second part of phase 1) and (3) assess the acceptability, general preferences, advantages and barriers across product types.

Methods

Study design

Data were collected using a convergent mixed methods approach that included quantitative and qualitative tools [180]. The value of gathering mixed methods data is the mutual validation of results through a process of triangulation. Throughout this research, the convergence of the different methods allowed for testing the same hypothesis and answering the same part of a research question through multiple lenses [181].

Recruitment and data collection

Data were collected over 3 weeks between May and June 2018 in Houndé district, Burkina Faso, West Africa. The district was selected because of the high percentage of SGA births and the study team's prior experience in the area, which facilitated study logistics. Houndé has one hospital and 31 health centers, of which five were identified for inclusion in this study because of their accessibility even during the rainy season and willingness to participate. All pregnant women aged 15 to 35 who visited one of the five health facilities for prenatal consultation were invited by their health care providers to participate.

The only exclusion criteria were allergies to product ingredients (soy, dairy products, eggs, gluten and nuts), none of which were reported during recruitment. Eight women per health center (40 in total) were targeted for inclusion in the study, and recruitment ceased when this number were enrolled. On the first day of data collection, which took place at each of the five health centers, data collectors explained the study and obtained informed consent from all participants. Women were provided with lunch in consideration of their participation.

Tested supplements

The nutritional composition of the specific BEP supplements was established during an expert consultation convened at the BMGF in September 2016 [146] and is set forth in **Table 2.1** (Chapter 2). The research partners liaised with private sector partners to develop supplements in seven different product formats to be evaluated in formative research: biscuits, bars, filled sticks, cold beverages, a soup, lipid-based pastes and 'pillows', a crispy puffed snack formulation. The pastes, bars, biscuits and pillows were available in both a primarily sweet and a primarily savory flavor profile. Several of the flavors were developed specifically to evoke familiar flavors from the Burkinabe diet, such as the fermented drink, the mango bars and the tomato/onion bars. **Table 3.1** presents the 12 products tested in the study and the flavor profiles and manufacturers of each (photos in **Annex 6**).

Table 3.1 Product groupings and manufacturers.

Product name	Product grouping	Product manufacturer
Sweet lipid-based paste	Sweet	Nutriset
Mango bar	Sweet	Nutriset
Vanilla-filled sticks	Sweet	Nutriset
Vanilla biscuits	Sweet	Nutriset
Vanilla drink	Sweet	Nutriset
Unseasoned pillows[a]	Sweet	Mars
Fermented drink[a]	Sweet	DSM
Tomato and onion lipid-based paste	Savory	Nutriset
Tomato and onion bar	Savory	Nutriset
Tomato and onion biscuits	Savory	Nutriset
Chicken soup	Savory	Nutriset
Seasoned pillows	Savory	Mars

[a] The unseasoned pillows and the fermented drink did not have a sweet taste but were grouped with the sweet products to distinguish them from products containing savory flavors.

Research tools

The quantitative (hedonic testing and product ranking exercises) and qualitative (focus group discussions) tools were pretested with a subset of volunteers (pregnant women) from the local community and were refined over the course of several weeks.

Quantitative tools

The quantitative data were collected electronically using the CSPro data management program (v. 7.1, Census Bureau, USA). Over two consecutive days, women were presented with samples (in the amount of 25% of the full daily portion) of each of the 12 products. Products were divided into sweet and savory groups, with seven characterized as sweet and five as savory. The products were moderately sweetened (**Annex 2**). Added sugars ranged from 13.7 to 21.6 g per 100 g serving for the sweet products and from 0 to 13 g per 100 g for the savory products. This level contributes 2.5-3.9% of energy from sugar in a 2200 kcal diet for the sweet products and 0-2.4% for the savory products. However, certain products were grouped as 'sweet' in order to distinguish them from those with a more savory taste profile.

Acceptability of five savory products was assessed on day one of data collection and of seven sweet products on day two. The decision to test on two separate days was based on practical reasons, i.e. to keep participant burden within reason. The amount of each product consumed (by weight) and the time taken were recorded; a limit of 20 min was allowed for the consumption of each individual product. In between all tastings, women were given water to rinse their mouth. Each woman was asked a series of questions about the acceptability and hedonic characteristics of each of the 12 products in turn after consumption, using a 7-point scale to answer from 1 (*I dislike it very much*) to 7 (*I like it very much*) [182]. The women were also presented with a series of statements regarding their potential use of the product and willingness to consume it during pregnancy, and the responses were scaled from 1 (*I do not agree at all*) to 7 (*I agree completely*). The 7-point scale was graphically depicted using a range of emoticon faces (very unhappy to very happy).

Following individual evaluation of each product grouping (i.e. sweet or savory), participants were asked to rank that group's products in order of preference from 'most liked' to 'least liked' for each of taste, texture, smell, color, portion size (full serving), ease of use and overall preference. Participants were also asked individually on day two to identify their overall 'top three' products out of all 12 products tasted. All quantitative data were collected from participants in individual sessions so that they were unable to hear (and potentially be

influenced by) others' responses to product acceptability, use and individual product ranking questions.

Qualitative tools

Complementary qualitative data were also collected on a third consecutive day of data collection in a series of 5 eight-person focus group discussions, composed of the women who had participated in the previous days' product testing. Women were grouped together roughly by age (younger women together and older women together) to reduce potential age-related impediments to free expression [183]. A structured framework was used to elicit contextual data relevant to women's general perspectives on product use and dietary practices. The framework included questions related to factors that might influence acceptability and consumption of flavor profiles, as well as sharing dynamics, local food practices and potential supplement use. The focus groups, which were audio recorded, were conducted with one moderator (a trained sociologist) and one note taker who supported with additional observational notes that would not be captured through the audio recording.

An additional ranking exercise was included in the focus group discussion to elicit further narratives around characteristics affecting the potential use of the products and how those characteristics related to each other. Participants were then asked to discuss and reach consensus on their top three products as a group.

Data analysis

The 7-point scale used for quantification of product acceptability and perceptions was treated as a continuous variable [184]. The mean (± SD) was calculated for the hedonic characteristics of acceptability, perception of product use and willingness to use for 12 months. Amount of money willing to pay and perception of portion size were recorded as categorical variables and displayed in numbers and relative percentages. CSPro data files were exported to Stata (v. 14.2, StataCorp, USA) for statistical analysis.

In order to analyze the product ranking data, a product was awarded three points every time it was ranked first, two points every time it was ranked second and one point every

time it was ranked third. If a product was not included in the top three, it received zero points. The points for each product were summed. For the individual 'top three' rankings, the maximum possible score was therefore 120 points (40 participants × 3 points maximum) and the minimum was zero (for a product that was never ranked in the 'top three'). For the focus group rankings, the maximum possible score was 15 points (five focus groups × 3 points maximum) and the minimum was again zero.

Qualitative data were analyzed using thematic analysis. This approach allows for the systematic identification and analysis of patterns and themes within a dataset [185]. Dominant, recurring themes were identified through the review of transcripts and field notes, and a thematic framework was iteratively developed. Salient concepts were then coded by hand and/or using Dedoose (v. 8.2.32, SocioCultural Research Consultants LLC, USA) and cross-referenced by the research team for quality assurance. The emerging trends were critically analyzed to ensure the emerging themes were relevant to the research objectives [186]: to assess which product types and varieties were preferred and why, what factors affected women's choice of preferred products, how those products would be incorporated into the local diet, the acceptability of snacking and sharing and the acceptability of at-home consumption of products. The quantitative and qualitative results were then compared and integrated with the final analysis.

Results

Key demographic data for the 40 study participants are presented in **Table 3.2**. The mean age was 25.4 year, with a mean gestational age of 5.2 months. Nearly all (95%, n = 38) were married, and 26 (65%) had never attended school.

Table 3.2. Demographic characteristics of study participants.

Characteristics of pregnant women (n = 40)	
Age (mean ± SD)	25.4 ± 4.7
Matrimonial status, n (%)	
Married	38 (95%)
Not married	2 (5%)
School attendance, n (%)	
None	26 (65%)
Primary	11 (27.5%)
Secondary	3 (7.5%)
Higher education	0 (0%)
Household size, number of people (mean ± SD)	8.7 ± 5.0
Religion, n (%)	
Christian	21 (52.5%)
Muslim	14 (35%)
Animist	5 (12.5%)
Gestational age in months (mean ± SD)	5.2 ± 1.9
First pregnancy, n (%)	7 (17.5%)
Number of children (mean ± SD)	1.9 ± 1.4
Number of pregnancy consultations (mean ± SD)	1.8 ± 1.3

Measures of overall preference

Detailed results of the product acceptability and the ranking of individual product characteristics are presented in **Table 3.3** (sweet product grouping) and **Table 3.4** (savory product grouping). **Table 3.5** presents (1) the results of the individual product ranking activity, (2) the results of the group product ranking activity and (3) the mean individual product acceptability score for the top five products along any of those three metrics.

Table 3.3 Hedonic testing, acceptability of sweet products, mean (SD), n (%).

	Sweet lipid-based spread	Vanilla biscuits	Filled sticks	Vanilla drink	Fermented drink	Sweet bar	Unseasoned pillows
Product consumption, mean (SD)							
Net weight consumed (g)	24.6 (0.9)	17.7 (0.8)[b]	24.6 (0.7)	67.0 (4.9)	63.5 (14.6)	15.4 (3.6)	15.2 (4.2)
Proportion of test portion consumed (%)[a]	98.4	98.3	98.4	95.7	90.7	96.3	89.4
Duration of consumption (min)	3.6 (1.6)	4.0 (1.4)	4.5 (1.4)	3.0 (3.0)	3.8 (4.1)	5.6 (4.5)	7.7 (5.5)
Appreciation of product (1= dislike very much to 7= Like very much), mean (SD)							
Color	6.7 (0.5)	6.5 (0.7)	6.5 (0.6)	6.3 (1.2)	6.3 (1.1)	6.0 (1.3)	6.2 (1.2)
Taste	6.5 (0.9)	6.6 (0.6)	6.6 (0.5)	6.3 (1.1)	5.9 (1.5)	6.2 (1.0)	5.8 (1.6)
Texture/consistency	6.4 (0.8)	6.4 (0.7)	6.2 (1.0)	6.2 (1.2)	5.9 (1.6)	5.8 (1.2)	5.8 (1.5)
Smell	6.2 (1.0)	6.3 (0.9)	6.2 (0.9)	6.0 (1.4)	6.2 (1.2)	5.5 (1.6)	5.5 (1.6)
Overall appreciation	6.5 (0.7)	6.4 (0.7)	6.4 (0.7)	6.1 (1.1)	6.0 (1.4)	5.8 (1.1)	5.7 (1.4)
Perceived child likeability	6.6 (0.6)	6.7 (0.5)	6.5 (0.6)	6.3 (0.9)	6.2 (1.2)	6.1 (1.0)	5.7 (1.2)
Perceived adult likeability	6.3 (0.7)	6.3 (0.8)	6.1 (0.8)	6.1 (0.9)	6.0 (1.2)	5.9 (1.0)	5.8 (1.1)
Perception of product use (1= not at all in agreement to 7= very in agreement), mean (SD)							
Product is convenient to eat	6.3 (0.9)	6.5 (0.7)	6.1 (1.0)	6.2 (1.1)	5.9 (1.5)	5.8 (1.5)	5.7 (1.6)
Product is convenient to eat between meals	6.4 (0.7)	6.6 (0.5)	6.3 (1.0)	6.2 (1.3)	6.0 (1.5)	6.2 (1.1)	5.8 (1.5)
Product is medicine	5.4 (1.8)	5.5 (1.8)	5.5 (1.7)	5.4 (1.8)	5.4 (1.8)	5.5 (1.8)	5.3 (1.7)
Feel full after full portion	5.0 (1.8)	5.1 (2.1)	5.3 (1.7)	4.9 (1.9)	4.7 (2.1)	5.3 (1.7)	5.1 (1.8)
Would share with others	3.4 (2.3)	3.4 (2.2)	3.5 (2.3)	3.4 (2.2)	3.5 (2.2)	3.4 (2.1)	3.6 (2.3)
Willingness to use daily for 12 months (1= not at all in agreement to 7= very in agreement), mean (SD)							
Would use daily if provided	6.3 (1.0)	6.4 (0.8)	6.2 (1.2)	6.0 (1.5)	6.0 (1.6)	5.6 (1.5)	5.7 (1.7)
Would use daily if purchased	5.8 (1.4)	5.8 (1.3)	5.6 (1.5)	5.5 (1.8)	5.5 (1.9)	5.2 (1.8)	5.1 (2.0)
Amount willing to pay, n (%)							
Would pay how much (CFA)							
1-100	23 (57.5%)	21 (52.5%)	21 (52.5%)	21 (52.5%)	22 (55%)	23 (57.5%)	24 (60%)
101-200	11 (27.5%)	12 (30%)	12 (30%)	11 (27.5%)	11 (27.5%)	11 (27.5%)	8 (20%)
201-300	4 (10%)	3 (7.5%)	4 (10%)	2 (5%)	1 (2.5%)	2 (5.0%)	1 (2.5%)
301-400	1 (2.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.5%)	1 (2.5%)
401-500	0 (0%)	2 (5%)	1 (2.5%)	5 (12.5%)	3 (7.5%)	2 (5.0%)	5 (12.5%)
> 500	1 (2.5%)	2 (5%)	2 (5%)	1 (2.5%)	3 (7.5%)	1 (2.5%)	1 (2.5%)
Acceptability of portion size (for a snack), n (%)							
Portion size is acceptable	39 (97.5%)	34 (85%)	37 (92.5%)	38 (95%)	34 (85%)	34 (85%)	37 (92.5%)
Too small	1 (2.5 %)	5 (12.5%)	1 (2.5%)	2 (5%)	5 (12.5%)	3 (7.5%)	0 (0%)
Too big	0 (0%)	1 (2.5%)	2 (5%)	0 (0%)	1 (2.5%)	3 (7.5%)	3(7.5%)

[a] net weight consumed/sample weight X 100; [b] n=38 for weight/duration of consumption for the vanilla biscuits. SD, standard deviation.

Table 3.4 Hedonic testing, acceptability of savory products, mean (SD), n (%).

	Savory lipid-based spread	Chicken soup	Bar	Biscuits	Seasoned pillows
Product consumption, mean (SD)					
Net weight consumed (g)	22.9 (5.7)	56.8 (22.6)	14.1 (4.7)	13.1 (5.6)	13.3 (6.0)
Proportion of test portion consumed (%)[a]	91.6	81.1	88.1	77.1	78.2
Duration of consumption (min)	5.8 (5.2)	6.8 (6.9)	8.3 (6.6)	9.4 (6.9)	10.5 (6.7)
Appreciation of product (1= dislike very much to 7= Like very much), mean (SD)					
Color	6.4 (1.0)	6.0 (1.5)	6.0 (1.2)	6.2 (1.4)	6.0 (1.6)
Taste	6.1 (1.4)	5.7 (1.7)	5.5 (1.6)	5.2 (1.7)	4.8 (2.1)
Texture/consistency	6.0 (1.3)	5.5 (1.8)	5.3 (1.5)	5.2 (1.8)	5.0 (2.1)
Smell	5.7 (1.8)	5.1 (2.2)	5.0 (2.0)	4.9 (2.1)	4.5 (2.4)
Overall appreciation	5.9 (1.5)	5.5 (1.8)	5.3 (1.8)	5.0 (2.1)	5.0 (2.2)
Perceived child likeability	6.2 (1.1)	5.8 (1.4)	6.1 (1.0)	5.9 (1.3)	5.7 (1.7)
Perceived adult likeability	6.0 (1.1)	5.8 (1.2)	5.8 (1.0)	5.5 (1.6)	5.3 (1.8)
Perception of product use (1= not at all in agreement to 7= very in agreement), mean (SD)					
Product is convenient to eat	6.1 (1.1)	5.8 (1.4)	5.6 (1.5)	5.7 (1.6)	5.5 (1.9)
Product is convenient to eat between meals	6.3 (1.2)	6.0 (1.5)	6.0 (1.4)	5.9 (1.6)	5.4 (1.8)
Product is medicine	5.5 (1.7)	5.5 (1.8)	5.2 (1.8)	5.4 (1.7)	5.1 (1.8)
Feel full after full portion	5.2 (1.6)	5.1 (1.7)	5.2 (1.6)	5.0 (2.0)	5.5 (1.7)
Would share with others	3.4 (2.2)	3.4 (2.2)	3.2 (2.2)	3.7 (2.3)	3.7 (2.4)
Willingness to use daily for 12 months (1= not at all in agreement to 7= very in agreement), mean (SD)					
Would use daily if provided	5.9 (1.4)	5.5 (1.9)	5.7 (1.6)	5.5 (1.9)	4.9 (2.2)
Would use daily if purchased	5.5 (1.6)	5.1 (2.1)	5.1 (2.0)	5.0 (1.8)	4.5 (2.3)
Amount willing to pay, n (%)					
Would pay how much (CFA)					
0	0 (0%)	0 (0%)	1 (2.5%)	2 (5%)	1 (2.5%)
1-100	18 (45%)	15 (37.5%)	22 (55%)	17 (42.5%)	14 (35%)
101-200	11 (27.5%)	15 (37.5%)	7 (17.5%)	13 (32.5%)	14 (35%)
201-300	4 (10%)	3 (7.5%)	2 (5%)	5 (12.5%)	6 (15%)
301-400	0 (0%)	0 (0%)	1 (2.5%)	0 (0%)	0 (0%)
401-500	4 (10%)	3 (7.5%)	4 (10%)	2 (5%)	2 (5%)
> 500	3 (7.5%)	4 (10%)	3 (7.5%)	1 (2.5%)	3 (7.5%)
Size of portion (for a snack or portion), n (%)					
Portion size is acceptable	35 (87.5%)	32 (80%)	33 (82.5%)	29 (74.3%)	27 (67.5%)
Too small	4 (10%)	4 (10%)	2 (5%)	3 (7.7%)	3 (7.5%)
Too big	1 (2.5%)	4 (10%)	5 (12.5%)	7 (18%)	10 (25%)

[a] net weight consumed/sample weight x 100. SD, standard deviation.

Table 3.5 Top 5 products across three primary metrics.

	Vanilla biscuits	Sweet lipid-based spread	Fermented drink	Vanilla drink	Filled sticks	Unseasoned pillows
Individual product ranking (points)	1 (55)	2 (50)	3 (39)	4 (33)	5 (26)	9 (5)
Group ranking (points)	2 (6)	1 (12)	4 (5)	N/A	2 (6)	5 (1)
Product acceptability (mean score, 7-point scale)	2 (6.4)	1 (6.5)	5 (6.0)	4 (6.1)	2 (6.4)	7 (5.7)

Sweet product preferences

The quantitative results suggested that participants strongly favored products they perceived as sweet. The sweet lipid-based paste and the vanilla biscuit were the top two products according to all three measurements, and the fermented drink, vanilla drink and filled sticks (all sweet products) were consistently in either third, fourth or fifth place. No savory product was ranked in the top five for any of these three measures.

Qualitative data gathered during the focus group discussions corroborated the quantitative findings regarding participants' preference for sweet products, and this preference was most apparent during direct comparisons between sweet and savory versions of the same products. When asked specifically to compare the sweet versus the savory bar, biscuit and lipid-based paste, the women were virtually unanimous in expressing a preference for the sweet versions of each product. During focus group discussions, women frequently commented on a product's sweet taste as one of its most favorable aspects, and they often said they disliked salty tastes and related products.

Data from focus group discussions also confirmed the women's specific preference for the sweet lipid-based paste and the vanilla biscuit. Women commented favorably on the paste's sweet taste ("When you put it in your mouth, its sweet and there is a good smell") and its 'milky' taste ("I like it, it's as though they've put milk inside"). In contrast to many of the other products, a large number of participants spoke positively about the smell of the lipid-based paste, whereas others commented favorably on its color or texture. The majority of participants said that the product was very good as currently formulated and had no changes to suggest. As one woman concluded, "When I eat it, I like the smell, the

taste, I like everything in this product". Comments regarding the vanilla biscuit were similar, although a small minority of women reported disliking the smell: One suggested "It's good when we eat it, but it contains an odor that I do not like" and another confirmed "It's as if we have put in garlic". Nonetheless, the overall response to the vanilla biscuits was positive, as many women noted, "I like everything about it".

Product odor

Odor was a particularly relevant factor for product preference and impacted several women's ability to tolerate a product. Several participants said that the smell and/or taste of certain products made them nauseous. For every product except one (the fermented drink), odor was the lowest mean score for a hedonic characteristic. Odor was also the only characteristic for which some products received scores of less than 5.0 out of 7.0. Many women raised the smell of a product as the reason why they disliked or could not eat several products, notably many of the savory products. For example, one focus group participant said of the seasoned pillows: "The odor makes it so that I can't even perceive the taste". In one focus group, participants discussed a pregnant woman's sensitivity to smell: "The smells that pregnant women smell other adults do not smell".

Familiarity

Focus group discussions revealed a positive correlation between the resemblance of a supplement to a known product and the appreciation the women had for that supplement. Some participants mentioned associations between products and specific local foods (such as couscous, porridge and others) or familiar ingredients or flavors (such as milk and peanut). In all these cases, the resemblance of products to favored familiar foods was perceived as a positive influence on their opinion of a study product.

Use during pregnancy

Women expressed an intention to use all of the tested products during pregnancy, provided they were able to tolerate them. Some women said that they simply could not eat one or more of the products. For example, although the savory version of the lipid-based paste was the highest-ranking product in the savory product testing, some women

reacted negatively to its smell, taste or texture. As one focus group participant put it, "It's good, but it's the salty taste that makes it so that I can't eat it". Another said, "When you eat it, it stays in your throat, it doesn't go down".

Participants repeatedly referred to the products' health benefits as a driver of their intention to use them during pregnancy and intended to eat the products even if they disliked them. One woman stated regarding the chicken soup, "If I know … that it can have benefits for my health I'm going to do everything [I can] to drink it". Another participant said she disliked the savory bar, but that she would still eat it: "I'll manage because it's a medicine". Although many women expressed dislike for the saltiness and odor of the savory biscuit, they generally agreed that they would eat it daily for the benefit of their baby. As one woman concluded: "A medicine can't always have a good taste".

Often women did not raise hedonic characteristics such as the taste or smell of the product as a factor influencing use or mentioned them only secondarily. In one focus group discussion, for example, only one of all the women who liked the product mentioned taste as a driver of her willingness to use it:

> Participant 6: Because it's a medicine, I will eat it during my pregnancy.
>
> Participant 4: I will eat it during my pregnancy because it's like a vitamin.
>
> Participant 7: I will eat it during my pregnancy because it will take care of my baby.
>
> Participant 1: For me, it's because it has a good taste.
>
> Participant 2: What will make me eat it during my pregnancy is that it will take care of my baby and make it strong.
>
> Participant 3: I will eat it during my pregnancy because it will make the baby in my belly grow.

With regard to the top two products, women consistently reported that they would eat both the lipid-based paste and the vanilla biscuit throughout pregnancy, although their reasons differed: For some women, daily consumption in pregnancy was directly linked to taste, whereas others emphasized that the products were perceived to be a medicine with health benefits.

Participants expressed a willingness to use both products daily during pregnancy even if they had to pay for it. The mean score for willingness to use the vanilla biscuit daily if provided for free was 6.4 (SD = 0.8); for willingness to use daily if they had to pay for it, the

mean score was 5.8 (SD = 1.3). Both of these were the highest scores received by any sweet product (if they had to pay, the vanilla biscuit was tied with the sweet lipid-based paste).

Ease of use

Participants were asked to evaluate on the 7-point scale the extent to which they agreed or disagreed that the products were easy to eat. The vanilla biscuit's score was higher than that of any other product at 6.5 (SD = 0.7). When discussed during focus groups, ease of consumption was found to relate specifically to the association of the vanilla biscuit with familiar products and to the health benefits perceived by the participants. For example, where all women agreed that the vanilla biscuit would be easy to consume, one participant explained, "It would be easy to eat because it resembles a biscuit and there are vitamins in it. It's good to eat". Participants also uniformly viewed the lipid-based paste as easy to use and to carry, including for consumption away from home.

Participants also associated a product's health benefits with ease of preparation and ease of use at home and elsewhere. When asked about why the product was easy to use or prepare, a recurring response was that it was easy because of its taste or its health properties. For the lipid-based paste, which requires no preparation and was well liked by participants, many women referred to the product's health benefits rather than ease of preparation or likeability as the main driver influencing their consumption. However, even products that required preparation (such as the vanilla drink and chicken soup) were characterized as easy to prepare and use at home and outside the home.

Sharing practices

The quantitative data demonstrated little variation in the scores regarding likelihood of sharing across the sweet products, with all scores ranging between 3.4 and 3.6 (where 1 indicates strong disagreement that they were likely to share and 7 indicates strong agreement). For the savory products, likelihood of sharing ranged from 3.2 (for the bar) to 3.7 (for the biscuit and seasoned pillows).

Focus group questions on sharing focused on both the perceived expectation to share and the likelihood of sharing. Despite widespread reference to household members' expectations that the pregnant woman would share her food, a majority of women reported

that they would not share the supplements. Reasons cited for why included “because it's reserved for pregnant women”, “because ‘it's not a normal food” and “because it has vitamins” for pregnant women.

A minority of women said that they would share the product with others, particularly with other pregnant women or with children. There was also some indication in the qualitative data that participants might be more likely to share products they disliked; as one woman concluded, “Because I don't like it, if nevertheless someone wanted it, I would give it to them”.

A number of women anticipated having to hide the lipid-based paste from children in order to avoid sharing. A participant described the intra-household sharing dynamic that she would face, explaining, “If it's me, you can't hide even if you're at home. There are people who if they see you eat, they will of course want it. There will be some who understand, they see the ‘burden’ that you carry [i.e. that you are pregnant], and at that time they'll think of that. But there are some who won't think, if they see you eat they'll think that it's for pleasure and they'll ask you. For me, that is the situation”. There was consensus among participants that they would not be expected to reduce their share of the household's food as a result of having the supplements. The following statement was representative: “It's not going to make me lose my share of family food because we know that it's because of the pregnancy that we were given [the vanilla biscuit]”.

Discussion

The mixed methods approach used in this study revealed a number of factors significant to product acceptability and use. The quantitative data provided insight into factors affecting product preference such as hedonic characteristics and factors relating to future use. The qualitative data provided valuable contextual data about the reasons for preferences and the factors affecting future use. Key themes were perceived sweetness, odor and resemblance to familiar products as factors influencing product preference, perceptions of health benefits as a driver of use and sharing as a concern for future monitoring and sensitization.

Factors influencing product acceptability

Research has shown that humans are attracted to sweet tastes, although this varies significantly among individuals as a result of factors such as age, race/ethnicity, nutritional deficiencies and more [187,188]. Preferred tastes may also vary during pregnancy and may change depending on stage of pregnancy; an increased preference for savory foods has been observed, for example, during the second and third trimesters of pregnancy [188]. Women in this study (mean gestational age, 5.3 months [SD = 1.9] had a strong preference for those products they perceived as sweet (compared with savory/spicy versions of the tested products.

Odor also proved to be an important factor in the present study. Physiologically, smell is closely linked to taste; increased olfaction during pregnancy has been documented, although the evidence is mostly anecdotal and conflicting [189]. In this study, sensitivity to smell appeared to be an important factor leading women to dislike or resist several products. Developers of nutritional supplements should thus be aware that it is important to test the hedonic properties, including odor, of any products in order to ensure acceptance of future users.

Finally, the familiarity of supplemental food flavors has been observed as a facilitator to product use [190]. As noted, several of the products developed for this study used locally familiar flavorings or ingredients. These products (mango flavored and tomato–onion flavored) were not among the most preferred by participants. However, other familiar flavors were singled out as influencing women's preference for certain products, such as the milk and peanut flavors identified in the top products. This points to the importance of hedonic testing and qualitative assessments of products and/or flavors that may be developed for use in the future.

Health benefits and product use and preferences

Previous studies have noted that perceived health benefits may influence acceptability and adherence in the context of maternal nutritional supplements [168,191]. Our analysis similarly revealed that participants valued the products' benefits for themselves and their unborn children. Women viewed the products as medicine more than food, and in focus groups, women indicated that this view was a significant factor in predicting product use.

Perceptions on sharing

Sharing has been widely recognized as a potential impediment to adherence in the context of nutritional supplements for children and adults [190,192–194], and cultural expectations around food sharing can impact supplement use [190]. Findings from other studies have indicated that the estimated energy intake from supplements was at times lower than anticipated when sharing with other family members appeared to be the norm [195]. Sharing dynamics were therefore considered crucial for understanding the pregnant woman's perception of use of a product, particularly when likelihood to share would affect daily consumption. The results here indicate widespread expectations that a pregnant woman will share her food, especially with children, and a degree of pressure on her to do so. Although most women reported that they would not share the supplements, a minority of women said that they might do so. Sharing behavior should thus be closely monitored, and additional data on sharing and expectations of sharing should be collected during the next phase of the study. Package design might also clearly state that the product is exclusively for pregnant women, in order to discourage sharing.

Study strengths and limitations

The present study provided valuable information for the remaining aspects of the overall study as well as for future product development. Study strengths included the consistency in findings across qualitative and quantitative data and the rich contextual information obtained to further explain quantitative results.

As is an inherent risk in rapid data collection, it is possible that participants expressed answers they perceived to be appropriate or socially desirable. Participants were encouraged to speak openly and honestly, and the frank and sincere dialogue elicited from participant discussions suggested that such bias was minimized. Findings were also triangulated across participant groups and with quantitative data to test the validity of answers.

Because of the volume of products, interactions with participants were sometimes quite long; despite the efforts of the facilitators, levels of participation engagement declined as the discussion continued. Where possible, focus group discussions were held in the morning to overcome issues of tiredness and fatigue.

It is important to acknowledge that the product preferences, and the women's intentions regarding future use and sharing, were expressed in the context of a single-meal rapid assessment of the 12 products. Further evaluation during the next study (the second part of phase 1), where the two most preferred products will be used at home for 10 weeks, will provide rich information regarding the longer-term acceptability of these products, as well as patterns of consumption and sharing within the family.

Conclusion

Women in this study had strong and relatively consistent opinions regarding product preference, clearly favoring products they perceived as sweet. The sweet lipid-based paste and the vanilla biscuit were best liked overall. Women also favored products that bore a resemblance to familiar, well-liked foods. Participants' ability to tolerate products as well as product preferences appear to be driven in part by their perceptions of the odor of the products. Results also support the notion that hedonic properties, rather than convenience, will influence women's perceptions of how easy a product is to use at home and away. Nonetheless, it emerged from both the quantitative and the qualitative data that women intend to eat any product they can tolerate – regardless of how much they like it – because of the perceived benefits for their unborn child. Information regarding women's intentions to share the selected products was mixed. Quantitative data suggested that women were somewhat unlikely to share any supplement they are provided in the future. Qualitative data suggest that women might be more likely to share with their children or other pregnant women or if they disliked the product. Women did express an understanding that the product was intended only for their use and an appreciation that it is to be considered a medicine, which also influenced expressed sharing intentions.

The results indicate that it may be advisable to focus future product development, and particularly the development of new flavor profiles, on favored local tastes. In addition, a focus on minimizing strong odors may be important for future prenatal product development. Understanding the challenges to targeting product use to pregnant women is essential for future use and distribution of nutritional supplements. This study demonstrates that potential sharing behaviors should be monitored and addressed in future parts of the MISAME-III project.

4

Acceptability of two fortified balanced energy-protein supplements among pregnant women in rural Burkina Faso: A mixed methods study

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Acceptability of two fortified balanced energy-protein supplements among pregnant women in rural Burkina Faso: A mixed methods study

Abstract

Background: Balanced energy protein (BEP) supplementation for pregnant and lactating women in low- and middle-income countries is a promising strategy to improve birth outcomes and child growth. The objective of this study was to assess and compare the acceptability of new formulations of two fortified BEP supplements, a lipid-based peanut paste and a vanilla biscuit, among 80 pregnant women in rural Burkina Faso, prior to an efficacy trial.

Methods: A 10-week individually randomized cross-over study was designed, in which women received a weekly supply of each supplement for 4 weeks, and a daily choice between the supplements in the last 2 weeks. Questionnaires to assess daily consumption and supplement acceptability (n = 80) and home observations (n = 20) were combined with focus group discussions (n = 6) and in-depth interviews with women (n = 80) and stakeholders (n = 24).

Results: Quantitative findings indicated high adherence (>99.6%) and high overall appreciation (score >6 out of 7) of both supplements. The assessment of preferred choice in weeks 9 and 10 indicated a slight preference for the vanilla biscuit. Qualitative findings indicated that perceived health benefits, support from household members and educational messages from health professionals were important drivers for acceptance and adherence. Sharing was not often reported but was identified during interviews as a possible risk.

Conclusion: Both the lipid-based peanut paste and vanilla biscuit were acceptable and likely to be well used in the future. Emphasis on the health benefits, informing and engaging family members, and carefully monitor potential sharing remain important factors to support acceptance and adherence.

Key messages

- To select the most optimal BEP supplement for administration in the efficacy trial, a 10-week home feeding trial was designed to assess the acceptability and utilization of two BEP supplements by pregnant women in Burkina Faso.
- The findings illustrate that both the lipid-based peanut paste and the vanilla biscuit were well accepted. Providing information on the use and benefits of food supplements by health care professionals, and engaging community leaders and family members appeared to be important promoting factors.
- For implementation in the trial, the research team finally decided to select the lipid-based peanut paste based on the fact that the supplement was well-accepted, the product is stable (no oxidation of vitamins), shelf life is guaranteed for a long period and the supplement production scheduling aligned with the project timeline.

Introduction

Low birth weight and small-for-gestational age affect an estimated 20 to 30 million infants every year [139,196]. These adverse birth outcomes lead to an increased risk of neonatal and infant mortality and can also have long-term consequences by increasing the risk of developing metabolic syndrome [41,52,197,198]. It is well known that poor maternal nutritional status can contribute to this restricted fetal growth (approximated by LBW and SGA) and is a major problem in many low-income countries [26,158,199].

To improve nutritional status, the WHO recommends BEP dietary supplementation during pregnancy in undernourished populations to reduce the risk of stillbirths and SGA newborns [108]. Although recent studies have demonstrated a positive effect of BEP supplements in pregnant women on birth outcomes, authors highlight the limited amount of available evidence and the need to evaluate the effect of this balanced supplement by large, well-designed randomized trials [100,130,144]. Such studies will help to generate more robust evidence on the impact to guide recommendations and decision making. For this reason, the MISAME-III study was designed. The study is a RCT to assess the effect of a fortified BEP supplement for pregnant and lactating women on birth and child health outcomes (ClinicalTrials Identifier: NCT03533712).

The results of the efficacy trial will depend to a large extent on the optimal use of the BEP supplements. In comparison with FBFs for pregnant and lactating women, BEP supplements provide the advantage that there is no preparation required. Although FBFs are often regarded as family foods, little is known about what type of ready-to-use product(s) pregnant and lactating women appreciate and how they are used. Understanding the factors that determine the acceptability of BEP supplements is however crucial to reduce the risk of poor adherence [166,176,191]. Hence, prior to testing the efficacy in the main trial, a formative study to assess acceptability and utilization of BEP supplements was designed. Twelve supplements of different types and flavors were rapidly assessed in terms of short-term acceptability using a single-meal test to inform the present study [147].

Here, we evaluate the two highest-ranking supplements from the meal test to assess (1) medium-term acceptability (defined for the present study as 4–6 weeks), (2) adherence during at-home consumption and (3) influencing factors for acceptability and adherence.

Materials and methods

BEP supplements

For this study, two forms of fortified BEP supplements were manufactured: a lipid-based peanut paste with a single serving of 72 g (389 kcal; 14.5 g protein) and a vanilla biscuit, served in a package of six biscuits, with a total portion size of 75 g (375 kcal; 16.5 g protein). Levels of folic acid and iron in the BEP supplements fall within the recommended intake range by WHO [108] and do not reach, even when combined with IFA tablets, the upper intake levels for folic acid.

Study design

The study followed a 2-arm cross-over design, testing the two BEP supplements for a period of 10 weeks (**Figure 4.1**). During the initial 8-week period, each of the two supplements were utilized for 4 weeks, and in the subsequent 2 weeks, a daily choice between the lipid-based peanut paste and vanilla biscuit was offered to all women. Women were individually randomized to determine which of the two supplements they

would use first, the lipid-based peanut paste (group 1) or the vanilla biscuit (group 2). A random allocation scheme was developed in Excel, and for each of the two health centers, 20 women were assigned to either group 1 or 2 by drawing an envelope containing the random group assignment.

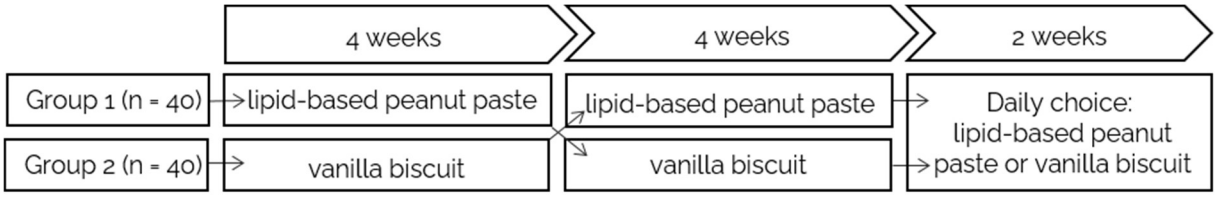

Figure 4.1 Study design.

Study area and participants

The study was conducted from December 2018 to March 2019 in Houndé, the district capital of the province of Tuy in the midwest area of Burkina Faso. Two health centers (Boni and Kari) were selected for this formative study on the basis of accessibility and are representative of the broader study area in terms of ethnicity and religion. A convenience sample of 80 pregnant women attending antenatal services at the selected health centers were invited to participate in collaboration with the health center officer-in-charge. Women had to be pregnant and aged between 15 and 40 for inclusion, which is in line with the inclusion criteria for the efficacy trial. Women were excluded if they reported an allergy to soy, dairy products, eggs, gluten or peanuts.

Data collection

A set of quantitative and qualitative research tools was developed to assess medium-term acceptability and consumption in the context of Burkina Faso. The French tools were translated into Mooré and Dioula with input from data collectors during a 2-week training and were refined based on feedback from the field research partner AFRICSanté. Prior to data collection, tools were pilot tested with pregnant women and revised for clarity prior to implementation.

Quantitative tools

Demographic questionnaire (n = 80)

An interview-based questionnaire was used to collect basic sociodemographic (age, school level, etc.) and obstetric history information.

Supplement acceptability questionnaire (n = 80)

Hedonic properties were assessed using a structured questionnaire at the end of week 4 and week 8. Women were asked to rate the supplement's color, taste, texture, smell and overall appreciation using a 7-point scale, ranging from 1 (dislike very much) to 7 (like very much). Subsequently, women were asked to respond to 11 statements regarding their perception of current and future supplement use during pregnancy. Responses were scaled from 1 (I don't agree at all) to 7 (I agree completely) when asked for their opinion. The scale was complemented with a range of emoticon faces (very unhappy to very happy), which has been used elsewhere to measure food acceptability in illiterate populations [172,174,200,201].

Daily consumption questionnaire (n = 80)

Women's consumption behavior, including supplement choice in weeks 9 and 10, was quantitatively assessed using a short multiple-choice questionnaire on a daily basis at home. Nine questionnaire items assessed the portion size consumed, timing, meal replacement and sharing. If the supplement was not consumed, the reason was registered.

Home observations (n = 20)

At-home consumption was directly observed twice among a random subsample of 20 women (10 per study arm) during weeks 3 and 4 and weeks 6 and 7. Participant activities were registered on paper using a standard set of codes every 5 min during 12-consecutive hours. All food-related activities (eating or drinking, preparing or cooking food, buying or storing food) and observations concerning the supplement during the 12-h observation period were described in more detail in an open text comment box. In case the participant left the house during this period, the home observer asked permission to follow her during the activities outside the household.

Qualitative tools

In-depth interviews (n = 80)

To identify factors influencing acceptability and consumption of the supplements, semi-structured interviews were held with women at four times over the course of the at-home tasting period: week 1 (initial interview), week 4 (supplement-specific interview 1), week 8 (supplement-specific interview 2) and week 10 (final interview with discussion of supplement choice). The interview guide contained general questions on household food practices and beliefs; diet during pregnancy and lactation, availability and access of supplements, and antenatal care-seeking behavior; and supplement-specific questions exploring attitudes and user experience (**Annex 3.1**).

In-depth interviews with stakeholders (n = 24)

To identify stakeholders' attitudes towards supplementation during pregnancy, interviews were held with family members (n = 8), health professionals (n = 8) and community leaders (n = 8) in week 6. Semi-structured interview guides were adapted to each stakeholder group.

Focus group discussions (n = 6)

To elicit additional information on women's experience with the BEP supplements and factors that influence acceptability and adherence, six focus group discussions (FGDs), each with eight women randomly selected from our study sample, were held using a semi-structured thematic guide in week 10 (**Annex 3.2**).

Procedures

Before the start of 10-week home feeding trial, women were visited at home to receive information about the study, as well as the risks, benefits and use of the supplements (**Annex 3.3**). The voluntary, confidential and anonymous nature of participation was emphasized. Informed consent was then obtained, and a sociodemographic questionnaire was administered. During the first 8 weeks, a 1-week supply of supplements was given to the pregnant women at home. Trained village-based project workers visited participants daily to complete the 'Daily Consumption Questionnaire' and collect the empty sachets.

During the last 2 weeks, the procedure was similar except that participants were given a daily choice to receive either the lipid-based peanut paste or vanilla biscuit. This daily choice was offered by the trained village-based project workers who had a supply of both products with them at each home visit. In a subsample, home observations were conducted from 6 AM to 6 PM by teams of two data collectors, each observing for a 6-h shift.

The interviews were held either at home (for pregnant women, family members and community leaders) or at the health center (for health professionals) and usually lasted no more than 60 min. The FGDs were held at the health center, led by one facilitator assisted by a note taker and lasted approximately 2-2.5 h. All interviews and FGDs were audio recorded and transcribed from local languages into French. In general, efforts were made to ensure the place used for data collection activities was as private and neutral as possible. The field team was familiar with the local context and languages use in each field site, and participants were encouraged to speak in the language in which they were most comfortable and confident.

Data analysis

Quantitative data were collected electronically using CSPro (v. 7.3.1, Census Bureau, USA). The CSPro data files were exported to Stata (v. 14.2, StataCorp LLC, USA) and R software for statistical analysis. Data are presented as median and interquartile range (Q1-Q3) or counts and percentages. The values of the 7-point scale used in the 'Supplement Acceptability Questionnaire' were treated as continuous variables [184]. Presence of a carryover effect due to the crossover study design was tested by computing carry-over, period, sequence and treatment effect using the pkcross Stata command. All registered activity codes from the home observation tool were copied to Excel and imported into R to calculate the counts and percentages.

For the interviews and FGDs, a framework with the most apparent themes was developed through systematic review of the transcripts [202]. In the next step, a codebook was developed in Dedoose analytic software (v. 8.2.32, SocioCultural Research Consultants LLC, USA), and all transcripts were imported for analysis by labelling phrases within the

transcripts. A selection of transcripts was reviewed by two separate researchers in order to cross-check codes and ensure consistency.

The results of the quantitative and qualitative methods were compared for a comprehensive analysis on the medium-term acceptability of the lipid-based peanut paste and vanilla biscuit. This allowed for triangulation of results on women's adherence, preference, contextual utilization and opinions on future use and messages.

Results

A total of 80 pregnant women participated in the 10-week home feeding trial, of which 28 women gave birth during the study period. All women completed the full study period. Demographic and socio-economic characteristics are presented in **Table 4.1**. In addition, eight health professionals, eight family members (four husbands, three mothers-in-law and one brother-in-law) and eight community leaders were interviewed.

Table 4.1 Characteristics of study participants.

Characteristics	**All** (n = 80)	**Group 1** (n = 40)	**Group 2** (n = 40)
Age (mean ± SD)	26.3 ± 6.0	26.3 ± 5.9	26.3 ± 6.1
Matrimonial status, n (%)			
Married	76 (95%)	38 (95%)	38 (95%)
Cohabitation	2 (2.5%)	2 (5%)	0 (0%)
Not married	2 (2.5%)	0 (0%)	2 (5%)
School attendance, n (%)			
None	52 (65%)	23 (57.5%)	29 (72.5%)
Primary	17 (21%)	11 (27.5%)	6 (15%)
Secondary	11 (14%)	6 (15%)	5 (12.5%)
Higher education	0 (0%)	0 (0%)	0 (0%)
Household size, number of people (mean ± SD)	6.2 ± 3.4	5.9 ± 3.9	6.5 ± 3.0
Household size, number of children <5 years old	1.2 ± 1.1	1.2 ± 1.0	1.2 ± 1.1
Village, n (%)			
Boni	40 (50%)	20 (50%)	20 (50%)
Kari	40 (50%)	20 (50%)	20 (50%)
Religion, n (%)			
Animist	34 (43%)	15 (37.5%)	19 (47.5%)
Christian	25 (31%)	14 (35%)	11 (27.5%)
Muslim	21 (26%)	11 (27.5%)	10 (25%)
Gestational age in months (mean ± SD)	5.4 ± 1.9[a]	5.5 ± 1.9	5.4 ± 2.0
First pregnancy, n (%)	18 (23%)	10 (25%)	8 (20%)
At least one previous fetal death, n (%)	11 (14%)	5 (12.5%)	6 (15%)
At least one previous child death, n (%)	15 (19%)	10 (25%)	5 (12.5%)
Number of children (mean ± SD)	1.9 ± 1.7	1.8 ± 1.8	2.0 ± 1.6
Number of children <5 years old	0.6 ± 0.6	0.7 ± 0.6	0.5 ± 0.5
Number of pregnancy consultations (mean ± SD)	1.5 ± 1.1	1.7 ± 1.1	1.3 ± 1.0

Note: eight women did not know their gestational age.

[a]Mean ± SD for 72 women.

SD, standard deviation.

The quantitative and qualitative research methods produced largely consistent and convergent results that both the lipid-based peanut paste and vanilla biscuit were well accepted. Results on the main study objectives (1) evaluation and preference, (2) adherence and (3) influencing factors of the BEP supplements are presented below.

Evaluation and preference

Both BEP supplements were positively evaluated. There was no difference in appreciation, convenience or intended daily use between the lipid-based peanut paste and vanilla biscuit after a consumption period of 4 weeks (**Table 4.2**). Both supplements scored ≥6 on a 7-point scale. A positive association between the supplements and other familiar foods such as peanuts, milk and chocolate appeared to influence both use and preference. Furthermore, participants gave a median score of 6 (I agree) out of 7 on the statement whether the supplements are a medicine (Table 2).

The final 2 weeks, where participants were offered a choice in supplements, revealed a slight preference for the vanilla biscuit over the lipid-based peanut paste. After having tried each product for 4 weeks, 60% of women indicated in the 'Supplement Acceptability Questionnaire' that they would opt for the vanilla biscuit during pregnancy and lactation if they were to continue after the 8-week test period. When the women were offered a daily choice between the two products in weeks 9 and 10, they also showed a preference for the vanilla biscuit, as it was chosen more often: 63% of all times (705/1120) versus 37% (415/1120) for the lipid-based peanut paste. The interviews and FGDs confirmed this small preference for the vanilla biscuit. Positive attributes of the vanilla biscuit were mainly the sweet taste and overall appreciation (I like it), whereas reasons for choosing the lipid-based peanut paste included a pleasant feeling in the mouth and easy-to-consume. A few women reported an aversion to the smell of the peanut paste as the reason to choose the vanilla biscuit.

Table 4.2 Acceptability of BEP supplements at week 4 and week 8.

	Lipid-based peanut paste			Vanilla biscuit		
	All (n = 80)	Group 1 (n = 40)	Group 2 (n = 40)	All (n = 80)	Group 1 (n = 40)	Group 2 (n = 40)
Appreciation of supplement (1 = I dislike it very much to 7 = I like it very much), median (Q1–Q3)						
Color	7 (6–7)	7 (7–7)	7 (6–7)	7 (6–7)	7 (6–7)	7 (7–7)
Taste	6 (6–7)	6 (6–7)	6 (6–7)	7 (6–7)	7 (6–7)	6.75 (7–7)
Texture/consistency	6 (6–7)	6 (5–7)	6.5 (6–7)	7 (6–7)	7 (6–7)	7 (6–7)
Smell	6 (3–7)	6 (3.75–7)	5.5 (2–7)	7 (6–7)	7 (6–7)	7 (7–7)
Overall appreciation	6 (5–7)	6 (5–7)	6 (5–7)	7 (6–7)	7 (6–7)	7 (6.75–7)
Perceived child likeability	7 (6–7)	7 (6–7)	7 (6–7)	7 (6–7)	7 (7–7)	6.5 (6–7)
Perceived adult likeability	6 (5–7)	6 (5–6.75)	6 (5–7)	6 (4–7)	6 (6–7)	6 (4–7)
Perception of supplement use (1 = I don't agree at all to 7 = I agree completely), median (Q1–Q3)						
Supplement is convenient to eat	6 (6–7)	6 (6–7)	6.5 (6–7)	7 (6.75–7)	7 (6–7)	7 (7–7)
Supplement is convenient to eat between meals	6 (5–7)	6 (5.75–7)	6 (4.75–7)	6 (5–7)	6 (6–7)	6 (5–7)
Supplement is medicine	6 (3.75–6)	5 (3–6)	6 (5–7)	6 (5–7)	6 (4.5–7)	7 (5–7)
Feel full after full portion	5 (3–6)	5 (3–6)	5 (3–6)	5 (3–6.25)	5 (3–6)	6 (5–7)
Would share with others	1 (1–2.25)	1 (1–2)	2 (1–3)	1 (1–3)	1 (1–2)	1 (1–3.25)
Willingness to use daily for 12 months (1 = I don't agree at all to 7 = I agree completely), median (Q1–Q3)						
Would use if provided	7 (6–7)	6.5 (6–7)	7 (6–7)	7 (6–7)	7 (6–7)	7 (6.75–7)
Would use if purchased	5 (3–7)	5 (3.75–7)	6 (3–7)	6 (4–7)	5.5 (5–7)	6 (3–7)
Would pay how much, per daily dose (CFA)a, n (%)						
0–100	47 (58.7)	24 (60)	23 (57.5)	58 (72.5)	30 (75)	28 (70)
101–200	16 (20)	8 (20)	8 (20)	13 (16.3)	7 (17.5)	6 (15)
201–300	3 (3.8)	1 (2.5)	2 (5)	5 (6.2)	1 (2.5)	4 (10)
301–400	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
401–500	6 (7.5)	5 (12.5)	1 (2.5)	2 (2.5)	2 (5)	0 (0)
>500	1 (1.3)	0 (0)	1 (2.5)	0 (0)	0 (0)	0 (0)
Do not know	7 (8.7)	2 (5)	5 (12.5)	2 (2.5	(0)	2 (5)
Acceptability of portion size (for a snack), n (%)						
Portion size is acceptable	75 (93.8)	37 (92.5)	38 (95)	72 (90)	37 (92.5)	35 (87.5)
Too small	1 (1.2)	1 (2.5)	0	3 (3.8)	2 (5)	1 (2.5)
Too big	4 (5)	2 (5)	2 (5)	5 (6.2)	1 (2.5)	4 (10)
Preference for continuation of consumption during pregnancy and breastfeeding at week 8, n (%)						
Choice of supplement	32 (40)	11 (27.5)	21 (52.5)	48 (60)	29 (72.5)	19 (47.5)

[a]The exchange rate for CFA during the study period was between 0.0017 and 0.0018 to 1 USD, retrieved from: www.OANDA.com.

Adherence

High adherence to both the lipid-based peanut paste (99.8% of all servings consumed) and vanilla biscuit (99.6% of all servings consumed) was recorded during the first 8 weeks, as well as during the choice period in weeks 9 and 10 (lipid-based peanut paste 100%; vanilla biscuit 99.9%). Only three women missed days of consumption. Of these, one woman

missed a period of 12 days because she gave birth. Two women missed a single day, one because she gave birth and one without indicating a reason. The full portion of both supplements was nearly always consumed. During the first 8 weeks, 95.8% of all portions of the lipid-based peanut paste were consumed in their entirety. In the last 2 weeks (when women specifically chose the lipid-based peanut paste), this was 99.8%. For the vanilla biscuit, this was 99.6% during the first 8 weeks and 100% during the last 2 weeks. Generally, the supplement was consumed in a single sitting (lipid-based peanut paste 79%; vanilla biscuit 77%) and in 2/3 of all cases it was consumed as a morning snack. No sequence nor period effect was observed for the cross-over model, indicating that there was no difference in portion consumed by temporal order of supplement use.

The home observations corroborated the findings on adherence for both supplements. Notes in the open text comment boxes of the home observation tool showed that women consumed the full portion of the supplement in the morning. Results from the interviews and FGDs illustrated factors that could explain this high adherence. Participants commented favorably on portion size, taste and perceived health benefits:

Interviewer: In-depth interview 2, HC Boni, participant 20:	"Why did you eat the full portion every day?" [lipid-based peanut paste]: "I find it the right amount and that is the reason I can finish the package every day."
FGD 1, HC Boni, participant 8:	[vanilla biscuit] "I still love it now like in the beginning because it's good."
FGD 4, HC Kari, participant 10:	[lipid-based peanut paste] "I consume it because it has been said that it is food for pregnant women, so I know that if I consume it, it will improve my body. That is the reason I consume it every day."

Adherence intention

Quantitative results showed high willingness to continue consumption of the food supplements during pregnancy and lactation with a median score of 7 (I agree completely) (**Table 4.2**). Similar results were found in the interviews and FGDs. Only five women specifically said they would find it difficult to continue supplementation throughout their pregnancy and 6 months after delivery because they did not like the supplement.

Factors influencing acceptability and adherence

All study tools, in particular the interviews and FGDs provided information on facilitating factors and barriers for acceptability and adherence: (1) dietary practices and beliefs for pregnant women, (2) perceived health benefits, (3) support from stakeholders, (4) sharing of the supplement, (5) educational messages from health professionals and (6) supplement choice. The combined data is presented per theme below.

Dietary practices and beliefs for pregnant women

Women and other stakeholders uniformly recognized the importance of a healthy diet to ensure the well-being of a pregnant woman and her fetus. Participants agreed that the quantity of food consumed during pregnancy and lactation should be increased. However, no other changes in the diet, such as intentional reduction of dietary intake to reduce the perceived risk of delivery complications due to large babies, were reported that could negatively influence the acceptance of BEP supplements. This was supported by experiences during the 10-week at-home consumption. Pregnant women and other stakeholders reported that the supplement was generally taken in addition to the normal diet and did not replace all or part of a meal. However, a small number of reports indicated that women were at times too full after having eaten the supplement to eat either their normal diet or additional snacks. This was more consistent in the context of the lipid-based peanut paste rather than the vanilla biscuit.

Perceived health benefits

Feeling good, stronger, healthier; having an improved appearance; and increased appetite were benefits of consuming the supplements identified by pregnant women in this formative study:

FGD 3, HC Boni, participant 7: "When you eat it every day it makes you shine and your child also shines, he is strong and healthy."

FGD 4, HC Kari, participant 14: "It improved my body posture, it boosted my appetite."

The perceived benefits for mother and child also appeared the main reason for their intention to continue supplementation:

In-depth interview 2,
HC Kari, participant 11: "I would continue to consume it to be healthy and for my child to be healthy."

FGD 4, HC Kari, participant 14: "Because by consuming it, it will stimulate the breast milk and if the child sucks, it will have vitamins."

Support from stakeholders

Family members, community leaders and health professionals expressed their willingness to support the use of the supplements for pregnant women and to encourage heads of households to permit use. The benefits of the supplement for mother and child and the recommendation by health professionals were the most important reasons to provide support. The pregnant women suggested that community leaders and family members, in particular the head of household and/or husband, should be engaged when introducing food supplements as they play an important role in the understanding and acceptance of the supplement.

Sharing of the supplement

Data captured by the quantitative questionnaire indicated that women's intention to share was very low for both supplements (mean score of 1 (1–3) out of 7, **Table 4.2**). This finding was confirmed by the home observations in which no sharing was registered (**Table 4.3**).

Table 4.3 Food related activities during 12-h home observation (total number of observations.

	Lipid-based peanut paste			**Vanilla biscuit**		
	All (n = 40)	Group 1 (n = 20)	Group 2 (n = 20)	All (n = 40)	Group 1 (n = 20)	Group 2 (n = 20)
Food related activities, n (%)						
Eating	299 (5.2)	157 (5.5)	142 (4.9)	319 (5.5)	174 (6.0)	145 (5.0)
Drinking	41 (0.7)	20 (0.7)	21 (0.7)	31 (0.5)	20 (0.7)	11 (0.4)
Preparing or cooking food	507 (8.8)	276 (9.6)	231 (8.0)	511 (8.9)	254 (8.8)	257 (8.9)
Purchasing food	2 (0.0)	2 (0.1)	0 (0.1)	3 (0.1)	2 (0.1)	1 (0.0)
Storing food	10 (0.2)	7 (0.2)	3 (0.0)	12 (0.2)	3 (0.1)	9 (0.3)
Sharing of the supplement	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Note: each observation represents the registration of an activity at a 5-min interval; some activities may have been recorded as more than one observation.

Furthermore, during the interviews, the majority of women revealed that they did not share the supplement with other adults or their children. These findings were corroborated by interviews with family members and did not differ between the supplement types. The main factors cited for not sharing included: the fact that the supplement is only intended for pregnant women; it is a medicine; instructions were given not to share; and women's belief that they would otherwise not receive the full benefit of the supplement. Despite the general tendency not to share, a handful of women indicated that they did share a small portion with their child(ren) because they were in their presence while eating. To avoid sharing, some women chose a separate room or took a moment alone to eat the supplement:

> In-depth interview 4, HC Kari, participant 33: "The reason I eat in my room is because of adults and children. If you eat outside, a child will see and cry for you to give it. If you refuse to give, they will say that it is you who is bad. They say not to give to children but there are some who give it to their child".

Women indicated that the instruction not to share the supplement helped to manage household members' expectations. Most participants agreed that family members would accept that sharing is not advised, as long they understand the underlying aim of daily supplementation:

> In-depth interview, community leader 4: "For those who understand things, will understand that it is made for pregnant women. But, for those who do not understand anything, even if the woman says that it is only for her alone, they will say that it is mean not to share with them."

Despite the majority's willingness to follow the instructions not to share, a small number of women and stakeholders suggested that there is a likelihood of the supplement being shared with children in the future. In general, participants mentioned that both the quantity of the supplement provided and women's personal and subjective approach to utilization and adherence were key determinants of whether or not a snack would be shared with others:

> In-depth interview, community leader 4: "Good! Eh! There are people when they buy, not everyone, and eat that alone. People don't have the same character, that's it! There are others when they buy, they do everything for the family to benefit."

Strategies to avoid sharing of the BEP supplements in the future focused on consuming the supplement alone and out of sight of others to avoid requests and conflicts. Women mentioned that for those with sufficient means, another option would be to buy alternative snacks for others to avoid sharing the provided supplement. Sensitization of household members and community leaders was put forward as a strategy to avoid sharing:

> FGD 3Boni:
> Participant 7: "I think it's better to explain to them the purpose of this work so that they understand well, otherwise they can take it without your knowledge, and it will cause other problems."
> Participant 37: "If you explain them well they will understand and not ask you to share."

Educational messages from health professionals

Women in this study uniformly agreed that the guidance and information provided at the onset was helpful. Especially the positive effects of the supplement 'good for mother and unborn child' and 'it's a medicine and contains vitamins' were perceived important messages. A small number of women indicated that they would have liked to receive additional information on the ingredients and how to use the supplement. Health professionals gave the suggestion to use pictures in communication to make sure all women understand the message. Both women and other stakeholders indicated that health centers are the most effective channel for providing information about nutritional supplements and for distribution, either through individual one-to-one meetings or in group discussions. Stakeholders agreed that further emphasis on promoting the supplements as a medicine would contribute to successful uptake and adherence.

Supplement choice

Data gathered during the daily choice period (weeks 9 and 10) highlighted that on average, women asked to change supplements two times over the course of the 2-week choice period. In total, 24 (30%) women did not request a change, 32 (40%) women asked to change one time and 24 (30%) women asked to change two times or more. The qualitative results were inconclusive: during the final in-depth interviews, a number of women indicated that they would prefer to have a choice between the two supplements for reasons including a desire for variety and the option to choose what they prefer at a given moment, whereas in the FGDs, participants did not express a strong interest in having a choice between supplement types or flavors.

Discussion

The present study provides evidence for high acceptability among pregnant women in Burkina Faso of two new fortified BEP supplements over a 10-week period during pregnancy and lactation. The quantitative data showed a positive assessment of hedonic characteristics, ease of use and adherence. The high acceptability and adherence appeared to be primarily influenced by the perceived benefits to the mother and child and

household support for supplement use. The majority of women indicated that they did not share the supplement, but qualitative results highlight the possibility that there may be some sharing with children. Results also gave insight that community sensitization and clear instructions by health professionals could play an important role in uptake and adherence.

The results on drivers of supplement use collected during this study are in line with other acceptability studies, in which perceived health benefits for women and their babies appeared to be an important motivating factor for daily consumption [166,168,169,176]. Moreover, positive attitudes of stakeholders have been found to be an important factor to promote acceptability and sustained use of supplementary foods by pregnant women in a study evaluating the acceptability of CSB in rural Cambodia [166]. Although we did not observe large difference in the responses to stratify the results per stakeholder group, the interviews provided valuable information that family members, community leaders and health professionals support the use of BEP supplements. We therefore recommend future studies or programs to take attitudes of all stakeholders into account when introducing nutritious food supplements.

Similar to another study on LNS in Burkina Faso, the women perceived both BEP supplements as a medicine. Scholars have previously suggested introducing supplementary foods as a medical treatment to increase adherence and decrease sharing [176,201]. We acknowledge that this could help to promote acceptability and adherence. Overall, the need for clear instructions and educational message is often mentioned as a key element to ensure appropriate utilization [173,203,204]. Although the present findings corroborate these previous observations, it remains essential to investigate context-specific factors that could affect acceptability and adherence.

In this formative study, sharing practices were not apparent and did not differ between the supplement types. In contrast to our findings, various studies report sharing of food supplements [166,176,177,205,206]. A reason for this difference could be that sharing is inherently difficult to assess due to the lack of objective measures. Research to better understand social dimensions of food and the optimal dose of supplements remains therefore important to ensure high adherence and reach the desired impact [207,208].

Hereby, it is important to take into account that in specific cultural contexts and food insecure areas, social and moral pressure to share can be high [168,209–211].

The cross-over design and 10-week duration are strengths of our study. All women were given each supplement for a period of 4 weeks and offered a choice in the final 2 consecutive weeks, allowing for a thorough evaluation of acceptability. The available evidence suggests that a 4-week period of daily consumption is sufficient to examine acceptability, as patterns of decreased motivation or sensory satiety appear after 2-3 weeks as a function of the number of times a food is consumed [212–214]. However, we extended the study period in order to generate a more rounded comparison between the supplements. Although the 7-point scale results did not show any substantial difference between the two supplements during the first 8 weeks of consumption, a slight preference for the vanilla biscuit was manifested during the last 2 weeks of the study when women were given a choice of supplements. Women's potential reluctance to give negative ratings to supplement characteristics or its use [169] might have played a role in the reported acceptability during the first 8 weeks of the study. We therefore suggest introducing a period in which a choice is offered to measure medium-term acceptability. Offering a choice between supplements in an intervention trial or program could also be an option, but more research is warranted to evaluate the added value, effect and practical feasibility.

The key limitation of this study is the potential for social desirability bias [215]. A mixed methods approach was adopted to mitigate potential bias of each single method and combined different approaches to triangulate findings and generate a more complete picture. Further, facilitators encouraged participants to speak openly and honestly in their local language, and they ensured the concept of confidentiality was well understood. Finally, we performed two home observations per household. The aim of this was to reduce change in behavior that may have emerged during the first observation because an outsider was in the home. Although it is nonetheless possible that women may have altered their behavior due to the presence of an observer [188], Harvey, Olórtegui, Leontsini and Winch [216] argue that the impact on validity is often minimal and propose to validate conclusions by collecting data in multiple ways. It is worth discussing that we did not find a noticeable difference between the adherence measured by participants' answers and

the home observations. This is in contrast to another study in Burkina Faso [175] in which adherence was higher in methods that relied on self-reporting. Although it is difficult to compare both studies, a possible explanation for the internal consistency between the two methods in this formative study could be that the MISAME study team is well known in the study area due to previous work. Their trustworthy reputation and the daily presence in the households could thus have lowered rates of misreporting.

For the planned efficacy study, the high acceptability and adherence rates reported in this preliminary research is a valuable result. Few women spoke however about explicit factors, such as palatability, flavor, form or packaging, that described why they liked or preferred a supplement. Even though our data collectors tried to trigger responses capturing 'why', the answers often remained minimal in terms of 'I like it', 'I don't like the other supplement', 'you asked me to choose, otherwise both are the same'. Finally, the high adherence rates in this 10-week home feeding trial are a good indicator for medium-term acceptably, but it is worth mentioning that this is a relatively short period to assess behavioral fatigue that could arise when the supplement is introduced during pregnancy and lactation, with daily consumption for a period of 6 months or more. In addition, the controlled study setting (incl. providing the supplements for free) could have influenced the results. Future studies should assess behavior throughout pregnancy to draw conclusions on the long-term acceptability and use of BEP supplements in real-world settings.

Conclusion

We conclude that both the lipid-based peanut paste and vanilla biscuit were acceptable and likely to be well used in the future. Women were positive about the taste and portion size of the supplements and willing to continue supplementation. Emphasis on the health benefits, informing and engaging family members and carefully monitor potential sharing remain important factors to support acceptance and adherence. As social context and individual preferences vary by setting and population [217], this study provides an example of how a mixed-method assessment can provide useful information on the acceptability of nutritious food supplements in research and programming.

For implementation in the RCT, the research team finally decided to select the lipid-based peanut paste. This decision was based on the fact that (1) the supplement appeared to be well accepted, (2) is stable (no oxidation of vitamins) and (3) shelf life is guaranteed for a long period, which are important factors for testing the efficacy of a BEP supplement. The production of the vanilla biscuits would require more time as additional stability tests were planned. To keep the project on track within the set timeline, the lipid-based peanut paste was the preferred option for the trial.

5

Fortified balanced energy-protein supplements increase nutrient adequacy without displacing food intake in pregnant women in rural Burkina Faso

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Fortified balanced energy-protein supplements increase nutrient adequacy without displacing food intake in pregnant women in rural Burkina Faso

Abstract

Background: In many low- and middle-income countries, the prevalence of energy and nutrient deficiencies is high among pregnant women. Balanced energy-protein (BEP) supplements are a promising strategy to cover nutritional requirements during pregnancy and improve birth outcomes. However, the displacement of nutrient-dense foods by BEP might attenuate the efficacy of supplementation. This cross-sectional study of participants in a randomized controlled trial evaluated the difference in energy and macro- and micronutrient intakes, food groups, and nutrient adequacy between a control and intervention group receiving either a daily iron–folic acid (IFA) tablet or IFA and BEP supplement during pregnancy, respectively.

Methods: We collected a single multiple-pass 24-h recall from 470 pregnant women from the MISAME-III study that investigates the efficacy of BEP supplementation on birth outcomes and infant growth. Dietary intake (median and IQR) and nutrient adequacy were assessed using individual recipes and preparation methods of mixed dishes for each participant. Linear regression models were fitted to compare energy and nutrient intakes.

Results: Dietary energy, and macro- and micronutrient intakes were significantly higher among women in the intervention group when including BEP [2329 kcal/d (1855, 3008 kcal/d) compared with 1942 kcal/d (1575, 2405 kcal/d) in the control group (all $P < 0.001$)]. The difference in median energy intake (448 kcal/d; 95% CI: 291, 605 kcal/d) was approximately equivalent to a daily dose of the BEP supplement (393 kcal). Nutrient adequacy ratios for both groups were low for all micronutrients (between 0.02 and 0.66), when excluding BEP (except iron and folic acid, due to standard supplemental doses) from analysis. However, nutrient intakes increased to the estimated average requirement for pregnant women when including BEP supplements.

Conclusions: BEP supplementation increases energy and macro- and micronutrient intakes among pregnant women and fills nutrient gaps without displacing food intake. This trial was registered at ClinicalTrials.gov as NCT03533712.

Key messages

- To assess whether a prenatal BEP supplement displaces part of the usual diet of pregnant women, which could attenuate the efficacy of supplementation, a cross-sectional food intake study of women from the MISAME-III trial was designed.
- Study results showed that BEP supplementation led to significantly higher intakes for energy, macro- and micronutrients as compared to control, indicating that BEP supplements did not displace foods part of the base diet.
- The BEP supplement improved nutrient adequacy ratios of pregnant women for all nutrients to at least the level of the respective estimated average requirement (EAR).

Introduction

During pregnancy, women have increased physiological needs for energy and nutrients to support fetal growth and development [68]. The average total cost of pregnancy is estimated to be 90 kcal, 290 kcal, 465 kcal per day, for the first, second and third trimester respectively [218]. Beside energy, protein and essential fatty acids, B-complex vitamins, vitamin A, vitamin D, iron and zinc are needed to regulate fundamental processes of fetal growth, and iodine is particularly important for brain development [18]. Optimal maternal nutrient intakes is also imperative to maintain maternal metabolism and support maternal tissue growth to ensure adequate lactation performance for the newborn [16]. Hence, inadequate diets among pregnant women can lead to adverse health outcomes in both mother and child [26].

In many LMICs, and in particular in food-insecure settings, the prevalence of micronutrient deficiencies is high, due to monotonous, nutrient-poor diets coupled with low bioavailability, and poor absorption due to infections [17,18,219]. Single micronutrient deficiency estimates among pregnant women range from 20 to 30% worldwide [16] and

up to 40% in Africa [18]. In Burkina Faso, a previous study has indicated that pregnant women do not meet any of the WHO/FAO recommended daily nutrient allowances, except for phosphorus [152]. Other studies have reported similar findings in Burkinabe mothers with young children [220,221], women of reproductive age [222] and women living in urban areas [223]. Furthermore, micronutrient needs of women cannot be met by a diet containing only commonly consumed local foods in Burkina Faso [224]. Nutrition supplements are thus advised to fill micronutrient gaps on a short-term basis, and possibly represent a lower cost compared to nutrient dense foods like animal-source foods. The following micronutrients were often found deficient by previous dietary intake studies in Burkina Faso: riboflavin, vitamin B6, vitamin B12, folate, calcium and iron [152,220–224]. The most recent reviews suggested that maternal MMN supplementation and supplements containing protein, energy and other nutrients [225–227] demonstrated a positive effect on several birth outcomes. Based on the available scientific evidence, the current WHO guidelines [108] therefore recommend providing nutritional supplements to cover nutritional requirements during pregnancy and lactation in undernourished populations.

BEP supplements provide less than 25% of total caloric content from protein and have the potential to improve maternal nutritional status and fetal growth [100,130,144,145] in undernourished populations, yet large trials are required to support the current evidence for future recommendations and decision-making. The MISAME-III study is an ongoing RCT assessing the efficacy of BEP supplementation on birth outcomes and infant growth. One important factor that may attenuate the efficacy of fortified BEP supplements is their displacement of (nutrient-dense) foods that are part of the base diet [228,229]. Therefore, the primary aim of this study was to assess whether a prenatal BEP supplement displaces part of the usual diet of pregnant women. A secondary aim was to assess the nutrient adequacy of pregnant women's diets with and without supplements.

Methods

Our research was reported using the STROBE-nut checklist [230].

Main trial

Details of the MISAME-III efficacy trial are described elsewhere [231]. In brief, 1,776 pregnant women were individually randomized to pre- and postnatal intervention or control groups. The intervention groups received a daily fortified BEP supplement and IFA tablet and the control groups received an IFA tablet alone. In a formative study, the most preferred and suitable BEP was selected for administration in the RCT [147,148]. The selected BEP supplement belongs to the family of LNS and consists of a peanut paste spread fortified with multiple micronutrients (**Table 5.1**). The advantage of this type of supplement is that it is an energy-dense, ready-to-consume product that does not require a cold chain, is highly stable, and has a long shelf-life. Anthropometric and socio-demographic data were collected at baseline.

Table 5.1 Nutritional values of the balanced energy-protein supplement

Ready-to-use supplementary food for pregnant and lactating women[a]	**Mean for 72g (serving size)**	**% EAR for pregnant women**[b]	**% RDA for pregnant women**[b]
Total energy (kcal)	393	NA	NA
Lipids (g)	26	NA	NA
Proteins (g)	14.5	28.4[d]	20.4
Carbohydrates (g)	23.3	17.3	13.3
Calcium (mg)	500	62.5	50.0
Iron (mg)	22	100	81.5
Zinc	15	158	136
Vitamin A (µg RE)[c]	770	140	100
Thiamin (mg)	1.4	117	100
Riboflavin (mg)	1.4	117	100
Niacin (mg)	15	107	83.3
Vitamin B6 (mg)	1.9	119	100
Folic acid (µg)	400	128	111
Vitamin B12 (mg)	2.6	118	100
Vitamin C (mg)	100	143	118

[a] Ingredients: vegetable oils (rapeseed, palm, soy in varying proportions), defatted soy flour, skimmed milk powder, peanuts, sugar, maltodextrin, soy protein isolate, vitamin and mineral complex, stabilizer (fully hydrogenated vegetable fat, mono and diglycerides).

[b] Pregnant women aged 19-30 year [81]. EAR is the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group. RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an EAR.

[c] 1 µg vitamin A RE = 3.333 IU vitamin A.

[d] Using the mean weight of all participating women in the trial: 58 kg.

EAR, estimated average requirements; NA, not applicable; RDA, recommended dietary allowances; RE, retinol equivalent.

Study population and recruitment

The study was conducted in six rural health center catchment areas located in the Houndé health district in the Hauts-Bassins region of Burkina Faso. Data were collected from September to October 2020, at the end of the pre-harvest season. According to a previous study conducted in the same area, the mean energy intake of pregnant women was 2050 with an SD of 623 kcal [232]. Based on that, a sample size of 234 subjects per study arm (468 in total) was required to detect a difference in energy intake of 196.5 kcal between arms, which corresponds to half the dose provided by the fortified BEP supplement, with $\alpha = 0.05$, $\beta = 0.90$ and 10% non-response. We randomly selected 595 pregnant women enrolled in the MISAME-III trial with a gestational age between 12 and 34 weeks, stratified per health center and intervention group, using the *runiform* function in Stata. The principal sample list consisted of 469 women. A back-up list of 126 women was generated in the event that women from the principal list were not available for an interview.

Dietary assessment

Dietary intake was assessed by means of a single 24-hour recall (24HR), conducted using a tablet-based multiple pass interactive strategy [233] implemented in SurveySolutions software (v. 20.10, World Bank Group, USA). Six enumerators and one supervisor with experience in 24h dietary recall assessment from other studies were recruited for this study. The field team received a one-week refresher training in September 2020 on interviewing techniques, portion size estimation and data entry on tablets. After the training, two days were scheduled for pilot testing the recall method with pregnant women not enrolled in the study.

Two days before the dietary assessment, standard plates and bowls were provided to the women to be used to serve their own food, instead of eating with other family members from a shared plate or individual plates that differ in size, which would hamper harmonized individual portion size estimation. The procedure was explained to the participants and it was emphasized not to change their eating habits in the interest of the study. Recall days were evenly distributed over the entire week, from Monday to Sunday.

The 24HR method consisted of five passes during which the woman was asked to recall all foods and drinks she consumed between waking up the previous day until waking up

on the day of the recall. In the first pass, the woman was asked to report all foods and drinks consumed. In the second pass, additional details about the foods and drinks she listed were collected, including time of consumption, preparation method (raw, boiled, grilled, etc.) and a detailed food name. In the third pass, details of mixed dishes were collected: a list of ingredients with its form (fresh, dry, etc.) and quantity, any leftovers, preparation method of the mixed dish (boiled, fried, etc.), and total volume of the prepared mixed dish. In the fourth pass, she was asked to estimate the portion size and any leftover of each food or drink consumed. Different pre-specified portion size estimation methods were used: actual foods and salted replicas for main staples (e.g. *tô* – a stiff cereal porridge and rice), playdough for pieces of food (e.g. meat, fish, snacks), volumetric measures for liquids (e.g. sauces, thin porridges, beverages), household measures using project utensils, photos using an atlas with common foods (e.g. vegetables, fruit, fish and mixed dishes), and prices (e.g. bread). A digital scale with 1 g precision was used. In the fifth pass, the enumerator reviewed the information recorded in the previous passes and checked for any missing foods. The 24HR also included questions on the consumption of BEP supplements, IFA tablets, or other supplements and qualitatively assessed whether the reported quantity and variety of intake was habitual compared to the woman's base diet.

Data preparation

A list of commonly consumed food and drinks in Burkina Faso, which were enumerated during two previous 24HR studies conducted by the SELEVER trial [234], were preloaded into the questionnaire and could be directly selected from a drop-down menu, each with a unique code. New foods could be added to the questionnaire throughout the study whenever needed.

Portion sizes measured by different methods were converted to edible portions into grams using conversion factors (food density or per unit measures) from context-specific conversion lists from the SELEVER trial [234], or collected by the supervisor after the study if the conversion factor was not available in this database. For mixed dishes, we calculated the amount of each ingredient consumed using the amount of raw ingredients used for preparation together with the total amount of the prepared dish and the amount consumed by the participant. For the few cases where a household recipe was not

reported, average recipe values from our study population were used or if these were absent, standard recipes values from the West African food composition table (FCT) were used [235].

Energy and nutrient intakes were calculated using food composition data from the West African FCT [235] with additional data from another FCT adapted to Burkinabe foods [236]. In case of missing data, values of similar food items in the West African FCT were used. This was mainly the case for a few micronutrients of local green leaves (e.g. Moringa) for which values from the food code "mixed/unknown green leaves" were used. For the shea tree caterpillar (*Cirina butyrospermi*) that was consumed by 12 participants, available mineral values from the literature were used [237]. Nutrient values for raw ingredients of recipes that were either boiled, steamed, grilled or fried were adjusted for nutrient losses using retention factors [238]. Because the BEP was peanut-based, we specifically looked into the intake of peanuts and peanut-based products. Finally, nutrient adequacy ratios (NARs) were calculated by dividing nutrient intakes by the estimated average requirements (EARs) for pregnant women by age group, truncated at 1 [81]. For iron, we used recommendations adjusted for low (5%), medium (10%) and high (16%) bioavailability [239]. For zinc, we used recommendations issued by the International Zinc Nutrition Consultative Group, considering 25% zinc absorption for women with an unrefined, cereal-based diet [240].

Statistical analysis

Data management and statistical analysis were performed in Stata (v. 14.2, StataCorp LLC, USA). Baseline characteristics of study participants were summarized by study group as mean ± SD for continuous variables and as percentages for categorical variables. Linear regression models were fitted on the crude observed intake values to compare energy and nutrient intakes between the control and intervention group (median and interquartile range [IQR] for values with a skewed distribution; or mean ± SD for normally distributed values), adjusted for health center to account for the study design and interviewer as fixed effect covariates [241]. The assumption of normality was checked by visual inspection of histograms and QQ-plots of the residuals. Crude values were used for normally distributed outcomes (% energy from protein and % energy from carbohydrates), and outcome

variables were log-transformed when these assumptions were violated to test the significance of differences between study groups, using the control as a reference. For outcomes that were not amenable to transformation (% energy from fat, vitamin B12 and C), we applied quantile regression to estimate median difference between study groups. We carried out sensitivity analysis by excluding potentially influential outliers with standardized residuals > |3|. A two-sided significance level of $P < 0.05$ was applied for all analyses.

Results

Characteristics of participants

A total of 470 recalls (including 36 women from the back-up list) were completed, of which 253 women in the control and 217 in the intervention arm. The mean duration of supplement intake was 117 days for the control group (IFA) and 122 days (IFA + BEP) for the intervention group at the time of the recall. Study arms were well-balanced in terms of socio-demographic and maternal characteristics at the time of interview, while slightly more women in the intervention group were underweight at the time of enrollment in the RCT (**Table 5.2**).

Food consumption pattern and supplement intake

Twelve percent of women (n = 56) reported having eaten less than usual, 76% (n = 357) as usual, and 12% (n = 57) more than usual on the day of the 24HR. Furthermore, women indicated that their food variety was less than usual in 5% of the recalls (n = 57), as usual in 80% (n = 376), and more varied than usual in 15% (n = 71) (**Table 5.2**).

Table 5.2 Characteristics of pregnant women, by study arm.[a]

	Control (IFA) n = 253	**Intervention (IFA + BEP)** n = 217
Age[b], yrs	25.5 ± 6.30	25.4 ± 6.63
Ethnicity[b]		
Bwaba	146 (57.7)	125 (57.6)
Mossi	91 (36.0)	71 (32.7)
Other	16 (6.32)	19 (8.76)
Education[b]		
None	127 (50.2)	93 (42.9)
Primary	107 (42.3)	97 (44.7)
Secondary	19 (7.51)	25 (11.5)
Gravidity[b]		
0	48 (19.0)	49 (22.6)
1-2	92 (36.4)	62 (28.6)
3 or more	113 (44.7)	106 (48.9)
Gestational age, weeks	27.5 ± 6.65	27.5 ± 6.86
Trimester		
First	1 (0.40)	0 (0.00)
Second	123 (48.6)	104 (47.9)
Third	129 (51.0)	113 (52.1)
Weight[b], kg	59.0 (7.93)	59.0 (9.53)
BMI[b], kg/m^2	22.3 ± 2.79	22.2 ± 3.15
BMI category[b], kg/m^2		
Underweight, <18.5	10 (3.95)	20 (9.22)
Normal, 18.5–24.9	212 (83.8)	165 (76.0)
Overweight, 25–29.9	28 (11.1)	27 (12.4)
Obese, ≥ 30	3 (1.19)	5 (2.30)
MUAC[b], mm	265 ± 25.5	263 ± 28.2
Nr of meals during recall day	4.06 ± 0.86	4.05 ± 0.86
Quantity of food intake during recall day		
Less than usual	32 (12.6)	24 (11.1)
As much as usual	194 (76.7)	163 (75.1)
More than usual	27 (10.7)	30 (13.8)
Variety of food intake during recall day		
Less than usual	13 (5.1)	11 (5.1)
As usual	205 (81.0)	170 (78.3)
More than usual	35 (13.8)	36 (16.6)
Special circumstances during recall day		
No appetite	4 (1.58)	3 (1.38)
Sick	5 (1.98)	4 (1.84)
Family visit/funeral	3 (1.19)	1 (0.46)
BEP intake		
Not consumed	251 (99.2)	5 (2.30)
1/2 portion consumed	0 (0.00)	4 (1.84)
3/4 portion consumed	0 (0.00)	8 (3.69)
Full portion consumed	2 (0.80)	200 (92.2)
IFA intake	252 (99.6)	215 (99.1)

[a] Values are mean ± SD or frequencies (%); [b] Baseline data were collected at inclusion in MISAME-III trial.

BEP, balanced energy-protein; BMI, body mass index; IFA, iron folic acid; MUAC, mid-upper arm circumference.

Figure 5.1 shows the contribution (in %) of each food group of the Minimum Dietary Diversity for Women (MDD-W) classification including the optional categories [242] to total energy intake of the base diet (BEP excluded), by study arm. The results show that the base diet is mainly cereal based. Almost all women (95%) consumed the main staple dish *tô* with a mean ± SD potion size of 543 ± 212 g and a corresponding 42.3% ± 23.3 contribution to total energy intake. *Tô* is a stiff cereal dough often served with a watery sauce containing green leafy vegetables (okra, hibiscus and baobab leaves) or other vegetables such as eggplant, with or without meat, fish, or caterpillars. Other nutritious food groups such as fruit, dairy, eggs, fish and meat contribute very small amounts to the total energy intake. No large differences or substitution of food groups was observed (**Figure 5.1**). Specifically, no meaningful difference was found in the base diet in the intake of sentinel food items, such as peanuts as a snack or dishes that contained peanuts between the intervention and control groups (**Annex 4**).

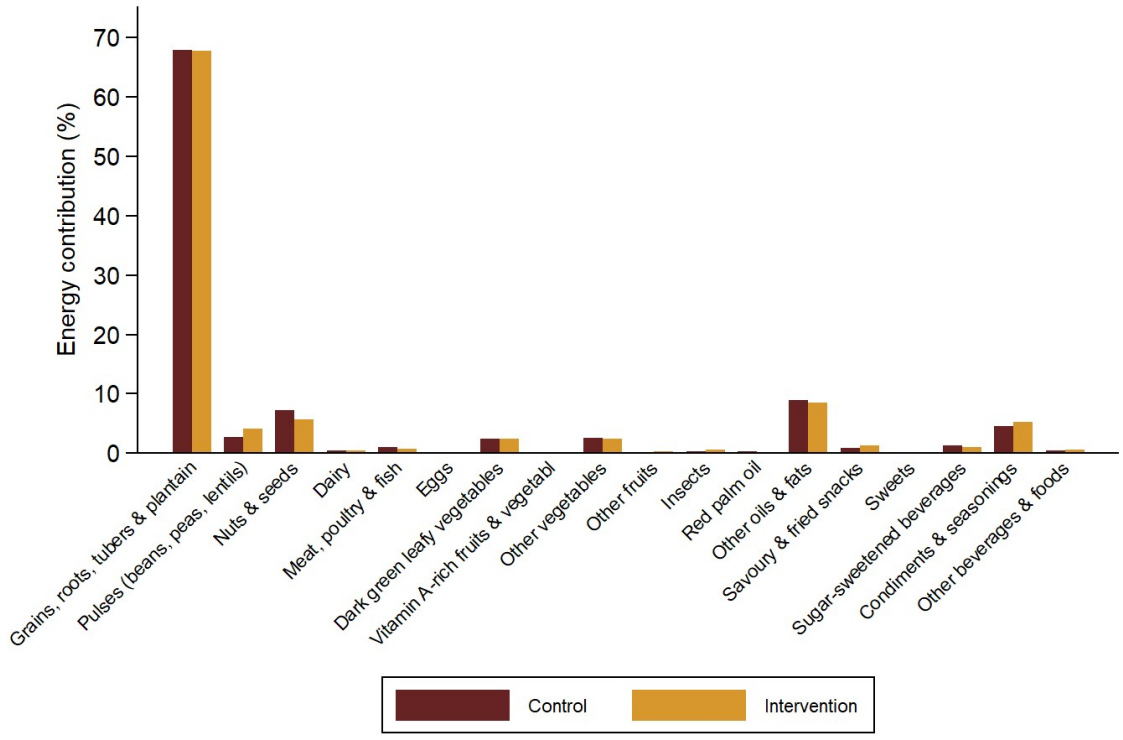


Figure 5.1 Energy contribution per food group of the base diet, according to classification of the MDD-W indicator, by control and intervention group.

MDD-W, Minimum Dietary Diversity for Women.

Among women in the intervention group, 92% (n = 200) consumed the complete portion of the fortified BEP supplement (72 g) the day prior to the assessment. Twelve women indicated that they consumed a part of it (½ or ¾ portion) and five women did not consume the BEP supplement at all. Reasons for not consuming the BEP supplement included: not feeling well, not feeling hungry, or not receiving the visit from the trial community support staff who provides the daily supplement. Two women from the control group indicated having consumed the BEP supplement. After verification by the field supervisors, these women were not provided and/or consuming BEP supplements on a daily basis. These two cases can thus either be considered a data entry or rare implementation error in the field. Concerning the IFA tablet, one woman in the control group and five women in the intervention group did not take the tablet. No women reported the consumption of any other type of nutritional supplement.

Energy and nutrient intakes

Median (IQR) energy intake on the recall day in the control group was 1942 kcal (1575, 2405) and 2329 kcal (1855, 3008) in the intervention group, including supplements (**Table 5.3**). Women in the intervention group had significant higher intakes of energy, macro-, and micronutrients. Comparing the base diet (without supplementation) between both groups indicates that almost all additional energy, macro-, and micronutrients in the intervention group can be attributed to the consumption of the BEP supplement (**Table 5.4**). No differences were found between trimesters. Sensitivity analyses excluding outliers (n = 10) did not change our findings (results not shown). Low energy intakes (min. 420 kcal/day) were observed in women who reported sickness, lack of appetite, or the consumption of rather nutrient-poor sauces with limited quantities of fresh green leaves and lots of added water. In contrast, high energy intakes (max. 5057 kcal/day) were observed in women who reported large portions of staple foods, peanuts, beans, legumes, shea butter, or oil; and high micronutrient contents were identified for diets which included sauces containing large amounts of nutrient-dense dried hibiscus/green leaves.

Nutrient adequacy

When omitting supplement intake (IFA or BEP) from the analysis, NARs from women in the control (IFA) and intervention (IFA + BEP) groups were low for zinc, calcium, thiamin, riboflavin, niacin, vitamin B6 and vitamin C; and very low for iron, vitamin A, folate and vitamin B12 (**Table 5.4**). The EARs for folate and iron, assuming medium (10%) and high (16%) bioavailability, were met in the control group by providing standard daily IFA tablets (**Table 5.3**). It is however likely that the bioavailability of iron is low (5%) in our setting due to a primarily plant-based diet [239]. In the intervention group, providing the BEP supplement in addition to the base diet increased the adequacy ratio for all nutrients to at least the level of the respective EAR (**Table 5.3**).

Table 5.3 Energy and nutrient intakes and adequacy ratios of diets including supplements, between control and intervention groups.

	Control (diet incl. IFA) n = 253[a]	**Intervention** (diet incl. IFA + BEP) n = 217[a]	**Difference** (95% CI)[b]	**P-value**	**NAR Control** (diet incl. IFA) n = 253	**NAR Intervention** (diet incl. IFA + BEP) n = 217
Energy (kcal/d)	1942 (1575, 2405)	2329 (1855, 3008)	448 (291, 605)	<0.01[d]	NA	NA
Fat (g/d)	33.9 (18.4, 57.6)	58.4 (42.1, 81.3)	25.6 (19.1, 32.0)	<0.01[d]	NA	NA
% energy from fat	16.2 (9.24, 23.4)	23.2 (18.8, 27.9)	6.74 (5.14, 8.34)	<0.01[e]	NA	NA
Protein (g/d)	50.6 (37.8, 67.9)	63.7 (49.6, 85.1)	15.2 (9.91, 20.5)	<0.01[d]	0.98 (0.74, 1.00)	1.00 (1.00, 1.00)
% energy from protein, mean ± SD	10.8 ± 2.43	11.4 ± 1.94	0.51 (0.10, 0.91)	0.01[c]	NA	NA
CHO (g/d)	340 (269, 424)	362 (280, 469)	37.5 (12.9, 62.0)	<0.01[d]	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
% energy from CHO, mean ± SD	69.8 ± 9.80	62.7 ± 7.41	-7.15 (-8.76, -5.54)	<0.01[c]	NA	NA
Calcium (mg/d)	407 (273, 587)	899 (744, 1149)	515 (449, 582)	<0.01[d]	0.50 (0.33, 0.71)	1.00 (0.90, 1.00)
Iron (mg/d)	81.9 (76.3, 90.6)	103 (97.1, 110)	20.5 (18.0, 23.0)	<0.01[d]	0.74 (0.69, 0.82)[f]	0.93 (0.87, 1.00)[f]
					1.00 (1.00, 1.00)[f]	1.00 (1.00, 1.00)[f]
					1.00 (1.00, 1.00)[f]	1.00 (1.00, 1.00)[f]
Zinc (mg/d)	8.96 (6.74, 11.4)	23.7 (21.4, 26.9)	14.6 (13.7, 15.5)	<0.01[d]	0.57 (0.43, 0.71)[g]	1.00 (1.00, 1.00)[g]
Vitamin A (RAE/d)	154 (75.7, 379)	910 (824, 1133)	764 (656, 873)	<0.01[d]	0.29 (0.14, 0.71)	1.00 (1.00, 1.00)
Thiamin (mg/d)	0.68 (0.47, 1.07)	2.13 (1.88, 2.49)	1.40 (1.29, 1.51)	<0.01[d]	0.57 (0.39, 0.89)	1.00 (1.00, 1.00)
Riboflavin (mg/d)	0.64 (0.46, 0.91)	1.99 (1.79, 2.32)	1.33 (1.24, 1.42)	<0.01[d]	0.53 (0.38, 0.76)	1.00 (1.00, 1.00)
Niacin (mg/d)	7.30 (5.48, 9.90)	22.3 (20.1, 24.9)	14.8 (13.8, 15.9)	<0.01[d]	0.52 (0.39, 0.71)	1.00 (1.00, 1.00)
Vitamin B6 (mg/d)	1.03 (0.80, 1.40)	2.90 (2.65, 3.28)	1.85 (1.75, 1.95)	<0.01[d]	0.64 (0.50, 0.88)	1.00 (1.00, 1.00)
Folate (µg/d)	605 (544, 754)	1006 (931, 1156)	399 (353, 444)	<0.01[d]	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Vitamin B12 (mg/d)	0.05 (0.01, 0.25)	2.63 (2.61, 2.79)	2.33 (2.02, 2.63)	<0.01[e]	0.02 (0.00, 0.11)	1.00 (1.00, 1.00)
Vitamin C (mg/d)	35.2 (19.0, 53.8)	133 (115, 156)	102 (91.6, 113)	<0.01[e]	0.50 (0.28, 0.77)	1.00 (1.00, 1.00)

[a] Values are median (interquartile range) unless otherwise indicated.

[b] Group differences were estimated using crude observed nutrient intake values by fitting linear regression models adjusted for health center and enumerator as fixed effect covariates.

[c] Significant differences between study groups were tested on crude values in case the data was normally distributed; or on log transformed values in case the assumption of normality was violated[d]; or by fitting quantile regression models based on the crude values in case the assumption of normality was violated but the outcomes were not amenable to transformation[e].

[f] Values, from top to bottom, are adjusted for low (5%), medium (10%) and high (16%) bioavailability for iron.

[g] Values are adjusted for 25% bioavailability for zinc in our setting of predominantly cereal-based diets.

BEP, balanced energy-protein; CHO, carbohydrates; CI, confidence interval; IFA, iron folic acid; NAR, nutrient adequacy ratio; RAEs, retinol activity equivalents.

Table 5.4 Energy and nutrient intakes and adequacy ratios of base diets, between control and intervention groups.

	Control (base diet) n = 253[a]	**Intervention** (base diet) n = 217[a]	**Difference** (95% CI)[b]	**P-value**	**NAR Control** (base diet) n = 253	**NAR Intervention** (base diet) n = 217
Energy (kcal/d)	1942 (1575, 2405)	1936 (1480, 2615)	70.8 (-86.0, 227)	0.47[d]	NA	NA
Fat (g/d)	33.9 (18.4, 57.6)	33.4 (17.1, 55.6)	0.63 (-5.83, 7.09)	0.97[d]	NA	NA
% energy from fat	16.2 (9.24, 23.4)	15.1 (8.76, 21.9)	-0.71 (-2.47, 1.05)	0.63[e]	NA	NA
Protein (g/d)	50.6 (37.8, 67.9)	49.3 (36.0, 72.2)	1.30 (-3.97, 6.58)	0.76[d]	0.98 (0.74, 1.00)	0.95 (0.73, 1.00)
% energy from protein, mean ± SD	10.8 ± 2.43	10.7 ± 2.26	-0.18 (-0.61, 0.25)	0.42[c]	NA	NA
CHO (g/d)	340 (269, 424)	338 (257, 447)	15.12 (-9.44, 36.7)	0.23[d]	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
% energy from CHO, mean ± SD	69.8 ± 9.80	70.8 ± 9.46	0.92 (-0.84, 2.69)	0.31[c]	NA	NA
Calcium (mg/d)	407 (273, 587)	409 (277, 664)	35.5 (-29.6, 101)	0.61[d]	0.50 (0.33, 0.71)	0.50 (0.34, 0.80)
Iron (mg/d)	16.9 (11.4, 25.7)	16.8 (11.1, 23.3)	-0.37 (-2.64, 1.90)	0.41[d]	0.15 (0.10, 0.23)[f]	0.15 (0.10, 0.21)[f]
					0.30 (0.20, 0.46)[f]	0.30 (0.20, 0.41)[f]
					0.50 (0.34, 0.76)[f]	0.49 (0.32, 0.68)[f]
Zinc (mg/d)	8.96 (6.74, 11.4)	8.94 (6.56, 11.9)	0.18 (-0.63, 1.01)	0.68[d]	0.57 (0.43, 0.71)[g]	0.56 (0.42, 0.76)[g]
Vitamin A (RAE/d)	154 (75.7, 379)	148 (65.2, 373)	25.7 (-81.3, 132)	0.65[d]	0.29 (0.14, 0.71)	0.28 (0.12, 0.70)
Thiamin (mg/d)	0.68 (0.47, 1.07)	0.75 (0.50, 1.10)	0.06 (-0.05, 0.16)	0.17[d]	0.57 (0.39, 0.89)	0.62 (0.41, 0.91)
Riboflavin (mg/d)	0.64 (0.46, 0.91)	0.60 (0.46, 0.92)	-0.16 (-0.10, 0.07)	0.45[d]	0.53 (0.38, 0.76)	0.50 (0.34, 0.76)
Niacin (mg/d)	7.30 (5.48, 9.90)	7.39 (5.24, 9.90)	0.42 (-0.57, 1.42)	0.63[d]	0.52 (0.39, 0.71)	0.53 (0.37, 0.71)
Vitamin B6 (mg/d)	1.03 (0.80, 1.40)	1.05 (0.80, 1.40)	0.03 (-0.06, 0.12)	0.72[d]	0.64 (0.50, 0.88)	0.66 (0.50, 0.88)
Folate (µg/d)	205 (144, 354)	209 (145, 356)	16.6 (-27.1, 60.3)	0.76[d]	0.39 (0.28, 0.68)	0.40 (0.28, 0.68)
Vitamin B12 (mg/d)	0.05 (0.01, 0.25)	0.04 (0.01, 0.19)	-0.17 (-0.47, 0.13)	0.70[e]	0.02 (0.00, 0.11)	0.02 (0.00, 0.09)
Vitamin C (mg/d)	35.2 (19.0, 53.8)	34.1 (18.0, 57.4)	6.47 (-4.16, 17.1)	0.88[e]	0.50 (0.28, 0.77)	0.50 (0.26, 0.85)

[a] Values are median (interquartile range) unless otherwise indicated.

[b] Group differences were estimated using crude observed nutrient intake values by fitting linear regression models adjusted for health center and enumerator as fixed effect covariates.

[c] Significant differences between study groups were tested on crude values in case the data was normally distributed; or on log transformed values in case the assumption of normality was violated[d]; or by fitting quantile regression models based on the crude values in case the assumption of normality was violated but the outcomes were not amenable to transformation[e].

[f] Values, from top to bottom, are adjusted for low (5%), medium (10%) and high (16%) bioavailability for iron.

[g] Values are adjusted for 25% bioavailability for zinc in our setting of predominantly cereal-based diets.

BEP, balanced energy-protein; CHO, carbohydrates; CI, confidence interval; IFA, iron folic acid; NAR, nutrient adequacy ratio; RAEs, retinol activity equivalents.

Discussion

We report that the distribution of BEP led to significantly higher intakes for energy, macro- and micronutrients as compared to women from the control group. Our results indicate that in case the full portion of BEP was consumed there was little to no displacement of the food intake among pregnant women in Burkina Faso. In fact, nutrient intakes in the base diet were remarkably similar across study groups, and all micronutrient intakes were inadequate relative to the EAR. The BEP supplement filled all the nutrient gaps and was able to account for the additional energy requirement during pregnancy, making it a valuable contribution to the diet of undernourished pregnant women.

For 13 supplementation studies that reported small to no impact on birth outcomes, authors speculated that the supplement might have displaced at least part of the usual diet [100,145]. Therefore, it is essential that food supplementation trials are accompanied by well-conducted dietary intake studies to assess possible substitution effects to aid in the assessment of the trial's outcomes. To our knowledge, this is one of the few studies that investigated the displacement of food by BEP supplementation in pregnant women in low- and middle-income countries. In the MISAME-II trial, a locally produced LNS based on soy flour, peanut butter, oil and sugar with a premix of multiple micronutrients was compared with a MMN tablet. In this study, the LNS appeared to be an extra allowance for women during pregnancy [232]. The few other studies on maternal food supplementation, date from more than 30 to 40 years ago [232,243]. Other peer-reviewed literature focuses on dietary intake differences between study arms in supplementation trials in children aged 3 to 60 months. The general conclusion of trials in Burkina Faso [229], Malawi [244–246], Ghana [247], Uganda [248], Honduras [249] and Bangladesh [250,251] is that LNS improved energy, macro-, and micronutrient intakes without displacing other foods in the diet of young children. For supplements based on cereals (e.g. CSB), some level of substitution was found [229,245,246].

Without considering the BEP supplements, pregnant women's nutrient intakes were inadequate. The nutrient inadequacies reported in this study are comparable to other countries in Africa. A systematic review from 20 studies in resource-poor settings in sub-Saharan Africa reported inadequate micronutrient intakes of pregnant women for vitamin

C, niacin, vitamin B6, folate, vitamin B12, iron and zinc [252]. In Mali, the NARs for vitamin A and calcium were also below recommended levels in 70-80% of households [253]; and in Niger, usual dietary intakes of vitamin A, thiamin, riboflavin, niacin, folate and vitamin C were inadequate for >50% of pregnant and lactating women with calcium and vitamin B12 intakes inadequate for all women, comparable to our study [254]. These diets typically show imbalances in macronutrients, inadequate micronutrient levels and are predominantly plant-based [255], which is most likely the cause of inadequate iron and zinc intakes [256].

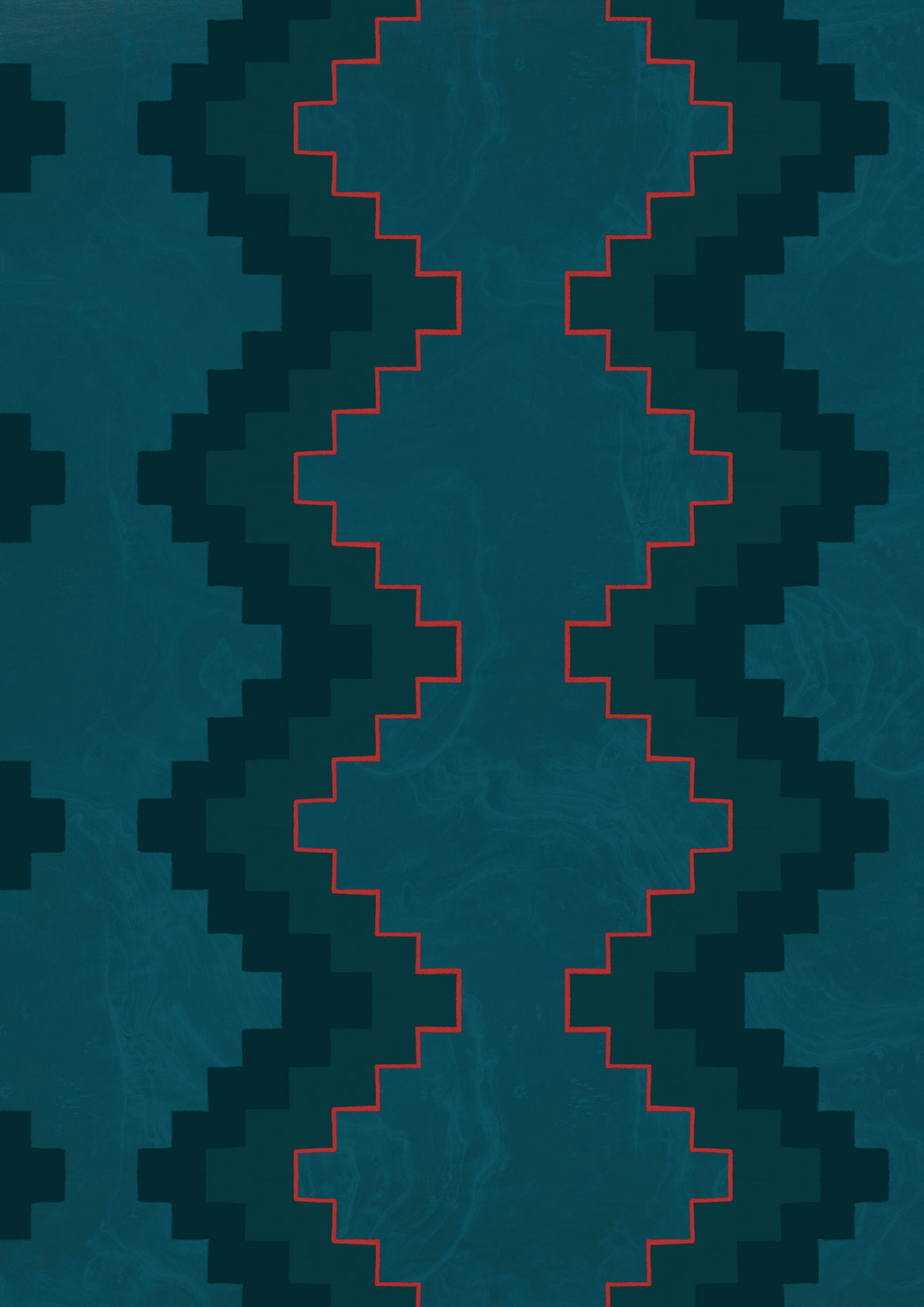
Addressing the nutritional needs of pregnant and lactating women is key to reach the UN 2030 Sustainable Development Goals [257]. In addition to food-based programs, our results show that fortified food supplementation to improve nutrient intakes can be a promising strategy. The mean energy intake of 1942 kcal in the control group does not seem to address the increasing energy requirement in the second (290 kcal) and third trimester (465 kcal) of pregnancy, considering for example a daily average energy requirement of 2360 kcal for non-pregnant women aged 18-30 year with a PAL of 1.75 and mean weight of 59 kg [218,258]. BEP could play a crucial role in addressing increasing energy and nutrient requirement throughout pregnancy.

Strengths of this study include the detailed semi-structured 24HR collection tool, in which almost all commonly consumed foods in Burkina Faso could be selected from a drop-down menu and included questions on type or preparation method. This way, a detailed list of food items was collected using a standardized five-pass method for all participants. Instead of using mean or standard recipe data, we collected information on all individual ingredients and the preparation method of mixed dishes to improve the quality of our findings by not only capturing potential differences across groups due to differences in portion sizes; but also setting us up to capture potential differences due to displacement in the type of ingredients cooked.

Our study has some limitations. First, the 24HR method is inherently prone to measurement error [233]. While this is an inevitable limitation, our goal was to compare differences between the two groups and estimate population average dietary intakes. Any possible systematic error of estimation might therefore be present in both study arms as women were randomly allocated to the study groups and it is unlikely that there would be any

different behavior in under- or overreporting. With intensive training, close supervision, recalls on all days of the week, a standardized multiple-pass interview technique and the use of multiple measurement aids (e.g. photos, clay, salted replicas) specifically designed for the study area allowing for visual and individual portion size estimations, we limited potential errors as much as possible [259]. Second, since the main study objective was a comparison between group means of intakes, we performed only one recall per woman. With this information, we were able to calculate adequacy ratios at a population level, but are unable to calculate individual level (mean) probability of adequacy across nutrients in this trial due to a lack of estimates of the intra-person or day-to-day variance of the observed nutrient intakes. Values from other studies may vary from our study population and incorrect use may influence the accuracy of prevalence estimates [260]. Additionally, we cannot entirely rule out group imbalances in unmeasured prognostic factors of dietary intake (e.g. physical activity level or basic metabolic rate). Any influence from the IFA tables on the diet, e.g. due to nausea, can neither be ruled out. Third, due to the cross-sectional nature of the study with data collection at the end of the pre-harvest season (Sep-Oct), there is a possibility that a certain level of substitution might exist during the lean season (June to Aug). Fourth, analyses were limited to available information in the West African FCT and additional tables from Burkina Faso. Even though the West African FCT was updated in 2019, not all locally consumed foods could be found in the database. Consensus was reached between the researchers on which alternative food items to use.

In conclusion, our study results show that BEP supplements improved nutrients intakes of pregnant women without displacing foods part of the base diet. Findings of the MISAME-III efficacy trial are critical to determine if the observed positive dietary contribution leads to improved birth outcomes and infant growth.



6

Prenatal fortified balanced energy-protein supplementation and birth outcomes in rural Burkina Faso: A randomized controlled efficacy trial

Redrafted from:

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Prenatal fortified balanced energy-protein supplementation and birth outcomes in rural Burkina Faso: A randomized controlled efficacy trial

Abstract

Background: Providing balanced energy-protein (BEP) supplements is a promising intervention to improve birth outcomes in low- and middle-income countries, however evidence is limited. We aimed to assess the efficacy of fortified BEP supplementation during pregnancy to improve birth outcomes, as compared to iron-folic acid (IFA) tablets, the standard of care.

Methods: We conducted an individually randomized controlled efficacy trial (MISAME-III) in six health center catchment areas in rural Burkina Faso. Pregnant women, aged 15-40 years with gestational age <21 completed weeks, were randomly assigned to receive either fortified BEP supplements and IFA (intervention) or IFA (control). Supplements were provided during home visits and intake was supervised on a daily basis by trained village-based project workers. The primary outcome was prevalence of small-for-gestational age (SGA) and secondary outcomes included large-for-gestational age, low birth weight, preterm birth, gestational duration, birth weight, birth length, Rohrer's ponderal index, head circumference, thoracic circumference, arm circumference, fetal loss and stillbirth. Statistical analyses followed the intention-to-treat principle.

Results: From October 2019 to December 2020, 1897 pregnant women were randomized (960 control, 937 intervention). The last child was born in August 2021 and birth anthropometry was analyzed from 1708 pregnancies (872 control, 836 intervention). Twenty-two women were lost to follow-up in the control group and 27 women in the intervention group. BEP supplementation led to a mean 3.1 percentage points (pp) reduction in SGA with a 95% confidence interval of -7.39 to 1.16 (P = 0.151), indicating a wide range of plausible true treatment efficacy. Adjusting for prognostic factors of SGA, and conducting complete cases (1659/1708, 97%) and per protocol analysis among women with an observed BEP adherence ≥75% (1481/1708, 87%), did not change the results. The

intervention significantly improved the duration of gestation (+0.20 weeks, 95% confidence interval (CI) 0.05 to 0.36, P = 0.010), birth weight (50.1 g, 8.11 to 92.0, P = 0.019), birth length (0.20 cm, 0.01 to 0.40, P = 0.044), thoracic circumference (0.20 cm, 0.04 to 0.37, P = 0.016), arm circumference (0.86 mm, 0.11 to 1.62, P = 0.025) and decreased low birth weight prevalence (-3.95 pp, -6.83 to -1.06, P = 0.007) as secondary outcomes measures. No differences in serious adverse events (fetal loss [21 control, 26 intervention] and stillbirth [16 control, 17 intervention]) between the study groups were found. Key limitations are the non-blinded administration of supplements and the lack of information on other prognostic factors (e.g. infection, inflammation, stress and physical activity) to determine to which extent these might have influenced the effect on nutrient availability and birth outcomes. The trial was registered on ClinicalTrials.gov with identifier: NCT03533712.

Conclusions: The MISAME-III trial did not provide evidence that fortified BEP supplementation is efficacious in reducing SGA prevalence. However, the intervention had a small positive effect on other birth outcomes. Additional maternal and biochemical outcomes need to be investigated to provide further evidence on the overall clinical relevance of BEP supplementation.

Key messages

- Previous studies on the impact of BEP should be interpreted with caution due to the large heterogeneity in supplement types, study populations and quality of study designs. Hence, there is a critical need for well-designed trials with adequate sample sizes to assess the efficacy of BEP during pregnancy to support fetal growth and improve birth outcomes.
- We performed an individually randomized controlled efficacy trial (MISAME-III) to assess the effect of fortified BEP supplementation during pregnancy on birth outcomes in rural Burkina Faso. Early enrolment, high adherence rates and reliable measurements of birth outcomes add to the quality of the study and robustness of the findings.
- The trial did not provide evidence that BEP is efficacious in reducing SGA prevalence, but gestational duration was slightly longer, prevalence of LBW babies lower, and birth weight, birth length, thoracic and arm circumference higher.

Introduction

Improving fetal and newborn health remains a global challenge with an estimated 20 million infants a year born LBW [38], 14 million born preterm [261] and 23 million born SGA [41]. Preterm birth and SGA babies have an increased mortality risk in the first year of life [30] and a higher likelihood to develop non-communicable diseases in adulthood [31]. Undernutrition during pregnancy is an important risk factor for intra-uterine growth restriction, poor fetal development and suboptimal newborn health [26]. In many LMICs, inadequate dietary intakes and increased physiological demands result in maternal macro- and micronutrient deficiencies [255].

At present, the WHO recommends daily oral IFA supplementation as part of routine antenatal care to mitigate the risks for neural tube defects, LBW, maternal anemia and iron deficiency [108]. Prenatal MMN and BEP supplementation are alternative strategies proposed to address maternal nutrient deficiencies and to improve subsequent birth outcomes [227].

Previous evidence indicates that prenatal BEP supplementation led to a reduction in the risks of SGA, LBW, stillbirth and increased birth weight, in particular among malnourished women [100,144]. Based on this evidence, WHO recommends prenatal BEP supplements in populations with a prevalence of >20% underweight pregnant women (BMI <18.5 kg/m^2) [108].

More recent studies, however, show mixed results on the effectiveness of nutritional supplements. The Women First trial, a multi-country RCT, indicated a positive effect of SQ-LNS (118 kcal) before conception or late in the first trimester on mean birth size and SGA [116]. However, an intervention in Niger, that offered MQ-LNS (237 kcal), did not report an impact on birth weight [122]. Similarly, an RCT in Malawi that provided a LQ-LNS (920 kcal) to malnourished pregnant women found no effect on birth weight and length [120].

The huge heterogeneity in the type of supplements (i.e., either MMN-fortified, ready-to-use, lipid-based or not), study designs, inclusion criteria and comparison or control groups make it difficult to draw firm conclusions on BEP supplementation. Specifically designed efficacy studies are therefore needed to assess the importance of investing in prenatal BEP supplements to support fetal growth and improve birth outcomes.

The previous efficacy study MISAME-II assessed the effect of a prenatal fortified BEP, with plant-based protein, as compared to a MMN tablet on birth size in rural Burkina Faso. Although a positive effect was observed for birth length (0.6–6.7 mm increase), the intervention did not reduce SGA prevalence or preterm delivery [132]. The use of an active comparator group with MMN could have potentially led to a masking effect of BEP on SGA, as it has been reported that MMN supplementation decreased LBW, SGA and preterm birth [111]. As many countries provide IFA as the standard of care, the effect of BEP should be assessed relative to this standard. In the MISAME-III study, we assess the effect of a daily prenatal fortified BEP supplement – a large quantity LNS with milk protein – and IFA tablet on SGA prevalence and other birth outcomes, as compared to a daily IFA.

Methods

Our research was reported using the Consolidated Standards of Reporting Trials (CONSORT) 2010 checklist (**Annex 5.1**) [262].

Study design and participants

The MISAME-III protocol was published previously (Chapter 2) [231]. In brief, the study was a community-based, non-blinded individually randomized 2×2 factorial RCT, with directly observed daily supplement intake, conducted in the Houndé health district situated in the Hauts-Bassins region of Burkina Faso. The present manuscript details the primary and secondary birth outcomes only. The maternal and post-natal study outcomes will be reported separately. The study protocol was approved by the ethics committee of Ghent University Hospital in Belgium (B670201734334) and Centre Muraz in Burkina Faso (N°2018–22/MS/SG/CM/CEI).

Women aged between 15 and 40 years and living in the study catchment villages were identified through a census in the study area (n = 10,165). A network of 142 trained village-based project workers visited all eligible women at their homes every five weeks to identify pregnancy early, by screening for self-reported amenorrhea. Potential cases were referred to the health center for a urinary pregnancy test. Once gestation was confirmed, the MISAME-III study purpose and procedures were explained in the local languages Mooré,

Dioula or Bwamu. Study eligibility criteria were: i) pregnancy confirmed by a urinary pregnancy test and ultrasound examination; ii) written informed consent. Exclusion criteria were: i) gestational age ≥21 completed weeks; ii) women who planned to leave the area during their pregnancy or deliver outside the study area; iii) women allergic to peanuts. Study inclusion ran from 30 October 2019 to 12 December 2020 and the final child was born on August 7, 2021.

The climate of the study setting is Sudano-Sahelian, with one dry season conventionally running from September-October to April. Malaria transmission is perennial, with seasonal variations. Regional health statistics from the six health care centers showed that 1.8% of adults suffered from hookworm or another parasitic infection and 0.9% from a sexually transmitted disease in 2021. The prevalence of pregnant women that suffered from a HIV infection was estimated to be 0.7% [263].

Randomisation and masking

We randomly allocated women to the prenatal control or intervention group. A stratified permuted block randomization schedule was used to allocate women to the study groups. These blocks were generated per health center in blocks of eight (4 control, 4 intervention) before the start of the study using Stata (v. 15.1, Statacorp LLC, USA) by a research analyst who was not involved in the study. The allocation was coded with the letters A for the prenatal control and B for the prenatal intervention group and concealed in sequentially numbered sealed opaque envelopes by study employees, not in direct contact with participants. The study midwives, who enrolled the participants, assigned the women to the study groups by drawing a next sealed envelope with the letter code. Post-randomisation, we excluded women without a confirmed pregnancy using the ultrasound examination, women with gestational age ≥21 completed weeks and multi-fetal pregnancies [264].

It was not possible to blind the supplement allocation from study participants and trained village-based project workers because the products are readily identifiable (photos in **Annex 6**). Outcome assessors (study physician, midwives and field supervisors) were different from study collaborators (trained village-based project workers) who distributed the study supplements. However, given the non-blinded nature of the study, outcome

assessors could have been aware of the study group allocation by asking the mother. Researchers who analyzed the data were not blinded.

Procedures

Women in the intervention group received a daily BEP supplement and IFA tablet for the duration of their pregnancy. In a formative study, the most preferred and suitable fortified BEP supplement was selected for administration in the MISAME-III efficacy trial [147,148]. The BEP supplement is an LNS in the form of an energy-dense peanut paste fortified with multiple micronutrients. The product is ready-to-consume, does not require a cold chain and is highly stable with a long shelf life. On average, the 72 g fortified BEP provided 393 kcal and consisted of 36% lipids, 20% protein and 32% carbohydrates. Protein came from soy (61%), milk (25%) and peanut (15%). Furthermore, the MMN content covered at least the daily Estimated Average Requirements (EARs) of micronutrients for pregnant women, except for calcium, phosphorous and magnesium which were lower [265]. The complete nutritional composition of the fortified BEP is provided in **Table 6.1**. Women in the control group received daily only an IFA tablet (65 mg iron [form: FeH2O5S] and 400 µg folic acid [form: C19H19N7O6]; Sidhaant Life Sciences, India), in accordance with the standard of care in Burkina Faso.

Both supplements were delivered on a daily basis and, to the extent possible, consumed under supervision by our trained village-based project workers during home visits. When women had a short and scheduled absence of home, supplements were given to the women in advance and intake was considered non-observed for the respective days. The trained village-based project workers also encouraged pregnant women to attend at least four ANC consultations. Study participants were designated as lost to follow-up if they moved from the study area, withdrew their participation, or if they could not be reached for more than three months.

At enrolment (i.e., first ANC visit), pregnancy antecedents were collected and maternal height, weight, MUAC and hemoglobin concentration were measured. Maternal height was measured to the nearest 1 cm with a ShorrBoard Infant/Child/Adult (Weigh and Measure, USA) and weight to the nearest 100 g with a Seca 876 scale (Seca, Germany); the accuracy of the scales was verified on a weekly basis.

Table 6.1. Nutritional values of the balanced energy-protein supplement for pregnant women[a]

	Mean for 72g (serving size)	**% EAR for pregnant women[b]**	**% RDA for pregnant women[b]**
Total energy (kcal)	393	NA	NA
Lipids (g)	26	NA	NA
Linoleic acid (g)	3.9	NA	30
α-Linoleic acid (g)	1.3	NA	92.9
Proteins (g)	14.5	28.4[f]	20.4
Carbohydrates (g)	23.3	17.3	13.3
Calcium (mg)	500	62.5	50
Copper (mg)	1.3	163	130
Phosphorus (mg)	418	72.1	59.7
Iodine (μg)	250	156	114
Iron (mg)	22	100	81.5
Selenium (μg)	65	133	108
Manganese (mg)	2.1	105[g]	105[g]
Magnesium (mg)	73	25.2	20.9
Potassium (mg)	562	12.0[g]	12.0[g]
Zinc (mg)	15	158	136
Vitamin A (μg RE)[c]	770	140	100
Thiamin (mg)	1.4	117	100
Riboflavin (mg)	1.4	117	100
Niacin (mg)	15	107	83.3
Vitamin B5 (mg)	7	117[g]	117[g]
Vitamin B6 (mg)	1.9	119	100
Folic acid (μg)	400	128	111
Vitamin B12 (mg)	2.6	118	100
Vitamin C (mg)	100	143	118
Vitamin D (μg cholecalciferol)[d]	15	150	100
Vitamin E (mg α-tocopherol)[e]	18	150	120
Vitamin K (μg)	72	80.0[g]	80.0[g]

[a] Ingredients: vegetable oils (rapeseed, palm, soy in varying proportions), defatted soy flour, skimmed milk powder, peanuts, sugar, maltodextrin, soy protein isolate, vitamin and mineral complex, stabilizer (fully hydrogenated vegetable fat, mono- and diglycerides).

[b] Pregnant women aged 19-30 year [81]. EAR is the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group. RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an EAR.

[c] 1 μg vitamin A RE = 3.333 IU vitamin A.

[d] 1 μg cholecalciferol = 40 IU vitamin D.

[e] 1 mg α-tocopherol = 2.22 IU vitamin E.

[f] using the mean weight of all participating women in the trial: 58 kg.

[g] using AI for pregnant women aged 19-30 year [81]. If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed.

AI, adequate intake; EAR, estimated average requirement; IU, international unit, NA, not applicable; RE, retinol equivalent.

Maternal MUAC was measured to the nearest 1 mm with a Seca 212 measuring tape. Hemoglobin concentration was assessed again between 30 and 34 weeks of gestation (i.e., third ANC visit) using a HemoCue Hb 201+ (HemoCue, Sweden); a calibration check was done weekly. Furthermore, a comprehensive socio-economic and demographic questionnaire was administered at enrolment [231].

During each subsequent ANC visit, the study midwives measured all anthropometrics and screened for potential adverse events by checking blood pressure, urine protein, body temperature, edema and fetal activity. Following Burkinabe guidelines, enrolled women received preventative malaria prophylaxis (three oral doses of sulfadoxine-pyrimethamine) at the relevant ANC visits.

Within 14 days of enrolment date, a woman's pregnancy was confirmed by the study physician using a portable ultrasound (SonoSite M-Turbo, FUJIFILM SonoSite Inc., USA). Gestational age was estimated by measuring crown-rump length (7–13 weeks) or by calculating the mean of three to four measurements: bi-parietal diameter, head circumference, abdominal circumference and femur length (12–26 weeks) [154]. In addition to the ultrasound, the physician performed maternal subscapular and tricipital skinfold measurements in triplicate using a Harpenden calliper.

At birth, anthropometry of all neonates was assessed in duplicate within the first 72 hours by study midwives (in practice, all were within 12-h) at the health center. Newborn length was measured to the nearest 1 mm with a Seca 416 Infantometer, whereas birth weight was measured to the nearest 10 g with a Seca 384 scale. Newborn head circumference, thoracic circumference and MUAC were measured to the nearest 1 mm with a Seca 212 measuring tape (photo in **Annex 6**). If there was a large discrepancy between measures (e.g., >10 mm for birth length and >200 g for birth weight), a third measurement was taken. The average of the two closest measures were used for analyses. The accuracy and precision of anthropometric measurements were established regularly through standardization sessions organized by an expert in anthropometry [266].

MISAME-III data were collected using SurveySolutions (v. 21.5, World Bank Group, USA) on tablets by the study physician and midwives and were transferred to a central server at Ghent University on a weekly basis. Questionnaire assignments were sent to the field team once a week including preloaded data collected at the previous ANC visit. We

programmed generic validation codes to avoid the entry of implausible values and improve the quality of data collection in the field. Additionally, data quality checks and missing or inconsistent data were sent back to the field for revision every two weeks.

The quality of ultrasound images and estimation of gestational age was checked for 10% of the examinations on a regular basis by an external gynecologist, using a quality checklist and scoring sheet. The MISAME-III trained village-based project workers collected data on the supplement adherence in both prenatal study groups using smartphones with computer-assisted person interviewing programmed in CSPro (v. 7.3.1, Census Bureau, USA) on a daily basis. Six field supervisors performed monthly quality checks by verifying a trained village-based project worker's work, at random, using a Lot Quality Assurance Sampling system [267].

All field staff received extensive training on all standard operating procedures (incl. Good Clinical Practices) and data collection tools before the start of the trial, with a dry-run period of ±3 months for testing and evaluation in the field. The MISAME-III data collection forms are publicly available [268].

Outcomes

The primary study outcome was the prevalence of SGA, defined as the proportion of newborns with a birthweight below the 10th percentile of the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) newborn size standards for a given gestational age at delivery [150].

The secondary outcomes of the prenatal BEP intervention were prevalence of large-for-gestational age (LGA; >90th percentile of INTERGROWTH-21st reference), LBW and preterm birth, gestation duration (weeks), birth weight (g), birth length (cm), Rohrer's ponderal index at birth [weight/length3 (g/cm^3) ×1000], head circumference (cm), thoracic circumference (cm), arm circumference (mm), fetal loss (<28 weeks of gestation) and stillbirth (died ≥28 weeks of gestation, before or during birth). Fetal loss is further categorized in: i) <22 weeks of gestation; ii) between ≥22 weeks and <28 weeks of gestation, according to the 'Maternal BEP studies Harmonization Initiative'. Birth length and Rohrer's ponderal index were measured to distinguish between short and thin newborns.

To assess safety and SAEs, all field staff was trained to recognize pregnancy related health issues to actively refer participants to the health center. All SAEs (i.e. miscarriage, stillbirth and maternal death) were recorded on a case-by-case basis, and verbal autopsies were conducted for infant and/or maternal deaths that occurred outside a health center.

Statistical analysis

All analyses were documented in the MISAME-III statistical analysis plan prior to analysis, which was validated on October 24, 2019 and published online on November 3, 2020 [268].

We calculated a sample size of 652 pregnant women per prenatal study group (total 1,304 participants) to detect a decrease in SGA of 7 pp between groups, with a power of 80% and a two-sided significance level (i.e., type I error) of 5%, assuming a SGA prevalence of 32% (estimated from Huybregts and colleagues [132]). In MISAME-I [269] and MISAME-II [132], a ~26% loss of information occurred, due to a combination of abortions, miscarriages, stillbirths, multifetal pregnancies, out-migrations, maternal deaths and incomplete data. Hence, the sample size was increased to 888 pregnant women per prenatal study group to accommodate for these potential losses (total 1,776 participants).

Only singleton pregnancies were included in the analysis, as anthropometric measures and fetal loss at birth in multifetal pregnancies are often not primarily nutrition-related. The primary analysis followed the intention-to-treat (ITT) principle. Therefore, we conducted multiple imputation by chained equations of missing outcome measures at birth under the 'missing at random' assumption. Fifty imputations of missing values were done for the lost to follow-up cases to estimate the regression coefficients using the predictors maternal height, BMI, MUAC, hemoglobin, age, gestational age and primiparity at baseline, and month of inclusion.

Descriptive data are presented as percentages or means ± standard deviation (SD). Unadjusted and adjusted group differences were estimated by fitting linear regression models for continuous outcomes to estimate the mean group difference. For binary outcomes, linear probability models with a robust variance estimator were used to estimate risk difference in pp. All models contained health center and randomisation block as fixed effect to account for any possible clustering by the study design. The adjusted models contained a priori defined known prognostic factors of study outcomes at birth,

including maternal height (cm), BMI (kg/m^2), MUAC (mm), hemoglobin (g/dl), age (years) and gestational age at inclusion (weeks), and primiparity. Due to balanced baseline characteristics across prenatal study groups (i.e., < |2.5| pp difference), no other socio-demographic variables were adjusted for in sensitivity analyses.

We conducted the following sensitivity analyses to assess the robustness of the primary findings: i) complete case analysis (i.e., excluding women lost to follow-up); ii) per protocol analysis restricting the intervention sample to women with BEP adherence of ≥75%. The strict adherence rate was calculated by dividing the total number of BEP supplements effectively taken under direct observation of a trained village-based project worker by the theoretical maximum number of prenatal BEP supplements, i.e., the number of days between study inclusion and delivery.

Furthermore, as an exploratory analysis, we tested an interaction term between the intervention group and pre-specified subgroups, including maternal BMI (<18.5 kg/m^2), MUAC (<23 cm), hemoglobin (<11 g/dl), height (<155 cm), age (<20 years), completion of primary education, possible and probable prenatal depression (Edinburgh depression scale ≥10 points and ≥13 points), primiparity, household food insecurity (Household Food Insecurity Access Scale), newborn sex, season of delivery (lean season: June-September) and inter-pregnancy interval (<18 mo). Lastly, we used the approach by Katz and colleagues [270] and Roberfroid and colleagues [269] to assess whether the treatment effect on birth weight and length was constant over percentiles of children's birth weight, birth length and maternal BMI distributions. In this method, differences (and CIs) in birth outcomes between intervention and control groups are estimated as non-linear smooth functions of the percentiles of birth weight, birth length or maternal BMI distributions.

Statistical significance was set at $P < 0.05$ for all tests, except for exploratory interactions tests ($P < 0.10$) as specified in the statistical analysis plan. All analyses were conducted with Stata (v. 17.1, StataCorp LLC, USA).

All SAEs reported by the study physician were evaluated on a continuous basis by the principal study investigators and reported to an independent DSMB when considered related to the supplement. The DSMB (established prior to the start of the efficacy trial) comprised an endocrinologist, two pediatricians, a gynecologist, and a medical ethicist of both Belgian and Burkinabe nationalities. Two virtual DSMB meetings were organized, at

month 9 and 20 after the start of the trial, to review the study progress and discuss all documented SAEs.

Data availability statement

The informed consent form does not allow sharing of personal data outside the research team. Request to access data need to be directed to the ethics committee of Ghent University Hospital through ethisch.comite@uzgent.be. Supporting study documents, including the study protocol and questionnaires, are publicly available on the study's website: misame3.ugent.be.

Results

From 30 October 2019 to 12 December 2020, 2,016 women were assessed for eligibility, of whom 1,897 were randomized (960 control, 937 intervention). Nine women refused to continue participation after randomisation and were excluded. Subsequently, 110 women were excluded post-randomisation, because pregnancy was not confirmed during the ultrasound examination. This resulted in a slight imbalance in the number of women allocated to the control and intervention groups, i.e. women who commenced IFA or IFA + BEP supplementation. Another 59 women were ≥21 completed weeks of gestation at inclusion and 50 women had a multifetal pregnancy and were therefore excluded from the analysis (**Figure 6.1**). The baseline characteristics of mothers included in the study (909 control, 879 intervention) are presented in **Table 6.2.** The control and intervention groups were well-balanced regarding household, maternal and pregnancy characteristics (i.e., < |2.5| pp difference across groups). At baseline, 54.7% of households were food insecure, whereas 7.1% of women were underweight and 37.7% anemic.

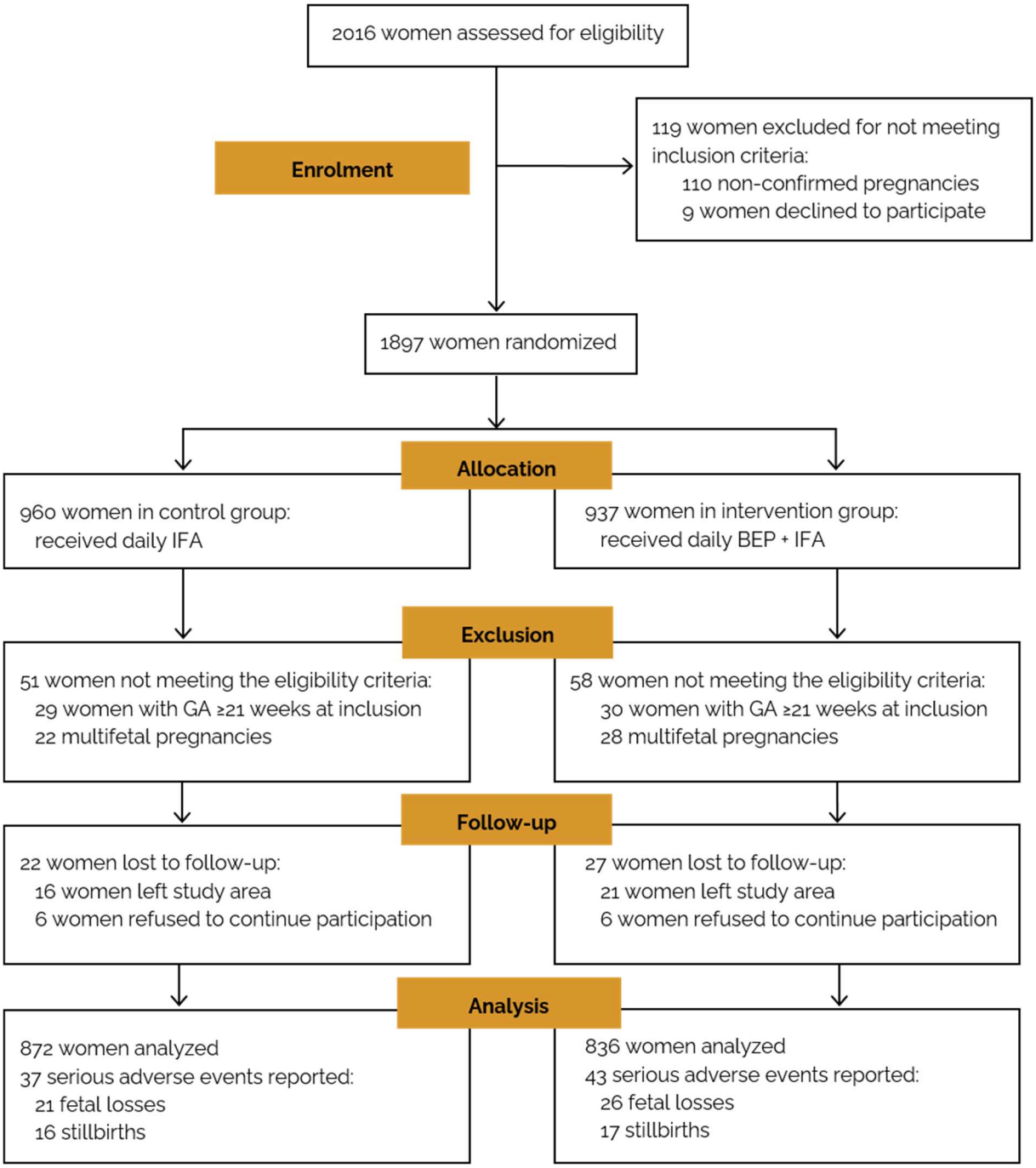

Figure 6.1 CONSORT flowchart.

Table 6.2 Baseline characteristics of study participants

Characteristics	**Control** (n = 909)	**Intervention** (n = 879)
Health center catchment area		
Boni	200 (22.0)	192 (21.8)
Dohoun	95 (10.5)	97 (11.0)
Dougoumato II	172 (18.9)	154 (17.5)
Karaba	93 (10.2)	94 (10.7)
Kari	167 (18.4)	164 (18.7)
Koumbia	182 (20.0)	178 (20.3)
Household level		
Wealth index, 0-10 points	4.51 ± 1.74	4.67 ± 1.75
Household food insecurity[a]	490 (53.9)	488 (55.5)
Improved primary water source[b]	565 (62.2)	551 (62.7)
Improved sanitation facility[c]	539 (59.3)	533 (60.6)
Household size	6.19 ± 4.45	6.20 ± 4.21
Polygamous households	289 (31.8)	287 (32.7)
Head of household		
Age, years	33.4 ± 9.16	33.8 ± 9.33
Male	906 (99.7)	877 (99.8)
Completed primary education	544 (59.8)	519 (59.0)
Maternal		
Age, years	25.1 ± 6.20	25.0 ± 6.18
Ethnic group		
Bwaba	521 (57.3)	506 (57.6)
Mossi	321 (35.3)	303 (34.5)
Other	67 (7.37)	70 (7.96)
Religion		
Muslim	383 (42.1)	372 (42.3)
Animist	213 (23.4)	200 (22.8)
Protestant	147 (16.2)	162 (18.4)
Catholic	131 (14.4)	115 (13.1)
No religion, no animist	35 (3.85)	30 (3.41)
Completed primary education	385 (42.4)	364 (41.4)
Weight, kg	57.9 ± 8.65	58.4 ± 8.69
Height, cm[d]	162 ± 5.91[d]	163 ± 6.05
BMI, kg/m^2	22.0 ± 2.87	22.0 ± 2.87
<18.5 kg/m^2	64 (7.05)	63 (7.17)
Mid-upper arm circumference, mm	262 ± 26.8	262 ± 26.4
Subscapular skinfold, mm	11.9 ± 5.47	12.1 ± 5.58
Tricipital skinfold, mm	11.8 ± 4.76	12.0 ± 4.86
Hemoglobin (Hb), g/dl	11.4 ± 1.47	11.3 ± 1.52
Anemia (Hb <11g/dl)	334 (36.7)	340 (38.7)
Severe anemia (Hb <7g/dl)	2 (0.22)	2 (0.23)
Gestational age, weeks	11.4 ± 4.08	11.5 ± 4.04
Trimester of gestation		
First	574 (63.1)	545 (62.0)
Second	335 (36.9)	334 (38.0)
Parity		
0	198 (21.8)	203 (23.1)
1-2	326 (35.9)	294 (33.4)
≥3	385 (42.4)	382 (43.5)

Data are frequencies (%) or means ± standard deviation.

[a] Assessed using FANTA/USAID's Household Food Insecurity Access Scale [271].

[b] Protected well, borehole, pipe or bottled water were considered improved water sources.

[c] Flush toilet connected to local sewage or septic tank, or pit latrine with slab and/or ventilation were considered improved sanitation facilities.

[d] Height of one woman with a physical disability could not be measured.

BMI, body mass index; FANTA, Food and Nutrition Technical Assistance; Hb, hemoglobin.

Of the 1,788 women who were enrolled at baseline and met the inclusion criteria, 22 (2.4%) women in the control and 27 (3.1%) women in the intervention group were lost to follow-up (**Figure 6.1**). Among the 1,739 pregnancies (887 control, 852 intervention) that were followed up, there were no significant differences (all P >0.1) in fetal loss (21 control, 26 intervention) or stillbirth prevalence (16 control, 17 intervention) across the groups (**Table 6.3**). No maternal deaths occurred in either the control or intervention group. The observed supplement adherence rate was 83.1% for BEP in the intervention group, and 88.8% and 90.6% for IFA in the control and intervention group, respectively.

Table 6.3. Fetal loss and stillbirth prevalence, by prenatal study group

Definition	Control[a] (n = 909)	Intervention[a] (n = 879)	Δ[b] (95% CI)	p value
Fetal death <22 wks of gestation	13 (1.43)	21 (2.39)	1.00 (-0.25, 2.25)	0.12
Fetal death ≥22 wks and <28 wks of gestation	8 (0.88)	5 (0.57)	-0.30 (-1.10, 0.48)	0.45
Stillbirth[c]	16 (1.76)	17 (1.93)	0.18 (-1.08, 1.44)	0.78

[a] Values are frequencies (%).

[b] Risk differences (Δ) in percentage points were estimated using linear probability models with robust variance estimation, adjusted for health center and randomization block as fixed effect to account for clustering by the study design.

[c] Child died ≥28 weeks of gestation, before or during birth.

CI, confidence interval.

BEP supplementation led to a mean 3.1 pp reduction in SGA with a 95% CI of -7.39 to 1.16 (P = 0.151) (**Table 6.4**). The main finding was confirmed by adjusting the regression model for prognostic factors of SGA (-2.93 pp, -7.04 to 1.17, P = 0.161), by (un)adjusted complete cases (850 control, 809 intervention) analyses (-3.15 pp, -7.41 to 1.12, P = 0.148; **Annex 5.1**), and by (un)adjusted per protocol (850 control, 631 intervention) analyses (-3.30 pp, -7.89 to 1.29, P = 0.160; **Annex 5.2**).

The (un)adjusted ITT analyses of secondary outcomes showed that the MISAME-III intervention led to significantly longer gestational duration (+0.20 weeks, 0.05 to 0.36, P = 0.010), birth weight (50.1 g, 8.11 to 92.0, P = 0.019), birth length (0.20 cm, 0.01 to 0.40, P = 0.044), thoracic circumference (0.20 cm, 0.04 to 0.37, P = 0.016) and arm circumference (0.86 mm, 0.11 to 1.62, P = 0.025). Moreover, prenatal BEP and IFA supplementation significantly decreased LBW prevalence (-3.95 pp, -6.83 to -1.06, P = 0.007), as compared to receiving IFA tablets only (**Table 6.4**).

There was no significant difference between the study groups in the prevalence of LGA (0.24 pp, -0.98 to 1.46, P = 0.700) or preterm birth (-1.72 pp, -3.56 to 0.13, P = 0.069), thinner newborns (Rohrer's ponderal index: 0.15, -0.09 to 0.38, P = 0.226) or newborns with larger head circumferences (0.10 cm, -0.05 to 0.25, P = 0.178; **Table 6.4**).

Table 6.4 Efficacy of prenatal BEP supplementation on birth outcomes.

Birth characteristics	**Control**[a] (n = 872)	**Intervention**[a] (n = 836)	**Unadjusted Δ**[b] (95% CI)	**P value**	**Adjusted Δ**[b] (95% CI)	**P value**
Small-for-gestational age	243 (27.9)	207 (24.8)	-3.11 (-7.39, 1.16)	0.153	-2.93 (-7.04, 1.17)	0.161
Large-for-gestational age	14 (1.55)	15 (1.75)	0.24 (-0.98, 1.46)	0.700	0.20 (-1.01, 1.40)	0.747
Low birth weight	107 (12.3)	69 (8.27)	-3.95 (-6.83, -1.06)	0.007	-4.07 (-6.86, -1.28)	0.004
Preterm delivery	40 (4.65)	25 (2.95)	-1.72 (-3.56, 0.13)	0.069	-1.82 (-3.67, 0.02)	0.052
Gestational age, weeks	39.9 ± 1.78	40.1 ± 1.48	0.20 (0.05, 0.36)	0.010	0.22 (0.06, 0.37)	0.006
Birth weight, g	2986 ± 450	3038 ± 427	50.1 (8.11, 92.0)	0.019	49.7 (10.8, 88.7)	0.012
Birth length, cm	48.2 ± 2.25	48.4 ± 2.13	0.20 (0.01, 0.40)	0.044	0.20 (0.01, 0.39)	0.037
Ponderal index[c]	26.5 ± 2.67	26.7 ± 2.67	0.15 (-0.09, 0.38)	0.226	0.15 (-0.08, 0.38)	0.208
Head circumference, cm	33.4 ± 1.64	33.5 ± 1.53	0.10 (-0.05, 0.25)	0.178	0.10 (-0.04, 0.24)	0.154
Thoracic circumference, cm	31.7 ± 1.84	31.9 ± 1.67	0.20 (0.04, 0.37)	0.016	0.20 (0.05, 0.36)	0.011
Arm circumference, mm	100 ± 8.43	101 ± 8.18	0.86 (0.11, 1.62)	0.025	0.89 (0.18, 1.60)	0.014

[a] Values are frequencies (%) or means ± standard deviation.

[b] Unadjusted and adjusted group differences (Δ) were estimated by fitting linear regression models for the continuous outcomes, to estimate the mean group difference, and using linear probability models with robust variance estimation for the binary outcomes, to estimate risk difference in percentage points. All models contained health center and randomization block as fixed effect to account for clustering by the study design. Adjusted models additionally contained a priori set known prognostic factors of birth outcome including maternal age, primiparity, gestational age, height, mid-upper arm circumference, body mass index and hemoglobin level at study enrolment.

[c] Ponderal index calculated as birth weight in g / (birth length in cm)3 × 1000.

CI, confidence interval.

Furthermore, (un)adjusted subgroup analyses (P interaction <0.10) indicated larger intervention-related reductions in SGA prevalence among women with a baseline MUAC >23 cm, women >20 years of age, non-anemic (Hb ≥11 g/dl) women at baseline and among female newborns (-6.73 pp, -12.6 to -0.81, P = 0.026; **Annex 5.3**).

Daily BEP and IFA supplementation had a stronger positive effect on birth weight and length at lower percentiles of the birth weight and length distributions, respectively (**Figure 6.2 and 6.3**). We did not find evidence that the treatment effect on birth weight or length was modified by maternal BMI at baseline (**Annex 5.4 and 5.5**).

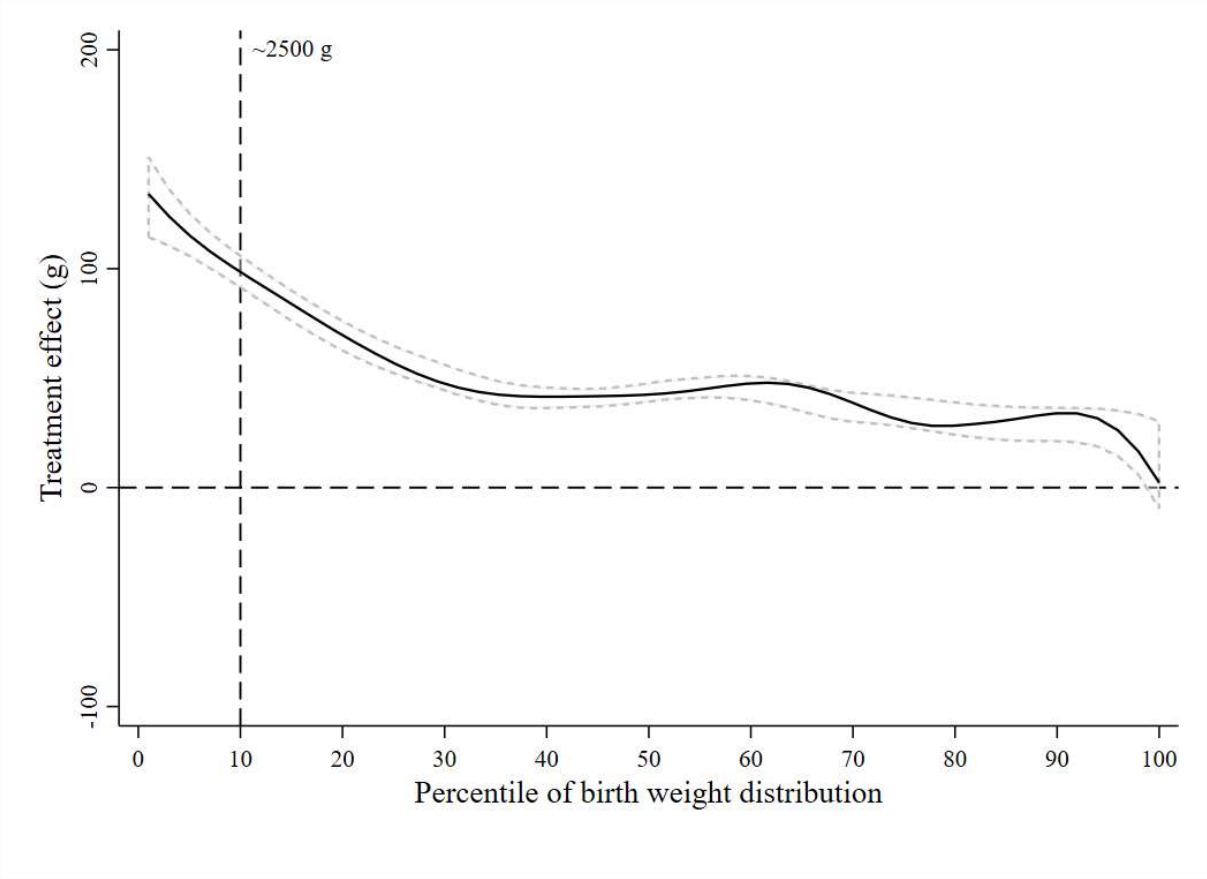

Figure 6.2 Treatment efficacy on birth weight across the distribution of birth weight. The estimated difference in birth weight between the women who received the balanced energy-protein (BEP) supplement and iron folic acid (intervention) and those who received only iron and folic acid (control) is shown as a function of the percentiles of birth weights. The zero line indicates no efficacy of BEP. The positive y values indicate a higher birth weight in the intervention group, and the negative y values indicate a lower birth weight. The central solid black line represents the smoothed treatment efficacy, with upper and lower dashed 95% confidence bands, using complete cases.

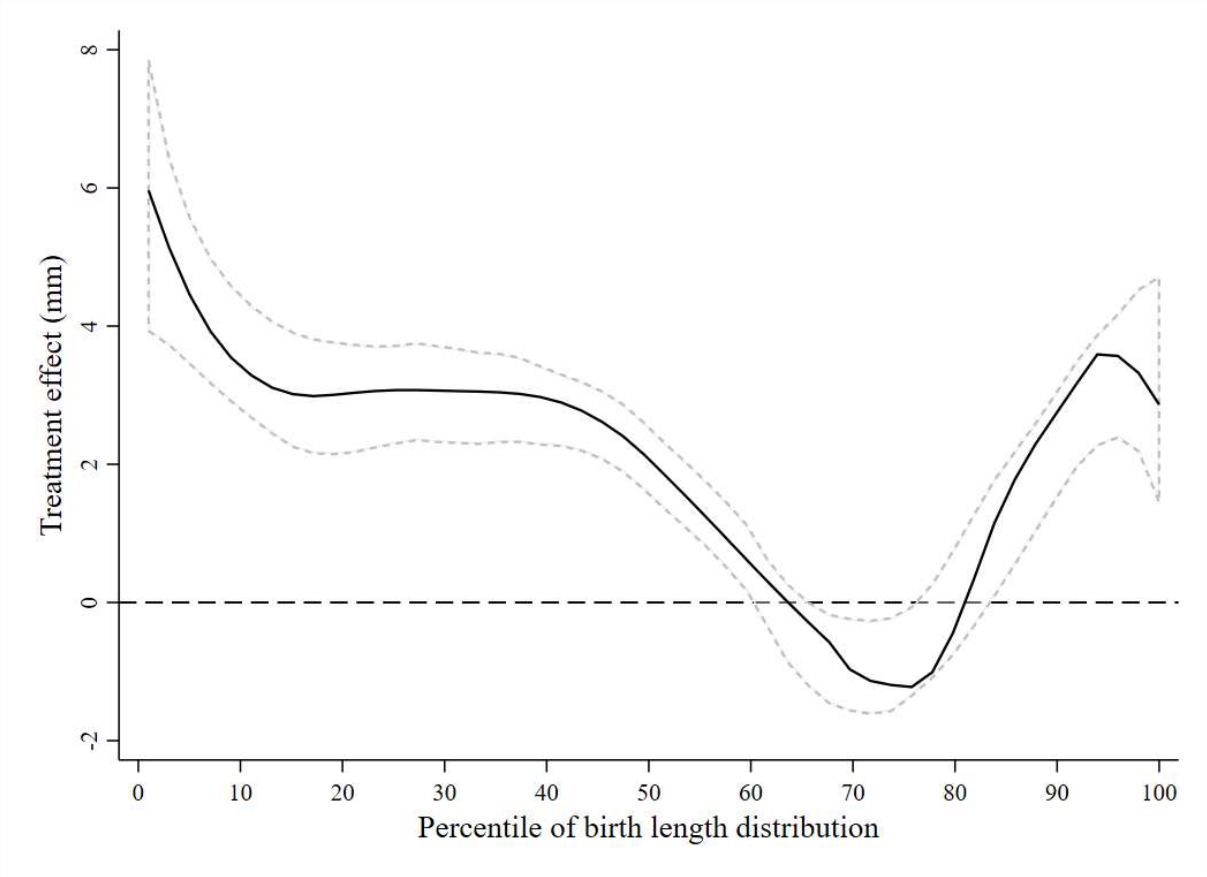

Figure 6.3 Treatment efficacy on birth length across the distribution of birth length. The estimated difference in birth length between the women who received the balanced energy-protein (BEP) supplement and iron folic acid (intervention) and those who received only iron and folic acid (control) is shown as a function of the percentiles of birth lengths. The zero line indicates no efficacy of BEP. The positive y values indicate a higher birth length in the intervention group, and the negative y values indicate a lower birth length. The central solid black line represents the smoothed treatment efficacy, with upper and lower dashed 95% confidence bands, using complete cases.

Discussion

The MISAME-III trial did not provide evidence that prenatal fortified BEP supplementation was efficacious in reducing SGA prevalence. However, the intervention led to improvements in gestational length, birth weight, birth length, thoracic and arm circumference, and decreased LBW prevalence.

This study was primarily designed to reduce the prevalence of SGA. A meta-analysis of (quasi-) RCTs concluded that prenatal (fortified) BEP supplementation resulted in a 11–51% (95% CI) reduction in SGA infants [144], whereas a Cochrane review of RCTs concluded that BEP led to a 10–31% decrease in the risk of SGA [100]. However, the supplements studied

varied tremendously in terms of energy (417–1017 kcal), protein (7–40 g) and micronutrient composition. In addition, various comparison groups and timing of supplementation were applied [272]. A direct comparison between results from these trials and our findings is therefore difficult. The previous MISAME-II trial, conducted in the same health district, can be considered the most comparable as a similar LNS type supplement was used [132]. Huybregts and colleagues reported no meaningful effect on SGA, neither on preterm birth nor a list of anthropometric measures at birth. However, the intervention led to a positive effect on birth length (+0.6–6.7 mm). An important difference to take into account for comparison of the results is the use of MMN (MISAME-II) vs. IFA tablets (MISAME-III) in the control group. Similarly, compared to IFA, prenatal LNS in The Gambia was not associated with SGA, birth weight, length or head circumference [121]. Trials offering reduced amounts of LNS, so-called small-quantity LNS (20 g/d; 118 kcal/d), compared to IFA, found no effects on SGA prevalence, but reported increases in birth weight (3–166 g) and reduced risk of LBW (-4–61%) in Ghana [118], lower offspring stunting (-3–29%) in Bangladesh [117] and higher newborn MUAC (0.1–0.3 mm) in Malawi [119].

The observed effect of BEP on birth weight, with an increase of 8–92 g (95% CI), is comparable to earlier studies, reporting increments of 30–117g [144] and 5–77 g [100]. This effect on birth weight and the 1-40 mm effect in birth length observed by our study can, at least partially, be attributed to the concurrent 0.4–2.5 days increment in gestational age at birth. Also, we speculate that the modest improvements in birth anthropometry is the result of the MMN compartment of the supplement, as previous research has shown that MMN led to an increase in birth weight resulting in lower proportion of LBW and SGA births [111].

Our data suggest that there was no risk in providing BEP to women that were not underweight at early gestation. The BEP did not impact the prevalence of LGA and no increase in C-sections was observed.

Subgroup analyses revealed that the intervention was efficacious in reducing SGA prevalence among mothers with a more adequate baseline nutritional status (e.g., non-anemic, higher MUAC). Similarly, in The Gambia, subgroup analysis indicated that the efficacy of BEP and/or MMN supplements might potentially be mediated through larger gestational weight gain (i.e., well-nourished women) [121]. These results are in contrast with

previous findings showing that nutritional supplementation had larger treatment effects among inadequately nourished pregnant women at early gestation, including underweight mothers [132], women with negative energy balance [273], food insecure households [117] and primiparous mothers [118]. A possible explanation for our findings could be that the additional nutrients provided by the BEP supplement are used to meet the demands of pregnancy by malnourished women and do not reach the fetus. Furthermore, our subgroup analysis showed that the impact was more profound among female newborns, while other studies found a larger effect of nutritional supplementation in males [274,275].

Some explanations for the lack of strong efficacy of fortified BEP as compared to IFA can be put forward. First, frequent acute and chronic infections during pregnancy, which are often prevalent in LMICs [276], can lead to nutrient losses and nutrient sequestration in the mother, which in turn may have limited the quantity of nutrients available to the fetus. Our trial did not collect data on maternal infection during gestation, but if prevalent in this setting, acute or chronic infection may have reduced the efficacy of the BEP supplements provided. Likewise, acute or chronic infection in the child could have limited the potential benefits from the nutrients received by the fetus during pregnancy. Second, starting fortified BEP supplementation during early pregnancy alone might not be sufficient to prevent adverse birth outcomes. Our subgroup analyses indicated that the BEP intervention was potentially more efficacious among women who started pregnancy with a better nutritional status; hence, preconception supplementation may confer greater benefits on birth outcomes. Although the Women First trial found that providing LNS and BEP at least 3-mo prior to conception did not yield additional benefits on child linear growth at birth relative to starting BEP supplementation during gestation [116], compelling evidence remains scarce and supplementation during the preconception period may warrant further exploration.

A major strength of our study was the high acceptability of the fortified BEP supplement, evaluated in a two-phase formative study (Chapters 3 and 4) [147,148], and strong emphasis on daily observed intake. The high adherence rate reported in this trial (~90% for IFA in both study groups and ~83% for BEP in intervention group) ensures the reliability of our results, compared to those from studies that rely on maternal recall of adherence. Moreover, the daily observed supplementation reduced the possible risk of sharing the

supplement with other household members and supported micronutrient adequacy following existing requirements. A cross-sectional dietary intake assessment showed that BEP did not displace energy and nutrient intake from the usual diet (Chapter 5) [277]. Hence, we can almost rule out a substitution effect that could have limited the efficacy of the BEP to support fetal growth and reduce SGA. Another strength was the early enrolment of participants, as a result of a monthly visiting schedule at home by trained village-based project workers, who received refresher trainings and close supervision by the MISAME-III field team. Finally, in almost all cases, birth weight was measured almost immediately after birth.

Our study also had some limitations. First, it was impossible to blind mothers or MISAME-III collaborators to the intervention allocation. Though care was taken to blind the study midwives measuring birth anthropometry, we cannot rule out that intervention allocation was unveiled by asking the mother which supplement she received. Second, it is possible that improvements are not visible through birth anthropometry and maternal biomarkers (to demonstrate any micronutrient deficiencies) or placental indicators are needed to assess an intermediate effect of the fortified BEP supplement on maternal nutritional status and placental function (e.g., fetal hypoxia might inhibit fetal growth) [132,278]. Ongoing multi-omics sub-studies will provide insight into the biochemical profiles of mother infant dyads to address this current limitation. Third, we lacked data on maternal infection, inflammation, stress and physical activity levels and could not determine the extent to which these prognostic risk factors may have influenced nutrient availability or poor birth outcomes [279].

In conclusion, we did not observe a statistically significant effect of fortified BEP supplements and IFA tablets on SGA prevalence, as compared to IFA tablets alone in rural Burkina Faso. Although, small positive effects were noticed on birth weight, gestational age and LBW prevalence. Exploratory analyses suggests that prenatal BEP supplementation was more beneficial for mothers that enter pregnancy more adequately nourished. MISAME-III sub-studies will evaluate the efficacy of prenatal BEP and IFA tablets on additional maternal and child biochemical parameters to provide more insight in mechanisms of action and the clinical relevance of providing BEP supplementation.

7

General discussion

Despite the significant effort over the last decades to meet the Sustainable Development Goals to prevent all forms of malnutrition in women and children, there are still major challenges to be overcome to eliminate malnutrition. Poor maternal nutritional status not only affects a woman's health, but also influences that of her child – from the early stages of fetal development throughout childhood, and persisting into adulthood. It is estimated that 32.4 million infants are born SGA [37], 20.5 million have a low birth weight and 14.9 million are preterm [39]. These infants are more vulnerable to illness and have a high risk of dying in their early years. Impaired growth and cognitive development and metabolic diseases later in life are also more frequent [24]. At present, the COVID-19 pandemic, climate shocks and ongoing conflicts is expected to exacerbate malnutrition in mothers and children [8,9].

Pregnancy is a window to future health, and nutrition interventions have the potential to deliver additional energy and nutrients which are required during pregnancy to meet maternal needs and support fetal development. In the context of global efforts to implement effective nutrition interventions to address maternal and child malnutrition, this present PhD work provides robust evidence on the acceptability and effectiveness of fortified BEP supplementation in a rural setting in Burkina Faso. This chapter presents an overview of the key findings, their implications and methodological issues of the studies conducted as part of the MISAME-III research project. Finally, recommendations for future research and policy are discussed. As the interpretation of the results of each individual study has been discussed in previous chapters, this general discussion will focus on the broader perspective of this PhD research.

7.1 Main research findings

The first two studies of the PhD thesis present the formative study to select the most suitable BEP supplement for administration in the RCT. The evaluation of 12 product formulations provided insight that products with a sweet flavor and resemblance to familiar foods were preferred (**Chapter 3**). Perceived health benefits were identified as a promoting factor, and an unpleasant odor of a product as a limiting factor for daily consumption. Sharing the BEP supplement was raised as a possible concern for the impact of the intervention. Second, the two highest-ranked products, a lipid-based peanut paste and vanilla biscuit, were evaluated to assess medium-term acceptability and adherence (**Chapter 4**). Both BEP supplements were well accepted by pregnant women. Structured and easy-to-understand communication by health care professionals on the benefits, and engaging community leaders and family members were identified as important promoting factors for adherence. For implementation in the main trial, the lipid-based peanut paste fortified with MMN was selected.

The goal of any supplement is to provide additional nutrients to the usual dietary intake. As such, one key investigation was to assess the effect of prenatal BEP on dietary intake (**Chapter 5**). Study results showed that the BEP supplement significantly increased dietary energy and macro- and micronutrient intakes, with a difference in median energy intake equivalent to a daily dose of the BEP supplement. Nutrient adequacy of the usual diet was low for all micronutrients and increased to the EAR for pregnant women when including the BEP supplement. In conclusion, this study showed that BEP supplementation improved nutrient intakes of pregnant women without displacing foods that are part of the base diet.

The lipid-based peanut paste fortified with MMN was selected for the main trial of the MISAME-III research project. In a large-scale randomized controlled efficacy trial, the effect of prenatal BEP supplementation on birth outcomes was assessed (**Chapter 6**). Early enrolment, high adherence rates and reliable measurements of birth outcomes contributed to the quality and robustness of the findings. The trial did not provide evidence that BEP supplementation is efficacious in reducing SGA prevalence, but gestational duration was slightly longer, prevalence of LBW babies lower, and birth weight, birth length, and thoracic and arm circumference higher.

7.2 Implications of the research findings

7.2.1 Provision and acceptability of food supplements

The world's largest humanitarian organization WFP is using food assistance "to build a pathway to peace, stability and prosperity for people recovering from conflict, disasters and the impact of climate change". For decades, WFP has been providing CSB Plus (a maize or wheat blended with soya beans, fortified with vitamins and minerals, processed into a flour) as a supplement to pregnant and lactating women with malnutrition (usually identified by low MUAC) or living in highly food insecure settings [280]. This food supplement is provided in 25 kg bags and despite the instruction not to share the supplement, CSB Plus is often regarded by beneficiaries as family food and is associated with sharing amongst household members and replacement of other foods of the usual diet [195]. Both situations could result in a lower than intended impact of the supplement. A ready-to-use supplement has the advantage that it is individually packed and does not require any time or resources. This makes it a convenient, compact vehicle to provide energy, compared to a porridge that requires preparation (e.g. women have to collect and prepare firewood to boil water) and consists for 90% of water. For this reason, the present research on a ready-to-use fortified food supplement for pregnant women is highly relevant.

In the formative study, we showed that BEP supplementation is suitable for daily consumption during pregnancy. A broad range of product formulations was tested in Burkinabe pregnant women living in study area. We selected an acceptable supplement for implementation in the main trial, which allowed us to obtain high adherence levels. We consider the formative study key for the trial, as it provided useful insights into product preferences and context-specific drivers and barriers. As an example, in our setting, the mango/tomato onion bar, tomato onion biscuits or (un)seasoned pillows received lower acceptability scores, making them a less favorable choice. If we would have selected any of these products, the adherence rates would most probably have been lower. During the 2016 BMGF expert consultation however, a 'bar' type supplement was ranked in the top 3 preferred forms for a BEP supplement [146]. This discrepancy highlights the importance of formative research.

In different geographical regions, other product preferences might exist. In Nepal, two similar acceptability studies were conducted to evaluate the acceptability of BEP supplements in the South Asian context [281,282]. Some flavors of the supplements were adjusted to regional food customs (e.g. a 'savory masala bar' and 'savory curry biscuit' were tested). The results indicate, similar to our study, that an aversion to a certain (strong) smell or taste of supplements could hamper daily consumption. In Nepal, the lipid-based peanut paste and vanilla biscuit also received high acceptability ratings and were tested over an 8-week period [281]. Both supplements appeared to be well accepted during pregnancy. These initial findings are encouraging for the potential of both supplement types. To assess the generalizability of the findings, similar studies in other countries are needed.

In addition, results from the daily choice period (biscuit or paste) indicated that 70% of women requested to change supplement type over the course of two weeks, which might suggest that variety in BEP supplements could potentially increase adherence in real-life settings.

Key implication: The findings of our study show that prenatal fortified BEP supplements in the form of a lipid-based peanut paste and vanilla biscuit are well accepted in a rural west-African setting. In other regions of the world, food habits and preferences might however differ. We therefore want to highlight the importance of involving the target population in selecting a suitable food supplement to promote optimal use.

7.2.2 BEP supplementation to fill nutrient gaps

Globally, looking at data between 2000 and 2015, progress has been made to improve women's nutrition in terms of low BMI, but very little change in short height or anemia is observed [14]. Despite hotspots in sub-Sahara Africa, where the prevalence of low BMI (<18.5 kg/m2) is more than 20%, we also observed an improvement over time in women with a low BMI in the study area (~12% in MISAME-II in 2006-2007 vs. ~7% in MISAME-III in 2019-2020). The idea that the women are nutritionally better off could be a possible explanation for the lower than hypothesized effect on SGA. It might be that the added energy and protein of the BEP supplement did not provide an additional benefit to support fetal development. Looking beyond nutritional status based on maternal weight and

height, the dietary intake assessment did show that women's intakes were low for zinc, calcium, thiamine, riboflavin, niacin, vitamin B6 and vitamin C, and very low for iron, vitamin A, folate and vitamin B12. Thus, while less women in the study area were underweight compared to 13-14 years ago, they were still at risk of micronutrient deficiency.

Despite the lack of a significant effect on SGA births, our other research findings do suggest that fortified BEP supplementation has the potential to provide pregnant women nutritional support. First, data on mean energy intake indicated that women's diet did not account for increased energy demands in the second and third trimester. Second, longitudinal data of the MISAME-III trial that measured usual food group diversity showed that the mean number of food groups (<5) and the proportion of women achieving MDD-W (<45%) were low, with modest seasonal variation [283]. Third, more than half of the households participating in the trial appeared to experience food insecurity (~55% in the overall sample, well-balanced over the study groups).

Key implication: Pregnant women in a rural setting in Burkina Faso are at risk of micronutrient deficiencies and many experience household food insecurity. We provide evidence that fortified BEP supplementation is able to provide additional energy and nutrients without displacing (nutrient-dense) foods from the usual diet.

7.2.3 Nutrient composition of BEP supplements

The composition of the BEP supplement used in the MISAME-III trial was established during an expert consultation convened at the BMGF in 2016 [146]. Specific requirements were set for macro-and micronutrients corresponding to the needs of pregnant and lactating women.

It was proposed that the supplement provided approximately 50% of the additional protein requirements in the third trimester (14-18g). The requirement for protein quality indicated that the BEP supplement should include, at least partially, animal-source protein as many pregnant women in LMICs have difficulties meeting the recommended intake of essential amino acids as most intake comes from plant sources [255]. Animal-source protein is considered high-quality as it contains all essential amino acids and resemble the proteins of the human body in their composition so it can be readily digested and used for growth,

repair and maintenance [16]. The lipid-based peanut paste used in MISAME-III contained 14.5 g protein per portion, with 25% from milk. All essential amino acids are present in milk in relatively high amounts, including sulfur-containing amino acids, such as methionine and cysteine, which play a crucial role in maintenance and integrity of cellular systems [284].

The dietary intake assessment confirmed that the diet consisted of mainly plant-based protein, but despite a difference in protein source used (plant-based in MISAME-II vs. partially animal-based in MISAME-III), similar modest effects were found on birth size. Based on the results of the current research, it is therefore difficult to draw conclusions on whether it is necessary to include (animal) protein in the food supplement to support in utero growth.

Our acceptability study did point out another important aspect to consider, which is the milk sugar lactose when using milk solids in supplements. While the lactose content of the lipid-based peanut paste was within the tolerable limit of 12 g [285], the study team received complaints from some women about bloating and more frequent stools during the 10-week test period. We therefore decided to continue with an adjusted product formulation containing only 6 g lactose in the main trial, taking into account the high prevalence lactose maldigestion in Africa (estimated between 70-90%) [286].

Concerning the fat content, a broad range of 10-60% of energy was proposed. The BEP supplement selected for the main trial was an LNS type and contained 26 g fat (59.5% of total energy of one serving). Addition of omega-3 fatty acids (α-linolenic acid), specifically DHA – which is potentially important for fetal development – was considered optional. The selected supplement contained 3.9 g α-linolenic acid, with the specific goal to boost the ratio omega-3/omega-6 in the context of growing evidence. A 2018 Cochrane review concluded that omega-3 supplementation during pregnancy reduced the prevalence of preterm birth with 11% and LBW with 10% [70]. On the contrary, an RCT in Mexico that supplemented pregnant women (n = 1,094) with DHA capsules (from an algal source) did not find any improvement in birth outcomes compared to the control group [287], while follow-up of the children showed a positive impact on general cognitive abilities at the age of 5 [288].

The effect on LBW (−3.95 pp) and modest non-significant effect on preterm birth (−1.72 pp) found in the MISAME-III trial, could thus potentially be linked to the addition of α-linolenic acid in the supplement.

Irrespective of the BEP supplement selected for the main trial, the specific composition of any BEP supplement (i.e. carbohydrate or fat-rich products) could influence the impact on birth outcomes. The metabolism of carbohydrates in different tissues is more tightly regulated than fat metabolism, as carbohydrate body stores are more limited [289]. During pregnancy, both metabolic processes could play a role in fetal growth. Glucose crosses the placenta easily, while lipids cross more difficult. Yet, it is suggested that maternal metabolism plays a major role in fetal adiposity and growth. The precise mechanism remains however to be elucidated, so no conclusions can be drawn with regard to the hypothesized impact of the other supplement types tested in the formative study [290].

Finally, concerning the mineral content of the supplement, the addition of calcium (500 mg) differs from the widely available UNIMMAP formulation that does not contain calcium. Whether the inclusion of calcium in the BEP supplement has added value is difficult to identify from our study results. Calcium requirements are higher during pregnancy and calcium supplementation is recommended by WHO for the prevention of pre-eclampsia in areas where dietary calcium intake is low [83]. While, calcium might block the uptake of iron and zinc [291], research has shown this could be a short-term effect and compensated over time [292,293]. Vitamin C, enhancing iron absorption, might also play a compensating role. Overall, the more components are in one (food) matrix, the more complicated it becomes to identify single effects and draw conclusions on the nutrient composition of prenatal food supplements.

Key implication: Our study results do not offer clear compositional guidance for BEP supplementation. Additional studies are needed to test the added value of including milk protein or omega-3 fatty acids (DHA) in a fortified BEP supplement for pregnant women. This would address some of the challenges on the manufacturing and consumer side, such as high (dairy) ingredient prices or lactose maldigestion in women.

7.2.4 Effect of BEP supplementation on birth outcomes

The benefit of BEP supplementation on birth outcomes was lower than hypothesized. Improvements in fetal growth is regulated by several pathways linking maternal nutrition to birth outcomes as explained in the introduction of this PhD thesis. Different risk factors may play an important interfering role in the effect of BEP supplementation on birth outcomes. This view is supported by a recent systematic review on estimating population attributable fraction for risk factors for SGA birth in LMICs. The investigators calculated that in sub-Sahara Africa three main factors play a role, namely nutrition (25.05%), environment and other exposure (13.01%), and general health issues or morbidity (10.72%) [294].

Impact on SGA

The prevalence of SGA is essentially based on the estimation of pregnancy duration. Compared to the previous MISAME-II study, gestational age in MISAME-III, was solely estimated by the golden standard of ultrasound examination. In MISAME-II, LMP was used for 21% of women to estimate gestational age as ultrasound measurement was not possible. Using LMP for gestational age determination is however imprecise, which might explain the difference between our findings on birth size and those of the previous MISAME trial and other studies that often rely on LMP.

A second possible explanation for the difference in the observed effect, is the use of another fetal growth reference to identify an infant as SGA. In the MISAME-II study, the population-based Canadian Reference for Birth Weight for Gestational Age [295] was used to define birth weight lower than the 10th centile, while in MISAME-III, we applied the INTERGROWTH-21st reference [150], a more recent international anthropometric standard for newborn size. If the INTERGROWTH-21st reference is applied to data from MISAME-II, we see that the prevalence of SGA is 23.8% compared to 34.1% originally reported. For MISAME-I, we calculated 29.6% of all infants SGA versus 39.7% originally reported. Although our study was still powered to identify a significant reduction, it is expected that it may be difficult to achieve a large reduction in SGA with a lower baseline prevalence.

Impact on (low) birth weight

BEP supplementation improved mean birth weight by 50.1 g, with an average birth weight of 2,986 g (±450) in the control and 3,038 g (±427) in the intervention group. The clinical importance of this improvement of 50.1 g is subject to debate. To date, there is no data available to determine whether this translates into better growth and survival. For the children that already have a low weight at birth, a slight improvement could potentially make a difference in survival. The birth weight distribution (Chapter 6, **Figure 6.2**) showed that infants on the left tail of the distribution benefited more, which is an interesting and promising finding from a public health perspective. Furthermore, the intervention significantly reduced the prevalence of LBW with 3.95 pp, which can translate into a meaningful decrease in mortality and morbidity risk as this category includes both premature and growth-restricted infants [196,296]. Follow-up of the newborns of MISAME-III will provide more insight to estimate the clinical importance of higher weight at birth.

Impact on preterm birth

The prevalence of preterm birth in the MISAME-III trial was found to be low (3.8% in total sample). While this substantially differs from the prevalence of 15% found in MISAME-II, other African studies have found similar low prevalence rates for preterm birth: 7.2% in Tanzania (n = 4,226) [297] and 3.9% in Ghana (n = 1,288) [298]. Both studies used ultrasound examination for the estimation of gestational age. As discussed earlier, gestational age of 21.4% of women in MISAME-II was estimated using LMP as no ultrasound was available. If the prevalence rate of preterm birth is calculated in this particular subsample of women, the prevalence of preterm delivery is 46.5%, indicating an overestimation of preterm births using LMP. For MISAME-III, we also calculated GA at birth using both methods. The mean GA in weeks using ultrasound was 39.8 ± 1.9 in the control group and 38.2 ± 4.0 using LMP, respectively. Calculating preterm delivery using only LMP results in a prevalence of around 32% versus 5% using ultrasound measurements. This means the use of LMP to estimate gestational age is overestimating preterm delivery.

We thus want to highlight that LMP is a more imprecise measure and systematic error leading to a wider distribution of the values (i.e. larger SD) overestimates the problem (i.e. more infants are below the cut-off value). This could be a possible explanation for higher

prevalence rates and a relative larger intervention impact in terms of pp on preterm birth found in other studies, that often rely on a combination of ultrasound and LMP or solely LMP to estimate gestational age of women, compared to this trial.

Impact on stillbirth

We did not observe any effect of BEP on stillbirth. In both groups, the number of stillbirths was very low (n = 16 control group, n = 17 intervention group). Therefore, no conclusions can be drawn on a possible reduction of stillbirths as a result of the nutrition intervention. First, because the biological mechanism underlying a risk reduction in stillbirth is still unclear [100], and second, because the incidence of stillbirths should also be viewed in the context of the health care system. In MISAME-III, we had a very good support system of trained midwifes and a large community of village-based project workers who received regular training on pregnancy health care services. For a rare outcome such as stillbirth, a meta-analysis, combining evidence from several studies, is the golden standard and is a better indicator to assess the effect of BEP supplementation on stillbirth.

Impact on vulnerable women

Our subgroup analysis showed larger intervention-related reductions in SGA prevalence among women with a higher baseline MUAC, above 20 years of age, and non-anemic status. This lays a basis for the idea that infants of well-nourished women benefited more of daily BEP supplementation, which is in contrast to the findings in MISAME-II and other supplementation trials, showing that nutritional supplementation had larger treatment effects among inadequately nourished pregnant women [117,132,273]. This finding should be interpreted with caution, as subgroup analysis may not have sufficient power to draw firm conclusions. Meanwhile, the identification of subgroups of mothers who experience greater benefits from BEP is useful to inform public health programs and policies. Hence, we discuss explanations for the discrepancy with earlier findings. First, due to the small group of women with a low BMI (7.1%) in our sample, we are limited in drawing strong conclusions if BEP exerted strong effects in this subgroup. Our study population might thus potentially not have been (sufficiently) vulnerable to macronutrient deficiencies (i.e., WHO antenatal care guidelines suggest the use of BEP where the population-level prevalence of low BMI (<18.5) is greater than 20% [108]).

Second, distinguishing between potential to *benefit* from potential to *respond*, could help to explain the results. The former is more likely when the mother is more vulnerable, for example when already experiencing undernutrition. However, some mothers who are undernourished may actually be less likely to *respond* to a nutrition intervention due to other constraints on growth, e.g. infection, inflammation, inadequate health care. It could be speculated that the mother and fetus compete for the same nutrients during pregnancy and undernourished women use the nutrients from BEP supplements for own maintenance, while nutritionally-better off women can pass it on to the fetus [299]. In MISAME-III, women who were nutritionally better off seemed to demonstrate more potential to *respond* to the BEP supplement.

Key implication: The MISAME-III efficacy trial showed a moderate positive effect of prenatal fortified BEP supplementation on birth outcomes. Given the robust study design and high adherence rates to daily supplementation, our findings suggest that restriction in fetal growth is unlikely to be reduced by BEP supplementation alone in this population. The prevalence of infants born SGA was 26% in our total study population, representing a large vulnerable group. To tackle the long-term consequences of SGA and other poor birth outcomes, there is an urgent need for a better mechanistic understanding of the effect (or lack of effect) of BEP supplementation on fetal development. In addition, longer follow-up of the children is required to evaluate the importance of improved size at birth (mean weight +50 g), with a larger treatment effect for infants with a lower weight at birth, on linear growth, cognitive and motor development to determine the overall importance of BEP supplementation.

7.3 Methodological issues

In this section, several general methodological strengths and limitations are discussed.

7.3.1 Study methods to elucidate factors influencing acceptability

In the formative study, there was a hypothetical question on the willingness to pay for supplements as a proxy for acceptability and product use. There is however a risk of hypothetical bias (overestimation of real willingness-to-pay) which is associated with this assessment method. The results on the amounts in CFA indicated by the women should therefore be interpreted with caution. Other strategies to investigate willingness-to-pay, such as face-to-face interviews, auction or choice experiments are other strategies that could be used in future research that provide a more sophisticated approach to elucidate a true willingness-to-pay for food supplements [300].

7.3.2 Data gaps

Currently there are huge data gaps worldwide regarding pregnant women's nutritional requirements and status, their dietary intake, and reliable pregnancy-related outcomes, such as gestational age and infant anthropometry at birth. This complicates the comparison of the present research findings and their generalizability to other contexts.

To illustrate, the National Academy of Medicine (NAM; formerly the Institute of Medicine, IoM) EAR values for pregnant women used as a reference in the dietary intake study are based on US/Canadian population and were developed over 20 years ago. For some micronutrients, the EAR could not be formulated due to adaptations during pregnancy to increased nutrient demand or loss of nutrients due to physiological mechanisms [69]. Overall, studies on requirements (optimal timing and dosage) for pregnancy are limited as exact mechanisms are often unclear [16]. Human nutrient requirements and recommended intakes are therefore largely based on expert opinion or intervention studies [301]. An RCT in Guinea-Bissau that assessed the effects of prenatal multi-micronutrient supplementation on birth weight and perinatal mortality (n = 2,100 pregnant women) compared a one or two Recommended Dietary Allowances (RDA) of 15 micronutrients vs. control (IFA) [302]. Mean birth weight was higher and the proportion of LBW was lower in the 2 RDA group compared to the 1 RDA and control group, suggesting

that 1 RDA of multi-micronutrients is not sufficient to positively influence pregnancy outcomes in a West-African setting. In Tanzania, two trials, one involving HIV-positive women (n = 1129) [303] and one among HIV-negative women (n = 8468) [304], investigating the efficacy and optimal dose of multivitamin supplements on decreasing the risk of adverse pregnancy outcomes, showed mixed results. The first study comparing single vs. multiple RDAs multivitamin supplements, concluded that both are as efficacious in decreasing the risk of adverse pregnancy outcomes [303], while the latter found positive effects for multiple RDAs on LBW and SGA births [304]. As the RDA is the level of recommended intake for healthy women in North America, even in absence of HIV infection, a single dose may be inadequate to meet the requirements during pregnancy in many LMICs due to the high burden of undernutrition and parasitic infections [304]. The fact that we could not determine whether the BEP supplement, with the RDA recommendation by the NAM as the maximum for all nutrients in one serving, actually met the nutritional needs of the pregnant women in our study can thus be considered a general methodological limitation.

In addition, there is little information on the prevalence of micronutrient deficiencies among pregnant women. Nutrition experts identified the biochemical assessment of micronutrient status of women, particularly in Africa, as the most pressing data gap [14,88]. In the present study, we also did not estimate the effect of BEP supplementation on maternal nutritional status. The lack of practical, affordable, reliable biomarkers to measure micronutrient status was a limitation in the design of this study.

Finally, global data on birth anthropometry and gestational age remain very limited. In many LMIC settings, there are no antenatal ultrasounds to accurately assess gestational age and determine the prevalence of SGA and preterm birth. Furthermore, low rates of facility-based deliveries hamper immediate measurement of birth weight prior to significant weight loss in the first days of life [305], by which the magnitude of the problem remains unknown. Even for research trials in which we refer to in the discussion of Chapter 6, ultrasound measurement was often not used for all study participants to estimate SGA prevalence. Putting our research results into perspective, therefore, requires regular and timely data collection to estimate and monitor the global health burden of poor birth outcomes and evaluate the impact of BEP supplementation as a targeted nutrition

intervention [14]. New technologies, as fully automated 3D imaging for child anthropometry, as evaluated in an effectiveness study in South Sudan, might also play a future role if performance is optimized with sufficient accuracy for widespread use [306].

7.3.3 Clinical trial setting

MISAME-III is a well-designed and implemented trial with sufficient statistical power to demonstrate an effect of BEP supplementation on birth outcomes. The focus on a strong methodology with pilot-tested standard operating procedures, initial and refresher trainings, and electronic data collection that allowed regular data quality checks, improved the quality of the data. The strong commitment and motivation of the field team allowed for optimal pregnancy care, accurate measures, and a high daily observed supplement intake. While the trained village-based project workers visited women every five weeks for early pregnancy detection, mean GA at enrolment was between 11 and 12 weeks, which is at the end of the first trimester. The moderate effects on birth outcomes may thus be a result of supplementation implemented too late [109]. Certain nutrients may be required before or during conception, or in the first weeks of pregnancy to promote placental and embryotic development. However, the effect of intervening earlier remains unknown and is a very difficult methodological issue to tackle due to socio-cultural barriers of early pregnancy announcement [16]. Enrolling women very early in their pregnancy would require a strong community-based support and health system, as a real-world setting is substantially different from our clinical trial setting that included a large network of community-based field workers who conducted frequent home visits. Finally, the selection of health centers for our trial could affect the external validity of the results to very rural/remote areas in Burkina Faso, as we selected villages that were accessible during the rainy season and relatively close to agricultural fields.

7.3.4 Performing research during the COVID-19 pandemic

The main MISAME-III intervention study started end of October 2019, just a few months before COVID-19 was declared a global pandemic. In contrary to many countries which had to suspend surveys and studies, we were able to continue data collection and ensure high quality of measurements. The following lessons learned could be useful for designing future studies and data collection instruments to mitigate risks in a crisis situation:

- The study was implemented via the government healthcare centers and as a result, our work was largely unaffected by COVID-19 restrictions. Even in this crisis situation, women were offered health care during pregnancy. In addition, our research was in collaboration with a well-established local research organization (AFRICSanté) which closely monitored the situation, adapted quickly (e.g. weekly instead of daily supplement distribution during a period of approximately two months) and distributed soap, masks and hand sanitation to the health centers and participating women to reduce transmission of the virus.
- Prior to implementation, detailed Standard Operating Procedures were developed and sufficient time was allocated for training and pilot testing. This way, data collection procedures could meet the established quality requirements. In our case, this process was luckily completed before the COVID-19 pandemic. Regular refresher trainings could thereafter be implemented by our local principal investigator and consultants.
- Careful development and execution of our data management flow allowed for continuous monitoring and data quality assurance, even when travels were suspended. We collected data using SurveySolutions developed by the World Bank Group (USA), a very intuitive digital data collection platform. For almost all questions in the surveys, we programmed data entry checks. In addition, we developed analysis scripts in Stata (StataCorp LLC, USA) to detect possible errors on a weekly basis, which were sent directly to the field staff for verification.

7.4 Recommendations for future research

Based on the study findings of this PhD research and taking into account the methodological gaps, several recommendations for future research can be put forward.

7.4.1 Investigating the effect during early postnatal period

Poor linear growth and development, reflected in childhood stunting, is associated with severe short- and long-term health consequences. Particularly in the first 1000 days, from conception until the age of two, impaired growth has serious consequences for the cognitive and physical development of a child, that can last into adulthood resulting in reduced productive capacity and poor health [307]. Childhood stunting usually begins in utero and continues after birth [308]. Hence, nutrition interventions during pregnancy and/or lactation could potentially reduce childhood stunting.

Previous trials conducted in West Africa that investigated the impact of LNS during pregnancy and lactation on infant growth showed mixed results. Follow-up of participants in the MISAME-II trial did not show a positive effect of the intervention during pregnancy on length-for-age growth [309]. In addition, a trial in Malawi (n = 869) evaluating the impact of SQ-LNS during pregnancy, lactation and infancy did not show a positive impact on child growth [310], while a similar trial in Ghana showed increased length at 18-m of age as a result of the nutrition intervention [311]

Follow-up of our study participants during the postnatal period, i.e. analyzing the growth of children during the first year of life, will add evidence on the longer-term benefits of improved birth outcomes. This future analysis will provide an answer to whether the effect at birth is sustained during infancy. As part of the MISAME-III study protocol, LAZ at 6 and 12 months of age is a second primary outcome measure. The results (expected towards the end of 2022) on linear growth trajectories, including stunting, wasting and underweight prevalence, will provide a more complete picture of the benefits of BEP supplementation during pregnancy. Extension of the follow-up period until the age of five would provide further useful insights on whether the effects at birth are sustained and result in positive linear growth, cognitive and motor development outcomes.

Key recommendation: Future research should invest in the follow-up of children to determine whether the moderate effects found at birth are sustained and result in a beneficial impact on linear growth, cognitive and motor development at the age of 6 and 12 months, preferably up until the age of 5 years.

7.4.2 Investigating the effect beyond birth anthropometry

As birth anthropometry, including SGA, is a crude indicator of fetal growth, there are several other indicators of interest for future research that would allow a more mechanistic understanding of the effect of BEP supplementation. By application of omics methods to placenta and maternal blood samples, potential markers, such as changes in maternal RNA, could be identified linked to fetal growth restriction [312].

Maternal weight gain

Maternal weight gain, as an intermediate outcome, may shed additional light on the advantage of prenatal supplementation as insufficient gestational weight gain has been associated with adverse maternal and birth outcomes [60]. Other than the assessment of SGA and other poor birth outcomes, the assessment of sufficient maternal weight gain (~7.5-8.5 kg) – required for the increase in maternal tissues (uterus, breast, blood) and the fetal-placental unit – could help to estimate the clinical relevance of BEP supplementation [313]. The analysis of this secondary outcome, led by our colleague Hanley-Cook, showed that women in the intervention group tend to have a higher, but non-significant, gestational weight gain [0.28 kg (95% CI: -0.05, 0.58); P = 0.099] compared to the control group. In addition, no significant differences were found for gestational weight gain rate, adequacy, or incidence of inadequate or excessive weight gain [314].

Maternal and neonatal body composition

The impact of BEP supplementation on maternal and neonatal body composition (lean and fat mass), assessed using double labelled water, might also provide a more complete understanding of the effect and its consequences within a life course context. This study was not part of this PhD thesis, but is an important future research as part of the overall MISAME-III trial. As evidence suggests that lower birth weights reflect increased relative

adiposity and higher birth weights reflect higher lean mass, information on these indicators could help to explain the hypothesis that small babies are more prone to the development of metabolic syndrome [315]. Also, this future research could test the hypothesis that additional maternal or neonatal fat mass provides energy reserves to support fetal development and/or postnatal infant growth.

Maternal nutritional status and nutrient biomarkers

An important methodological gap is the assessment of the effect of BEP supplementation on maternal nutritional status. Over the past years, the use and interpretation of nutrient biomarkers received growing attention on the global research agenda. As an example, the Biomarkers of Nutrition for Development (BOND) program developed evidence-based advice regarding nutrient biomarkers that reflect nutrient exposure, status and functional effect, with a focus on iodine, vitamin A, iron, folate, vitamin B12 and zinc [316]. For each individual micronutrient, the strengths and limitations are discussed in separate reports within the series. For example, several biomarkers for vitamin B12 status are available, yet the exact cut-off to classify clinical and subclinical deficiency remain debated [317,318]. Besides the six micronutrients covered by the BOND initiative, identified as high priority for public health, there is a need for reliable biomarkers for other micronutrients as well to evaluate the effect of the complete MMN matrix in the BEP supplement. To illustrate, it is currently unclear which biomarker is best to identify thiamin deficiency, as biomarkers do not correlate well with clinical signs [319].

In addition, when measuring nutritional status in a population with a known or suspected moderate-to-high prevalence of inflammation, which is presumed to be the case in our rural Burkina Faso setting, the effect of inflammation on nutrient biomarkers cannot be neglected. However, to date, there is no single preferred method to account for inflammation, which might lead to higher or lower nutrient biomarker concentrations [320]. The Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE) expert group published a summary overview of current key nutrient biomarkers and highlights the need for new reliable biomarkers and the establishment of deficiency cut-off values, as the interpretation (i.e. correlation with clinical outcomes) is often uncertain [320].

The BOND and INSPIRE research initiatives serve as great examples to achieve progress, but continuing effort is required. Especially to determine micronutrient status in pregnant women, as physiological changes during pregnancy complicate the interpretation of optimal biomarkers [317], or in young infants, requiring a minimally invasive approach. New investments are needed to collect samples and establish affordable and practical biomarkers in low-resource settings.

Key recommendation: There is an urgent need for research investments in the discovery and use of biochemical markers for a better mechanistic understanding of the effect of BEP supplementation on fetal development beyond birth anthropometry. Priority should be given to biomarkers for the assessment of women's nutritional status in order to determine the effect and optimal composition of BEP supplements.

7.4.3 Investigating potential effect modifiers

Infectious diseases and inflammatory markers

There is also a need for a better understanding on micronutrient functioning during pregnancy (through maternal-placental-fetal axis) and the role of infections and inflammations, for a mechanistic understanding of the hypothesized effect of BEP supplementation on fetal growth [16]. In MISAME-III, 38% of women did not have an improved primary water source (protected well, borehole, pipe, or bottled water) and 40% did not have an improved sanitation facility (flush toilet connected to local sewage or septic tank or pit latrine with slab and/or ventilation). These poor WASH services can increase the risk of diarrheal and helminthic infections which is associated with maternal malnutrition [63].

Infections causing inflammatory responses, either acute or chronic, can directly impact nutrient absorption or homeostasis as a result of environmental enteric dysfunction, reducing the effect of the BEP supplement [320]. It may be an important mediating link in rural low-resource settings that warrants further investigation.

To understand mechanisms relevant to gut health (i.e. chronic gut inflammation) and birth outcomes, a bio-specimen study, was added to the original MISAME-III study protocol to cover this knowledge gap. Biochemical parameters in blood (capillary and umbilical cord),

breastmilk, saliva, stool and urine are collected from a subsample of women in the trial. The use of multi-omics analysis on these mediating determinants may provide new insights into possible treatment effects of BEP supplementation or assist in the interpretation of the current effect seen on birth size by revealing mechanistic pathways [318,321].

A lack of access to safe WASH increasing the risk of parasitic infections and diarrhea and other infectious diseases such as malaria and HIV often converge in resource-poor settings. Especially sub-Saharan Africa carries a high share of the global malaria burden with approximately 25 million pregnant women at risk of malaria infection every year [56]. Previous studies estimate that one in four women has a placental malaria infection at the time of delivery, which approximately doubles the risk of LBW deliveries [56]. In MISAME-III, following Burkinabe guidelines, most women received preventive treatments for malaria (three oral doses of sulfadoxine-pyrimethamine) at the relevant ANC visits. In addition, 74% of women answered they were sleeping under a bed net for protection against malaria. While the link between LBW and infant mortality has been studied widely, the effect of infection in the first trimester and the longer-term benefits remain unknown [56]. Next to socio-demographic questionnaires, collecting information within a trial on intestinal parasites, malaria and HIV should receive future research attention to gain more insight into the prevalence and consequences for nutrient requirements.

Environmental hazards

Besides nutrition, other environmental risk factors play a role in fetal growth and development. In our setting, maize and peanuts contaminated with mycotoxins – toxic secondary metabolites of fungi due to poor storage conditions in a warm and humid climate – might be an important confounding factor in the effect of BEP supplementation on birth outcomes. Research has shown that mycotoxins might potentially have a negative impact on pregnancy, breastmilk and growth of children [322]. Other hazards, such as exposure to black carbon due to cooking over open fires [323] or exposure to pesticides in e.g. cotton fields as a result of agricultural work (the primary source of income for 73% of women in our study population), might also influence gestational quality and birth outcomes [321]. Future research within the MISAME-III project will investigate levels of exposure and the relationship with birth outcomes.

Key recommendation: New studies should examine the influence of infection and inflammation on energy and nutrient stores required to support fetal development, and the role of hazardous exposure on pregnancy and birth outcomes.

7.4.4 Investigating the effect before conception: a life-course perspective

As our results from the subgroup analysis showed that well-nourished mothers benefited more from the BEP intervention compared to mothers with a low BMI, it is recommended that future interventions should focus on what happens in the life-course and investigate effects beyond short-term outcomes. In many countries, early adolescent pregnancies might threaten the growth potential and nutritional status of young women, with consequences that extent to the next generation [109,299]. The 2022 *Lancet* series on adolescent nutrition highlights that research on adolescence nutrition in LMICs is scarce and that improvements in nutrition provide an opportunity to interfere with the current trends of malnutrition and negative consequences on the long-term. Research has shown that infants from adolescent mothers are at increased risk of LBW, short birth height and premature delivery [324,325]. As micronutrient deficiencies among women between the ages of 10-19 years persist into later pregnancies and shape fetal programming and development, future studies into adolescence growth and nutrition are warranted. Experts highlight two main research aims closely related to the present PhD research: (1) increased knowledge on the biological mechanisms between pubertal development and nutrition and (2) better dietary intake data to understand which nutritional needs of adolescent girls are essential to address. Overall, there is emphasis on integrated system-wide approaches to address the growing burden of adolescent malnutrition [299].

Key recommendation: Future research should evaluate the effect of providing BEP supplements before conception, by targeting adolescent girls, and during the first trimester of pregnancy which is a crucial period for the development of major organs and body systems.

7.4.5 Combining evidence to evaluate the impact of BEP supplementation

Meta-analysis

To assess the overall impact of BEP supplementation, it is necessary to look at the bigger picture and include other trials. Parallel to MISAME-III, a large RCT, Maternal and Infant Nutrition Trial (MINT), is being conducted to test the efficacy of a similar fortified BEP supplement during pregnancy on birth outcomes in rural Nepal (ClinicalTrials.gov identifier: NCT03668977). Study findings will be available in 2022-2023 and will be useful to compare our results with. Furthermore, the "Maternal BEP studies Harmonization Initiative" includes additional RCTs studying the effect of balanced-energy protein supplementation in Ethiopia, India and Pakistan that will allow future meta-analysis in order to combine effect sizes and establish public health and clinical recommendations. While our subgroup analysis was not sufficiently powered to identify vulnerable or risk groups, combining evidence should give more confidence on which women potentially benefit most from BEP supplementation.

Key recommendation: Pooling data from well-designed RCTs that investigate the effect of prenatal fortified BEP supplements will increase the statistical power to evaluate the impact of this nutrition intervention on birth outcomes in vulnerable subgroups which can be policy relevant to target BEP supplementation.

7.5 Recommendations for policy

Based on our findings, several suggestions for the best course of action in policy are provided.

7.5.1 Multi-sectoral approach

The MISAME-III research project showed modest effects of fortified BEP supplementation during pregnancy which can partly be explained by the multifactorial etiology of poor birth outcomes. Many underlying and contextual factors play a role in both maternal nutritional status and fetal development [199]. Therefore, BEP supplementation is only one part of the solution and a multi-sectoral approach is believed to offer the best approach to effectively address maternal malnutrition and improve birth outcomes. To achieve sustainable improvements, both direct and indirect social determinants of malnutrition should be targeted. It is recommended that indirect nutrition strategies, as malaria prevention, preconception care, improved WASH and rural health care, parental education and women's empowerment complement a supplementation strategy to address underlying causes [14,227]. 'Real world' evidence from 11 countries (including Burkina Faso [153]) and 4 Indian States showed that multisector approaches, including nutrition-sensitive interventions, accounted for ~50% reduction in child stunting, serving as an example for efforts to address maternal malnutrition [8]. Essential elements of a multi-sectoral approach, of which BEP supplementation can be one aspect, are being discussed in the next paragraphs.

Key recommendation: A multi-sectoral approach that targets both direct (insufficient dietary intake) and indirect determinants (such as malaria infections and poor WASH conditions) of malnutrition is needed to tackle the complex etiology of poor birth outcomes.

7.5.2 BEP Supplementation

In certain circumstances, e.g. emergency situations, ready-to-use fortified BEP supplements might be a good vehicle for delivering macro- and micronutrients as these provide the advantage of not requiring any preparation and having a long shelf life [326].

Geographical targeting

As our study results do not offer sufficient evidence to identify vulnerable groups (e.g. undernourished women identified by low BMI or short stature) that would benefit most from BEP supplementation, geographical targeting would probably be the best programmatic approach. By selecting settings with high food insecurity and inequality (e.g. affected by conflict, frequent droughts or seasonal food shortages), it is most likely that nutritionally vulnerable women can benefit from prenatal BEP supplementation. To facilitate this selection, an analysis of the food system and food environment that pertain specific typologies and provide insight into the availability, affordability and acceptability of nutritious foods could offer a way forward [327].

Implementation strategy

If future policies include BEP supplementation as a strategy to improve maternal nutritional status and birth outcomes, it is important to put sufficient efforts to ensure optimal use. Our preliminary study showed that investments in health communication on the benefits of BEP supplementation for pregnant women and babies and engaging family members are very important to support high adherence. In our RCT setting, we had a strong social and professional health care support system which resulted in a high adherence.

This policy recommendation is in line with the current debate on why progress on nutrition targets in many countries is insufficient and slow, which mainly focuses on the fact that "we still do not deliver at scale the interventions we know", e.g. IFA supplementation of 90 or more tablets during pregnancy (estimates of ~30% coverage in LMICs) and at least four ANC visits (estimates of ~67% coverage in LMICs) [8]. The bottlenecks that have been identified by the Micronutrient Initiative in 7 countries in Africa and Asia: supply chain, delayed and variable attendance at ANC and lack of adherence by pregnant women,

could be potential barriers to BEP supplementation as well. Implementation research is therefore crucial to ensure optimal use and reach the maximum potential effect of BEP supplementation.

Delivery platform

The health system is a good delivery platform to support nutrition interventions and deliver BEP supplements, as identified by our home consumption study and two recent reviews on barriers and enablers for effective delivery of nutrition (supplementation) programs [328,329]. Similar to our trial setting, health care professionals should receive training on fortified food supplementation guidelines and counselling skills to encourage daily consumption and prevent sharing of the food supplement. It is advised to create an information sheet about the benefits of the supplements and intended use, which can be consulted at any time. The WHO guidelines for a positive pregnancy experience advice 8 ANC visits during pregnancy, which is 4 more than the current standard in Burkina Faso [108]. If BEP supplements would be provided during each visit (e.g. a month supply), it could be both an incentive to attend each ANC visit and reinforce the effect of supplementation by improved health care. Also, programs could consider a community-based delivery platform as this promoted high adherence in MISAME-III and allowed for close monitoring and support throughout pregnancy. This is however a costly approach which requires a sustainable monitoring and evaluation system.

Local production and packaging

Policies that include BEP supplementation should investigate the possibility of producing the BEP supplement locally. This will require a coordinated approach to deal with different challenges along the supply chain, e.g. local technical capacity, quality-assured monitoring and surveillance, guidelines and government oversight, competition with imported brands [330]. Furthermore, attention should be paid to collect empty packages to prevent waste or open burning of sachets, posing a risk to the environment and public health. In MISAME-III, all packages were collected by the village-based project workers (for verification of supplement intake in case directly observed intake was not possible) and the packages were burned in an incinerator, as recycling was unfortunately not possible due to the combination of aluminum and plastic. As this package is the best way

to preserve the content and keep the MMN content stable for a long period, re-using the energy from an incinerator would be the only sustainable solution for the moment (personal communication prof. dr. Kim Ragaert, Ghent University).

Cost-effectiveness

Before implementing BEP supplementation as a nutrition intervention at scale, the cost-effectiveness of BEP supplementation should be considered. In our study, the mean duration of study participation was 199 (± 30) days, which means a cost of approximately $50 (ranging between $42-57; using the August 2022 exchange rate 1:1 for Euro to US dollar) per pregnancy if we apply the current production cost of €0,25 per portion BEP. Besides the product costs, program costs for distribution (mainly labor costs), training, promotional campaigns, administration, etc. will add another estimated 50-100% to the total price [331]. This means it is an expensive intervention if we compare it with the domestic general government health expenditure per capita of $51 in 2019 [332]. The investment in BEP supplementation therefore requires a high level of political and donor commitment to be sustainable and improve maternal nutritional status and newborn growth, health and survival. If national budgets for maternal and child health are shifted to support supplementation programs, investment losses in other public health components must be taken into account when assessing the total sum of benefits for the quality of life.

Key recommendation: Our findings did not elucidate subgroups of women that are likely to benefit most for a targeted approach of BEP supplementation. For the moment, we therefore recommend geographical targeting, i.e. identifying (seasonal) food insecure areas, to support nutritionally vulnerable women. Health centers are the desired delivery platform to (1) provide information on the benefits of BEP supplements and (2) encourage early and frequent antenatal care attendance. Programs should explore local production options and ensure proper waste management. Regardless, we strongly recommend a thorough analysis of the cost-effectiveness to inform decision makers prior to implementation.

7.5.3 Food system and diets

Our dietary intake study showed that the usual energy and nutrient intake of pregnant women is below the EAR for pregnancy. Also, household food insecurity appeared to be high and dietary diversity was low among study participants [283]. The diet is very similar and particularly monotonous, consisting of staple foods (mainly maize) with small amounts of green leaf or vegetable sauce. Pulses (beans, peas and lentils) are eaten much less often, as well as foods of animal origin (dairy, eggs, meat, poultry and fish). Fruits, outside the mango season (typically from April to June), are barely eaten by pregnant women. A change in dietary habits towards a diet with local, fresh foods is needed, but is complex and not expected in the short-term. Nutrient-rich foods need to be available, affordable and culturally acceptable. The COVID-19 pandemic and ongoing conflicts cause record high food prices that trigger a global crisis that will drive millions more into extreme poverty, increasing hunger and malnutrition [333]. In addition, LMICs bear a considerable burden of the financial costs of climate change with more droughts and floods every year increasing food insecurity [334]. It is therefore important to strengthen the agricultural system for supply of healthy foods, alongside counselling and supplementation policies. When several nutrition interventions within policies are put into place, one needs to be careful of reaching upper intake levels of some nutrients, e.g. iron, folic acid and vitamin A [335]. Policy, based on evidence, should thus be coherent across sectors.

Key recommendation: Policies should strengthen agricultural development to build a resilient food system for the supply of healthy foods, alongside nutritional counselling and supplementation, to support women in their increased needs for energy and nutrients during pregnancy. Continuous efforts from the public and private sector are required to improve women's dietary diversity and diet quality.

7.5.4 Health care services

The quality of, and access to health care services is an important determinant for pre- and postnatal care, as well as newborn health and survival, especially of preterm and LBW babies. For this policy recommendation, we refer to the recently launched WHO global

guidelines to support women and newborns in the postnatal period – the first six weeks after birth that advocates for a stronger health care and support system [336].

Within the present study, the delivery rate at the health center was very high (86%). Each of the six health centers had a trained project midwife to attend deliveries. Using budget from the MISAME-III project funding, we renovated each health care facility making it a more comfortable and safer place to deliver (photos in **Annex 6**). Though, the basic rural conditions in the health centers do not allow surgical care (e.g. C-sections) or special care that requires medical equipment (e.g. incubators) as this is lacking. Alongside nutrition interventions, policies should invest in strengthening the performance of health services. Examples drawn from our clinical trial experience in rural Burkina Faso include the need to contract skilled and motivated health personnel, provide regular training, upgrade facility infrastructure (incl. water and power supplies), modernize data systems, and ensure the availability of equipment and sufficient medical supplies over time. These improvements are needed to reinforce the impact of BEP supplementation on maternal and child health. When scaling up the intervention in 'real-world' settings, these enabling factors need to be in place to avoid a lower than estimated effect under optimal conditions in the MISAME-III clinical trial.

Future programs should encourage early first ANC visits, 8 ANC contact moments [108], and appropriate information on healthy diet during pregnancy and the benefits of nutritional supplementation at every ANC visit. A nationally representative nutrition survey carried out in Burkina Faso in 2018 estimated that only 56% of women received information on nutrition and diet at an ANC visit, supports this need for improved policy efforts [337].

Key recommendation: Policies should invest in improving rural health care services to support women during pregnancy, facilitate safe delivery, and offer good-quality care for (vulnerable) infants during the postnatal period to tackle poor infant growth and development and reinforce the impact of BEP supplementation.

7.6 Conclusion

High rates of maternal malnutrition and poor birth outcomes in low- and middle-income countries, exacerbated by the COVID-19 pandemic, conflicts and climate change, require investment in nutrition to meet global targets. For future guidance, this PhD research concludes that:

- Fortified BEP supplementation in the form of a lipid-based peanut paste was well accepted by pregnant women with high adherence to daily consumption in the MISAME-III efficacy trial.
- Nutrient adequacy was low in pregnant women in rural Burkina Faso. BEP supplementation was shown to fill nutrient gaps without displacing food intake and has potential to deliver nutritional support for healthy fetal growth and development.
- Fortified BEP supplementation modestly reduced the prevalence of low birth weight babies and increased birth weight compared to iron-folic acid tablets. No statistically significant effect was found on SGA. Combining evidence from other high-quality trials will help assessing the external validity of our findings before developing policy recommendations.
- There is a need for reliable biomarkers for a mechanistic understanding of the impact of BEP supplementation beyond birth anthropometry. Priority should be given to the discovery and use of biomarkers to assess women's nutritional status in order to determine the effect and optimal composition of BEP supplements.
- BEP supplementation is not an alternative to healthy diets and should be regarded as a complementary solution. A multi-sectoral approach that addresses underlying and contextual determinants of malnutrition is required to tackle the complex problem of poor birth outcomes.

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Curriculum vitae of the author

Brenda de Kok was born on April 27, 1988 in Hoeven, the Netherlands. She finished pre-university education in 2006 and started to study Nutrition and Health at Wageningen University. To broaden her horizons, she organized a semester exchange at the University of Guelph in Canada to study international nutrition and development. For her MSc thesis, she joined the INSTAPA research project and investigated the acceptability of biofortified yellow cassava in Kenya. In 2012 she finished her Master's degree in Public Health Nutrition.

Brenda started working as a researcher and project manager in the field of nutrition and public health at the Municipal Health Service for the Utrecht region and Nestlé. In 2013, she joined the TNO Trainee Program and contributed to research projects in the fields of personalized nutrition, workplace health promotion and school-based nutrition education. After finishing the Trainee Program at TNO end of 2015, she fulfilled her dream to travel the world and volunteer as public health nutritionist in Indonesia.

In 2018, she started her PhD at the Department of Food Technology, Safety and Health at Ghent University. This PhD research within the MISAME-III framework was financed by the Bill & Melinda Gates Foundation. She joined the doctoral training program of Ghent University and supervised MSc students during their internship and thesis. She attended several on-site and online national and international conferences in the field of nutrition. Her PhD work involved a combination of working from Belgium and visits to Burkina Faso for training and coordination of the field work.

Training

Ghent University

Statistics:	Multilevel analysis for grouped and longitudinal data (2021)
	Applied linear regression (2019)
Research skills:	Academic posters (2020)
	Good Clinical Practice (2019)
	Doing research in Africa (2018)
Transferable skills:	Creative thinking (2020),
	Communication skills (2019)

TNO

	Basic project management (2014)
	Customer-orientation (2014)
	Personal effectiveness (2014)

Publications in peer-reviewed journals

Hanley-Cook G, Toe LC, Tesfamariam K, **de Kok B**, Argaw A, Compaoré A, … & Huybregts L. (2022). Fortified balanced energy-protein supplementation, maternal anemia, and gestational weight gain: a randomized controlled efficacy trial among pregnant women in rural Burkina Faso. The Journal of Nutrition; nxac171.

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Kocken PL, Scholten AM, Westhoff E, **de Kok B**, Taal EM, Goldbohm RA. (2016) Effects of a theory-based education program to prevent overweightness in primary school children. Nutrients; 8(1).

Talsma EF, Melse-Boonstra A, **de Kok B**, Mbera GNK, Mwangi AM, Brouwer ID. (2013) Biofortified cassava with pro-vitamin A is sensory and culturally acceptable for consumption by primary school children in Kenya. PLoS ONE; 8(8).

Presentations

MISAME-III Study results on birth outcomes. Panelist at the BEP Harmonization Initiative; Grand Challenges Annual Meeting 2022. 23th October 2022 at Warwick, Brussels.

The burden of malnutrition and MISAME-III study. Coalition against Hunger. Workshop "Food systems at the crossroads of today's global challenges". May 17th 2022, at Maison des Associations Internationales, Brussels.

MISAME-III Study protocol. 9th Annual Belgian Nutrition Society Meeting. Hot topic seminar: Nutrition research in the context of meeting the Sustainable Development Goals. May 3rd, 2019 at the Royal Library, Brussels.

Annexes

Annex 1.1: SPIRIT Checklist 2013

Reporting checklist for protocol of a clinical trial

Based on the SPIRIT guidelines.

		Reporting Item	Page nr publication
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	20
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 / 20
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	20
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating center, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	17
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (e.g. parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g. superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (e.g. community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centers and individuals who will perform the interventions (e.g. surgeons, psychotherapists)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g. drug dose change in response to harms, participant request, or improving / worsening disease)	n/a
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g. drug tablet return; laboratory tests)	7 /
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a

Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g. systolic blood pressure), analysis metric (e.g. change from baseline, final value, time to event), method of aggregation (e.g. median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	14
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enroll participants or assign interventions	8
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enroll participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	17
Statistics: outcomes	#20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17
Statistics: additional analyses	#20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	n/a
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomized analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	17
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17

Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	18
Protocol amendments	#25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	18
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorized surrogates, and how (see Item 32)	18
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	18
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18
Ancillary and post-trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	18
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	18
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorized surrogates	18
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

The SPIRIT checklist is distributed under the terms of the Creative Commons Attribution License CC-BY-ND 3.0. This checklist was completed on 06. March 2020 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

Annex 1.2: Informed consent

1. PARTICIPANT INFORMATION SHEET

THE EFFECT OF BALANCED ENERGY-PROTEIN SUPPLEMENTATION DURING PREGNANCY AND LACTATION ON BIRTH OUTCOMES AND INFANT GROWTH IN RURAL BURKINA FASO

Coordinating Investigator :	Prof. Dr. Patrick Kolsteren
Principal Investigator:	Dr. Laeticia Celine TOE
Sponsor of the study:	Ghent University
Participant Number:	|__||__||__||__||__| _ |__||__|

Dear madam,

You are invited to participate in a study that wants to study the effect of providing a food supplement during pregnancy and lactation on the growth of your baby. The study is a collaboration between the Institut de Recherche en Sciences de la Santé (IRSS) and AFRICSanté in Burkina Faso, Ghent University of Belgium and the International Food Policy Research Institute (IFPRI) in the USA. Before you decide to participate in this study, it is important that you read/are read and explained to this form, because it explains your rights and our responsibilities to you. In this information and consent form, the purpose, examinations, advantages, risks and inconveniences related to this study are explained. The available alternatives and the right to withdraw your consent to participate at any time are also described below. No promises or guarantees can be made about the results of the study. You have the right to ask questions at any time, for example about the possible and/or known risks related to this study.

Purpose and description of the study

This research study will provide more evidence and insight into how we can improve pregnancy outcomes , birthweight of babies and the growth of newborns. With this study, we aim to assess whether the provision of the food supplement under investigation can improve the growth of fetuses during pregnancy and that of infants during their first 6 months of life. In addition, we will evaluate how the food supplements that are offered to you improve the nutritional quality of the human milk produced for your child, as well as your and your child's body composition.

We will thus compare the effect of 2 different approaches. One is to give you a tablet during your pregnancy and with the other approach participants will also receive a food supplement next to the tablet. The tablet is what you normally received for any pregnancy according to the guidelines of the ministry of health of Burkina Faso.

We will provide the supplement for the entire duration of your pregnancy. After birth some mothers will continue to receive a food supplement for six months. Who receives what is entirely determined by chance. In total, 1776 pregnant women living in the catchment area of the health centers of de Boni, Dohoun, Dougmato II, Karaba, Kari and Koumbia will be recruited to participate in this study.

How the study is done

If you are confirmed pregnant and accept to participate in this study, the midwife will do a full medical check-up of you and your baby according to medical standards. She will also inform you about a good dietary practices for you during your pregnancy.

After the check-up we will provide you either with the tablets, or the food supplements.

Thereafter, you will receive a daily visit from the community health worker who will ask you to take the tablet or eat the food supplement. A ultrasound examination will be planned for you within 2 weeks after your first consultation and will be performed by the project doctor. We will also arrange for you regular check-ups of your pregnancy by the midwife or the medical doctor.

Examinations in the context of the study

If you accept to participate in the study and if you and your child meet all the conditions for participating in the study, the following tests and examinations will be performed:

- We will take your weight t and height and ask you your age at inclusion.
- The midwife will invite you for antenatal check-ups according to the guidelines of the ministry of health and the medical doctor will perform an echography of your baby. This is not invasive and has no known risks. It is like taking a picture of your baby. As part of the routine check-up we will take fingerpick blood to test whether you are anemic and give you treatment when necessary.
- We will also ask you questions about your mental wellbeing, e.g. how you are living your pregnancy and if something bothers you in your everyday life.
- After delivery we will again take fingerpick blood to test whether you are anemic and give you treatment when necessary.
- When you deliver we will weigh your baby and measure his/her length and head and chest circumferences.
- After birth we will visit you or ask you to come to the health center every month to have the weight and the length of you baby taken. At that time we will also ask you question on illnesses your child might have had in the previous week and on what he/she has been eating.
- We will also perform a finger/heel prick on your baby to see if he/she is anemic and a treatment will be provided if necessary

- Halfway through pregnancy in some of the participating women, we will ask you questions about what and how much they have eaten the day before the interview. This should take about half an hour.

When the doctor identifies a medical problem he will see to it that you receive the appropriate information and necessary treatment, and will refer you when necessary.

If your child suffers from a disease or undernutrition, he/she will be treated in the best way possible. In such case, your child will be referred to the local health center or the district hospital, for further physical examination by a medical doctor.

Voluntary participation

You participate entirely voluntarily in this study. You have the right to refuse to participate in the study. Your decision to participate or not in this study, or to stop your participation in this study will have no influence whatsoever on present and future medical consultations and possible treatments. You also have the right yourself to stop your participation in the study at any time, even after you have signed this informed consent form. The withdrawal of your consent will not cause any disadvantage or loss of advantages/privileges.

Risks and inconveniences

Finger-prick blood will be taken from you at the first ante-natal consultation. You will experience a prick in your finger, however the prick is not invasive. Antiseptic measures will be taken to prevent any inflammation/infection of your finger.

The food supplement used in this study is safe for you and your baby. The food supplement contains milk, sugar, oil, peanut butter and a mix of vitamins. Previous studies in Houndé that provided similar food supplements during pregnancy have not documented any complications during pregnancy or delivery. However, the supplement contains milk, which can cause bloating, flatulence and other digestive discomfort in some people. We encourage you to notify us, should you experience these effects, so we can take measures to insure you the best comfort possible.

All other investigation are routinely done as part of the follow-up of pregnancy.

Some of you will be asked to donate a small amount of breast milk at two time points during the study (between 1-2 months and 3-4 months age of your child). This will not diminish the amount of milk for your child or influence lactating performance. We will ask your permission for this donation again when the time comes.

In a random sample of all participants, we will assess body composition after delivery in the mother and the infant, using a special water that has been proven safe for such use. If you are chosen for that examination, we will provide detailed explanations of the procedure to you and a specific consent will be ask before we perform the assessment. Your privacy will be respected at all times.

Benefits

We expect to show that taking food supplements during pregnancy and lactation will help children grow better and improve the quality of breastmilk. If this would be the case we will have the possibility to change policy to provide supplements to pregnant and lactating women.

Protection of your private life

Your identity and your participation in this study will be treated strictly confidential. The specific information we obtain from you will not be shared with anybody, except the study investigators and partner institutes. Your identity remains secret since your personal information will only be designated by a unique participant number. Your name will not appear in any reports or publication resulting from this study. After the study is completed, you may request information about the study results. As soon as possible (maximum 5 years) after the study is completed, all personal information from participant shall be deleted from all databases to ensure complete anonymity. Those anonymized databases shall be shared with other researchers to advance research on mother and child health. This shall be done in strict accordance with international laws and regulations about privacy.

Ethics committee

Before starting, this study has been reviewed and approved by an independent Ethics Committee in Belgium, namely the Ethics Committee of the University Hospital in Ghent and it has been reviewed and approved by the Ethics Committee Centre Muraz in Burkina Faso. These committees also perform continuous reviews of the study during its progression to make sure the study is carried out in the safest possible way.

Compensations and incidents that may occur in this study

All costs related to sickness occurring during the study will be reimbursed to you. Investigators shall seek to provide a compensation and/or the best possible treatment in the event of damages/ injuries that may occur as a result of your participation in this study. For any other damages thought to be related to your participation in this study, and occurring after the study has been completed, participant may file a complaint to the relevant jurisdiction, which will treated in accordance with applicable laws in Burkina Faso.

Contact persons in case you have questions about this study

If you have any questions concerning your participation in this study, or think you have been injured as a result of the study or have a reaction to the study food, inform the village health worker who visits you. S/he will bring you immediately in contact with the project coordinator or the project doctor. All village health workers have cell phones to contact these persons directly. You may also contact, now, during or after the study:

- The principle investigator in Burkina Faso: Dr. Laeticia Celine Toe
- The president of the ethic committee: Dr. Adama Dembélé,
- The local project Coordinator : Mr. Moctar Ouédraogo
- The project medical doctor: Compaoré S. Anderson Casimir

Informed consent

Before you agree to participate in the informal consensus activity, you need to be aware that:

- This study was presented and cleared by the Ethical Review Board of the Ghent University and the ethics committee of Centre Muraz, Burkina Faso.
- This clearance is not to be taken as an obligation to take part in this study.
- Your participation is only voluntary and will be confirmed by signing this form. If you wish, you can withdraw from this study at any point, even after signing this form; you can withdraw by contacting the researcher (below) through email or telephone. You do not have to motivate or explain the decision of withdrawal. In case of withdrawal, your data already collected will be used to assess study outcomes.
- You can revise your answers to the questions if you wish so.
- Your input will be stored anonymously; researchers not involved in the data collection will not have access to your personal data and name. Anonymized databases shall be shared with other researchers for to advance research on maternal and child health.
- You can contact the researcher or the coordinator of the project at any time if you wish to obtain more information regarding this study
- There are very limited risks related to your participation in this study. Those are mentioned under the section " risks and inconveniences" of the information sheet. However, in accordance with the Directive concerning experiments on humans (07/05/2004), a civil liability insurance has been foreseen in the event of any injury or damage occurring and deemed due to your participation in this study.

Part intended only for the participant

I, the undersigned, _____ (name and surname) confirm that I have been informed about the MISAME-III clinical trial and that I have received a copy of the Participant Information Sheet and a copy of the consent form. I confirm that I have read and / or understood the four pages of the information sheet for participants. The study responsible gave me enough information about the conditions and duration of the study, and the possible risks and disadvantages. In addition, I had enough time to review the information and to ask questions, and I received satisfactory answers.

- I understand that I can terminate my participation in this study at any time after notifying the study responsible and this decision will not cause any inconvenience to me or my child.
- I am aware of the purposes for which the data is collected, processed and used in this study.
- I am ready to give information about my medical history and that of my child, or about any medication taken or participation in another study.
- I voluntarily consent to participate in this study and to cooperate with the required examinations and tests.
- I consent to my data being shared anonymously for the benefit of research and maternal and child health.
- I understand that auditors, employee representatives, the Commission for Medical Ethics or competent authorities may inspect my data in order to verify the information collected. By signing this document, I give permission for this control. At all times my privacy will be respected.
- I understand that the biological samples collected from me and / or my child will be sent to Europe or the USA for analysis, and this in strict respect of my confidentiality.
- I am aware that this study has been approved by an independent commission for medical ethics linked to the University Hospital of Ghent, examined by the Ethics Committee of the Center Muraz in Burkina Faso and that this study will be carried out in accordance with the guidelines Good Clinical Practice Guidelines and the Declaration of Helsinki, established for the protection of people participating in clinical trials.

Signature (or fingerprint) of the participant

_____

In case of minority

Informed consent of the legal representative

I, the undersigned, _____ (name and surname), legal representative of _____ (name and surname) confirm that I have been informed about the MISAME-III clinical trial and that I have received a copy information sheet for participants and a copy of the consent form. I confirm that I have read and / or understood the 6 pages of the information sheet for participants. The study responsible gave me enough information about the conditions and duration of the study, the procedures, the advantages and possible disadvantages. In addition, I had enough time to review the information and to ask questions, and received satisfactory answers.

- I understand that I can withdraw my consent at any time after having informed the study managers and this decision will not cause any inconvenience to my child / wife / ward or to myself.
- I am aware of the purposes for which the data is collected, processed and used in this study.
- I voluntarily consent to my child / wife / ward participating in this study.
- I understand that auditors, employee representatives, the Commission for Medical Ethics or competent authorities may inspect their data in order to verify the information collected. By signing this document, I give permission for this control. At all times his privacy will be respected.

Date: (dd / mm / yyyy)

Signature (or fingerprint) of the participant's legal representative

_____

Part intended for the investigator

I, the undersigned, _____

confirm that I have informed, _____ (full name of the participant)

and that she has:

□ Consent to participate in the study Refused to participate in the study

□ Reason for refusal (mark not filled in if no reason provided)

Date: (dd / mm / yyyy)

Signature:

_____

If the participant is unable to read and / or write, an impartial witness should be present during the consent discussion. After having read and explained the information sheet and the informed consent form to the participant. After she has verbally consented to her participation in the study, and has affixed her fingerprint, the witness should complete the name of the participant, add the date, and personally sign and date the consent form. By signing the consent form, the witness certifies that the information in the information sheet and the consent form have been precisely explained and understood by the participant and that the participant has freely given her consent.

Name and first name (s) of witness: _____

Signature of witness:

_____

Date: (dd / mm / yyyy)

Contact Principal investigator

Toe Laeticia Celine

Contact of project medical doctor

Compaoré S. Anderson Casimir

Annex 2: Added sugars in 12 BEP supplements

Product Name	Added sugars (g)/100g
Sweet lipid-based spread	18.0
Mango bar	21.6
Vanilla Filled sticks	20.0
Vanilla biscuits	19.9
Vanilla drink	13.7
Unseasoned pillows†	3.9
Fermented drink†	17.0
Tomato and onion lipid-based spread	2.0
Tomato and onion bar	13.0
Tomato and onion biscuits	0
Chicken soup	0
Seasoned pillows	5.1

Annex 3.1: In-depth interview guide for pregnant women

Initial interview (week 1)

Role of pregnant women in the household:

- Daily activities and adaptations due to pregnancy
- Announcement of pregnancy

Household food practices and beliefs:

- Food practices such as decision making, serving and sharing
- Beliefs and traditions regarding foods in general and during pregnancy

Diet during pregnancy and lactation:

- Importance of diet during pregnancy and lactation
- Dietary intake and behavior changes during pregnancy and lactation

Availability and access to supplements:

- Knowledge of supplements for pregnant women
- Current intake and availability of vitamin supplements

Antenatal consultations (ANC):

- Attendance
- Attitudes towards ANC

Supplement-specific interview (week 4 and 8)

Evaluation of the supplement:

- Appreciation in terms of taste, texture, smell and color
- General appreciation: overall judgement, portion size, resemblance to other foods

User experience:

- Timing, food replacement, sharing and changes over time

Future use:

- Willingness to continue using the supplement for the duration of the pregnancy
- Willingness to pay for the supplement

Final interview (week 10)

Product preference:

- Preferred choice for Peanut paste or Vanilla biscuit

User experience:

- Experience after using the supplements for a longer period in terms of general feeling, satiety, weight changes, etc.
- Experience and perception of household and community members

Future use:

- Willingness and reasoning to continue supplementation during pregnancy and lactation
- Opinion regarding having a choice between the supplements
- Opinion regarding sharing in the future; and suggestions to prevent sharing

Distribution and information:

- Preferred distribution channel
- Preferred information on the supplements
- Opinion regarding the involvement of household and community members

Conclusion

Annex 3.2: Semi-structured thematic FGD guide for pregnant women

User experience of the two BEP supplements:

- Overall evaluation and perception over time
- Use of the supplements and (changes in) meal pattern
- Portion size
- Timing of consumption
- Opinion of other people

Preference:

- Preference for one supplement over the other, incl. reasons
- Opinion regarding having a choice between the two supplements

Future use:

- Intention and reasons for continued use in the future

Distribution and information:

- Preferred supply channel
- Preferred information
- Facilitating factors or barriers to use the supplements

Conclusion

Annex 3.3: Participant information on BEP supplements

Informed consent

Benefits

We hope to prove that supplementation during pregnancy and lactation will improve pregnancy outcomes, help the child grow better, and also improves the quality of breast milk and body composition of the mother and child. If proven, it will be possible to change policies regarding supplementation of pregnant and lactating women. This will benefit children in Burkina Faso and other countries.

Risk and inconveniences

The dietary supplements that will be used in this study are made from foods that are normally consumed in Burkina Faso to which vitamins and minerals have been added. They are therefore safe and will be provided in the recommended daily doses. The study staff will ask you about allergies (or related symptoms) to one of the ingredients, which is a potential risk.

Information sheet: daily consumption of supplements

- 1 Full sachet should be consumed per day.
- The supplement has been specially developed for your well-being and that of the baby in your belly.
- The supplement is therefore only for you and should not be shared with others (adults or children).
- The supplement should not replace local food but should be added to it.
- Empty packets may not be thrown away after the supplement is consumed and must be returned to the study staff after consumption.

Home Storage

- Keep the complete packets in a clean, dry, cool place in your home (in the box).

Usage

- Clean the package and your hands before consumption (with soap and water).
- The supplement can be eaten as it is and requires no preparation. It should not be heated or added to hot foods.
- The supplement must be consumed at one time.
- If the supplement cannot be consumed at one time, seal the bag if possible and keep it in a clean, dry, cool place for later.

Annex 4: Percent energy contribution by food groups and important food items within group[a]

	Control (IFA) n = 253	Intervention (IFA + BEP) n = 217
Grains, white roots, tubers, and plantains	68.0	68.0
Maize	53.5	53.2
Rice	8.88	9.61
Beignet	1.61	1.22
Bread	1.16	0.91
Pasta	0.78	0.36
Sorghum	0.97	1.30
Cassava	0.49	0.55
Pulses (beans, peas, and lentils)	2.55	3.95
Beans	1.70	2.43
Cowpea	0.53	1.29
Nuts and seeds	7.15	5.61
Peanuts	7.08	5.61
Dairy	0.39	0.39
Meat, poultry, and fish	0.90	0.61
Eggs	0.11	0.07
Dark green leafy vegetables	2.40	2.28
Hibiscus leaves	0.93	0.70
Baobab leaves	0.62	1.23
Wild jute leaves	0.58	0.54
Vitamin A-rich fruits & vegetables	0.01	0.00
Other vegetables	2.47	2.37
Okra	1.21	0.92
Eggplant	0.58	0.73
Other fruits	0.07	0.27
Insects	0.26	0.46
Red palm oil	0.17	0.08
Other oils and fats	8.83	8.52
Vegetable oil	4.79	4.39
Shea butter	3.29	3.09
Peanut oil	0.74	0.86
Savoury and fried snacks	0.79	1.26
Cake	0.26	0.55
Sweets	0.07	0.05
Sugar and sweetened beverages	1.25	0.96
Condiments and seasonings	4.27	4.92
Sugar	1.91	2.00
African locust bean	0.75	0.99
Maggi	0.70	0.66
Other beverages and foods	0.38	0.44
Sorghum beer	0.51	0.36
Coffee	0.35	0.58

[a]Food items with at least 0.5% energy contribution were considered as important.

Annex 5.1: CONSORT checklist

Checklist of information to include when reporting a cluster randomized trial*.

Section/Topic	Item No	Checklist item	Reported on page nr publication
Title and abstract			
	1a	Identification as a randomized trial in the title	Title
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Abstract
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	Introduction: paragraph 1-5
	2b	Specific objectives or hypotheses	Introduction: paragraph 6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Methods: "Study design and participants", paragraph 1 & "Randomisation and masking", paragraph 1
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	Methods: "Study design and participants", paragraph 2
	4b	Settings and locations where the data were collected	Methods: "Study design and participants", paragraph 1, 3
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Methods: "Procedures", paragraph 1-2
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Methods: "Procedures", paragraph 3-6 & "Outcomes", paragraph 1-2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	Methods: "Statistical analysis", paragraph 2
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation: Sequence generation	8a	Method used to generate the random allocation sequence	Methods: "Randomisation and masking", paragraph 1
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Methods: "Randomisation and masking", paragraph 1

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Methods: "Randomisation and masking", paragraph 1
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Methods: "Randomisation and masking", paragraph 1
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Methods: "Randomisation and masking", paragraph 2
	11b	If relevant, description of the similarity of interventions	Methods: "Procedures", paragraph 1
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	Methods: "Statistical analysis", paragraph 3-4
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Methods: "Statistical analysis", paragraph 3-4
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	Results, paragraph 1 & Fig. 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Results, paragraph 1-2 & Fig. 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Methods: "Study design and participants", paragraph 2
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 2
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Results, paragraph 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Results, paragraph 3-4 & Table 4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Results, paragraph 3-4 & Table 4
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Results, paragraph 5-6
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Results, paragraph 3 & Table 3
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Discussion, paragraph 8
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	Discussion, paragraph 2-3
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	Discussion, paragraph 5-6
Other information			
Registration	23	Registration number and name of trial registry	

Protocol	24	Where the full trial protocol can be accessed, if available	Methods: "Statistical analysis", paragraph 8
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Metadata of publication

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Annex 5.2: Complete cases analysis of primary and secondary outcomes

Birth characteristics	Control[a] (n = 850)	Intervention[a] (n = 809)	Unadjusted Δ[b] (95% CI)	P value	Adjusted Δ[b] (95% CI)	P value
Small-for-gestational age	236 (27.8)	199 (24.6)	-3.15 (-7.41, 1.12)	0.148	-2.91 (-7.01, 1.19)	0.164
Large-for-gestational age	13 (1.53)	14 (1.73)	0.23 (-0.99, 1.44)	0.716	0.18 (-1.02, 1.37)	0.771
Low birth weight	103 (12.1)	66 (8.16)	-3.95 (-6.83, -1.07)	0.007	-4.13 (-6.91, -1.35)	0.004
Preterm delivery	39 (4.59)	24 (2.97)	-1.90 (-3.80, 0.01)	0.051	-2.04 (-3.94, -0.13)	0.036
Gestational age, weeks	39.9 ± 1.78	40.1 ± 1.48	0.25 (0.09, 0.42)	0.003	0.26 (0.10, 0.42)	0.002
Birth weight, g	2988 ± 450	3039 ± 427	50.7 (8.60, 92.7)	0.018	50.5 (11.5, 89.5)	0.011
Birth length, cm	48.2 ± 2.25	48.4 ± 2.13	0.21 (0.01, 0.41)	0.039	0.21 (0.02, 0.40)	0.028
Ponderal index[c]	26.6 ± 2.67	26.8 ± 2.67	0.14 (-0.09, 0.38)	0.232	0.14 (-0.09, 0.37)	0.232
Head circumference, cm	33.4 ± 1.64	33.5 ± 1.53	0.10 (-0.04, 0.25)	0.159	0.11 (-0.03, 0.25)	0.118
Thoracic circumference, cm	31.7 ± 1.84	31.9 ± 1.68	0.21 (0.04, 0.37)	0.016	0.20 (0.05, 0.36)	0.010
Arm circumference, mm	100 ± 8.42	101 ± 8.13	0.88 (0.13, 1.64)	0.022	0.94 (0.23, 1.65)	0.010

[a] Values are frequencies (%) or means ± standard deviation.

[b] Unadjusted and adjusted group differences (Δ) were estimated by fitting linear regression models for the continuous outcomes, to estimate the mean group difference, and using linear probability models with robust variance estimation for the binary outcomes, to estimate risk difference in percentage points. All models contained health center and randomization block as fixed effect to account for clustering by the study design. Adjusted models additionally contained a priori set known prognostic factors of birth outcome including maternal age, primiparity, gestational age, height, mid-upper arm circumference, body mass index, and hemoglobin level at study enrolment.

[c] Ponderal index calculated as birth weight in g / (birth length in cm)^3 × 1000.

CI, confidence interval.

Annex 5.3: Per protocol analyses of primary and secondary outcomes

Birth characteristics	Control[a] (n = 850)	Intervention[a,b] (n = 631)	Unadjusted Δ[c] (95% CI)	P value	Adjusted Δ[c] (95% CI)	P value
Small-for-gestational age	236 (27.8)	155 (24.6)	-3.30 (-7.89, 1.29)	0.16	-3.16 (-7.59, 1.26)	0.161
Large-for-gestational age	13 (1.53)	10 (1.58)	0.07 (-1.20, 1.35)	0.90	0.08 (-1.19, 1.35)	0.90
Low birth weight	103 (12.1)	52 (8.24)	-3.66 (-6.69, -0.63)	0.018	-3.85 (-6.78, -0.92)	0.010
Preterm delivery	39 (4.59)	21 (3.33)	-1.31 (-3.33, 0.71)	0.20	-1.47 (-3.49, 0.56)	0.16
Gestational age, weeks	39.9 ± 1.78	40.0 ± 1.54	0.20 (0.02, 0.38)	0.033	0.20 (0.02, 0.38)	0.027
Birth weight, g	2988 ± 450	3037 ± 430	45.3 (-0.50, 91.0)	0.05	45.0 (2.52, 87.5)	0.038
Birth length, cm	48.2 ± 2.25	48.3 ± 2.17	0.18 (-0.04, 0.40)	0.11	0.18 (-0.02, 0.39)	0.08
Ponderal index[d]	26.6 ± 2.67	26.8 ± 2.65	0.14 (-0.11, 0.40)	0.27	0.14 (-0.12, 0.39)	0.29
Head circumference, cm	33.4 ± 1.64	33.5 ± 1.56	0.12 (-0.04, 0.28)	0.14	0.13 (-0.03, 0.28)	0.10
Thoracic circumference, cm	31.7 ± 1.84	32.0 ± 1.71	0.22 (0.04, 0.40)	0.019	0.22 (0.05, 0.39)	0.013
Arm circumference, cm	100 ± 8.42	101 ± 8.38	0.92 (0.10, 1.75)	0.029	0.98 (0.20, 1.76)	0.014

[a] Values are frequencies (%) or mean ± standard deviation.

[b] Subsample of women meeting a strict adherence rate ≥75%. Strict adherence was defined as the number of days with observed balanced energy-protein supplement intake over the total days between study inclusion and delivery.

[c] Unadjusted and adjusted group differences (Δ) were estimated by fitting linear regression models for the continuous outcomes, to estimate the mean group difference, and using linear probability models with robust variance estimators for the binary outcomes, to estimate risk difference in percentage points. All models contained health center and randomization block as fixed effect to account for clustering by the study design. Adjusted models additionally contained a priori set known prognostic factors of birth outcome including maternal age, primiparity, gestational age, height, mid-upper arm circumference, body mass index and hemoglobin level at study enrolment.

[d] Ponderal index calculated as birth weight in g / (birth length in cm)^3 × 1000.

CI, confidence interval.

Annex 5.4: Subgroup analysis by potential treatment effect modifiers of small-for-gestational age

Subgroup factor	Control[a] (n = 850)	Intervention[a] (n = 809)	Unadjusted Δ[b] (95% CI)	Pvalue	Adjusted Δ[b] (95% CI)	P value
Maternal BMI[c]				0.14		0.26
<18.5 kg/m^2 (underweight)	55 (6.48)	57 (7.05)	-	-	-	-
≥ 18.5 kg/m^2	795 (93.5)	752 (93.0)	-	-	-	-
Maternal hemoglobin level[c]				0.050		0.036
<11 g/dl (anemic)	309 (36.4)	309 (38.2)	2.06 (-5.41, 9.53)	0.59	3.27 (-4.02, 10.6)	0.38
≥11 g/dl	541 (63.7)	500 (61.8)	-7.14 (-12.4, -1.90)	0.008	-6.77 (-11.8, -1.71)	0.009
Maternal MUAC[c]				0.095		0.078
<23 cm	461 (54.2)	460 (56.9)	0.22 (-5.87, 6.32)	0.94	1.16 (-4.70, 7.01)	0.70
≥23 cm	389 (45.8)	349 (43.1)	-6.47 (-12.4, -0.50)	0.034	-6.07 (-11.9, -0.32)	0.042
Maternal height[c]				0.36		0.28
<155 cm	81 (9.53)	80 (9.89)	-	-	-	-
≥155 cm	769 (90.5)	729 (90.1)	-	-	-	-
Maternal age[c]				0.062		0.050
<20 years	185 (21.8)	191 (23.6)	2.70 (-8.05, 13.5)	0.62	5.08 (-5.41, 15.6)	0.34
≥20 years	665 (78.2)	618 (76.4)	-5.68 (-10.3, -1.07)	0.016	-5.23 (-9.74, -0.72)	0.023
Primiparity[c]				0.19		0.28
Yes	176 (20.7)	181 (22.4)	-	-	-	-
No	674 (79.3)	628 (77.6)	-	-	-	-
Household food insecurity[c]				0.64		0.29
Food insecure	462 (54.4)	445 (55.0)	-	-	-	-
Food secure	388 (45.7)	364 (45.0)	-	-	-	-
Depression possible[c]				0.25		0.35
Yes	19 (2.2)	14 (1.7)	-	-	-	-
No	831 (97.8)	795 (98.3)	-	-	-	-

Depression probable[c]				0.73		0.99
Yes	69 (8.1)	60 (7.4)	-	-	-	-
No	781 (91.9)	749 (92.6)	-	-	-	-
Child sex[c]				0.16		0.08
Female	437 (51.4)	50.3	-	-	-6.73 (-12.6, -0.81)	0.026
Male	413 (48.6)	49.7	-	-	1.13 (-4.78, 7.04)	0.71
Season of delivery[c]				0.50		0.46
Lean (June - September)	267 (31.4)	260 (32.1)	-		-	
Plenty	583 (68.6)	549 (67.9)	-		-	
Inter-pregnancy interval[c]				0.88		0.98
<18 mo	18 (2.12)	27 (3.34)	-	-	-	
≥18 mo	832 (97.9)	782 (96.7)	-	-	-	

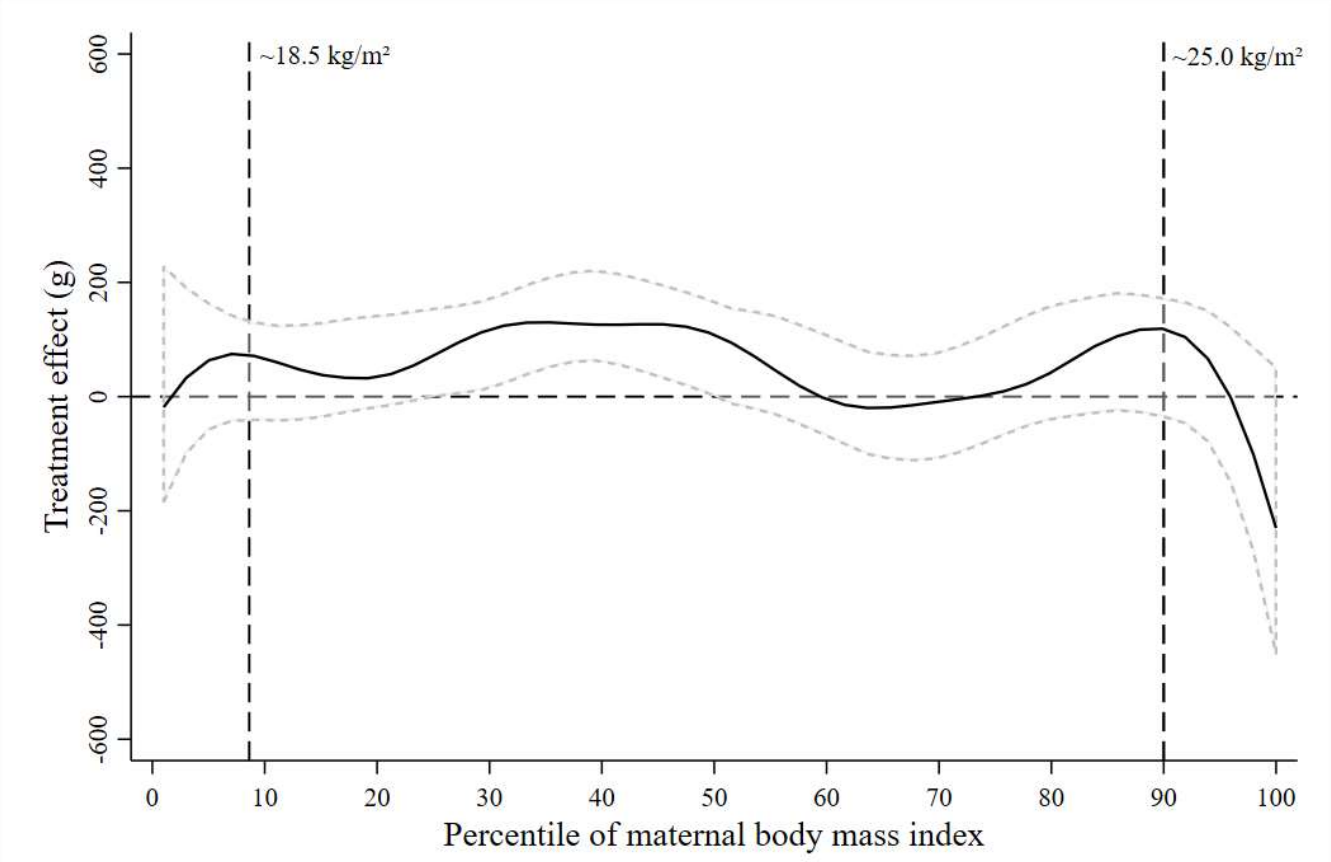
[a] Values are frequencies (%).

[b] Unadjusted and adjusted group differences (Δ) were estimated by fitting linear probability models with robust variance estimators for the binary small-for-gestational age outcome, to estimate risk difference in percentage points. All models contained health center and randomization block as fixed effect to account for clustering by the study design. Adjusted models additionally contained a priori set known prognostic factors of birth outcome including maternal age, primiparity, gestational age, height, mid-upper arm circumference, body mass index and hemoglobin level at study enrolment

[c] Statistical significance was set at P <0.10 for interaction.

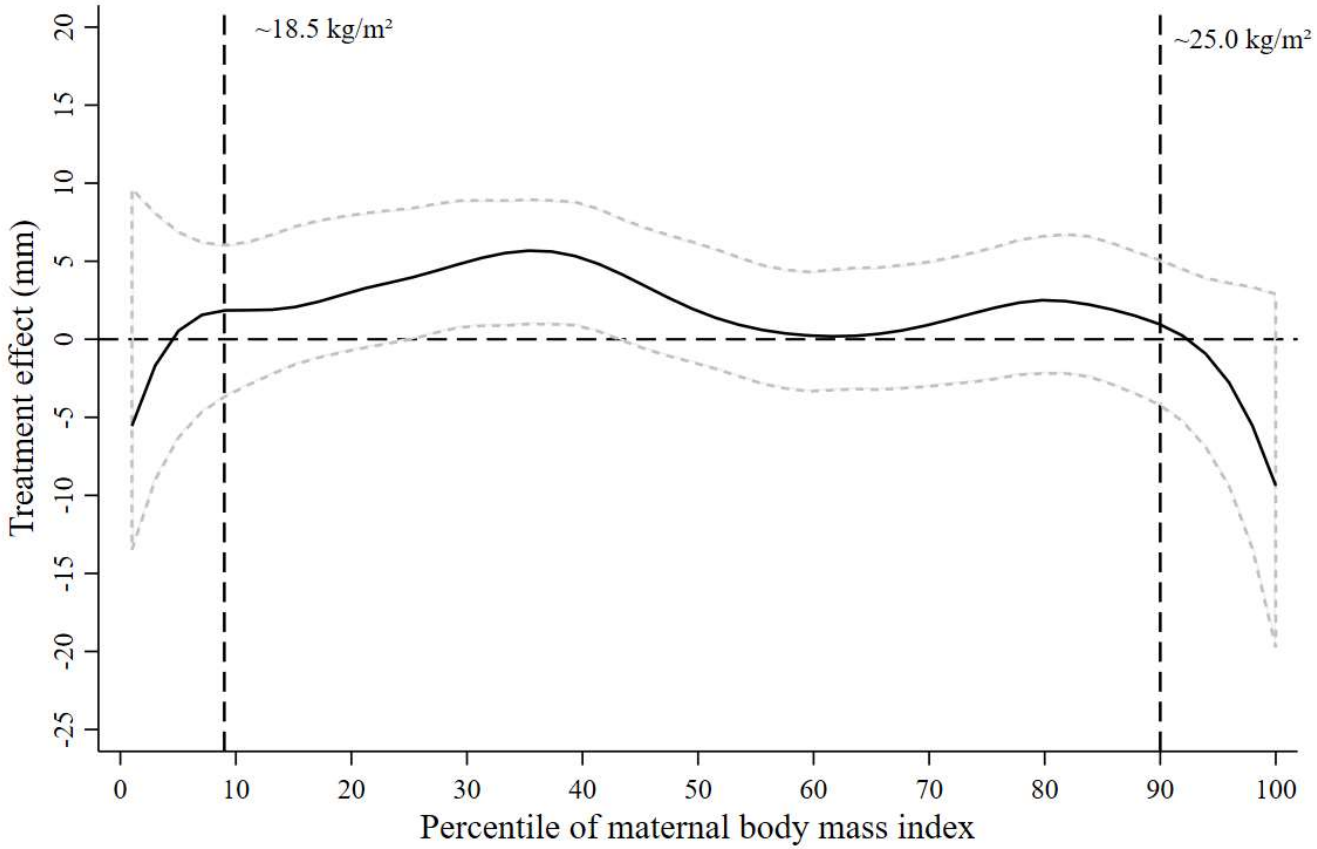
BMI, body mass index; CI, confidence interval; MUAC, mid-upper arm circumference.

Annex 5.5: Figure. Treatment efficacy on birth weight across the distribution of maternal body mass index



The estimated difference in birth weight between the women who received the BEP supplement and IFA (intervention) and those who received only IFA (control) is shown as a function of the percentiles of maternal BMI. The zero line indicates no efficacy of BEP. The positive y values indicate a higher birth weight in the intervention group, and the negative y values indicate a lower birth weight. The central solid black line represents the smoothed treatment efficacy, with upper and lower dashed 95% confidence bands, using complete cases.

Annex 5.6: Figure. Treatment efficacy on birth length across the distribution of maternal body mass index



The estimated difference in birth length between the women who received the BEP supplement and IFA (intervention) and those who received only IFA (control) is shown as a function of the percentiles of maternal body mass index. The zero line indicates no efficacy of BEP. The positive y values indicate a higher birth length in the intervention group, and the negative y values indicate a lower birth length. The central solid black line represents the smoothed treatment efficacy, with upper and lower dashed 95% confidence bands, using complete cases.

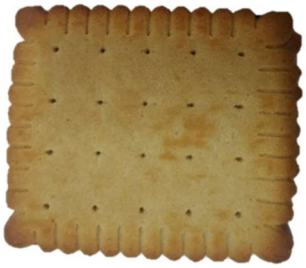
Annex 6: Photos of the research

Chapter 3: Test portions of 12 BEP product formulations

Left plate, clockwise from top center: vanilla biscuit, unseasoned pillows, vanilla-filled sticks, chicken soup, mango bar, lipid-based peanut paste, fermented drink.

Right plate, clockwise from top center: tomato and onion lipid-based paste, tomato and onion biscuit, seasoned pillows, tomato and onion bar, chicken soup.

Chapter 4: Vanilla biscuits (6 biscuits per serving)



Chapter 4 and 6: Lipid-based peanut paste

Chapter 5: 24-Hour recall data collection

Chapter 6: Trial activities and health center renovation

Renovation of all 6 health care centers, examples of Koumbia, Karaba and Dohoun.

Renovation of all 6 health care centers, example of Kari.