Drivers of the trend in child stunting and the role of omega-3 long-chain polyunsaturated fatty acids in relation to child growth and development in rural Ethiopia

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List of abbreviations

AA: Arachidonic acid
ALA: Alpha-linolenic acid
ASQ-SE: Ages and Stages Questionnaire: Social Emotional
BSID: Bayley Scales of Infant Development
C: A study group in which mother and child received a control supplement
CI: A study group in which child received fish-oil supplementation while the mother received control supplement
CNS: Central nervous system
COX: Cyclooxygenase
CRP: C-reactive protein
Denver II: Denver Developmental Screening Test version II
Denver II-Jimma: A culturally adapted and standardized Denver II Developmental Screening Test for use in Ethiopian children
DEVCO: European Commission's Directorate-General for International Cooperation and Development
DHA: Docosahexaenoic acid
DHS: Demographic and Health Survey
EED: Environmental enteric dysfunction
EPA: Eicosapentaenoic acid
FA: Fatty acid
FADS: Fatty acid desaturase enzyme
FAO: Food and Agriculture Organization
GDP: Gross Domestic Product
GPR: G-coupled protein receptor
HDSS: Health and Demographic Surveillance System
HC: Head circumference
HIC: High-income country
HM: Human milk
IL: Interleukin
iNOS: Inducible nitric oxide synthase
IYC: Infants and young children
IYCF: Infant and young child feeding
LA: Linoleic acid
LAZ: Length-for-age *z* score
LCP: Long-chain polyunsaturated fatty acid
LIC: Low-income country
LMIC: Low- and middle-income countries
LOX: Lipoxygenase
LT: Leukotriene
MCI: A study group in which both lactating mother and her child received fish-oil supplementation

MI:	A study group in which lactating mother received fish-oil supplementation while the child received a control supplement
MUAC:	Mid-upper arm circumference
n-3:	Omega-3
n-6:	Omega-6
NFkB :	Nuclear transcription factor kappa B
PG:	Prostaglandin
PPAR:	Peroxisome proliferator activated receptor
PUFA:	Polyunsaturated fatty acid
RCT:	Randomized controlled trial
SDGs:	Sustainable Development Goals
TNFα:	Tumor necrosis factor α
UNICEF:	United Nations Children Fund
URI:	Upper respiratory infection
VEP:	Visual evoked potentials
WHA:	World Health Assembly
WHO:	World Health Organization
WLZ:	Weight-for-length *z* score

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Summary

Despite the significant progress the world has made over the past few decades, millions of children in low- and middle-income countries still suffer from poor growth and development. Growth faltering during the critical window period of the first 1,000 days after conception is associated with multiple adverse consequences limiting human potential and economic growth. Recognizing the magnitude of the problem and its severe consequences, the World Health Assembly in 2012 endorsed a Comprehensive Implementation Plan on Maternal, Infant, and Young Child Nutrition with six global targets, chief among which is the target to reduce the number of stunted children by 40% in 2025.

Identifying priority areas of action in high nutrition-burden countries is the first step to accelerate current progress and achieve the global nutrition targets. For the purpose of facilitating evidence-based decision-making, the first part of this PhD thesis explores nutrition-specific and -sensitive factors that can contribute to a reduction in chronic child undernutrition in low- and middle-income countries. We pooled data from 50 Demographic and Health Surveys conducted in 14 low- and middle-income countries to explain the trend in under-five stunting prevalence over the past two decades. A four-level mixed-effects linear probability model, accounting for clustering of data by sampling clusters, survey-rounds and countries, was fitted to estimate the association between the change in a range of distal to proximal determinants at a country-level and stunting risk for an individual child while adjusting for time trend and child-level covariates.

Furthermore, innovative approaches that maximize the impact of existing nutrition interventions are highly required to assist the progress in low- and middle-income countries. Complementary feeding interventions in low-income settings to date have been focused on energy and micronutrient content of diets and yielded only small to moderate effects on growth and development. Increasing evidence support the hypothesis that environmental enteric dysfunction, a chronic gut inflammation with morphological and functional derangements and systemic inflammation occurring at a high prevalence in children living in poor settings, could be an important missing link that mediates and reduces the expected benefits from interventions. There is evidence that omega-3 long-chain polyunsaturated fatty acids (n-3 LCPs) may

improve gut integrity, reduce inflammation and enhance maturation of the immune system, which could lead to amelioration of this condition and the associated growth impairment. Additionally, some studies in high-income populations showed that n-3 LCPs may have benefits for infant neurocognitive development. However, there is limited evidence from studies testing these potential benefits in infants and young children in a low-income setting.

Therefore, in the second part of the PhD thesis, we hypothesized that an increased intake of n-3 LCPs would result in reduced morbidity and inflammation, and improved growth and development of children aged 6-24 months in a low-income setting. To test these proposed hypotheses, we conducted the OME^{3}JIM project involving a 2 x 2 factorial randomized controlled trial of n-3 LCPs-rich fish-oil supplementation (500 mg/day n-3-LCPs) through lactation (MI), complementary feeding (CI), or a combination of both (MCI). We enrolled 360 pairs of lactating mothers and their infants 6-12 months old from three rural communities in Jimma district, southwest Ethiopia. The primary study outcomes were child linear growth, i.e., monthly changes in length-for-age *z* score (LAZ) over the 12 months intervention follow-up, and the evolution of developmental performance from baseline through 6 and 12 months of the intervention, using the Denver II and the Ages and Stages Questionnaire: Social Emotional tools. Secondary outcomes included LCP concentrations in maternal milk and child blood, anthropometry measurements of weight-for-length *z* score (WLZ), head-circumference and mid-upper arm circumference (MUAC), nutritional status (stunting, wasting and anemia), common childhood morbidities, and inflammation using C-reactive protein.

Chapter 3 presents results of the study on the trend in child stunting. Stunting followed a declining trend in all the 14 countries studied at an average annual reduction rate of 1.04 percentage points (pp). Among the distal factors assessed, a decrease in the Gini coefficient, an improvement in women's decision-making, and an increase in urbanization over time within a country were significantly associated with a lower risk of stunting. Improvements in households' access to improved sanitation facilities and drinking water sources, and children's access to basic vaccinations were the important intermediate service-related drivers of stunting risk identified, whereas an improvement in early initiation of breastfeeding and a decrease in the prevalence of low birthweight were the important proximal drivers.

Our findings indicate that although there has been progress in reducing stunting, the rate of reduction in the studied countries was below the average 3.9 pp annual reduction rate required to meet the global target set for 2025. Furthermore, our findings reinforce the need for a combination of nutrition-specific and -sensitive interventions on top of economic development to tackle the problem of chronic childhood undernutrition. The identified drivers will help to guide global efforts to further accelerate stunting reduction and monitor progress against chronic child undernutrition.

Results of the OME^{3}JIM study are presented and discussed in **Chapters 4-6**. From the total of 360 mother-infant pairs enrolled, 87% completed all the 12 months study follow-ups and the mean (SD) duration of supplementation was 11.0 (2.9) months. Compliance rate for the child and the maternal interventions were ~80% and ~70%, with no difference between study arms. All statistical analyses were conducted following the intention-to-treat principle. In **Chapter 4,** we present the efficacy of fish-oil supplementation of lactating mothers on human milk LCP concentrations using a random sub-sample of 154 study participants. Fish-oil supplementation during lactation increased maternal milk concentrations of docosahexaenoic acid (DHA) by 39.0% ($P < 0.001$) and eicosapentaenoic acid (EPA) by 36.2% ($P < 0.001$), whereas the ratio of arachidonic acid (AA)/(DHA + EPA) decreased by 53.5% ($P < 0.001$), compared to the control. However, the maternal milk DHA concentration still remained lower than international norms after the intervention. The results demonstrate that fish-oil supplementation during lactation improves n-3 LCPs status of the maternal milk. In these mothers with a very low baseline breastmilk DHA status, which further declines over the course of lactation, a higher dose of supplementation may be required to attain optimal breastmilk DHA levels.

Chapter 5 presents the independent and combined effects of the fish-oil intervention through lactation and complementary food on child n-3 LCPs status, health and growth. Fish-oil supplementation significantly increased child blood n-3 LCPs concentrations ($P < 0.01$) and decreased the AA/(DHA + EPA) ratio ($P < 0.001$) in all the MI, CI and MCI intervention arms as compared to the control. Fish-oil intervention also resulted in a better ponderal growth of children, as indicated by the small, but statistically significant, positive effects on monthly WLZ changes in the CI (effect size: 0.022/month; 95% CI: 0.005, 0.039/month; $P = 0.012$) and MCI arms

(effect size: 0.018/month; 95% CI: 0.001, 0.034/month; *P* = 0.041). We also noted a non-significant trend towards larger monthly MUAC increments in the CI and MCI arms compared to the control. No further effects were detected on the primary study outcome linear growth or on the other secondary outcomes of growth, nutritional status, morbidity, and inflammation. **Chapter 6** presents the effects of the same intervention on child development performance. There was no difference between study arms on the evolution of overall and social-emotional developmental performance over time (intervention by time interaction: F = 1.09; *P* = 0.35, and F = 0.61; *P* = 0.61, respectively). Overall, the findings from the OME^{3}JIM trial did not support our primary study hypotheses that dietary n-3 LCPs supplementation through lactation and/or complementary feeding improves linear growth and development of infants and young children from a rural setting in Ethiopia. n-3 LCP supplementation given directly to children or in combination with maternal supplementation was found to modestly increase relative weight gain.

In conclusion, this PhD research provides evidence on a set of potentially important proximal to distal factors that can contribute to reduction in chronic childhood undernutrition in low- and middle-income countries. It also contributes to the limited literature on the effects of n-3 LCP supplementation in infants and young children 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. It is underlined that economic development and nutrition-sensitive interventions, on top of nutrition-specific programs, could play an important role in further reduction of the high stunting burden in low- and middle-income countries. Future follow-up of the OME^{3}JIM cohort is also recommended to determine whether there are long-term effects of the fish-oil intervention.

Samenvatting

Ondanks de aanzienlijke vooruitgang die de wereld afgelopen decennia heeft gemaakt, zijn miljoenen kinderen in lage- en middeninkomenslanden nog steeds belemmerd in hun groei en ontwikkeling. Een slecht verloop van de groei tijdens de kritieke periode, van de eerste 1000 dagen na conceptie, gaat gepaard met meerdere nadelige gevolgen die het menselijk potentieel en de economische groei beperken. De Wereldgezondheidsassemblee herkende de omvang van het probleem en de ernstige gevolgen ervan en keurde in 2012 een alomvattend uitvoeringsplan voor de voeding van moeders, zuigelingen, en jonge kinderen goed, met zes wereldwijde doelstellingen, waaronder het hoofddoel om het aantal kinderen met groeiachterstand te verminderen met 40% tegen 2025.

Het identificeren van prioritaire actiegebieden, in landen waar het risico op ondervoeding groot is, is de eerste stap om de huidige vooruitgang te versnellen en de wereldwijde voedingsdoelstellingen te halen. Het eerste deel van dit doctoraatswerk heeft als doel het faciliteren van evidence-based besluitvorming en is gericht op het onderzoeken van voedingsspecifieke en -gevoelige factoren die kunnen bijdragen aan het verminderen van chronische ondervoeding in lage- en middeninkomenslanden . We hebben gegevens verzameld van 50 Demographic and Health Surveys uitgevoerd in 14 lage- en middeninkomenslanden om de trend in de prevalentie van groeiachterstand in kinderen, jonger dan vijf jaar, in de afgelopen twee decennia te verklaren. Een lineair waarschijnlijkheidsmodel met vier niveaus voor gemengde effecten, dat rekening houdt met de clustering van gegevens door steekproefclusters, enquêterondes en landen, werd opgesteld om de associatie tussen de verandering in een reeks distale en proximale determinanten op landenniveau en de kans op groeiachterstand voor een individueel kind te schatten, met een correctie voor tijdtrend en covariabelen op kindniveau.

Bovendien zijn innovatieve benaderingen die de impact van bestaande voedingsinterventies maximaliseren vereist om de vooruitgang in lage- en middeninkomenslanden te ondersteunen. Complementaire voedingsinterventies in lage- en middeninkomenslanden waren tot nu toe eerder gericht op het energie- en micronutriëntengehalte van diëten en leverden slechts kleine tot matige effecten op groei en ontwikkeling op. Steeds meer bewijs ondersteunt de hypothese dat

omgeving gerelateerde enterische dysfunctie, een chronische darmontsteking met morfologische en functionele verstoringen en systemische ontsteking die veel voorkomen bij kinderen in slechte omstandigheden in arme regio's, een belangrijke ontbrekende schakel is die de verwachte voordelen van interventies bemiddelt en vermindert. Er zijn aanwijzingen dat omega-3 meervoudig onverzadigde vetzuren met lange keten (n-3 LCPs) de darmintegriteit kunnen verbeteren, ontstekingen kunnen verminderen en de rijping van het immuunsysteem kunnen verbeteren, wat zou kunnen leiden tot verbetering van deze aandoening en de daarmee samenhangende groeiachterstand. Bovendien is aangetoond dat n-3 LCPs de neurocognitieve ontwikkeling van zuigelingen in ontwikkelde landen ten goede komt. Er is echter beperkt bewijs uit studies die deze potentiële voordelen testen bij zuigelingen en jonge kinderen uit ontwikkelingslanden.

Daarom werd in het tweede deel van het doctoraatswerk de hypothese opgesteld dat een verhoogde inname van n-3 LCPs zou leiden tot verminderde morbiditeit en ontsteking en tot verbeterde groei en ontwikkeling van kinderen 6-24 maanden in een laag inkomen gebied. Om deze voorgestelde hypothesen te testen, hebben we de OME^{3}JIM-studie uitgevoerd. De studie is een gerandomiseerde gecontroleerde studie met een 2 x 2 factorieel ontwerp van n-3 LCPs-rijke visolie-supplementatie (500 mg/dag n-3-LCPs) via borstvoeding (MI), aanvullende voeding (CI) of beide (MCI), door zogende moeders en hun zuigelingen 6-12 months uit drie rurale gemeenschappen in het district Jimma, in het zuidwesten van Ethiopië. De primaire uitkomsten van het onderzoek waren lineaire groei van het kind (d.w.z. maandelijkse veranderingen in lengte-voor-leeftijd *z*-score (LAZ) over 12 months) en ontwikkelingsprestaties op 6 en 12 months met behulp van de Denver II en de Ages and Stages Questionnaire: Social Emotional hulpmiddelen. Secundaire uitkomsten waren onder meer LCP-concentraties in moedermelk en bloed van de kinderen, antropometriemetingen van de gewicht-voor-lengte *z*-score (WLZ), hoofdomtrek en midden bovenarmomtrek (MUAC), voedingsstatus (groeiachterstand, 'wasting', en bloedarmoede), veelvoorkomende morbiditeiten bij kinderen en ontstekingen met behulp van C-reactief proteïne.

Hoofdstuk 3 presenteert de resultaten van de groeiachterstand trendstudie. Groeiachterstand volgde een dalende trend in alle 14 onderzochte lage- en middeninkomenslanden met een gemiddeld jaarlijks reductiepercentage van 1,04

procentpunt (pp). Onder de beoordeelde distale factoren waren een afname van de Gini-coëfficiënt, een verbetering van de besluitvorming van vrouwen, en een toename van verstedelijking in de tijd binnen een land significant geassocieerd met een lagere kans op groeiachterstand. Verbeteringen in de toegang van huishoudens tot sanitaire voorzieningen en drinkwaterbronnen, en de toegang van kinderen tot basisvaccinaties waren de belangrijke tussenliggende service gerelateerde drijfveren, terwijl verbetering in het vroeg starten met borstvoeding en een afname in de prevalentie van laag geboortegewicht de belangrijke proximale drijfveren waren. De resultaten geven aan dat, hoewel er vooruitgang is geboekt in het verminderen van groeiachterstand, het huidige reductiepercentage in de bestudeerde landen lager is dan de jaarlijkse vermindering van 3,9 pp die nodig is om de doelstelling van 2025 te halen. De resultaten versterken de behoefte aan een combinatie van voedingsgevoelige en -specifieke interventies bovenop economische ontwikkelingen om het probleem van chronische ondervoeding bij kinderen aan te pakken. De geïdentificeerde drijfveren helpen de wereldwijde inspanningen te begeleiden om de groeiachterstand reductie verder te versnellen en de voortgang tegen chronische ondervoeding bij kinderen te volgen.

De resultaten van de OME^{3}JIM-studie worden gepresenteerd en besproken in **Hoofdstukken 4-6**. Van de in totaal 360 ingeschreven moeder-kindparen, voltooide 87% de follow-up periode van 12 months van het onderzoek. De gemiddelde (SD) duur van supplementatie was 11,0 (2,9) months. De nalevingspercentages voor kind en maternale interventies waren 80% en 70%, zonder verschil tussen studiearmen. Alle statistische analyses zijn uitgevoerd volgens het intention-to-treat-principe. In **Hoofdstuk 4** hebben we de werkzaamheid van supplementatie met visolie van zogende moeders op LCP-concentraties in moedermelk (HM) in een willekeurig deelmonster van 154 deelnemers gepresenteerd. Supplementatie met maternale visolie verhoogde de HM-concentraties van docosahexaeenzuur (DHA) met 39,0% (*P* <0,001) en eicosapentaeenzuur (EPA) met 36,2% (*P* <0,001), terwijl het arachidonzuur (AA)/(DHA + EPA) ratio afnam met 53,5% (*P* <0,001), vergeleken met de controlegroep. We vonden significante verbanden tussen de verandering in de verhouding tussen HM en kinderbloed (DHA + EPA)/AA na de supplementatie (*P* <0.001). De HM DHA-concentraties bleven na de interventie echter nog steeds lager dan de internationale normen. De resultaten tonen aan dat supplementatie met

visolie tijdens borstvoeding de status van HM n-3 LCPs verbetert. Bij deze moeders met een zeer lage HM DHA-baseline-status, die verder afneemt tijdens het geven van borstvoeding, kan een hogere dosis supplementatie nodig zijn om adequate HM-spiegels te bereiken.

Hoofdstuk 5 presenteert de onafhankelijke en gecombineerde effecten van de visolie-interventie door borstvoeding en complementaire voeding op de status van kind n-3 LCPs, en gezondheids- en groeiresultaten. Supplementatie met visolie verhoogde significant de bloedconcentraties van n–3 LCPs van kinderen (*P* <0,01) en verlaagde de AA/(DHA + EPA) -ratio (*P* <0,001) in alle interventiearmen. De visolie-interventie resulteerde in een klein positief effect op maandelijkse veranderingen in WLZ in de CI- (effectgrootte: 0,022/month; 95% CI: 0,005, 0,039/month; *P* = 0,012) en MCI-armen (effectgrootte: 0,018/month ; 95% CI: 0,001, 0,034/month; *P* = 0,041). We merkten ook een niet-significante trend op van grotere maandelijkse MUAC-verhogingen in dezelfde interventiearmen. Er werden geen verdere effecten gedetecteerd op de primaire uitkomst lineaire groei of op de andere secundaire uitkomsten van groei, voedingstoestand, morbiditeit en ontsteking.
Hoofdstuk 6 presenteert het effect van dezelfde interventie op de ontwikkelingsprestaties van kinderen. Er was geen verschil tussen de onderzoekarmen wat betreft de evolutie van de algemene en sociaal-emotionele ontwikkelingsprestaties over tijd (interventie × tijd: F = 1,09; *P* = 0,35 en F = 0,61; *P* = 0,61, respectievelijk). Over het algemeen ondersteunden de resultaten van de OME^{3}JIM-studie de hypothesen van de primaire studie niet dat supplementatie met n-3 LCPs via lactatie en/of complementaire voeding de lineaire groei en ontwikkeling van zuigelingen en jonge kinderen in een landelijke Ethiopische omgeving verbetert. n–3 LCP-supplementatie direct aan kinderen gegeven, verhoogde in bescheiden mate de relatieve gewichtstoename.

Samenvattend, dit doctoraatsonderzoek leverde bewijs op over belangrijke drijfveren voor de vermindering van chronische ondervoeding in kinderen in lage- en middeninkomenslanden en heeft bijgedragen aan het beperkte bewijs over n-3 LCP-supplementatie van zuigelingen en jonge kinderen in een rurale sub-Sahara Afrikaanse setting. In **Hoofdstuk 7** worden de implicaties van de bevindingen besproken en worden aanbevelingen voor toekomstig onderzoek gedaan. Benadrukt wordt dat economische ontwikkeling en voedingsgevoelige interventies, naast

voedingsspecifieke programma's, een belangrijke rol zouden kunnen spelen bij het verder verminderen van groeiachterstand in lage- en middeninkomenslanden . Opvolging van het OME^{3}JIM-cohort wordt aanbevolen om te bepalen of er langdurige effecten van de interventie zijn.

1

CHAPTER 1

GENERAL INTRODUCTION

1.1. The epidemiology of childhood undernutrition and developmental delay

1.1.1. Magnitude and consequences

Poor child growth and development are major public health problems in low- and middle-income countries (LMICs). According to the 2018 estimates of the global burden of malnutrition, 149 million (22%) children younger than five years old suffer from stunted growth, indicating chronic undernutrition (1). During the same period, an estimated 49 million (7.3%) children were affected by acute malnutrition (wasting) from which nearly 17 million were severely wasted, seriously jeopardizing their survival. South Asia and sub-Saharan Africa are the two most affected regions, contributing to 55% and 39% of the global burden of stunting, and 68% and 28% of the wasting burden, respectively.

Although there is a lack of data to directly estimate the global burden of childhood developmental problems, children in LMICs have increased risk of poor developmental outcomes because of a combination of risk factors such as poverty, poor health and nutrition, and inadequate caring practice. Estimates based on proxy measures of stunting and extreme poverty in 2010 indicated that 250 million (43%) children younger than five years in LMICs are at risk of not reaching their developmental potential, with Sub-Saharan Africa (66%) and South Asia (53%) being the regions with the highest percentage of disadvantaged children (2).

Undernutrition and poor development during early-life have far reaching negative consequences on the health and wellbeing of individuals as well as on the human and economic development of societies and countries at large (3–5). Undernutrition increases children's susceptibility to illness and mortality (6). It has been estimated that undernutrition in the aggregate, including intrauterine growth retardation, stunting, wasting, and deficiencies of vitamin A and zinc along with suboptimal breastfeeding, underlies 45% of the global burden of under-five mortality, resulting in 3.1 million deaths in 2011, and contributes to 80% of the neonatal mortality occurring among low birthweight babies (7,8).

Children surviving from malnutrition encounter short-, medium- and long-term adverse consequences. Children with suboptimal nutritional status and development will subsequently have diminished cognitive performance and school achievement, which have a knock-on effect for adult wages and productivity (5,9–15). Early

nutritional insult also has irreversible negative effects on physical strength and work capacity, resulting in reduced adult earnings (16). The loss of human potential associated with stunted growth in early-life has been estimated to be more than a 20% deficit in adult income, which has substantial implications for national development (11). Furthermore, evidence shows that physiological changes related to early-life nutritional insults could contribute to increased risk of nutrition-related chronic diseases and related mortality into adulthood (5,14,17–19).

Undernutrition is a cyclical process because children who are stunted usually grow-up to be stunted mothers and likely to transfer this status to their offspring, creating an intergenerational cycle of poor health, poverty and reduced human capital (20–23). Short statured mothers have increased risk of cephalopelvic disproportion leading to obstructed labor, maternal morbidity and mortality, and stillbirths (5,7,14,24). Newborns from stunted mothers are more likely to face intrauterine growth restriction that increases their risk of perinatal mortality and their later risks of morbidity and mortality, and impaired growth and development (5,8,21,24–26).

1.1.2. The determinants of child nutritional status and development

A complex interrelationship of multiple factors determines children's risk of suboptimal health, nutrition and development in LMICs. The United Nations Children's Fund (UNICEF) (27) in 1990 proposed a conceptual framework for 'the causes of child malnutrition and death in developing countries', explaining how the hierarchical relationship of multiple factors operate at the immediate (individual child), underlying (household), and basic (societal) levels. The more recent Lancet 2013 Framework for Action (7) also reflected on this framework to elucidate the potential means to achieve optimum fetal and child nutrition and development (**Figure 1.1**).

In short, the framework identifies the dietary, health, and caregiving determinants of child nutrition, growth and development, which manifest themselves at the level of the individual child, and how these factors are influenced by underlying resources of household food security, caregiving resources, and the health environment determinants, which are in turn shaped by structural and contextual factors at the wider national and global level (7,27). Accordingly, it has been emphasized that combinations of nutrition-specific interventions, that address the immediate causes

of suboptimum growth and development, and nutrition-sensitive interventions, that address the underlying factors, on top of structural actions to create enabling environment for policy changes and to support interventions and programs are required to improve the wellbeing of children in LMICs (7,28–30).

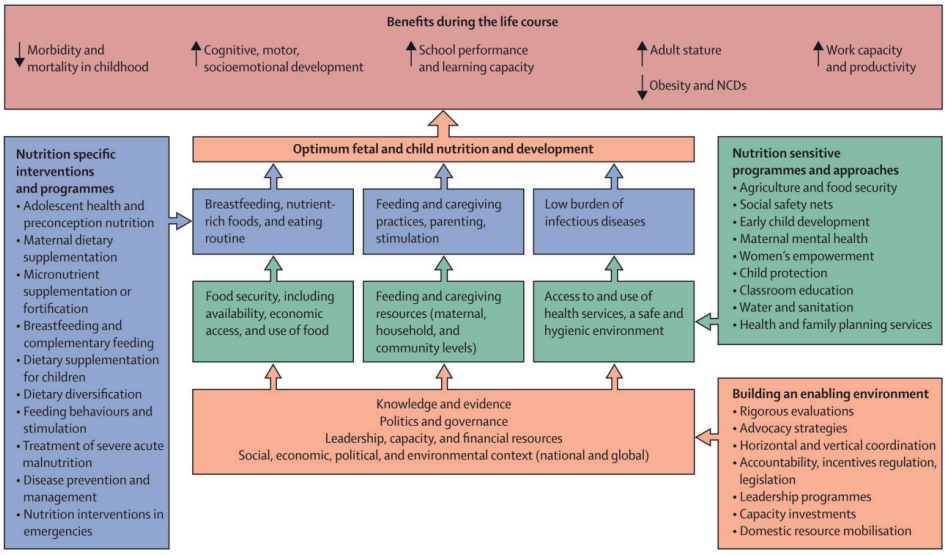

Figure 1.1. Conceptual framework of the immediate, underlying, and basic determinants, and relevant actions to achieve optimum child nutrition and development (from Black et al. (7)**).**

Inadequate dietary intake and poor health are the immediate causes of suboptimal child growth and development. Optimal breastfeeding and complementary feeding are crucial to secure the high energy and nutrients requirements of infants and young children (IYC) who are in rapid growth and development (31,32). The World Health Organization (WHO) (33) in 2010 proposed global recommendations for infant and young child feeding (IYCF), which included eight core and seven optional indicators of optimal feeding practices. Subsequently, several studies showed that adherence to the recommended IYCF indicators and interventions targeted at improving optimal IYCF practices resulted in improved growth and nutritional status of children in LMICs (34–39). Prenatal nutrition, which is mainly determined by maternal health and nutritional status at the time of conception and during pregnancy, also plays a critical role in the status of children at birth and their subsequent growth and development. It has been estimated that 20% of the stunting

and 30% of the wasting burden in under-five children have prenatal origins (26).The high burden of infectious diseases like diarrhea in low-income settings compromise the healthy growth and development of children (40,41). Furthermore, in children living under poor environmental and food hygienic conditions, even when there are no obvious symptoms, physiological conditions associated with persistent asymptomatic gut inflammation can result in growth faltering by impairing absorption of nutrients, increasing nutrient losses and diverting nutrients away from growth and development towards the requirements for immune response (17,41–43).

The three underlying determinants that influence child nutrition and development through the immediate causes are the availability, economic accessibility, and utilization of resources including household food security; resources for care to children and women; and the health environment. Food security is crucial to ensure access to sufficient amounts of safe and nutritious food for healthy growth and development (44,45). Household food insecurity could be caused by unavailability of food, insufficient purchasing power, inappropriate intrahousehold distribution, or inadequate body utilization of food (46). Care is the provision in the household and the community of adequate time, attention, and support to meet the physical, mental, and social needs of the growing child and other household members (47). Examples of caring practices are child feeding (including feeding during illness), child cognitive stimulation, health-seeking behavior, and caring and support for mothers during pregnancy and lactation. Resources for care for children and women include women's empowerment through education, work opportunity, autonomy and control of resources, caregiver knowledge and beliefs, caregiver physical health and nutritional status, caregiver mental health and protection from abuse, and family and community social support (47–50). The third underlying determinant is the health environment surrounding a child, including hygiene and sanitation conditions and access to health care services, which determine a child's exposure to pathogens and risk of morbidity as well as utilization of preventive and curative health care services. Important aspects of the health environment include access to safe water, sanitation facilities, safe housing and food hygiene, and access to basic health services such as child immunization and growth monitoring, deworming, treatment of sick child, and maternal health care services (51–53).

Finally, the basic determinants that impact child nutrition and development through the underlying and immediate determinants, are structural and contextual factors at the wider national and global level. They form the economic, political, environmental, social, and cultural context in which children's health, nutrition and development is determined. Economic progress in a country, for instance, can influence child nutrition and health through different routes. Higher national income is related to poverty reduction and thus, can be considered as a proxy indicator of a household's expenditure on health and nutrition inputs such as food, water, sanitation, and health care (28,54). On top of this, increasing national income can be exploited for investment in pro-poor public services such as health services, social protection and education (52). The political, social, and cultural contexts of a country, as well as the leadership taken, also play numerous roles as basic drivers of the wellbeing of children and women. For instance, responsiveness and accountability of the government and the bureaucracy are important in designing, implementing, and monitoring policies addressing the needs of vulnerable groups such as the health and nutrition needs of children and women (29,52,55).

1.1.3. Global trends in child undernutrition

Recently, reducing the scourge of childhood undernutrition has been gaining increasing priority on the international development agenda both as a maker and a marker of sustainable development in LMICs. In this regard, stunting, defined as length/height-for-age falling below 2 standard deviations from the WHO Child Growth Standards median, emerged as a key indicator to follow progress on the situation of children globally (56,57). Stunting is not only the most prevalent form of undernutrition globally, but it is also the best overall proxy index of children's physical and developmental well-being and has concurrent short- and long-term health and economic consequences (11,56).

The World Health Assembly (WHA) in 2012 adopted a resolution to reduce by 40% the number of under-five children who are stunted between 2010 and 2025 (58). Significant progress has been made towards reducing the global burden of stunting over the past few decades. The prevalence of childhood stunting decreased from about 33% in 2000 to less than 22% in 2018 (**Table 1.1**) (1). However, the prevalence rates and trends vary greatly across countries and world regions and while there has been progress, millions of children are still suffering from stunted

growth and its functional consequences. In South Asia and sub-Saharan Africa, stunting still remains unacceptably high, affecting more than one-third of under-five children. In Africa, where population growth outpaces the reduction in stunting prevalence, the absolute number of stunted children is increasing. At the current rates of stunting reduction, it is estimated that there will be 127 million stunted children globally by 2025, a reduction of only 26% in contrast to the global target of 40% reduction (56).

Table 1.1. Global trends in stunting prevalence among under-five children, 2000-2018[1]

Regions	2000 % (nr in million)	2018 % (nr in million)	Decrease since 2000 (percentage points)	Relative decrease from 2000 (%)
South Asia	51.4 (89.4)	34.4 (58.7)	17.0	33.1
Sub-Saharan Africa	43.0 (50.0)	33.3 (57.9)	9.7	22.6
East Asia & the Pacific	24.5 (36.9)	8.4 (13.0)	16.1	65.7
Middle East & North Africa	22.8 (8.5)	14.7 (7.1)	8.1	35.5
Latin America & the Caribbean	16.7 (9.6)	9.0 (4.8)	7.7	46.1
Europe and Central Asia	-	-	-	-
Low- and middle-income countries	35.9 (195.0)	23.9 (145.9)	12.0	33.4
Global	32.5 (198.2)	21.9 (149.0)	10.6	32.6

[1]Source: UNICEF, WHO, The World Bank. Levels and trends in child malnutrition: key findings of the 2019 Edition of the Joint Child Malnutrition Estimates; WHO: Geneva, Switzerland, 2019 (1).

Several studies have evaluated which of the determinants in the UNICEF's conceptual framework of childhood undernutrition were the most important drivers for the stunting reduction observed over the past three decades. It has been shown that both progress in specific interventions and changes in general developmental factors were associated with the improvements in child undernutrition in LMICs (45,51–54,59–65). The important proximal and underlying drivers of stunting reduction that were reported across settings included improvements in access to sanitation infrastructure (52,53,59,60), safe water (45,52,61), child immunization (45,51), deworming medication (51), antenatal care service (53,59,60), maternal iron supplementation (51), increases in women's education (45,52–54,59–61,65), improvements in household income or asset ownership (53,54,59,60,65), improvements in women status such as gender equality (52,61) and maternal nutrition (59,60), and improvements in food availability (i.e., per capita national energy supply (45,52,61).

The studies also noted the important facilitating role of basic factors such as economic growth (45,52,54,61), good governance and democracy (52,61), and urbanization (45) for the observed reduction in child undernutrition. On the other hand, whether general economic growth alone can result in a rapid reduction in undernutrition is questionable (62,63,65,66). Economic growth may not necessarily result in improvements in nutrition when income distribution is very unequal and benefits of increased national income do not adequately reach the poor. Analysis of the trends in stunting prevalence and gross domestic product (GDP) in 85 LMICs found equitable income distribution to be a necessary condition for increases in GDP to have an impact on stunting reduction (45). Furthermore, a higher national income may not be efficiently directed towards nutrition-specific and -sensitive investments. Harttgen et al. (65) showed that GDP growth in 28 Sub-Saharan African countries had a smaller contribution in explaining the differential improvements in child undernutrition across countries as compared to other factors such as progresses in women's education and access to health services, and reduction in socioeconomic inequality.

1.2. Omega-3 long-chain polyunsaturated fatty acids as essential nutrients

1.2.1. Metabolism and dietary sources

Essential fatty acids (FAs) are FAs that are required for important body functions but cannot be synthesized by the human body and, as such, must be provided in the diet. Linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3) are the two polyunsaturated fatty acids (PUFA) that are considered essential in the diet and the parent FAs for the omega-6 (n-6) and omega-3 (n-3) PUFA families, respectively. The distinction between the n-6 and n-3 PUFA families is based on the location of the first unsaturated bond from the methyl end (i.e. delta (Δ) position) of the FA molecule, which also determines their metabolic pathways. Mammals cannot synthesize the two essential FAs because they lack the FA desaturase enzymes (FADSs) introducing double bonds beyond the Δ9 positions, i.e. the Δ12- and Δ15-FADSs catalyzing the synthesis of LA from oleic acid and ALA from linoleic acid (67).

Much of the principal biological roles of LA and ALA in the body come from their conversion into the more biologically active long-chain PUFA (LCP) derivatives through a series of FA elongation, desaturation, and beta-oxidation steps which are

summarized in **Figure 1.2** (67). The most important n-6 LCP is arachidonic acid (AA; 20:4n-6) for which LA serves as the parent FA. ALA is converted to eicosapentaenoic acid (EPA; 20:5n-3) and further to docosahexaenoic acid (DHA; 22:6n-3), which are the two major n-3 LCPs.

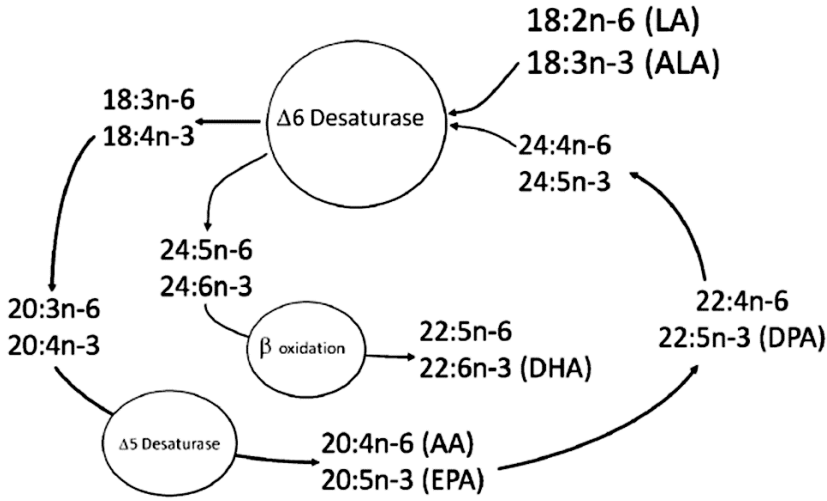

Figure 1.2. Metabolism of essential FAs to their LCP derivatives (from Gibson et al. (67)**)**. DPA; docosapentaenoic acid.

The n-3 and n-6 PUFA families compete with one another for shared enzymes in the metabolic pathway and no interconversion occurs between the two families. Therefore, the balance between LA and ALA in the diet determines the balance between their biologically active n-6 and n-3 LCP derivatives in body tissues (67–70). Despite desaturase enzymes show better affinity for ALA over LA, the metabolism of LA is generally favored over ALA due to the greater abundance of LA in human diets (67,71).The Δ6-desaturase is thought to be the rate limiting enzyme in the metabolic pathway and is required twice in the conversion of DHA from ALA, i.e., for desaturation of ALA to 18:4n-3 and 24:5n-3 to 24:6n-3 (67). The later use of the enzyme for desaturation of 24:5n-3 which subsequently leads to DHA synthesis thus faces competition from both LA and ALA in the diet. As a result, DHA synthesis may be inhibited not only by high dietary LA: ALA ratio, but also when diets contain very high amounts of ALA. Dose-response studies showed that while dietary ALA intake and tissue EPA content have a linear relationship, DHA status followed a curvilinear relationship with increasing dietary ALA intake (72,73). The synthesis of

DHA from ALA in weaning rats was inhibited when their diet contained high amounts of PUFA (LA + ALA), and the maximum conversion to DHA occurred when the diet contained low LA: ALA ratio and the total PUFA intake did not exceed 2% of the total dietary energy intake (%E) (74). Furthermore, the two PUFA families may also compete for incorporation into tissue phospholipids (75,76).

LA and ALA can be synthesized *de novo* only in plants and thus their dietary sources are principally plants (77). LA is the most abundant PUFA in human diet and rich in the four main vegetable oils (palm, soybean, rapeseed and sunflower). It is also obtained from various other oils, such as safflower, corn and cottonseed oils, as well as poultry and certain grains and cereals. ALA is produced by terrestrial plants and also cold water vegetation such as algae and phytoplankton. The chloroplast membranes of green leaves contain ALA, often representing more than 50% of the total FAs, but green leaves are not high in total fat and therefore, not considered as major dietary sources of ALA. Major sources of ALA in human diet are oils such as flaxseeds, rapeseeds and soybeans oil, and nuts like walnuts. The n-6 LCP AA is found in most animal tissues and can be obtained from animal-based foods including eggs, poultry, meat, and milk. In contrast, the n-3 LCPs DHA and EPA are not widely distributed in the food chain and obtained almost exclusively from marine foods such as fish, shellfish, and fish-oils particularly those derived from cold water fatty-fish. Organ meats like brain and some egg yolks, depending on the content of the chicken feed, are the only other important dietary sources of the n-3 LCPs (78).

The fact that the two PUFA families compete for metabolic conversion and incorporation into tissue phospholipids, subsequently influencing their distinct and sometimes competing physiological effects, makes the n-6 to n-3 ratio an important feature in PUFA status (67–70,75,76). Several sources of information suggest that humans evolved on a diet with a balanced ratio of n-6 to n-3 FAs, whereas great changes that took place in the human diet resulted in an increase in n-6 and a decline in n-3 PUFAs consumption (79–81). The balanced n-6/n-3 FA ratio provided by early human diet (a ratio of 1:1 to 4:1) is believed to be more beneficial to human health than the ratios provided by the typical modern day human diet of between 15:1 and 20:1 (79,80,82–84). Particularly, a concern has been raised that n-3 LCPs

may be one of the limiting nutrients for optimal development of the central nervous system (CNS) and visual system in contemporary populations.

DHA and AA are major structural components of the CNS and are rapidly incorporated into the brain from the last trimester of pregnancy up to two years after birth (85,86). Although infants can synthesize DHA and AA from their precursor essential FAs, due to the low and rate limited enzymatic conversion and the high rates of PUFA oxidation, most evidence indicate that this endogenous synthesis cannot sufficiently meet infants' CNS accretion needs. This appears to be particularly true for DHA, and therefore, the consumption of preformed n-3 LCPs is suggested to be essential (67,87–89). Isotopic tracer studies estimated that 15-35% of dietary ALA is rapidly catabolized to carbon dioxide for energy, and that only a small proportion (~1%) is converted to DHA (90–94). Furthermore, diets high in ALA appear to increase the rate of ALA oxidation, limiting its accumulation in plasma and reducing its conversion rate to EPA and DHA, suggesting that a higher intake of ALA does not necessarily lead to higher EPA and, particularly, DHA concentrations in the body (74,90). Consistent with the isotopic tracer studies, blood and human milk (HM) DHA concentrations in adults increase with increased dietary intake of DHA, which is less the case with consumption of precursors (87,95,96). Similarly, infant formulas containing ALA appear ineffective in improving infant DHA status in blood and cerebral cortex as compared to when infants receive formulas containing pre-formed DHA or maternal milk which normally contains DHA (86,97). More recent studies also indicate that the genetic make-up in the FADS gene cluster of individuals may have substantial effect on the extent to which endogenous synthesis contribute to tissue DHA accumulation (98,99).

1.2.2. Mechanisms of action

LCPs have a range of physiological roles that relate to optimal cell membrane structure and optimal cell function and responses. These include being a component of cell membrane phospholipids influencing membrane order and lipid raft formation, being a precursor of lipid mediators with different biological activities and potency, being a potent activator of a number of gene transcription factors, and acting as signaling molecules and modulators of membrane protein functions that are summarized in **Figure 1.3** (71,100,101). Through these mechanisms, n-3 LCPs play

several important roles in the body including the development of neurocognitive and visual functions, controlling inflammation and modulating other immune functions, and optimizing cellular differentiation and metabolism.

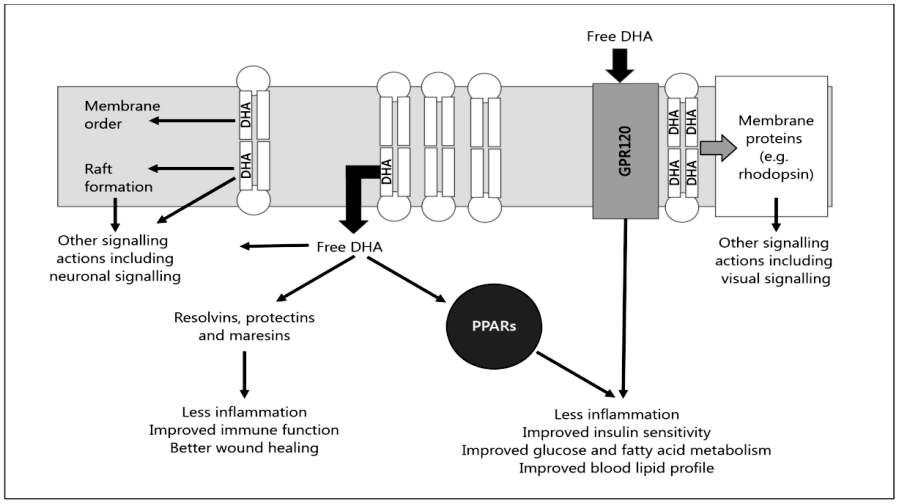

Figure 1.3. Summary of n-3 LCP mechanisms of action (from Calder et al. (71)**).**

DHA is relatively protected from beta-oxidation and preferentially incorporated into cell membrane phospholipids throughout the body. Due to its highly unsaturated structure, DHA containing cell membranes show increased membrane dynamics (fluidity and flexibility) and a more compact lipid bilayer compared to membranes containing saturated and mono unsaturated FAs (100,102,103). The incorporation of DHA in the membrane lipid bilayer displaces saturated and n-6 FAs, facilitating a variety of membrane functions requiring membrane flexibility such as the processes of cell fusion and fission, endocytosis and exocytosis, ion channels and transport, and the activities of membrane-bound proteins (71,100,104–107).

DHA embedded in cell membrane phospholipids grants a conducive environment for membrane protein function, such as lateral diffusion and conformational changes of membrane proteins, and play a major role in altering the size and stability of membrane signaling platforms termed “lipid rafts” (71,100,104–108). Lipid rafts are specialized subdomains in plasma membranes that are comprised of tightly packed combinations of sphingolipids, cholesterol and saturated FAs, and contain many of the proteins involved in signal transduction (109). They facilitate molecular

interactions and response with sensing protein motifs influencing numerous signaling actions. Although not directly incorporated into raft lipids, DHA found in non-raft regions of the cell membrane modulate raft structure and function, by displacing key signaling proteins and altering membrane protein trafficking (108,110)

n-3 LCPs released from membrane phospholipids act as cell surface and intracellular signaling molecules and ligands for gene transcription factors such as peroxisome proliferator activated receptors (PPARs) (71,111,112). PPAR-α and PPAR-γ are among the several PPAR isoforms that are well investigated. PPAR-α is involved in regulating gene expression encoding key enzymes of β-oxidation and lipoprotein metabolism, facilitating partitioning of FAs towards hepatic oxidation and away from triglyceride synthesis (71,101,113). PPAR-γ is involved in regulating adipocyte differentiation and metabolic responses of adipocytes including promoting insulin sensitivity. PPAR-γ, when expressed in immune cells, is involved in regulating the production of inflammatory mediators having anti-inflammatory actions (71,114). DHA and EPA can induce and activate PPARs and upregulate PPAR target genes, thus influencing metabolic and inflammatory responses. This may explain some of the effects of dietary n-3 FAs such as reducing inflammation, lowering plasma triglyceride, and increasing insulin sensitivity (71,115–119).

n-3 LCPs also influence the nuclear transcription factor kappa B (NFkB) pro-inflammatory signaling pathway, leading to suppression of apoptotic pathways and the production of various pro-inflammatory compounds (101,120). NFkB is a key transcription factor involved in inducing the expression of genes encoding a range of pro-inflammatory proteins including several cytokines, adhesion molecules, and enzymes, such as cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) (120,121). In response to inflammatory stimuli, NFkB is activated by phosphorylation reaction and subsequently translocate to the nucleus (120). Activated PPAR, through receptor-ligand interaction with n-3 LCPs, can inhibit NFkB pro-inflammatory gene expression by preventing translocation of NFkB to the nucleus and downregulating NFkB target genes (101,120,122). Additionally, n-3 LCPs interact with membrane associated G-coupled protein receptors (GPRs) promoting GPR120-mediated gene activation that has an inhibitory action on phosphorylation of NFkB (101,123). Furthermore, NFkB can be activated by pro-inflammatory cytokines such as interleukins (IL) and tumor necrosis factor α (TNFα),

which their production has been shown to decrease with dietary n-3 LCPs (101,124–126).

Free LCPs from membrane phospholipids are substrates for biosynthesis of bioactive lipid mediators. These signaling molecules act in diverse physiological and pathological processes such as inflammation and immunity, platelet reactivity, smooth muscle contraction and controlling blood pressure, and regulation of cell growth, pregnancy and normal child birth among others (119,127–129). Historically, most attention has been given to prostaglandins and thromboxanes, which are cyclo-oxygenase products, and leukotrienes, lipoxygenase products (**Figure 1.4**). The lipid mediators derived from AA have predominantly pro-inflammatory actions and are involved in vasoconstriction and platelet aggregation, and leukocyte chemotaxis and adhesion. The less potent eicosanoids from EPA have inflammation-lowering effects and are involved in attenuation of platelet aggregation and vasoconstriction, and inhibit the production of AA-derived mediators (127,129). More recently, new families of bioactive lipid mediators were also discovered that have potent anti-inflammatory and pro-resolving actions. They include the resolvins produced from DHA (D-series) and EPA (E-series), and the protectins and maresins produced from DHA (71,128,130). These mediators acting through specific GPRs are able to induce resolution of inflammation (i.e., 'turn inflammation off') and to promote immune functions, resulting in improved host defense and diminished pathological effect of inflammation (131,132).

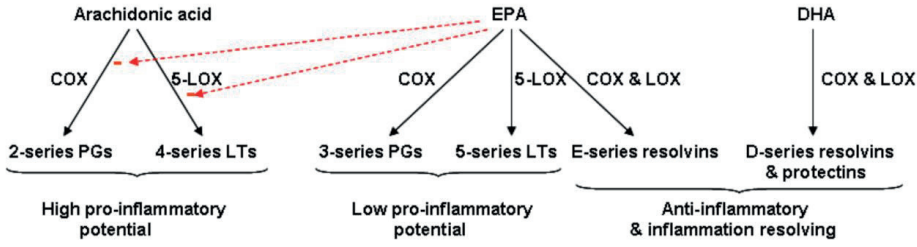

Figure 1.4. Overview of the synthesis and actions of lipid mediators (Calder et al. (128)**).**

The essentiality of preformed n-3 LCP in the diet during early-life is mainly in consideration of the uniquely high DHA incorporation in the CNS, although DHA is also incorporated in most other tissues where it has important functional effects (98).

Lipids make up 50-60% of the dry brain matter and DHA is one of the most abundant FAs, comprising 10-20% of the total brain FAs, with amounts reaching as high as 35% of the FAs in phospholipids of neural synapses (133–135). Its accumulation in the fetal brain takes place mainly during the last trimester of pregnancy and continues at very high rates up to the end of the second year of life, when the rate of brain growth is maximal and therefore vulnerable to the effects of nutritional deficiencies (85,86). Over this period, the total brain DHA content is normally increased by 35-fold, signifying its critical role in the substantial structural and functional development of the CNS occurring during the brain growth spurt (86).

Although much remains to be understood, DHA can influence brain function by modifications of neuronal membrane fluidity, regulating the activity of membrane bound proteins and function of neuronal ionic channels, and acting as a ligand to gene transcription factors involved in inflammation, oxidative stress, neurogenesis, differentiation and FA metabolism, and influencing neurotransmitters and brain peptides production that can reasonably be considered fundamental for the synthesis, functionality, and protection of neural tissues (85,98,134,136–140).

As a ligand for nuclear hormone transcription factors regulating the patterns of gene expression throughout the CNS, DHA is involved in neurogenesis, synaptogenesis, synaptic plasticity, endocytosis, neurochemistry, and synaptic vesicle recycling (141). Although it is rarely found in the adult brain, PPARγ is expressed abundantly in the developing brain in association with the rapid brain DHA accretion and appears to be important in regulating stem cell proliferation in early CNS development (142). DHA and AA also act as ligands affecting the activity of brain retinoid X receptor (RXR), which has important roles in neurogenesis, neuronal differentiation, and neurotransmission specifically within the hippocampus (112,143).

DHA in neural membrane phospholipids optimizes membrane fluidity and may positively impact the speed of signal transduction that theoretically lead to improved cognitive development (137). Memory and processing speed, two specific processes thought to underlie early cognitive development, are influenced by synaptic efficiency and transmission speed (137). DHA is especially enriched in synaptic terminal membranes of the prefrontal cortex where it has important roles in enhancing neurogenesis, neurite outgrowth, dendritic complexity, and metabolism of several

neurotransmitters including dopamine, serotonin and acetylcholine (134,144,145). DHA may also influence the timing of neural tissue myelination, potentially influencing the speed of signal transduction (146).

In addition, DHA enhances the function of N-methyl-D-aspartate channels, which may in turn alter long-term potentiation in the hippocampus, a plasticity process thought to be essential in the establishment of an explicit memory trace (137,147,148). In animal models, DHA-depleted rats performed poorly in hippocampal- and frontal-based memory and learning tasks, while DHA-supplemented rats experienced improvements in memory and learning (138,149–152). Cortical neuroplasticity, which is key to learning and memory, was shown to be reduced in children born preterm, which might have resulted from a lower prenatal DHA accretion (153). DHA supplementation in older boys aged 8-10 years was also shown to increase prefrontal cortex activation in Magnetic Resonance Imaging (154). Therefore, it is plausible that DHA has a role in increasing the speed at which information is acquired and the efficacy with which such information is retained, subsequently facilitating early cognitive development.

In addition to neurogenesis and signal transduction, n-3 LCPs also play important roles in neuroinflammation and neuronal cell survival (140,155–158). Pro-inflammatory COX-2 expression, and the production of IL-6, TNFα and PGE2 have been associated with cognitive impairment (159–161), where n-3 LCPs provide protections by downregulating NFκB pro-inflammatory signaling pathway and COX-2 mediated inflammation (101,120,155,162). Several brain specialized pro-resolving derivatives of DHA such as neuroprotectin D1 were also identified (140). In brain injury models, neuroprotectin D1 has been shown to have potent neuroprotective and pro-resolving effects reducing damages to neuronal tissues and synaptic transmission dysfunction resulting in improvements in cognitive function and neurobehavioral scores (163–167).

1.2.3. n-3 PUFA intake and status in low-income settings

Dietary fats contribute to 45-55 percent of energy (%E) in human milk (HM) and increase energy density of complementary foods, which makes them vital for meeting the high energy requirements of IYC to support their rapid growth and physical activity (168–170). In addition to the total fat content, there is interest in the

FA composition of diets including essential FAs which have specific physiological roles that cannot be replaced by other FAs in the diet.

Various authorities have proposed dietary recommendations on total fat and specific PUFA intakes for vulnerable groups (89,95,171–173). A joint expert consultation group organized by the Food and Agriculture Organization (FAO) and the WHO recommended that fats should constitute 40-60%E in young infants less than 6 months old with gradual reduction to 35%E in older IYC 6-24 months (171). The same group recommended an adequate intake of DHA at 0.1-0.18%E (equivalent to a mean of 102 mg DHA/d) for young infants and 10-12 mg/d/kg body weight for older IYC. The European Food Safety Authority recommended a DHA content of 0.3% total fat (%TF) in infant formula and an adequate intake of 100 mg DHA/d for older IYC (172). For pregnant and lactating women, an adequate intake of 300 mg/d DHA + EPA of which at least 200 mg as DHA is recommended to support optimal transfer to the fetus during pregnancy and to the infant through lactation (95,173).

There is a lack of data on the PUFA status of populations in low-income countries (LICs) in general, and in Ethiopia in particular. Unlike cellular proteins which are genetically determined, the FA composition of cell membranes is largely dependent on dietary intake (81). In many LIC populations, particularly those living far from coastal areas, diets are too low in n-3 LCPs to meet the recommended intake levels by vulnerable groups. A review of PUFA intakes in 13 selected LICs showed that the total n-3 FA supply in the diet was below the recommended intake range for IYC and below the minimum recommended level for pregnant and lactating women in the nine countries with the lowest GDP including Ethiopia (174). A more comprehensive review of LCPs availability in 175 countries also disclosed that the availability of fish and seafood in the food supply of several LICs is too low to meet the recommended intake levels (175). The average per capita DHA availability in the 47 LICs assessed was estimated at 96 mg/d, in contrast with the range of 184-473 mg/d in 128 high-income countries (HICs), and the consumption rate was even negligible in the countries with the lowest GDP such as Ethiopia (7.0 mg/d) (**Figure 1.5)**.

As HM is one of the best sources of ALA and DHA, infants in low-income settings who are usually breastfed for longer period may be considered to have a relative protection from insufficient intakes. However, it should be noted that DHA

concentration in HM varies widely, mainly depending on the maternal dietary intake of preformed n-3 LCP (176–178). In Ethiopia where the availability of DHA in the food supply is reported to be the lowest globally (175), breastfed infants also could be susceptible to a suboptimal intake of n-3 LCP. More importantly, the period of transition from exclusive breastfeeding seems to be important for n-3 LCP status of infants in low-income settings as most local complementary diets are very low in n-3 LCP content (169,179,180). The average per capita DHA intake from both breastfeeding and complementary foods among older IYC in 78 LMICs has been estimated to be 50 mg/d which is well below the recommended intake level in this group (100 mg/d) and with the cessation of breastfeeding, the per capita consumption rate further falls to 14.6 mg/d (172,179,180). In Ethiopia, the estimated contribution of complementary foods for DHA intake was found to be the lowest (1.1 mg/d) of the 78 LMICs evaluated.

During pregnancy, the substantial LCP requirement to support accretion to the developing fetus via preferential placental transfer and later to the infant via lactation (86,181,182) may lead to maternal n-3 FA depletion if these requirements are not met by adequate dietary intake (183). Studies found that maternal DHA status declines starting from late pregnancy and continues over the course of lactation (181,184–187). DHA status was significantly lower in multigravidae mothers and their newborns compared to primigravidae, and in mothers who were breastfeeding compared to those that did not (185,187,188). Although DHA status may normalize between pregnancies (189), the high fertility rate in LICs like in rural Ethiopia (5.2 births/woman) (190) may limit the time to replenish maternal stores between closely spaced pregnancies, and in the presence of very low dietary intake can increase the susceptibility of multigravidae women for DHA depletion, which could then be reflected in a lower neonatal and infant status (181).

In addition to the low n-3 LCP intake from complementary foods and the possible higher risk of deficiency in those born from multigravidae mothers, children in low-income settings who live under poor environmental and hygienic conditions may have increased n-3 LCPs requirements. Recurrent infections and prevalent asymptomatic chronic immune stimulation could divert LCPs that are normally used to support growth and development towards the increased requirements for inflammatory and other immune responses (128,191,192). Furthermore, in nutritionally vulnerable

populations, the synthesis of LCPs from precursors in the body could be hampered by other dietary factors. Iron, zinc, vitamin B6 and vitamin E are required as co-factors for elongation enzymes in PUFA metabolism and therefore, it is possible that micronutrient deficient populations may have a lower LCPs conversion rate and status than well-nourished populations (71,193). Additionally, when total energy intake (especially fat) in the diet is low, ALA could be used for energy expenditure rather than conversion to EPA and DHA (194).

In general, despite the lack of evidence from biochemical studies of n-3 FA status, a combination of very low dietary availability and potential stressors that might increase n-3 FA requirements discussed above suggest that Ethiopian mothers and their IYC in rural areas are likely to have a sub-optimal n-3 LCP status. Detrimental child outcomes that might be caused by poor n-3 LCP status include diminished neurodevelopment and cognitive performance, and poor health with associated growth failure, which might benefit from dietary supplementation of preformed n-3 LCP. The transition from exclusive breastfeeding to complementary feeding, starting from around 4-5 months of age, typically puts Ethiopian infants at risk as this is the period where they are introduced to foods with very low n-3 LCP content. The introduction of unhygienically prepared complementary foods can also lead to intestinal inflammation and associated local and systemic immune stimulations, which presents an additional burden to the infant.

Together with the n-3 LCPs, the n-6 LCP AA also plays important roles for early-life CNS development, growth, and immune function (195,196). Unlike DHA, maternal tissue and HM AA contents are relatively stable and less affected by maternal diet including in most cases of supplementation with DHA (176–178,197,198), and it is thus expected that AA status is less likely to be compromised in children who receive breastfeeding. Currently, there is no indication that women of childbearing age with an adequate dietary intake of the precursor LA would require an additional dietary supply of preformed AA (95,173).

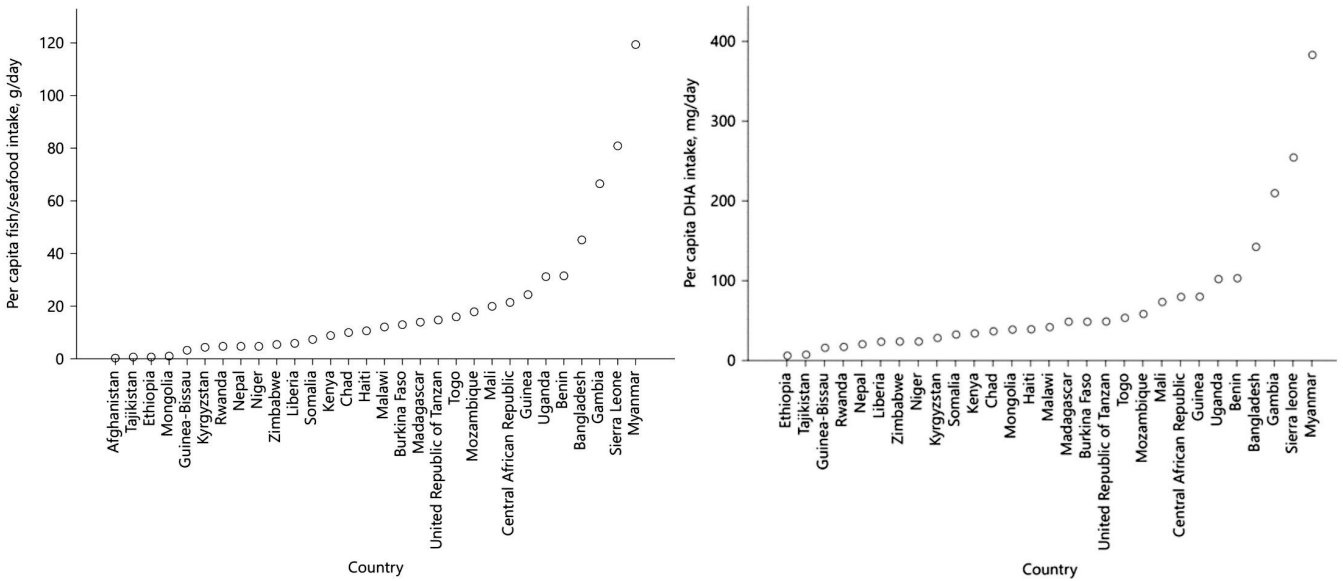

Figure 1.5. Estimated per capita consumption of fish and sea food (left) and DHA (right) in countries with the lowest GDP (from Forsyth et al. (175)).

1.3. Dietary n-3 LCP in relation to healthy growth and development

1.3.1. Neurodevelopment

The evidence for the importance of dietary n-3 LCP for early cognitive and visual development in humans first emerged from epidemiological studies. In prospective observational studies, the children of mothers with higher consumption of seafood during pregnancy have exhibited better cognitive outcomes (199–201). Studies demonstrated higher DHA concentration in the cerebral cortex of breastfed infants than their counterparts who received formulas that did not contain LCPs (135,202), and several individual studies and meta-analysis of these studies also found the former group having cognitive and visual advantages over the latter group (203–207). Earlier experiments in animals also demonstrated that changes in brain DHA concentrations are positively associated with changes in cognitive and behavioral performance in rodents and nonhuman primates (85,138).

Neurodevelopmental benefits observed in breastfed infants and infants with higher prenatal seafood exposure have been attributed to the biologically plausible roles of DHA for early development and maturation of the CNS and visual system. However, as these studies were not randomized, potential confounding from several factors precluded the causality of evidence for the observed association between dietary n-3 LCPs and neurodevelopmental benefits in humans (208,209). Subsequently, randomized controlled trial (RCT) of n-3 LCP supplementation in relation to infant neurocognitive and visual development has remained the subject of intense investigation over the past two decades including several reviews of these experimental trials (210–220).

Systematic reviews and meta-analyses in the Cochrane library summarized the effects of LCP supplementation of formula-milk in term (211) and preterm infants (213), and maternal n-3 LCP supplementation during lactation (221). The latest 2017 Cochran review (211) of formula-milk LCP supplementation in term infants included 15 RCTs of which eleven measured neurodevelopmental outcomes (222–234). From 9 studies that used Bayley Scales of Infant Development (BSID) (222,223,227–233), only 2 showed benefits of LCP supplementation for mental development index (MDI) scores at 18 months of age (232,233). One study, using the Brunet-Lezine developmental quotient, reported a positive effect of supplementation at age of 4

months (234), but not in later follow-up of the same cohort at 12 and 24 months of age (225). Meta-analysis of 4 studies (230,231,235,236) that used BSID at 18 months of age did not find significant benefits of LCP supplementation for MDI or psychomotor development index (PDI). Some trials assessed specific tests of infant cognitive development, with only few finding positive results (224–226,228,231,234,237). In one study (224), LCP supplementation improved quality of attention (a cognitive index derived from the convergence of behavioral and cardiac responses) in infants at the age of 4-9 months, and another study (237) found better novelty preference measured by the Fagan test of infant intelligence at 9 months of age. Willatts et al. (226) demonstrated LCP supplementation improved infant problem-solving skills at 10 months of age.

Few of the above trials reported follow-up data on long-term effects of LCP supplementation (238–243). The further follow-up of the study sample used by Willatts et al. (240), cited above, found a positive effect on information processing at 6 years of age, but not on IQ score. Colombo et al. (242) evaluated the effect of infant formula fortified with LCP on later cognitive development between 18 months and 6 years of age. LCP supplementation did not influence standardized developmental measures at 18 months of age using the BSID and MacArthur-Bates Communicative Development Inventory. However, between 3 to 6 years of age, positive effects were observed on specific tests of executive functions, vocabulary, and IQ measures. de Jong et al. (238,243) found no consistent long-term effect of a two-month postnatal LCP supplementation on cognitive development and neurological functions at 9 years of age. The results, however, suggested a beneficial effect on cognitive function in the subgroup of children who were exposed to maternal smoking during pregnancy. Two other studies failed to detect long-term effects on tests of IQ, language development, or school readiness at 3 years of age (239,241).

With regard to visual development, the review included 6 RCTs that assessed visual acuity using visual evoked potentials (VEP) (222,223,235,236,244,245), 2 using Teller acuity cards (228,246), and 1 using both (227). Five of these studies reported beneficial effects on visual acuity (223,235,236,244,245), while others did not (222,227,228,246). Meta-analysis of 3 RCTs showed significant benefit for sweep VEP acuity at 12 months of age, whereas meta-analysis of 3 other RCTs found no

benefit for visual acuity measured using Teller acuity cards at the same age. The review authors concluded that most of the included RCTs reported no beneficial effects or harms of LCP supplementation on neurodevelopmental outcomes of formula-fed full-term infants and no consistent beneficial effects on visual acuity. Therefore, routine supplementation of full-term infant milk-formula with LCP cannot be recommended at this time.

Dietary LCP may be particularly important for infants who are born preterm and had a limited exposure to the rapid in utero DHA accretion that occurs during the last weeks of pregnancy. The latest 2016 Cochrane review (213) of formula-milk LCP supplementation in preterm infants examined 17 RCTs with 7 studies having neurodevelopmental outcomes (247–254). Three of the 7 studies reported benefits of LCP using BSID at different infant ages (252–254). However, meta-analysis of 4 studies (247,250,251,253) at 12 months of age and 3 studies (249,252,254) at 18 months of age found no significant effect of LCP supplementation on MDI or PDI. The authors of this review therefore concluded that there are no clear long-term benefits of LCP supplementation of formula-milk for visual or intellectual development in preterm infants.

HM is an important source of both DHA and AA for breastfed infants. Compared to other dietary sources, it is safe from environmental contaminants and contains a higher fraction of LCPs in phospholipid form which increases their bioavailability (255,256). The high variability in HM n-3 LCPs levels which mainly depend on the maternal diet led some investigators to evaluate the effects of maternal supplementation during lactation on child outcomes. The latest 2015 Cochrane review (221) of maternal n-3 LCP supplementation summarized 8 RCTs that provided supplementation either starting from pregnancy and continued postpartum (257–261), or starting at the beginning of lactation (262–264). When pooling 5 RCTs having neurodevelopmental outcomes, except a beneficial effect detected on child attention scores at the age of 5 years based on data from only one study (265), the review failed to find evidence of a long-term benefit beyond 24 months of age on other neurodevelopmental outcomes, including language (262,263), intelligence or problem-solving skills (257,261–263), psychomotor development (261,263,264), motor development (262,263), general movements (261), working memory and inhibitory control (262), neurological optimality score (261), or on measures of visual

acuity (262–264). Results were inconsistent within a trial when outcomes were assessed at different age. Helland et al. (257), for instance, found no effect of fish-oil supplementation starting from pregnancy until 3 months after birth on infant cognitive development at 6 months of age using the Fagan Test of Infant Intelligence. However, in a follow-up study on this sample using the Kaufman Assessment Battery for children, the authors found improved cognitive function at the age of 4 years (266), but this effect was not sustained at the age of 7 years of age (267). Jensen et al. (263) similarly reported a beneficial effect of maternal DHA supplementation during lactation on child neurodevelopment at 30 months of age despite this effect not being detected during infancy. On further follow-up of the same cohort at 5 years of age, they also found a benefit using the test of sustained attention (265). Overall, the authors of this review concluded that there is inconclusive evidence at this time to support or refute the practice of giving n-3 LCP supplementation to breastfeeding mothers in order to improve child neurodevelopment or visual acuity at this time.

Shulkin et al. (210) reported relatively encouraging evidence of benefit in their most recent comprehensive systematic review and meta-analysis of n-3 LCP supplementation via supplements, fortified foods or diet in pregnant and/or lactating women and children below 24 months of age. The review included 38 RCTs across the critical period of CNS development, with13 trials in mothers (262–265,267–278), 18 in term infants (222,223,225,228,229,231–233,236,239,240,242,244,246,279–286) and 7 in preterm infants (250–254,287–289), and evaluated standardized measures of cognitive and visual development reported up to the age of 18 years. In the meta-analysis stratified by intervention, MDI was significantly improved in preterm infants without statistically significant effects for term infants and for maternal supplementation. No significant effect was found for PDI and overall IQ in the stratified analyses. Visual acuity, measured as the logarithm of the minimum angle of resolution, was significantly improved by supplementation in preterm and in term infants, but not in mothers. In the main analysis pooling all supplementation periods, n-3 LCP supplementation significantly improved MDI (n = 21 trials), PDI (n = 21), and visual acuity (n = 24), but not overall IQ (n = 7). Potential publication bias was identified only for the MDI outcome. The authors therefore concluded that n-3 LCP supplementation improves childhood psychomotor and visual development,

without significant effects on later global IQ, although the latter conclusion is based on fewer studies.

In general, while some studies provide evidence that n-3 LCP may benefit early-life visual and neurocognitive development, systematic reviews of numerous RCTs in HICs remain inconclusive about whether or not infants should receive n-3 LCP supplementation (210–221). On the other hand, research investigating n-3 LCP supplementation in LMIC settings remains largely absent. However, possible benefits might be more noticeable in populations with high exposure to developmental risk factors, such as intrauterine growth restriction, stunting, inadequate stimulation, and infections (98,194,277,290). Only one trial in a low-income setting reported on a postnatal n-3 LCP supplementation in Gambian breastfed infants which failed to detect any benefits on cognitive function (291). However, the authors found that the Gambian study mothers had considerably high HM DHA concentrations similar to those reported in populations with high fish consumption and thus, the intervention appears to be targeted to infants who already had an adequate dietary intake via breastfeeding. If gains in neurocognitive development from n-3 LCP supplementation could be detected in Ethiopian IYC, it would provide important evidence for future research and interventions in populations in low-income setting, where the availability of n-3 LCP in the diets is very low.

1.3.2. Physical growth

LCP supplementation has also been investigated extensively with child physical growth as an endpoint. The Cochrane review (211) of formula supplementation in term infants cited 13 RCTs with child growth measurements. None of the included trials reported a beneficial or harmful effect of LCP supplementation on weight, length, or head circumference (HC) measurements until 9 years of age (223,227–231,234–236,244,292–294). Meta-analysis of 5 RCTs (227,228,234,236,244) showed that the LCP supplemented group had a lower weight *z*-score at 12 months of age, whereas no significant differences found with respect to length or HC. Meta-analyses at 18 and 24 months of age found no effect on any child growth outcome. The authors concluded that even though meta-analysis of pooled data revealed a

marginally lower weight *z*-score in the LCP supplemented group at 12 months of age, these differences are small and unlikely to be of clinical significance.

Similarly, the Cochrane review (221) of maternal n-3 LCP supplementation during pregnancy and/or lactation found no evidence of a long-term growth benefit in any of the trials included (257,258,262,265,267,295–299) as well as in the meta-analysis of pooled data. At short term follow-up, n-3 LCP supplementation significantly increased weight and BMI at birth in 1 trial (297), and weight and HC at 12 months of age in a meta-analysis of 3 trials (257,260,262); whereas child length was not affected during the follow-up period. The fact that some of the included trials started supplementation prenatally might explain the positive results at short-term follow-ups as other studies also detected a longer gestation period and a small increase in birthweight resulting from increased maternal dietary n-3 LCP intake during pregnancy (300,301).

Studies on LCP supplementation in preterm infants produced mixed results on child growth, which is possibly due to differences in combinations of LCPs used in different test formulas. From 15 RCTs evaluated in the latest Cochrane review (213), 4 reported positive effects on growth (child weight and/or length) at different child ages (252,254,302,303), 2 reported negative effects on growth (weight-for-age, weight-for-length and/or HC-for-age) using formulas containing only n-3 LCP and no AA (247,248), 1 reported mild reductions in weight and length values using a formula supplemented with both n-3 LCP and AA (249), and the rest found no effect (250,253,288,304–309). Meta-analysis of 5 trials (248,288,302,303,307) having measurements at 2 months post-term showed increased length and weight as a result of LCP supplementation. Meta-analysis of 4 trials (247,248,252,306,307) at child age of 12 months and 2 trials (249,254) at age of 18 months, however, found no significant effect on any growth indicator. The authors concluded that this meta-analysis of trials does not support a long-term growth benefit of LCP supplementation of formula in preterm infants, while the authors also recognized that most trials enrolled relatively mature and healthy preterm infants.

The most likely reason for the impaired growth experienced by supplemented preterm infants in few trials was a reduction in body AA levels resulting from supplementation using only n-3 LCP (213). Considering that exclusively formula-fed

infants may not secure an adequate supply of AA depending entirely on metabolic conversion from LA, some investigators recommend a balanced combination of DHA and AA in infant formulas (310). A risk of n-3 LCP supplementation-induced AA inadequacy is, however, unlikely in the case of breastfed infants who receive preformed AA through breastmilk.

Overall, the current body of literature does not support any growth benefit of LCP supplementation in term infants. However, the existing evidence is exclusively based on trials in infants from HICs consisting of child populations that are generally well-nourished and follow the optimal growth trajectory. Therefore, the potential to reveal any benefit on child growth of an intervention is expected to be limited. In contrast, IYC in low-income settings who have a high risk of impaired growth and a suboptimal dietary intake of essential FAs, may respond differently to n-3 LCP supplementation. Furthermore, in children living under poor environmental conditions, persistent subclinical inflammations and recurrent infections contribute substantially to their growth failure. n-3 LCP can modulate inflammation and other immune functions (discussed in the next section), and through this pathway, there might be a stronger effect of dietary n-3 LCP on growth of children living in such settings.

1.3.3. Immune function and inflammation

The multiple mechanisms through which n-3 LCPs modulate several aspects of the immune system are detailed in previous reviews (119,311,312) and summarized in section 1.2.2. Notably, n-3 LCPs exert partly inhibitory effects on inflammatory responses including on the activation of different immune cells and also on the production of various proinflammatory chemical mediators such as cytokines and eicosanoids derived from AA (119,311). EPA also gives rise to less potent alternative eicosanoids that compete with proinflammatory eicosanoids derived from AA. Novel groups of DHA and EPA derived anti-inflammatory and inflammation resolving mediators such as resolvins, protectins, and maresins were also identified by previous work (119,311). These compounds are involved in alleviating the degree of inflammatory responses and self-limiting inflammation that are important in limiting damages to the host tissues and facilitating resolution of inflammation. On the other hand, some specific immune functions are promoted by n-3 LCPs, such as phagocytosis and bactericidal capacity of macrophages and neutrophils, and T

regulatory cells differentiation, indicating that n-3 LCPs are not non-specific immune suppressors in general (312).

Clinical studies of n-3 LCP largely focused on a range of clinical conditions collectively referred as inflammatory diseases. These conditions typically share characteristics of excessive, inappropriate or on-going inflammation. They are subject to investigation based on the hypothesis that the ability of n-3 LCPs to down regulate several aspects of inflammation may exert therapeutic effects in these conditions. While several positive results were reported by both animal and human studies, systematic reviews of human trials found inconclusive evidence for clinical benefits of n-3 LCPs in different inflammatory conditions (313–318). Calder (119) concluded that dietary n-3 LCPs inducing anti-inflammatory actions may have therapeutic use in a variety of acute or chronic inflammatory conditions, whereas current evidence on clinical efficacy is reasonably strong for some conditions like rheumatoid arthritis, but inconsistent in others like asthma and inflammatory bowel diseases (IBD).

The potential clinical benefits of dietary n-3 LCP in inflammatory conditions is of particular interest in relation to the gut damage with underlying chronic immune stimulation- called environmental enteric dysfunction (EED)- that commonly occurs in children raised in the typically unhygienic conditions of LICs (43,319–321). EED is a persistent subclinical enteropathy characterized by villous atrophy, crypt hyperplasia, inflammatory cell invasion of the lamina propria and damage to tight junctions, resulting in nutrient maldigestion and malabsorption, and a leaky gut that allows translocation of bacteria and potentially toxic or allergenic substances which then stimulates local and systemic inflammation (43,320,321). The enteropathy generally starts early in life, coinciding with the first exposures to complementary foods at the age of 4-9 months, and has been strongly implicated as a driver of growth failure (322–324). It has been hypothesized that prevalent EED among children in LICs could be the reason for previous dietary and health interventions have been only partially successful in preventing stunted child growth (319,323).

In-vitro and animal experiments suggest that dietary (n–3) LCPs may improve small intestinal barrier functions (325), and enhance recovery of intestinal tissues after inflammation in severe malnutrition and chemically-induced damages (326,327). This

potential for n-3 LCPs to improve intestinal wall integrity and resolve mucosal inflammation might help in alleviating the persistent enteropathy and associated local and systemic inflammation with a potential resultant effect on child growth in LICs. Most clinical studies of n-3 LCP on intestinal inflammation focused on IBD (328,329). Clinical benefits in IBD may have a role in treating EED, as these conditions share key features of enteric inflammation, loss of intestinal architecture and systemic inflammation, despite their differences in etiologies and affected population (330,331). n-3 LCPs proved to be protective in animal models with experimental IBD (332–337). In patients with IBD, fish-oil supplementation resulted in incorporation of n-3 LCPs into the affected gut mucosal tissue thereby modifying inflammatory mediators profile (338–342). However, clinical effects on gut histology, disease activity and relapse are inconsistent (119,317,329,343–347). A meta-analysis including 6 RCTs of n-3 LCP therapy in patients with IBD found a marginally significant benefit in preventing relapse over 12 months (317). However, the authors cautioned about the low overall quality of evidence from this review due to the heterogeneity among included studies and two of the well-designed large trials showing no benefit and thus, suggested future well-designed large clinical trials.

In contrast to the bulk of studies on n-3 LCP in relation to IBD and other inflammatory conditions, only two trials evaluated the effects of dietary n-3 LCPs on early-life EED and related child growth failure. Supplementing fish-oil to Gambian breastfed infants between age of 3-9 months did not improve EED and infant growth (291). Only a larger increase in linear growth in the intervention group was noticed, albeit not statistically significant. Multimicronutrient supplementation with or without fish-oil in Malawian children 12-35 months old also found no added benefit of fish-oil for the transient modest improvement in EED detected with the multimicronutrient intake (348). Overall, both trials did not report beneficial effects of fish-oil supplementation on EED and growth. However, biochemical measures of PUFA in child blood and maternal milk were not suggestive of a sub-optimal n-3 LCPs status of children in both studies. Furthermore, studies of n-3 LCPs as anti-inflammatory agents usually used much higher doses than the doses provided in these studies (119,317).

In addition to the anti-inflammatory effects with potential benefits for EED and related child growth failure, n-3 LCPs may also contribute to infant immune development.

Studies showed that early n-3 LCP exposure influences different immune markers that are indicative of a faster maturation of infant immune system (349–352), which may result in improved resistance to infection (351). Dietary (n-3) LCPs supplementation was also shown to have clinical benefits in both inflammatory and infectious conditions in formula-fed infants in Europe and the US (353–355), in US toddlers (356) and Thai schoolchildren (357), and in Mexican infants who were exposed to prenatal maternal supplementation (358). However, there is limited evidence on the effects of dietary n-3 LCP among breastfed children in low-income settings who live under poor environmental conditions with high burden of infectious diseases.

1.4. The rationale for this research

As detailed earlier, poor child growth and development is one of the most pressing public health problems in LMICs. Linear growth retardation during the critical period of 1,000 days after conception is a marker of multiple pathological changes that are associated with increased morbidity and mortality, reduced physical, neurocognitive and economic capacity, and an elevated risk of metabolic diseases in adulthood. Linear growth failure is the most common form of undernutrition globally, and the severe irreversible physical and neurocognitive damage that accompanies stunted growth is a major barrier to the development of human capital and economic growth. Increasing awareness of its high magnitude and devastating consequences has led stunting to receive high priority in the post-MDGs era development agenda.

The United Nation's Sustainable Development Goals (SDGs) adopted the resolution endorsed by the WHA for maternal, infant and young child nutrition that included six global targets, prominent among which is the ambitious target to reduce the number of stunted children by 40% in 2025 (58). Although there has been progress in stunting reduction globally, many countries are not on track to achieve the stunting target. At the current rate of progress, only 26% of the global burden of child stunting can be averted by 2025 (56). It has been emphasized that acceleration of current progress in LMICs requires effective large-scale nutrition-sensitive programs that address key underlying and basic determinants of undernutrition, and scaling up the coverage and effectiveness of evidence-based nutrition-specific interventions (28,30,32,359). The challenge, however, is identifying which set of priority actions

are the most effective to be implemented at scale within the context of limited development budgets (56).

Understanding the proximal, underlying and basic determinants that most explain the trend in stunting prevalence in high-burden countries is useful for supporting evidence-based decision-making by policy makers and program planners. Previous studies tried to address this important question and produced empirical evidence on the important drivers behind the observed improvements in childhood undernutrition over three decades (detailed in section 1.1.3). Adding to these previous efforts, timely revision of the evidence is strongly required to understand the important drivers behind the more recent trend for current decision-making. The strength of the relationship between stunting and important drivers in the past could change over time. In addition, some determinants that were important in past may saturate for current and future changes. An updated analysis using newly emerging data is also required to provide a more comprehensive list of important drivers, including those potential determinants that were not considered in previous analyses due to unavailability of data. Furthermore, most of the previous efforts to assess drivers of the trend of child stunting have taken a country focus and there are limited studies of cross-country trend analysis. However, comprehensive evidence on the drivers of child stunting across high burden countries is equally important for decision-making at the global and regional levels. Therefore, the first part of this PhD research explores a range of basic, underlying, and immediate determinants that explain the more recent trend in chronic childhood undernutrition in a set of LMICs.

One important bottleneck in promoting healthy growth and development of children in LMICs is identifying interventions that effectively impact child linear growth. Evidence from systematic reviews showed that existing interventions have yielded only small to moderate effects on growth which are not large enough to fully compensate for the substantial growth deficit that children experience in poor settings (360,361). A review of an optimal package of nutrition interventions for high stunting burden countries also revealed that recommended nutrition solutions combined, even if universally applied, will only partially avert stunting and the estimated death and disability due to undernutrition (30). Therefore, while existing evidence-based interventions should be implemented at scale, new approaches are needed to maximize the impact of nutrition interventions in preventing growth failure.

Among others, understanding the range of growth-limiting nutrients and their potential for intervention has paramount importance (362).

Studies of complementary feeding interventions to date have focused on energy and micronutrient content of diets and achieved only modest impact on child growth and development (361,363). EED occurring at high prevalence among children in LMICs has been implicated as an important missing link that mediates and reduces the expected benefits from interventions (319,323). The mechanisms contributing to growth failure in EED include small intestinal villus atrophy, decreased gut integrity, and a chronic gut inflammation that lead to dysbiosis and bacterial translocation, systemic inflammation, and nutrient malabsorption. Results from laboratory experiments and studies in HICs suggest that n-3 LCPs improve gut integrity, reduce inflammation, and enhance maturation of immune system in early-life (discussed in section 1.3.3). These reported beneficial effects of n-3 LCPs could result in even more important physiological implications for children from LMICs and will ultimately contribute to their healthy growth.

In addition, due to the role of DHA in neurogenesis, synaptogenesis, neurite outgrowth and its high incorporation into the brain during early-life, this n-3 LCP is considered crucial for early cognitive development. Most evidence suggest that endogenous synthesis of DHA is insufficient to support the rapid brain accretion during fetal and early postnatal life and therefore, adequate dietary supply of preformed n-3 LCP is required to support optimal structural and functional development of the CNS during this period (85,88,89,97,173).

Global estimates of dietary LCPs intakes showed that the n-3 PUFAs supply in several LICs are far below the recommended intake levels for pregnant and lactating women, and IYC (174,175,180). Furthermore, various factors may increase the risk of n-3 FA deficiency among mothers and their infants in vulnerable populations. These include depleted maternal stores due to multiparity, higher requirements for immune responses facing recurrent infections and persistent subclinical inflammations, and deficiency of micronutrients required for PUFA metabolism. Previous reviews on FA status and associated functional outcomes among children in low-income settings indicated that limited data suggest n-3 FA interventions in these settings may yield benefits for health, growth, and development of young

children 6-24 months of age, although randomized trials are needed for a conclusive association (194,364).

In Ethiopia, the per capita n-3 LCP intakes from diets and complementary foods are reported to be one of the lowest globally (175,179), which provides a favorable setting to generate evidence on the impact of n-3 LCP supplementation in young children. In the second part of the PhD work, using a randomized controlled trial design, we evaluate the efficacy of n-3 LCP supplementation through lactation and/or complementary food on growth, morbidity, nutritional status, and development of IYC form a rural Ethiopian setting.

2

CHAPTER 2

AIMS OF THE RESEARCH

2.1. Aims of the Research

The overall aim of the PhD study is to generate empirical evidence on important proximal to distal factors that can contribute to reduction in child undernutrition in LMICs, and assess the role of improved n-3 LCPs intake in supporting healthy growth and development of IYC in a rural sub-Sahara African community.

The hypotheses underlying the studies conducted in the framework of this PhD thesis are:

- Different nutrition-specific interventions and nutrition-sensitive development actions may contribute to alleviate the high burden of childhood undernutrition in LMICs with different level of impact on the trend in stunting prevalence.
- Due to the very low availability in the food supply, Ethiopian mothers and their IYC in rural areas would have a suboptimal n-3 LCP status that can be improved by dietary fish-oil supplementation.
- Supplementing lactating women with fish-oil capsules leads to higher n-3 LCP concentration in their breastmilk.
- Increased n-3 LCPs intake by IYC through lactation and/or complementary food will result in reduced morbidity and inflammation, and improved growth and nutritional status.
- Increased n-3 LCPs intake by IYC through lactation and/or complementary food will result in higher scores on overall development in the Denver-II test and social-emotional development, indicating enhanced neurocognitive development.

2.2. Overview of the study settings

The results in the current PhD work were generated from two independent studies: i) The drivers of stunting reduction in LMICs, and ii) n-3 LCPs supplementation for healthy growth and development of IYC in rural Ethiopia (The OME^{3}JIM project).

2.2.1. The drivers of stunting reduction in LMICs

This study was subscribed to the call by the European Commission's Directorate-General for International Cooperation and Development (DEVCO) to generate

evidence on priority areas of action for stunting reduction in 42 LMICs that are partners for nutrition support. For this purpose, we modeled the stunting trend in the target countries over the last two decades to understand the important drivers behind the observed reduction in chronic child undernutrition. Secondary data were secured by pooling the Demographic and Health Surveys (DHS) conducted in these countries since 2000. The DHS, which has been conducted in several countries for more than three decades, is typically characterized by standardized data collection procedures across countries and over time, allowing for comparison of results across-countries and within-country over time (365). Analysis of potential drivers was based on the UNICEF conceptual framework for childhood undernutrition (27) and its recent Lancet revision on relevant actions for optimum child nutrition and health shown in **Figure 1.1** (7). Details on the various indicator variables and their definition and analysis approach are presented in the relevant chapter.

2.2.2. The OME^{3}JIM project

The OME^{3}JIM project involves a factorial 2X2 RCT aimed at evaluating the effects of n-3 LCPs supplementation to lactating mothers and their IYC. The trial was carried out from November 2013 to February 2015 in three small districts located under Jimma Zone of Oromia Regional State in Ethiopia.

Country profile

Ethiopia is a large landlocked country located in the Horn of Africa bounded by Eritrea and Djibouti in the north and northeast, Somalia in the east and southeast, Kenya and South Sudan in the southwest, and Sudan in west and northwest. The country is the second most populous in Africa with an estimated population of 108,386,391 in 2018, and covers a total area of 1,104,300 km^2 (366). Ethiopia is one of the least developed countries, globally ranked 173th according to the 2018 United Nation's human development index ranking of 189 countries (367). Agriculture is the dominant economy sector employing more than 70% of the labor force and about 30% of the total population lives below the poverty line earning less than $1.90 a day.

Childhood undernutrition is a major public health problem in Ethiopia with 13% of children born with low birthweight and 38% of under-five children stunted in 2016 (190). One in ten under-five children suffer from acute malnutrition (wasted), and

infant and under-five mortality rates are high at 48 and 67 deaths per 1,000 live births, respectively. Maternal undernutrition is also high, with 22% of women aged 15-49 years were found to be exposed to chronic energy deficiency (BMI<18.5 kg/m^2).

In Ethiopia, almost all children (97%) receive breastfeeding at some point, but only 58% and 79% of infants under 6 months receive exclusive and predominant breastfeeding, respectively (190). Continued breastfeeding is also relatively high with 92% and 76% of children continue to receive breast milk until their first and second birthdays. The feeding practices of only 7% of children aged 6-23 months meet the minimum acceptable dietary standards, and only 14% of children had an adequately diverse diet.

Study area

The OME^3JIM project was implemented in three rural subsistence farming districts, named Assendabo (total population *n* = 40,266), Serbo (*n* = 7,938) and Deneba (*n* = 10,428), which are located at ~300 km to the southwest of the country's capital Addis Ababa. The districts are located under the catchment of Gilgel Gibe Health and Demographic Surveillance System (HDSS) which is one of the six HDSS sites in the country. The HDSS center was established since 2005 with the primary purpose of monitoring basic vital events indicators to generate relevant health, demographic and socioeconomic information for policies and programs. The center is comprised of 11 districts surrounding the reservoir of Gilgel Gibe hydroelectric dam covering the area within latitudes of 07.4253 and 07.5558°N and longitudes of 037.1153 and 037.2033°E with midland agroclimatic zone. Study districts were selected because their location under the catchment of HDSS, which facilitated obtaining basic household and child information, their population size to secure adequate number of study subjects, and their proximity and accessibility for the intervention.

Childhood malnutrition is prevalent in the study area and IYC receive complementary diets with low dietary diversity and quality (368,369). Infant and under-five mortality rates were reported to be 76 and 104 deaths per 1,000 live births in 2015, and the commonest causes of mortality were premature birth, pneumonia, malaria and diarrheal diseases (370).

Descriptions of the implementation of the OME[3]JIM trial including enrollment and follow-up of study participants, the study interventions, and outcome measurements are detailed in the relevant chapters.

Figure 2.1. Map of Ethiopia with Jimma Zone (green arrow) under Oromia region

2.3. Outline of the dissertation

This thesis can be divided into two parts reporting on two major areas of the PhD work. In the first part of the thesis (**Chapter 3**), using data from 14 LMICs, we provide empirical evidence on important nutrition-sensitive and -specific drivers for the reduction trend in under-five stunting observed since the turn of the Millennium.

The second part of the thesis (**Chapters 4–6**) presents results from an RCT of n-3 LCPs supplementation of lactating mothers and their children in a rural setting in Ethiopia. **Chapter 4** reports on the effect of fish-oil supplementation of lactating mothers on HM LCPs concentrations and its association with blood concentrations in their breastfed children. **Chapter 5** evaluates the effect of n-3 LCPs supplementation of IYC through lactation and/or complementary food on systemic inflammation, morbidity for common childhood illnesses, and growth outcomes. **Chapter 6** investigates the effects of the same intervention on child developmental performance outcomes.

Finally, a general discussion of the major findings in the various chapters as well as recommendations for future research are formulated in **Chapter 7**. The schematic structure of the thesis is presented in **Figure 2.2**.

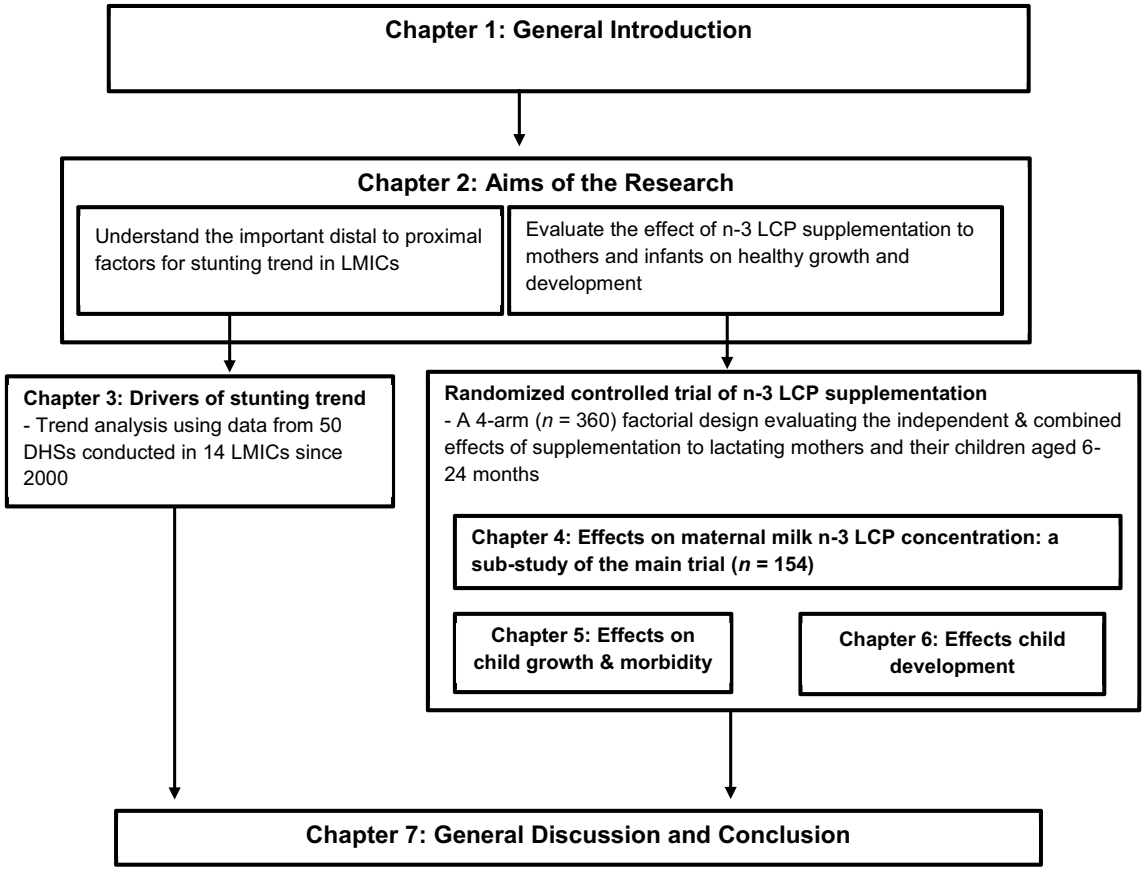

Figure 2.2. Schematic presentation of the PhD research

3

CHAPTER 3

DRIVERS OF UNDER-FIVE STUNTING TREND IN 14 LOW- AND MIDDLE-INCOME COUNTRIES SINCE THE TURN OF THE MILLENNIUM

Redrafted from Argaw, A., Hanley-Cook, G., De Cock, N., Kolsteren, P., Huybregts, L. & Lachat, C. (2019) Drivers of Under-Five Stunting Trend in 14 Low- and Middle-Income Countries since the Turn of the Millennium: A Multilevel Pooled Analysis of 50 Demographic and Health Surveys. Nutrients, 11, 2485.

3.1. Abstract

Background: Understanding the drivers contributing to the decreasing trend in stunting is paramount to meeting the World Health Assembly's global target of 40% stunting reduction by 2025.

Methods: We pooled data from 50 Demographic and Health Surveys since 2000 in 14 countries to examine the relationships between the trends in stunting and potential factors at the distal, intermediate, and proximal levels. A multilevel pooled trend analysis was used to estimate the association between the change in potential drivers at a country level and stunting probability for an individual child while adjusting for time trend and important covariates for the individual child, mother and household. A four-level mixed-effects linear probability regression model was fitted, accounting for the clustering of data by sampling clusters, survey-rounds, and countries.

Results: Stunting followed a declining trend in all countries with an average annual rate of reduction of 1.04 percentage points. Among the distal factors assessed, a decrease in the Gini coefficient, an improvement in women's decision-making, and an increase in urbanization over time within a country were significantly associated with a lower probability of stunting. Improvements in households' access to improved sanitation facilities and drinking water sources, and children's access to basic vaccinations were the important intermediate service-related drivers, whereas improvements in early initiation of breastfeeding and a decrease in the prevalence of low birthweight were the important proximal drivers.

Conclusions: The results reinforce the need for a combination of nutrition-sensitive and -specific interventions to tackle the problem of stunting. The identified drivers help to guide global efforts to further accelerate stunting reduction and monitor progress against chronic childhood undernutrition.

3.2. Introduction

Stunting occurs in more than one in five children of age under-five years, affecting 149 million children globally (1). Childhood stunting is strongly associated with an array of short- and long-term health and economic consequences, including greater risks of child morbidity and mortality, developmental deficits resulting in poorer school performance and lower productivity and earnings in adulthood, and early-life physiological changes contributing to an increased risk of nutrition-related chronic diseases into adulthood (3,9,17). Stunting also leads to undesired outcomes in the following generation, where newborns from stunted mothers are more likely to be small-for-gestational-age with an increased risk of later growth faltering, and morbidity and mortality (25).

In recognition of stunting as an important outcome in itself and as a marker of several adverse outcomes, global attention has been directed towards accelerated reduction of the high burden of child growth faltering in LMICs (371). In 2012, the WHA endorsed global nutrition targets for 2025, including a 40% reduction in the number of children under-five years old who are stunted (57). While stunting has declined over the last three decades, the current trend in many LMICs is insufficient to reach the target for 2025 (56). To reach this target, it is essential to understand the important drivers behind the observed trend in stunting reductions in these countries.

While a great number of studies relied on cross-sectional variations to explore the determinants of child stunting (40,372–378), only a few tried to understand which of these time-variant factors influence the trend in stunting prevalence over time (53,54,59,60,64,379). Moreover, most efforts to assess the trend in stunting reduction have taken a country focus and examined contextual drivers (53,54,59,60,64). However, comprehensive evidence of stunting drivers across high burden countries is equally important for decision-making at the global and regional levels. Only a few studies have used a cross-country analysis to explore the association between stunting trends and potential drivers over the past three decades (45,52,61,63). However, previous analyses were limited by the unavailability of data on some important nutrition-related determinants. For instance, the use of per capita energy supply may be a distant proxy for the underlying IYCF practice indicators (45,52,63). Furthermore, timely revision of evidence on the drivers of the current stunting trend is

relevant for decision-making as the strength of the relationship between stunting and potential drivers could change over time (59,64).

In this study, we used a large cross-country dataset of the Demographic and Health Surveys (DHS) to understand the association between changes in a range of distal to proximal factors and stunting prevalence among under-five children in 14 LMICs since 2000.

Table 3.1. Characteristics of included Demographic and Health Surveys

Characteristics	Statistics
Surveys, *n*	50
Countries, *n*	14
Surveys per country, *n*	
3 rounds	6
4 rounds	8
Countries with survey years	
Bangladesh	2004, 2007, 2011, 2014
Cambodia	2000, 2005, 2010, 2014
Ethiopia	2000, 2005, 2011,2016
Haiti	2000, 2005, 2012, 2016
Kenya	2003, 2008, 2014
Malawi	2000, 2004, 2010, 2015
Mali	2001, 2006, 2012
Nepal	2001, 2006, 2011, 2016
Nigeria	2003, 2008, 2013
Rwanda	2000, 2005, 2010, 2014
Tanzania	2004, 2010, 2015
Uganda	2000, 2006, 2011, 2016
Zambia	2001, 2007, 2013
Zimbabwe	2005, 2010, 2015
Total sample size, *n*	322,320
Children per survey, mean (range)	6457 (2070–24,505)
Child age (month), mean ± SD	28.6 ± 17.2
Child sex (female), %	49.8
Maternal age (y), mean ± SD	28.7 ± 6.86

3.3. Materials and methods

3.3.1. Data source

We considered data from DHSs conducted in 42 LMICs that are partners for nutrition support by the DEVCO. Our trend analysis required data from at least 3 time-points. Therefore, the present analysis only utilized data from 14 countries with an adequate number of standard DHS rounds since 2000. Countries included in our analysis were Bangladesh, Cambodia, Ethiopia, Haiti, Kenya, Malawi, Mali, Nepal, Nigeria, Rwanda, Tanzania, Uganda, Zambia, and Zimbabwe (**Table 3.1**).

The DHS has been conducted in several countries for over three decades, providing nationally representative cross-sectional data on demographic, health, and nutrition information, among others (365). The DHS uses standardized data collection procedures across countries and consistent content across survey rounds, allowing for comparison of data across-countries and within-country over time. We pooled data from 50 DHS rounds in the 14 countries. There were a different number of surveys per country and different time spans between surveys for the countries. Data were compiled from the official website of the DHS Program accessed with permission in November 2018 (https://dhsprogram.com/). Children of under-five years who had data on length/height measurements and other relevant information available were included in the analysis.

3.3.2. Variables

The outcome of interest was the probability of being stunted in under-five children. Stunting here is described as binary (stunted/not stunted), defined as a length/height-for-age *z* score below 2 SD from the WHO 2006 Child Growth Standards median value for the same age and sex (27). Potential drivers for stunting reduction were identified based on the UNICEF (27) conceptual framework for the determinants of childhood undernutrition and the more recent 2013 Lancet (7) framework for actions to improve child nutrition and development. A variable was selected for analysis when it was available in the DHS datasets for enough survey rounds, and used as a determinant in more than one paper, or mentioned in reviews and/or were found to be significant in previous country analyses. We considered three groups of variables operating at different levels as distal factors, intermediate health and related services utilization factors, and proximal factors (**Table 3.2**). We considered variables such as women's education, decision-making power and work opportunity as distal factors because these factors can influence access to and utilization of the intermediate service-related variables. Definition of indicators used in our potential drivers list is in line with the DHS statistics guideline (380). Estimates for the association between drivers and stunting were adjusted for important demographic and socioeconomic covariates for the child, mother, and household, including child age, sex, birth-order and birth-interval, maternal age and marital status, household wealth status, and place of residence (urban/rural).

Table 3.2. Description of variables used in the study[1]

Variables	Definition
Outcome	
Stunting	Height/length-for-age *z* score value 2.0 SD below the median based on the WHO 2006 Child Growth Standards.
Distal determinants	
Gini coefficient	A coefficient calculated from the DHS household wealth index score indicating the level of concentration of wealth among households in a country. Possible values range from 0 (being an equal distribution) to 1 (a totally unequal distribution) with higher values indicating more unequal distribution with a lower proportion of households controlling more of the wealth in the country.
Total fertility rate	The average number of births women (15–49 years) would have by the time they reach age 50 years based on the current age-specific fertility rate during the 3 years preceding the survey (excluding 1–36 months before the survey).
Urbanization	Percentage of total population living in urban areas.
Female primary education coverage	Percentage of women (15–49 years) who attended any level of primary education.
Male secondary education coverage	Percentage of men (15–49 years) who attended any level of secondary education.
Women's decision making power	Percentage of currently married women (15–49 years) who usually make final decisions (alone or jointly with their husband) about their own healthcare, large household purchases, and visits to family or relatives.
Women work opportunity	Percentage of women (15–49 years) who worked in the past 7 days (including women who did not work in the past 7 days, but who are regularly employed and were absent from work for leave, illness, vacation, or any other reason) or worked in the past 12 months, but not currently.
Intermediate health and related service determinants	
Improved sanitation facility coverage	Percentage of total households with improved sanitation following the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply, Sanitation and Hygiene.
Improved drinking water sources coverage	Percentage of total households with improved drinking water source following the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply, Sanitation and Hygiene.
Antenatal care follow-up with ≥4 visits coverage	Percentage of women with a live birth in the 5 years preceding the survey who received ≥ 4 antenatal care visits for the most recent birth.

Table 3.2 continued

Variables	Definition
Delivery at health facility coverage	Percentage of live births to interviewed women in the 5 years preceding the survey delivered in a health facility.
Iron supplementation during pregnancy coverage	Percentage of women with a birth in the 5 years preceding the survey who took iron tablets or syrup (given or bought) during the pregnancy for the most recent live birth.
Children with all 8 basic vaccinations coverage	Percentage of living children (12–35 months) who received BCG[5], 3 doses of DPT[6]-containing vaccine, 3 doses of polio vaccine (excluding polio vaccine given at birth), and 1 dose of MCV[7] at any time, according to vaccination card or mother's report.
Proximal determinants	
Initiation of breastfeeding in ≤1 day	Percentage of last-born children who were born in the 2 years preceding the survey put to the breast within one day of birth .
Median duration of exclusive breastfeeding	Median duration (in months) of exclusive breastfeeding among children born in the past three years.
Complementary feeding between ages 6–9 months	Percentage of children (6–8 months) who were both breastfed and received complementary food (solid or semi-solid foods) in the 24 h preceding the interview.
Prevalence of reported low birthweight	Percentage of live births to interviewed women in the 5 years preceding the survey where the mother's estimated baby's size at time of birth as smaller than average.
Prevalence of acute respiratory infection (ARI)	Percentage of living children (0–59 months) with symptoms of ARI at any time in the 2 weeks preceding the survey.
Prevalence of diarrhea	Percentage of living children (0–59 months) with diarrhea at any time in the 2 weeks preceding the survey.

[1]BCG, Bacille Calmette Guerin vaccine against tuberculosis; DPT, diphtheria-pertussis-tetanus vaccine; DHS, Demographic and Health Survey; MCV, Measles antigen-containing vaccine; SD, standard deviation; UNICEF, United Nations Children's Fund; WHO, World Health Organization

3.3.3. Statistical analysis

Data management and statistical analyses were conducted in Stata version 14.1 (StataCorp LLC, Texas, USA) using the High Performance Computing infrastructure at Ghent University. For all analyses, associations with a *P*-value of less than 0.05 were regarded as statistically significant. Missing indicators for certain survey-rounds were imputed based on linear interpolation of the available time-points within a country. The aggregate value of stunting and explanatory indicators for each survey-round in a country was calculated by taking into account the DHS sampling weight, using the *svyset* command in Stata. The pooled annualized rate of change in stunting and indicators were estimated by fitting mixed-effects linear regression models using country as a random intercept and survey-round as a random slope, and by applying weightings based on the population size of each country.

We followed the trend analysis approach suggested by Fairbrother (381) for analysis of outcome at individual-subject level using repeated cross-sectional survey datasets. The method allowed us to examine the association between the trend in an indicator at aggregate country-level and the risk of stunting at an individual child, while adjusting for time trends (survey year) and important demographic and socioeconomic covariates for the individual child, mother, and household mentioned above.

We fitted mixed-effects linear probability models with robust variance estimation. A four-level random intercept was considered to account for potential sources of clustering in our data where individuals (level-1) are nested within the DHS sampling clusters (level-2), which in turn are nested within survey-rounds (level-3), and finally, survey-rounds nested within countries (level-4). Since an indicator varies both within-country over time and across countries, we sought to identify separate within-country (longitudinal) and between-country (cross-sectional) components for the association between an indicator variable and stunting. For this purpose, we simultaneously specified in the model the average value of an indicator per country (i.e., representing cross-country differences) and the average value of an indicator per survey-round within a country (i.e., representing within-country change over time). This within-between model specification has the additional advantage of minimizing potential endogeneity problems in mixed-effects models (382). In order to avoid over-

adjustment from the inclusion of distal, intermediate, and proximal indicators in a single model, we specified three separate multivariable regression models for each group of indicators.

The relationship between an explanatory variable and stunting risk in our four-level model is represented the equation below:

$$y_{iktj} = \beta_0 + \beta_{1xtjM} + \beta_{2\bar{x}j} + \beta_{3xiktj} + \beta_{4timetj} + u_k + u_{kj} + u_{ktj} + \varepsilon_{iktj;}$$

where y_{iktj} is the risk of stunting in child *i* who is from a DHS sampling cluster *k* nested within a survey for country-year *tj*, which in turn is nested within a country *j*. β_1 and β_2 give estimates for stunting-indicator associations decomposed into between-country (which is estimated from the value of an indicator at a country level using the average of all survey-rounds in a country $\bar{x}_j$) and within-country effects (which is estimated from the average value of an indicator for each survey-round per country x_{tjM}), respectively. Country-year level variables were mean-centered by subtracting the value of the country-level variable from the value at survey-round within the same country. This allows us to the cross sectional component and the longitudinal component to be orthogonal (independent) to each other by construction and their effects can be estimated simultaneously with in a model. β_3 gives the estimate for the association of individual-subject level covariates used for adjustment in the model. β_4 gives the estimate for the time variable as a set of year dummies, which adjust for possible simultaneous but unrelated time trends in both x_j/x_{tjM} and stunting. Random effect components modeling clustering of data by the DHS sampling cluster (u_k), by country-years (u_{kj}), and countries (u_{ktj}), and the residual term for individual child (ε_{iktj}) are assumed to follow a Gaussian distribution with a mean value of zero.

3.4. Results

Our final dataset included 322,320 children of age under-five years from 50 survey-rounds in 14 countries that were conducted between 2000 and 2016 (**Table 3.1**). The mean ± SD child age was 28.6 ± 17.2 months and 49.8% of children were female. The mean ± SD maternal age was 28.7 ± 6.86 years. The number of survey-rounds per country were three in six countries and four in eight countries. The average sample size per survey-round was 6,457 (range: 2,070–24,505).

The trend over survey-rounds for stunting prevalence and the indicator variables within the 14 study countries are presented in **Figure 3.1** and **Annex 1**. Stunting prevalence followed a declining trend over time in all countries assessed. The overall average annual rate of stunting reduction was 1.04 percentage points (pp) (β (95% CI): −1.04 (−1.24, −0.84); $P < 0.001$) with differing slopes among countries (**Table 3.3**). Similarly, most of the indicator variables also showed improvements over time except in some variables, like women's work opportunity.

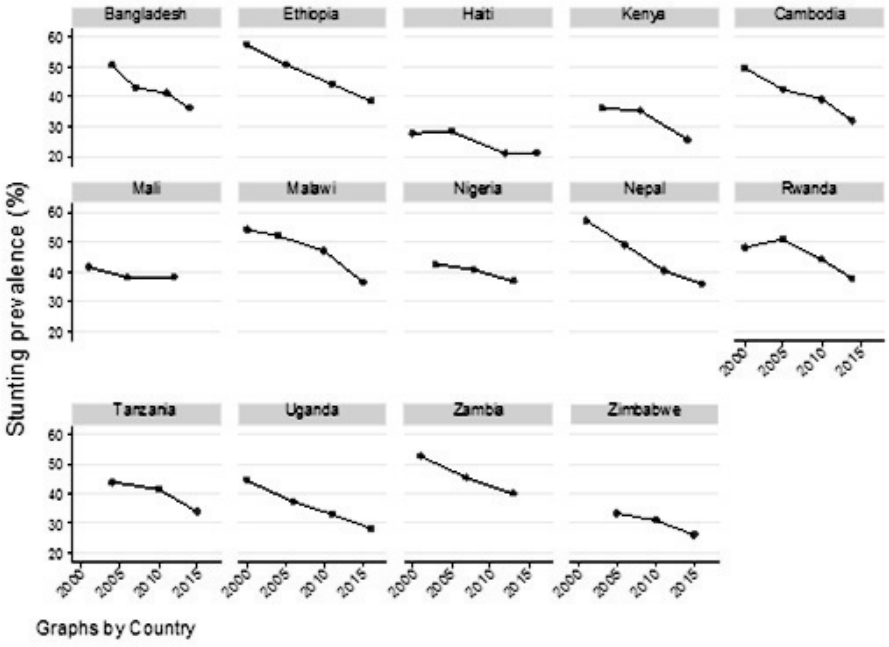

Figure 3.1. Trends in under-five stunting prevalence by country

We estimated the associations between the probability of stunting and the variations in potential drivers within- and between-countries with the estimate of the within-country association reported as a proxy measure of the effect of an indicator on stunting trend over time (**Table 3.4**). The Gini coefficient, urbanization, and women's decision-making power were the distal-level indicators found to be significantly associated with the change in stunting risk within a country. A SD decrease in the Gini coefficient within a country over time was associated with a decreased probability of stunting by 1.10 pp ($P = 0.008$). A 10% increase in the percentage of

the population living in urban areas and women with decision-making power was associated with a 0.67 pp (P = 0.013) and a 0.36 pp (P = 0.040) decreases in the probability of being stunted, respectively.

Households' access to improved sanitation facilities and improved drinking water sources, and children's access to all basic vaccinations are the intermediate level health and related service determinants that were found to be significantly associated with stunting risk overtime. Within a country, a 10% increase in the coverage of improved sanitation facilities, improved drinking water sources, and basic child vaccinations were associated with decreases in the probability of stunting by 1.40 pp (P < 0.001), 1.48 pp (P = 0.003), and 1.74 pp (P < 0.001), respectively.

Table 3.3. Trends in stunting prevalence and potential determinants across survey-rounds (n = 50)[1]

Variables	Average annualized rate of change	
	Beta (95% CI)	*P*
Stunting prevalence in under-five children (%)	−1.04 (−1.24, −0.84)	<0.001
Gini coefficient	−0.01 (−0.02, −0.01)	<0.001
Total fertility rate (average number of births per women)	−0.07 (−0.09, −0.05)	<0.001
Urbanization (%)	0.61 (0.21, 1.00)	0.003
Female primary education (%)	0.21 (−0.49, 0.91)	0.560
Male secondary education (%)	0.13 (−0.05, 0.30)	0.163
Women's decision-making power (%)	2.31 (1.36, 3.26)	<0.001
Women working (%)	−0.30 (−0.84, 0.25)	0.286
Improved sanitation facilities (%)	1.87 (1.02, 2.72)	<0.001
Improved drinking water sources (%)	1.25 (0.66, 1.84)	<0.001
Antenatal care follow-up with ≥4 visits (%)	1.13 (0.53, 1.72)	<0.001
Delivery at health facility (%)	1.82 (1.17, 2.47)	<0.001
Iron supplementation during pregnancy (%)	0.39 (−2.59, 3.37)	0.798
Children with all 8 basic vaccinations (%)	1.18 (0.98, 1.38)	<0.001
Initiation of breastfeeding in ≤1 day (%)	1.00 (0.66, 1.33)	<0.001
Median duration of exclusive breastfeeding (months)	0.08 (0.04, 0.12)	<0.001
Complementary feeding between ages 6–9 months (%)	0.32 (−0.21, 0.86)	0.239
Prevalence of reported low birthweight (%)	−0.18 (−0.58, 0.23)	0.392
Prevalence of acute respiratory illness (%)	−1.00 (−1.14, −0.86)	<0.001
Prevalence of diarrhea (%)	−0.47 (−0.69, −0.26)	<0.001

[1]Average annualized rates of change (beta coefficients (95% CI)) were estimated using a mixed-effects linear model with country as random intercept and time as random slope and using weighting for each country's population size.

As for the proximal nutrition and health factors, prevalence of reported low birthweight and percent of children with early initiation of breastfeeding were significantly associated with changes in stunting risk overtime. A 10% decrease in the prevalence of low birthweight over time was associated with a 3.91 pp ($P < 0.001$) decrease in the probability of stunting. A 10% increase in children with early initiation of breastfeeding within a country was associated with a 1.17 pp ($P < 0.001$) decrease in the probability of stunting.

Table 3.4. Modeling distal, intermediate, and proximal drivers of the trend in stunting prevalence[1]

Indicators	Stunting	
	Beta (95% CI)	*p*
Distal determinants		
Gini coefficient (SD)	**1.10 (0.29, 1.91)**	**0.008**
Total fertility rate (SD)	0.39 (–0.78, 1.56)	0.513
Urbanization (10%)	**−0.67 (−1.21, −0.14)**	**0.013**
Women primary education (10%)	−1.12 (−2.42, 0.18)	0.092
Male secondary education (10%)	−1.95 (−5.47, 1.56)	0.275
Women's decision making (10%)	**−0.36 (−0.69, −0.02)**	**0.040**
Women's working (10%)	−0.72 (−2.58, 1.15)	0.450
Intermediate service related determinants		
Improved sanitation facilities (10%)	**−1.40 (−2.17, −0.62)**	**<0.001**
Improved drinking water sources (10%)	**−1.48 (−2.45, −0.52)**	**0.003**
Antenatal care follow-up with ≥4 visits (10%)	0.38 (−0.46, 1.23)	0.377
Delivery at health facility (10%)	0.08 (−1.20, 1.36)	0.907
Iron supplementation during pregnancy (10%)	0.21 (−0.14, 0.56)	0.240
Children with all 8 basic vaccinations (10%)	**−1.74 (−2.53, −0.94)**	**<0.001**
Proximal determinants		
Initiation of breastfeeding in ≤1 day (10%)	**−1.17 (−1.62, −0.73)**	**<0.001**
Median duration of exclusive breastfeeding (SD)	0.18 (−0.32, 0.68)	0.473
Complementary feeding b/n age 6–9 months (10%)	0.75 (−0.53, 2.03)	0.250
Prevalence of reported low birthweight (10%)	**3.91 (2.40, 5.43)**	**<0.001**
Prevalence of acute respiratory illness (10%)	1.28 (−1.40, 3.96)	0.349
Prevalence of diarrhea (10%)	−2.73 (−6.12, 0.65)	0.113

[1]Beta coefficients (95% CI) are estimated using a mixed-effects linear probability regression model with a robust variance estimator and using four-level random intercept accounting for clustering of individuals by sampling clusters, survey-rounds, and countries. Separate models were fitted for each group of the distal, intermediate, and proximal variables. Models were adjusted for time trend and important individual-level covariates including child age, sex, birth-order and birth-interval, maternal age and marital status, household wealth status, and place of residence (urban/rural). Bold values indicate $p < 0.05$. SD, standard deviation.

3.5. Discussion

This study examined a range of distal to proximal factors that might explain the observed trend in stunting reduction in 14 LMICs since the turn of the millennium. Stunting prevalence followed a declining trend in all countries assessed, with an average annual rate of reduction of 1.04 pp. However, this observed reduction is below the 3.9% average annual rate of reduction that is expected to meet the WHA 2025 stunting target, indicating the need for further efforts by the countries and their international partners (57).The key indicators that explained the observed trend in stunting were improvements in income inequality, urbanization, and women's decision-making power from the distal factors assessed; improvements in households' access to improved sanitation facilities and improved drinking water sources, and child immunization rate for basic vaccinations from the intermediate service related factors; and a decline in the prevalence of reported low birthweight and an improvement in early initiation of breastfeeding from the proximal factors.

The current finding that investments narrowing economic inequalities among households and empowering women are key drivers of stunting reduction is in line with the findings from previous studies. In a cross-country analysis of stunting trend from 1970–2001, Milman et al. (45) reported that countries with more equitable income distribution achieved better stunting reduction. Others also reported that increases in household asset accumulation was an important driver of stunting reduction in a cross-country study (44) as well as in studies of the trend in individual countries with substantial stunting reduction (53,59,60). Improvements in women's decision-making as a key stunting driver in this study is consistent with a previous review by Carlson et al. (48) that suggested raising maternal autonomy is an important goal for improving children's nutritional status in LMICs. It is widely acknowledged that mothers play a vital role as a primary caregiver of the child, and their control over household resources and decision-making is expected to facilitate the level of childcare and feeding practices as well as the utilization of health care services which are important underlying determinants of child nutritional status and health. On the other hand, women's education, which has been reported as a key driver of stunting reduction for over the past three decades (45,52,53,59–61), was not found to be important driver in our analysis. In this study, although countries with

a better coverage of women's primary education had significantly lower stunting burden, the within-country association was not significant. This may suggest that although women's education has been responsible for the changes in stunting prevalence that occurred over the long-term, it has a lesser contribution for the more recent changes over the last two decades. This may be due to a lack of substantial gains in women's education coverage in the more recent period in the studied countries, as it is indicated by the small nonsignificant change observed in this indicator compared to most other indicators in our data (**Table 3.3**). Another possible explanation is that the potential impact of women's education on stunting trend might be partially explained by other closely related indicators in our model, such as women's decision-making power and work opportunity. However, the within-country association for women's education remains nonsignificant after excluding the mentioned indicators from the model, suggesting that our previous explanation holds.

This study found that within-country change in stunting risk was associated with improvements in households' access to improved sanitation facilities and improved drinking water sources and increased coverage of child immunization, which is consistent with findings from previous studies of stunting trend (52,53,59–61). These findings are also supported by the growing body of literature that demonstrate environmental enteric dysfunction in children living under poor water, sanitation, and hygienic conditions is among the most important factors for the high burden of child growth faltering in low-income settings (17,42,383).

This study extends previous trend analysis studies by including more direct measures for the proximal determinants of child undernutrition, including the WHO IYCF indicators, and prevalences of low birthweight and common childhood illnesses. The finding that reductions in the rate of low birthweight was an important driver of the changes in stunting prevalence concurs with a previous estimate showing intrauterine growth restriction (estimated by low birth weight) accounts for 20% of the stunting burden in LMICs (26). From the IYCF indicators assessed, only improvements in early initiation of breastfeeding was found to be significantly associated with a reduction in the probability of stunting in the 14 countries. We did not find a statistically significant association of the trends in stunting with the percentage of breastfed children 6-8 months old who started complementary foods or with the

median duration of breastfeeding. The mere introduction of solid and semi-solid foods may not be a good indicator of the quality of children's diet which could explain the lack of association with this indicator. In this regard, indicators such as dietary diversity and the consumption of animal source foods might have been better indicators to evaluate dietary quality and its association with child linear growth (34–37,384–386). However, such indicators are available only in the most recent DHS rounds. The lack of association between longer duration of breastfeeding and stunting reduction has also been reported previously. It is possible that higher reliance on breastfeeding may occur in low-income settings where infants have lesser access to diverse diet as well as mothers of infants with poor health and growth may decide to continue breastfeeding for a longer duration (39,387,388).

One important limitation of the current study is that generalizability of the findings is limited by the facts that our analysis did not include all relevant countries, such as India, which holds almost a third of the world's stunting burden. Given the cross-sectional nature of the study, we cannot assure observed associations are causal. It was also not possible to consider a time-lag between the indicators and stunting risk as our analysis was entirely based on the DHS data, which collects both the predictors and the outcome at the same time. However, it should be noted that experimental impact evaluations, such as controlled interventions, are extremely challenging, if not impossible, to address such holistic research questions addressing several drivers of chronic child undernutrition at the level of multiple countries. It should be noted that the indicators that were found to be important drivers in the past may not necessarily be the ones that will be important in the future due to possible changes in indicator-stunting associations and a saturation of some indictors for future improvements. For this reason, unlike most previous trend analysis studies, we aimed at evaluating the more recent trends since the year 2000. Furthermore, we note that most of the indicators found to be important in our analysis have also been targeted by the United Nations' Millennium Development Goals set for 2015 and its continuation of the SDGs for the year 2030 (58). Thus, it is possible that with future investment, substantial progress can be made on these indicators with a potential resultant effect on child stunting reduction. Finally, some of the potentially expected drivers, like complementary feeding practice, were not found to be significantly associated with stunting reduction in the current analysis, which could be due to the

limited progress in these indicators in most of the countries studied. Therefore, we cannot exclude the potential that some of these nonsignificant indicators will contribute to stunting reduction with future investments improving their status in a population.

3.6. Conclusion

In conclusion, the current study identified important distal to proximal drivers for the observed trend in stunting reduction in 14 LMICs, which is an important input for future decision-making to further accelerate stunting reduction and monitor progress against chronic childhood undernutrition. Our findings indicate that stunting drivers were present both at the distal and intermediate, as well as at the proximal levels, suggesting that economic development and nutrition-sensitive interventions, on top of nutrition-specific programs, play an important role in reducing the high stunting prevalence in these countries.

4

CHAPTER 4

FISH-OIL SUPPLEMENTATION AND MATERNAL MILK N-3 LCP CONCENTRATIONS

Redrafted from Argaw, A., Bouckaert, KP., Wondafrash, M., Kolsteren, P., Lachat, C., De Meulenaer, B., Hanley-Cook, G., Huybregts, L. (2020) Effect of fish-oil supplementation on breastmilk n-3 long-chain polyunsaturated fatty acid concentration: a randomized controlled trial in rural Ethiopia. European Journal of Clinical Nutrition. doi: 10.1038/s41430-020-00798-x.

4.1. Abstract

Background: For infants and young children in low-income settings, human milk (HM) is the main source of omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPs), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). However, the n-3 LCPs concentrations of HM show wide variability, largely depending on the maternal intake of marine foods. This may put children living far from coastal areas at risk of inadequate intake. We evaluated the efficacy of fish-oil (FO) supplementation of lactating mothers on HM n-3 LCPs concentrations in a rural setting from Ethiopia.

Objective: In a sub-study of an individually randomized controlled trial, we evaluated the efficacy of fish-oil supplementation of lactating mothers on HM n-3 LCPs concentrations in a rural setting in Ethiopia.

Methods: Mothers (n=360) with children 6-12 months old were randomized to receive either intervention FO capsules (215 mg DHA + 285 mg EPA) or control corn-oil capsules (without n-3 LCPs). In a random subsample of 154 participants, we analyzed LCPs in HM and child capillary blood using gas chromatography..

Results: Compared to the control, FO supplementation increased HM concentrations of DHA by 39.0% (95% CI: 20.6, 57.5%; P<0.001) and EPA by 36.2% (95% CI: 16.0, 56.4%; P<0.001), whereas the arachidonic acid (AA)/(DHA + EPA) ratio decreased by 53.5% (95% CI: -70.2, -36.7%; P<0.001). We also found statistically significant association between the changes in (DHA + EPA)/AA ratio in HM and child capillary blood (P<0.001). However, HM DHA concentrations remained lower than international norms after FO supplementation..

Conclusions: FO supplementation improves n-3 LCPs content of HM. Future studies should evaluate different doses of n-3 LCPs and consider potential effect modifiers such as genetic polymorphism and diet. This trial was registered at clinicaltrials.gov as NCT01817634.

4.2. Introduction

Adequate supply of the LCPs of the n-3 series DHA and EPA, and of the n-6 series AA are essential for normal growth and development (85,195). DHA and AA accumulate rapidly in the cerebral cortex and retina during the brain growth spurt which takes place during the first 1,000 days after conception (146,389,390). DHA status of infants was shown to relate with visual and neurocognitive development (210). In addition, LCPs are precursors of eicosanoids and other lipid mediators modulating the expression of genes involved in inflammation processes and other immune functions and metabolic control (311,351,391,392).

HM remains the most affordable source of LCPs for IYC in LMICs as complementary diets in these settings are usually very low in preformed LCPs (174,179). The global average concentrations of DHA and AA in HM are estimated at 0.37% (SD 0.11) and 0.55% (SD 0.14) in w/w of total lipids (%TL) (178). The concentration of DHA in HM is highly variable and mainly explained by maternal dietary intake of preformed DHA found in fish and other marine foods, whereas the concentration of AA is relatively stable and less affected by AA in the maternal diet (176–178). Expert groups recommend that lactating women should aim for an average daily dietary intake of 300 mg DHA + EPA from which 200 mg should come from DHA (95,173). There are no indications that lactating women with an adequate dietary intake of the precursor LA need an additional dietary supply of AA (95). The recommended intake of n-3 LCPs during pregnancy and lactation can be attained by a weekly consumption of 1-2 portions of sea fish (95). For those women without access to marine foods, n-3 LCP supplementation may be required to ensure this recommended intake.

In Ethiopia, the LCPs status of HM have not been documented. The per capita DHA availability in Ethiopian diet was estimated at 7.0 mg/d, which is one of the lowest globally and extremely low to meet the recommended intake during lactation (175). Furthermore, the contribution of complementary foods to DHA intake by Ethiopian children 6–36 months old was estimated to be negligible (1.1 mg/d), compared to the worldwide average (14.6 mg/d) (179).

We conducted the OME^{3}JIM trial, a 2x2 factorial randomized controlled trial to evaluate the effects of n-3 LCPs supplementation of lactating women and/or their children 6-24 months of age on child health, growth and development in rural

Ethiopia. In a subsample of this trial, we collected HM and child capillary blood samples. The purpose of this sub-study was to evaluate the effect of maternal fish-oil (FO) supplementation on n-3 and n-6 LCPs concentrations in maternal milk and the association between changes in maternal milk and child blood concentrations.

4.3. Methods

The OME³JIM trial was conducted from November 2013 to February 2015 in three districts of Jimma Zone in southwest Ethiopia. Subsistence farming is the main form of livelihood in the study districts, and staple crops in the area are very low in n-3 LCPs. Mother-infant pairs with singleton infants of age 6-12 months were enrolled in the main trial if the infant was breastfeeding and not acutely malnourished (weight-for-length *z* score ≥-2 SD and no bilateral pitting edema), and the mother had no plan to leave the study area for more than one month during the study period and was willing to participate in the trial. Mother-child pairs were excluded from the trial when the mother or child had a known chronic illness, was taking other nutritional supplements, when the child had a congenital abnormality or severe anemia (hemoglobin <7.0 g/dL) at enrolment or during study follow-up.

Details of the main OME^{3}JIM trial are reported in **Chapter 5**. In brief, from a total of 413 mother-infant pairs screened, 360 eligible pairs were enrolled in the main trial (**Figure 4.1**). Study mothers were randomly assigned to either an intervention group that received fish-oil capsules (FO, *n* = 180) or a control group that received placebo corn-oil capsules (CO, *n* = 180). A random subsample of 168 mother-infant pairs from both study arms was selected for the HM sub-study. HM samples at baseline were available from 154 mothers (*n*: CO = 82; FO = 72), who were finally considered for this study.

Both the fish-oil and corn-oil capsules were produced as identical airtight soft-gel capsules (Biover NV, Belgium). A daily dose of two intervention fish-oil capsules provided 500 mg/d n-3 LCPs (215 mg DHA + 285 mg EPA) whereas the control corn-oil capsules contained no n-3 LCPs. Each capsule additionally contained 5 mg of the antioxidant d-α-tocopherol to limit oxidative loss of LCP. The intervention was provided for 12 months, with supplements distributed on a monthly schedule and compliance monitored through weekly counts of remaining capsules.

In addition to the maternal intervention, infants aged 6-12 months of the same mothers were individually randomized to either an intervention group that received a food supplement fortified with fish-oil (n = 181) or a control group that received the same food supplement without fish-oil (n = 179) during the same period. The intervention food supplement contained a daily dose of 500 mg n-3 LCPs (169 mg DHA + 331 mg EPA), whereas the control food supplement contained no n-3 LCPs.

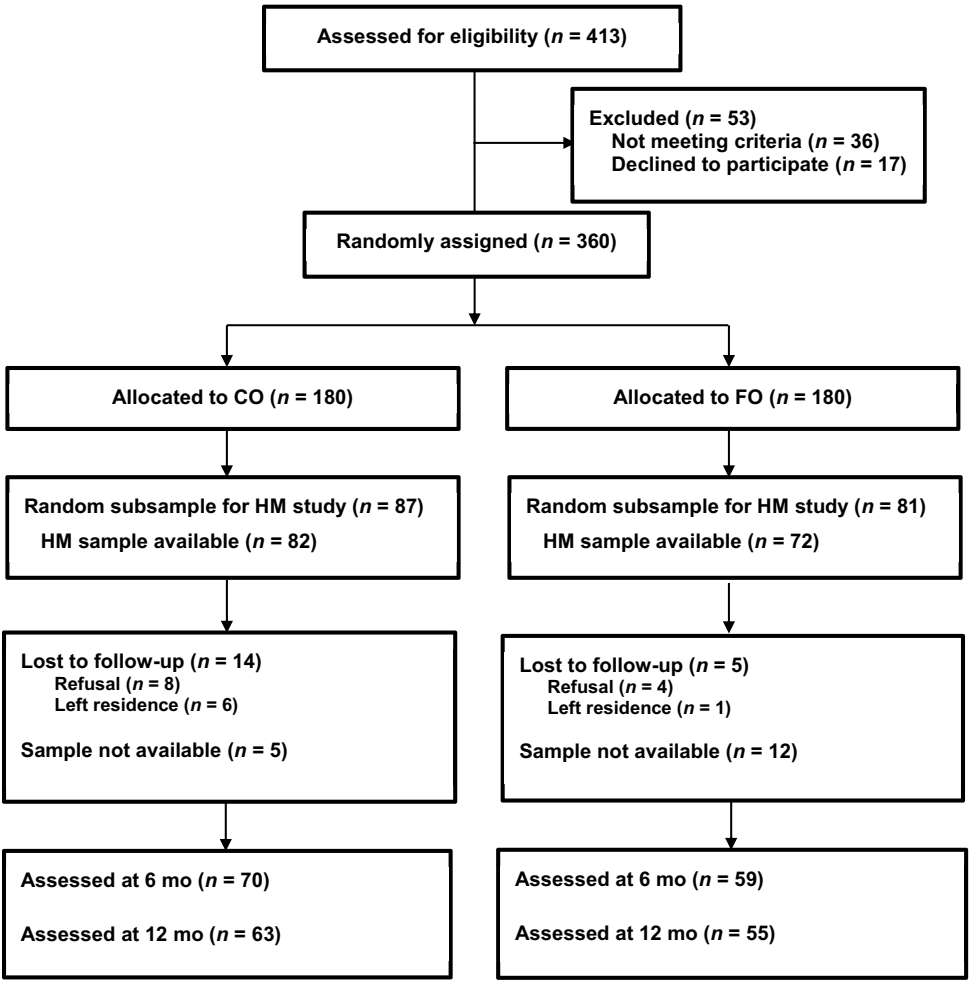

Figure 4.1. Trial flowchart

A random subsample of 168 mothers was drawn from the main trial (*n* = 360) and asked to participate in the HM sub-study. A total of *n* = 154 mothers from whom samples were available at baseline were finally considered for the HM sub-study. CO, control mothers who received a placebo corn-oil capsules; FO, intervention mothers who received fish-oil capsules; HM, human milk.

4.3.1. Sample collection and LCP analysis

HM and child capillary blood samples were collected at baseline, midline (after 6 months) and endline (after 12 months) of the intervention to determine DHA and EPA concentrations. The concentration of AA was additionally considered to evaluate any potential influence of n-3 LCP supplementation on n-6 LCP levels. Before collecting HM samples, mothers were asked to breastfeed their child for a few minutes to establish breastfeeding. Then, study nurses expressed breast milk samples (10-15 mL) manually or by using manual breast pumps into sterile plastic containers with lids. An aliquot of 9 mL homogenized milk sample was pipetted into a 10 mL cryovial containing 1 mL of an 0.01% BHT (2,6-Di-tert-butyl-4-methylphenol) acetone solution for storage, so as to limit lipolytic and oxidative degradation of milk lipids before extraction. Child blood samples were collected using dried blood spot cards. Prior to sample collection, blood spot cards (TFN, Munktell) were impregnated with BHT (2,6-di-tert-butyl-4-methylphenol) to minimize the oxidation of LCPs as previously described by Ichihara et al. (393). A large drop of blood from a finger prick was collected on preprinted circles on the spot cards and then dried overnight at room temperature, and, once dry, inserted into aluminum-coated airtight envelopes with dry desiccants before storage. HM samples and dried blood spot cards were collected in the field in the morning up to noon (between 9:00 AM and 1:00 PM), and transported in cold-chain using cooled bags before storage at -80°C in a central laboratory at Jimma University. Samples were later air-shipped on dry ice to a laboratory in Belgium and stored at -80°C upon arrival until analyses.

A total lipids extract was prepared from each HM sample using an aliquot of 50 µL homogenized HM according to the Bligh-Dyer method (394), and from each child whole blood sample using a disc of 8 mm diameter punched from the blood spot cards (corresponding to ±21.8 µL blood) (395) according to the method detailed by Bailey-Hall et al. (396). A prior lipid extraction step was performed to guarantee the extraction of all lipids from the filter paper. We performed saponification with NaOH and methylation into FA methyl esters with BF3 in methanol. Then, FA methyl esters were separated by gas chromatography with cold-on column injection (0.1 µL, GC-FID 6890N, Agilent Technologies) and a FA methyl ester column (CP-Sil 88, 60 m length, 0.25 mm ID, 0.20 µm film thickness, Agilent Technologies). We used helium as carrier gas (BIP Plus-X50S, Air Products) and applied a programmed temperature

gradient (hold at 50°C for 4 min, increase temperature by 25°C/min to 225°C, hold at 225°C for 25 min). Retention times were compared to the standard (GLC-68 D, Nu-Chek-Prep), and FAs were quantified relative to the 19:0 FA internal standard.

4.3.2. Statistical analysis

A sample size of 144 participants (72 participants/group) was sufficient to detect effect size of ≥0.44 SDs on HM LCP concentrations between groups using power of 80%, type I error of 5%, anticipated attrition of 20%, and ρ=0.50 for the correlation between midline and endline measurements. The FA data are presented in mg/L HM or blood, as the overall fat content of HM and capillary blood samples could not be determined. Data were assessed for equality of variance between groups and normality of distribution and log transformation used when necessary. Data were summarized using mean (SD) or median (P^{25}, P^{75}) for the continuous variables and percentages for the nominal variables. Participant characteristics were compared between the HM sub-study sample and the main study sample using t-test and chi-square test.

Group differences in HM LCPs concentrations were estimated using a mixed-effects linear regression model with the mother as a random intercept to account for repeated measurements at the midline and endline assessment. A similar mixed-effects model was used to assess the relationship in the changes in (DHA + EPA)/AA ratios between HM and child blood following the FO intervention. For the latter analysis, results are presented separately by the child intervention arms and the difference in slope between the two groups was assessed using an interaction term between child intervention arm and HM ratios. Models were adjusted for baseline HM LCPs concentrations and additional covariates, including household wealth, child age and sex, frequency of breastfeeding, maternal age, height, parity and the occurrence of pregnancy during study follow-up. For the log-transformed outcomes, the antilog of coefficients and CIs were used to express group difference as percent of the control group value.

We followed a modified intention-to-treat analysis which included all mother-infant pairs sampled for the HM sub-study and from whom a baseline HM sample was available. For this purpose, a multiple imputations procedure under the missing at random assumption was employed using chained equations of 50 imputations for the

lost-to-follow-up cases. Analysis was conducted using Stata version 14.1 and a two-sided statistical significance was considered at *P* value <0.05.

4.4. Results

Of the total of 154 mothers who were considered for the HM sub-study at baseline, breastmilk samples were available from 129 (83.8%) and 118 (76.6%) mothers at the subsequent midline and endline follow-up measurements, respectively (**Figure 4.1**). Baseline characteristics of mothers in the HM sub-study (*n* = 154) were similar to those of mothers in the main trial (*n* = 360) except that mothers in the sub-study had slightly younger infants (**Annex 2**). Within the HM sub-study, the CO and FO groups were comparable in most baseline characteristics. Mothers in the CO group had better household wealth status and were slightly taller (**Table 4.1**). At baseline, mothers were on average 25.9 years old (range: 17 - 40 years), had been nursing for 7.66 months (range: 5.75 - 12.1 months), and 26.6% were primiparous. Median (IQR) compliance to daily intake of the maternal capsules (i.e., percent of the actual capsules consumed over the prescribed amount of capsules) in the HM sub-study was 71.0% (IQR: 51.3, 80.1%) with no significant difference between study groups [median (IQR): CO = 67.6% (51.1, 79.6%) vs. FO = 71.7% (54.6, 82.6%); $P_{Wilcoxon\ rank\text{-}sum\ test}$ = 0.45] (see **Table 5.3 in Chapter 5**).

Table 4.1. Baseline maternal characteristics and HM LCP concentrations[1]

Characteristics	CO (*n* = 82)*	FO (*n* = 72)*
Maternal age, year	25.8 ± 5.43	25.9 ± 4.67
Child age, month	7.6 ± 1.1	7.7 ± 1.3
Child sex, female	37 (45.1)	42 (58.3)
Primiparous	20 (24.4)	21 (29.2)
Marital status, married	78 (95.1)	70 (97.2)
Maternal education		
No formal education	36 (43.9)	41 (56.9)
Primary education	34 (41.5)	19 (26.4)
Secondary and above	12 (14.6)	12 (16.7)
Household head education		
No formal education	26 (31.7)	24 (33.3)
Primary education	37 (45.1)	33 (45.8)
Secondary and above	19 (23.2)	15 (20.8)
Household wealth tertiles		
Lowest	20 (24.4)	33 (45.8)
Middle	35 (42.7)	22 (30.6)
Highest	27 (32.9)	17 (23.6)
Breastfeeding frequency		
4-6 times/day	10 (12.2)	6 (8.33)
7-9 times/day	22 (26.8)	16 (22.2)
≥10 times/day	50 (61.0)	50 (69.4)
Maternal anthropometry		
Height, cm	158 ± 5.06	156 ± 5.36
Weight, kg	50.6 ± 6.48	50.5 ± 7.58
Body Mass Index, kg/m^2	20.3 ± 2.33	20.8 ± 2.86
HM LCP		
DHA, mg/L	70.1 (50.1, 94.3)	74.9 (54.4, 116)
EPA, mg/L	24.9 (15.8, 37.3)	24.9 (16.9, 32.8)
AA, mg/L	315 (224, 442)	334 (238, 443)
AA/∑(DHA, EPA)	3.29 (2.83, 3.59)	3.22 (2.71, 3.70)

[1]Data are presented as means ± SDs, n (%), or median (P^{25}, P^{75}). *The control group (CO) included 41 (50.0%) children, and the intervention group (FO) included 40 (55.6%) children who received an n-3 LCP fortified food supplement as part of the child intervention.
AA, arachidonic acid; CO, control mothers who received placebo corn-oil capsules; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, intervention mothers who received fish-oil capsules; HM, human milk; LCP, long-chain PUFA.

At baseline, there was no significant difference in HM LCP concentrations between the study groups ($P > 0.10$) (**Table 4.1**). After 6 and 12 months of supplementation, however, the DHA concentration in HM was 39.0% (95% CI: 20.6, 57.5%; $P < 0.001$) and EPA concentration was 36.2% (95% CI: 16.0, 56.4%; $P < 0.001$) higher in the FO group compared to the CO group, whereas the AA concentration decreased by

17.5% (95% CI: -31.4, -3.74%; *P* = 0.013) and the AA/(DHA + EPA) ratio by 53.5% (95% CI: -70.2, -36.7%; *P* < 0.001) (**Figure 4.2**).

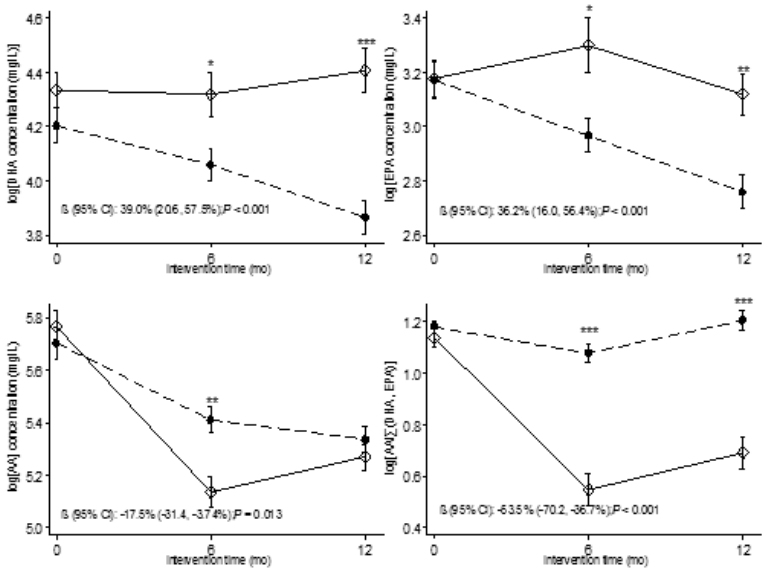

Figure 4.2. HM LCP concentrations at baseline, midline and endline of intervention in the CO (- - ●- -; *n* = 82) and FO (—◇—; *n* = 72) groups.

Values are expressed as means with their standard error indicated by vertical bars. β (95% CI) for group differences in HM LCPs concentration were estimated by fitting a mixed-effects linear model with random intercept mother to account for clustering of the midline and endline measurements. Asterisks (*P < 0.05; **P < 0.01; ***P < 0.001) indicate significant group differences at each measurement-point using a linear regression model. Models were adjusted for baseline LCPs values and additional covariates including household wealth, duration of lactation, breastfeeding frequency, and maternal age, height, parity and occurrence of subsequent pregnancy during follow-up. LCP values were log transformed before analysis and the antilog of coefficients and CIs were used to express group differences as percent of the control value. AA, arachidonic acid; CO, control mothers who received placebo corn-oil capsules; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, intervention mothers who received fish-oil capsules; HM, human milk; LCPs, long-chain PUFAs.

The changes in HM and child capillary blood (DHA + EPA)/AA ratios following FO supplementation were significantly associated. The association was marginally stronger in mother-child pairs where children received no FO in the child intervention (β (SE) = 0.25 (0.03); *P* < 0.001) compared to those who received a FO-fortified food supplement in the child intervention (β (SE) = 0.16 (0.03); *P* < 0.001) (β (SE) for interaction = 0.09 (0.04); *P* = 0.043) (**Figure 4.3**).

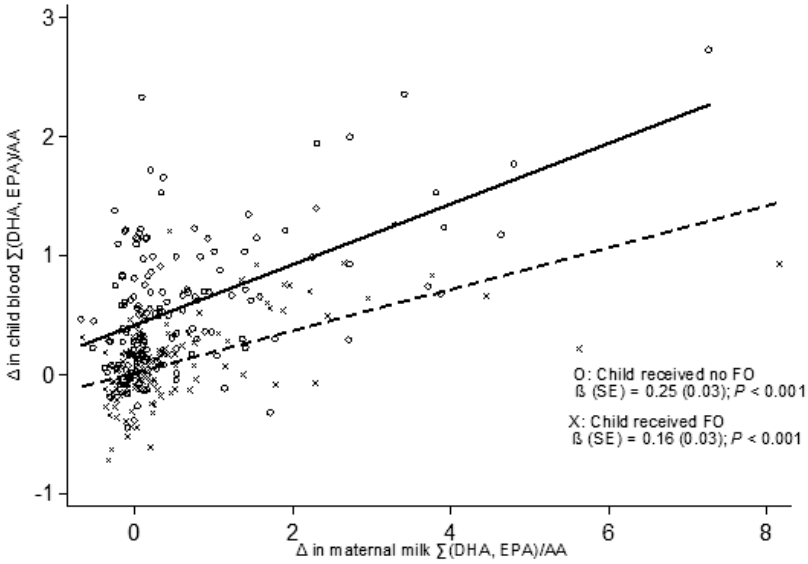

Figure 4.3. Relationship between the change in HM and child capillary blood (DHA + EPA)/AA ratios following the intervention.

Values are expressed as the difference between midline/endline and baseline as a ratio of baseline values. A mixed-effects linear model was fitted to estimate the relationship between HM and child capillary blood ratios, with random intercept mother-child pair to account for clustering of the midline and endline measurements. The model was adjusted for covariates child age and sex, household wealth, breastfeeding frequency, and maternal age, height, parity and occurrence of subsequent pregnancy during follow-up. Data are presented separately for the group of mother-child pairs where children did not receive (o & solid line; n = 72) and received (x & dashed line; n = 81) FO as part of the child intervention. AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish-oil; HM, human milk.

Compared with the global average estimates and other studies (**Table 4.2**), the average DHA concentration in HM of the study mothers was lower both at baseline [median (IQR): 74.0 (50.4, 104)] and after FO supplementation [median (IQR): 89.5 (45.9, 122)]. In contrast, the AA concentration in HM was high at baseline [median (IQR): 328 (230, 442)] and normalized towards the global average estimate after FO supplementation [median (IQR): 189 (134, 248)].

Table 4.2. HM DHA and AA concentrations in study mothers compared to global estimates provided by meta-analysis of other studies[1]

Studies	Age range (months)	DHA		AA	
		Conc. (mg/L)	%TL	Conc. (mg/L)	%TL
Brenna et al. (177) (65 studies from 33 countries)	0.1-18	134*	0.32	197[a]	0.47
Fu et al. (178) (78 studies from 41 countries)	0.5-18	147*	0.35	231[a]	0.55
Yuhas et al. (176) (9 countries)	1-12	156	0.41	151	0.41
Current study					
Before supplementation (FO & CO)	6-12	74.0	na	328	na
After supplementation (FO)	18-24	89.5	na	189	na

[1]Data are average HM concentrations expressed as the concentration in HM as mg/L (conc. (mg/L)) and as percent total lipids as g/100 g HM total lipid (%TL).
*Values are calculated from percent of total milk FAs assuming 4.2% of HM is composed of FAs(397).
AA, arachidonic acid; CO, control mothers who received placebo corn-oil capsules; DHA, docosahexaenoic acid; FA, fatty acid; FO, intervention mothers who received fish-oil capsules; na, not available.

4.5. Discussion

We investigated the effect of n-3 LCPs-rich fish-oil supplementation of mothers during lactation (6-24 months postpartum) on HM LCP concentrations. Fish-oil supplementation resulted in significantly higher HM DHA and EPA concentrations and a lower AA/(DHA + EPA) ratio compared to the placebo group. Although fish-oil supplementation prevented the decline in HM n-3 LCP concentrations over time, which was observed in the placebo group, the daily dose of 500 mg n-3 LCPs (215 mg DHA + 285 mg EPA) did not result in HM DHA concertation comparable to the worldwide norms. A review of previous trials on maternal supplementation using fish-oil (i.e., DHA + EPA) or DHA alone during lactation showed similar results of improved HM n-3 LCPs status (197).

Due to the lack of a clear consensus on the optimal concentrations in HM, current recommendation on DHA intake by lactating mothers is based on observed intake levels in previous studies that were required to enrich HM to the global mean DHA value of 0.32% TL (~ 134 mg/L) (95,173). A dose of 200 mg DHA/d increased DHA in HM from 0.21% TL to the global average when consumed during 4-6 weeks (198) and 5 days-12 weeks postpartum (398). HM DHA concentration (89.5 mg/L) in our study mothers remained below the global average after receiving a similar dose. The relatively smaller increase in our study may relate to different factors. Mainly, the stage of lactation is an important difference between the current study and most previous studies with stronger impact. Compared to this study, supplementation in the other studies usually started no later than a few weeks postpartum and lasted for a shorter period. Decline in HM DHA levels over the course of lactation has been

described previously including in mothers enrolled in supplementation studies (184,186,399–402). Therefore, the relatively lower HM DHA enrichment in our study might be due to maternal depletion concurring with the intervention period; i.e., the relatively later start at 6 to 12 months postpartum as well as the longer follow-up period of up to 24 months postpartum.

Our results also raise the question of whether current intake recommendation for lactating women – which is mainly based on dose-response curves of maternal DHA intake and HM levels derived from short-term studies (398) – may need reconsideration using data from studies covering the whole period of lactation. A higher dose than the recommended intake may be required to achieve optimum HM concentrations in this and similar populations, especially during the period of late lactation. A previous study using two different doses of DHA supplementation also supports this supposition (403). In this study, although dosages of both 200 and 400 mg DHA/d significantly increased HM DHA compared to a placebo, an average HM DHA concentration closer to the global mean was achieved only in the group of mothers who received the higher dose of 400 mg/d (HM DHA in placebo vs. 200 vs. 400 mg DHA/d groups: 58.7 vs. 88.3 vs. 131 mg/L). On the other hand, the safety of a very high n-3 LCPs intake through consumption of sea fish or fish-oil supplements should be considered carefully with respect to a potential dietary exposure of contaminants such as methylmercury and dioxins in the mother and her child during pregnancy and lactation (173).

The extent of HM enrichment following supplementation may also be influenced by factors that can affect PUFA metabolism and their transfer to breastmilk or elsewhere. Competition between the n-3 and n-6 FA families occurs not only in the synthesis of the LCPs from their respective precursors, but also in their incorporation into tissues (67,404). The basal AA concentration in HM of the study mothers was very high compared to the global mean, albeit within the range of values reported in different populations (177,178). The reason for this high value is not clear as intake of AA sources – like meat and eggs – is limited in the study area. Alternatively, milk AA mainly comes from maternal AA stores (405,406), which in turn could have been influenced by long-term high dietary LA intake, possibly from vegetable oils and cereals like maize in the staple foods. Studies showed that a high dietary LA intake can inhibit the incorporation of preformed DHA into milk lipids (67), which might have

potentially attenuated the uptake of supplementary DHA. Additionally, effects of an intervention could be modulated by single nucleotide polymorphisms that are commonly observed in the FADS gene cluster, suggesting the need for consideration of possible differences in the distribution of these gene variants while comparing results from different populations. A study in the Netherlands showed that the FADS genotype modifies the effect of maternal fish and fish-oil intake on HM DHA proportions, possibly due to limited incorporation into breastmilk in women with some gene variants (407). Currently, there is no information available on the distribution of polymorphisms in the FADS gene cluster in the Ethiopian population. However, the very high milk AA in our mothers is not indicative of an important role of genetic polymorphism as these gene variants are generally expected to have more effects on HM levels of the n-6 than the n-3 PUFAs (98).

The significant association observed in the changes in n-3/ n-6 LCPs ratio between the maternal milk and child capillary blood following the intervention shows that the increased maternal dietary n-3 LCP intake improved the status of their breastfed IYC. Other studies also noted positive correlations between HM concentrations and child n-3 LCP status (402,408) as well as improved child status following supplementation of their lactating mothers (409,410).

The low maternal DHA status in this setting corroborates the findings of previous reports on the very low availability of n-3 FA in the Ethiopian diet (174,175). It is also an indication of potential risk of a suboptimal status in their breastfed IYC who live in a setting where the complementary foods have a negligible contribution to n-3 LCPs intake (179). The present study showed the potential of consumption of additional DHA by lactating mothers for improving the content in HM and the status of their breastfed children. Maternal supplementation with fish-oil or other sources of preformed n-3 LCP is one potential intervention approach in Ethiopia considering the good coverage of breastfeeding (190) and the challenges of securing adequate access to marine foods in one of the world's most populous land-locked country.

This study is limited by the absence of maternal dietary intake data which would have aided in the interpretation of the effect of the studied doses of DHA and EPA on HM concentrations and our understanding of the unexpectedly high AA concentration in HM in this population. Furthermore, we were unable to provide the overall fat content

of samples due to laboratory procedures and interference from fatty acids coming from plastic test tubes. On the other hand, our study had a large sample size and relatively low dropout rate compared to similar studies (197).

4.6. Conclusion

We demonstrated that fish-oil supplementation of mothers during lactation increased HM DHA and EPA content and improved the status of their breastfed children. However, HM DHA concentrations of lactating mothers remained lower than international norms even after supplementation. It is recommended that future studies evaluate different doses of n-3 LCP covering a longer period of lactation as well as the impact of potential effect modifiers such as genetic polymorphism and diet.

5

CHAPTER 5

N-3 LCP SUPPLEMENTATION AND CHILD GROWTH AND MORBIDITY

Redrafted from Argaw, A., Wondafrash, M., Bouckaert, KP., Kolsteren, P., Lachat, C., Belachew, T., De Meulenaer, B., Huybregts, L. (2018) Effects of n–3 long-chain PUFA supplementation to lactating mothers and their breastfed children on child growth and morbidity: a 2 × 2 factorial randomized controlled trial in rural Ethiopia. Am J Clin Nutr, 107:454–464.

5.1. Abstract

Background: Recurrent infections and inflammation contribute to growth faltering in low-income countries. n-3 LCPs may improve immune maturation, resistance to infections, and growth in young children who are at risk.

Objective: We evaluated the independent and combined effects of fish-oil (500 mg n–3 LCPs/d) supplementation to lactating mothers and their breastfed children, aged 6–24 months, on child morbidity, systemic inflammation, and growth in southwest Ethiopia.

Design: A 4-arm double-blind randomized controlled trial was conducted by enrolling 360 mother-infant pairs with infants 6–12 months old. Study arms were both the lactating mother and child receiving fish-oil intervention (MCI), only the lactating mother receiving fish-oil intervention and child receiving placebo control (MI), only the child receiving intervention and mother receiving placebo control (CI), and both mother and child receiving a placebo supplement or control (C). The primary study outcome was linear growth using monthly changes in length-for-age *z* score. Anthropometric measurements were taken monthly, and hemoglobin, C-reactive protein, and blood LCPs were measured at baseline and after 6 and 12 months of follow-up. Weekly morbidity surveillance was conducted throughout the study.

Results: Fish-oil supplementation significantly increased blood n-3 LCP concentration ($P < 0.01$) and decreased the AA/(DHA + EPA) ratio ($P < 0.001$) in all intervention arms. No significant intervention effect was found on linear growth, morbidity, or systemic inflammation. Compared to the control group, a small positive effect on monthly changes in weight-for-length *z* scores was found in the CI arm (effect size (95% CI): 0.022/month (0.005, 0.039/month); $P = 0.012$) and the MCI arm (effect size (95% CI): 0.018/month (95% CI: 0.001, 0.034/month); $P = 0.041$).

Conclusions: n-3 LCP supplementation of lactating mothers and children did not affect child linear growth and morbidity in a low-income setting. n-3 LCP supplementation given directly to children modestly increased relative weight gain. This trial was registered at clinicaltrials.gov as NCT01817634.

5.2. Introduction

Stunting occurs in more than a third of children in sub-Saharan Africa and accounts for 21% of disability-adjusted life years in the region (3,7). Recurrent infections affect energy and nutrient intake and expenditure, compromising child growth (411,412). Stunting is associated with environmental enteric dysfunction (EED), a chronic inflammation of the small intestine that impairs absorptive capacity and barrier functions with subsequent immunostimulation (413,414). The high prevalence of EED in children in LICs may explain the lack of large effects from the various nutrition-specific interventions to prevent child stunting (323).

n-3 LCPs are known to modulate immune cell functions and inflammatory processes (349–351,415), which may lead to a more rapid maturation of the immune system, and improved intestinal wall integrity and resistance to infectious diseases in young children. In *vitro* and animal experiments have shown that n-3 LCPs may increase intestinal barrier function (325) and enhance recovery of intestinal tissues after inflammation (326,327). Dietary LCP supplementation of formula-fed infants (353–355), older children (356,357), and infants with prenatal maternal supplementation (358) led to clinical benefits in both inflammatory and infectious conditions. However, there is limited evidence on the impact of n-3 LCP supplementation on breastfed children in LICs characterized by very poor hygienic conditions and a high burden of infectious diseases.

The evidence of the impact of n-3 LCP supplementation on child growth is mainly based on studies in HICs (212,221,416). Only one study, to our knowledge, evaluated the efficacy of fish-oil supplementation to infants in an LIC and found no notable effect on growth and morbidity (291). However, the trial was conducted in infants with a high intake of n-3 LCPs from breastmilk. Yet a trial in Ghana found that a lipid-based nutrient supplement containing ALA, the precursor of n-3 LCPs, had an effect on child linear growth that was attributed to the higher plasma n-3 PUFA in the intervention group (417). Observational studies in the Congo and Brazil also showed that infant growth was positively associated with breastmilk n-3 PUFA concentration (418,419). In settings where the intake of n-3 LCP is low and chronic immunostimulation contributes to poor growth, an increased n-3 LCP intake could improve child growth.

HM is the predominant source of n-3 LCPs for infants in LICs (169,174). However, the breastmilk concentration of n-3 LCPs is influenced by maternal intake of marine foods, which are unavailable to rural populations living far from coastal areas as in Ethiopia (95,174,176,177,404,420). In Ethiopia, the total dietary n-3 PUFA supply is below the recommended amount for IYC, which poses a further risk as children transfer to complementary diets (174). In this study, we hypothesized that increased intake of n–3 LCPs through HM and/or complementary food would result in reduced morbidity and inflammation and improved growth in children aged 6–24 months in southwest Ethiopia.

5.3. Methods

5.3.1. Participants

The trial was carried out from November 2013 to February 2015 in 3 districts of Jimma zone in southwest Ethiopia located ~300 km from the capital, Addis Ababa. The area lies in a midland agroclimatic zone where subsistence farming is the predominant form of livelihood for the population. The main crops produced in the area are maize, hot pepper, and teff (a species of *Eragrostis* native to Ethiopia), and staple foods are very low in n-3 LCPs.

Study participants were identified from the Gilgel Gibe HDSS, which registers all births in the study area, and through an additional census conducted by the study team. Mothers with infants were invited to a nearby school in each of the 3 study districts to be screened for study eligibility. Mothers with a singleton infant of age 6–12 months were eligible if the infant was not wasted (weight-for-length *z* score (WLZ) ≥ −2 SD and presented no bilateral pitting edema), was being breastfed, and the mother declared having no intention of leaving the study area for >1 month over the coming year. Exclusion criteria included infants or mothers with known chronic illness or medical treatment for this purpose, infants with a congenital abnormality that could affect feeding or growth, or with severe anemia (hemoglobin <7.0 g/dL), and children or mothers taking a nutritional supplement other than the ones provided by the project at enrollment or during the follow-up period.

5.3.2. Study design

This study was a randomized, double-blind, placebo controlled trial involving child n-3 LCP supplementation through lactation (maternal intervention) and/or through

supplementary feeding (child intervention). Mothers were randomly allocated to receive either intervention capsules containing fish-oil or placebo capsules of identical appearance containing corn oil. Children of the same mothers were randomly allocated to receive either an intervention food supplement containing fish-oil or the same food supplement without fish-oil as control. Thus, there were 4 study arms: lactating mother and child both receiving fish-oil intervention (MCI); only the lactating mother receiving fish-oil intervention and child receiving placebo control (MI); only the child receiving fish-oil intervention and mother receiving placebo control (CI); and both mother and child receiving a placebo supplement or control (C).

A statistician who was not part of the research team generated a randomization scheme for the allocation of the maternal and child interventions. Each unique randomization number was randomly allocated to 1 of 6 letter codes for the maternal intervention (A, E, I, J, L, T)- 3 letters each for the intervention and the control group- using a computer program in permuted blocks. The randomization procedure was repeated for the child intervention by randomly allocating each randomization number to 1 of 6 letter codes representing the child intervention (B, K, P, V, Y, Z). The maternal capsules and the child food supplements were prepared in packages containing monthly supplies, and labeled with one of their corresponding letter codes by a person not involved in the study. Information regarding the assignment of the letter codes to the intervention and control supplements was sealed in an opaque envelope and sent to the study director (PK), who locked the envelope away until analysis was completed. Before the study started, 2 researchers (AA, MW) sealed each of the randomization numbers with the corresponding letter codes of the maternal and child interventions into separate opaque envelopes that were marked with unique participant numbers to be attributed at study inclusion. At study inclusion, a researcher opened the next sealed envelope in the presence of an eligible mother-child pair (in order of arrival), provided them with the first monthly dose of the maternal and child supplements corresponding to the letter codes, and filled out the study participation card.

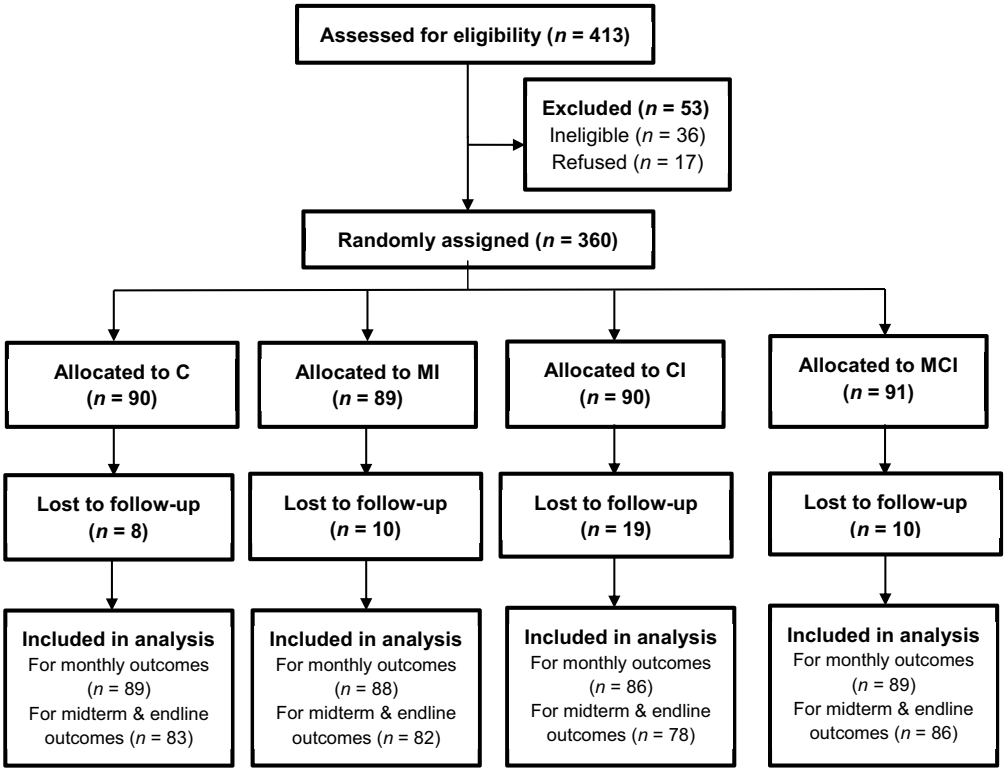

Figure 5.1. Trial flowchart

C, both mother & child received placebo; CI, child received fish-oil & mother received placebo; MCI, both mother & child received fish-oil; MI, mother received fish-oil & child received placebo.

5.3.3. Interventions

The maternal intervention consisted of identical airtight gel capsules containing either fish-oil (intervention) or corn oil (placebo). Both the intervention and placebo maternal supplements were provided by Biover NV. A daily dose of the intervention capsules contained 500 mg n-3 LCPs [215 mg DHA + 285 mg EPA]. Each capsule additionally contained 5 mg of d-α-tocopherol, an antioxidant. Supplements were packaged in small nontransparent polystyrene containers containing a monthly supply of 60 capsules.

The child food supplements were produced by Michiels Fabrieken NV and consisted of an extruded corn-soy blend fortified with 19 micronutrients. Both the intervention and control food supplements were designed to provide energy and fat equivalent to half of the estimated average requirement and micronutrients at a dose of the recommended nutrient intake from complementary foods for an 11-months-old infant (**Table 5.1**). Iron was added at a lower dose of 12.5 mg/d. The intervention food supplement was additionally enriched with microencapsulated fish-oil, providing a daily dose of 500 mg n-3 LCPs (169 mg DHA + 331 mg EPA). The microencapsulation of the fish-oil reduced the fishy smell and taste, and minimized oxidation of n-3 LCPs. Monthly supplies of the food supplements were packaged using nontransparent bags with a protective aluminum coat and with nitrogen injected into the internal atmosphere to prevent oxidation.

The dosage of n-3 LCP in the intervention supplements was designed to achieve a substantial increase in blood n-3 LCP (291,421) and took into consideration the factorial design where we preferred children not to be exposed to doses >1 g n-3 LCP. The provision of multimicronutrient-fortified food supplements for children in both the control and intervention groups was considered to avoid any hampering effect of energy and micronutrients deficiencies on the impact of n-3 LCP supplementation.

Study supplements were transported in a cold chain (6°C) and stored in a cold room (<16°C) at Jimma University until distribution. Supplementation was provided for a total duration of 12 months through monthly distribution schedules. Mothers were instructed to take a daily dose of 2 capsules and provide their child a daily dose of 2 spoons (±40 g) of the food supplement mixed with preboiled water. They were advised to keep the supplement packages closed in a cool and dark place and avoid direct cooking of the food supplements.

Weekly home-visits were conducted by trained project community workers to provide counseling on breastfeeding and preparation of supplementary foods, and monitor compliance with supplementation. Compliance with the maternal intervention was measured by counting the remaining capsules in the supplement container and asking mothers about their frequency of breastfeeding during the day prior to the home-visit. Child compliance was assessed by collecting weekly data on the amount

of supplementary food remaining in the food bag, i.e., full, three-quarters full, one-half full, or empty. Leftover supplements from the previous month were collected during the monthly distributions of new supplies.

Table 5.1. Nutritional composition of daily rations (40 g) of food supplements

Nutrients	Intervention supplement	Control supplement
Energy (kcal)	165	160
Energy (kJ)	693	671
Protein (g)	5.9	5.1
Carbohydrates (g)	24.4	26.1
Fat (g)	4.7	3.6
DHA (mg)	169	0.0
EPA (mg)	331	0.0
Calcium (mg)	421.8	412.4
Selenium (µg)	12.3	12.4
Magnesium (mg)	82.6	79.6
Zinc (mg)	8.8	8.4
Iron (mg)	12.7	12.3
Iodine (µg)	94.4	94.3
Vitamin C (mg)	30.7	29.8
Thiamine (mg)	5.7	6.1
Riboflavin (mg)	0.4	0.5
Niacin (mg NE)	5.5	5.0
Vitamin B6 (mg)	0.4	0.3
Pantothenic acid (mg)	1.9	1.8
Biotin (µg)	6.7	6.3
Vitamin B12 (µg)	0.7	0.7
Folate (µg DFE)	109.5	112.4
Vitamin A (µg RE)	447.0	458.2
Vitamin D (IU)	208.5	216.9
Vitamin E (mg α-TE)	4.2	4.5
Vitamin K (µg)	9.1	9.9

[1]Intervention supplement, corn-soy blend fortified with 19 micronutrients and fish-oil; control supplement, corn-soy blend fortified with multiple micronutrients and without fish-oil.
DFE, dietary folate equivalents; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NE, niacin equivalents; RE, retinol equivalents; TE, tocopherol equivalents.

Children in all study arms received deworming medication (500 mg mebendazole) at the age of 12 and 18 months. Children and mothers with any illness were referred to the nearby health post with a referral slip for the local health extension workers, whereas the presence of any danger sign or suspected potential adverse event was immediately reported to the researchers.

5.3.4. Ethical consideration

The study protocol was approved by the Ethics Committee of Ghent University Hospital in Belgium (registration number B670201214299), the Institutional Review Board of Jimma University in Ethiopia, and the National Health Research Ethics Committee of Ethiopia. The use of study supplements was approved by the Food, Medicine and Health Care Administration and Control Authority of Ethiopia. At the start of the trial, written consent was obtained from eligible mothers after an individual information session detailing the study, voluntary participation and study withdrawal in the presence of a community member. The trial was registered at clinicaltrials.gov as NCT01817634.

5.3.5. Outcomes

The primary study outcome was linear growth as measured by the monthly change in length-for-age *z* score (LAZ) over the 12-months intervention follow-up. Secondary outcome measures included monthly changes in WLZ, midupper arm circumference (MUAC), and head circumference (HC); the longitudinal prevalence of diarrhea, vomiting, fever, acute respiratory illnesses, and any illness that required medical attention during the intervention period; the longitudinal development of stunting (LAZ <–2), wasting (WLZ <–2), anemia, and systemic inflammation, and blood LCPs concentrations at midterm (after 6 months of intervention) and at endline (after 12 months of intervention).

5.3.6. Field measurements

Child anthropometry was recorded monthly at the 3 study sites by teams of trained data collectors using calibrated equipment and standardized techniques (422). All measurements were done in duplicate by 2 different teams and written down on separate forms so that the first measurement could not influence the second. A study data-quality manager then compared the duplicate measurements and both teams repeated the measurement when there was a difference of ≥0.5 kg for weight, ≥1.0 cm for length, and ≥0.5 cm for MUAC and HC measurements. Length was measured in the recumbent position to the nearest 1 mm using a portable foldable length board (SECA 417). Weight measurements were taken together with the mother using an electronic scale (SECA 876) to the nearest 50 g. Flexible, non-stretchable measuring tapes were used to measure HC (SECA 212) and MUAC (MUAC S0145610,

UNICEF) to the nearest 1 mm. Child age was estimated using date of birth data from the Gilgel Gibe HDSS, which typically records the dates of birth during the neonatal phase. For study sites outside the catchment of the HDSS (16% of the participants), date of birth was either copied from birth certificate or recalled using a local events calendar.

Hemoglobin and C-reactive protein (CRP) concentrations were measured on site from a single finger prick blood sample by using a QuickRead go instrument (Orion Diagnostica) that was calibrated using control kits at the start of every measurement day. Anemia was defined as having a hemoglobin concentration <11.0 g/dL and the presence of systemic inflammation by a CRP concentration ≥5 mg/L (423).

Structured questionnaires and observation forms were used to collect morbidity data through weekly home-visits. Trained project community workers interviewed mothers to estimate the number of days since the last visit, typically during the previous 7 d, that the child experienced diarrhea, cough, and vomiting. Diarrhea was defined as the occurrence of ≥3 liquid or semisolid stools within a day. The occurrence of an illness that required medical attention (hospital or health center visits) was also recalled as a proxy for serious illness. Children were assessed for runny nose, fever, tachypnea, and difficulty of breathing (grunting or chest indrawing) during each home visit. Fever was defined as having an armpit body temperature of ≥37.5°C; tachypnea as having a respiratory rate of ≥50 breaths/min for infants and ≥40 breaths/min for older children (>12 months). Pneumonia was diagnosed by the concurrent presence of cough and tachypnea. Household wealth and hygiene status were assessed by a structured questionnaire and observation checklist adapted from the Ethiopian DHS wealth index items (190) and a simple hygiene index tool (424).

Community workers, all female high-school graduates from the local communities, were recruited to conduct the weekly follow-up home visits for compliance assessment and morbidity surveillance. They received intensive training at the start of the study and refresher trainings during the monthly distribution schedules. The research team supervised community workers through unannounced monthly home visits applying the Lot Quality Assurance Sampling approach (425). Every mother-child dyad had a study participation card that community workers would sign off after a home visit. During the supervision home visits, the number of capsules and the

amount of supplementary food would be counted and estimated and compared to the weekly report of the community worker. The morbidity record of the community workers was also compared against morbidity data collected by study nurses at the monthly distribution schedules.

5.3.7. Child LCPs status

The procedures for child whole blood samples collection using dried blood spot cards and determination of LCPs concentration by gas chromatography are described in **Chapter 4**.

5.3.8. Statistical analyses

A sample size of 360 subjects (90/study arm) was required to detect an effect size of 0.027 on average monthly LAZ changes over the 12 months of follow-up with a statistical power of 80%, a type I error of 5%, an anticipated attrition of 20%, and assuming a $\rho = 0.50$ correlation among the repeated monthly measurements (426). For the blood LCP analysis, a subsample size of 168 subjects (42 for each study arm) enables us to detect an effect size of ≥0.58 on blood LCP concentrations with a statistical power of 80%, a type I error of 5%, an anticipated attrition of 20%, and assuming a $\rho = 0.50$ correlation between the midterm and endline measurements.

Continuous double data entry was performed using EpiData version 1.4.4.4 (EpiData Association). Consistency checks and data analyses were conducted using Stata version 13.1 (StataCorp LLC, Texas, USA). The average value of the duplicate anthropometry measurements was used for analysis, and LAZ and WLZ scores were calculated based on the WHO 2006 Child Growth Standards (427).

Growth curves were fitted for the difference from baseline of the monthly anthropometric measurements using a linear mixed-effects model that included child as random intercept and intervention exposure time as random slope. Fixed effects included intervention group, intervention time, quadratic time, and the interaction between intervention group and time; these compare each intervention arm with the control arm on monthly linear growth over time. For the outcomes assessed at baseline, midterm, and endline, we fitted a mixed-effects linear probability model with child level as a random intercept, whereas fixed effects included intervention group, intervention time, and the interactions between intervention group and time to

compare study arms by the development of an outcome over the 3 measurement points. For LCP measurements, a mixed-effects linear model with random intercept child was used to estimate the main effect of intervention group on the midterm and endline measurements adjusted for the baseline measurement. The main effect of time and time by group interactions were not considered in this model because blood concentrations reach a level of saturation after only few weeks of supplementation. Percentage change from the baseline measurement was also calculated for LCPs measurements. We applied log transformations to normalize the heavily skewed LCP distributions before analysis. We preferred the use mixed-effects model because it allows the use of observations with different length of time between follow-ups; can take into account subjects with incomplete data where measurements were missed for some follow-up points; and its more robust assumption regarding missing data where missing observations are assumed to be missing at random as compared to other approaches for analysis of longitudinal data such as multivariate analysis of variance and generalized estimating equation .

Longitudinal prevalence of morbidity outcomes during follow-up were calculated using as numerator the total number of days that the outcome was positive, and as denominator the total days reported or assessed (428). We used a negative binomial regression model to compare the impact of the interventions between study arms, adjusting for the total days reported or assessed. Compliance and breastfeeding frequency during the study follow-up were compared among study arms using Fisher's exact test for the categorical variables and the Kruskal-Wallis test for the continuous variables.

In the presence of a statistically significant overall group difference, pairwise comparisons of each intervention arm against the control arm were conducted, adjusting for false discovery rate using the Benjamini-Hochberg method (429). Analyses were done based on the intention-to-treat principle, using all children initially enrolled into the study. For this purpose, a multiple chained equations data imputation was conducted under the "missing at random" assumption. Fifty imputations of missing data for cases lost to follow-up were run to estimate the regression coefficients. The use of the intention-to-treat principle is one of the most accepted analysis strategy for randomized controlled trials recommended by the CONSORT statement for clinical trials.. Analysis of all individuals initially randomized

allows maintenance of comparability between study groups obtained through the randomization, maintains sample size and prevents potential bias due to study withdrawal that might relate to the intervention or study outcomes. Compared to the per-protocol analysis (i.e. restricting analysis to subjects who complied with the intervention), the intention-to-treat analysis can be more conservative and may underestimate the potential effect of an intervention because of the inclusion of subjects who did not adhere to the intervention. However, the results obtained from intention-to-treat analysis can be considered as a representative of what would have been found from an intervention in a practical setting. The use of multiple imputation approach to the problem of missing data is a proven method of imputation taking into account the uncertainty of the estimates by creating several plausible imputed datasets and appropriately combining results obtained from each dataset.

All models were adjusted for sex of the child due to the imbalance among study arms at baseline. We also assessed for interactions between child sex and intervention group on the study outcomes. All statistical tests were 2-sided with statistical significance set at $\alpha < 5\%$.

Table 5.2. Baseline characteristics of study participants[1]

Characteristics	C (*n* = 90)	MI (*n* = 89)	CI (*n* = 90)	MCI (*n* = 91)
Sex, Females	42 (46.7)	49 (55.1)	36 (40.0)	54 (59.3)
Age (months)	8.89 ± 2.16	9.18 ± 2.09	8.93 ± 2.10	8.68 ± 2.00
Maternal age (yrs)	26.0 ± 5.04	25.8 ± 4.82	26.1 ± 5.48	26.3 ± 5.28
Maternal education				
No formal education	42 (47.2)	44 (50.6)	45 (54.2)	47 (53.4)
Primary education	36 (40.5)	29 (33.3)	25 (30.1)	31 (35.2)
Secondary and above	11 (12.4)	14 (16.1)	13 (15.7)	10 (11.4)
Household wealth tertiles				
Lowest	26 (29.2)	33 (37.9)	27 (32.5)	30 (34.1)
Middle	30 (33.7)	25 (28.7)	30 (36.1)	31 (35.2)
Highest	33 (37.1)	29 (33.3)	26 (31.3)	27 (30.7)
Hygiene score tertiles				
Lowest	30 (34.1)	26 (30.2)	29 (34.5)	30 (34.5)
Middle	31 (35.2)	29 (33.7)	30 (35.7)	25 (28.7)
Highest	27 (30.7)	31 (36.1)	25 (29.8)	32 (36.8)
Maternal height (cm)	157 ± 4.67	157 ± 5.55	157 ± 5.95	157 ± 5.74
Maternal BMI (kg/m^2)	20.2 ± 2.41	20.3 ± 2.50	20.1 ± 2.57	21.0 ± 3.31
Breastfeeding during previous day				
4-6 times/day	10 (11.1)	6 (6.74)	11 (12.2)	8 (8.89)
7-9 times/day	21 (23.3)	28 (31.5)	26 (28.9)	23 (25.6)
> 10 times/day	59 (65.6)	55 (61.8)	53 (58.9)	59 (65.6)
Anthropometric indices				
Length-for-age z-score	-0.99 ± 1.07	-1.03 ± 1.10	-1.08 ± 1.15	-0.97 ± 1.11
Weight-for-length z-score	0.26 ± 0.94	0.18 ± 1.04	0.06 ± 0.99	0.09 ± 0.93
Head circumference (cm)	44.1 ± 1.79	43.9 ± 1.68	44.0 ± 1.70	43.7 ± 1.71
Mid-upper arm circumference (cm)	14.1 ± 1.06	14.0 ± 1.04	13.9 ± 1.15	13.9 ± 1.07

[1]Values are expressed as n (%) or mean ± SD.
BMI, body mass index; C, both mother and child received placebo; CI, child received fish-oil and mother received placebo; MCI, both mother and child received fish-oil; MI, mother received fish-oil and child received placebo.

5.4. Results

From a total of 413 mother-infant pairs screened, 360 were randomly assigned into the C (*n* = 90), MI (*n* = 89), CI (*n* = 90), and MCI (*n* = 91) intervention arms (**Figure 5.1**). Three-hundred thirteen (87%) participants completed all 12 months of intervention follow-up (C: 91%, MI: 89%, CI: 79%, MCI: 89%). Analyses were based on all children with ≥1 follow-up measurement for an outcome, which included 352 (98%) of children initially randomly assigned into the study for the monthly anthropometry outcomes and 329 (91%) children for the outcomes assessed at midterm and endline. Study arms were comparable on baseline characteristics of participants except that there was an imbalance in child sex (**Table 5.2**).

Table 5.3. Compliance to maternal and child interventions by study arms

Variable	C	MI	CI	MCI	*P*[1]
Breastfeeding frequency, n(%)					
1-3 times/day	0 (0.00)	2 (2.25)	0 (0.00)	5 (5.49)	
4-6 times/day	12 (13.3)	13 (14.6)	17 (18.9)	8 (8.79)	0.084
7-9 times/day	61 (67.8)	50 (56.2)	54 (60.0)	57 (62.6)	
≥ 10 times/day	17 (18.9)	24 (27.0)	19 (21.1)	21 (23.1)	
Capsules compliance[2], n(%)					
0-25	6 (6.67)	9 (10.1)	13 (14.4)	8 (8.79)	
26-50	9 (10.0)	10 (11.2)	14 (15.6)	15 (16.5)	0.440
51-75	41 (45.6)	40 (44.9)	29 (32.2)	31 (34.1)	
76-100	34 (37.8)	30 (33.7)	34 (37.8)	37 (40.7)	
Median (IQR)	70.5 (60.6, 79.6)	65.3 (54.7, 81.0)	67.4 (41.8, 81.0)	72.8 (51.4, 82.6)	0.340
Food supplement compliance[2], n(%)					
0-25	1 (1.11)	3 (3.37)	5 (5.56)	2 (2.20)	
26-50	8 (8.89)	9 (10.1)	9 (10.0)	12 (13.2)	0.700
51-75	22 (24.4)	24 (27.0)	25 (27.8)	29 (31.9)	
76-100	59 (65.6)	53 (59.6)	51 (56.7)	48 (52.8)	
Median (IQR)	84.1 (67.4, 91.8)	81.0 (62.3, 92.4)	79.0 (62.3, 89.9)	77.4 (59.6, 91.8)	0.450

[1]*P*-values for group difference were obtained from Fisher's exact test for the categorical variables and Kruskal-Wallis test for the continuous variables.
[2]Compliance is defined as the ratio of the total number of capsules or estimated amount of food supplement actually consumed over the total number of capsules or amount of food supplement prescribed.

5.4.1. Compliance and effects on LCPs status

Overall mean ± SD supplementation duration was 11.0 ± 2.88 months, with 11.2 ± 2.68 months in C, 11.2 ± 2.65 months in MI, 10.4 ± 3.56 months in CI, and 11.3 ± 2.45 months in MCI intervention arms. Compliance, i.e., the ratio of actual supplement consumption over prescribed supplement consumption, was similar among study arms for both the child and the maternal supplements (**Table 5.3**). Median (IQR) compliance was 79.7% (IQR: 62.6%, 91.4%) for the child food supplements and 69.9% (IQR: 52.2%, 80.4%) for the maternal capsules. The average frequency of breastfeeding during the study follow-up was ≥7 times/d in 84.2% of the study participants with no significant difference among intervention arms.

Table 5.4. Blood LCPs concentration (mg/L) at midterm and endline by study arms[1]

Outcomes	Midterm	Endline	*P*[1]	Effect Size (95% CI)[1]	*P*[1]	% change[2]
	Mean ± SD	Mean ± SD				
DHA						
C (*n* = 46)	51.0 ± 18.2	42.7 ± 16.9		Reference group		1.73
MI (*n* = 35)	58.8 ± 23.1	62.6 ± 23.5		13.4 (5.35, 21.4)	0.001*	16.3
CI (*n* = 41)	62.57 ± 24.4	53.3 ± 22.4	0.001	10.5 (2.51, 18.5)	0.010*	19.9
MCI (*n* = 46)	63.5 ± 23.1	61.0 ± 18.6		14.2 (6.63, 21.7)	<0.001	14.9
EPA[3]						
C (*n* = 46)	5.44 (4.25, 8.67)	5.23 (3.82, 7.89)		Reference group		35.1
MI (*n* = 35)	8.03 (5.67, 10.5)	7.13 (5.70, 11.3)		1.88 (0.96, 3.68)	0.064	58.9
CI (*n* = 41)	10.0 (6.68, 18.7)	9.78 (7.08, 12.3)	<0.001	2.97 (1.55, 5.67)	0.001*	116
MCI (*n* = 46)	13.3 (7.55, 22.9)	10.9 (6.70, 16.0)		4.58 (2.44, 8.57)	<0.001	129
N-3 LCP (DHA + EPA)						
C (*n* = 46)	58.1 ± 20.7	49.1 ± 19.1		Reference group		11.7
MI (*n* = 35)	67.85 ± 26.7	71.3 ± 27.0		15.1 (5.32, 25.0)	0.003*	21.5
CI (*n* = 41)	76.5 ± 32.4	63.9 ± 27.0	<0.001	15.9 (6.17, 25.6)	0.001*	26.6
MCI (*n* = 46)	80.1 ± 30.9	74.6 ± 25.3		21.5 (12.4, 30.7)	<0.001	30.7
AA						
C (*n* = 46)	233 ± 72.9	211 ± 62.7		Reference group		-3.16
MI (*n* = 35)	215 ± 63.6	237 ± 84.6		1.19 (-20.2, 22.6)	0.913	-9.35
CI (*n* = 41)	216 ± 62.0	191 ± 79.5	0.022	-22.9 (-43.4, -2.31)	0.029*	-13.3
MCI (*n* = 46)	200 ± 58.8	210 ± 55.7		-23.3 (-43.6, -3.01)	0.024*	-16.9
AA/(DHA+ EPA)						
C (*n* = 46)	4.19 ± 0.86	4.42 ± 1.12		Reference group		1.29
MI (*n* = 35)	3.36 ± 0.83	3.42 ± 0.84		-0.92 (-1.30, -0.54)	<0.001	-21.7
CI (*n* = 41)	3.08 ± 0.84	3.26 ± 0.97	<0.001	-1.17 (-1.54, -0.80)	<0.001	-29.1
MCI (*n* = 46)	2.71 ± 1.10	3.04 ± 0.91		-1.45 (-1.81, -1.09)	<0.001	-34.6

[1]Mixed-effects linear model with child as random intercept and fixed effects child sex, baseline measurement, and intervention group.
[2]Percent change (median) from baseline value to midterm/endline values.
[3]Values are expressed as median (IQR) and because analysis was based on log transformed values, effect sizes (95% CI) are expressed as the antilog of coefficients indicating the ratio of the geometric means in an intervention arm over the control arm.
*Significant after correction for multiple testing using the Benjamini-Hochberg method.
AA, arachidonic acid; C, both mother and child received placebo; CI, child received fish-oil and mother received placebo; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LCP, long-chained polyunsaturated fatty acids; MCI, both mother and child received fish-oil; MI, mother received fish-oil and child received placebo.

In the subsample with LCP analysis, compared to the control arm, fish-oil supplementation resulted in significantly higher blood DHA concentration in all the MI (P = 0.001; increase from baseline = 16.3%), CI (P = 0.010; increase from baseline = 19.9%), and MCI (P < 0.001; increase from baseline = 14.9%) arms (**Table 5.4**). Blood EPA concentration was also significantly higher in the CI (P = 0.001; increase from baseline = 116%) and the MCI arms (P < 0.001; increase from baseline =

129%). Blood AA significantly decreased in the CI arm (P = 0.029; decreases from baseline = 13.3%) and the MCI arm (P = 0.024; decreases from baseline = 16.9%) compared to the control arm. The ratio of AA/(DHA + EPA) also showed significant decreases in all the intervention arms ($P \leq 0.001$; decreases from baseline = 21.7–34.6%).

5.4.2. Effects on growth and nutritional status

We found no significant effect in any of the intervention arms on the primary study outcome linear growth (LAZ) (**Figure 5.2**). However, a statistically significant group difference was detected on ponderal growth expressed as WLZ (P = 0.021). compared to the control arm, a small positive effect on monthly WLZ changes were detected in the CI arm (effect size (95% CI): 0.022 *z* scores/month (0.004, 0.039 *z* scores/month); P = 0.012) and the MCI arm (effect size (95% CI): 0.018 *z* scores/month (0.001, 0.034 *z* scores/month); P = 0.041); whereas no significant difference was found in the MI arm. We also found a trend towards larger monthly MUAC increases in the CI and the MCI arms compared to the control arm, though the differences were not statistically significant. No significant effect of any of the interventions was found on the nutritional status of children using stunting, wasting, and anemia (**Table 5.5**).

5.4.3. Effects on morbidity and inflammation

The occurrence of systemic inflammation, as determined by CRP concentration (**Table 5.5**), and common childhood illnesses (**Table 5.6**) were not statistically different between study arms. We did not find any adverse effect related to the study supplements in all participating mothers and children.

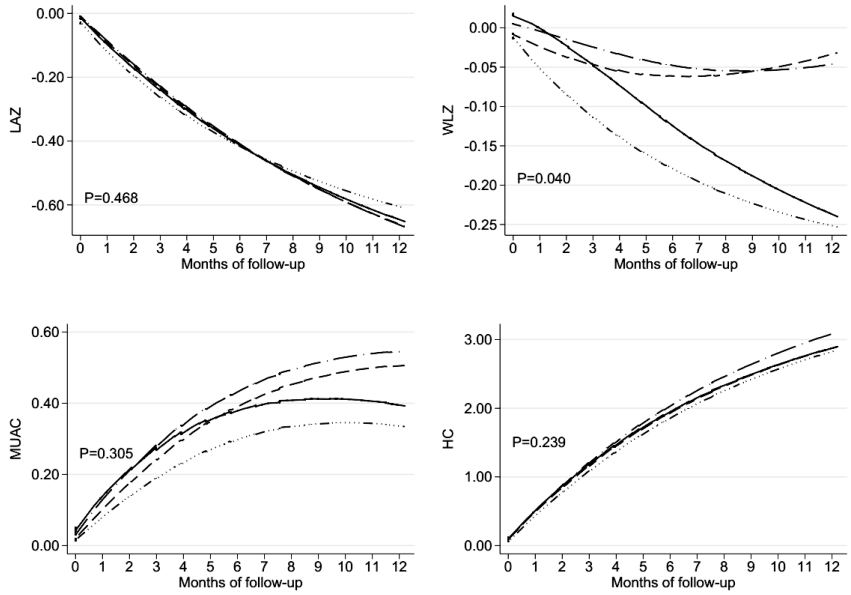

Figure 5.2. Monthly anthropometric growth over 12 months in the C (——), MI (·····-·····-·····-), CI (– – –) and MCI (— · — · —) arms.

Plotted values for the difference from baseline of the monthly measurements are estimated from a linear mixed-effects model with random intercept child and random slope intervention time, with fixed effects that include time, quadratic time, intervention group, and time × group interactions adjusted for the sex of the child. P values are for group difference on monthly changes of the outcome over time. Effect sizes (95% CI) on monthly changes in WLZ were 0.005 (-0.012, 0.022) *z* scores/month; *P* = 0.538 in the MI, 0.022 (0.005, 0.039) *z* scores/month; *P* = 0.012 in the CI, and 0.018 (0.001, 0.034) *z* scores/month; *P* = 0.041 in the MCI.

C, both mother and child received placebo; CI, child received fish-oil and mother received placebo; HC, head circumference; LAZ, length-for-age *z* score; MCI, both mother and child received fish-oil; MI, mother received fish-oil and child received placebo; MUAC, midupper arm circumference; WLZ, weight-for-length *z* score.

Table 5.5. Nutritional status and systemic inflammation by study arms

	Baseline				Midterm				Endline				*P*[1]
Outcomes	**C (*n* = 90)**	**MI (*n* = 89)**	**CI (*n* = 90)**	**MCI (*n* = 91)**	**C (*n* = 90)**	**MI (*n* = 89)**	**CI (*n* = 90)**	**MCI (*n* = 91)**	**C (*n* = 90)**	**MI (*n* = 89)**	**CI (*n* = 90)**	**MCI (*n* = 91)**	
Stunting, *n*(%)	15 (16.7)	11 (12.4)	23 (25.6)	21 (23.1)	20 (22.2)	26 (29.2)	26 (28.9)	27 (29.7)	28 (31.1)	29 (32.6)	38 (42.2)	30 (33.0)	0.243
Wasting, *n*(%)	6 (6.67)	6 (6.74)	11 (12.2)	7 (7.69)	7 (7.78)	2 (2.25)	9 (10.0)	6 (6.59)	7 (7.78)	6 (6.74)	9 (10.0)	6 (6.59)	0.465
Anemia[2], *n*(%)	49 (54.4)	39 (43.8)	55 (61.1)	47 (51.7)	24 (26.7)	25 (28.1)	36 (40.0)	29 (31.9)	15 (16.7)	17 (19.1)	18 (20.0)	13 (14.3)	0.170
Inflammation[2], *n*(%)	22 (24.4)	22 (24.7)	21 (23.3)	22 (24.2)	22 (24.4)	25 (28.1)	29 (32.2)	33 (36.3)	16 (17.8)	24 (27.0)	21 (23.3)	15 (16.5)	0.937

[1]Mixed-effects linear probability model with random intercept child and fixed effects including child sex, intervention group, intervention time, and groupXtime interaction; *P*-values are for groupXtime interaction indicating groups' difference on development of the outcome over time.
[2]Anemia, hemoglobin<11.0 g/dL; inflammation, CRP>5 mg/L.
C, both mother & child received placebo; MI, mother received fish-oil capsules & child received placebo; CI, child received fish-oil fortified supplementary food & mother received placebo; MCI, mother received fish-oil capsules and child received fish-oil fortified supplementary food.

Table 5.6. Morbidity outcomes during the intervention follow-up by study arms[1]

Outcomes	C	MI	CI	MCI	*P*
Diarrhea					
Child wks with event/total child wks followed	264/4511	238/4414	223/4526	202/4501	
Longitudinal prevalence (95% CI)	5.85 (5.59, 6.13)	5.38 (5.13, 5.65)	4.93 (4.69, 5.18)	4.48 (4.25, 4.72)	0.214
Cough					
Child wks with event/total child wks followed	275/4511	259/4414	245/4526	253/4501	
Longitudinal prevalence (95% CI)	6.09 (5.82, 6.36)	5.88 (5.61, 6.15)	5.42 (5.16, 5.68)	5.61 (5.36, 5.88)	0.864
Vomiting					
Child wks with event/total child wks followed	82/4511	78/4414	74/4526	75/4501	
Longitudinal prevalence (95% CI)	1.82 (1.68, 1.98)	1.77 (1.62, 1.92)	1.64 (1.50, 1.79)	1.66 (1.52, 1.81)	0.775
Runny nose					
No. of visits with diagnosis/total no. of visits	1566/4520	1600/4417	1596/4532	1605/4514	
Longitudinal prevalence (95% CI)	34.6 (33.0, 36.4)	36.2 (34.5, 38.0)	35.2 (33.5, 37.0)	35.6 (33.8, 37.3)	0.983
Tachypnea					
No. of visits with diagnosis/total no. of visits	220/4520	176/4417	210/4532	215/4514	
Longitudinal prevalence (95% CI)	4.87 (4.25, 5.55)	3.98 (3.42, 4.62)	4.61 (4.01, 5.28)	4.76 (4.15, 5.44)	0.854
Fever					
No. of visits with diagnosis/total no. of visits	12/4520	13/4417	10/4532	13/4514	
Longitudinal prevalence (95% CI)	0.27 (0.14, 0.46)	0.29 (0.16, 0.50)	0.22 (0.11, 0.41)	0.29 (0.15, 0.49)	0.975
Pneumonia					
No. of visits with diagnosis/total no. of visits	31/4520	18/4417	20/4532	19/4514	
Longitudinal prevalence (95% CI)	0.69 (0.47, 0.97)	0.41 (0.24, 0.64)	0.44 (0.27, 0.68)	0.42 (0.25, 0.66)	0.639
Illness requiring medical attention					
Child wks with event/total child wks followed	47/4511	42/4414	49/4526	47/4501	
Longitudinal prevalence (95% CI)	1.05 (0.94, 1.17)	0.95 (0.85, 1.07)	1.08 (0.97, 1.20)	1.05 (0.94, 1.17)	0.764

[1]Longitudinal prevalence was calculated using as numerator the total number of days that the child presented a positive outcome and as denominator the total number of days observed or assessed. *P*-values for group difference from a negative binomial regression model comparing intervention groups by number of days with morbidity adjusted for child sex and log number of days observed or assessed.

5.5. Discussion

In this study, fish-oil supplementation increased blood n-3 LCPUFA concentrations and lowered the AA:(DHA + EPA) ratio, indicating tissue uptake of the study supplements. We found no impact of the supplementation on child linear growth, systemic inflammation, or morbidity except that a small but statistically significant positive effect on monthly relative weight gain and a trend towards larger MUAC increments were detected in the CI and MCI arms.

The lack of impact on child linear growth is consistent with previous studies in HICs that evaluated n-3 LCP supplementation of lactating mothers or infants (212,221,416). We expected a better impact of supplementation on growth and morbidity in our setting due to the potential for n-3 LCP to modulate the child's immune response to infectious challenges and inflammation. Nonetheless, we were unable to detect an effect on reported morbidity or systemic inflammation using CRP. This finding is in agreement with the Gambian study where fish-oil supplementation to breastfed infants had no effect on growth, morbidity, inflammation, or gut integrity (291). The addition of fish-oil to multimicronutrient supplementation starting from a mean age of 24 months also did not improve EED or common illnesses in Malawian children (348). In contrast, in 2 RCTs in the United States, the supplementation of DHA and AA to infant formula resulted in a delayed onset and lower incidence of allergic symptoms and upper respiratory infections (URIs) compared to a similar formula without DHA and AA (355). In 2 nonrandomized studies in Spain and France, infant formula containing DHA and AA was associated with lower incidence of respiratory symptoms and diarrhea (353,354). A lower incidence and shorter duration of URI episodes was also reported among Thai schoolchildren (357) and US toddlers (356) who received milk containing fish-oil or DHA.

A number of hypotheses can be put forward to explain the lack of impact of fish-oil supplementation on children's growth and morbidity. It could be argued that n-3 LCP status in the study children was already satisfactory and hence the added effect of supplementation would be negligible. This hypothesis was proposed by the Gambian study where breastmilk substantially contributed to n-3 LCP intake. The lack of reference values for LCP status hampers a direct assessment of children's status. However, baseline n-3 LCP concentrations in our children were significantly lower

compared to the children from the Gambia. Unlike the Gambian study, our intervention increased baseline DHA status by 14.9–19.9% and EPA by 58.9–129%, and decreased the AA/(DHA + EPA) ratio by 21.7–34.6%. Thus, it is less likely that the children in our study had an optimal n-3 LCP status and possible functional benefits associated with increased n-3 LCP status should have been observed in this study.

We anticipated an important impact of n-3 LCP supplementation on morbidity symptoms and inflammation in a child population with high exposure to recurrent infections. However, the period prevalence of diarrhea in our children was rather low (5.2%) compared to previous studies from LICs (2.4–16.3%) (430). The proportion of children with systemic inflammation was also much lower in our study (24.2%) compared to the n-3 LCPUFA study in the Gambia (44.4%) (291). Hence, we cannot exclude that the relatively lower prevalence of morbidity and systemic inflammation in the study population might have attenuated the impact of the intervention.

Impacts on children's outcomes could also be conditional on the intake of n-3 LCPs from other sources. In the control children, blood concentrations of n-3 LCPs remained rather stable during the 12 months of follow-up. This was an unexpected finding given that breastmilk is the only important source of preformed n-3 LCPs and one would expect that breastmilk is gradually replaced by complementary foods, which are typically very low in n-3 LCPs. A possible reason for the stable n-3 LCP concentrations in this group could be the high frequency of breastfeeding throughout the follow-up.

Finally, it should be noted that the children in both the intervention and control groups received multimicronutrient fortified food supplement and deworming medication to avoid any hampering of the efficacy of the n-3 LCPs by micronutrient deficiencies and intestinal parasite infections. It therefore cannot be excluded that both the micronutrients and deworming might have exerted a positive, albeit small, effect on child linear growth, thereby possibly diluting the intervention effect.

The heterogeneous findings on child morbidity between our and the Gambian studies and other studies conducted in affluent countries may relate to differences in background n-3 LCPUFA status of the study populations and the clinical endpoints assessed. It is possible that LCP intake from breastmilk might have provided some

protection in our and the Gambian children compared to the exclusively formula-fed infants and older children in the other studies. The clinical benefits reported in the latter studies might also have been due to n-3 LCPs' modulating immune response to inflammatory conditions rather than infectious diseases. Even in the studies that reported benefits for URIs, symptoms associated with allergic and inflammatory sensitization could have contributed to URIs being diagnosed at an early age (355). Differential effects may also arise from differences in etiologies as animal experiments found that dietary n-3 fatty acids can improve or impair the host response to infectious challenges depending on the type of pathogen (415). Moreover, the effects of n–3 fatty acids on the immune system may vary according to dose, age of exposure, and the polarization and profile of the involved immune system (T helper, Th1/Th2) (351).

Our study found that direct fish-oil supplementation to children or combined with maternal fish-oil supplementation resulted in higher ponderal growth, indicated by increased WLZ rates, and a trend to larger MUAC increments over time as compared to the control group. Other RCTs also reported positive impacts of n-3 LCP supplementation on BMI, MUAC, skinfold thickness, and waist circumference, suggesting changes in body composition (291,431). The competitive roles of n-3 and n-6 FAs on the regulation of genes encoding for fat oxidation, thermogenesis, and adipose differentiation, demonstrated in animal studies, have been suggested as plausible mechanisms for effects on the rate and/or composition of weight gain (391,392). On the other hand, the clinical significance of the observed intervention effect on ponderal growth of our children requires careful consideration because of the small effect size (432). The difference in the rate of weight gain from the C arm was 0.5 and 0.6 g/d in the CI and MCI arms, whereas the recommendation for nutritionally important weight gain is >3 g/d (433).

The study has a few limitations that need to be considered. We did not include markers of immune cell function and intestinal inflammation, nor indicators of intestinal integrity, which impedes a more mechanistic understanding of the intervention's effect on child immune functions. Close surveillance and referral for treatment might have an effect of obscuring the usual occurrence of morbidity outcomes in the study population. It is also important to note that we had limited power to detect small differences in morbidity outcomes with low prevalence

estimates. We did not monitor dietary intake other than the food supplements. However, previous dietary surveys in the study area indicated that intake of fish and other seafoods, the only important sources of n-3 LCPUFA, is rare. Only 1 out of 291 infants consumed fish during the pre- and post-harvest seasons as measured by repeated 24-h dietary recalls (369).

5.6. Conclusion

In conclusion, this study adds to the growing body of evidence on the lack of growth benefits from n-3 LCP supplementation to IYC. Despite an improvement in n-3 LCP status, we did not find a benefit on linear growth or morbidity. n-3 LCP supplementation seems to modestly increase body adiposity when given in sufficiently large doses.

6

CHAPTER 6

N-3 LCP SUPPLEMENTATION AND CHILD DEVELOPMENT

Redrafted from Argaw, A., Huybregts, L., Wondafrash, M., Kolsteren, P., Belachew, T., Worku, BN., Abessa, TG., Bouckaert, KP. (2019) Neither n–3 Long-Chain PUFA Supplementation of Mothers through Lactation nor of Offspring in a Complementary Food Affects Child Overall or Social-Emotional Development: A 2 × 2 Factorial Randomized Controlled Trial in Rural Ethiopia. J Nutr,149:505–512.

6.1. Abstract

Background: The n-3 LCP DHA is essential for optimal brain development. There is a lack of evidence on the effect of postnatal n-3 LCP supplementation on child development in LICs.

Objective: We evaluated the efficacy of fish-oil supplementation through lactation or complementary food supplementation on the development of children aged 6–24 months in rural Ethiopia.

Methods: We conducted a double-blind randomized controlled trial of n-3 LCP supplementation for 12 months using fish-oil capsules [maternal intervention: 215 mg DHA + 285 mg EPA] or a fish-oil–enriched complementary food supplement (child intervention: 169 mg DHA + 331 mg EPA). In total, 360 pairs of mothers and infants aged 6–12 months were randomly assigned to 4 arms: maternal intervention and child control, child intervention and maternal control, maternal and child intervention, and maternal and child control. Primary outcomes were overall developmental performance with the use of a culturally adapted Denver II test that assesses personal-social, language, fine-motor, and gross-motor domains, and social-emotional developmental performance using the Ages and Stages Questionnaire: Social Emotional at baseline and at 6 and 12 months. We used mixed-effects models to estimate intervention effects on developmental performance over time (intervention × time interaction).

Results: The evolution in overall and social-emotional developmental performance over time did not differ across study arms (intervention × time: F = 1.09, *P* = 0.35, and F = 0.61, *P* = 0.61, respectively). Effects did not change after adjustment for child sex, age, birth-order, and nutritional status; maternal age and education; wealth; family size; and breastfeeding frequency. Children's developmental performance significantly decreased during study follow-up (β (95% CI): −0.03 SD/month (−0.04, −0.01 SD/month); *P* < 0.01).

Conclusions: n-3 LCP supplementation does not affect overall or social-emotional development of children aged 6–24 months in a low-income setting. Follow-up of the cohort is recommended to determine whether there are long-term effects of the intervention. This trial was registered at clinicaltrials.gov as NCT01817634.

6.2. Introduction

DHA is an n-3 LCP essential for the structural and functional development of the brain (138,434). DHA deficiency during the brain growth spurt has been shown to exert deleterious effects on learning, mood, and motor development in animals (435,436).

HM is the predominant source of n-3 LCPs for IYC in LMICs (169,174,404). However, the adequacy of the DHA supply remains a concern because breastmilk DHA concentrations, which largely depend on the maternal diet, show considerable variations across and within populations (176,177,408). Mothers living far from coastal areas have limited access to dietary DHA sources to ensure the recommended intake of 200 mg DHA/d during lactation, which could be achieved by consuming 1–2 portions of sea fish/wk (95,173–175). Furthermore, with the introduction of complementary foods, children in LMICs gradually shift from dependence on breastmilk to a complementary diet that is often very low in DHA (174,179). Although DHA can be synthesized de novo from its precursor, ALA (18:3n−3), the conversion is typically very low and is further hampered by a high dietary n-6 to n-3 PUFA ratio and common genetic polymorphisms (67,437). Studies have confirmed that infant DHA status can be more efficiently improved by supplementation with preformed DHA compared with ALA (97).

Observational studies have shown that breastfed compared with formula-fed children have better neurodevelopment, which has been attributed to the lack of preformed n-3 LCPs in previous infant formulas (135,203). However, it remains uncertain whether n-3 LCP supplementation of children born at term can have neurodevelopmental benefits because systematic reviews of RCTs did not produce any conclusive evidence (210,211,214,217,221). Furthermore, it is currently still unclear whether these findings from predominantly HICs can be extrapolated to children living in LMICs. LMICs have a higher prevalence of other developmental risk factors, such as intrauterine growth restriction, stunting, inadequate stimulation, infections, and poor water and sanitation conditions (438). Some studies have reported differential responses to n-3 LCP supplementation in which subgroups with a higher developmental risk– such as very-low-birth-weight infants and children with reduced caregiver interactions and psychomotor stimulation– benefited more from

supplementation (277,290,439). This might suggest that any neuroprotective effects of n-3 LCP supplementation could be more pronounced among children living in LMICs. To date, only one clinical trial in The Gambia evaluated n-3 LCPUFA supplementation of breastfed infants in LMICs and found no benefit on cognitive development (291). However, the infants had a rather adequate dietary n-3 LCP supply, and breastmilk DHA concentrations of mothers were surprisingly high and matched those of populations with high fish consumption.

Ethiopia is a landlocked country and has been estimated to have the lowest dietary DHA intake in the world (i.e., 7.0 mg/d per capita) (175). The median DHA intake from complementary foods in children aged 6-36 months is estimated at 1.1 mg/d, which is negligible compared with the worldwide median of 14.6 mg/d (179,180). Thus, breastfed children's DHA status is expected to be low due to suboptimal DHA intakes from complementary foods and breastmilk. In this study, we hypothesized that an increased intake of n-3 LCPs would improve developmental performance of breastfed children aged 6-24 months in a rural setting in Ethiopia. The intervention was delivered through 2 channels: 1) supplementation of children with a complementary food supplement enriched with fish-oil and/or 2) supplementation of their lactating mothers with fish-oil capsules.

6.3. Methods

6.3.1. Subjects and measurements

This study was a randomized, double-blind, placebo-controlled trial that assessed the independent and combined effects of fish-oil (n-3 LCP) supplementation of lactating mothers using fish-oil capsules (maternal intervention) and their breastfed children using a fish-oil-enriched complementary food supplement (child intervention). The trial was conducted from November 2013 to February 2015 in the districts of Deneba, Assendabo, and Serbo of Jimma Zone, Ethiopia. Previous dietary surveys conducted in the study area showed that a negligible number of IYC consumed fish, whereas 95% of children continued breastfeeding throughout the second year of life (369). Children and their lactating mothers were randomly assigned to receive either the fish-oil (intervention) or a placebo control without fish-oil. Thus, there were 4 study arms: both mother and child received the fish-oil intervention (MCI), only the mother received the fish-oil intervention and her child received a placebo control (MI), only

the child received the fish-oil intervention and the mother received a placebo control (CI), and both mother and child received the placebo control (C). Study participants, development assessors, and researchers remained blinded to the intervention allocation until the end of data analysis. The maternal supplements were airtight soft-gel oil capsules, which, at a daily dose of 2 capsules, provided either 500 mg n-3 LCPs (intervention: fish oil providing 215 mg DHA + 285 mg EPA) or no n-3 LCPs (control: corn oil). The intervention and control capsules were identical in appearance (Biover NV). The child complementary food supplements were extruded corn-soy blends fortified with 19 micronutrients that were either enriched with a daily dose of 500 mg n-3 LCPs (intervention: fish oil providing 169 mg DHA + 331 mg EPA) or not enriched with n-3 LCPs (control: corn oil) (Michiels Fabrieken NV and Fortitech, Inc.) (see details in **Chapter 5**).

The primary study outcomes were the performance score on overall development using a culturally adapted and standardized Denver II Developmental Screening Test (Denver II-Jimma), which assesses personal-social, language, fine-motor and gross-motor developmental domains, and the performance score on social-emotional development using an adapted Ages and Stages Questionnaire: Social Emotional (ASQ-SE) (440). Secondary outcomes included performance scores on the individual Denver II developmental domains (i.e., personal-social, fine-motor, language, and gross-motor development) to evaluate effects on different aspects of child development and the risks of suspected global developmental delay (using Denver II-Jimma) and social-emotional developmental delay (using ASQ-SE) to evaluate effects on the proportion of children with poor developmental performance.

Outcomes were assessed at baseline, midline (after 6 months), and endline (after 12 months) of the intervention. The Denver II-Jimma has 125 test items that evaluate a child's skills in 4 domains of development (personal-social, fine-motor, language, and gross-motor). The number of test items to be administered per domain depends on the age and performance of the child as described in the official Denver II manual (441). Some of the test items were assessed by maternal report ("report item"), whereas others required an observation of the child carrying out the task ("test item"). Test items were scored as "pass," "fail," "refusal," or "no opportunity." "Refusal" indicates that a child refused to attempt a "test item" 3 times, and "no opportunity" indicates that a child did not have the chance to perform a "report item" at home.

Children's skills in overall development and for each developmental domain were then evaluated by applying a scoring approach on a continuous scale. Children's performance scores were calculated as the ratio of the total number of passed test items to the total number of test items expected to be passed by ≥75% of same-age children in the Ethiopian benchmark (440). Children were also assessed for risk of suspected global developmental delay by categorizing each child as "normal," "suspect," or "untestable" according to the criteria in the official Denver II manual (**Table 6.1**).

Table 6.1. Screening criteria for global developmental delay and social-emotional problems

Outcomes	Cutoff criteria
Global development (Denver II- Jimma, 125 items)[1]	
Normal	No delay and a maximum of one caution
Suspect	≥ 2 cautions and/or ≥ 1 delay
Untestable	≥ 1 refusal scored as delay or ≤1 refusal scored as caution
Social-emotional development (ASQ: SE)[2]	
Age 3-8 months (19 items)	Total score >45
Age 9-14 months (22 items)	Total score >48
Age 15-20 months (26 items)	Total score >50
Age 21-26 months (26 items)	Total score >50

[1]Delay, test item failed/refused and the child is older than the age corresponding to the 90th percentile of success for the item in the Ethiopian benchmark; Caution, test item failed/refused and the child's age falls on or between the 75th and the 90th percentile.
[2]Each item is scored according to how frequently the child performs the behavior: most of the time (=0), sometimes (=5) or rarely/never (=10), with an additional 5 points if there is maternal concern for the behavior being performed sometimes (=10) or rarely/never (=15).

The ASQ-SE evaluates a child's skills in social-emotional development and therefore complements the Denver II-Jimma. The ASQ-SE tool contains age specific questionnaires including for the age ranges of 3-8, 9-14, 15-20, and 21-26 months (35) (442). Each questionnaire contains a set of questions that refer to critical adaptive and maladaptive behaviors for the target age interval and focuses on skills related to attachment, autonomy, and self-development. The ASQ-SE questionnaire was completed by asking the mother how frequently her child performed a given behavior (i.e., "most of the time," "sometimes," or "rarely/never"), which were each assigned a score. Social-emotional performance scores were then calculated as the ratio of the sum of all scores over the maximum attainable score on the age-specific questionnaire, with a higher value indicating poor developmental performance. Each

child was also screened for suspected social-emotional developmental delay using the recommended age-specific cutoffs (**Table 6.1**) (442).

Two clinical nurses conducted the developmental tests throughout the study after receiving 1 month of intensive training, with practical sessions and standardization exercises, and a refresher training before the start of midline measurements. Two experienced developmental psychologists provided the training and alternately supervised data collection in the field. Both nurses worked in all of the 3 study districts and were randomly assigned to perform an assessment in order of children's arrival at the sites. Assessments were conducted in quiet and convenient rooms and took ~30–45 min/child. Assessments were postponed to the next day when a child was sick or refused to start the test.

6.3.2. Statistical Analysis

A sample size of 360 subjects (90 subject/study arm) was required to detect an effect size of 0.038 SDs in monthly change of the overall and social-emotional developmental performance score over 12 months of intervention follow-up, assuming an autocorrelation of 0.50, 80% statistical power, and a type I error of 5% and taking into account an anticipated 20% attrition rate (426). This effect size on the monthly change in child development is equivalent to an effect size of 0.12 SDs after 12 months of intervention using an estimated SD of 0.03 for the mean monthly change in child development and an SD of 0.12 for the mean developmental score (443). These estimates are derived from a study conducted in a similar population in the study area using the same Denver II-Jimma tool (444). Data were entered in duplicate using EpiData version 1.4.4.4 (EpiData Association), and consistency checks and statistical analysis were conducted using Stata version 13.1 (StataCorp LLC, Texas, USA). Developmental performance scores were standardized to *z* scores on the basis of the distribution of the data. The Denver II-Jimma-derived risks of suspected developmental delay were categorized as either "normal" or "suspect," with the latter including the "untestable" category.

The effect of the interventions was assessed with mixed-effects linear regression models for the continuous outcomes and mixed-effects linear probability models for the binary outcomes, with child identifier as random intercept. The use of linear probability models for binary outcomes is well established and allows for a

straightforward interpretation of the average intervention effect expressed as risk difference using percentage points (445). Fixed effects in the models included study arm (C, MI, CI, or MCI), intervention time (in month), and child sex. Child sex was added due to an imbalance among study arms at baseline. We evaluated the interventions' effects on the evolution of child developmental performance taken over the 3 measurement rounds. For this purpose, we tested interaction terms between study arms and intervention time that estimate the difference between each intervention arm and the control arm on monthly changes in an outcome over time.

As a secondary analysis, we analyzed the intervention effect adjusted for relevant time-invariant covariates such as child age, birth order, and LAZ score at enrollment; maternal age and education; household wealth and family size; and frequency of breastfeeding at baseline. We further explored if there were any effect modifications of the intervention by our chosen covariates by testing triple interaction terms between study arm, intervention time, and a covariate. Analyses were performed by the intention-to-treat principle (i.e., including all children initially enrolled into the study). For this purpose, we conducted multiple imputations of missing data using chained equations under the missing-at-random assumption. Fifty imputations of missing data were conducted to estimate the regression coefficients. All of the tests were 2-sided, and the level of significance (α) was set at 0.05.

6.4. Results

The numbers of mother-child pairs who were screened for eligibility, randomly assigned, and lost to follow-up are presented in **Figure 6.1**. A total of 360 mother-child pairs were randomly assigned to the study arms and 329 (91%) attended either the midterm or the endline developmental test. Eighty-seven percent of the participants received all 12 distributions of the supplements. Baseline characteristics of study participants were comparable across the study arms, except for an imbalance in child sex (**Table 6.1**).

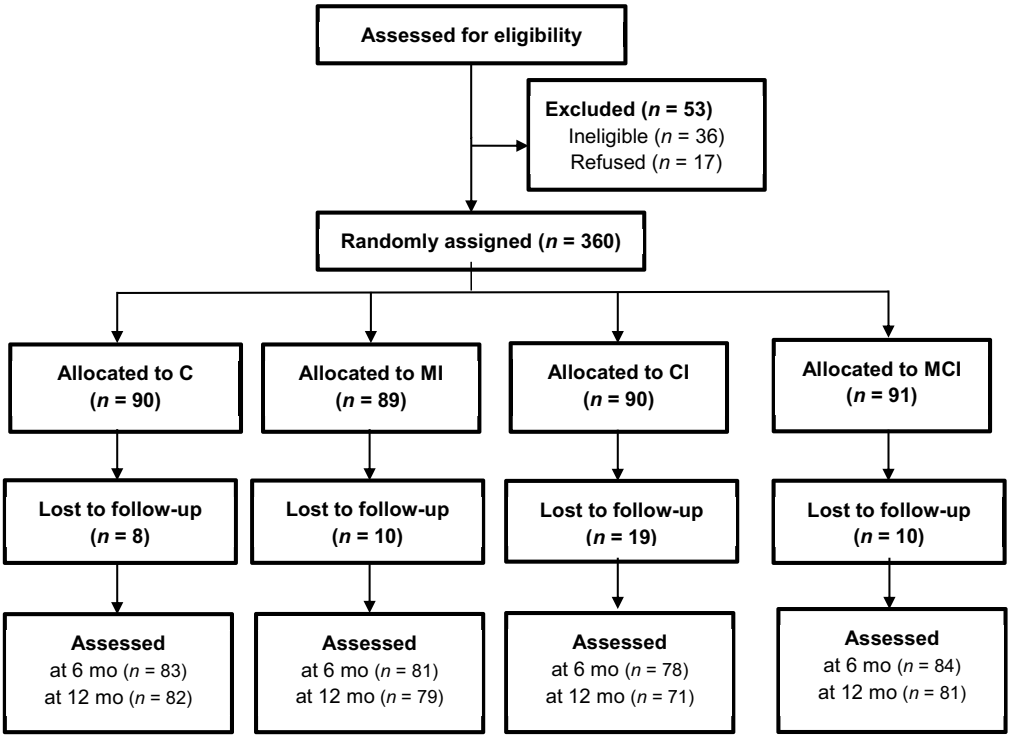

Figure 6.1. Trial flowchart.
C, control - both mother and child received placebo; CI, child intervention - child received fish-oil and mother received placebo; MCI, maternal and child intervention - both mother and child received fish-oil; MI, maternal intervention - mother received fish-oil and child received placebo.

Table 6.1. Baseline characteristics of study children and their mothers by study arm[1]

Characteristics	C (*n* = 90)	MI (*n* = 89)	CI (*n* = 90)	MCI (*n* = 91)
Child sex, female	46.7	55.1	40.0	59.3
Child age, month	8.89 ± 2.16	9.18 ± 2.09	8.93 ± 2.10	8.68 ± 2.00
Maternal age, year	26.0 ± 5.04	25.8 ± 4.82	26.1 ± 5.48	26.3 ± 5.28
Maternal education				
No formal education	46.7	49.4	51.1	52.7
Primary education	41.1	34.8	34.4	36.3
Secondary and above	12.2	15.7	14.4	11.0
Household wealth tertiles				
Lowest	30.0	38.2	31.1	34.1
Middle	32.2	25.8	38.9	36.3
Highest	37.8	36.0	30.0	29.7
Child weight, kg	8.18 ± 1.15	8.15 ± 1.10	8.05 ± 1.08	7.93 ± 1.10
Maternal height, cm	157 ± 4.67	157 ± 5.55	157 ± 5.95	157 ± 5.74
Maternal BMI, kg/m^2	20.2 ± 2.41	20.3 ± 2.50	20.1 ± 2.57	21.0 ± 3.31
Breastfeeding frequency, times/d				
4-6	11.1	6.74	12.2	8.89
7-9	23.3	31.5	28.9	25.6
≥10	65.6	61.8	58.9	65.6

[1]Values are means ± SDs or percentages.
BMI, body mass index; C, control - both mother and child received placebo; CI, child intervention - child received fish-oil and mother received placebo; MCI, maternal and child intervention - both mother and child received fish-oil; MI, maternal intervention - mother received fish-oil and child received placebo.

There was no significant difference between the study arms on the evolution of children's performance score over time for both overall development using the Denver II-Jimma (intervention × time: F = 1.09, *P* = 0.35) and social-emotional development using the ASQ-SE (intervention × time: F = 0.61, *P* = 0.61) (**Table 6.2**). Similarly, we found no significant effect of any of the interventions on developmental performance scores on the personal-social, fine-motor, language, and gross-motor domains as well as on the risks of suspected global developmental delay (using Denver II-Jimma) and social-emotional developmental delay (using ASQ-SE). The interventions' effects on developmental outcomes remained unaffected when we adjusted the analysis for relevant covariates such as child sex, age, birth order, and LAZ score at enrollment; maternal age and education; household wealth and family size; and frequency of breastfeeding at baseline. There was no significant interaction between the study arms and our chosen covariates on the developmental outcomes.

The age-adjusted overall developmental performance of children significantly decreased with time during the study follow-up (β (95% CI): −0.03 SD/month (−0.04, −0.01 SD/month); $P < 0.01$). The prevalences of suspected global developmental delay and social-emotional developmental delay were 19.2% and 67.5% at baseline, 19.7% and 60.6% at midline, and 20.6% and 41.7% at the endline of the study, respectively.

6.5. Discussion

We previously showed that supplementation with n-3 LCP–rich fish-oil through lactation and/or a child complementary food supplement significantly increased child blood DHA concentrations by 15–20% and decreased the ratio of arachidonic acid (20:4n−6) to DHA+EPA by 22–35% (**Table 5.2**). However, the results of the current study showed no apparent benefit of the supplementation on child developmental performance assessed using the Denver II-Jimma and ASQ-SE tests.

The current study is 1 of 2 trials conducted in an LMIC setting that reports on the impact of postnatal n-3 LCP supplementation on child development. Another trial in The Gambia found no benefit of direct fish-oil supplementation of breastfed infants from ages 3–9 months on cognitive performance using the Willatts' infant planning test (291). Several RCTs in developed countries have examined the effects of n-3 LCP or DHA supplementation of lactating mothers or infant formulas on different aspects of child neurocognitive and behavioral development. Although some of these studies reported beneficial effects of supplementation on some aspects of child development, others studies that used similar or other developmental tools found no differences between intervention and control groups (210,211,214,217,221). Campoy et al. (217) and Meldrum et al. (421) proposed that the mixed results from previous trials could be due to heterogeneity among studies with regard to the timing, duration, and dose of supplementation; the methods used for outcome assessment; the age of children at outcome measurement; inadequate sample size; or potential differences in the populations studied, such as genetic polymorphisms possibly affecting DHA requirements and concentrations in breastmilk.

Table 6.2. Developmental performance score and suspected developmental delay in children, by measurement round and study arm[1]

Outcomes	Group	Baseline	Midterm	Endline	*P*[2]	*P*[3]
Developmental performance scores						
Overall development	C	1.14 ± 0.12	1.14 ± 0.09	1.10 ± 0.07		
	MI	1.12 ± 0.12	1.12 ± 0.09	1.10 ± 0.07	0.351	0.312
	CI	1.12 ± 0.12	1.12 ± 0.08	1.08 ± 0.06		
	MCI	1.13 ± 0.13	1.13 ± 0.10	1.11 ± 0.07		
Social-emotional	C	0.35 ± 0.23	0.32 ± 0.20	0.22 ± 0.15		
	MI	0.38 ± 0.24	0.27 ± 0.22	0.23 ± 0.17	0.607	0.648
	CI	0.35 ± 0.24	0.32 ± 0.20	0.25 ± 0.15		
	MCI	0.35 ± 0.22	0.30 ± 0.23	0.21 ± 0.15		
Personal-social	C	1.31 ± 0.23	1.28 ± 0.23	1.17 ± 0.11		
	MI	1.28 ± 0.23	1.23 ± 0.18	1.18 ± 0.11	0.796	0.747
	CI	1.27 ± 0.22	1.25 ± 0.19	1.17 ± 0.10		
	MCI	1.32 ± 0.23	1.29 ± 0.21	1.20 ± 0.10		
Fine-motor	C	1.15 ± 0.17	1.09 ± 0.09	1.07 ± 0.07		
	MI	1.12 ± 0.18	1.08 ± 0.10	1.07 ± 0.07	0.720	0.771
	CI	1.11 ± 0.16	1.07 ± 0.09	1.06 ± 0.07		
	MCI	1.11 ± 0.21	1.09 ± 0.09	1.08 ± 0.07		
Language	C	1.14 ± 0.20	1.10 ± 0.11	1.08 ± 0.16		
	MI	1.10 ± 0.16	1.08 ± 0.10	1.08 ± 0.16	0.124	0.131
	CI	1.13 ± 0.20	1.08 ± 0.12	1.03 ± 0.13		
	MCI	1.16 ± 0.20	1.09 ± 0.12	1.09 ± 0.16		
Gross-motor	C	1.04 ± 0.12	1.13 ± 0.11	1.08 ± 0.08		
	MI	1.04 ± 0.12	1.12 ± 0.13	1.08 ± 0.08	0.787	0.851
	CI	1.04 ± 0.11	1.12 ± 0.10	1.07 ± 0.08		
	MCI	1.04 ± 0.10	1.11 ± 0.14	1.09 ± 0.08		
Suspected developmental delays						
Global DD	C	21.1	12.2	22.2		
	MI	16.9	22.5	18.0	0.235	0.258
	CI	18.9	22.2	26.7		
	MCI	19.8	22.0	15.4		
Social-emotional DD	C	67.8	67.8	38.9		
	MI	71.9	52.8	42.7	0.777	0.811
	CI	64.4	64.4	44.4		
	MCI	65.9	57.1	40.7		

[1]Values are means ± SDs of developmental performance score, and proportions of children with suspected developmental delay. Sample size in C (*n* = 90), MI (*n* = 89), CI (n = 90), and MCI (*n* = 91).
[2]*P*-values for the intervention effect on the evolution of an outcome over time (intervention X time interaction), estimated from mixed-effects linear models for the continuous outcomes and mixed-effects linear probability models for the binary outcomes with child identifier as random intercept and adjustment for child sex.
[3]*P*-values for the intervention effect, estimated from models adjusted for child sex and fixed-effect covariates including child age, birth-order and length-for-age Z-score at enrollment, maternal age and education, household wealth and family size, and frequency of breastfeeding.
C, control - both mother and child received placebo; CI, child intervention - child received fish-oil and mother received placebo; Global DD, suspected global developmental delay assessed using Denver II-Jimma; MCI, maternal and child intervention - both mother and child received fish-oil; MI, maternal intervention - mother received fish-oil and child received placebo; Social-emotional DD, suspected social-emotional developmental delay assessed using ASQ:SE.

The interpretation of the results of the current study should therefore consider the age at which supplementation was provided (i.e., 6-24 months). The intervention did not cover the entire period of expected higher brain sensitivity to dietary DHA. Rapid brain DHA accretion coincides with the brain growth spurt that spans from the beginning of the third trimester of pregnancy to the second year of life, with the majority accumulating before the first 12 months of life (146,390). This may suggest that supplying DHA prenatally and to younger infants could influence brain DHA status and associated functional outcomes more. On the other hand, potential impacts in older IYC cannot be excluded because the human brain remains sensitive to dietary DHA throughout its protracted developmental phase (446). Increased brain DHA exposure during this time may benefit the late-maturing dopamine system of the prefrontal cortex, which is selectively influenced by DHA (447) and which reaches peak maturation toward the end of the first year and reaches functional maturity at ~12–15 months of age and beyond (448,449). For instance, a DHA supplementation study in older children aged 8–10 years showed a significantly higher functional activity of the prefrontal cortex in the supplemented group, and erythrocyte DHA composition was inversely correlated with reaction time in a sustained attention task (154).

Another important point requiring consideration is that the Denver II-Jimma and ASQ-SE tools might not be adequately sensitive to detect subtle effects of dietary DHA on specific brain functions (137,450). DHA selectively concentrates in the prefrontal cortex of the brain region, where cognitive processes involving attention regulation and components of short-term and working memory take place (447,449). Thus, tools assessing specific cognitive abilities mediated by the prefrontal cortex, such as attention and problem solving, might have been more informative about the effects of increased dietary DHA intake on brain function (450). Furthermore, the impacts of early DHA exposure could be more evident on cognitive abilities that emerge at a later age. On the other hand, global developmental tests taken before the age of 2 years have been shown to have limited predictive validity for later childhood cognitive and behavioral performance (451,452). Jensen et al. (263,265) reported benefits of maternal supplementation with 200 mg DHA/d during lactation on child development at 2.5 and 5 years of age but did not find any impact during infancy compared with a control group. Others found that the addition of DHA to infant formula was associated

with better performance on several tasks, reflecting discrete aspects of cognitive functions between the ages of 3 and 6 years. However, no impact of this enriched infant formula was detected using standardized developmental tasks at the age of 18 months (242).

Children from the control arm of this study still received DHA through breastmilk. As such, this could have diluted the differential impact between the intervention and control arms. This contextual element is important to highlight when comparing the study results with previous findings from RCTs conducted in weaned infants in which control infants received formulas devoid of preformed n-3 LCPs. A review by Lauritzen et al. (85) suggested that DHA supplementation is more likely to result in an impact on developmental outcomes when children's basal DHA intake is <70 mg/d. From the analysis of baseline breastmilk samples we derived a mean DHA concentration of 74.0 mg/L (See **Table 4.1** in Chapter 4). With HM being the sole predominant source of DHA in this setting and assuming an average breast-milk intake of 650 mL/d (453), we estimate that the study children's average DHA intake from breastfeeding would not exceed 50 mg/d. We therefore do not expect that this low HM DHA concentration would have masked any potential effects of n-3 LCP supplementation in the current study.

Often, the inadequacy of the studied dose or poor compliance to the intervention is an argument to explain the lack of impact of supplementation trials on functional outcomes. In the current study, the median (IQR) compliance (i.e., the ratio of actual supplement consumption over prescribed consumption) was 79.7% (62.6–91.4%) for the child complementary food supplement and 69.9% (52.2–80.4%) for the maternal capsules, with no significant difference between the study arms (See **Table 5.3 in Chapter 5**). The frequency of breastfeeding remained high throughout follow-up, with an average frequency of ≥7 times/d in 84.2% of the participants. The n-3 LCP dose amounts for the maternal and child supplements used were determined on the basis of the trade-off between supplying an adequate amount of n-3 LCPs and avoiding theoretically possible adverse effects of a high-dose of n-3 LCPs, including the risk of immunosuppression among the children exposed to both the maternal and the child interventions. Reviews of previous RCTs of infant formula supplementation recommend a dose of ≥0.32% DHA of total fat (TF; grams per 100 g TF) to target developmental outcomes, an amount based on the global average HM concentration

(218,421). The dose of DHA in the child intervention complementary food supplement in our study was estimated to be 0.56% TF using the total dietary fat requirement of 30 g/d for infants aged 7-12 months (453). Even when considering compliance in the CI arm (median: 79.0%; IQR: 62.3–89.9%), the dose was ≥0.35% TF in 75% of the children. The dose of DHA in the maternal intervention capsules was also aimed at achieving an optimum breastmilk DHA content because a previous dose-response study showed that a dosage of 167 mg DHA/d was able to enrich breastmilk to 0.32% TF in lactating mothers consuming a very low n-3 LCP diet similar to a vegetarian diet (398). Furthermore, we addressed the potential for a lack of impact due to an inadequate dose by including a study arm who received the combined interventions.

We found a high risk of suspected developmental delay among children in our study sample. In addition, the age-adjusted developmental performance scores of children declined during the study follow-up. Poor nutrition and suboptimal child care and stimulation practices have been identified as important contributors of poor developmental performance in LMICs (9,454). In a recent randomized controlled study in our study area, a home-based stimulation intervention was shown to significantly improve developmental performance in high-risk children aged <5 years (444). Therefore, there is a critical need for identifying appropriate interventions that integrate both nutrition and stimulation interventions to mitigate the developmental risks in this and other child populations.

This study has a few strengths and limitations that need to be addressed. First, we used the Denver II instrument because it has been culturally adapted and standardized to the local context by a previous study conducted in the same study area (440). Work by Rubio-Codina et al. (455) showed that this shorter tool can be used as a valid and highly feasible substitute of the more-lengthy Bayley Scales of Infant and Toddler Development for large-scale field-setting studies in LMICs. On the other hand, tests of specific cognitive tasks that can be mechanistically and theoretically related to the role of DHA on brain function may have provided more understanding of an impact of the intervention. However, the feasibility of administering such tests in young children in our study was limited by the field setting and the relatively large sample size. Second, we did not monitor dietary intake other than compliance to the intervention supplements. However, previous dietary surveys found that the consumption of fish and other sea foods was very rare in the study

area (369). Finally, we did not assess the impact of n-3 LCP supplementation in infants aged <6 months because of the universal recommendation of exclusive breastfeeding in this age group and the specific design of this study (i.e., the assessment of the concomitant impact of n-3 LCP supplementation through complementary foods and breastmilk enrichment).

6.6. Conclusion

In conclusion, this study did not find evidence of a positive impact of n-3 LCP supplementation on child developmental performance in breastfed children in a low-income setting. Assessing long-term effects of early dietary n-3 LCP exposure on later cognitive and behavioral skills in this population is warranted.

7

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Despite the significant steps the world has taken towards improving nutrition and the associated burden of disease and impaired quality of life over recent decades, millions of children in LMICs are still suffering from the severe consequences of poor health and nutrition. According to the global burden of maternal and child undernutrition estimates in 2018, more than 149 million under-five children were suffering from stunted growth (1). Growth faltering in early-life is associated with an elevated risk of mortality, poor neurocognitive development, poor learning capacity and productivity in later life, and increased risk of nutrition related chronic diseases during adulthood (4). The lifelong consequences that follow poor nutrition in early-life is not only a problem that matters to the quality of life of the victims, rather it is a cross-cutting agenda impeding economic and societal growth.

There is a growing consensus that good nutrition during the window period of the first 1,000 days of life is critical to achieve full human potential throughout life, and a means to break the intergenerational cycle of poverty and inequity. Accordingly, the WHA in 2012 endorsed a Comprehensive Implementation Plan on Maternal, Infant and Young Child Nutrition with six global targets, which have also been embraced in the post-2015 sustainable development agenda (58). Despite this encouraging global momentum, recent estimates showed that current progress in reducing child undernutrition in many LMICs is inadequate to achieve the WHA global targets (56).

In the context of the global efforts to improve the health and nutritional status of children in LMICs, the current PhD work contributes to two important aspects. First, we explored nutrition-specific and -sensitive factors that can contribute to reduction in chronic childhood undernutrition in a set of LMICs. Second, in an effort to find a new approach that maximizes effectiveness of existing nutrition interventions, we evaluated the efficacy of n-3 LCPs supplementation on healthy growth and development of IYC from a rural setting in Ethiopia. This chapter presents an overview of the major findings, their implications and methodological issues of the studies conducted in the PhD thesis, and thoughts for future research and policy recommendations to be considered. Various specific aspects of the studies, results, and discussions in relation to the literature have already been detailed in the previous chapters. Therefore, here the general discussion will be limited to considerations from a broader perspective.

7.1. Main research findings

The first study of the PhD thesis examined the relationship between the trends in under-five stunting prevalence in 14 LMICs and determinants at the distal, intermediate and proximal levels (**Chapter 3**). Stunting prevalence followed a declining trend in all the countries studied at an average annual rate of reduction of 1.04%. Narrowing income inequalities (i.e. decreases in Gini coefficient), urbanization, and women's empowerment (i.e. improvements in women's decision making in the household) were the determinants at the distal level that explained the observed trend in stunting reduction over time. Improvements in households' access to improved sanitation facilities and drinking water sources, and increases in child immunization coverage were the intermediate determinants having significant contribution for stunting reduction. The important stunting drivers identified at the proximal level were improvements in prenatal nutrition (i.e. decreases in prevalence of low birthweight) and increases in early initiation of breastfeeding.

The effects of n-3 LCPs-rich fish-oil supplementation in lactating mothers and their IYC 6-24 months old are evaluated in three chapters of the PhD thesis. Fish-oil supplementation of lactating mothers resulted in significantly higher maternal milk DHA and EPA concentrations and a lower ratio of n-6/ n-3 LCPs compared to the control group (**Chapter 4**).

Fish-oil supplementation through lactation (maternal supplementation), complementary food (child supplementation), and combination of both successfully increased child n-3 LCPs status as reflected in the significantly higher blood n-3 LCPs concentrations and the lower n-6/n-3 LCPs ratios compared to the control group. Direct fish-oil supplementation to children or combined with maternal fish-oil supplementation also resulted in higher ponderal growth, as indicated by the significant increases in WLZ rates and the trends to larger MUAC increments over time as compared to the control group. No further effects of the fish-oil intervention were detected on the outcomes child morbidity, systemic inflammation (CRP), linear growth (LAZ), HC, nutritional status (stunting, wasting and anemia), or on overall and social-emotional developmental performance (**Chapters 5 & 6**).

7.2. Implications of the research findings

7.2.1. Drivers of the trend in child stunting

Achieving the WHA global target of a 40% stunting reduction between 2010 and 2025 requires an average annual reduction by 3.9% (57); whereas the average annual rate of stunting reduction observed in the 14 LMICs in the current study is 1.04%. This finding that current trends in these countries is not rapid enough to meet the global stunting target calls for the critical need to scale up efforts by the individual countries and their international partners. Experiences of countries with success stories show that rates of stunting reduction can be accelerated with deliberate actions (456–458). The global stunting target was also established using the actual experience of emerging countries as a benchmark (57). An important question however is which important actions are required to accelerate current progress in high burden countries to meet the stunting target for 2025 and beyond.

Despite its high global prevalence, the mechanisms underlying childhood growth faltering and the most tractable pathways for interventions to effectively prevent stunting remain a work in progress (362,459,460). From epidemiological studies, it is apparent that stunting represents the outcome of a complex array of causal and contextual factors as captured in the WHO Conceptual Framework of Childhood Stunting (32). Prenatal and postnatal nutritional deficits and poor health such as infectious diseases and EED are the proximal determinants of stunting. Linear growth failure also occurs within a complex interplay of more distal factors such as agriculture and food systems, political stability and economy, access to healthcare and education, water and sanitation infrastructure, social support networks, and demographics. Preventing childhood growth faltering therefore requires multifaceted and multisectoral approaches combining direct nutrition interventions together with nutrition-sensitive development that address the underlying and contextual determinants of undernutrition (28,32,359). The range of important proximal to distal stunting drivers detected in the countries included in this study also affirm this prior knowledge on the need for fostering multisectoral efforts.

On top of existing trends, enhanced investment in proven nutrition interventions and delivery strategies to reach populations at greatest risk can make a substantial contribution to meeting the global stunting target. The Lancet Series (30) on

nutrition-specific interventions showed that accelerated gains are possible by expanding existing evidence-based interventions in the 34 countries housing 90% of the world's stunted children. The review estimated that about a fifth of the existing stunting burden can be averted if a package of ten interventions to address undernutrition and micronutrient deficiencies can be scaled up from existing low population coverage to 90%.

Given the complex nature of the problem, it is conceivable that direct nutrition interventions alone are insufficient to fully avert the high stunting burden in LMICs. A long-term and sustainable improvement in nutrition requires development approaches in LMICs address the underlying and contextual factors in which undernutrition operates (32). The findings from this study also entail the important role of improvements in basic and underlying factors such as income inequalities, urbanization, women's empowerment, water and sanitation infrastructure, and child immunization. Investments in development interventions spanning multiple sectors, such as agriculture, education, health care, social safety nets, and water and sanitation infrastructure, may improve nutrition through boosting agricultural production and access to diverse diets, increasing income and purchasing power of poor households, fostering women's empowerment, and improving health status (461–463). The central role of nutrition-sensitive development has been highlighted in the experience of countries with remarkable achievements in stunting reduction. To demonstrate, stunting prevalence in Brazil fell by almost three-quarters between 1996 and 2006 with socioeconomic inequalities in stunting also reduced (456,457,464). This dramatic decline has been associated with concurrent improvements in purchasing power of low-income families through targeted conditional cash transfer program, improved maternal schooling, virtually universal basic health care, and expanded public water supply and sanitation system. Similarly, Mexico achieved a substantial stunting reduction which has been linked to better targeting and enhanced coverage of a conditional cash-transfer program and increased access to health care facilities (458,465). These experiences suggest that the scourge of chronic child undernutrition can be reduced rapidly if income among the poor rises simultaneously with increased access to basic services.

Despite the evidence for the potential to reach the global stunting target through combination of nutrition-specific and -sensitive approaches, translating this potential

into results on the ground seems to be constrained by various factors. There is a critical funding gap to expand key nutrition-specific interventions from their current negligible coverage in high burden countries. It has been estimated that the scale up and maintenance of key nutrition interventions to reach the WHA stunting target will cost about US$50 billion over ten years (466). Mobilizing this additional cost requires current annual spending to increase from US$2.6 to 7.4 billion, including governments in high burden countries on average allocating 3% of their health budgets to stunting-related nutrition activities. Current encouraging momentum of prioritizing nutrition in the international development agenda should be further strengthened to make the case for additional resource mobilization. The strong case for investment in nutrition is well-established and nutrition interventions are among the most cost-effective development actions. For instance, every dollar invested in programs to reduce stunting has been estimated to have a long-term economic return of US$18 (467). However, there is a critical need for a greater appreciation by decision makers at national and international levels that stunting is not solely a consequence of poverty, which is rather a critical barrier to economic development. It should also be emphasized that improving nutrition will be a catalyst to achieve progress across the other SDGs. Furthermore, accountability mechanisms should be put in place to ensure that previous commitments made by all stakeholders for their globally endorsed stunting target are acted upon.

Current efforts on stunting reduction should also be complemented by exploring low-cost, more effective and scalable solutions within the context of limited funding. Delivery platforms and models that increase effectiveness and reduce cost should be identified and adopted for large-scale implementation of nutrition interventions. Nutrition-sensitive programs can be used to enhance the scaling up and sustainability of nutrition-specific interventions. For instance, micronutrient interventions such as supplements and specialized fortified food products have traditionally been delivered through the health facility platforms. However, an integrated approach using additional platforms, such as agriculture, market-based, and social protection programs, may help to increase the coverage and cost effectiveness of such individual interventions (468).

Governments and donors invest more in nutrition indirectly, i.e., on nutrition-sensitive development spending, than they do on nutrition interventions directly. However, the

limited priority for nutrition-related goals and actions, and the gap in evidence-based decision-making have constrained development programs from having the desired impact on nutritional status. The Lancet Series (28) on nutrition-sensitive interventions found that programs in agriculture, social safety nets, schooling, and early child development improve several of the underlying determinants of undernutrition. However, robust evidence for their impact on nutritional status across settings is still scarce. According to this review, the fact that most development programs are not originally designed to improve nutrition has resulted in a lack of explicit nutrition goals and actions, poor quality of services and/or weak program evaluation of nutritional impact. Therefore, there is a critical need for efficient multisectoral collaboration to maximize the nutrition sensitivity of development activities in LMICs.

Achieving nutrition impact in more distal interventions is often complex and operates through multiple pathways (461–463). To illustrate, the potential impact of improved household income on child nutritional status can be warranted in the presence of various conditions such as women's control of household resources, optimal feeding practices, and access to basic health services. This implies that in order to reach their full potential on nutritional impact, development programs needs to integrate nutrition education, health service utilization and women's empowerment components among others. Therefore, future development programs across sectors should be proactively reoriented to integrate strong nutrition goals and targeted actions grounded in program theory of impact pathways. Furthermore, informing investments across development sectors is constrained by the lack of effective mechanisms to track the impact of development spending on health and nutrition outcomes. Currently, the fraction of stunting that can be averted via nutrition-sensitive actions alone is unknown as well as the combined or synergistic impact of nutrition-sensitive and -specific interventions. In addition to their main program outcomes, development programs should understand the program impact pathways responsible for impacts on key nutrition and health indicators such as child linear growth using appropriate implementation research.

Further efforts should also be made to fill the gaps in clearly understanding the etiology of growth faltering during early-life and related effective nutrition solutions. Among others, increasing evidence support the hypothesis that EED, a persistent

asymptomatic enteropathy occurring at a high prevalence among children in poor settings, plays a central role in the pathogenesis of stunting (324,469,470). However, this plausible pathway has been less clearly understood and thus, largely ignored in current nutrition interventions. The prevalent EED among IYC in LICs has been indicated as the missing link that could hamper the effectiveness of existing nutrition and health interventions to promote child growth (470,471). Therefore, research exploring effective interventions to prevent and ameliorate the persistent enteropathy and its associated growth impairment in children is paramount to achieve a rapid stunting reduction in LICs.

Structural and functional disturbances in EED are associated with nutrient malabsorption and a leaky-gut stimulating local and systemic inflammation, which are thought to underlie the impaired growth of children in poor settings (43,320–324,469,470). Studies from laboratory experiments and human subjects in HICs indicate that n-3 LCPs may reduce inflammation, improve gut integrity, and enhance maturation of the immune system in early-life (as detailed in section 1.3.3). Considering the role of intestinal and systemic inflammation in the pathways from EED to stunted growth, these potential benefits of n-3 LCP may have important implications for optimal growth of IYC in LICs. Furthermore, the importance of an adequate supply of n-3 LCP for optimal development of the brain and visual systems during pregnancy and infancy is well established (134).

The fortification of infant formulas with LCPs has been recommended by different authorities in HICs, whereas IYC living in LICs relay on breastfeeding as the only important source of LCPs. However, the n-3 FAs consumption in several LICs like Ethiopia is negligible to meet the adequate intake level recommended during pregnancy and lactation to support adequate transfer to the fetus and the infant (175). Moreover, IYC in vulnerable populations may have additional n-3 FA requirements due to different stressors such as deficiency of micronutrients required for essential FA metabolism, and malabsorption and chronic immune stimulation incurred by high rates of EED and diarrhea (71,128,191–194). Therefore, n-3 FAs may be one dietary limiting factor for optimal child health and development in these settings, especially when complementary foods very low in n-3 FAs content are introduced. Nonetheless, whether the supplementation of n-3 LCP is required for children in low-income settings is one of the least investigated research questions.

7.2.2. n-3 LCP supplementation of infants and young children

A large number of infant n-3 LCP supplementation studies in HICs have been conducted in relation to child growth and development as discussed in Chapter 1. Briefly, n-3 LCP supplementation did not improve the growth of term infants in all studies considered, while inconsistent results were found in preterm infants. Similarly, evidence from RCTs did not show a consistent benefit of n-3 LCP supplementation for neurocognitive and behavioral development outcomes. However, participants in latter studies were mainly formula-fed infants from HICs that cannot be considered comparable with the breastfed IYC studied presently including with respect to growth and developmental risks.

In the present study, we hypothesized that n-3 LCP-rich fish-oil supplementation would have an enhanced effect on growth of children in poor settings by modulating immune function, resulting in alleviation of intestinal inflammation and chronic immune stimulation associated with EED and enhancing maturation of the immune system. We also hypothesized that an improved DHA status would enhance neurocognitive and behavioral development in IYC. Furthermore, we expected a more pronounced effect of the fish-oil intervention on these study outcomes working in a country with one of the lowest dietary n-3 FA supply reported globally.

However, empirical data from the present trial produced remarkably little evidence to support the proposed hypotheses that increased n-3 LCPs intake by IYC in a rural Ethiopian setting would lead to reduced morbidity and inflammation, and improved growth, nutritional status and development. The only positive finding detected on functional outcomes is the better ponderal growth in children who received fish-oil, as indicated by the small, but statistically significant, increase in relative weight gain and the trend towards larger MUAC increments over time. It should however be noted that the effect size attained on weight gain is too small to have meaningful impact on growth. Similarly, a fish-oil intervention was associated with higher MUAC and skinfold thickness in Gambian infants (291) and weight gain in Malawian children without improvements in linear growth or nutritional status (348).

The current study adds to the only two previous efforts in a rural African setting that also failed to produce evidence for a substantial benefit of fish-oil intervention on child gut integrity, inflammation, morbidity, growth, or cognitive development. A

Gambian study evaluated a direct supplementation of fish-oil in infants from 3 to 9 months old (291), and a trial in Malawian children 12-35 months of age used supplementation of multimicronutrient powder with fish-oil (348). This study further adds to these previous trials by testing different modes of delivery, i.e., supplementation through complementary feeding, lactation, and combination of both, allowing for a dose-response analysis by comparing the factorial combinations of interventions.

The possible explanation that has been given as to why fish-oil intervention did not achieve substantial benefits in the trials in Gambia and Malawi was that most children appeared to already have an adequate n-3 FA intake, although this is less likely in the present study. Red blood cell and plasma LCP analyses in the former trials did not show important n-3 FA deficiencies in the study children when compared to normative data from other settings. The average HM DHA level (0.77%TF) in the Gambian study mothers was also considerably higher than reported in most other populations. A review of 78 studies from 41 different countries estimated a global mean HM DHA concentration of 0.35%TF with country level values ranging from 0.10 to 0.84%TF (178). The semi-quantitative method used in this study to measure blood LCP does not allow us to measure the absolute LCP status in children. Our method collect dried blood spots and quantified LCP per volume extracted blood. These values are not comparable to values from literature reporting the relative LCP status in erythrocyte or plasma phospholipids as percent total fat. Given this methodological difference, the ratio of AA/(EPA + DHA) may provide a relatively better comparison of our results with others. The ratio of AA/(EPA + DHA) in our children at baseline (6-12 months old) as compared to plasma FA levels in the Gambian control infants at the age of 9 months show values that are almost three times higher (mean ± SD: 4.44 ± 0.73 vs. 1.28 ± 0.31).

HM DHA level is one way of assessing n-3 FA status in lactating women and DHA levels below 0.2%TF (~84 mg/L) have been suggested as indicative of increased risk of not supporting optimal infant development (404). From the analysis of baseline samples, the average HM DHA level in this study was 74 mg/L, indicating a very low maternal status with a potential risk of suboptimal development in their IYC. Dietary intake was not monitored in IYC of the current study. However, our earlier cross-sectional dietary surveys during both the pre- and post-harvest seasons in the same

population found extremely rare consumption of preformed n-3 LCP sources in the complementary diet, with only 1 out of 291 IYC who were studied consumed fish (369). In contrast, small fish consumption was reported to be 2.2 times/wk throughout the study period in the Malawian children, and 80% of the Gambian infants at the age of 9 months consumed some egg yolk or fish. Therefore, neither an adequate n-3 LCP intake from breastfeeding nor from complementary diet can be a likely explanation for the null results in the current study and potential impact of fish-oil intervention, if any exists, should have been noticed better.

The doses used in this study are very high compared to the adequate intake level recommended for older infants and young children, especially considering the children who received additional n-3 LCP through both complementary food and breastmilk. The dose of DHA in the child food supplement, even after taking into account compliance data, was also adequate compared to the dose that has been recommended for infant formulas to achieve definitive results on cognitive development (detailed in Chapter 6). The optimum dose of n-3 LCP taking into account the presence of EED and associated growth faltering remains unknown. It is noteworthy that studies of n-3 LCP as a therapeutic agent in chronic inflammatory conditions, such as inflammatory bowel disease, typically used much higher doses (119,317). However, in this and other similar child populations suffering from recurrent infections, the use of such high therapeutic doses raises safety concerns in relation to a potential excessive anti-inflammatory responses (472). A review of studies in animal models suggested that reduced pro-inflammatory responses due to increased n-3 LCP intake can improve or impair resistance to infectious challenge depending on the type of pathogen (415). Diminished inflammation due to n-3 LCP may reduce tissue damage, which could result in improved resistance against gram-negative bacteria. In contrast, it may also suppress cell-mediated immunity, which could in turn compromise resistance to gram-positive intracellular pathogens.

On the other hand, it should be recognized that the concentrations of n-3 LCP in HM found in our intervention mothers remained below our expectations when considering results from previous studies. A dose-response study of maternal supplementation during early lactation, which was also used as the basis for developing the study dose for our sample of lactating women, showed that DHA enrichment of HM to the global average of 0.32%TF (~134 mg/L) is possible through

intake of at least 167 mg DHA/d (398). The fish-oil intervention using a daily dose of 500 mg n-3 LCPs (215 mg DHA + 285 mg EPA) in our study significantly increased HM DHA concentration by 39% compared to the control. However, the average maternal milk DHA enrichment achieved with the supplementation was only 90 mg/L. Possible explanations for the relatively smaller effect in our intervention mothers may include the later stage of lactation, compliance to supplementation, and other factors requiring future investigation.

A number of factors might have diluted the anticipated impact of the fish-oil intervention. Children in both the control and intervention groups have received a close surveillance of morbidity and referral to primary health care, a preventive deworming medication for intestinal parasites, and a multimicronutrient fortified complementary food supplement. Although it cannot be determined in the present study design, these intervention components provided by the study setting might have some positive effects on the studied outcomes, thereby possibly diluting the potential effects of n-3 LCP supplementation in children living under the usual setting. The intervention components were considered in the study design for the purpose of avoiding any hampering of the efficacy of n-3 LCP by deficiency of other nutrients and intestinal parasite infections as well as some of the services usually delivered by the routine health care system. More importantly, it is unlikely that a single nutrient solution exists to improve the health and nutritional status of children in poor settings and from a program point of view, the potential of n-3 LCP intervention should be seen within the context of existing health and nutrition programs.

The interpretation of the lack of impact of the fish-oil intervention on child development outcomes has been detailed previously (Chapter 6). These include the timing and duration of the fish-oil supplementation which did not cover the entire period of expected high brain sensitivity to dietary DHA and the chosen assessment tools that might not be sensitive enough to detect effects on specific brain functions that are thought to be influenced by DHA. However, the Gambian trial also found no effect of their fish-oil intervention on early child cognitive development. This trial was started at an earlier age and used a wide range of tests of infant attention and problem-solving behaviors. Yet the infants in this study were reported to have adequate basal n-3 FA status. No other study assessing the effect of fish-oil on

development of IYC from LICs was found to compare our results to. Only a few studies conducted in HICs reported on observed benefits of n-3 LCP supplementation on development. However, the infants in these trials were exposed to different contextual conditions than IYC in the present study. Children in HIC consumed mainly child formula, while children in the present study did not have access to such products and were predominantly breastfed. Other contextual factors include a different nutrient intake and level of stimulation of the child that can affect neurocognitive development.

An important point for future consideration is that potential impacts of early DHA exposure on brain function may become more evident on cognitive abilities that emerge at a later age. Jensen et al. (263), for instance, supplemented lactating women with 200 mg DHA/d during four months postpartum and found no effect on neurocognitive development of study infants at 12 months of age. However, on later follow-up at the age of 30 months and 5 years, children of mothers who received the DHA intervention had significantly better cognitive development than children of the control mothers (263,265). Similarly, a longitudinal follow-up study of children who received infant formulas with different doses of DHA from birth to 12 months of age, found no effect of the DHA intervention using standardized tests of cognitive development at 18 months of age, but later at the age of 3 to 6 years, positive effects were detected on specific tests of executive functions, vocabulary, and IQ measures (242). These findings, although not consistent across all trials, imply the need for continued follow-up of the present study cohort to evaluate any long-term effects of the fish-oil intervention on cognitive development.

Most studies in HICs have been focused on LCPs supplementation during infancy and there is limited data relating to older infants and young children. Although not successful at having the anticipated impact, this study contributes to the evidence during this period when most children in LICs may be vulnerable to n-3 FA insufficiency due to the low content in the traditional complementary foods. On the other hand, considering the potential correlation between maternal DHA status during pregnancy and lactation (189), there is a concern that children in the present study might have also had inadequate fetal and early postnatal DHA exposure. During this period brain DHA accretion is the most rapid, as such, the potential for any impact on cognitive development potentially the largest. Therefore, future

interventions starting from pregnancy or early lactation may result in more observable impacts of n-3 LCP in this population. Furthermore, limited data from LICs suggest that a higher n-3 LCP status or supplementation during pregnancy is associated with reduced risk of preterm delivery and modest improvements in birthweight and length (473–476), which remains a research question for investigation in the current population.

The very low HM DHA concentration observed in our lactating mothers could be an indication of a suboptimal child cognitive development (404), which might not be captured by the currently tested domains. DHA rapidly accumulates in the brain perinatally, and involved in neurogenesis, neurotransmission and protection against oxidative stress (85,98,134,136–140). Animal studies show unequivocally that dietary n-3 FA deficiency during pregnancy and lactation severely impairs cognitive and behavioral performance (138). It has also been shown that maternal n-3 LCP intake during pregnancy and lactation and breastmilk DHA content are associated with child cognition (477). An international consensus recommends that the daily intake of DHA by pregnant and lactating women should reach 200 mg, which can be met by weekly consumption of 1-2 portions of fatty-fish (95,173). The HM DHA status in this study is consistent with the very low availability of fish and other seafood in Ethiopian diet reported previously (175). Therefore, while more trials evaluating the effects of an n-3 LCP intervention are required in the future, there should also be efforts exploring strategies to increase the n-3 FA intake among Ethiopian women during pregnancy and lactation.

Despite the health and nutrition interventions with or without fish-oil supplementation mentioned above, growth and nutritional status of the study children remained poor when compared to the WHO standards. The risk of developmental delay was also found to be prevalent based on Ethiopian benchmark. Previous energy and micronutrient supplementation and health interventions in similar settings generated inconsistent results on the prevention of child stunting. Therefore, some authors argue that this poor growth is not pathological for these children, but may be a beneficial adaptation mechanism allowing the use of limited resources to the best advantage (476). However, this claim is unlikely to be true given that child growth during the first few months of life is relatively good in most poor settings when infants are receiving exclusive breastfeeding and have a limited exposure to adverse

environmental factors. Several studies have shown that while some children follow the optimal growth pattern, others in the same population do not, and this variation in growth pattern has been explained by variations in exposure to different factors (40). Intraindividual variations in growth patterns have also been shown to depend on seasonal changes in food availability and other environmental stressors (478,479). Moreover, evidence shows that not only are the stunted children who have an elevated risk of mortality, but also the apparently healthy looking children with mild growth restrictions (i.e. *z*-scores between -2 and -1 SD) (480). Therefore, efforts exploring effective interventions to prevent the growth restriction and developmental delay experienced by children in poor settings should be continued. There is a critical need to better understand the causes and mechanisms behind growth faltering to assist in the development of effective nutrition interventions. The complex interactions between nutrition including essential FAs, the immune system, infection, and gut health in relation to growth faltering should be studied using more rigorous and mechanistic approaches.

Finally, there is still limited evidence on the potential impact of n-3 LCP intervention in low-income populations under different contextual settings and using different intervention approaches. For instance, observed effects of the fish-oil intervention may differ in a child population with a different degree of EED, infection and associated growth faltering as well as using a different timing, duration, or dose of supplementation. Therefore, further RCTs addressing the gaps in the few available studies are required before a conclusive remark can be made regarding the potential for n-3 LCP supplementation of IYC in low-income settings. Methodological considerations and thoughts for future research are discussed in the next sections.

7.3. Methodological considerations

In addition to the strengths and limitations that have been mentioned earlier in the relevant chapters, we address here general methodological issues requiring consideration while interpreting the findings.

The stunting trend analysis was limited to only potential drivers for which data were available in all DHS rounds for all the countries considered. Accordingly, the analysis did not consider some important potential drivers for which data were not available in the earlier DHS rounds, e.g. all of the IYCF indicators. Including additional indicators

from other data sources was also constrained by the difficulty of matching the year of data in other sources and the DHS rounds which also varied across countries. Furthermore, because of the focus on the countries that are partners for nutrition support by the DEVCO, this study evaluated the trend in child stunting in only 14 LMICs. As such, the generalizability of our findings to all relevant countries should be considered carefully.

The OME^{3}JIM study is a well-designed and implemented trial with sufficient statistical power to demonstrate a clinically relevant effect on child growth and development. The treatment was randomly allocated and concealment strategies were well implemented. All outcome assessments were performed blinded to treatment allocation and clearly defined study inclusion criteria were used. RCTs, by virtue of design, allow for elimination of multiple sources of bias and are therefore considered the gold standard for testing a causal hypothesis that a certain exposure affects an outcome of interest. On the other hand, even if variability is distributed evenly across treatment groups, sample heterogeneity may contribute to nuisance variability in study outcomes and potential heterogeneity of treatment effect (481). In this respect, participants in this study were recruited from 3 small rural communities sharing similar demographic characteristics, livelihoods, diets, IYCF practices, and other risk factors. Yet, sample homogeneity might have been affected by the recruitment of infants with a wider age interval. We had sufficient statistical power to detect clinically important group differences for most outcomes and no secondary analysis suggested effect-modification by age. However, we would have been more confident about the absence of any age-related differences masking the intervention effect provided that all infants were recruited starting from the age of 6 months.

Another important point requiring consideration is the extent to which the empirical data collected in a trial address the theoretically hypothesized effects of an intervention (482). Despite various dimensions of growth being measured, the present study used morbidity signs and CRP as the only proxy outcomes to evaluate effects on child immune function and health. A recent systematic review of evidence on the possible pathways from EED to stunting suggests the need for considering multiple indicators including markers of gut integrity, intestinal and systemic inflammation, and immune cell functions (324). The lack of such indicators in this

study hampered a more mechanistic understanding of the hypothesized effects of the fish-oil intervention. Furthermore, data on the burden of EED and inflammation, and the degree to which these conditions contribute to growth faltering in IYC in this study would have facilitated extrapolation of current results to children in other settings.

The sensitivity of the chosen methods for development assessment is one frequently cited possible reason for the inconsistent results from previous trials. Cheatham et al. (137) argue that tests of specific cognitive functions, such as memory, attentional control and higher-order cognition, may be more sensitive than global development measures to detect effects of DHA on brain function. Empirical data from n-3 LCP trials in general remain inconsistent for both the specific and the global cognitive tests with beneficial effects also detected in few studies using global development tools such as the BSID (232–234,252–254,263). In this study, we decided to use the Denver II and ASQ-SE tools of global development because none of the suggested specific cognitive tests are adapted for use in Ethiopian IYC and due to the lack of previous experience in administering such tests. We found the Denver II and ASQ-SE practical choices in our research setting as they were culturally adapted and validated in the same population as well as Ethiopian benchmark is available for the Denver II (440). These tools are also easily standardized for use in the field by non-expert staff and carried out on a large sample over short time, improving precision of measurements and statistical power of the study.

Finally, although IYC 6-24 months old in this study can be considered representative of similar aged children in the studied communities, the generalizability of the findings across different circumstances and populations should be carefully interpreted. It is not clear whether current results might differ if the fish-oil intervention was provided starting from prenatally or early lactation. The burden and etiology of EED and associated growth faltering, which were not measured in these IYC, may differ across child populations living in different contextual settings. Thus, to what extent the present results may be extrapolated to apply to children in other populations with a different degree of gut damage and growth faltering is less clear. The results might also differ if the doses were altered in magnitude or composition (e.g. DHA/EPA ratio) or the study was conducted in a population with a different basal diet (e.g. n-6/n-3 FAs ratio) or desaturase enzyme activity.

7.4. Thoughts for future research

A few points can be proposed for future research involving n-3 LCP intervention in mothers and children based on the previously described study findings and gaps in this PhD research, and in relation to existing literature.

Different intervention: timing and setting

The current study was aimed at addressing the timing of increased risk of enteric infections, and intestinal and systemic inflammation in relation to introduction of unhygienic complementary foods and a child's increased exploration of its surroundings. We also opted to limit the possibility that a high rate of exclusive breastfeeding in this population may mask effects of a fish-oil intervention provided during early infancy. On the other hand, our HM LCP data at baseline suggest that the current child population might also have had inadequate DHA exposure to support the rapid brain accretion during the fetal and early postnatal periods. Therefore, a future study in this population may find a different impact on infant cognitive development by conducting the n-3 LCP intervention starting from pregnancy or birth.

The burden of EED and its contribution to impaired growth of children, including the different possible pathways, could vary across settings. In this regard, the lack of data on the burden of EED in our study population limits extrapolation of the findings to children in other rural African settings. The studies in the Gambia and Malawi found no substantial benefits of fish-oil intervention despite the presence of a very high burden of EED. Yet, children in these studies were reported to have an optimal n-3 LCP status. Therefore, it is recommended that future studies replicate these trials in a child population with a known combination of a high burden of EED and a suboptimal n-3 LCP status.

Future follow-up of the study cohort

Potential impacts of n-3 LCP supplementation in early-life may change over time. As discussed earlier, effects on brain function may become more evident in cognitive and behavioral skills that emerge when these IYC get older. The fact that the global development tools used presently may have limited predictive validity for future cognitive performance is a further justification warranting future follow-up (452). Such a follow-up will also provide insights into any long-term implications of the modest

effects detected on ponderal growth, including whether these will be sustained, fade away, or become more pronounced with time, and relate to other functional outcomes in the future. Nutritional interventions, when applied at critical periods in early-life, may have the potential to remotely influence or program future health. Epigenetic modification may occur with n-3 LCP supplementation in early-life. Recent literature suggests that prenatal n-3 LCP supplementation may alter DNA methylation in genes involved in diverse biological processes including inflammatory and metabolic pathways, which may have a potential implication for inflammatory conditions and metabolic syndrome later on (483–485). Although not consistent, a few studies conducted in HICs also noted that n-3 LCP supplementation in infancy or maternal milk content during lactation may affect later childhood body composition and other markers of metabolic risk such as blood pressure and insulin sensitivity (486–488). The Gambian study also noted a delayed effect of the fish-oil intervention on body composition as shown by the significant increases in MUAC and skinfold thicknesses with the latter effect seen only at a continued follow-up after the intervention ended (291). It is therefore recommended that the present study cohort be followed to examine whether benefits which were not seen in IYC may manifest at a later age.

Additional measurements to be considered in the future

Future studies of n-3 LCP in the current and similar populations may provide more robust evidence by addressing current gaps with regard to various measurements mentioned earlier.

In addition to global measures of child development, specific cognitive skills that might be more sensitive to the role of DHA on brain function should be considered in future studies as well as in follow-up of the present study cohort. For this purpose, current challenges in administering such tests need to be addressed through cultural adaptation and validation of various age-specific tests for discrete aspects of cognitive function that have been applied in previous n-3 LCP trials in HICs (242). Furthermore, variability in relevant factors such as child rearing practices and the quality of home environment may modify the potential effects of n-3 LCP intervention on child development. In evaluating the effect of a prenatal DHA supplementation on child development at the age of 18 months, Ramakrishnan et al. (277) found a differential effect of the intervention by the quality of home environment. Therefore,

future studies should also consider measuring the level of child stimulation practices and the quality of home environment for a more rigorous analysis of the intervention effect.

Future studies of maternal fish-oil supplementation in this population should give attention to the relatively modest increase in HM DHA concentrations with the current intervention. A more mechanistic study in a small sample of lactating women may provide a better insight on the optimal dose before conducting a large-scale intervention study. For this purpose, a dose-response study applying stable isotope tracers and a more rigorous method for monitoring intake from both supplementation and basal diet should be considered in the future.

The potential impacts of n-3 LCP on child immune function and EED could have been understood better by using a variety of relevant biomarkers. These include markers of intestinal damage and repair, permeability and absorption, microbial translocation, and intestinal and systemic inflammation (324). It has also been suggested that a combination of biomarkers of intestinal inflammation and permeability may explain linear growth deficits better than the use of single biomarker (489). Furthermore, as mentioned earlier, n-3 LCP supplementation may result in changes in body composition. Therefore, in addition to gross assessments of child growth, future studies can capture this potential effect better by using more reliable measurements of body composition such as bioelectrical impedance, air displacement plethysmography and dual-energy X-ray absorptiometry.

Finally, emerging evidence on potential interpersonal variations in LCP requirements introduced new variables to be considered in the interpretation of n-3 LCP effects on child health and development. Lauritzen et al. (98), in a review of evidence from recent studies, emphasized that effects of DHA supplementation on the infants' brain may depend on gender and genotype of genes involved in the endogenous synthesis of DHA. No statistical evidence was found for differential effects between boys and girls in this study. However, to what extent heterogeneity in FADS genotype pattern affected the results on development or the other outcomes cannot be ascertained at this time. Thus, it would be of great interest to secure data on FADS genotype of our subjects in a future study to examine any effect-modification of the fish-oil intervention. More importantly, future studies may provide a more clear

answer on the impact of n-3 LCP supplementation by taking into account these factors at the design stage including a priori hypothesized subgroup effects with adequate sample size. The use of the Mendelian randomization approach was also recommended for evaluation of the role of FADS gene clusters in the association between dietary n-3 LCP and functional outcomes (98).

Future studies of trend analysis

The double-burden of malnutrition is an emerging public health concern in LMICs. In populations undergoing rapid nutrition transition, childhood stunting and overweight coexist (490), and evidence showed that nutritionally stunted children may have an increased risk of excessive weight gain during nutrition interventions and in later life (491). In emerging countries, the challenge ahead is achieving a rapid stunting reduction while keeping child overweight/obesity at bay, which has also been echoed in the WHA global targets. Thus, it is of great interest that future studies involving trend analysis monitor not only the trend in child stunting in LMICs, but also the trend in the double-burden of malnutrition at the level of individuals, households and countries. This is also critical to understand the shared drivers and identify double-duty actions for interventions, programs, and policies that eradicate child undernutrition without contributing to overweight/obesity and the associated risk of nutrition-related chronic diseases (492).

Other nutrition studies in this population

This study noted a high risk of growth faltering and developmental delay among Ethiopian IYC in rural areas, requiring attention of future research and programs. It is also a concern that women have potentially inadequate dietary n-3 LCPs intake during pregnancy and lactation, as indicated by the maternal milk DHA content in this study which is also in agreement with a previous country level estimates of n-3 FA consumption (175). Before recommendations on context specific interventions, in-depth epidemiological research is required to gain more insight into the important health and nutritional contributors underlying the poor growth and development of IYC in these settings. The contribution of poor hygiene and sanitation, and contaminants in complementary diet to enteric infections and EED as well as the different pathways that link EED and growth faltering should be studied in the context of the current population. In addition to nutrition and health interventions, studies have also shown that child stimulation may have an effect on child development

(493–495). Therefore, the role of suboptimal child care and stimulation practices for child development performance in the current population should be closely understood. Furthermore, future studies exploring the association between dietary essential FA and various outcomes in pregnant women and IYC will provide more insight into the health consequences of the very low n-3 LCP concentration in the Ethiopian diet.

7.5. Policy recommendations

7.5.1. Acceleration of the trend in reducing chronic child undernutrition

Meeting the WHA global target of childhood stunting reduction requires acceleration of the current progress in many LMICs. Evidence suggests that accelerated gains are possible in these countries if the coverage of direct nutrition interventions can be scaled-up extensively along with large-scale implementation of nutrition-sensitive development programs. However, the realization of this potential requires addressing various challenges at different levels.

Ensuring political and policy commitment for the WHA nutrition targets

The nutrition society has made great achievements in the past few years in building the political and policy commitment that placed nutrition at the center of the SDGs agenda. This encouraging momentum should be maintained and further strengthened for the globally endorsed nutrition targets to be translated into results on the ground. One of the main challenges for a rapid progress in child nutrition is the lack of adequate funding to expand key nutrition-specific interventions from their current very low coverage in LMICs. Therefore, further political and policy commitment should be garnered at all levels to fill the current funding gap through rapid expansion and strong coordination of investment from both domestic financing and development assistance. Another important challenge is the lack of adequate nutrition-sensitive approaches in various development activities in LMICs. It is well recognized that sustainable progress in nutrition requires a multisectoral approach, and not just the up-scaling of nutrition-specific interventions. However, this requires a strong coordination and collaboration between various development actors and stakeholders at the international and national level. Decision makers and program planners at different levels should be informed that integrating nutrition goals and actions across development sectors is a critical input to achieve progress in other development targets. Furthermore, there is a need for strong accountability

mechanisms that will enforce national governments and their development partners to meet their commitments for the nutrition targets endorsed globally.

Maximizing the impact of current interventions

Acceleration of the progress in reducing child undernutrition requires strategies that maximize the impact of existing direct and indirect investment in nutrition. A large-scale implementation of the proposed package of nutrition-specific interventions and reaching populations at most risk should be made feasible by identifying various delivery strategies that are effective, low-cost and sustainable. In addition to the traditionally used health and nutrition programs, diversification of delivery platforms should be considered through the integration of nutrition-specific interventions into various development programs. Furthermore, investments in various development activities should be exploited to contribute to better progress in nutrition. There is a need for enhancing the nutrition sensitivity of distal development programs in LMICs so that their potential to deliver on maternal and child nutrition outcomes is unleashed. It has been suggested that the nutrition-sensitivity of development programs can be enhanced through better targeting of program beneficiaries on the basis of nutritional vulnerability, and by incorporating nutrition goals and actions as well as strengthening their effective implementation. Development programs should also engage women actively by including interventions that protect and promote their nutritional and health status, social status and decision making. Additionally, investment across different development sectors should follow evidence-based decision-making processes that take into account health and nutritional outcomes among indicators of overall progress. This can be made possible through establishing effective mechanisms to track the impact of investment in various development programs on health and nutritional outcomes.

7.5.2. Improving n-3 fatty acid status in Ethiopian women and children

The limited available studies from rural African settings did not provide substantial evidence to support the routine supplementation of n-3 LCP as an intervention strategy to improve child growth and development at this time. However, the importance of an adequate supply of n-3 LCP for ensuring optimal development of brain and visual systems during early-life is well-established. There is also evidence that the dietary supply of n-3 LCP influences pregnancy outcome and a range of metabolic and immune functions. The FAO food balance sheet data show that the n-

3 LCP supply in the Ethiopian diet is far below the recommendations for optimal health. However, the consequences of this very low intake have received little attention so far, despite potential major implications for child development and subsequent learning capacity. Policies and programs in nutrition and other relevant sectors should take various measures in the future to protect vulnerable groups from potential adverse health and functional outcomes due to n-3 FA insufficiency. These are discussed in the following sections.

Evidence on n-3 fatty acid status of vulnerable groups

Investigating the burden of n-3 FA insufficiency and its health implications is the first step to inform and guide relevant policy, strategy and programs. Therefore, the dietary intake and status of Ethiopian women of child bearing age and IYC should be defined for various PUFAs, and the associated impact on health and nutrition outcomes should be estimated. This can be facilitated through existing platforms of health and nutrition surveys that are conducted at a regular interval. The Ethiopian National Food Consumption Survey in the future may consider reporting dietary consumption of various PUFAs in addition to total fat intake. For this purpose, the current Ethiopian food composition table, which reports only total fat content of food items, should be revised to include FAs profile including PUFA contents. Information on PUFA profile of Ethiopian foods is also important for informing consumers and various stakeholders, including in the design of food-based dietary guidelines and other strategies aimed at improving n-3 FA status. Additionally, measuring PUFA status in children and women, including the content in breastmilk, can be incorporated into the National Micronutrients Survey.

Breastfeeding promotion, support and protection

A key strategy to improve the adequacy of n-3 LCP intake in IYC in low-income settings is promotion of universal exclusive breastfeeding in the first 6 months and continued breastfeeding up to at least the age of 2 years. Promotion of optimal breastfeeding practices is also one of the most effective strategies identified to improve child survival in LICs. Ethiopia has made limited progress in this regard. For instance, the National Nutrition Program in its second phase (2016-2020) has set a target to increase the rate of exclusive breastfeeding by 22% from 58% in 2016 to 80% in 2020. In contrast, only a 1% increase in the rate of exclusive breastfeeding achieved nationally between 2016 and 2019. Therefore, there is a critical need for

scaling up the coverage and effectiveness of interventions for breastfeeding promotion, protection and support in the country. Current programmatic and implementation gaps responsible for the limited progress should be identified and addressed in the future. A combination of health facility- and community-based intervention approaches has been identified as particularly effective in bringing sustainable behavioral change in breastfeeding. In this regard, the Community Based Nutrition program implemented in the country should be used as a platform for a large-scale breastfeeding promotion and support intervention. The outreach strategy involving health extension workers and local women peer educators can be exploited to reach mothers living in remote areas with limited access to health facilities and media, and to mobilize local community participation and support for mothers to breastfeed. This approach can also create the opportunity to deliver effective messages that are tailored to the specific context including the local social norms, and enablers and barriers to breastfeeding.

Furthermore, breastfeeding promotion should be complemented by strategies that create an enabling environment and community support for mothers. Women working both in the formal and informal sectors need to be empowered to exclusively breastfeed by enacting appropriate maternity protection laws as well as policies that encourage breastfeeding in the workplace. Current Ethiopian labor proclamation allows only a 3-month postpartum leave, which may require amendment to cover at least the exclusive breastfeeding period of 6 months. Furthermore, the use of infant formula products may increase in the future with urbanization and increasing women's involvement in the formal work sector. The Ethiopian Government recently took an encouraging step by endorsing a directive on infant and follow-up formula that will provide the opportunity to encourage and protect breastfeeding if implemented effectively. In the future, the directive should also consider protecting exclusively formula-fed infants from a potential risk of inadequate brain DHA accretion. This can be made feasible by considering the fortification of infant formulas with preformed DHA as one of the mandatory criteria for appropriate standard of formula products.

n-3 fatty acid in other nutrition interventions and strategies

It should be noted that the n-3 LCP composition of breastmilk as well as the transfer to the fetus during pregnancy are vulnerable to influence by the maternal diet. Thus,

even breastfed infants may not be protected from a risk of suboptimal development when the maternal dietary intake of n-3 LCP during pregnancy and lactation is very low. Furthermore, children become more vulnerable to n-3 FA insufficiency with the introduction of a very low content complementary foods. Therefore, additional strategies to promotion of breastfeeding are required to improve dietary fat composition in support of n-3 FA intake in women and IYC. However, current nutrition programs in Ethiopia lack strategies that specifically target the adequacy of n-3 FA. Strategies and interventions to improve the quality of complementary foods and women's diet largely focused on energy and micronutrients content with little attention given to quality of the FA supply. This is in contrast to the attention that has been given to some micronutrients such as iron, zinc, iodine and vitamin A. Future efforts for identifying nutrient gaps and developing dietary strategies should look beyond total fat intake and consider the adequacy of n-3 FA in the diet. Fish and other seafood are the important sources of n-3 LCP in human diet, while the precursor ALA can also be obtained from some oils, green leafy vegetables, and legumes. Food-based dietary guidelines and nutrition recommendations should encourage the consumption of both ALA and preformed n-3 LCP sources by women and IYC. Dietary recommendations for Ethiopian women may also consider adopting the international consensus on recommended fish consumption during pregnancy and lactation. Furthermore, dietary essential FA balance has important implications for n-3 LCP adequacy, especially in populations with very low fish consumption. Diets high in LA content compromise the endogenous conversion of ALA to n-3 LCP and the incorporation of n-3 LCPs into infant and maternal tissues including breastmilk. Therefore, formulation of food-based dietary guidelines and nutrition recommendations in the future should also take into account the recommended LA and ALA balance in the diet.

The few available intervention strategies that give attention to quality of the FA supply such as lipid-based nutrient supplementation (LNS) also miss important aspects of n-3 FA metabolism. It should be noted that increasing dietary ALA alone may not be an effective strategy to improve the status of n-3 LCP, DHA in particular. The metabolic conversion rate of ALA to n-3 LCP is typically low and also influenced by genetic profile and background diet including the LA content. The conversion of ALA from the diet could be further decreased in populations with inadequate energy

and fat intake and prevalent micronutrient deficiencies such as iron and zinc. Therefore, effective interventions to improve the DHA status of populations at risk require a combined approach involving an increased intake of fish and ALA sources together with ensuring an optimal balance of essential FAs. LNS products, including ready-to-use therapeutic foods as well as the various preventive LNS products for IYC and pregnant and lactating women, provide the essential FAs ALA and LA, but not preformed n-3 LCP. These products usually contain marginal amounts of ALA and a high LA content due to the LA-rich vegetable oils predominantly used to increase energy density. As a result, it is unlikely that currently available LNS products provide effective protection against n-3 LCP insufficiency and associated health outcomes. It is therefore important that future LNS formulations consider products fortified with preformed DHA and a lower LA content which could lead to enhanced impact of LNS interventions on pregnancy outcomes and child health and development.

n-3 fatty acid supply in the food system

A sustainable approach to improve n-3 LCP status of vulnerable groups is improving the availability, accessibility, affordability, and desirability of fish and other n-3 FAs sources in the food system. Ethiopia is a land-locked country depending on inland water bodies for domestic fish supply, with an estimated annual yield potential of around 94,500 metric tons (496). However, only about 20% of this potential is harvested presently and fish consumption contributes to only 1% of the animal protein intake in the country. Furthermore, aquaculture production is negligible in Ethiopia despite the favorable ecological conditions available for fish farming. It has been estimated that year-round farming of coldwater fish species can be achieved in the country's highlands covering 11% of the surface area and there is also additional potential for farming an array of coldwater and warmwater species in other parts of the country (497). This currently underexploited potential for fish production leaves significant room for further development to improve the very low n-3 LCP consumption rate in the country. For this purpose, fishery and aquaculture should be fully integrated into Ethiopia's food security policies and plans including in the National Agricultural Sector Policy and Investment Framework, and the National Water Sector Strategy. The current predominantly artisan fishery production system should be improved by providing inputs and transfer of technologies to producers.

Development of aquaculture should be considered in existing irrigation, farming and water harvesting schemes. Although the Ethiopian water bodies support diverse aquatic life including more than 180 identified fish species, only few species are commercially important. The species with better n-3 LCP content should be identified and their production and consumption should be encouraged in the future.

It is also important to address various challenges in the fish market chain. There is a wide spatial variation within the country in terms of the production and market availability of fish products, which are mainly driven by access to infrastructure such as roads and transportation. Furthermore, most of the population is yet to integrate fish into their diet and consumption pattern is seasonal, limited to only few fasting months per year. These important limitations in the fish market chain should be addressed through expansion of infrastructure, adoption of appropriate post-harvest management technologies, and promotion of fish consumption in the population.

7.6 Conclusive remarks

Finally, the following remarks are made from this PhD work:

- The current trend in stunting reduction in LMICs requires further acceleration to meet the WHA global target.
- Current trend in stunting reduction could be further accelerated by a combination of economic development and nutrition-sensitive interventions, on top of nutrition-specific programs.
- There is a need for more evidence on the potential impact of n-3 LCP supplementation on healthy growth and development of IYC in low-income settings.
- There is a critical need to improve the essential fatty acids adequacy in the diets of Ethiopian pregnant and lactating women and infants and young children.

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References

Curriculum vitae of the author

Alemayehu Argaw Alemayehu was born on September 25, 1982 in Arba Minch, Ethiopia. He started schooling in Shashemene Catholic Church Primary School and took the Ethiopian School Leaving Certificate Examination (ESLCE) in 2001. The following year he started studying Public Health Science in Alemaya University and received his Bachelor of Science Degree in 2005. He started working in Menschen fur Menschen and a private health science college for two years. Then, he studied his second degree in Applied Human Nutrition at Hawassa University for two years. Starting from 2011 till now, he is working at the Department of Population and Family Health, Jimma University.

In 2015, he started his PhD study at the Department of Food Technology, Safety and Health, Ghent University, under the VILR-OUS scholarship program in collaboration with Jimma University. Alemayehu speaks Amharic and English languages.

Peer reviewed publications

1. Workicho A, Belachew T, Argaw A, Roba A., Ghosh S, Kershaw M, Lachat C and Kolsteren P. Maternal nutritional status mediates the association between maternal age and birth outcomes. Matern Child Nutr. 2020; e13015.
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Services

- **Assistant Professor**

October 2016 - to date: Department of Nutrition and Dietetics, Jimma University.

- **Lecturer**

January 2010 - September 2016, 2016 to date: Department of Population and Family Health, Jimma University.

September 2009 - December 2010: Department of Public Health Science, Arba Minch University.

- **Head of Department and Instructor**

January 2006 - September 2007: Department of Public Health, Harambe College

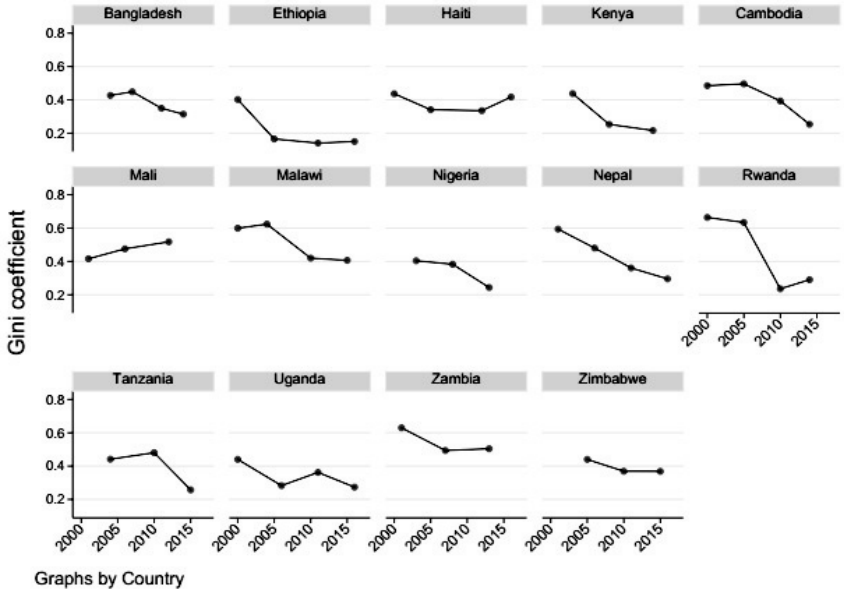
- **Program Officer of HIV/AIDS Care and Prevention Program**

January 2005 - December 2007: Menschen fur Menschen

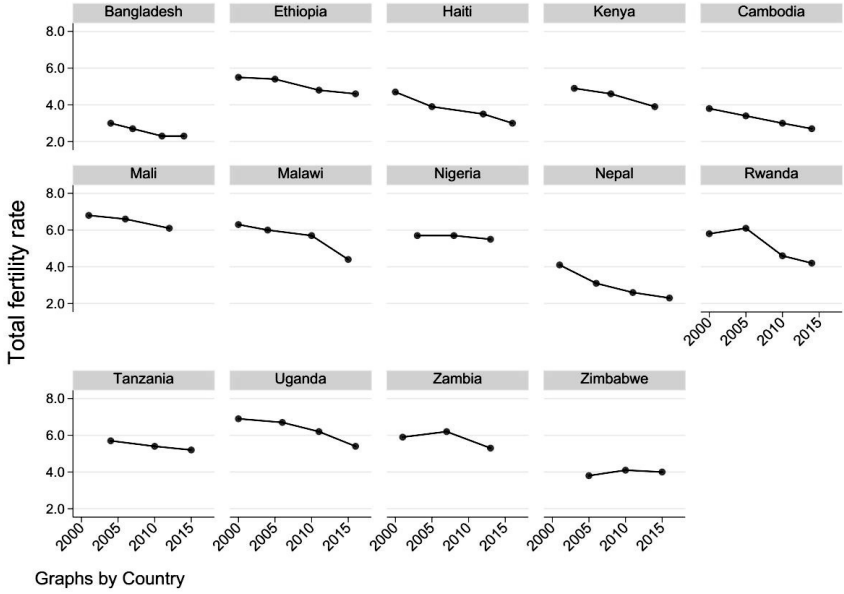
Annexes

Annex 1: Trends in stunting drivers by country

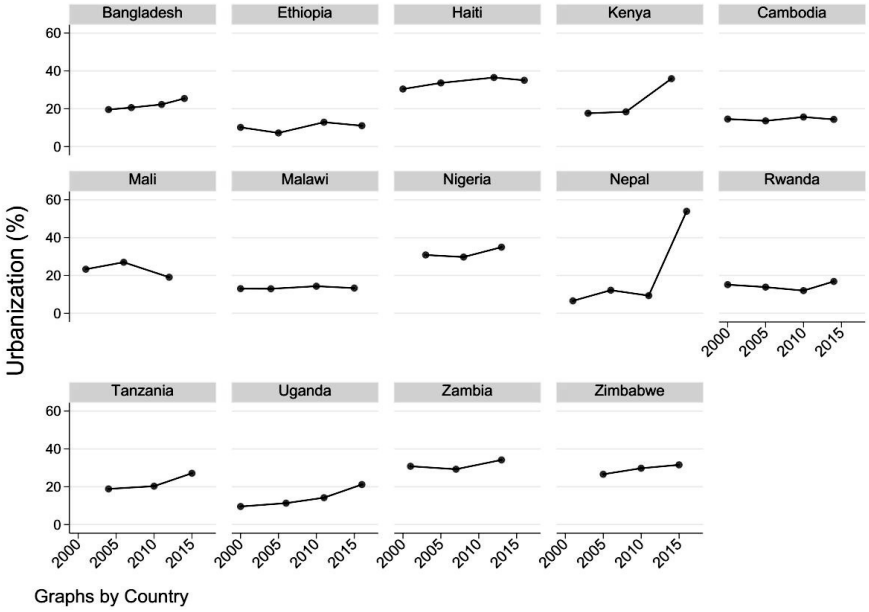
1.1 Gini coefficient



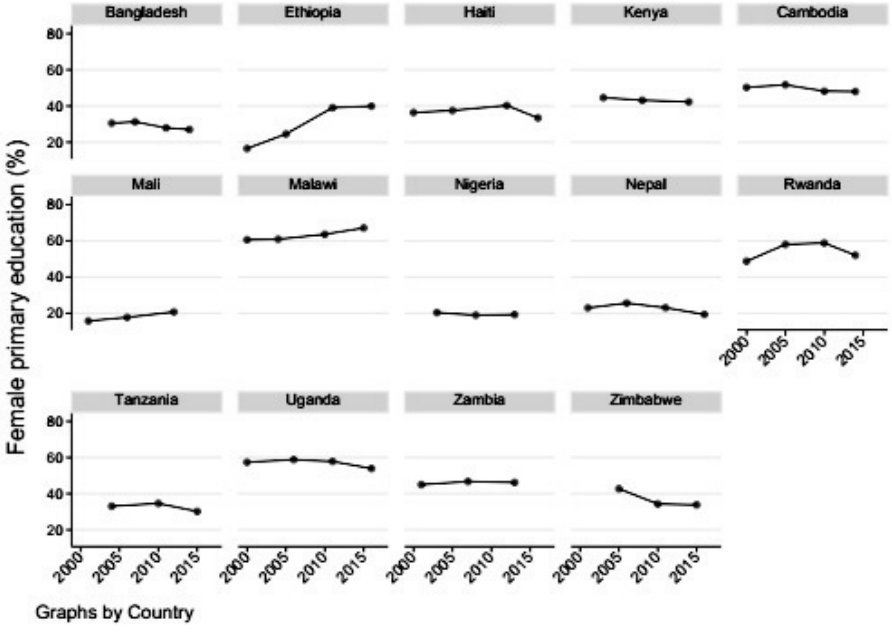
1.2 Total fertility rate



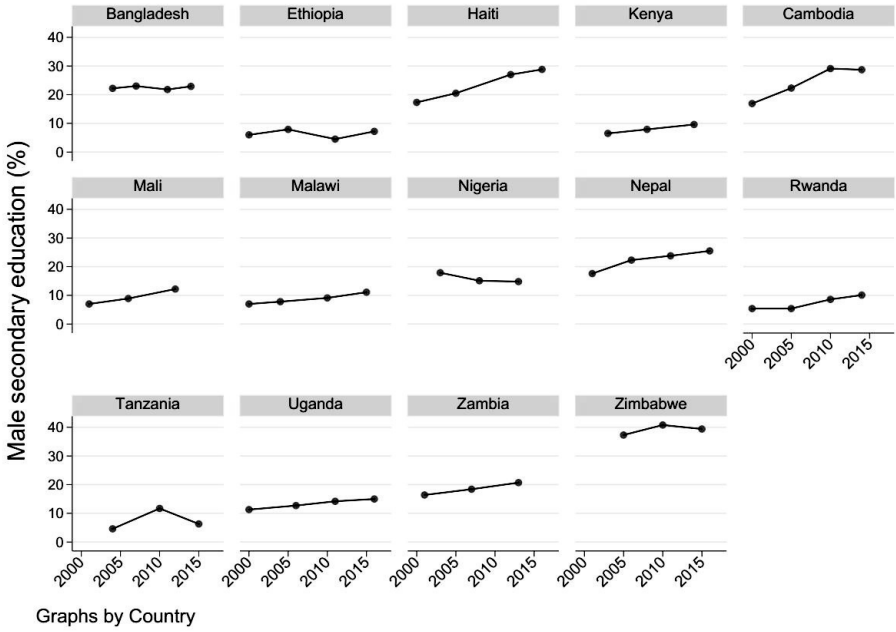
1.3 Urbanization



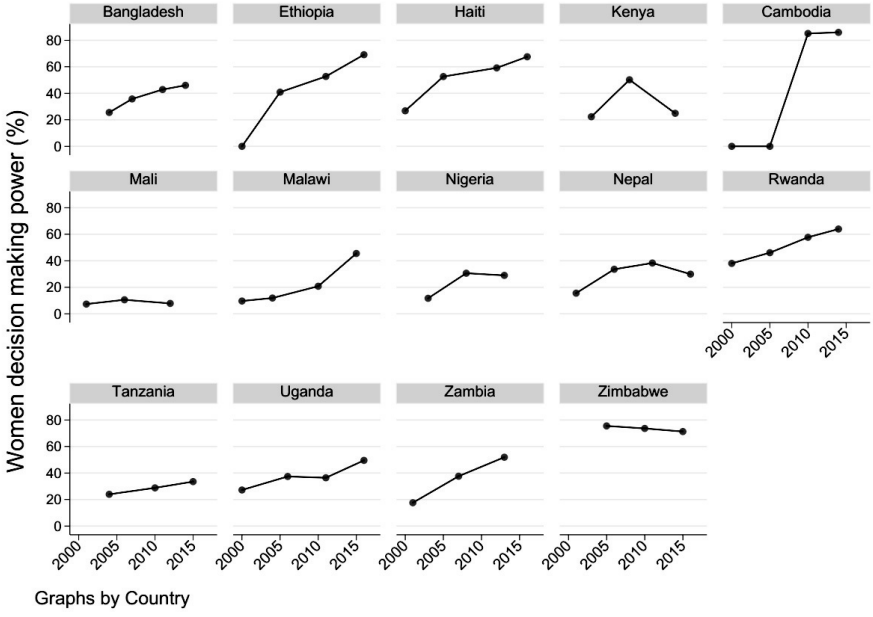
1.4 Female primary education



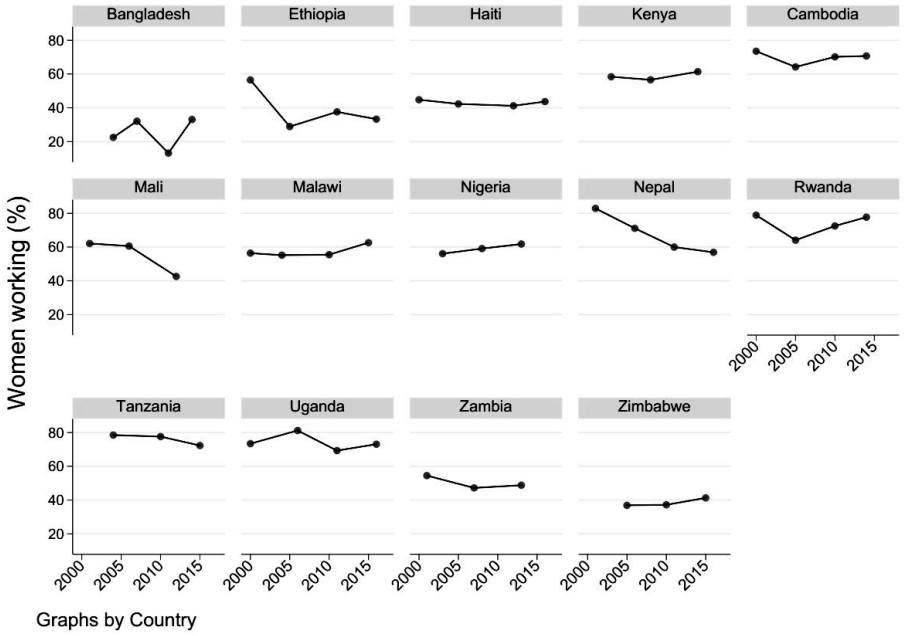
1.5 Male secondary education



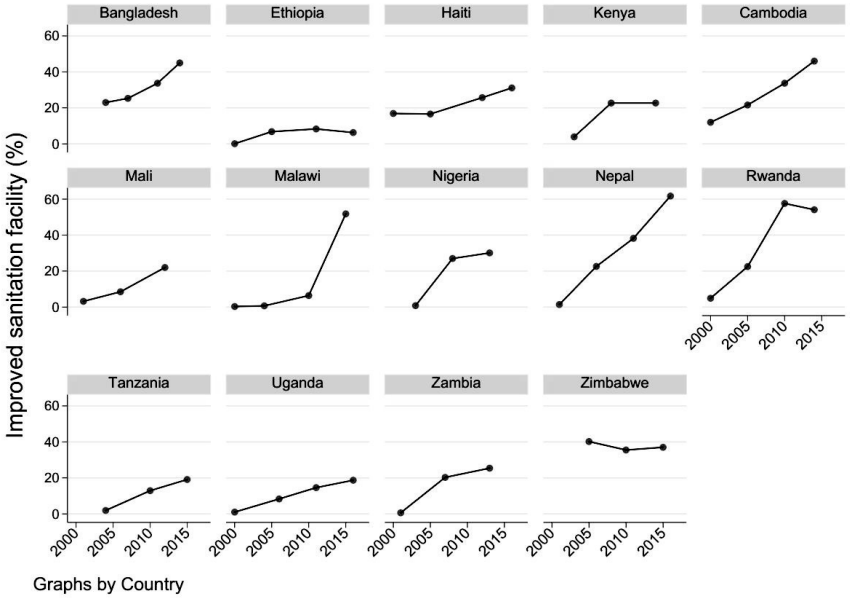
1.6 Women's decision-making power



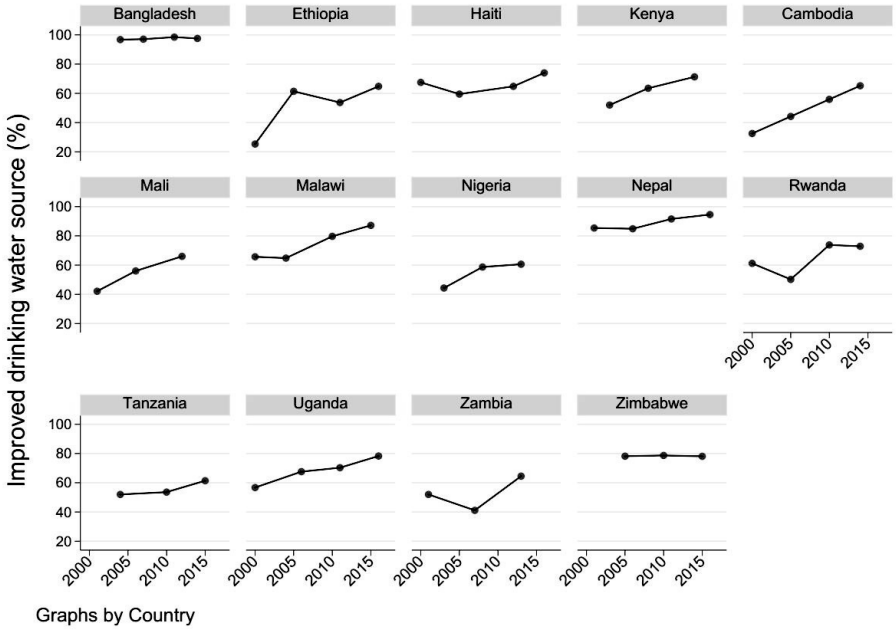
1.7 Women working



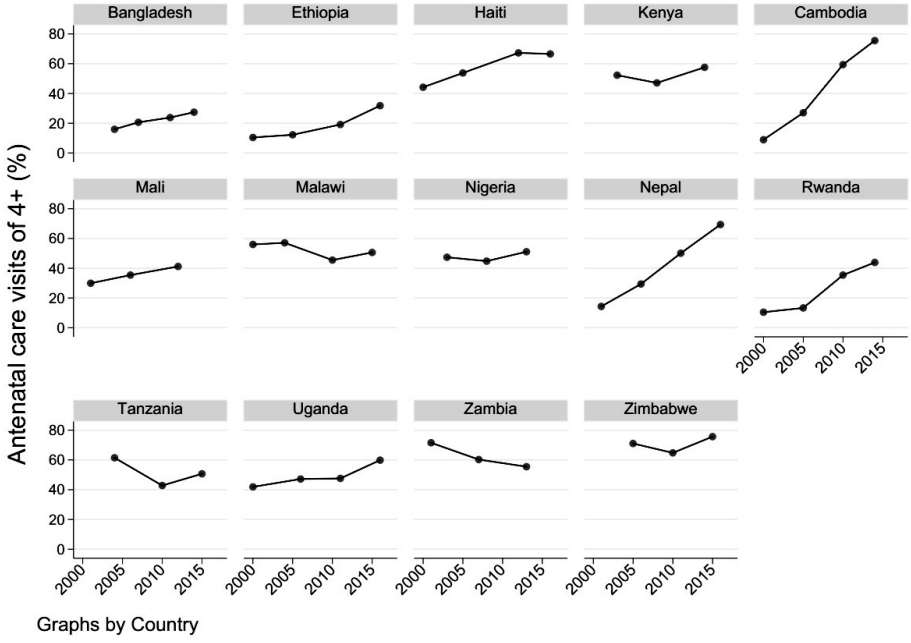
1.8 Improved sanitation facility



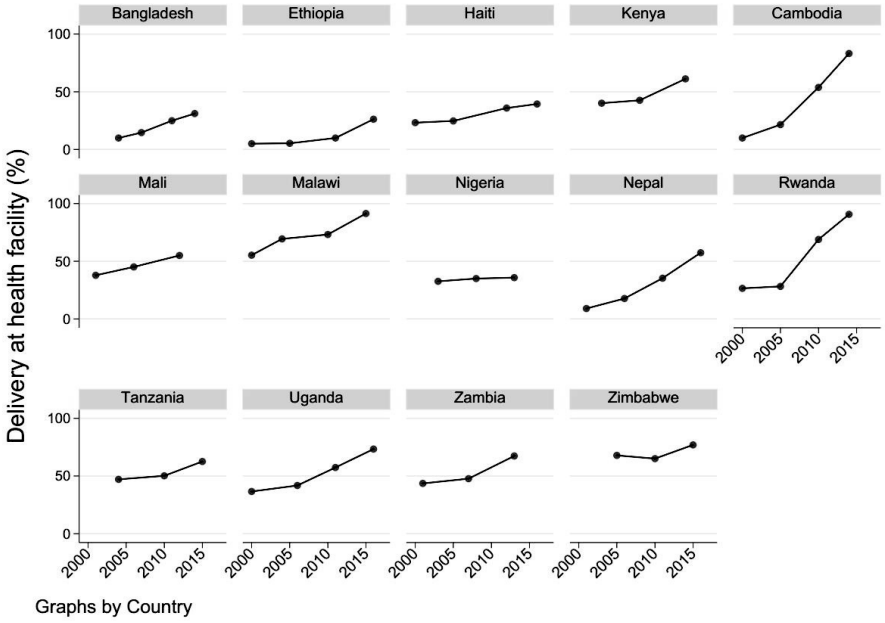
1.9 Improved drinking water source



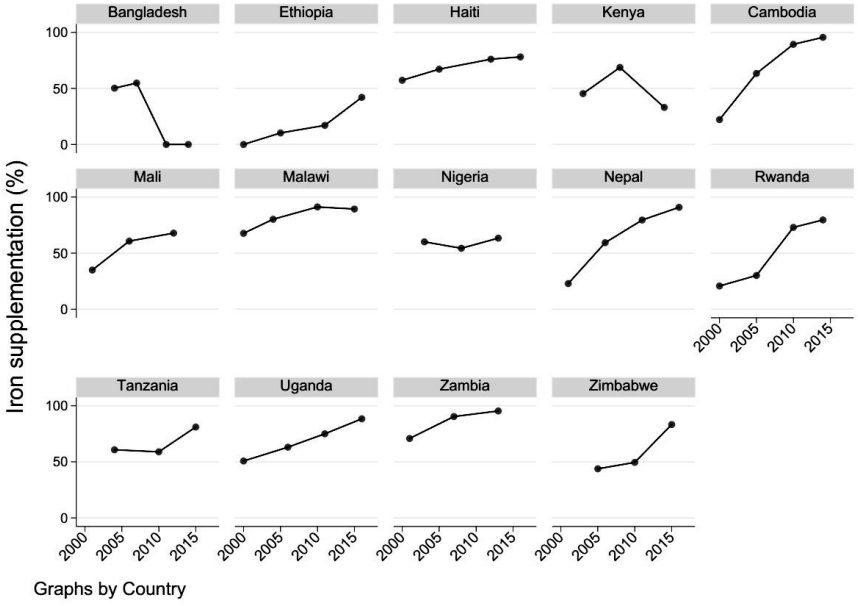
1.10 Antenatal care follow-up of 4+



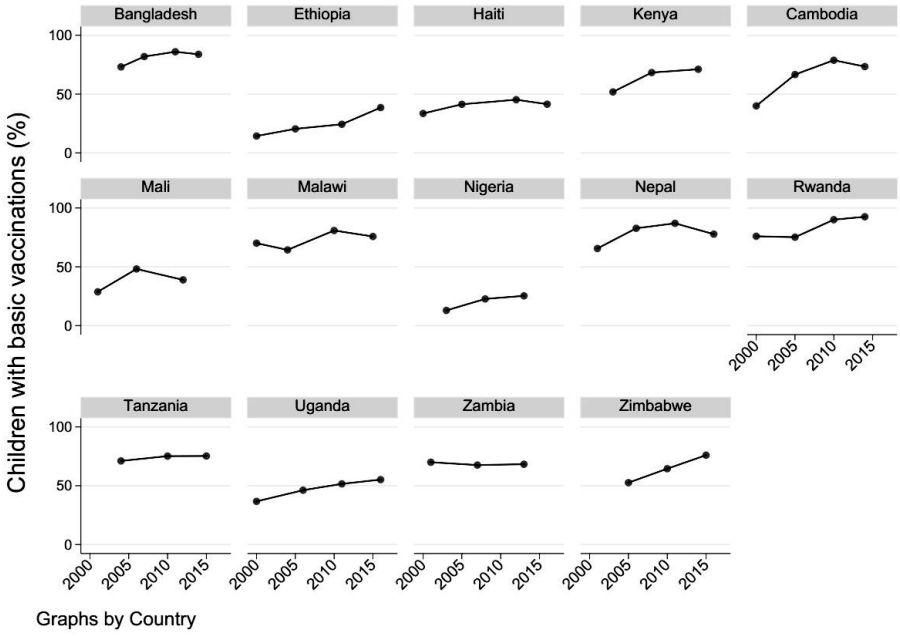
1.11 Delivery at health facility



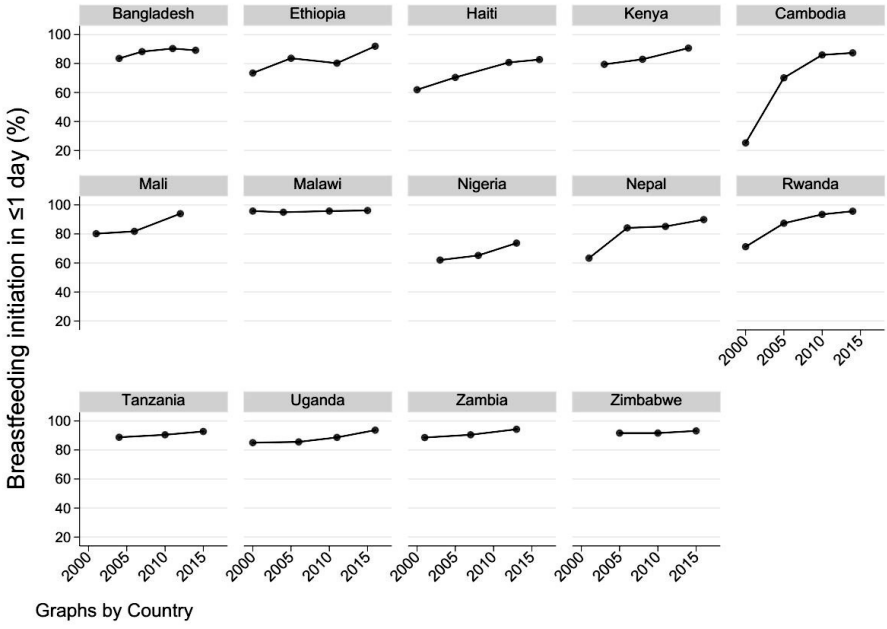
1.12 Iron supplementation during pregnancy



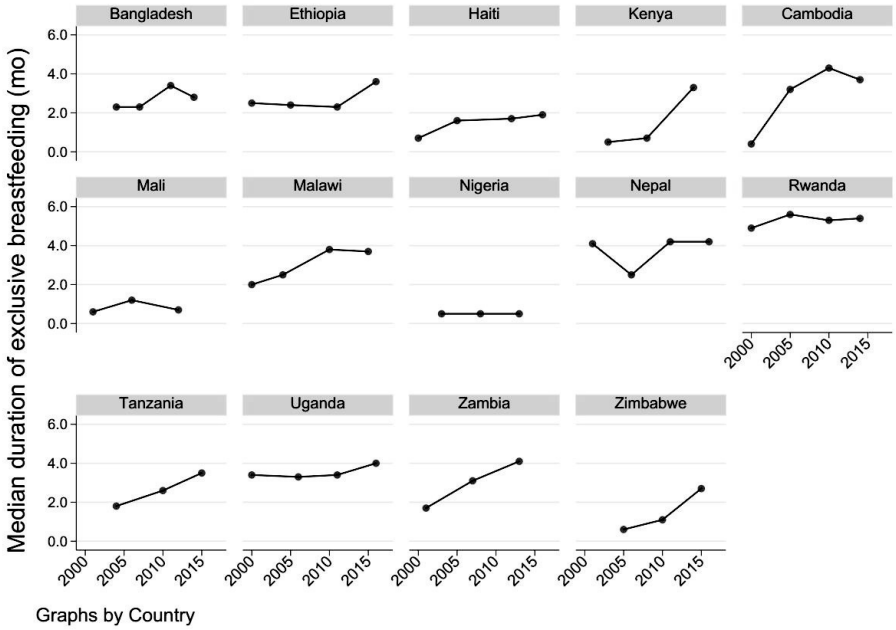
1.13 Children with basic vaccinations



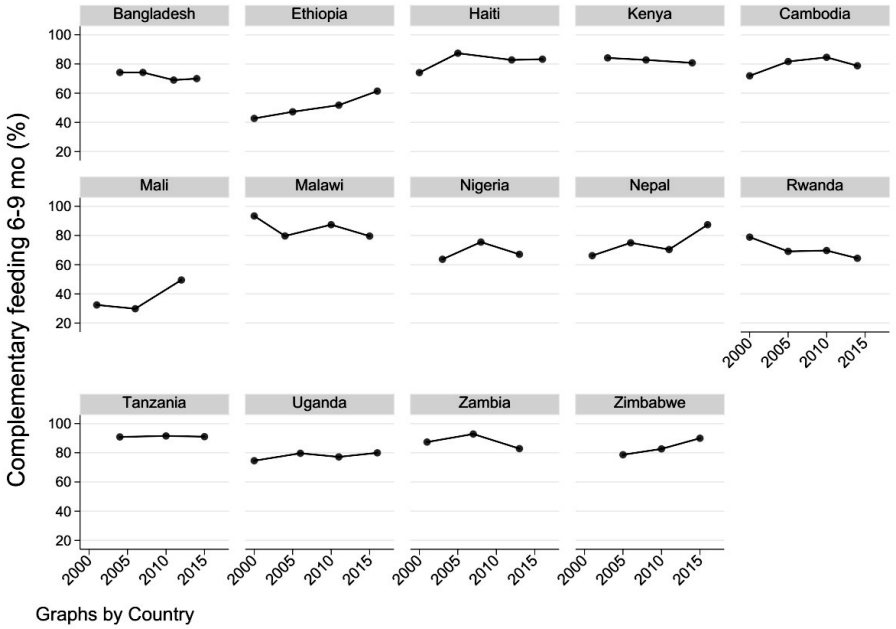
1.14 Breastfeeding initiation within ≤ 1 day



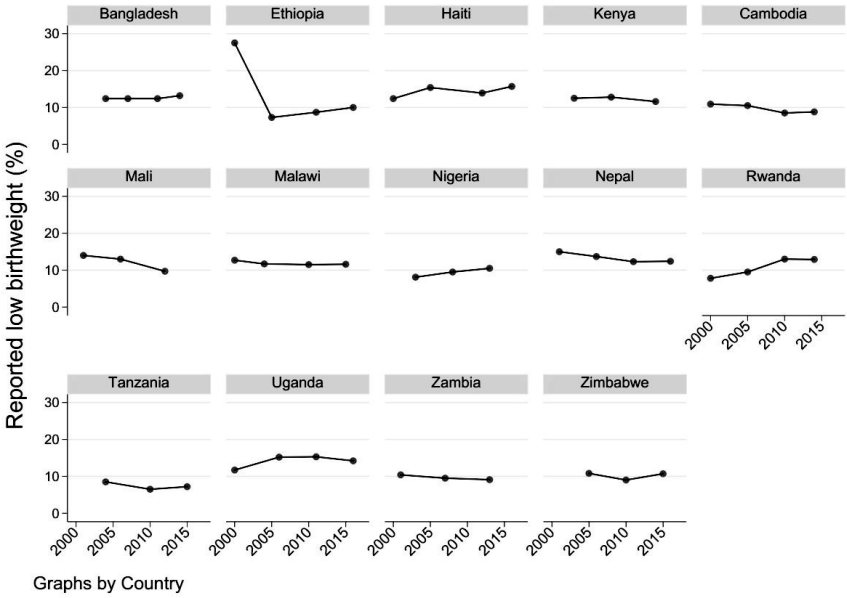
1.15 Median duration of exclusive breastfeeding



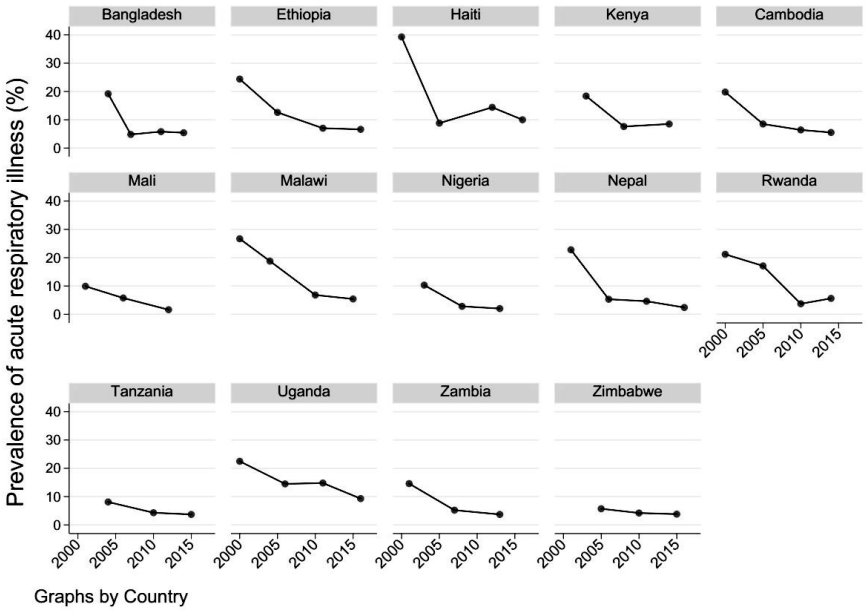
1.16 Complementary feeding 6-9 months age



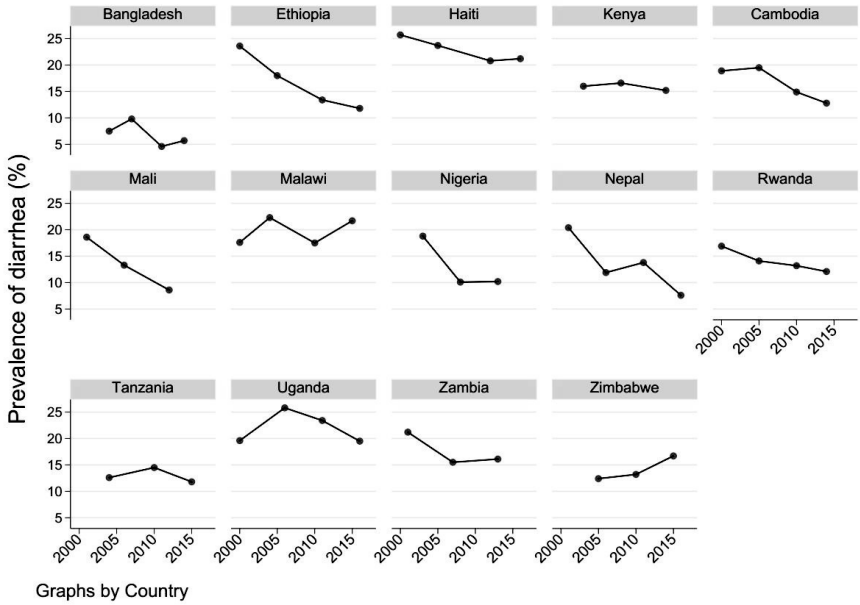
1.17 Reported low birthweight



1.18 Prevalence of acute respiratory illness



1.19 Prevalence of diarrhea



Annex 2: Comparing characteristics of participants in the HM sub-study and all participants in the main trial[1]

Characteristics	HM subsample (*n* = 154)	Main sample (*n* = 360)	*P*
Maternal age, y	25.9 ± 5.08	26.1 ± 5.14	0.68
Child age, mo	7.7 ± 1.2	8.9 ± 2.1	<0.001
Primiparous	41 (26.6)	99 (27.5)	0.84
Marital status, married	148 (96.1)	345 (95.8)	0.89
Maternal education			0.75
No formal education	77 (50.0)	179 (49.7)	
Primary education	53 (34.4)	133 (36.9)	
Secondary and above	24 (15.6)	48 (13.3)	
Household head education			0.99
No formal education	50 (32.5)	117 (32.5)	
Primary education	70 (45.5)	165 (45.8)	
Secondary and above	34 (22.1)	78 (21.7)	
Household wealth tertiles			0.54
Lowest	53 (34.4)	120 (33.3)	
Middle	57 (37.0)	120 (33.3)	
Highest	44 (28.6)	120 (33.3)	
Breastfeeding frequency			0.84
4-6 times/d	16 (10.39)	35 (9.72)	
7-9 times/d	38 (24.68)	98 (27.22)	
≥10 times/d	100 (64.94)	227 (63.06)	
Maternal height, cm	157 ± 5.26	157 ± 5.48	0.97
Maternal weight, kg	50.6 ± 6.99	50.3 ± 7.61	0.67
Maternal BMI, kg/m^2	20.5 ± 2.59	20.4 ± 2.74	0.61

[1]Values are presented as means ± SDs or n (%). *P*'s comparing study arms using independent sample t-test or Pearson's chi-squared test. HM, human milk.