ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Investigation of the exposure to low-iron multiple micronutrient powders (MNPs) on development, anaemia and growth of infants and young children in Sidama Zone, Southern Ethiopia

By:

Ashenafi Geletu Hayi

Dissertation Submitted to the Center for Food Science and Nutrition in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (Ph.D.) in Food Science and Nutrition

Advisor

Dr. Kaleab Baye (Asso. Prof)

Co-Advisor

Dr. Seid M. Adem (Asso.Prof)

June 2018,

Addis Ababa

Center for Food Science and Nutrition
College of Natural Sciences
Addis Ababa University

This is to certify that the thesis prepared by Ashenafi Geletu Hayi entitled: ″ **Investigation of the exposure to low-iron multiple micronutrient powder (MNPs) on development, anaemia and growth of infants and young children in Sidama Zone, Southern Ethiopia**″ submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition, complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Prof. Judith Kimiywe__________	______________________	06/28/2018__
Examiner	Signature	Date
Dr. Afework Mulugeta_________	_____________________	06/28/2018__
Examiner	Signature	Date
Dr. Kaleab Baye______________	______________________	06/28/2018__
Advisor	Signature	Date
Dr. Seid M. Adem_____________	______________________	06/28/2018__
Co-Advisor	Signature	Date
Dr. Poulos Getachew_______	______________________	06/28/2018__
Chair of Department or Graduate Program Coordinator		

Investigation of the exposure to low-iron multiple micronutrient powder (MNPs) on development, anaemia and growth of infants and young children in Sidama Zone, Southern Ethiopia

Ashenafi Geletu Hayi, Ph.D. Candidate in Food Science and Nutrition
Addis Ababa University, 2018

ABSTRACT

Micronutrient malnutrition is estimated to affect 2 billion people worldwide. The deficiency is more prevalent in infants and young children during complementary feeding period. Inappropriate complementary feeding contributes to growth faltering, anemia, and cognitive impairments. Among the various strategies designed to prevent and treat the nutritional deficiency home fortification with micronutrient powders (MNP) is a novel alternative for delivering micronutrients with foods. Limited programmatic evidence exists on the effectiveness of low-iron micronutrient powders (MNPs) on anemia, growth and development when provided every other day in the first year of life.

This study investigated associations between exposure of low-iron MNPs with anemia, growth, and development of infants in Southern Ethiopia. Using a retrospective cohort design, the associations between MNP exposure (98 MNP exposed; 102 unexposed) and anemia, growth and motor development were assessed among infants 9-11 months of age. This was followed by a prospective follow-up of the same groups six months after the intervention was interrupted. Children's developmental milestone acquisition like language, social skills, and fine- and gross-motor skills were assessed. The retention of fat-soluble vitamins in the home-fortified foods was evaluated using HPLC-DAD.

Socio-demographic characteristics and dietary patterns were similar between MNP exposed and unexposed groups. Provision of low-Fe (6 mg) MNP every other day, for three months, was associated with reduced risk of anaemia and stunting, and increased likelihood of standing alone (AOR=3.1; 95% CI: 1.53, 6.46) or walking alone (4.9; 95% CI 2.12, 11.37). Dietary diversity and mothers' education were also associated with motor development. The prospective follow-up (cohort) after the interruption of the intervention revealed that difference-in-difference (DID) values between the two groups remained significant and positive, meaning that earlier differences between the two groups widened with time even in the absence of continued intervention. The risk of stunting and anemia in the MNP exposed infants were still reduced by 32.6 % (RR: 0.67; 95% CI: 0.48, 0.94) and 42.6% (RR: 0.57; 95% CI: 0.44, 0.75), respectively. Similarly, children exposed to MNP intervention in their first year of life had a lower risk of deficits in the ability to walk alone (RR: 0.47; 95% CI: 0.38, 0.57) or standing alone (RR: 0.60; 95% CI: 0.46, 0.79) compared to those that were unexposed to MNP. Exposure to MNPs was positively associated with various indicators of cognitive development like gross motor (β=0.59; 95% CI: 0.27, 0.91), fine motor (β=0.48; 95% CI: 0.17, 0.79), language (β=0.48; 95% CI: 0.10, 0.85) and social skills (β= 0.16; 95% CI: 1.09, 2.24). Morbidity, head-circumference, and LAZ were all significantly associated with development milestone acquisition ($P<0.05$). However, concentrations of retinol, cholecalciferol and α –tocopherol were much lower than the expected values based on theoretical calculations.

Earlier exposure to improved nutrition can have long-term effects even when interventions are stopped. Low-Fe MNPs provided on alternate days were associated with improved growth, lower risk of anemia and better motor milestone acquisition. This should however be validated using a community-based clinical trial. Improving the retention of fat soluble vitamins in MNPs would be critical to maximize impact.

***Keywords*:** Micronutrient powder, undernutrition, Infant development, anaemia, stunting,

DEDICATION

This work is dedicated to my father, the late ***Geletu Hayi Kassa, who*** *passed away when I was a child. It is also dedicated to our beloved family member, the late* ***Fisseha Beza.***

ACKNOWLEDGMENT

First and foremost, I would like to thank the Almighty God, our mother St. Mary the Virgin and St. John the Baptist for giving me the strength, knowledge, ability and opportunity to undertake this research study, persevere and complete it.

First of all, I don't have enough words to express my deepest gratitude to my advisor **Dr. Kaleab Baye.** This study would not have been possible without his diligent expert guidance and his consistent availability. **Dr. Kaleab Baye**, whom I began to work with as a student with a limited knowledge about research, has extended his tireless support and encouragement to improve my research skills. The fact that he shared his knowledge in his field of expertise, his steady and timely feedback throughout the study process, and his strong determination and passion has helped me to complete the study successfully. He has taught me another aspect of life that, "goodness can never be defied and good human beings can never be denied". Thank you Dr**. Kaleab!**

I would also like to express my deepest gratitude to my co-advisor **Dr. Seid M. Adem.** His cooperation, motivation, support and positive outlook have always kept me going ahead. I will not forget the extra miles he went to make my research become a reality by facilitating the entire experimental instrument, equipment and chemicals for the analysis part of my study. During my study visit, **Dr. Adem's** family happily welcomed me into their home. Thus, I would like to thank his entire family, his wife Rehan and his sons Nebil, Malek and Kerim for making me feel at home and supporting me during my stay with them.

My sincere thanks also goes to Prof. Stephen Angel, Chemistry Department Head of Washburn University and Prof. Sam Leung who gave me permission to work in their laboratory and use the facilities available during my study visit at Washburn University.

I am also grateful for the staff members of the center: Mr. Tilahun, Dr. Ashagre, Dr. Dawd, Mr. Aynadis, Mr. Yohanis, Mr. Habtamu, Ms. Mesert and Ms. Tensae for their support, feedback and encouragement.

My special thanks go to my best friend Dr. Paulos Getachew for his unfailing support, encouragement and good wishes. I also want to acknowledge Mr. Yonatan Matewos's genuine support and assistance during my field work.

I am grateful for the following organizations and people; Addis Ababa University (AAU), Kotebe Metropolitan University (KMU), all staff members of the Chemistry Department of KMU, Chemistry Department of Washburn University, Nutrition International (NI), Terebeza Development Association (TDA), International Orthodox Christian Charities (IOCC), Ethiopian Public Health Institute (EPHI), SNNPR Health Bureau, the study participants, health extension workers and data collectors.

My thanks also goes to my seniors PhD student Abera B, Abebe A, Tadesse A, Tesfaye D, Yenenesh A, Zeweter A, and Tilahun A, my classmates Abenet T and Erkyehun M and my junior Woyneshet, for their encouragement, moral support and help in any difficulties or queries I had regarding my study.

Words cannot express how grateful I am to my mother Tsige Lemma, I am speechless! I can barely find the words to express all the wisdom, love and support you've given me from my birth to date. You are my greatest support and for that I am eternally grateful. Your prayers are what sustained me thus far. A very special gratitude to my siblings Tsehay G, Mulu G(PhD), Sinkenesh G, Tewdros G, Rahel F, Fasik F, Bethelehem G. and brother in-law Sintayehu W, Abebe M. and Asefa N. for all of the sacrifices that you have made on my behalf, and love and care.

I also want to thank my father-in-law Ato Muhe Surur, mother -in-law W/ro Guday Zeleke sister and brothers' in-law Mesert M, Solomon M and Berhanu M for your prayer, unreserved support and encouragement. Special thanks to my brother-in-law Biniyam M. words fail me to express my heartfelt gratitude for all that you have done; I cannot count the many ways you have supported me throughout my journey, thank you Bini. My sincere appreciation goes to Serkalem for editing the language of parts of my dissertation.

Last but by no means least, I would like to express my immeasurable appreciation and deepest affection to my soul mate, my best friend and my beloved wife Eyerusalem Muhe (Emu), who spent sleepless nights with me and was always supportive. For the last two years, she has endured great sacrifices for our family. Emu has been my inspiration and motivation to pursue my studies and move my career forward. I thank God for lightening up my life with her presence. I would like to express my deepest gratitude and love to my beloved children Hennon A, Michael A. and Salem A. for being such an amazing sons and daughter. They have always been a reason for me to smile and I am forever thankful for their tolerance and patience during the last four years.

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ABBREVIATIONS/ACCRONYMS

ASF	Animal source food
DAD	Diod array detector
DHS	Demographic and health survey
Fe	Iron
GMRS	Gross motor reference study
HC	Head circumference
HPLC	High performance liquid chromatography
IDA	Iron deficiency anaemia
IDD	Iodine deficiency disorders
IOWA	Infant oriented with attention
IYC	Infants and young children
IYCF	Infant and young child feeding
LAZ	Length for age Z score
LMICs	Low and middle income countries
MD	Mental Development
MDAT	Malawian developmental assessment tool
MGRS	Multi-growth reference study
MND	Micronutrient deficiency
MMN	Multiple micronutrient
MNP	Micronutrient powders
MUAC	Middle upper arm circumference
NI	Nutrition International

PD	Psychomotor development
SMD	Standard mean deference
SNNPR	Southern nation nationality people region
SQ-LNS	Small quantity lipid based nutrient supplement
RCT	Randomized control trial
RSD	Relative standard deviation
TDA	Terebeza Development Association
VAD	Vitamin A deficiency
VA	Vitamin A
VD	Vitamin D
VE	Vitamin E
WAZ	Weight for age Z score
WHO	World Health Organization
WLZ	Weight for length Z score

Chapter 1

Introduction

1. Introduction

1.1 Background

Undernutrition covers a range of disorders including impaired growth and micronutrient deficiencies. Globally, about 795 million people suffer from undernutrition and about 780 million of these live in developing countries (McGuire, 2015). Over 150 million children suffer from one or more forms of malnutrition including stunting, underweight and wasting (R. E. Black et al., 2008). Deficiencies of iron, vitamin A, iodine, B-vitamins and zinc are among the common types of micronutrient malnutrition worldwide affecting millions in low-income countries (Khor & Misra, 2012) and may affect the risk of illness or death from infectious diseases.

Children less than two-years of age are at a higher risk of malnutrition because of the high nutrient needs to support the rapid growth and development. This is further exacerbated by the small gastric capacity (30 g/kg body weight/meal) that is even not met in many countries (Baye, Tariku, & Mouquet-Rivier, 2018; Kathryn G. Dewey, 2013). Furthermore, complementary foods consumed by infants and young children in many developing countries are nutritionally inadequate (Henry, Whiting, & Regassa, 2015) because of low dietary diversity, low meal frequency (Mitchodigni et al., 2017), consuming bulky foods that are low in nutrient density and low bioavailability (K. G. Dewey & Adu-Afarwuah, 2008) and the presence of infections (Usha Ramakrishnan, 2002b). Consequently, putting children at a higher risk of growth faltering and micronutrient deficiencies (Taghizade Moghaddam, Khodaee, Ajilian Abbasi, & Saeidi, 2015).

Various strategies have been put in place to improve complementary feeding in low and middle income countries (LMIC). These include dietary diversification, biofortification, fortification and supplementation(C. Berti, M. Faber, & C. M. Smuts, 2014b). Although dietary diversification is considered the most sustainable interventions, home-fortification is often considered because it is easier and quicker to implement and is considered to be cost-effective (Allen, Peerson, & Olney, 2009). Fortification was often done in a central unit, but this has been ineffective in LMIC where such central unit processing units are rare (de Barros & Cardoso, 2016). Consequently, home-fortification with MNPs are novel alternatives for delivering micronutrients to enrich complementary foods (De-Regil, Suchdev, Vist, Walleser, & Peña-Rosas, 2013).

1.2 Statement of the problem

Micronutrients are important for infant and young children brain development and have a critical role in brain nutrition, maintaining its structure and functionality (Prado & Dewey, 2014) and improvement of growth faltering of infant (U Ramakrishnan, Goldenberg, & Allen, 2011). Any damage caused by nutritional deficiencies during the first two years of life could lead to impaired cognitive development, compromised educational achievement, and low economic productivity (Cesar G Victora et al., 2008). Although several meta-analyses of RCTs exist on the efficacy of MNPs, little is known about the best timing and dosage to impact not only micronutrient status, but also growth and cognitive function. This is unfortunate as such information would have played a great role in maximizing benefits, while reducing potential risks of excessive intake.

In Ethiopia, dietary diversity is one of lowest in the world. Only 13.8% of children 6-23 months meet the minimum dietary diversity score. As a result, intake assessments conducted in Ethiopia have highlighted several micronutrient deficits (Mengistu et al., 2017, Baye et al., 2013). Irrespective of such poor dietary quality, iron intake is also often found to be significantly high (Abebe et al., 2018), suggesting that alternative regimens other than the ones recommended by WHO need to be tested. Besides, studies assessing the effect of poor quality complementary feeding on cognitive outcomes and how they respond to micronutrient interventions are non-existent in Ethiopia and scare internationally.

1.3 Objectives

General objective

The main objective of this study was to investigate the associations between micronutrient powder (MNP) exposure in the first year of life on anemia, growth and developmental milestone acquisition in rural Ethiopia

Specific objective

- ✓ To investigate whether exposure to a low-iron MNP provided on alternate days in the first year of life is associated with reduced risk of anemia, stunting, and deficit in motor milestone development
- ✓ Evaluate if motor, anemia and stunting changes associated with MNP exposure in the first year of life are sustained in the second year of life, six months post-intervention
- ✓ Identify predictors of child developmental milestone acquisition including, language, social, gross and fine motor development
- ✓ Investigate the retention of fat-soluble vitamins in home-fortified complementary foods
- ✓ Inform micronutrient programs and policies on timing, dosage, and delivery modalities to improve their effectiveness

Organization of the thesis:

Chapter two presents literature review that covers a brief description of undernutrition, its causes and consequence. It presents the conceptual framework of the determinants of undernutrition, and outlines the prevalence of global and regional undernutrition. The review highlights the importance of complementary feeding and highlights different public health strategies that can prevent stunting and micronutrient deficiencies. A systematic review and meta analyses on the effect of micronutrient interventions and cognitive outcomes (draft article) is presented, to then briefly review literature from Ethiopia.

Chapter three presents the description of the materials and methods applied in this study.

Chapter four presents the findings of the different studies in the form of, submitted or draft manuscripts. The chapter is organized as follows:

Section 4.1 *Provision of low-iron micronutrient powders on alternate days is associated with lower prevalence of anemia, stunting, and improved motor milestone acquisition in the first year of life: A retrospective cohort study* (Under review submitted to *Maternal and Child Nutrition*). This section presents the result of a retrospective cohort study that evaluated association between exposure of MNPs on anemia, stunting, and motor development acquisition**.**

Section 4.2 *Consuming MNP fortified complementary food in the first year of life reduce the risk of anaemia, stunting and motor millstone acquisition delay of young children (15-18 month): prospective cohort study in rural Ethiopia* (draft article). This section evaluates whether MNP-related benefits continue or attenuate after discontinuation of the intervention.

Section 4.3 *Early child nutrition is associated with achievement of development milestones in young children aged 15-18 months in rural Ethiopia* (draft article). This section looks at the association of early child nutrition to achievement of development milestones in its broadest sense by assessing language, social, gross and fine motor development and their predictors.

Section 4.4 *Retention of fat-soluble vitamins in complementary foods home-fortified with micronutrient powder*

This section evaluates the quality and homogeneity of vitamin A, D and E in home-fortified complementary foods by determining their concentration using HPLC DAD and sheds light on the improvements needed to maintain the retention of vitamins for maximal benefit.

Chapter five gives a general discussion on the findings of the studies.

Chapter six presents conclusions and perspectives.

Chapter 2

Literature Review

2. Literature review

2.1 Worldwide prevalence and distribution of undernutrition

Malnutrition, including undernutrition and micronutrient deficiencies is a worldwide problem that affects millions of unborn, infant and young children mainly during their vulnerable age (Warthon-Medina et al., 2015). Undernutrition covers a range of disorders including impaired growth and micronutrient deficiencies. The first 1,000 days is a critical time in which the body is quickly laying down its fundamental building blocks for brain development and future growth (Smith & Haddad, 2015). Thus, undernutrition within this time range is a fundamental wasting of human potential (Smith & Haddad, 2015). At this age, any dietary disruption leaves a long-lasting mark such as brain damage due to undernutrition in early life which is largely irreversible.

Child undernutrition is identified by comparing height (length) and weight at specific age and sex with the distribution of observed weights or heights in a reference population, and then calculating z-scores (WHO, 2006a). It includes stunting (HAZ>-2), wasting (WHZ>-2), underweight (WAZ>-2), and deficiencies of essential vitamins and minerals (Waterlow, 1973).

On the basis of the WHO Child Growth Standards, globally 165 million children younger than 5 years had a height-for-age *Z* score (HAZ) < –2 (stunted) ((World Bank, 2012). The higher prevalence stunting among children under-five years of age is found in Africa (36%) and Asia (27%) (Figure 2:1) (World Bank, 2012). More than 100 million (16%) of children younger than 5 years, were underweight, (weight-for-age *Z* score [WAZ] <–2) and the highest prevalence of undernutrition recorded was 30% and 22%, in southcentral Asia and western Africa respectively.

Wasting in children globally (weight-for-height *Z* score [WHZ] <–2) is estimated to be 8% (52 million) and 70% of the world's children with this condition live in Asia (Black et al., 2013).

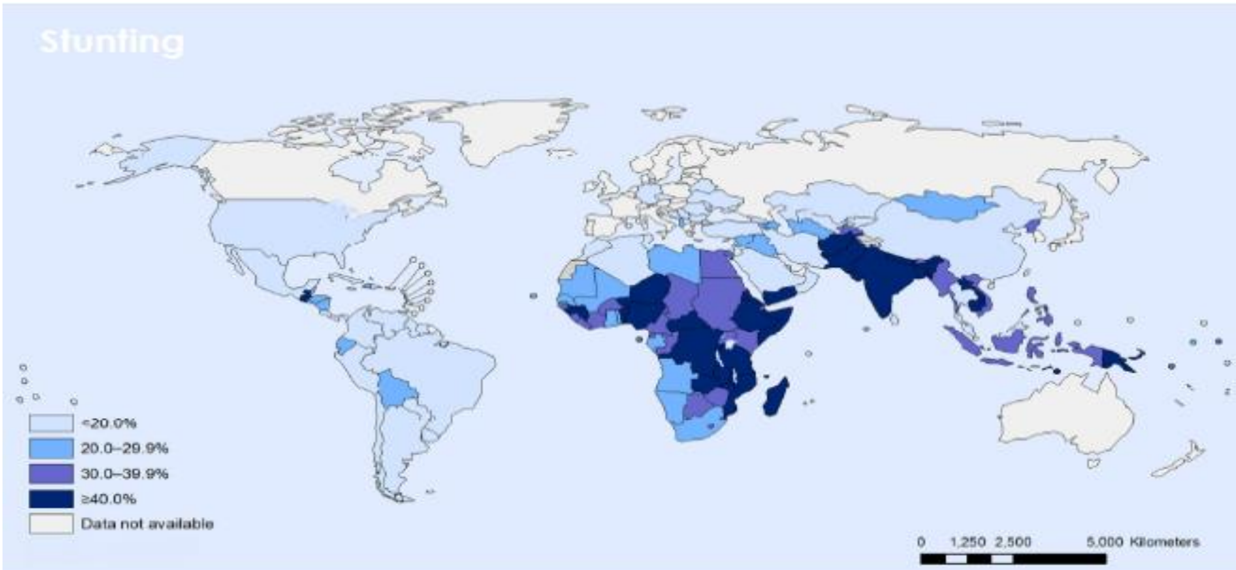


Figure 2.1 Latest country prevalence estimates for stunting among children under-five years of age.

Source : WorldBank (2012)

Furthermore, micronutrient malnutrition remains a public health concern in most developing countries, partly due to monotonous, cereal-based diets that lack diversity. Roughly more than one-third of the world's population is at risk of one or more micronutrient deficiencies (Tulchinsky, 2010). Micronutrient deficiencies of iron, zinc, iodine, and vitamin A are major public health problems among children in the form of multiple or single deficiency in most low and middle income countries. In general, vitamin and mineral deficiencies are seen worldwide but the prevalence is higher in low and middle income countries (LIMCs) (Usha Ramakrishnan, 2002b).Thus, all the burden of early nutritional deficiencies lead to a negative impact on infant and young children's linear growth and consequently affect a wide range of physical, cognitive, educational, health, and economic productivity outcomes throughout adulthood.

2.2 Timing of growth faltering

Child undernutrition remains one of the main public health challenges of the 21st century, particularly in low and middle-income countries. Among the different forms of undernutrition,stunting (low length/height for age) or linear growth retardation continues to be a major global health problem (Black et al., 2013). Worldwide, more than one in four children under the age of five years are too short for their age (Figure 2.2) (UNICEF, 2016). Faltering in linear growth begins before birth and continues until at least 2 years of age (Cesar Gomes Victora, de Onis, Hallal, Blössner, & Shrimpton, 2010).

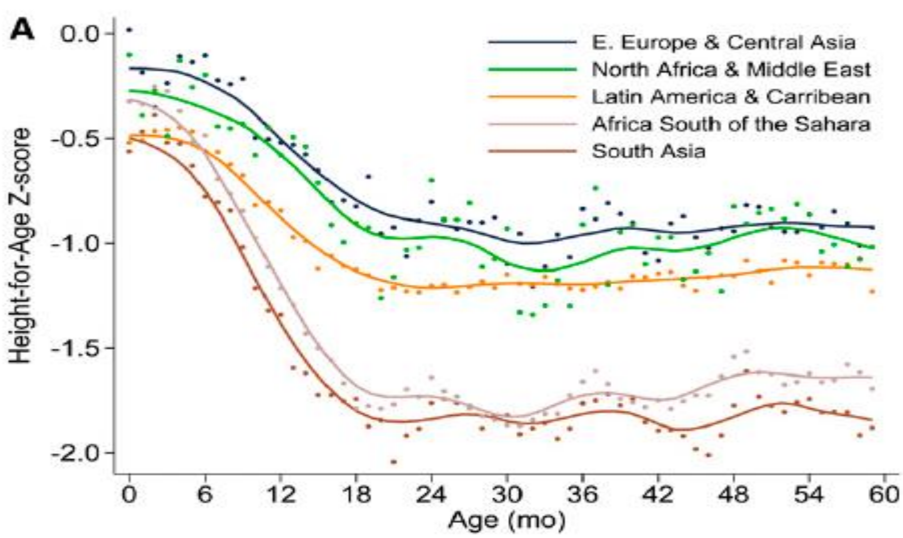


Figure 2.2 Mean HAZ relative to the WHO standard (1–59 mo) Results are presented by region (n = 12,397–154,379).

Sources**:** Leroy, Ruel, Habicht, and Frongillo (2014)

Sub-Saharan Africa and South Asia suffer the heaviest burden, with 75% of the world's stunted children (UNICEF, 2015). Stunting prevalence increases rapidly between 12 to 24 months (40% to 54%) and continues increasing until 36 months of age (58%). It then remains fairly stable until the children are five years old (55%) (Bhutta et al., 2013).

Moreover, growth failure results from the combination of intrauterine growth retardation,(Salam, Das, & Bhutta, 2014) inappropriate feeding practices and inadequate nutrition (WHO, 2014) and the increased burden of infection, coupled with suboptimal child care practices (Black et al., 2008). Stunting early in life can have long-term effects later in life on cognitive development, school achievement, economic productivity in adulthood and maternal reproductive outcomes (Dewey & Begum, 2011). In addition, growth failure can also be a predictor of poor survival and development of adult human capital in countries with the highest affected children (Black et al., 2008).

2.3 Cause and consequences of undernutrition

2.3.1 Cause of undernutrition

The causes of undernutrition are complex and multidimensional (Müller & Krawinkel, 2005). According to a conceptual framework by UNICEF(2013), the causes of undernutrition are classified as basic, underlying and immediate cause. The basic cause consists of social, economic, and political contexts. The underlying causes are income and poverty, which can lead to household food insecurity, inadequate care, unhealthy household environment and lack of health services. The immediate cause is related to inadequate dietary intake and diseases. Child undernutrition occurs as a result of one or more of these causes.

More specifically, the WHO's conceptual framework on childhood stunting developed by Stewart, Iannotti, Dewey, Michaelsen, and Onyango (2013) builds on UNICEF's framework on the context, cause and long and short term consequences of stunting and development. The framework illustrates the pattern of childhood stunting as context (basic), causes, and consequences, specifically emphasizing the effects of inadequate complementary feeding on stunting and development (Figure 2.3).

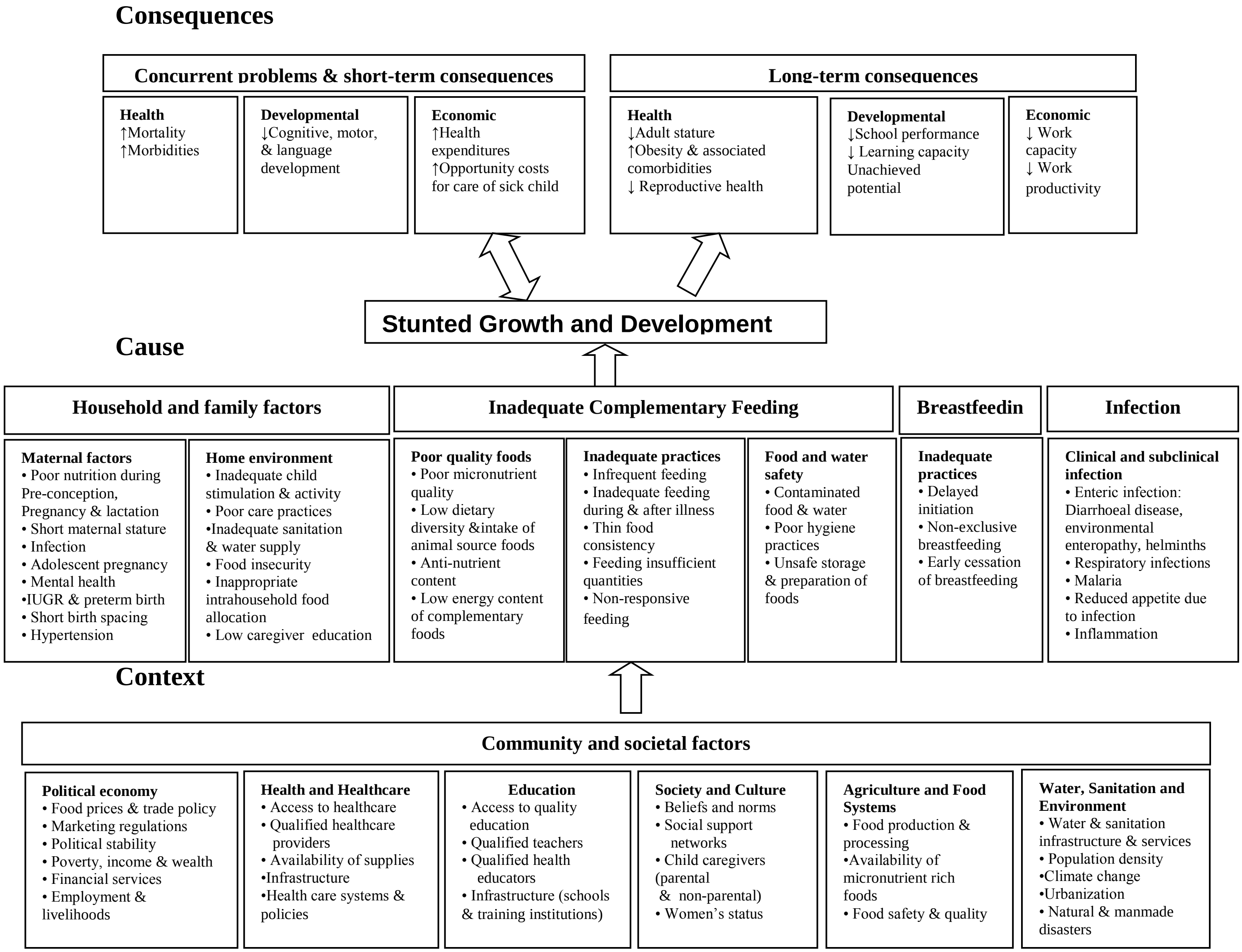


Figure 2.3 WHO conceptual framework on Childhood Stunting: Context, Causes, and consequences, with an emphasis on complementary feeding.

Source: Stewart et al. (2013)

Similarly with the UNICEF(2013), the context (basic) of childhood stunting center around community and societal factors which includes political economy, healthcare, education, society and culture, agriculture and food system and water, sanitation and environment. These context influence household and family factors, inadequate complementary feeding, lack of breastfeeding and infections. However, in this framework, emphasis is placed upon complementary feeding because it is a central pillar for the healthy growth and development of infants and young children (Stewart et al., 2013).

According to UNICEF (2013), the causes and consequences of undernutrition, inadequate dietary intake and disease factors themselves are interdependent. A child with inadequate dietary intake is more susceptible to diseases; diseases in turn depress appetite, inhibit the absorption of nutrients in food, and drains (weakens) the child's energy (Smith & Haddad, 2015). Infectious diseases can also cause under nutrition in children (UNICEF,1990). This is because a sick child may not eat or absorb enough nutrients, or may lose nutrients from the body due to vomiting or diarrhea, or have increased nutrient needs which are not met (UNICEF, 2003). As a result of this burden, the children are susceptible to long and short term health problems such as (mortality, morbidity, short adult stature and increased obesity), development (lower cognitive, motor, & language development and poor school performance) and economic (medical expenditure and lower work and production capacity).

2.3.2 Consequences of undernutrition

Globally, it is estimated that undernutrition is responsible for at least 45% (3·1 million) of deaths in children less than five years of age (Black et al., 2013). Among those who survive, an estimated 165 million (26%), more than 100 million (16%) and 52 million (8%) children are stunted, underweight and wasted respectively. High prevalence of undernutrition is widely observed in LMICs (Black et al., 2013; World Bank, 2012). In general, an episode of undernutrition during the first 1000 days of a child can lead to serious health and developmental problems including, developmental delays (mental and motor development), growth faltering (stunting), susceptibility to infections, poor psychosocial development, reduced learning capacity and productivity.

It is a fact that brain is one of the organs that continuously develops for many years after birth (Figure 2.4) (Anjos et al., 2013). Its development begins 18 days after fertilization (Prado & Dewey, 2014). Between 24 and 42 weeks of gestation, the developing brain is vulnerable to nutritional impacts because of the rapid trajectory of several neurologic processes, including synapse formation and myelination (Georgieff, 2007a).

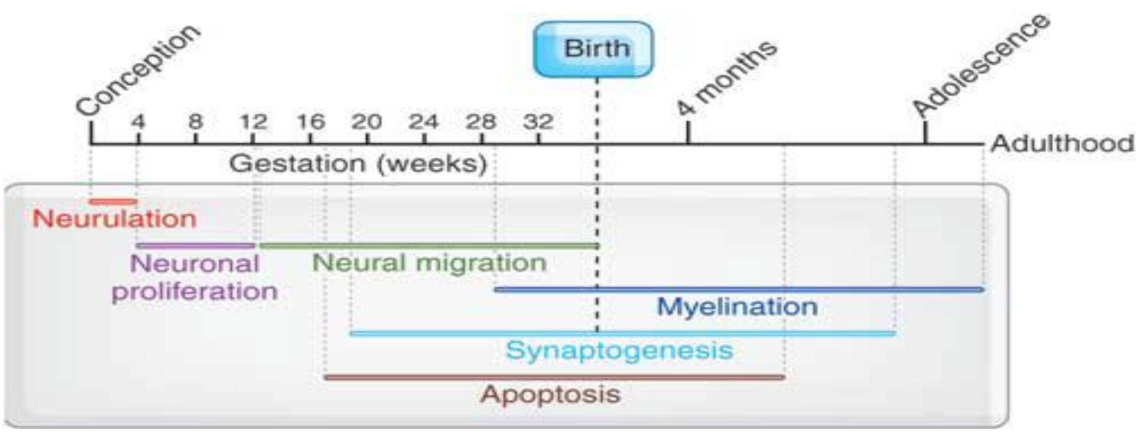


Figure 2.4 Timeline of major events in brain development.

Source: Tau and Peterson (2010)

Despite prenatal development, dramatic change in brain structure and function occurs at early postnatal period. That is, the newborn brain at 2–4 weeks of age is approximately 36% the size of an adult's brain, about 70% by 1 year of age and about 80% by age 2, which increases in volume by an impressive 88% in the first year, 15% in the second year, and then more modestly but steadily thereafter (Figure 2.5) (Tau & Peterson, 2010).

Figure 2.5 Brain myelination across development. Showing age related increase in brain size and white matter intensity.

Source : Tau and Peterson (2010)

Thus, malnutrition during this vulnerable period (the first 1000 days) of brain development leads to a reduction in brain cells, myelin production and number of synapses and alterations in neurotransmitter systems (Figure 2.6). (Keunen, van Elburg, van Bel, & Benders, 2014). Research conducted on animals also showed that early undernutrition affects brain structure and functions, and has lasting cognitive and emotional effects (Sally Grantham-McGregor et al., 2007).

Figure 2.6 Brain scan from two 3 years old children

Source: Barros (2007)

Moreover, a severely stunted child faces a 5.5 times higher risk of dying than a normal child (McDonald et al., 2013). In the long term, stunting in children may affect their physical size as an adult, intellectual ability, learning difficulties in school (poor school achievement and poor school performance), earn less as adults, may increase the risk of metabolic disorders and have an increased likelihood of being overweight and develop associated chronic illnesses such as cardiovascular diseases, diabetes and cancer, and suffer from mental health issues (K. G. Dewey & Begum, 2011; Cesar G Victora et al., 2008; Walker et al., 2007). Therefore, these children begin their lives at a marked disadvantage (W. UNICEF, 2012).

2.4 Complementary feeding

Complementary feeding is the process of introducing complementary foods to children aged 6–23 months (Stone-Jimenez, 2014) which are any non-breast milk foods or nutritive liquids that are given to infant and young children (WHO, 2008) because breast milk alone is no longer sufficient to meet the nutritional requirements of infants (K. Dewey, 2002). On the other hand, complementary feeding is described as a transition from exclusive breastfeeding to family foods. The start of complementary feeding is a very vulnerable period in which malnutrition begins in many infants worldwide (Menon, 2012).

2.4.1 Inadequate complementary feeding

Complementary feeding is an effective child survival strategy and is ranked among the top life-saving interventions for children under 5 years and can prevent about 6% of under-five mortality (G. Jones et al., 2003). However, during this period, children consume small amounts of foods, because of their small gastric capacity, Consequently, complementary foods need to have high nutrient density and be given to children frequently to support optimal physical growth and brain development (K. G. Dewey, 2016).

On the other hand, infants have high nutritional requirements relative to their body size and consume small amounts of food, thus requiring nutrient-dense foods. Yet, the opposite is often the case in low-income settings where they are typically fed nutrient-poor, watery porridges (Vitta & Dewey, 2012) with monotonous and low dietary diversity (R. S. Gibson et al., 2009). In general, complementary foods given in many developing countries are nutritionally inadequate (R. Gibson, Ferguson, & Lehrfeld, 1998) thus, mineral and vitamin deficiencies occur in infants and young children (Faber, 2005).

2.4.2 Inadequate micronutrient intake

Micronutrients are essential vitamins and minerals required from diets to sustain virtually all normal cellular and molecular functions (Merson, Black, & Mills, 2012). Micronutrient deficiency (MND) can have wide-ranging negative health impacts and if untreated, could ultimately result in death. MNDs often occur as part of a cycle of malnutrition (Bailey, West Jr, & Black, 2015).

In many developing countries, deficiency of micronutrients is common, due to consistent consumption of monotonous diets (provide large amounts of energy but relatively low amounts of essential vitamins and minerals) (McEvoy, Temple, & Woodside, 2012), inadequate food intake and poor dietary quality (Kathryn G Dewey, 2013), poor bioavailability (because of the presence of inhibitors like phytate, mode of preparation, and interactions) (K. G. Dewey & Vitta, 2013) low dietary intakes of animal source foods (K. G. Dewey & Vitta, 2013), and the presence of infections (Bailey et al., 2015; Usha Ramakrishnan, 2002a). As a result, micronutrient deficiencies hamper both human and economic development.

2.4.3 Consequences of micronutrient deficiencies

Micronutrient deficiency is generally correlated with overall undernutrition (Horton, Alderman, & Rivera, 2008). Inadequate intake of micronutrient is now recognized as an important contributor to the global burden of diseases through increased rates of illness and death from infectious diseases, and of disability such as mental impairment (R. Black, 2003).

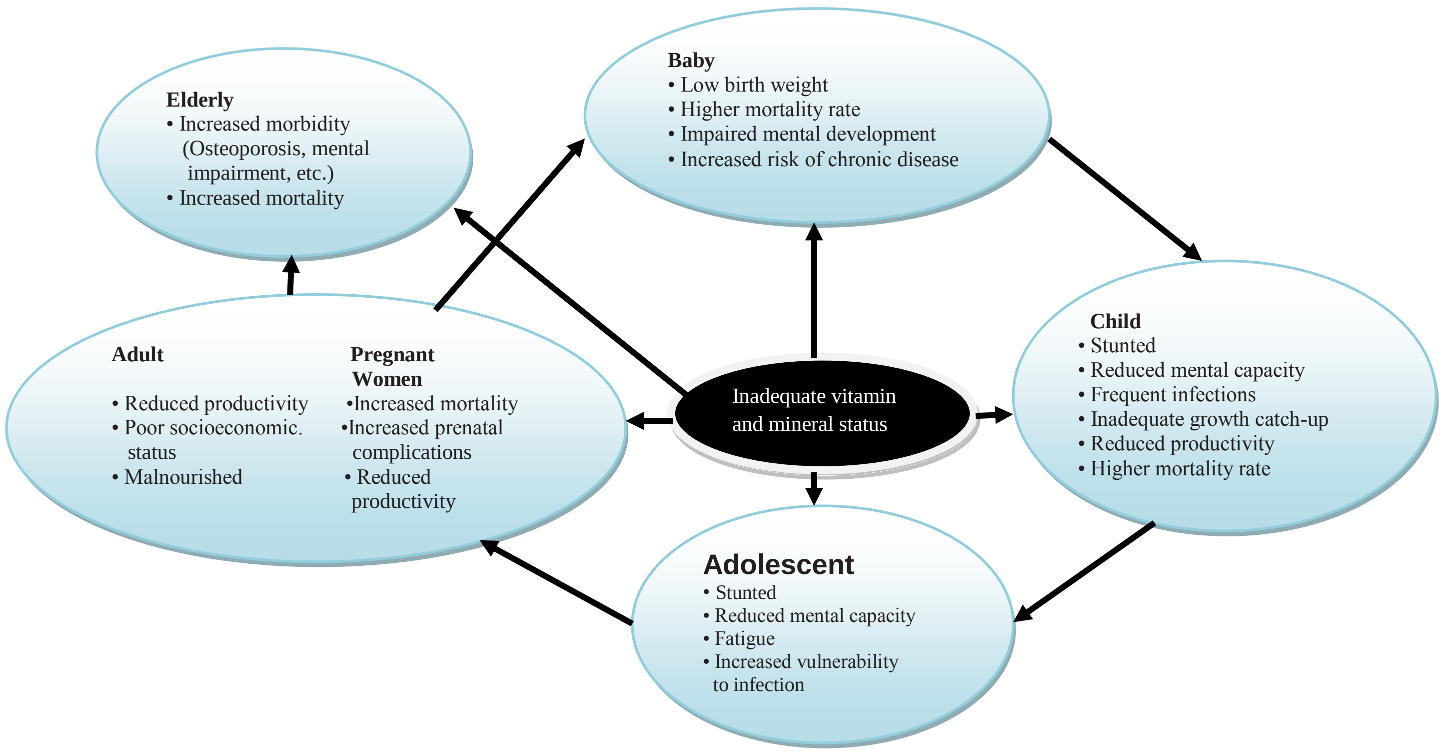


Figure 2.7 Micronutrient deficiencies have consequences throughout an individual's life span and are perpetuated across the generation.

Source: Bailey et al. (2015)

Furthermore, growth retardation and developmental delays that could result from micronutrient malnutrition during the first 1000 days might be irreversible (Lozoff et al., 2006). In general, micronutrient deficient children that survive are likely to suffer from frequent illness, which adversely affects their nutritional status and locks them into a vicious cycle of recurring sicknesses, faltering growth and diminished learning ability, as shown in the Figure 2.7. Deficiencies of iron, zinc, iodine, vitamin A, and folate are among the common types of micronutrient malnutrition worldwide (Table 2.1) (Khor & Misra, 2012) and lack of these nutrients is affecting millions of infants and young children in low income countries. Therefore, the 1,000 days period is considered the key window of opportunity to prevent undernutrition and its long-term consequences. (Prado, Abbeddou, Adu-Afarwuah, et al., 2016)

Table 2.1 Summary of the roles of key micro nutrients in biological function prevalence of deficiency and effect of deficiency

Nutrients	Biological function of the nutrient	Prevalence of the deficiency	Consequence of the deficiency
Iron	• Oxygen transport, production of ATP, DNA synthesis, and protection of cells from oxidative damage (McCann & Ames, 2007) • Hemoglobin synthesis (Atamna, Walter, & Ames, 2002; Iannotti, Tielsch, Black, & Black, 2006) • Enzymatic activity for brain function : myelination (Martínez, Knappskog, & Haavik, 2001; Nagatsu, 1995) , synthesis of the neurotransmitters (Szajewska, Ruszczynski, & Chmielewska, 2010)	▪ Worldwide affecting 2 billion people (Lanzkowsky, 2016) ▪ Risk of highest prevalence is age 6–24 mo of infant and children (Balarajan, Ramakrishnan, Özaltin, Shankar, & Subramanian, 2011)	▪ Reduced oxygen carrying capacity and can impact immunity, growth and development (M. M. Black, 2003) ▪ Iron deficiency anaemia (Georgieff, 2011; Grantham-McGregor & Ani, 2001; Hulthén, 2003) ▪ Poor cognitive and motor development and behavioural problems, poor school achievement (Balarajan et al., 2011; Grantham-McGregor & Ani, 2001; Lozoff et al., 2006)
Zinc	• Adequate growth, immunocompetence and neurobehavioral development of young children (Brown et al., 2004) • Recognized action on more than 300 enzymes by participating in their structure or in their catalytic and regulatory actions structural ion of biological membranes and closely related to protein synthesis (Salgueiro et al., 2002)	▪ 82% of the pregnant women (Pathak, Kapil, Dwivedi, & Singh, 2008) ▪ Global prevalence of inadequate zinc intake at 48.9% and 20.5%, respectively (Wessells, Singh, & Brown, 2012)	• Over half a million deaths per year in infants and children (Krebs, Miller, & Michael Hambidge, 2014) • Prenatal zinc deficiency in animals model study associated with abnormal cortical electrophysiology and reduced brain mass in the cerebellum, limbic system, and cerebral cortex; behaviorally, decrements in short-term memory, learning (Spann et al., 2015)
Iodine	• To produce thyroid hormones thyroxine (T4) and triiodothyronine (T3) (Zimmermann, 2011) throughout life (Morse, 2012) • These hormones are needed to normal development of the brain and nervous system during gestation and early life (Zimmermann, 2009) • Thyroid hormone also required for normal neuronal migration, myelination, and synaptic transmission and plasticity during fetal and early postnatal life (De Escobar, Obregón, & Del Rey,	▪ Nearly one-third of the world's population is at risk for iodine deficiency (Andersson, Karumbunathan, & Zimmermann, 2012) ▪ The prevalence of IDD is highest in the Eastern Mediterranean region (32%), followed by Africa (20%), Europe (15%) and Southeast Asia (12%) (Usha Ramakrishnan, 2002a)	• Hypothyroxinemia in infant and young children cause irreversible brain damage with mental retardation and neurologic abnormalities (Melse-Boonstra & Jaiswal, 2010) • In utero or postnatal, it leads to hypothyroidism and irreversible neurological and cognitive deficits manifested as cretinism (R. E. Black, 2014). • Neurological cretinism includes mental retardation, primitive reflexes, visual problems, facial deformities,

	2004; Dong et al., 2005)		stunted growth and diplegia (M. M. Black, 2003).
Selenium	• Found in two unusual amino acids i.e. selenocysteine and selenomethionine which are the principal functional components of selenoenzymes • It is an essential cofactor in approximately 50 enzymes (Gómez-Galera et al., 2010) • Selenium mediate their effects through thyroid hormone metabolism (Georgieff, 2007a)	Selenium deficiency affect up to 1billon people (Haug, Graham, Christophersen, & Lyons, 2007)	▪ Deficiency of this mineral with other problem like anaemia and stunting were associated with poor cognitive performance (Gashu et al., 2016).
Copper	• Copper is an essential divalent cation for proteins involved in brain-energy metabolism, dopamine metabolism, antioxidant activity, and iron accretion in the fetal and neonatal brain (Georgieff, 2007a)	copper deficiency is relatively infrequent in humans but, more frequent in preterm infants, especially those with very-low birth weights (Uauy, Olivares, & Gonzalez, 1998)	▪ The most frequent clinical manifestations of copper deficiency are anemia, neutropenia, and bone abnormalities (Uauy et al., 1998) ▪ Animal studies show that gestational copper deficiency with long-term effects on motor function, balance, and coordination (Georgieff, 2007a).
Vitamin A	• Vitamin A essential in the immune response and in eye health(Caulfield, Richard, & Black, 2004)	It has been estimated that 127 million preschool-aged children are vitamin A deficient (David Benton, 2012).	▪ Severe vitamin A deficiency typically entails clinical signs of xerophthalmia and very deficient serum levels of vitamin A (0.35 mol/dL)(R. E. Black, 2014) ▪ Poor vitamin A nutrition status lead decline in cognitive ability (Olson & Mello, 2010)
Folate and vitamin B-12	• Both vitamin B_{12} (cobalamin) and folic acid Essential for neurodevelopment and function in the antenatal and early postnatal periods (Veena et al., 2010). ▪ Cobalamin important for a rapidly developing nervous system (Torsvik, Ueland, Markestad, & Bjørke-Monsen, 2013) • Folate plays a crucial role in neurotransmitter synthesis (Fekete et al., 2012) and folic acid is necessary during early fetal development to prevent NTD, and cobalamin deficiency may interfere with early development through disruptions in myelination and dendritic	▪ Deficiencies of folate and vitamin B_{12} are prevalent across the globe (ref). ▪ Deficiency is certainly more prevalent in strict vegetarians, but lacto-ovo vegetarians are also at higher risk for inadequate intakes (de Benoist, 2008)	▪ Deficiency of these nutrients during pregnancy has been linked to fetal growth restriction and neural tube defects (Veena et al., 2010) ▪ Vitamin B_{12} status related with delayed neurodevelopment in infants born to mothers with pernicious anaemia and also poor cognitive outcomes in children of vitamin B_{12}–deficient mothers (Veena et al., 2010). ▪ Severely cobalamin-deficient infants are feeding difficulties, developmental delay, and progressive

	formation or inflammation (M. M. Black, 2008a).		neurologic symptoms (Torsvik et al., 2013)
Vitamin D	• Vitamin D is inherently important for muscle development and motor function (Ceglia & Harris, 2013)	▪ Global public health problem in all age groups, even in countries with sun exposure all year round particularly in those from the Middle East (Palacios & Gonzalez, 2014)	▪ Maternal deprivation of vitamin D affects the foetal brain at the gross morphological, cellular and molecular levels (Eyles, Brown, Mackay-Sim, McGrath, & Feron, 2003)
Vitamin E	Vitamin E is indispensable for reproduction and prevents diseases associated with oxidative stress, such as cardiovascular disease, cancer, chronic inflammation and neurologic disorders (Gathwala, Yadav, & Lal, 2013)	▪ Prevalence of vitamin E is highly in developing countries. Children and the elderly are more vulnerable age groups and that men may be at higher risk for deficiency(Dror & Allen, 2011)	▪ Ataxia, peripheral neuropathy, muscle weakness, slow growth in children(Bhandari & Banjara, 2015)

2.5 Strategies to improve complementary feeding

Multiple strategies exist for preventing malnutrition in infant and young children in short and long term (Caulfield, Richard, Rivera, Musgrove, & Black, 2006). In developing countries, the long term strategy is the most ideal and sustainable solution. Increasing the consumption of a diversified diet and biofortification are among the common long term strategies (Mayer, Pfeiffer, & Beyer, 2008). Fortification and supplementation (Mitchodigni et al., 2017; Ortiz-Monasterio et al., 2007) are a couple of the potential short-term public health strategies to improve the quality of complementary food of infants and young children. Current evidence suggests that effective interventions to reduce undernutrition should be targeted to mothers and children during the first ‘‘1000 days’’ of life. An increasing number of health and nutrition programs currently focus on this ‘‘window of opportunity” to prevent undernutrition.

i. Dietary diversification

Infant and young child feeding (IYCF) practices directly affect the nutritional status of children (UNICEF, 2008). In developing countries, dietary diversity has been shown to be associated with increased nutrient adequacy, nutrient intake or better child nutritional status (Arimond & Ruel, 2004; WHO, 2005). Minimum dietary diversity is proportion of children 6–23 months of age who received foods from 4 or more food groups during the previous day (Jones et al., 2014). Consumption of a minimum acceptable diet with dietary diversity reduced the risk of both stunting and underweight, whereas minimum meal frequency was associated with lower risk of underweight (Marriott, White, Hadden, Davies, & Wallingford, 2012).

ii. Biofortification

Biofortification is the process of enhancing the nutrient content of staple crops through traditional breeding and modern technology (Nestel, Bouis, Meenakshi, & Pfeiffer, 2006). This process is a novel and sustainable agricultural approach to reducing micronutrient malnutrition among people at high risk (i.e., resource-poor women, infants and children) by enriching major staple food crops (e.g., rice, wheat, maize, beans and cassava) with micronutrients through plant-breeding strategies (C. Berti, M. Faber, & C. Smuts, 2014a; Welch, 2002). Biofortified crops are developed with conventional breeding being the primary focus to increase the content of iron, zinc, and provitamin A carotenoids (Hotz & McClafferty, 2007). The potential benefit of biofortification depends on the amount of the staple food consumed per day (Hotz & McClafferty, 2007).

iii. Improving processing methods

At the household level, food processing and preparation techniques are most likely to promote the content, form and bioavailability of micronutrients of foods (Tontisirin, Nantel, & Bhattacharjee, 2002). Processing increasing bioavailability and accessibility of micronutrients and decreasing absorption inhibitors , or increase bioavailability enhancing compounds (Tontisirin et al., 2002). Typical food processing methods such as germination, malting and fermentation have been found to enhance iron absorption by increasing vitamin C content or by lowering the tannin or phytic acid content, or both (Tontisirin et al., 2002). Germinated flours can be used to enhance the energy and nutrient density of cereal-based porridges for infant feeding, while simultaneously reducing their phytate content to some degree (R. S. Gibson & Hotz, 2001). Fermentation also improves the protein quality and digestibility, vitamin B content, microbiological safety and keeping quality, while at the same time enhancing the iron and zinc bioavailability (R. S. Gibson & Hotz, 2001).

iv. Nutrition Education for mother/ caregiver of the child

Nutrition education programmes are recommended to improve complementary feeding through food diversity and proper use of local food (Zhang et al., 2016). However, poor feeding practices, such as low dietary diversity, feeding frequency, and inadequate energy intake (DIOUF & SHEERAN, 2010) may correlate with caregivers' poor knowledge on nutrition and food diversity in their environment (Saloojee, De Maayer, Garenne, & Kahn, 2007). A study on complementary feeding practices among infant and young children in southern Ethiopia proposed that nutrition-based educational intervention is an important strategy to improve caregivers' knowledge and complementary feeding practices (Henry et al., 2015). Furthermore, nutrition training for mothers or caregivers to the child can improve healthy feeding behaviors (Sripaipan et al., 2002) and dietary diversity (Penny et al., 2005) during the complementary feeding period (Sunguya et al., 2013).

v. Fortification /Supplementation

The most conventional and widely practiced strategies used to address micronutrient malnutrition are supplementation and food fortification. The effect of food fortification with single, dual, or multiple micronutrients in women and children led to the conclusion that food fortification is potentially an effective strategy to address micronutrient malnutrition (Berti et al., 2014a). Micronutrient powders (MNP) are single-dose packets containing multiple vitamins and minerals in powder form that can be sprinkled onto any semi-solid food prior to consumption

(De-Regil et al., 2013). It improves the quality of the typically plant based complementary food in developing countries (WHO, 2011a).The product formulations can differ in terms of the number and content of micronutrients included; however, standard formulations usually contain 15 vitamins and minerals which are essential for a child's development and survival. One sachet is equivalent to 1 Reference Nutrient Intake (RNI) –an amount which can fulfill the nutritional needs of 97.5% of the target population as indicated in (Table 2.2).

Table 2.2 The composition of multiple micronutrient supplements for children from 6 to 59 months of age, designed to provide the daily recommended intake of each nutrient

Micronutrients	Amount of micronutrient in one sachet
Vitamin A	400 µg
Vitamin E	5.0 mg
Vitamin D	5.0 µg
Vitamin C	30.0 mg
Thiamine (vitamin B1)	0.5 mg
Riboflavin (vitamin B2)	0.5 mg
Niacin (vitamin B3)	6.0 mg
Vitamin B6	0.5 mg
Vitamin B12	0.9 µg
Folic acid	150 µg
Iron	12.5 mg
Zinc	4.1 mg
Copper	0.56 mg
Selenium	17.0 µg
Iodine	90.0 µg

Sources: WHO (2007a)

This strategy has an effective solution for acute malnutrition, however, most nutritional fortification (central or at the point of fortification) programs mainly focus on evaluating challenges like coverage, adherence and modest biological impacts such as anemia status and nutritional status. However, no attention is given to the retention/ stability of degradable component (vitamins) of the fortificant before and after it mixes with the food of the end user.

It is known that component of nutritional fortificant through time may degrade in various ways. Particularly, vitamins in the fortificant are easily degraded and its content is reduced as it is exposed to heat, light (UV) (Lešková et al., 2006), unfavorable storage conditions (Hemery et al., 2018) and lack of mother/caregiver knowledge and practice on use of fortification at home.

Therefore, there must be a need of random and easy analytical method to analyze the amount of the fortificant (easily degradable such as vitamins) before and after it mixes with the food matrix. Several methods exist for the determination of fat soluble vitamins in foods. For instance, the most common methods are HPLC with UV and fluorescence detector simultaneously (Gimeno, Castellote, Lamuela-Raventos, De La Torre, & Lopez-Sabater, 2000) for vitamin A and E respectively and Vitamin D3 separately with HPLC-UV (Indyk & Woollard, 1984) by either gradient elution or isocratic separation techniques.

On the other hand, the emphasis of a number of studies (RCT or systematic review and meta analysis) regarding the effect of MNP fortification of complementary food has been placed on growth, anaemia and serum micronutrient status of infant and young children. Little attention has been drawn to implications on the effects of MNP fortification on the development of infant and young children. However, globally there is insufficient or little information on the effects of nutrition intervention (fortification/ supplementation) on infant and young children's mental and motor development. Therefore, one of the components of the literature review of this study is a "systematic review and meta-analysis on the effects of nutrition intervention on mental and motor development". The next section will present a systematic review on nutrition intervention on mental and motor development.

Micronutrient interventions improve mental and motor development of infants and young children in low-and middle income countries: *A systematic review and Meta-analysis*

(Draft paper to be submitted)

2.6 Systematic review and meta-analysis

Micronutrient interventions improve mental and motor development of infants and young children in low-and middle income countries: *A systematic review and Meta-analysis*

Ashenafi Geletu[1,2] Taddes Alemu Zerfu[3,4,] and Kaleab Baye[1]

[1]Center for Food Science and Nutrition, College of Natural Sciences Addis Ababa University, Addis Ababa, Ethiopia

[2]Department of Chemistry, College of Natural and computational Science, Kotebe Metropolitan University, Addis Ababa, Ethiopia

[3]College of Medicine and Health Sciences and Referral Hospital, Dilla University, Gedeo, Ethiopia

[4]Maternal and Child Well Being Unit, African Population and Health Research Center

Abstract

Background: Diets in many of the low and middle income countries are poorly diversified, low in quality and the possibility of multiple micronutrient deficiencies is high. Micronutrient malnutrition could impair development potential of infants and young children. However; compelling and conclusive evidence on the benefit (s) of similar interventions for wider public health use is lacking.

Objective: This study aims to systematically review the current literature and to quantify the effects of micronutrient interventions on mental and motor development of infants and young children in low-and middle income countries.

Methods: Cochrane, PubMed, Medline and Google scholar databases were searched for trials published up to the end of December, 2017. Strictly following a protocol, all randomized controlled trials investigating the effects of micronutrient interventions (single or multiple) on the mental and motor development of healthy infants and young children were included. Data were extracted by 2 independent researchers. The developmental tests used in the trials were grouped into mental and motor development domains, and pooled effect size estimates were calculated per domain. Heterogeneity was explored using I^2 test and sub-group analysis techniques.

Results: We retrieved a total of eight studies reporting the effects of micronutrient interventions on mental development and eleven studies reporting the effects of micronutrient interventions on motor development of infants and young children. The pooled random-effect estimates (SMD) of the intervention on mental and motor development of infants was 0.10 (95% CI: 0.04, 0.17) and Z= 2.92; P=0.003 and 0.06 (95% CI: 0.01, 0.11) and Z=2.29; P=0.02 respectively. The pooled SMD on mental and motor development of younger children was even stronger, with an effect sizes of 0.3 (95% CI: -0.03, 0.09 P =0.03) and 0.03 (95% CI: -0.03, 0.08 P =0.34), respectively.

Conclusions and interpretations: Multiple micronutrient supplementations only during infancy had a moderate but significant effect in improving cognitive and motor development in low and middle income settings. It is time to consider this intervention for public health recommendations in such settings, after evaluating its safety and potential adverse effects.

Key words: Mental and motor development, micronutrient, , Iron, Zinc

INTRODUCTION

Malnutrition, in all its forms, remains a leading global concern and one of the greatest challenges of our time (Dora et al., 2015). Chronic and recurrent malnutrition leads to longstanding consequences that significantly impair physical and cognitive development potentials of a child. Today, Even though the world produces enough food to feed everyone, over 156 million children under five around the globe are stunted; close to 250 million are vitamin A deficient; 40% are anemic and 51 million are wasted (Haddad et al., 2016; UNICEF, 2017). Worrisomely, close to 11% (795 million) people worldwide, particularly from the southern Asia and sub-Saharan Africa, go to bed on an empty stomach each night, and nearly one in three people suffer from some form of malnutrition (Haddad et al., 2016).

Diets in many of the low income countries are monotonous plant-based food sources with little energy content and diversity. This indicates that diets in these regions are deficient in many of the micronutrients and there is a high possibility of coexistence of multiple micronutrient deficiencies (Haddad et al., 2016; Onyango, 2003). Likewise, supplementation with multiple micronutrients seems to be an effective short term strategy in such settings (Zeng, Yan, Cheng, & Dibley, 2011).

From 2009 onwards, five different systematic reviews (Aboud & Yousafzai, 2015; D Benton, 2001; Eilander et al., 2009; Larson & Yousafzai, 2017; Pasricha, Hayes, Kalumba, & Biggs, 2013) were published in the literature concerning the effect (s) of single (iron or zinc) and multiple micronutrients in children. The first review was published in 2009 by Eilander et al

(Eilander et al., 2009). The study pooled the effect of multiple-micronutrient interventions on cognitive performance of children from 20 randomized controlled trails in children aged 5 - 16 years. Eilander et al. (2009) also reported that supplementing multiple-micronutrient to healthy schoolchildren, may be associated with a marginal increase in fluid intelligence and academic performance, but not with crystallized intelligence.

The latest review and meta analysis was reported by Larson and Yousafzai (2017) and showed limited benefits with minimal effect of nutrition interventions on a child's motor development and not affecting physical growth and cognitive development. Similarly, Pasricha et al. (2013) has also reviewed the effects of a single micronutrient supplement and reported a non-conclusive finding, a small non-significant effect on growth, mental and motor development of children.

Recognizing their strengths, all authors reported several limitations recommending more research before proceeding to public health recommendations. The major limitations reported were: lack of rigor in the evidence generated, limited availability of data from developing countries, analysis of wide age range (preconception to school age), high level of heterogeneity among studies, and didn't pool the effects on additional domains (psychomotor development).
Since the last review by Larson and Yousafzai (2017) and Pasricha et al. (2013), some trials have been published (Christian, Kim, Mehra, Shaikh, Ali, Shamim, Wu, Klemm, Labrique, & West Jr, 2016; Locks et al., 2017) in the literature. These publications have several compelling quantities (facts) with a potential to fill the critical limitations and evidence gaps identified by previous reviews: both examined the effect of MMN (than single), were reported from

developing countries, focused on infants and young children, and examined both outcome domains of interest; i.e. cognitive and motor development.

In the present analysis, we aim to perform a more rigorous, but focused (children 0-23 months) analysis, through literature review of updated literature. We particularly aim to report a more elaborated and updated pooled analysis on the effects of micronutrient interventions on both mental and psychomotor development of infants and young children. As observed in previous reviews and anticipated in our current analysis, we aim to explore whether subgroups and factors such as age, number of micronutrients, presence of iron in the supplement, and other related factors could predict (yield) vivid and informative answers to our research question of the effects of micronutrients on mental and psychomotor development of children.

METHOD

Information sources

A literature search of titles and abstracts of trials reporting the effects of MMN supplement on the cognitive and motor development of infants and young children was conducted using electronic databases: Cochrane database, PubMed, Medline and Google scholar. We reviewed the references of the identified articles and previous systematic reviews. The search was restricted to articles published in the English language. A search string consisting of a combination of the following terms was used: vitamin, mineral, micronutrient, multiple micronutrients; with cognition, memory, mental performance, moteor development, millstone acquisition, child development, infant development, psychomotor; and with child, infant, young children, toddler. Titles and abstracts from the initial search results were examined to identify potentially relevant studies; references of identified relevant studies and reviews were screened for further pertinent studies. We performed the searches in December, 2017.

Inclusion criteria

Type of trials

We took into consideration randomized controlled trials and cluster randomized trials that included infants (0-23mo) who were randomly assigned to micronutrient supplementation or controlled group to assess its effects on cognitive performance and/or motor skill development. Both prenatal and postnatal nutritional supplementation trials were included.

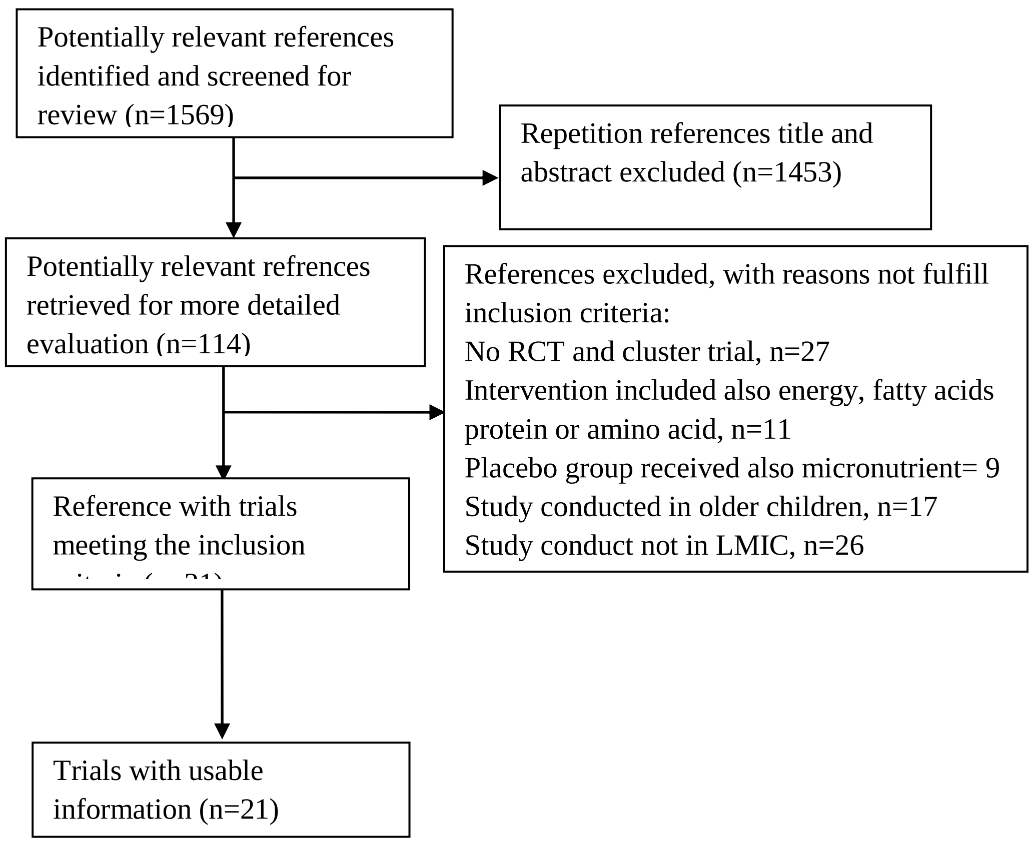


Figure 1: Flow diagram for inclusion of randomized control trials

We included trials involving participants from all low and middle income countries and socioeconomic backgrounds. Authors were contacted to obtain outcome statistics if they were not included in the published article.

Type of participants

Trials conducted in healthy infants were included. Trials conducted in selected subjects with learning disabilities, neuropsychiatric disorders, or chronic psychiatric health conditions were excluded.

Types of intervention

We included trials in which infants and young children were supplemented with one or more different micronutrients compared with a placebo/control as well as studies that compared foods fortified with one or more micronutrients to non-fortified foods. Moreover, trials that had used other interventions along with multiple micronutrients, such as psychosocial stimulation (PS) deworming tablets, malaria & diarrhea treatment, were also included provided that the addition of multiple micronutrients was the sole difference between the intervention and control groups.

Type of Outcomes

The outcomes primarily include mental and psychomotor development.

Data extraction

Each author independently assessed the titles and abstracts of potential papers identified according to the above-described search strategy. All potentially relevant articles were retained, and the full text of these studies was examined to determine which studies satisfied the inclusion criteria. Data extraction was carried out independently by all of the reviewers using standard data extraction forms. If needed and wherever possible, authors were contacted to retrieve missing data. We compared the extracted data to identify errors. Discrepancies between the reviewers were resolved by discussion. To avoid multiple counting of the control group in trials with multiple intervention groups and a single control group, we divided the sample size of the control group equally between the numbers of intervention groups, whereas retaining the mean change and it's SD. This method will reduce the probability of type 1 error.

Measures of treatment effect

The data was analyzed using Review Manager ((RevMan) [Computer program] version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014.). The standard mean difference (SMD) between the treatment and control groups was selected to represent the difference in continuous outcomes (effect size measure) with the study confidence interval and total confidence interval at 95% CIs. The overall mean effect size was calculated by using the fixed-effects model.

Assessment of heterogeneity

We used a chi-square test to assess heterogeneity and the Higgins I^2 statistic to determine the percentage of total variation across studies due to heterogeneity. A value of 0% indicates no observed heterogeneity, and larger values show increased heterogeneity. For simplicity, if heterogeneity (I^2 =50%) was not shown, we present the results of the fixed effects model only.

Assessment of reporting biases

To test for publication bias, we used a test for asymmetry of the funnel plot proposed by Egger et. al (Egger, Smith, Schneider, & Minder, 1997). The reviewers independently, but without being blinded to the authors or journals, assessed the risk of bias in the studies that met the inclusion criteria.

Stratified analyses

We pooled data reported for subgroups. Stratified analyses were conducted for the following variables: quality of methods [concealment of allocation (adequate compared with unclear, inadequate, or not used], blinding (double blinding compared with single blinding, no blinding, or unclear), attrition (<20% compared with >20% or unknown), comparability of intervention groups at baseline (yes compared with no or unclear), type of analysis (intention- to-treat compared with others), supplementation vehicle (fortification compared with tablet), number of micronutrients provided (single compared with multiple), duration of supplementation (<12 wk compared with >12 wk), and inclusion of either iron or zinc in the intervention (yes compared with no).

RESULTS

Search flow

The search identified 1569 potentially relevant references for inclusion in the meta-analysis; of which 1453 papers were excluded during initial screening because of duplication and irrelevance (none were directly referring to the effect of micronutrient supplementation on mental and psychomotor development). On further more detailed evaluation, 95 papers were excluded for different reasons (Figure 1). Two additional recent reviews were added and a final full text review resulted with twenty-one publications eligible for inclusion for final analysis.

Study characteristics

From twenty-one articles that met the inclusion criteria and were selected for final analysis, two studies (Prado et al., 2015; Prado, Maleta, et al., 2016b), were used for review purpose only. Though both fulfilled all the inclusion criteria, they were excluded from poling to meta-analyses by the authors' decision. This was mainly due to the studies' more complex design with additional arms containing macronutrient (20g sachet of small-quantity lipid based nutrient supplement (SQ-LNSs) supplementation to mothers and children. The authors believed that such studies could potentially affect comparability between studies and decided to exclude them from the final analysis. Briefly, the first of these two was a Malawian study (Prado, Maleta, et al., 2016b) that examined the effects of providing small-quantity lipid-based nutrient supplements (SQ-LNSs) to mothers during pregnancy and during the first 6 months postpartum. They also gave the supplementation to children aged 6–18 months. Similarly, the second study by the same authors (Prado et al., 2015) used similar approaches, but the supplementations were given to infants aged 9 to 18 months .

A total of 21 publications were included in the final review and 19 publications for Meta analysis, of which; three were conducted in African ((Adu-Afarwuah et al., 2007a; Faber, Kvalsvig, Lombard, & Benadé, 2005b; Manno et al., 2012)), three ((Ashworth, Morris, Lira, & Grantham-McGregor, 1998; Castillo-Durán et al., 2001; Colombo et al., 2014a)) in Latin America, one in Mexico(Rosado, López, García, Alatorre, & Alvarado, 2011) and twelve in Asian ((M. M. Black, Baqui, et al., 2004; M. M. Black, Sazawal, et al., 2004; Christian, Kim, Mehra, Shaikh, Ali, Shamim, Wu, Klemm, Labrique, & West Jr, 2016; Hamadani et al., 2001; Li et al., 2009; Lind et al., 2004; Nahar et al., 2012; Singla, Shafique, Zlotkin, & Aboud, 2014; Surkan et al., 2013; Taneja, Bhandari, Bahl, & Bhan, 2005; Tofail et al., 2008; Yousafzai, Rasheed, Rizvi, Armstrong, & Bhutta, 2014)) countries. Eight trials were conducted in infants that are 0 - 12 months of age, and eleven trials in young children, 12-23 months of age. Eight trials in mental and nine trials in motor development assessed the effects of a single micronutrient and eleven trials in mental and twelve in motor development assessed the effects of multiple micronutrients. Sixteen papers reported data on the effects of micronutrient supplementation on mental development and eighteen papers reported data on the effects of micronutrient supplementation on motor development. The duration of the interventions varied from four to seventy two weeks. The number of multiple micronutrients provided ranged from single (one) to multiple micronutrients (22MN).

Type of micronutrients used for the intervention

Eight trials in mental and nine trials in motor development (Table 1) used a single micronutrient; while eleven trials in mental and twelve in motor development used double and/or multiple micronutrient interventions. Besides, some also used micronutrient in the form of powder or a syrup and others were fortified with food (Aburto, Ramirez-Zea, Neufeld, & Flores-Ayala, 2010; Faber et al., 2005b; Manno et al., 2012) or food supplement (Manno et al., 2012). In addition, few others (Nahar et al., 2012) provided food supplement with and without psychosocial stimulation and (Prado et al., 2015; Prado, Maleta, et al., 2016b) micronutrient supplement with small- quantity lipid-based nutrient supplement (Table 1 for an overview of the trial characteristics).

Table 1 Characteristics of included trials

Author's name	Country	Design	Exposure	Main outcomes	Sample size	Attrition	Study participant	Mental development (Mean ±SD)	Moter development (Mean ±SD)	Remark
(Ashworth et al., 1998)	Brazil	double-blind, part-randomized efficacy trial	1 mg zinc 5 mg zinc or placebo -starting at birth daily except Sunday for eight weeks	**MD and PD**	Initial N=205 **At 6mo** nc=53 n1mg=56 n5mg=54 **At 12mo** nc=44 n1mg=48 n5mg=46	At 6mo 20% At 12mo 15% Overall 32.68%	Infants (LBW) from birth to 12 mo of age Meas. At 6& 12mo	**BSID** **At 6mo** Cont 91.5 ± 5.4 Int1.89.6 ±6.9 Cont 91.5 ± 5.4 Int5.90.1± 7.4 **At 12mo** Cont 109.1±12.2 Int1.106.7 ±11.1 Cont 109.1 ±12.2 Int5106.9 ±12.1	**BSID** **At 6mo** Cont 93.6 ±6.6 Int1. 91.3 ±8.7 Cont 93.6 ±6.6 Int5. 93.7 ±8.4 **At 12mo** Cont 100.4 ±11.3 Int1. 101.1 ±11.0 Cont 100.4 ±11.3 Int5. 100.0 ±11.6	No sign. d/f in the score of MD &PD on the 3 group -5 mg zinc may reverse some of the poor responsiveness (+effect)
(Hamadani et al., 2001)	Bangladesh	randomized, double-blind, controlled trial	5 mg elemental Zn or placebo -daily for 5 mo	MD and PD	Initial N=272 **At 7mo** nc=109 ni=103 **At 13mo** nc=101 ni=97	**At 7mo** 22% **At 13mo** 27%	Infants from 1mo to 13 mo of age Meas. At7& 13mo	**BSID** **At 7mo** Cont 98.4±6.5 Int. 98.4±7.0 **At 13mo** Cont 106.4±9.3 Int. 103.1±11.0	**BSID** **At 7mo** Cont 102.1±14.0 Int. 101.8±13.3 **At 13mo** Cont. 90.6±18.9 Int. 88.0±18.9	Zn treated group slightly but significantly lower MD score than placebo
(Castillo-Durán et al., 2001)	Chile	double-blind randomized control trials	5mg of Zn/d or placebo for 4wk After 5mo Fe drop 1-2mg/kg/d	MD and PD	Initial N=150 nc=52 ni=57	**Overall 27.33%**	Infant from birth to 12mo Meas. At 6& 12mo	**BSID II** **At 6mo** **Cont.** 90.8 ± 7.8 Int. 90.1 ± 5.6 **At 12mo** Cont. 88.9 ± 9.1 Int. 90.9 ± 10.5	**BSID II** **At 6mo** **Cont.** 87.7 ± 8.9 Int. 87.4 ± 8.5 **At 12mo** Cont. 87.6 ± 9.9 Int. 84.5 ± 11.5	Zn supp. has a beneficial effect on mental & motor quality behavior of healthy term infants. No significant differences in the orientation/ engagement and emotional regulation factors
(Lind et al., 2004)	Indonesian	RCT	10 mg Fe, 10 mg Zn , 10 mg Fe and 10 mg Zn , or placebo daily for 6mo	MD and PD	Initial N=680 nc=165 nFe=163 nZn=167 nFe+Zn=160	4%	infants from 6 to 12 mo of age Measure at 12mo	**BSIDII** Cont 99±10.0 Int. Fe 101±9.7 Cont 99±10.0 Int. Zn 101±9.3 Cont 99±10.0 IntFe+Zn 100±9.8	**BSID II** Cont103±10.8 IntFe106±11.0 Cont103±10.8 IntZn 105±10.6 Cont103±10.8 IntFe+Zn 103±10.3	PD higher in Fe group at 12mo old no significant difference in MD or BRS between the groups

Author's name	Country	Design	Exposure	Main outcomes	Sample size	Attrition	Study participant	Mental development (Mean ±SD)	Moter development (Mean ±SD)	Remark
(M. M. Black, Sazawal, et al., 2004)	India	RCT	Micronutrient mix with or without 5 mg of zinc sulfate or placebo daily for 8mo	MD and PD	Initial N=200 **At 6mo** nc=71 ni=79 **At 10mo** nc=77 ni=85	**At 6mo** 25% **At 10mo** 19% Overall 19%	infants from 1 to 9mo of age (SGA) Measure. at 6 &10mo	**BSID II** **At 6mo** Cont.82.0 ± 9.1 Int.MNZn82.7± 8.6 **At 10mo** Cont.86.4 ± 5.1 Int.MNZn 86.2±4.9	**BSID II** **At 6mo** Cont.(87.8 ± 12.3) IntMNZn(89.6±11.2) **At 10mo** Cont.(91.5 ± 14.2) Int.MNZn (91.7± 9.8)	At 6 &10 mo No direct effect of Zn supp on both devpt & behavior
(M. M. Black, Baqui, et al., 2004)	Bangladeshi	double-blind trial	Fe 20mg Zn 20mg Fe&Zn MM (16 vitamins & minerals, or riboflavin (Placebo) weekly for 6mo	MD and PD	Initial N=346 At 12mo nfe=49 nzn=49 nfezn=43 nmm=35 nc=45	Overall 36%	infants from 6 to 12 mo of age Measure. at 6 &12mo	**BSID II** **At 12mo** Int. Fe (104.3 ±9.5) Cont(102.7 ± 13.5) Int.Zn(104.7 ± 8.3) Cont(102.7 ± 13.5) IntFe+Zn(105.4±11.6) Cont(102.7 ± 13.5) IntMM(104.3± 13.0) Cont (102.7± 13.5)	**BSID II** **At12mo** Int. Fe 99.5± 16.6 Cont (95.4± 16.3) Int. Zn (101.2± 16.6) Cont (95.4± 16.3) IntFe+Zn (103.7±16.2) Cont (95.4± 16.3) Int MM(104.5±16.5) Cont (95.4± 16.3)	Fe&Zn together &with other MN have a + effect on PD & exploration (orientation-engagement)
(Faber, Kvalsvig, Lombard, & Benadé, 2005a)	South Africa	RCT	3mg β-car. 11mg Fe & 3mg Zn 10µg Cu, 10 µg Se, 0.4mg rib. 0.15mgB_6,0.25µgB_{12}2.5 mg Vit E daily for 6mo	Gross motor milestones	Initial N=361 ni=128 nc=138	26%	Infants aged 6–12 mo Measure at 12mo		**Gross motor milestones** (Interview of the mother) **At 12mo** Int (15.5±4.0) Cont.(14.4±3.7)	low-cost fortified maize-meal porridge can potentially have a significant effect on. improving motor devt.
(Taneja et al., 2005)	India	Double-blind, RCT	10 mg of Zn for infants & 20 mg for older child. or placebo daily for 4mo	MD and PD	Initial N=650 ni=283 nc=288	12%	Infants aged 6-30mo Measure at 12-18 mo	**BSID II** **At12-18mo** Int. Zn(92.8 ±10.9) Cont(91.3 ± 10.8)	**BSID II** **At12-18mo** Int. Zn (93.9 ±11.8) Cont.(92.2 ± 11.4)	Zn supp. did not affect MD & PD in setting in which Zn deficiency is common
(Tofail et al., 2008)	Bangladesh	RCT	MMs,60 mg Fe (fumarate) +400µg folate and 30 mg Fe (fumarate) + 400 µg folate Daily until delivery	PD	Initial N=2853 **L-BMI E** nFe30=29 nFe60=29 nMN=27 **L-BMIU** nFe30=32 nFe60=30 nMN=24 **EFG** nFe30=29 nFe60=29 nMN=27 **UFG** nFe30=32 nFe60=30 nMN=24	25%	Pregnant women and their Infant Measur at 7mo	-	**BSID II** **At 7mo** **H-BMI-EFG** Fe-30g-103.6±16.1 Fe-60g-104 ±14.7 MMg-102.8 ±16.2 **H-BMI-UFG** Fe-30g-103.2±15.4 Fe-60g-103.5 ±15.3 MMg-104.3 ±16.4 **L-BMI-EFG** Fe-30g100.9±15.6 Fe-60g-101.9 ±15.4 MMg-102.6 ±17.3 **L-BMI-UFG** Fe-30g-98.4±14.3 Fe-60g-99.3 ±15.1 MMg-105.3±16.4	Supp. In early or usual food with MM or Fe-F no overall benefit for child development Small benefit in motor score and activity rating in early food and MM supp. were found in infant of L-BMI but not H-BMI mother

Author's name	Country	Design	Exposure	Main outcomes	Sample size	Attrition	Study participant	Mental development (Mean ±SD)	Moter development (Mean ±SD)	Remark
(Li et al., 2009)	China	RCT	Multimicronutrient, folic acid plus iron or folic acid Daily until delivery	MD and PD	Initial n=1159 **At 12mo** nMM=351 nFF=393 nc=415	11.19%	Pregnant women and their Infant mesurement at 12mo	**BSID** **At 12mo** **IntFF(102.44**±45.26) Cont.(102.65±49.21) Int.MM(103.65±42.11) Cont.(102.65±49.21)	**BSID** **At 12mo** **IntFF(45.3**±22.15) Cont.(45.39±22.40) Int.MM(45.64±19.83) Cont. 45.39±22.40)	Supp. Of MN to pregnant women improve MD of the infant at 1y of age
(Rosado et al., 2011)	Mexico	RCT	Food supplement (OFS), powdered milk (PM) and placebo (PL) dally for 6mo	MD and PD	Initial N=224 nFS=58 nPM=65 nc.=60	16.9%	12-24 mo of children Measured at	**BSID-II** **Int. FS(135.9**±13.7 Cont. 137.6±12.6 Int. PM (138.3±13.6) Cont. 137.6±12.6	**BSID-II** **Int.FS 88.6**±9.2 Cont.90.0±9.3 Int.PM (89.6±9.2) Cont.90.0±9.3	Daily suppl. of 12–24-month-old children with OFS has no additional benefits cognitive performance
(Manno et al., 2012)	Zambian	RCT	basal fortified porridge and richly fortified porridge (vitamin A,Bs,C,D and Folet For 12mo	MD and PD	Initial N=502 **At 6mo** nb=213 nr=207 **At 12mo** nb=186 nr=178 **At 18mo** nb=175 nr=160	Over all 23%	infants aged 6 to 18mo Measured at 12 and 18mo	**BSID II** **At 6mo** Bas.(99.0±6.4) Rich(99.2±7.5) **At 12mo** Bas.(95.1±7.9) Rich(95.9±8.7) **At 18mo** Bas(88.4±7.4) Rich(89.8±7.4)	**BSID II** **At 6mo** Bas.(102.0±9.0) Rich(102.3±10.3) **At 12mo** Bas.(93.1±9.2) Rich.(93.4±10.4) **At 18mo** Bas.(91.4±6.1) Rich.(90.0±6.2)	1y rich MN forific. does not have benefit on child devt (MD &PD) compare with basal fortification
(Nahar et al., 2012)	Bangladesh	RCT	Psychosocial stimulation, Food supplementation and Micronutrient supplementation Daily for 6mo	MD and PD	Initial N= 507 nps=59 nfs=77 np+f=68 ncc=59 nch=59	36%	Severely underweight hospitalized children aged 6–24 months Measured at about 18mo	**BSID** **after 6moI** Int.FS(67.9±14.4) Cont.CC(66.6±14.4) Int.PS+FS(72.3±15.3) CC(66.6±14.4) CH 66.7±13.6	**BSID** **after 6moI** Int.FS(66.2±15.2) Cont.CC(67.4±16.2) Int.PS+FS(71.5±16.5) CC(67.4±16.2) CH(69.4±16.4)	PS+FS significantly benefitted the MD But not motor development
(Surkan et al., 2013)	Nepal	Cluster RCT	Zn 10mg, Fe 12.5 mg & folic acid 50 µg, Zn10mg +Fe12.5 mg +folic acid50 µg or placebo 3days per week	PD	Initial N=569 Zn+=285 NoZn=277 Fe+=285 NoFe=277		Infant 4-17 mo old Measured at 3times with 3mointerval at visit 2 and 5 moter millstone was missing		**Motor milestone** (adapted Griffiths Mental Development Scale and MacArthur CDI) Int.Zn (23.8±6.5) Cont.NoZn (24.2±6.5) Int.Fe (24.1±6.5) ConNoFe (24.0±6.5)	Found no effects of zinc or iron supplementation on motor milestones
(Singla et al., 2014)	Bangladeshi	cluster RCT	22element MNP plus NHHE and NHHE Daily for 5mo	MD	Initial n=231 ni-117 nc-114 At16-22m ni=82 nc=96	19%	Infant 0-12 mo Measer at 16-22mo (LBW)	**BSID III** **Total cognitive** Int.(51.20 ± 4.66) Cont.(50.67 ± 4.31) **Standard cognitive** Int.(9.26 ± 2.27) Cont.(8.98 ± 2.41)		MNP supp .not Significant effect on cognitive devt but + effect on expressive language

Author's name	Country	Design	Exposure	Main outcomes	Sample size	Attrition	Study participant	Mental development (Mean ±SD)	Moter development (Mean ±SD)	Remark
(Colombo et al., 2014a)	Peru	A double-blind, randomized clinical trial	10 mg/d Zn 10 mg/d Fe 0.5 mg/dCu or 10 mg/d Fe 0.5 mg/d Cu. Daily for 12mo	MD and PD	Initial N=251 ni6=128 ni12=101 ni18=98 nc6= 121 nc12= 106 nc18= 101	At12mo 17.5% At18mo 20.7%	Measure at infants at 6 mo, 12mo and 18mo	**BSIDII** **At 6 mo** Int.(102.0± 4.65) Cont.(101.6±4.42 **At 12 mo** Int.(103.4± 6.87) Cont.(103.2±7.61) **At 18 mo** Int.(95.0 ± 6.95) Cont(94.4±6.76)	**BSIDII** **At 6 mo** Int.(101.0± 7.89) Cont.(100.9±6.53) **At 12 mo** Int.(101.5± 6.56) Cont.(100.41±7.29) **At 18 mo** Int.(104.1 ± 5.77) Cont(103.9±6.10)	No differ. b/n the 2G In psychophysiology indices & the BSID or A not B error task But, MNP sign. affaect expressive language of children
(Yousafzai et al., 2014)	Pakistan	Cluster RCT	nutrition education and MMP and Control received standard care. MMP comprised of iron, folate, vitamin A and vitamin C	MD and PD	Initial N=680 At 12mo n both=338 n nut. =323 nc=342 At 24mo n both=344 n nut=335 nc=348	At 24 mo 7%	Infant age 6 to 24 mo Measured at 12 and 24mo	**BSID III** **At 12mo** Int. (95.9±12.7) Cont.(93.4±14.8) **At 24mo** Int. (78.4±14.6) Cont. (77.6±14.7)	**BSID III** **At 12mo** **Int.(83.2** ±13.4) Cont. (81.5±13.9) **At 24mo** Int. (89.4±16.8) Cont. (87.7±17.5)	Children received enhanced nutrition had significantly higher development scores(cognitive, language, and social-emotional scales) at 12mo of age but at 24mo of age only the language scores remained significantly higher.
(Locks et al., 2016)	Tanzania	RCT (Indivdual)	Zinc multivitamins , zinc and multivitamins or placebo at 6weeks of age daily for 15mo	MD and PD	Initial N= SubSample nZn+=121 nZn-=126 nMV+=119 nMV-=128	Subsample 0%	Infant age 6wk to 18mo Measure at 15mo	**BSID** Zinc Int. (50.03 ± 3.33) Cont.(50.19 ± 3.35) MV Int.(50.04 ± 3.19) Cont.(50.18 ± 3.47)	**BSID** **Zinc -Fine motor** **Int.(** 35.17 ± 2.84) Cont.(34.89 ± 3.02) **Zinc –Gross motor** **Int.(** 48.28 ± 2.41) Cont.(48.06 ± 2.33) MV-Fine motor Int.(35.14 ± 2.87) Cont.(34.92 ± 2.99) MV-Gross motor Int.(48.32 ± 2.33) Cont.(48.02 ± 2.40)	Neither daily zinc nor multivitamin supplements, alone or in combination, had a significant effect on developmental outcomes at 15months of age
(Christian, Kim, Mehra, Shaikh, Ali, Shamim, Wu, Klemm, Labrique, & West, 2016)	Bangladesh	RCT (Cluster)	IFA and MM to pregnant women 10wk to 3mo of postpartum	MD and PD	Initial N= SubSample nIFA=384 nMM=348	Subsample 0%	Pregnant women and their infant Mesure at 24mo	**BSID** Cognitive IFA(76.5± 7.8) MM(76.2 ±7.0)	**BSID** **IFA(**90.7 ±7.8) MM(90.1 ± 7.4)	had no impact on cognitive and motor function at 2 y

Author's name	Country	Design	Exposure	Main outcomes	Sample size	Attrition	Study participant	Mental development (Mean ±SD)	Moter development (Mean ±SD)	Remark
(Prado, Maleta, et al., 2016a)	Malawi	randomly assigned to 1 of 3 group	60 mg Fe & 400 µg folic acid + placebo(Ca) MMN SQ-LNSs + MMN During pregnancy and the 1st 6mo of postpartum and LNS children 6-18mo	PD	Initial n=743 nifa=222 nmm=225 nlns=223	9.8%	Pregnant women and Infant age 0 to18 mo	Executive function A-not-B correct	Kilifi developmental inventory(KDI) **Motor z score**	SQ-LNS to pregna. & infant +ve effect on devt milestone attainment but not motor language soci.emo or executive function at 18mo
(Prado, Abbeddou, Jimenez, et al., 2016)	Burkina Faso	partially masked, cluster-randomized controlled trial	20 g LNS 20 g LNS Zn5, 20 g LNS Zn10 20 g LNS TabZn5 Daily for 9mo	MD	Initial n=1426 nz0=255 nz10=248 ntz5=243 nnic=376	21.3%	Infant age 9-18mo		Developmental Milestones Checklist (DMC) II Motor z score	SQ-LNS + Malaria & Diarrhea treatment +vely affect motor, language &personal social devt at 18mo Regardless of added of Zn

Mental and motor development tests used

Most of the randomized trials included to this meta-analysis used direct assessment methods to determine mental and motor development of a child. The majority of the studies used Bayleys scale (version I, II, III) of mental development tool for assessing mental development of children. Similarly, sixteen studies used Bayleys scale (version I, II, III) , one study used the **Gross motor milestones** while another one used **motor milestone adapted** for the local setting from the Griffiths Mental Development Scale and MacArthur CDI method for assessing motor development (Table 1)

Effects of micronutrient supplementation/fortification on mental development of infants and young children

We pooled the results of sixteen trials to assess the effects of micronutrient (single or multiple) supplements on mental development of infants and young children. The funnel plot looked quite symmetrical (Figure 2) and there was no evidence of publication bias.

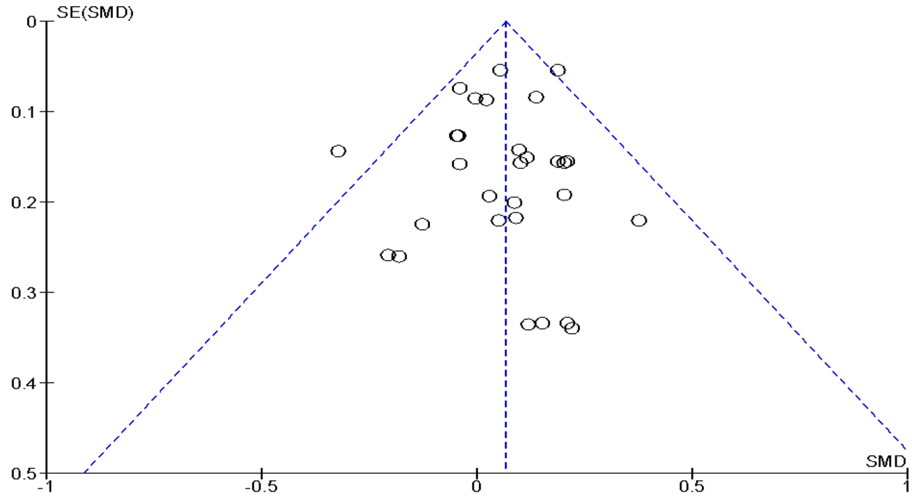


Figure 2 Funnel plot for mental development

The heterogeneity among trials was not significant $Chi^2 = 26.34$, ($P = 0.55$) and $I^2 = 0\%$. The overall effect size standard mean difference (SMD) of micronutrients on mental development of children was SMD= 0.07 (95% CI: 0.02, 0.11) and Z= 2.92; P=0.003 (Figure 3).

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
1.1.1 all studies								
Ashworth et.al 1998-1mg Zn 12mo	106.7	11.1	48	109.1	12.2	22	0.8%	-0.21 [-0.71, 0.30]
Ashworth et.al 1998-5mg Zn 12mo	106.9	12.1	46	109.1	12.2	22	0.8%	-0.18 [-0.69, 0.33]
Black, Baqui, et al., 2004 Fe 12mo	104.3	9.5	49	102.7	13.5	11	0.5%	0.15 [-0.50, 0.81]
Black, Baqui, et al., 2004 Fe+Zn 12mo	105.4	11.6	43	102.7	13.5	11	0.4%	0.22 [-0.44, 0.89]
Black, Baqui, et al., 2004 MMN 12mo	104.3	13	35	102.7	13.5	12	0.5%	0.12 [-0.54, 0.78]
Black, Baqui, et al., 2004 Zn 12mo	104.7	8.3	49	102.7	13.5	11	0.5%	0.21 [-0.45, 0.86]
Black, Sazawal, et al., 2004Mn10mo	86.2	4.9	85	86.4	5.1	77	2.1%	-0.04 [-0.35, 0.27]
Castillo-Durán et al., 2001 Zn 12mo	90.9	10.5	57	88.9	9.1	52	1.4%	0.20 [-0.18, 0.58]
Christian et al., 2016	76.2	7	348	76.5	7.8	384	9.3%	-0.04 [-0.19, 0.10]
Colombo et al., 2014 Fe+Cu+Zn 12mo	103.4	6.87	51	103.2	7.61	56	1.4%	0.03 [-0.35, 0.41]
Colombo et al., 2014 Fe+Cu+Zn18mo	95	6.95	49	94.4	6.76	51	1.3%	0.09 [-0.31, 0.48]
Hamadani et al., 2001 Zn 13mo	103.1	11	97	106.4	9.3	101	2.5%	-0.32 [-0.60, -0.04]
Li et al., 2009 FFA	102.44	45.26	393	102.65	49.21	207	6.9%	-0.00 [-0.17, 0.16]
Li et al., 2009 MMN	103.65	42.11	351	102.65	49.21	208	6.6%	0.02 [-0.15, 0.19]
Lind et al., 2004 Fe 12mo	101	9.7	163	99	10	55	2.1%	0.20 [-0.10, 0.51]
Lind et al., 2004 Fe+Zn 12mo	100	9.8	160	99	10	55	2.1%	0.10 [-0.21, 0.41]
Lind et al., 2004 Zn 12mo	101	9.3	167	99	10	55	2.1%	0.21 [-0.10, 0.52]
Locks et al., 2016-MM	50.04	3.19	119	50.18	3.47	128	3.1%	-0.04 [-0.29, 0.21]
Locks et al., 2016-Zinc	50.03	3.33	121	50.19	3.35	126	3.1%	-0.05 [-0.30, 0.20]
Manno et al., 2012 12mo	95.9	8.7	98	95.1	7.9	98	2.5%	0.10 [-0.18, 0.38]
Manno et al., 2012 18mo	89.8	7.4	80	88.4	7.4	88	2.1%	0.19 [-0.12, 0.49]
Nahar et al., 2012 FS + MN After6mo	67.9	14.4	77	66.6	14.4	29	1.1%	0.09 [-0.34, 0.52]
Nahar et al., 2012 PS +FS + MN After 6mo	72.3	15.3	68	66.6	14.4	30	1.0%	0.38 [-0.06, 0.81]
Rosado et.al 2011 FS	135.9	13.7	58	137.6	12.6	30	1.0%	-0.13 [-0.57, 0.31]
Rosado et.al 2011PM	138.3	13.6	65	137.6	12.6	30	1.0%	0.05 [-0.38, 0.48]
Singla, Shafique, Zlotkin, & Aboud, 2014 total	51.2	4.66	96	50.67	4.31	82	2.2%	0.12 [-0.18, 0.41]
Taneja, Bhandari, Bahl, & Bhan, 2005	92.8	10.9	283	91.3	10.8	288	7.2%	0.14 [-0.03, 0.30]
Yousafzai et.al 2014 12mo	95.99	12.7	658	93.4	14.8	699	17.1%	0.19 [0.08, 0.29]
Yousafzai et.al 2014 24mo	78.4	14.6	676	77.6	14.6	705	17.5%	0.05 [-0.05, 0.16]
Subtotal (95% CI)			**4590**			**3723**	**100.0%**	**0.07 [0.02, 0.11]**
Heterogeneity: Chi² = 26.34, df = 28 (P = 0.55); I² = 0%								
Test for overall effect: Z = 2.92 (P = 0.003)								
Total (95% CI)			**4590**			**3723**	**100.0%**	**0.07 [0.02, 0.11]**
Heterogeneity: Chi² = 26.34, df = 28 (P = 0.55); I² = 0%								
Test for overall effect: Z = 2.92 (P = 0.003)								
Test for subgroup differences: Not applicable								

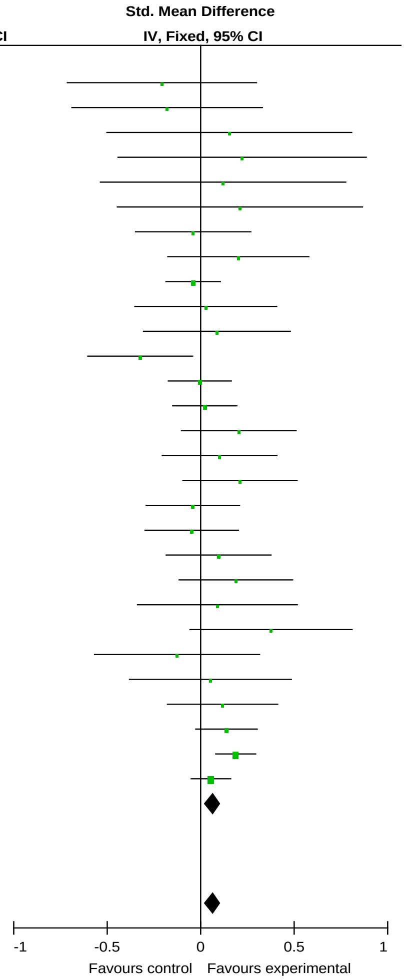


Figure 3 Forest plot for mental development

Subgroup analyses by age (using 12 months as a cutoff point) and number of micronutrient supplements (single versus multiple) showed that supplementation with multiple (than single) and during infancy (≤ 12 months) than at later ages (12 -23 months) significantly improved mental development of children. The effect size (SMD) of supplementation with single and multiple micronutrient was SMD = 0.04 (95% CI: -0.05, 0.14) and SMD=0.07 (95% CI: 0.02, 0.12), respectively (Figure 4 and Figure 5 respectively).

Study or Subgroup	Favours [experimental] Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
1.1.1 all studies								
Ashworth et.al 1998-1mg Zn 12mo	106.7	11.1	48	109.1	12.2	22	3.3%	-0.21 [-0.71, 0.30]
Ashworth et.al 1998-5mg Zn 12mo	106.9	12.1	46	109.1	12.2	22	3.3%	-0.18 [-0.69, 0.33]
Black, Baqui, et al., 2004 Fe 12mo	104.3	9.5	49	102.7	13.5	11	2.0%	0.15 [-0.50, 0.81]
Black, Baqui, et al., 2004 Zn 12mo	104.7	8.3	49	102.7	13.5	11	2.0%	0.21 [-0.45, 0.86]
Black, Sazawal, et al., 2004Mn10mo	86.2	4.9	85	86.4	5.1	77	9.0%	-0.04 [-0.35, 0.27]
Castillo-Durán et al., 2001 Zn 12mo	90.9	10.5	57	88.9	9.1	52	6.0%	0.20 [-0.18, 0.58]
Hamadani et al., 2001 Zn 13mo	103.1	11	97	106.4	9.3	101	10.8%	-0.32 [-0.60, -0.04]
Lind et al., 2004 Fe 12mo	101	9.7	163	99	10	55	9.1%	0.20 [-0.10, 0.51]
Lind et al., 2004 Zn 12mo	101	9.3	167	99	10	55	9.1%	0.21 [-0.10, 0.52]
Locks et al., 2016-Zinc	50.03	3.33	121	50.19	3.35	126	13.7%	-0.05 [-0.30, 0.20]
Taneja, Bhandari, Bahl, & Bhan, 2005	92.8	10.9	283	91.3	10.8	288	31.6%	0.14 [-0.03, 0.30]
Subtotal (95% CI)			**1165**			**820**	**100.0%**	**0.04 [-0.05, 0.14]**
Heterogeneity: Chi² = 13.55, df = 10 (P = 0.19); I² = 26%								
Test for overall effect: Z = 0.91 (P = 0.36)								
Total (95% CI)			**1165**			**820**	**100.0%**	**0.04 [-0.05, 0.14]**
Heterogeneity: Chi² = 13.55, df = 10 (P = 0.19); I² = 26%								
Test for overall effect: Z = 0.91 (P = 0.36)								
Test for subgroup differences: Not applicable								

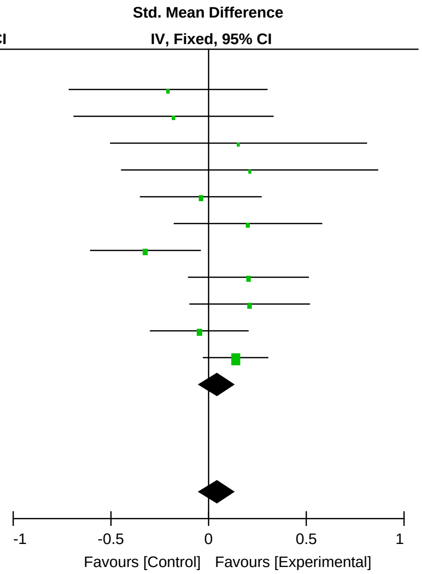


Figure 4 Forest plot for mental development single micronutrient

Study or Subgroup	Favours [experimental] Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
1.1.1 all studies								
Black, Baqui, et al., 2004 Fe+Zn 12mo	105.4	11.6	43	102.7	13.5	11	0.6%	0.22 [-0.44, 0.89]
Black, Baqui, et al., 2004 MMN 12mo	104.3	13	35	102.7	13.5	12	0.6%	0.12 [-0.54, 0.78]
Christian et al., 2016	76.2	7	348	76.5	7.8	384	12.0%	-0.04 [-0.19, 0.10]
Colombo et al., 2014 Fe+Cu+Zn 12mo	103.4	6.87	51	103.2	7.61	56	1.8%	0.03 [-0.35, 0.41]
Colombo et al., 2014 Fe+Cu+Zn18mo	95	6.95	49	94.4	6.76	51	1.6%	0.09 [-0.31, 0.48]
Li et al., 2009 FFA	102.44	45.26	393	102.65	49.21	207	8.9%	-0.00 [-0.17, 0.16]
Li et al., 2009 MMN	103.65	42.11	351	102.65	49.21	208	8.6%	0.02 [-0.15, 0.19]
Lind et al., 2004 Fe+Zn 12mo	100	9.8	160	99	10	55	2.7%	0.10 [-0.21, 0.41]
Locks et al., 2016-MM	50.04	3.19	119	50.18	3.47	128	4.1%	-0.04 [-0.29, 0.21]
Manno et al., 2012 12mo	95.9	8.7	98	95.1	7.9	98	3.2%	0.10 [-0.18, 0.38]
Manno et al., 2012 18mo	89.8	7.4	80	88.4	7.4	88	2.7%	0.19 [-0.12, 0.49]
Nahar et al., 2012 FS + MN After6mo	67.9	14.4	77	66.6	14.4	29	1.4%	0.09 [-0.34, 0.52]
Nahar et al., 2012 PS +FS + MN After 6mo	72.3	15.3	68	66.6	14.4	30	1.3%	0.38 [-0.06, 0.81]
Rosado et.al 2011 FS	135.9	13.7	58	137.6	12.6	30	1.3%	-0.13 [-0.57, 0.31]
Rosado et.al 2011PM	138.3	13.6	65	137.6	12.6	30	1.4%	0.05 [-0.38, 0.48]
Singla, Shafique, Zlotkin, & Aboud, 2014 total	51.2	4.66	96	50.67	4.31	82	2.9%	0.12 [-0.18, 0.41]
Yousafzai et.al 2014 12mo	95.99	12.7	658	93.4	14.8	699	22.2%	0.19 [0.08, 0.29]
Yousafzai et.al 2014 24mo	78.4	14.6	676	77.6	14.6	705	22.7%	0.05 [-0.05, 0.16]
Subtotal (95% CI)			**3425**			**2903**	**100.0%**	**0.07 [0.02, 0.12]**
Heterogeneity: Chi² = 12.49, df = 17 (P = 0.77); I² = 0%								
Test for overall effect: Z = 2.83 (P = 0.005)								
Total (95% CI)			**3425**			**2903**	**100.0%**	**0.07 [0.02, 0.12]**
Heterogeneity: Chi² = 12.49, df = 17 (P = 0.77); I² = 0%								
Test for overall effect: Z = 2.83 (P = 0.005)								
Test for subgroup differences: Not applicable								

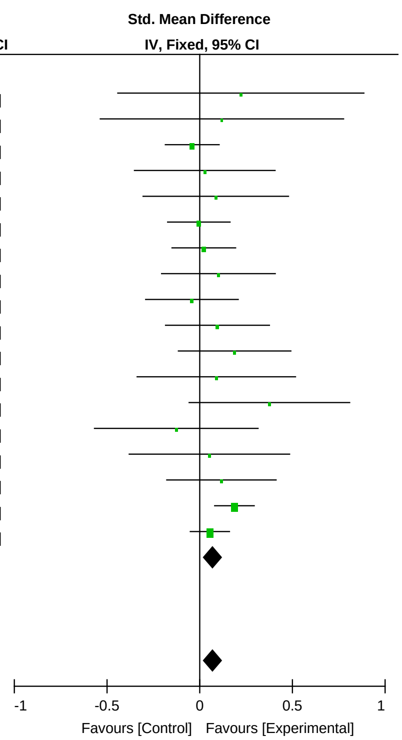


Figure 5 Forest plot for mental development multiple micronutrient

Similarly, micronutrient supplementation during infancy (<12 month) and during early childhood had a pooled mean effect size (SMD) of 0.10 (95% CI: 0.04, 0.17) and SMD= 0.02 (95% CI: -0.03, 0.09), respectively (Figures 6.and Figure7).

	Favours [experimental]			Control				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
1.1.1 all studies								
Ashworth et.al 1998-1mg Zn 12mo	106.7	11.1	48	109.1	12.2	22	1.6%	-0.21 [-0.71, 0.30]
Ashworth et.al 1998-5mg Zn 12mo	106.9	12.1	46	109.1	12.2	22	1.6%	-0.18 [-0.69, 0.33]
Black, Baqui, et al., 2004 Fe 12mo	104.3	9.5	49	102.7	13.5	11	1.0%	0.15 [-0.50, 0.81]
Black, Baqui, et al., 2004 Fe+Zn 12mo	105.4	11.6	43	102.7	13.5	11	0.9%	0.22 [-0.44, 0.89]
Black, Baqui, et al., 2004 MMN 12mo	104.3	13	35	102.7	13.5	12	1.0%	0.12 [-0.54, 0.78]
Black, Baqui, et al., 2004 Zn 12mo	104.7	8.3	49	102.7	13.5	11	1.0%	0.21 [-0.45, 0.86]
Black, Sazawal, et al., 2004Mn10mo	86.2	4.9	85	86.4	5.1	77	4.3%	-0.04 [-0.35, 0.27]
Castillo-Durán et al., 2001 Zn 12mo	90.9	10.5	57	88.9	9.1	52	2.9%	0.20 [-0.18, 0.58]
Colombo et al., 2014 Fe+Cu+Zn 12mo	103.4	6.87	51	103.2	7.61	56	2.9%	0.03 [-0.35, 0.41]
Li et al., 2009 FFA	102.44	45.26	393	102.65	49.21	207	14.5%	-0.00 [-0.17, 0.16]
Li et al., 2009 MMN	103.65	42.11	351	102.65	49.21	208	14.0%	0.02 [-0.15, 0.19]
Lind et al., 2004 Fe 12mo	101	9.7	163	99	10	55	4.4%	0.20 [-0.10, 0.51]
Lind et al., 2004 Fe+Zn 12mo	100	9.8	160	99	10	55	4.4%	0.10 [-0.21, 0.41]
Lind et al., 2004 Zn 12mo	101	9.3	167	99	10	55	4.4%	0.21 [-0.10, 0.52]
Manno et al., 2012 12mo	95.9	8.7	98	95.1	7.9	98	5.2%	0.10 [-0.18, 0.38]
Yousafzai et.al 2014 12mo	95.99	12.7	658	93.4	14.8	699	36.1%	0.19 [0.08, 0.29]
Subtotal (95% CI)			**2453**			**1651**	**100.0%**	**0.10 [0.04, 0.17]**
Heterogeneity: Chi² = 9.83, df = 15 (P = 0.83); I² = 0%								
Test for overall effect: Z = 3.15 (P = 0.002)								
Total (95% CI)			**2453**			**1651**	**100.0%**	**0.10 [0.04, 0.17]**
Heterogeneity: Chi² = 9.83, df = 15 (P = 0.83); I² = 0%								
Test for overall effect: Z = 3.15 (P = 0.002)								
Test for subgroup differences: Not applicable								

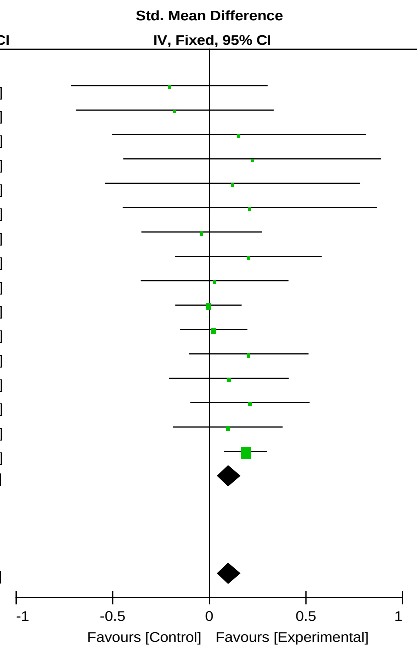


Figure 6 Forest plot for mental development <12mo

	Favours [experimental]			Control				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
1.1.1 all studies								
Christian et al., 2016	76.2	7	348	76.5	7.8	384	17.6%	-0.04 [-0.19, 0.10]
Colombo et al., 2014 Fe+Cu+Zn18mo	95	6.95	49	94.4	6.76	51	2.4%	0.09 [-0.31, 0.48]
Hamadani et al., 2001 Zn 13mo	103.1	11	97	106.4	9.3	101	4.7%	-0.32 [-0.60, -0.04]
Locks et al., 2016-MM	50.04	3.19	119	50.18	3.47	128	6.0%	-0.04 [-0.29, 0.21]
Locks et al., 2016-Zinc	50.03	3.33	121	50.19	3.35	126	6.0%	-0.05 [-0.30, 0.20]
Manno et al., 2012 18mo	89.8	7.4	80	88.4	7.4	88	4.0%	0.19 [-0.12, 0.49]
Nahar et al., 2012 FS + MN After6mo	67.9	14.4	77	66.6	14.4	29	2.0%	0.09 [-0.34, 0.52]
Nahar et al., 2012 PS +FS + MN After 6mo	72.3	15.3	68	66.6	14.4	30	2.0%	0.38 [-0.06, 0.81]
Rosado et.al 2011 FS	135.9	13.7	58	137.6	12.6	30	1.9%	-0.13 [-0.57, 0.31]
Rosado et.al 2011PM	138.3	13.6	65	137.6	12.6	30	2.0%	0.05 [-0.38, 0.48]
Singla, Shafique, Zlotkin, & Aboud, 2014 total	51.2	4.66	96	50.67	4.31	82	4.3%	0.12 [-0.18, 0.41]
Taneja, Bhandari, Bahl, & Bhan, 2005	92.8	10.9	283	91.3	10.8	288	13.8%	0.14 [-0.03, 0.30]
Yousafzai et.al 2014 24mo	78.4	14.6	676	77.6	14.6	705	33.3%	0.05 [-0.05, 0.16]
Subtotal (95% CI)			**2137**			**2072**	**100.0%**	**0.03 [-0.03, 0.09]**
Heterogeneity: Chi² = 14.04, df = 12 (P = 0.30); I² = 15%								
Test for overall effect: Z = 1.03 (P = 0.30)								
Total (95% CI)			**2137**			**2072**	**100.0%**	**0.03 [-0.03, 0.09]**
Heterogeneity: Chi² = 14.04, df = 12 (P = 0.30); I² = 15%								
Test for overall effect: Z = 1.03 (P = 0.30)								
Test for subgroup differences: Not applicable								

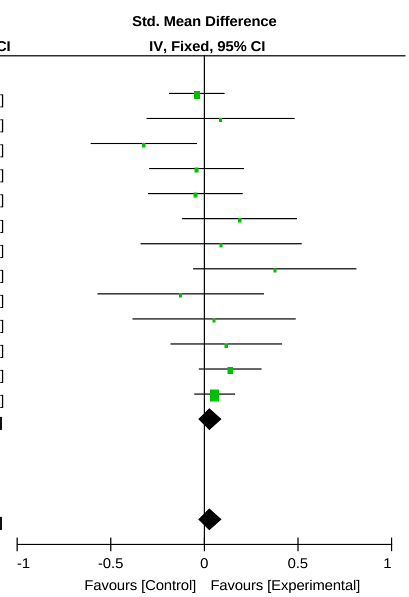


Figure 7 Forest plot for mental development >12mo

On sensitivity and subgroup analysis; allocation concealment, nature of blinding, attrition rate, comparability of groups at baseline, method of randomization, method of analysis, presence of iron in MMN supplements were significantly associated with the effects of micronutrient supplementation on mental development (Table 2).

Table 2 Subgroup analysis for mental and psychomotor development

	Mental development (MD)			Psychomotor development(PD)		
Stratification variables	No. of trials	Overall effect size MSD(95%CI); p value	Test for heterogeneity I^2 (%);Chi^2(p value)	No. of trials	Overall effect size MSD(95%CI); p value	Test for heterogeneity I^2(%);Q(p value)
Allocation concealment						
Adequate						
Others						
	9	0.10(0.05,0.16); <0.0001	0.00;9.71;(0.92)	10	0.07(0.03,0.12);0.002	0.00;17.64(0.55)
	7	-0.02(-0.01,0.06); 0.63	0.00;10.60(0.72)	8	-0.00(-0.06,0.05);0.89	0.00;14.12(0.72)
Blinding						
Double blinded						
Others	10	0.02(-0.05,0.08);0.70	0.00;14.95(0.60)	10	0.03(-0.03,0.09);0.37	0.00;15.86(0.67)
	6	0.15(0.08,0.21);<0.0001	0.00;4.25(0.94)	8	0.05(0.00,0.10);0.03	9.00;19.76(0.35)
Attrition rate						
<20%						
Others	10	0.07(0.02,0.12);0.004	0.00;13.27(0.65)	10	0.06(0.01,0.10);0.01	0.00;13.99(0.78)
	6	0.04(-0.07,0.16);0.46	15.00;12.93(0.30)	8	0.01(-0.05,0.08),0.71	13.00;20.76(0.29)
Comparability of group at baseline						
Yes						
Others	7	0.13(0.05,0.22);0.002	0.00;3.88(1.00)	9	0.07(0.01,0.14);0.03	9.00;19.68(0.35)
	9	0.04((-0.01,0.09);0.12	37.00;19.07(0.09)	9	0.03(-0.01,0.08)0.18	0.00;15.18(0.71)
Randomization method						
Individual						
Others						
	11	0.06(-0.01,0.14);0.08	0.00;17.50(0.62)	14	0.05(-0.01,0.10);0.08	0.00;27.79(0.63)
	5	0.07(0.01,0.12);0.02	21.00;8.84(0.26)	4	0.04((-0.01,0.09);0.13	26.00;8.08(0.23)
Method of analysis						
Intention to treat						
Others	4	0.09(0.03,0.15);0.003	3.00;7.21(0.41)	5	0.05(0.00,0.10);0.04	0.00;8.80(0.79)
	12	0.04(-0.03,0.10);0.28	0.00;17.50(0.62)	13	0.03(-0.02,0.09);0.27	10.00;26.78(0.31)
Supplementation vehicle						
Fortification	5	0.12((0.06,0.18);0.0002	0.00;4.55(0.71)	4	0.09(0.03,0.16);0.005	32.00;8.76(0.19)
Syrup/tablet/capsule	11	0.01(-0.05,0.07);0.71	0.00;16.25(0.70)	14	0.02(-0.03,0.06);0.39	0.00;23.76(0.82)

Stratification variables	No. of trials	Overall effect size MSD(95%CI); p value	Test for heterogeneity I^2 (%);Chi^2(p value)	No. of trials	Overall effect size MSD(95%CI); p value	Test for heterogeneity I^2(%);Q(p value)
Number of MN						
Single	3	0.04 (-0.05,0.14);0.36	26.00;13.55 (0.19)	3	-013 (-0.32,0.06);0.17	0.00;1.35 (0.72)
Multiple	13	0.07 (0.02,0.12);0.005	0.00;12.49 (0.77)	15	0.05 (0.01,0.09);0.009	0.00;31.15 (0.61)
Duration of intervention						
≤12wk	2	0.10 (0.04,0.17);0.002	0.00;9.83 (0.83)	2	0.06 (0.01,0.11);0.02	0.00;21.74 (0.48)
>12wk	14	0.03 (-0.03,0.09);0.30	15.0; 14.04 (0.30)	16	0.03 (-0.03,0.08);0.34	0.00;12.54 (0.34)
Iron in intervention						
Yes	10	0.08 (0.03,0.13);0.002	0.00;13.26 (0.83)	12	0.05 (0.01,0.09);0.02	0.00;26.29 (0.50)
No	6	0.02 (-0.07,0.11);0.62	33.00;11.97 (0.15)	6	0.02 (-0.07,0.11);0.62	0.00;9.38 (0.50)
Zinc in intervention						
Yes	14	0.03 (-0.03,0.09);0.31	0.00;19.05 (0.75)	16	0.03 (-0.02,0.07);0.24	0.00;28.96 (0.71)
No	2	0.12 (0.05,0.19);0.0007	7.00;3.21 (0.36)	2	0.11 (0.04,0.19);0.004	0.00;0.11 (0.74)

Effects of micronutrient supplementation/fortification on motor development of infants and young children

For this component of the analysis, we pooled a total of eighteen trials to see if supplementation of micronutrients affects motor development of infants and young children. Similar to the previous analysis, the funnel plot was quite symmetrical (Figure 8) and there was no evidence of publication bias.

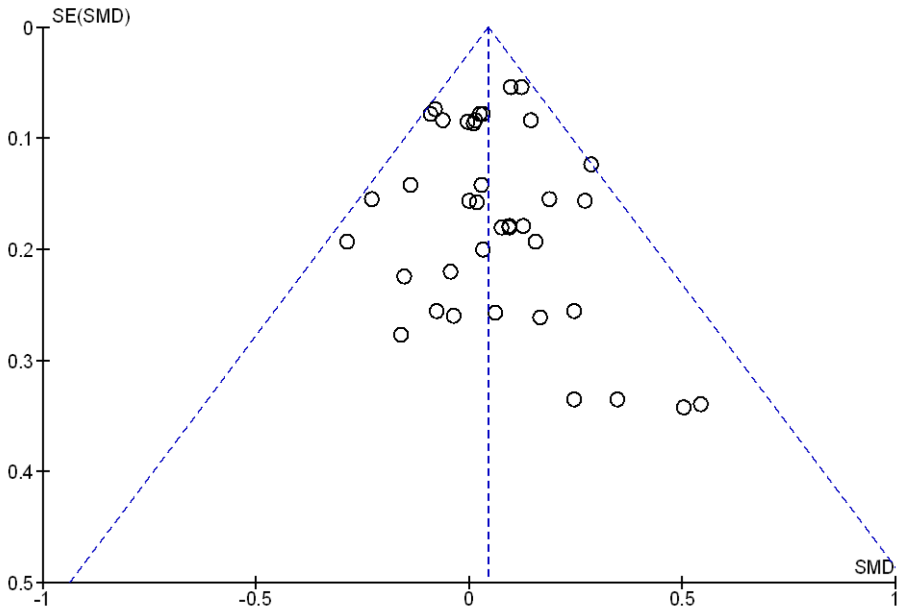


Figure 8 Funnel plot for motor development

The heterogeneity among trials was also not significant Chi² = 35.90, (P = 0.57) and I² = 0%. Overall, there is a significant effect (SMD = 0.04; 95% CI: 0.01, 0.08; P = 0.02) of micronutrient supplementation on motor development of infants and young children (Figure 9).

Study or Subgroup	Intervantion Mean	Intervantion SD	Intervantion Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
1.2.1 all studies								
Ashworth et.al 1998-1mg Zn 12mo	101.1	11	48	100.4	11.3	22	0.5%	0.06 [-0.44, 0.57]
Ashworth et.al 1998-5mg Zn 12mo	100	11.6	46	100.4	11.3	22	0.5%	-0.03 [-0.54, 0.47]
Black, Baqui, et al., 2004 Fe 12mo	99.5	16.6	49	95.4	16.3	11	0.3%	0.24 [-0.41, 0.90]
Black, Baqui, et al., 2004 Fe+Zn 12mo	103.7	16.2	43	95.4	16.3	11	0.3%	0.50 [-0.17, 1.17]
Black, Baqui, et al., 2004 MMN 12mo	104.5	16.5	35	95.4	16.3	12	0.3%	0.54 [-0.12, 1.21]
Black, Baqui, et al., 2004 Zn 12mo	101.2	16.6	49	95.4	16.3	11	0.3%	0.35 [-0.31, 1.00]
Black, Sazawal, et al., 2004Mn10mo	91.7	9.8	85	91.5	14.2	77	1.4%	0.02 [-0.29, 0.32]
Castillo-Durán et al., 2001 Zn 12mo	84.5	11.5	57	87.6	9.9	52	1.0%	-0.29 [-0.66, 0.09]
Christian et al., 2016	90.1	7.4	348	90.7	7.8	384	6.5%	-0.08 [-0.22, 0.07]
Colombo et al., 2014 Fe+Cu+Zn 12mo	101.5	6.56	51	100.41	7.29	56	1.0%	0.16 [-0.22, 0.54]
Colombo et al., 2014 Fe+Cu+Zn18mo	104.1	5.77	49	103.9	6.1	51	0.9%	0.03 [-0.36, 0.43]
Faber, Kvalsvig, Lombard, & Benadé, 2005 post	15.5	4	128	14.4	3.7	138	2.4%	0.29 [0.04, 0.53]
Hamadani et al., 2001 Zn 13mo	88	18.9	97	90.6	18.9	101	1.8%	-0.14 [-0.42, 0.14]
Li et al., 2009 FFA	45.3	22.15	393	45.39	22.4	207	4.9%	-0.00 [-0.17, 0.16]
Li et al., 2009 MMN	45.64	19.83	351	45.39	22.4	208	4.7%	0.01 [-0.16, 0.18]
Lind et al., 2004 Fe 12mo	106	11	163	103	10.8	55	1.5%	0.27 [-0.03, 0.58]
Lind et al., 2004 Fe+Zn 12mo	103	10.3	160	103	10.8	55	1.5%	0.00 [-0.31, 0.31]
Lind et al., 2004 Zn 12mo	105	10.6	167	103	10.8	55	1.5%	0.19 [-0.12, 0.49]
Locks et al., 2016-MM-fine	35.14	2.87	59	34.92	2.99	64	1.1%	0.07 [-0.28, 0.43]
Locks et al., 2016-MM-gross	48.32	2.33	60	48.02	2.4	64	1.1%	0.13 [-0.23, 0.48]
Locks et al., 2016-zinc-fine	35.17	2.84	60	34.89	3.02	63	1.1%	0.09 [-0.26, 0.45]
Locks et al., 2016-Zinc-Gross	48.28	2.41	61	48.06	2.33	63	1.1%	0.09 [-0.26, 0.44]
Manno et al., 2012 12mo	93.4	10.4	98	93.1	9.2	98	1.8%	0.03 [-0.25, 0.31]
Manno et al., 2012 18mo	90	6.2	80	91.4	6.1	88	1.5%	-0.23 [-0.53, 0.08]
Nahar et al., 2012 FS + MN After6mo	66.2	15.2	77	67.4	16.2	19	0.5%	-0.08 [-0.58, 0.42]
Nahar et al., 2012 PS +FS + MN After 6mo	71.5	16.5	68	67.4	16.2	20	0.6%	0.25 [-0.25, 0.75]
Rosado et.al 2011 FS	88.6	9.2	58	90	9.3	30	0.7%	-0.15 [-0.59, 0.29]
Rosado et.al 2011PM	89.6	9.2	65	90	9.3	30	0.7%	-0.04 [-0.48, 0.39]
Surkan et al., 2013 Fe	24.1	6.5	285	24	6.5	277	5.0%	0.02 [-0.15, 0.18]
Surkan et al., 2013 Zn	23.8	6.5	285	24.2	6.5	277	5.0%	-0.06 [-0.23, 0.10]
Taneja, Bhandari, Bahl, & Bhan, 2005	93.9	11.8	283	92.2	11.4	288	5.1%	0.15 [-0.02, 0.31]
Tofail et al., 2008 early Fe-30H	103.6	16.1	325	103.2	15.4	318	5.8%	0.03 [-0.13, 0.18]
Tofail et al., 2008 early Fe-30L	100.9	15.6	29	98.4	14.3	30	0.5%	0.16 [-0.35, 0.68]
Tofail et al., 2008 early Fe-60H	104	14.7	324	103.5	15.3	326	5.8%	0.03 [-0.12, 0.19]
Tofail et al., 2008 early Fe-60L	101.9	15.4	32	99.3	15.1	27	0.5%	0.17 [-0.35, 0.68]
Tofail et al., 2008 early MMH	102.8	16.2	322	104.3	16.4	330	5.8%	-0.09 [-0.25, 0.06]
Tofail et al., 2008 early MML	102.6	17.3	29	105.3	16.4	24	0.5%	-0.16 [-0.70, 0.38]
Yousafzai et.al 2014 12mo	83.2	13.4	658	81.5	13.9	699	12.1%	0.12 [0.02, 0.23]
Yousafzai et.al 2014 24mo	89.4	16.8	676	87.7	17.5	705	12.4%	0.10 [-0.01, 0.20]
Subtotal (95% CI)			**6253**			**5368**	**100.0%**	**0.04 [0.01, 0.08]**
Heterogeneity: Chi² = 35.90, df = 38 (P = 0.57); I² = 0%								
Test for overall effect: Z = 2.29 (P = 0.02)								
Total (95% CI)			**6253**			**5368**	**100.0%**	**0.04 [0.01, 0.08]**
Heterogeneity: Chi² = 35.90, df = 38 (P = 0.57); I² = 0%								
Test for overall effect: Z = 2.29 (P = 0.02)								
Test for subgroup differences: Not applicable								

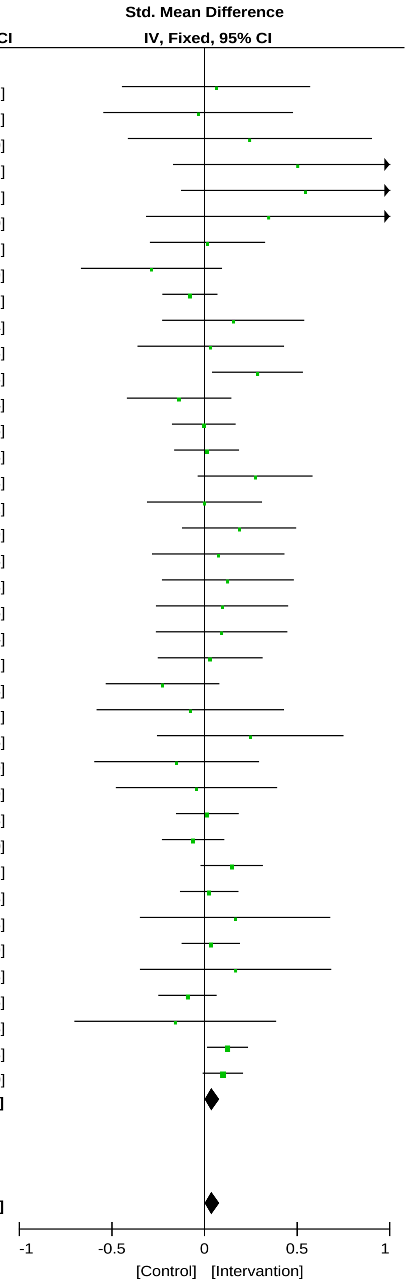


Figure 9 Forest plot for motor development

On further sub-group analysis however, micronutrient supplementation to young children (12 -23 months) after infancy, didn't affect motor activity (SMD = 0.03; 95% CI: -0.03, 0.08) but did affect motor development of infants ≤ 12 months (SMD = 0.06; 95% CI: 0.01, 0.11; p = 0.02) (Figure11 & 10 respectively).

	Intervantion			Control				Std. Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
1.2.1 all studies								
Ashworth et.al 1998-1mg Zn 12mo	101.1	11	48	100.4	11.3	22	1.0%	0.06 [-0.44, 0.57]
Ashworth et.al 1998-5mg Zn 12mo	100	11.6	46	100.4	11.3	22	1.0%	-0.03 [-0.54, 0.47]
Black, Baqui, et al., 2004 Fe 12mo	99.5	16.6	49	95.4	16.3	11	0.6%	0.24 [-0.41, 0.90]
Black, Baqui, et al., 2004 Fe+Zn 12mo	103.7	16.2	43	95.4	16.3	11	0.6%	0.50 [-0.17, 1.17]
Black, Baqui, et al., 2004 MMN 12mo	104.5	16.5	35	95.4	16.3	12	0.6%	0.54 [-0.12, 1.21]
Black, Baqui, et al., 2004 Zn 12mo	101.2	16.6	49	95.4	16.3	11	0.6%	0.35 [-0.31, 1.00]
Black, Sazawal, et al., 2004Mn10mo	91.7	9.8	85	91.5	14.2	77	2.6%	0.02 [-0.29, 0.32]
Castillo-Durán et al., 2001 Zn 12mo	84.5	11.5	57	87.6	9.9	52	1.8%	-0.29 [-0.66, 0.09]
Colombo et al., 2014 Fe+Cu+Zn 12mo	101.5	6.56	51	100.41	7.29	56	1.7%	0.16 [-0.22, 0.54]
Faber, Kvalsvig, Lombard, & Benadé, 2005 post	15.5	4	128	14.4	3.7	138	4.3%	0.29 [0.04, 0.53]
Li et al., 2009 FFA	45.3	22.15	393	45.39	22.4	207	8.9%	-0.00 [-0.17, 0.16]
Li et al., 2009 MMN	45.64	19.83	351	45.39	22.4	208	8.5%	0.01 [-0.16, 0.18]
Lind et al., 2004 Fe 12mo	106	11	163	103	10.8	55	2.7%	0.27 [-0.03, 0.58]
Lind et al., 2004 Fe+Zn 12mo	103	10.3	160	103	10.8	55	2.7%	0.00 [-0.31, 0.31]
Lind et al., 2004 Zn 12mo	105	10.6	167	103	10.8	55	2.7%	0.19 [-0.12, 0.49]
Manno et al., 2012 12mo	93.4	10.4	98	93.1	9.2	98	3.2%	0.03 [-0.25, 0.31]
Tofail et al., 2008 early Fe-30H	103.6	16.1	325	103.2	15.4	318	10.5%	0.03 [-0.13, 0.18]
Tofail et al., 2008 early Fe-30L	100.9	15.6	29	98.4	14.3	30	1.0%	0.16 [-0.35, 0.68]
Tofail et al., 2008 early Fe-60H	104	14.7	324	103.5	15.3	326	10.6%	0.03 [-0.12, 0.19]
Tofail et al., 2008 early Fe-60L	101.9	15.4	32	99.3	15.1	27	1.0%	0.17 [-0.35, 0.68]
Tofail et al., 2008 early MMH	102.8	16.2	322	104.3	16.4	330	10.6%	-0.09 [-0.25, 0.06]
Tofail et al., 2008 early MML	102.6	17.3	29	105.3	16.4	24	0.9%	-0.16 [-0.70, 0.38]
Yousafzai et.al 2014 12mo	83.2	13.4	658	81.5	13.9	699	22.1%	0.12 [0.02, 0.23]
Subtotal (95% CI)			**3642**			**2844**	**100.0%**	**0.06 [0.01, 0.11]**
Heterogeneity: Chi² = 21.74, df = 22 (P = 0.48); I² = 0%								
Test for overall effect: Z = 2.34 (P = 0.02)								
Total (95% CI)			**3642**			**2844**	**100.0%**	**0.06 [0.01, 0.11]**
Heterogeneity: Chi² = 21.74, df = 22 (P = 0.48); I² = 0%								
Test for overall effect: Z = 2.34 (P = 0.02)								
Test for subgroup differences: Not applicable								

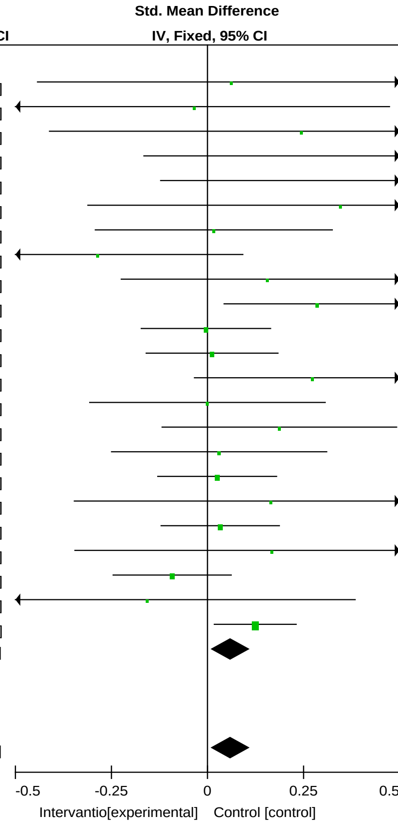


Figure 10 Forest plot for motor development <12mo

	Intervantion			Control				Std. Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
1.2.1 all studies								
Christian et al., 2016	90.1	7.4	348	90.7	7.8	384	15.0%	-0.08 [-0.22, 0.07]
Colombo et al., 2014 Fe+Cu+Zn18mo	104.1	5.77	49	103.9	6.1	51	2.0%	0.03 [-0.36, 0.43]
Hamadani et al., 2001 Zn 13mo	88	18.9	97	90.6	18.9	101	4.0%	-0.14 [-0.42, 0.14]
Locks et al., 2016-MM-fine	35.14	2.87	59	34.92	2.99	64	2.5%	0.07 [-0.28, 0.43]
Locks et al., 2016-MM-gross	48.32	2.33	60	48.02	2.4	64	2.5%	0.13 [-0.23, 0.48]
Locks et al., 2016-zinc-fine	35.17	2.84	60	34.89	3.02	63	2.5%	0.09 [-0.26, 0.45]
Locks et al., 2016-Zinc-Gross	48.28	2.41	61	48.06	2.33	63	2.5%	0.09 [-0.26, 0.44]
Manno et al., 2012 18mo	90	6.2	80	91.4	6.1	88	3.4%	-0.23 [-0.53, 0.08]
Nahar et al., 2012 FS + MN After6mo	66.2	15.2	77	67.4	16.2	19	1.2%	-0.08 [-0.58, 0.42]
Nahar et al., 2012 PS +FS + MN After 6mo	71.5	16.5	68	67.4	16.2	20	1.3%	0.25 [-0.25, 0.75]
Surkan et al., 2013 Fe	24.1	6.5	285	24	6.5	277	11.5%	0.02 [-0.15, 0.18]
Surkan et al., 2013 Zn	23.8	6.5	285	24.2	6.5	277	11.5%	-0.06 [-0.23, 0.10]
Taneja, Bhandari, Bahl, & Bhan, 2005	93.9	11.8	283	92.2	11.4	288	11.7%	0.15 [-0.02, 0.31]
Yousafzai et.al 2014 24mo	89.4	16.8	676	87.7	17.5	705	28.2%	0.10 [-0.01, 0.20]
Subtotal (95% CI)			**2488**			**2464**	**100.0%**	**0.03 [-0.03, 0.08]**
Heterogeneity: Chi² = 12.54, df = 13 (P = 0.48); I² = 0%								
Test for overall effect: Z = 0.95 (P = 0.34)								
Total (95% CI)			**2488**			**2464**	**100.0%**	**0.03 [-0.03, 0.08]**
Heterogeneity: Chi² = 12.54, df = 13 (P = 0.48); I² = 0%								
Test for overall effect: Z = 0.95 (P = 0.34)								
Test for subgroup differences: Not applicable								

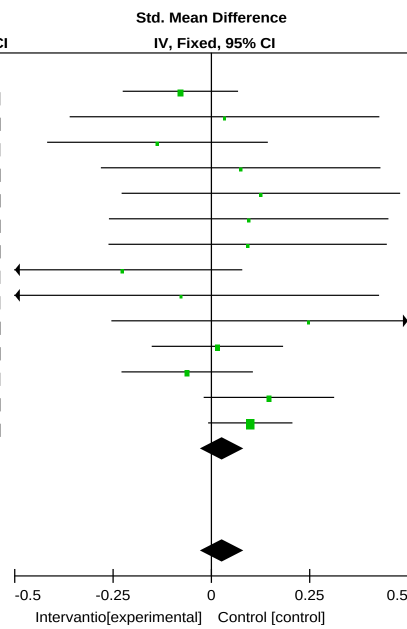


Figure 11 Forest plot for motor development >12mo

In the same way, a sub-group analysis by number of micronutrients supplemented (single versus multiple) has also showed that multiple micronutrient supplementation significantly (SMD = 0.04; 95% CI: 0.00, 0.09; p = 0.05) affected motor development of children than single micronutrient supplementation (d = 0.04; 95%CI: -0.03, 0.11; P = 0.25), (Figure13 & Figure12 respectively)

	Intervantion			Control				Std. Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
1.2.1 all studies								
Ashworth et.al 1998-1mg Zn 12mo	101.1	11	48	100.4	11.3	22	2.1%	0.06 [-0.44, 0.57]
Ashworth et.al 1998-5mg Zn 12mo	100	11.6	46	100.4	11.3	22	2.0%	-0.03 [-0.54, 0.47]
Black, Baqui, et al., 2004 Fe 12mo	99.5	16.6	49	95.4	16.3	11	1.2%	0.24 [-0.41, 0.90]
Black, Baqui, et al., 2004 Zn 12mo	101.2	16.6	49	95.4	16.3	11	1.2%	0.35 [-0.31, 1.00]
Black, Sazawal, et al., 2004Mn10mo	91.7	9.8	85	91.5	14.2	77	5.5%	0.02 [-0.29, 0.32]
Castillo-Durán et al., 2001 Zn 12mo	84.5	11.5	57	87.6	9.9	52	3.7%	-0.29 [-0.66, 0.09]
Hamadani et al., 2001 Zn 13mo	88	18.9	97	90.6	18.9	101	6.7%	-0.14 [-0.42, 0.14]
Lind et al., 2004 Fe 12mo	106	11	163	103	10.8	55	5.6%	0.27 [-0.03, 0.58]
Lind et al., 2004 Zn 12mo	105	10.6	167	103	10.8	55	5.6%	0.19 [-0.12, 0.49]
Locks et al., 2016-zinc-fine	35.17	2.84	60	34.89	3.02	63	4.2%	0.09 [-0.26, 0.45]
Locks et al., 2016-Zinc-Gross	48.28	2.41	61	48.06	2.33	63	4.2%	0.09 [-0.26, 0.44]
Surkan et al., 2013 Fe	24.1	6.5	285	24	6.5	277	19.2%	0.02 [-0.15, 0.18]
Surkan et al., 2013 Zn	23.8	6.5	285	24.2	6.5	277	19.2%	-0.06 [-0.23, 0.10]
Taneja, Bhandari, Bahl, & Bhan, 2005	93.9	11.8	283	92.2	11.4	288	19.5%	0.15 [-0.02, 0.31]
Subtotal (95% CI)			**1735**			**1374**	**100.0%**	**0.04 [-0.03, 0.11]**
Heterogeneity: Chi² = 12.15, df = 13 (P = 0.52); I² = 0%								
Test for overall effect: Z = 1.14 (P = 0.25)								
Total (95% CI)			**1735**			**1374**	**100.0%**	**0.04 [-0.03, 0.11]**
Heterogeneity: Chi² = 12.15, df = 13 (P = 0.52); I² = 0%								
Test for overall effect: Z = 1.14 (P = 0.25)								
Test for subgroup differences: Not applicable								

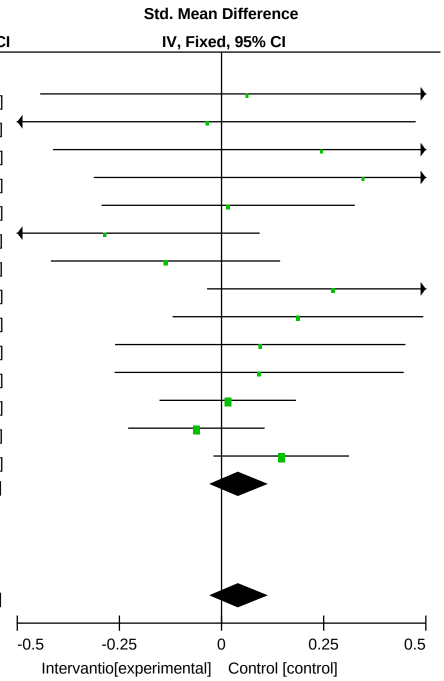


Figure 12 Forest plot for motor development single micronutrient

Study or Subgroup	Intervantion Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
1.2.1 all studies								
Black, Baqui, et al., 2004 Fe+Zn 12mo	103.7	16.2	43	95.4	16.3	11	0.4%	0.50 [-0.17, 1.17]
Black, Baqui, et al., 2004 MMN 12mo	104.5	16.5	35	95.4	16.3	12	0.4%	0.54 [-0.12, 1.21]
Christian et al., 2016	90.1	7.4	348	90.7	7.8	384	8.9%	-0.08 [-0.22, 0.07]
Colombo et al., 2014 Fe+Cu+Zn 12mo	101.5	6.56	51	100.41	7.29	56	1.3%	0.16 [-0.22, 0.54]
Colombo et al., 2014 Fe+Cu+Zn18mo	104.1	5.77	49	103.9	6.1	51	1.2%	0.03 [-0.36, 0.43]
Faber, Kvalsvig, Lombard, & Benadé, 2005 post	15.5	4	128	14.4	3.7	138	3.2%	0.29 [0.04, 0.53]
Li et al., 2009 FFA	45.3	22.15	393	45.39	22.4	207	6.6%	-0.00 [-0.17, 0.16]
Li et al., 2009 MMN	45.64	19.83	351	45.39	22.4	208	6.3%	0.01 [-0.16, 0.18]
Lind et al., 2004 Fe+Zn 12mo	103	10.3	160	103	10.8	55	2.0%	0.00 [-0.31, 0.31]
Locks et al., 2016-MM-fine	35.14	2.87	59	34.92	2.99	64	1.5%	0.07 [-0.28, 0.43]
Locks et al., 2016-MM-gross	48.32	2.33	60	48.02	2.4	64	1.5%	0.13 [-0.23, 0.48]
Manno et al., 2012 12mo	93.4	10.4	98	93.1	9.2	98	2.4%	0.03 [-0.25, 0.31]
Manno et al., 2012 18mo	90	6.2	80	91.4	6.1	88	2.0%	-0.23 [-0.53, 0.08]
Nahar et al., 2012 FS + MN After6mo	66.2	15.2	77	67.4	16.2	19	0.7%	-0.08 [-0.58, 0.42]
Nahar et al., 2012 PS +FS + MN After 6mo	71.5	16.5	68	67.4	16.2	20	0.7%	0.25 [-0.25, 0.75]
Rosado et.al 2011 FS	88.6	9.2	58	90	9.3	30	1.0%	-0.15 [-0.59, 0.29]
Rosado et.al 2011PM	89.6	9.2	65	90	9.3	30	1.0%	-0.04 [-0.48, 0.39]
Tofail et al., 2008 early Fe-30H	103.6	16.1	325	103.2	15.4	318	7.8%	0.03 [-0.13, 0.18]
Tofail et al., 2008 early Fe-30L	100.9	15.6	29	98.4	14.3	30	0.7%	0.16 [-0.35, 0.68]
Tofail et al., 2008 early Fe-60H	104	14.7	324	103.5	15.3	326	7.9%	0.03 [-0.12, 0.19]
Tofail et al., 2008 early Fe-60L	101.9	15.4	32	99.3	15.1	27	0.7%	0.17 [-0.35, 0.68]
Tofail et al., 2008 early MMH	102.8	16.2	322	104.3	16.4	330	7.9%	-0.09 [-0.25, 0.06]
Tofail et al., 2008 early MML	102.6	17.3	29	105.3	16.4	24	0.6%	-0.16 [-0.70, 0.38]
Yousafzai et.al 2014 12mo	83.2	13.4	658	81.5	13.9	699	16.4%	0.12 [0.02, 0.23]
Yousafzai et.al 2014 24mo	89.4	16.8	676	87.7	17.5	705	16.7%	0.10 [-0.01, 0.20]
Subtotal (95% CI)			**4518**			**3994**	**100.0%**	**0.04 [0.00, 0.09]**
Heterogeneity: Chi² = 23.75, df = 24 (P = 0.48); I² = 0%								
Test for overall effect: Z = 1.98 (P = 0.05)								
Total (95% CI)			**4518**			**3994**	**100.0%**	**0.04 [0.00, 0.09]**
Heterogeneity: Chi² = 23.75, df = 24 (P = 0.48); I² = 0%								
Test for overall effect: Z = 1.98 (P = 0.05)								
Test for subgroup differences: Not applicable								

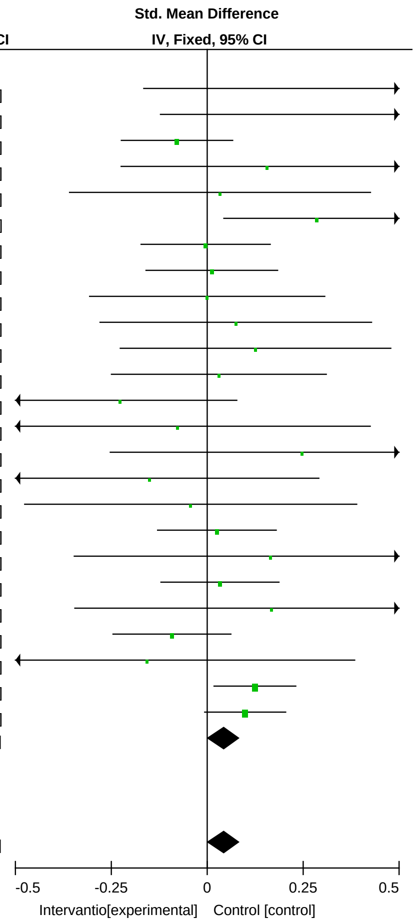


Figure13 Forest plot for motor development multiple micronutrient

Morbidity report

In this systematic review, morbidity report was also assessed and reviewed. From the selected published articles, six studies reported the presence of morbidity during the interventionperiod. The study by Ashworth et al. (1998) on zinc supplementation reported that infants who received 5 mg zinc had significantly less diarrhea than infants supplemented with 1 mg zinc and placebo. The other study on zinc supplementation also reported that the number of upper respiratory infections, pneumonias and diarrheal episodes were observed in the supplementing and placebo group (Castillo-Durán et al., 2001). A study conducted by Lind et al. (2004)

reported that the presence of illness during the supplementation of zinc, iron and a combination of the two indicated that there was an incidence of diarrhea. Morbidity was also recorded in a similar study in Ghana on the intervention of three types of micronutrients (Sprinkles, Nutritabs and Nutributter) but no significant difference was observed between the 3 intervention groups, except for a slightly higher prevalence of coughing in the Nutritabs group (Adu-Afarwuah et al., 2007a). A nutritional supplementation study in three groups (Oportunidades food supplement (OFS), powdered milk (PM) and placebo (PL)) also showed that no significant differences were found between groups in the number of episodes of gastrointestinal infections and in the number and duration of respiratory infections (Rosado et al., 2011). Relatively, a recently study in Bangladesh on 22-element MNP intervention reported the presence of sickness during the intervention period (Singla et al., 2014).

DISCUSSION

This systematic review and meta-analysis was conducted to quantify the effects of micronutrient supplements on mental and psychomotor development of infants and young children in low-and middle income countries. The results suggest the possibility of a small, but positive and statistically significant effect of multiple micronutrient supplementation on mental ($P = 0.003$) and motor ($P = 0.02$) development of children. The subgroup level analysis also showed that supplementation with multiple (than single) micronutrients and at earlier (<12 weeks) stages of life, than late (12 -23 months) significantly affected both mental and motor development of infants. Programmatically, our findings suggest that supplementing multiple micronutrients to infants in low and middle income countries is a promising strategy to foster cognitive and motor developments in those regions.

Cognitive development is influenced by several factors, including nutrition (SM Grantham-McGregor et al., 2007). There is an increasing body of literature suggesting a connection between improved nutrition and optimal brain function. In the present analysis, we observed that supplementation with multiple (than single) micronutrients benefited infants of LMIC for mental and motor development. This could mainly be attributed to the highly prevalent subclinical or biochemical deficiencies of multiple micronutrients in those countries (Bailey et al., 2015). Evidences show that multiple micronutrient deficiencies are not uncommon in the context of poverty and poor living conditions. Particularly, among families who are beset by multiple stressors and whose micronutrients are derived from the same source, micronutrient deficiencies often co-occur and interfere with the healthy development of children.

As nutrients are building blocks that play a critical role in cell proliferation, DNA synthesis, neurotransmitter and hormone metabolism and are important constituents of enzyme systems in the brain (Bhatnagar & Taneja, 2001; Lozoff & Georgieff, 2006; Zeisel, 2009) their deficiencies are related to impaired cognitive and motor development, particularly during the early period of life. For instance, zinc plays a significant role in support of memory formation and cognitive stability (Osredkar & Sustar, 2011). It may also play a part in controlling the devastating occurrence of epileptic seizures (Osredkar & Sustar, 2011). Iodine on the other hand, is required for the synthesis of thyroid hormones which in turn are required for brain development that occurs during fetal and early postnatal life (Delange, 2000). Folates and vitamin B12 have fundamental roles in CNS function at all ages, especially the methionine-synthase mediated conversion of homocysteine to methionine (Reynolds, 2006). Iron helps for the development of brain structures, neurotransmitter systems and myelination that protects acute brain dysfunction during the period of deficiency (M. M. Black, 2008b).

Our analysis has also indicated that supplementations with multiple micronutrients would benefit children during early life, i.e. infancy than later ages. This could be due to the fact that brain development is faster in the early years of life compared to the rest of the body (Benton, 2010a). Furthermore, a significant part of the brain development occurs either during pregnancy or at an early age of life. According to Levitt, by five to sixteen weeks after conception, the anterior-posterior and dorsal-ventral axes of the neural tube have already developed and the cortical plate with some inter-neuronal connections have been formed (Levitt, 2003) , which may make it more vulnerable to dietary deficiencies.

Comparisons with previous reviews

The most recent systematic reviews mainly focused on analyzing the effects of multiple micronutrient supplementation on the cognitive performance of healthy school-age children (Eilander et al., 2009) and nutrition supplementation interventions on mental (motor development) and physical growth of young children. Our current analysis focused on analyzing the effect of micronutrient supplements on mental and psychomotor development of infants and young children, but limited only to low-and middle income countries.

Unlike a previous review that showed weak associations between mental developments and nutrition interventions of under-two years children in low- and middle-income countries, our review showed the possibility of a small, but positive and statistically significant effect of multiple micronutrient supplementation on mental and motor development of young children.

Several previous reviews have tried to pool / synthesize findings of RCTs evaluating the effect of nutritional interventions including micronutrient supplementation on mental (motor or cognitive) and physical development of children. Nevertheless, almost all of these studies reviewed the effects of single and multiple micronutrient supplementations, separately. More importantly, they lack evidence from low income countries, as most pooled data from high and middle income countries.

On the other hand, a recent review by Larson and Yousafzai (2017), was unique in that it went beyond previous reviews by analyzing the effects of prenatal and postnatal nutritional interventions on mental development of children. It also focused on physical growth and motor development as mediators. Nonetheless, it mixed several aspects together; prenatal interventions with postnatal, micronutrients with macronutrients and mental development with physical development. As such, it revealed a very small (weak) and controversial benefits of nutritional interventions on mental development in children under-two years of age in low- and middle-income countries.

There are a number of strengths and some limitations to this analysis, which should be taken into account while interpreting the results. We have improved previous systematic reviews and meta-analyses by using a more comprehensive search strategy, incorporating more studies than previous meta-analyses. The analysis being restricted only to LMICs has dual implications, both strengthes and limitations. In many of the LMICs, both macro and micronutrient malnutritions are highly prevalent, which both contribute for mental development of infants.

On the other hand, our review was done in the context of growing uncertainty of weather micronutrient supplementation alone affects cognitive and motor development of children or not in the setup of LMICs. As such, it provides an up-to-date systematic review using rigorous selection methodology, but is limited to studies from developing countries. Furthermore, we performed a meta-analysis to see the effects of micronutrient supplementation on cognitive development of young children aligning with the mediator; i.e. motor development, which has been hardly shown before in literature. We also analyzed the effects of both single (iron or zinc) micronutrient and multiple micronutrients separately. We realize that our definition of multiple micronutrients is arbitrary; however, others have used the same definition as ours(Aboud & Yousafzai, 2015; D Benton, 2001; Eilander et al., 2009; Larson & Yousafzai, 2017; Pasricha et al., 2013). Furthermore, we examined weather age at the point of supplementation is a factor for cognitive and motor development of children; classifying early life or the period of infancy (≤ 12 months) and later (12 - 23 months) childhood ages. Likewise, we observed significant and positive benefits of multiple micronutrient supplementation at earlier ages than single micronutrient supplementation or supplementations that occur at later ages of growth and development.

We found no evidence of publication bias among trials assessing the cognitive and motor development, and influence analyses did not indicate that any of the trials had a significant effect on the overall effect sizes for these domains. We conducted a large number of subgroup and meta-regression analyses and given the increased risk of false-positive results, significant findings should be interpreted with caution. We have also reported morbidity data from studies.

In conclusion, this meta-analysis suggests that supplementation of multiple micronutrients to infants (<12 months) has a modest but positive impact on both motor and cognitive development of children in low and middle countries. Supplementation with either a single micronutrient or any of those at later ages of life has little or no effect on both motor and cognitive development of children. Therefore; it is time to think of routine multiple micronutrient supplementations to infants in low and middle income countries through the development of public health guidelines to improve cognitive performance of children in those setups.

Contributions

A.G. and K.B. compiled the data. A.G performed the analyses and A.G and T.A performed the literature review and wrote the manuscript. K.B and T.A revised the final draft manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of Interest

Reference

Aboud, F. E., & Yousafzai, A. K. (2015). Global health and development in early childhood. *Annual Review of Psychology*, *66*, 433-457.

Aburto, N., Ramirez-Zea, M., Neufeld, L., & Flores-Ayala, R. (2010). The effect of nutritional supplementation on physical activity and exploratory behavior of Mexican infants aged 8–12 months. *European journal of clinical nutrition*, *64*(6), 644.

Adu-Afarwuah, S., Lartey, A., Brown, K. H., Zlotkin, S., Briend, A., & Dewey, K. G. (2007). Randomized comparison of 3 types of micronutrient supplements for home fortification

of complementary foods in Ghana: effects on growth and motor development. *The American journal of clinical nutrition, 86*(2), 412-420.
Ashworth, A., Morris, S. S., Lira, P., & Grantham-McGregor, S. (1998). Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. *European journal of clinical nutrition, 52*(3), 223-227.
Bailey, R. L., West Jr, K. P., & Black, R. E. (2015). The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism, 66*(Suppl. 2), 22-33.
Benton, D. (2001). Micro-nutrient supplementation and the intelligence of children. *Neuroscience & Biobehavioral Reviews, 25*(4), 297-309.
Bhatnagar, S., & Taneja, S. (2001). Zinc and cognitive development. *British Journal of Nutrition, 85*(S2), S139-S145.
Black, M. M. (2008). Effects of vitamin B12 and folate deficiency on brain development in children. *Food and nutrition bulletin, 29*(2_suppl1), S126-S131.
Black, M. M., Baqui, A. H., Zaman, K., Persson, L. A., El Arifeen, S., Le, K., . . . Black, R. E. (2004). Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. *The American journal of clinical nutrition, 80*(4), 903-910.
Black, M. M., Sazawal, S., Black, R. E., Khosla, S., Kumar, J., & Menon, V. (2004). Cognitive and motor development among small-for-gestational-age infants: impact of zinc supplementation, birth weight, and caregiving practices. *Pediatrics, 113*(5), 1297-1305.
Castillo-Durán, C., Perales, C. G., Hertrampf, E. D., Marín, V. B., Rivera, F. A., & Icaza, G. (2001). Effect of zinc supplementation on development and growth of Chilean infants. *The Journal of pediatrics, 138*(2), 229-235.
Christian, P., Kim, J., Mehra, S., Shaikh, S., Ali, H., Shamim, A. A., . . . West Jr, K. P. (2016). Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: the JiVitA-3 Trial, 2. *The American journal of clinical nutrition, 104*(4), 1175-1182.
Christian, P., Kim, J., Mehra, S., Shaikh, S., Ali, H., Shamim, A. A., . . . West, K. P. (2016). Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: the JiVitA-3 Trial. *The American journal of clinical nutrition, 104*(4), 1175-1182.
Colombo, J., Zavaleta, N., Kannass, K. N., Lazarte, F., Albornoz, C., Kapa, L. L., & Caulfield, L. E. (2014). Zinc supplementation sustained normative neurodevelopment in a randomized, controlled trial of peruvian infants aged 6–18 months. *The Journal of nutrition, 144*(8), 1298-1305.
Delange, F. (2000). The role of iodine in brain development. *Proceedings of the nutrition society, 59*(1), 75-79.
Dora, C., Haines, A., Balbus, J., Fletcher, E., Adair-Rohani, H., Alabaster, G., . . . Neira, M. (2015). Indicators linking health and sustainability in the post-2015 development agenda. *The lancet, 385*(9965), 380-391.
Egger, M., Smith, G. D., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *Bmj, 315*(7109), 629-634.
Eilander, A., Gera, T., Sachdev, H. S., Transler, C., van der Knaap, H. C., Kok, F. J., & Osendarp, S. J. (2009). Multiple micronutrient supplementation for improving cognitive

performance in children: systematic review of randomized controlled trials–. *The American journal of clinical nutrition, 91*(1), 115-130.

Faber, M., Kvalsvig, J. D., Lombard, C. J., & Benadé, A. S. (2005a). Effect of a fortified maize-meal porridge on anaemia, micronutrient status, and motor development of infants. *The American journal of clinical nutrition, 82*(5), 1032-1039.

Faber, M., Kvalsvig, J. D., Lombard, C. J., & Benadé, A. S. (2005b). Effect of a fortified maize-meal porridge on anaemia, micronutrient status, and motor development of infants–. *The American journal of clinical nutrition, 82*(5), 1032-1039.

Grantham-McGregor, S., Cheung, Y., Cueto, S., Glewwe, P., Richter, L., & Strupp, B. (2007). Child development in developing countries. 1. Developmental potential inthe first five years for children in devloping countries. *Lancet, 69*, 6-7.

Haddad, L., Hawkes, C., Udomkesmalee, E., Achadi, E., Ag Bendech, M., & Ahuja, A. (2016). Global Nutrition Report 2016: From Promise to Impact: Ending Malnutrition by 2030. *Washington, DC.: International Food Policy Research Institute*.

Hamadani, J. D., Fuchs, G. J., Osendarp, S. J., Khatun, F., Huda, S. N., & Grantham-McGregor, S. M. (2001). Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. *The American journal of clinical nutrition, 74*(3), 381-386.

Larson, L. M., & Yousafzai, A. K. (2017). A meta-analysis of nutrition interventions on mental development of children under-two in low-and middle-income countries. *Maternal & child nutrition, 13*(1).

Levitt, P. (2003). Structural and functional maturation of the developing primate brain. *The Journal of pediatrics, 143*(4), 35-45.

Li, Q., Yan, H., Zeng, L., Cheng, Y., Liang, W., Dang, S., . . . Tsuji, I. (2009). Effects of maternal multimicronutrient supplementation on the mental development of infants in rural western China: follow-up evaluation of a double-blind, randomized, controlled trial. *Pediatrics, 123*(4), e685-e692.

Lind, T., Lönnerdal, B., Stenlund, H., Gamayanti, I. L., Ismail, D., Seswandhana, R., & Persson, L.-Å. (2004). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *The American journal of clinical nutrition, 80*(3), 729-736.

Locks, L. M., Manji, K. P., McDonald, C. M., Kupka, R., Kisenge, R., Aboud, S., . . . Duggan, C. P. (2016). The effect of daily zinc and/or multivitamin supplements on early childhood development in Tanzania: results from a randomized controlled trial. *Maternal & child nutrition*.

Locks, L. M., Manji, K. P., McDonald, C. M., Kupka, R., Kisenge, R., Aboud, S., . . . Duggan, C. P. (2017). The effect of daily zinc and/or multivitamin supplements on early childhood development in Tanzania: results from a randomized controlled trial. *Maternal & child nutrition, 13*(2).

Lozoff, B., & Georgieff, M. K. (2006). *Iron deficiency and brain development.* Paper presented at the Seminars in pediatric neurology.

Manno, D., Kowa, P. K., Bwalya, H. K., Siame, J., Grantham-McGregor, S., Baisley, K., . . . Filteau, S. (2012a). Rich micronutrient fortification of locally produced infant food does not improve mental and motor development of Zambian infants: a randomised controlled trial. *British Journal of Nutrition, 107*(04), 556-566.

Manno, D., Kowa, P. K., Bwalya, H. K., Siame, J., Grantham-McGregor, S., Baisley, K., . . . Filteau, S. (2012b). Rich micronutrient fortification of locally produced infant food does not improve mental and motor development of Zambian infants: a randomised controlled trial. *British Journal of Nutrition, 107*(4), 556-566.

Nahar, B., Hossain, M., Hamadani, J., Ahmed, T., Huda, S., Grantham-McGregor, S., & Persson, L. (2012a). Effects of a community-based approach of food and psychosocial stimulation on growth and development of severely malnourished children in Bangladesh: a randomised trial. *European journal of clinical nutrition, 66*(6), 701.

Nahar, B., Hossain, M., Hamadani, J., Ahmed, T., Huda, S., Grantham-McGregor, S., & Persson, L. (2012b). Effects of a community-based approach of food and psychosocial stimulation on growth and development of severely malnourished children in Bangladesh: a randomised trial. *European journal of clinical nutrition, 66*(6), 701-709.

Onyango, A. W. (2003). Dietary diversity, child nutrition and health in contemporary African communities. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 136*(1), 61-69.

Osredkar, J., & Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *J. Clinic. Toxicol. S, 3*, 2161-0495.

Pasricha, S.-R., Hayes, E., Kalumba, K., & Biggs, B.-A. (2013). Effect of daily iron supplementation on health in children aged 4–23 months: a systematic review and meta-analysis of randomised controlled trials. *The Lancet Global Health, 1*(2), e77-e86.

Prado, E. L., Abbeddou, S., Jimenez, E. Y., Somé, J. W., Ouédraogo, Z. P., Vosti, S. A., . . . Ouédraogo, J.-B. (2016). Lipid-Based Nutrient Supplements Plus Malaria and Diarrhea Treatment Increase Infant Development Scores in a Cluster-Randomized Trial in Burkina Faso. *The Journal of nutrition, 146*(4), 814-822.

Prado, E. L., Abbeddou, S., Yakes Jimenez, E., Somé, J. W., Ouédraogo, Z. P., Vosti, S. A., . . . Ouédraogo, J.-B. (2015). Lipid-Based Nutrient Supplements Plus Malaria and Diarrhea Treatment Increase Infant Development Scores in a Cluster-Randomized Trial in Burkina Faso–3. *The Journal of nutrition, 146*(4), 814-822.

Prado, E. L., Maleta, K., Ashorn, P., Ashorn, U., Vosti, S. A., Sadalaki, J., & Dewey, K. G. (2016a). Effects of maternal and child lipid-based nutrient supplements on infant development: a randomized trial in Malawi. *The American journal of clinical nutrition, 103*(3), 784-793.

Prado, E. L., Maleta, K., Ashorn, P., Ashorn, U., Vosti, S. A., Sadalaki, J., & Dewey, K. G. (2016b). Effects of maternal and child lipid-based nutrient supplements on infant development: a randomized trial in Malawi, 2. *The American journal of clinical nutrition, 103*(3), 784-793.

Reynolds, E. (2006). Vitamin B12, folic acid, and the nervous system. *The lancet neurology, 5*(11), 949-960.

Rosado, J. L., López, P., García, O. P., Alatorre, J., & Alvarado, C. (2011). Effectiveness of the nutritional supplement used in the Mexican Oportunidades programme on growth, anaemia, morbidity and cognitive development in children aged 12–24 months. *Public health nutrition, 14*(05), 931-937.

Singla, D. R., Shafique, S., Zlotkin, S. H., & Aboud, F. E. (2014). A 22-Element Micronutrient Powder Benefits Language but Not Cognition in Bangladeshi Full-Term Low-Birth-Weight Children. *The Journal of nutrition, 144*(11), 1803-1810.

Surkan, P. J., Siegel, E. H., Patel, S. A., Katz, J., Khatry, S. K., Stoltzfus, R. J., . . . Tielsch, J. M. (2013). Effects of zinc and iron supplementation fail to improve motor and language milestone scores of infants and toddlers. *Nutrition, 29*(3), 542-548.

Taneja, S., Bhandari, N., Bahl, R., & Bhan, M. K. (2005). Impact of zinc supplementation on mental and psychomotor scores of children aged 12 to 18 months: a randomized, double-blind trial. *The Journal of pediatrics, 146*(4), 506-511.

Tofail, F., Persson, L. Å., El Arifeen, S., Hamadani, J. D., Mehrin, F., Ridout, D., . . . Grantham-McGregor, S. M. (2008). Effects of prenatal food and micronutrient supplementation on infant development: a randomized trial from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study. *The American journal of clinical nutrition, 87*(3), 704-711.

UNICEF. (2017). WHO, World Bank Group. Levels And Trends in Child Malnutrition in UNICEF/WHO/World Bank Group Joint Child Malnutrition Estimates Key Findings of the 2016 edition. New York: UNICEF; Geneva: WHO; Washington DC: World Bank.

Yousafzai, A. K., Rasheed, M. A., Rizvi, A., Armstrong, R., & Bhutta, Z. A. (2014). Effect of integrated responsive stimulation and nutrition interventions in the Lady Health Worker programme in Pakistan on child development, growth, and health outcomes: a cluster-randomised factorial effectiveness trial. *The lancet, 384*(9950), 1282-1293.

Zeisel, S. H. (2009). Epigenetic mechanisms for nutrition determinants of later health outcomes–. *The American journal of clinical nutrition, 89*(5), 1488S-1493S.

Zeng, L., Yan, H., Cheng, Y., & Dibley, M. J. (2011). Modifying effects of wealth on the response to nutrient supplementation in pregnancy on birth weight, duration of gestation and perinatal mortality in rural western China: double-blind cluster randomized controlled trial. *International journal of epidemiology, 40*(2), 350-362.

2.7 Nutritional and micronutrient status of infant and young children in Ethiopia

Ethiopia has one of the largest rates of undernutrition in Sub-Saharan Africa. According to the resent EDHS report, 38 % of children less than five years of age are stunted, 10 % are wasted, and 24 % are underweight. Only 7% of children aged 6-23 months meet the minimum acceptable diet and only 14% had met the minimum dietary diversity score (CSA, 2016). To what extent this high rate of undernutrition relates to congnitive and developmental deficts remains unclear.

Among the very few available studies a relatively recent study in Southern Ethiopia Sidama, zone showed that nutritionally-disadvantaged children do not reach their full developmental potential (Bogale et al., 2013). In various studies where supplementation was provided to children who were already undernourished, follow-up after the intervention showed limited or no benefits to cognition or behaviour (Walker et al., 2007). Less or no information is available on low iron MNP provision in the first year of life and its effect on infant motor milestone development, anemia ad stunting. Even less information exist on whether potential benefits of interventions persist after discontinuation of an effective intervention. Therefore this study evaluate whether exposure to a low-iron MNP in alternate days in the first year of life is associated with reduced risk of anemia, stunting, and deficit in motor milestone development, evaluate if motor, anemia and stunting changes associated with MNP exposure in the first year of life are sustained in the second year of life, six months post-intervention, identify predictors of child developmental milestone acquisition including, language, social, gross and fine motor development, investigate the retention of fat-soluble vitamins in home-fortified complementary foods and inform micronutrient programs and policies on timing, dosage, and delivery modalities to improve their effectiveness

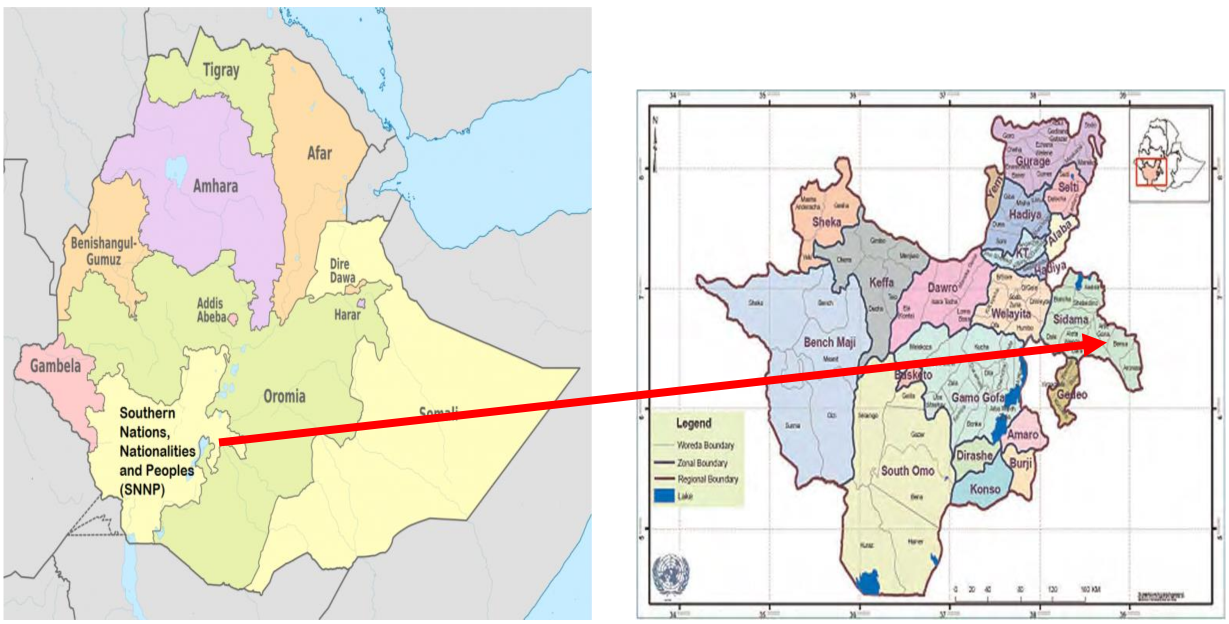
Chapter 3

Materials & Methods

3. Materials and Methods

3.1 Study Area

The study was conducted in Aleta Chuko district, Sidama Zone, Southern Nations, Nationalities, and People’s region (SNNPR) of Ethiopia. The prevalence of stunting (aged 6–59 months) in SNNPR is equal to the national average (Central Statistical Agency - CSA/Ethiopia & ICF, 2017).



a) b)

Figure 3.1 Map of regional stat of Ethiopia (a). Map of Southern Nations Nationalities and People’s Regional State (SNNPRS) administrative zones and special woreda (districts) (b) retrieved at: www.rippleethiopia.org/page/snnpr)

The population Sidama zone heavily depends on staples like ‘Enset’ (*Enset ventricosum*) and maize (*Zea mays L.*) (Haile, Belachew, Berhanu, Setegn, & Biadgilign, 2015). The area was characterized by delayed introduction of complementary foods, and low dietary diversity throughout the first two years of life (Arimond & Ruel, 2004; Regassa, 2014).

Home fortification with multiple micronutrients powders was implemented by Terebeza Development Association (TDA) with the support of Micronutrient Initiatives, now known as Nutrition International. The project was implemented in five districts, namely East Badewacho, Shebedino, Aleta Chuko, Geta, and Damot Pulasa. From these five districts, Aleta Chuko district were purposively selected for this study.

3.2 Study design

This study followed a mixed method design approach (a combination of community and laboratory based approach). It includes observational studies (retrospective and prospective cohort), developmental milestone acquisition (using WHO-GMRS), nutritional survey (dietary practice, dietary diversity and food frequency using IYCF practice), nutritional status (by anthropometry measurement) and anaemia status (using hemoglobin measure). Cross-sectional studies (infant widen development) and laboratory based analysis of fat soluble vitamins in a fortified complementary food. These activities were performed in the following two phases.

Phase one

At this stage data were collected on socioeconomic and demographic characteristics, feeding practice of infant (dietary diversity of infant using repeated 24 hours dietary recall method). In line with these motor development of infant was measured using WHO gross motor reference study method and hemoglobin was measured using a portable photometer (Hemocue HB 301). Anthropometric measurement of the infant such as length, weight, MUAC, and HC were measured using standard method. Finally, the most commonly consumed complementary foods were identified and collected for fat soluble vitamin analyses method.

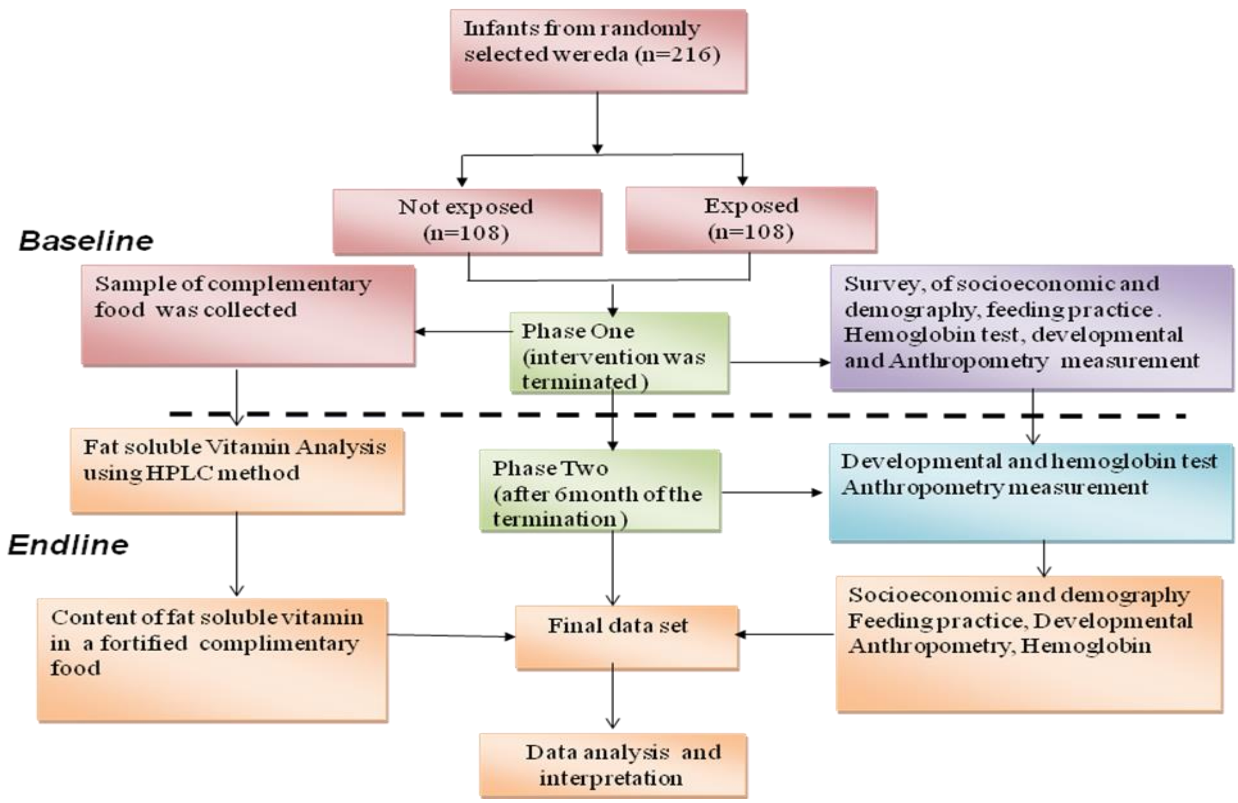


Figure 3.2 Study design

Phase two

In this phase (endline) the procedures and activities implemented in phase one were repeatd six-months post-intervention (interruption) to evaluate the long term effect of provision of low iron MNP (Figure 3.2).

3.3 Sample size determination

Statistically adequate sample size (n) was calculated using two independent proportion (exposed to MNP and unexposed to MNP) sample size formula. It was calculated using G*Power version

3.0.10 software by considering the following assumption: Effect size (d) 0.5, α err prob. is 0.05, powers (1-β err prob.) 0.9, Allocation ratio N2/N1 is 1. Based on the above assumptions and an attrition allowance of 20%, a total sample size of 216 (108 infant per arm) was needed.

3.4 Study population recruitment

Eligible infants were identified by the health extension worker from three kebele of the district. From 456 infants in 9-11 months of age, 149 were exposed to intermittent (every other day) low-iron (6 mg) MNP in the three months preceding the survey. A sub-sample (n= 216) of eligible infants, 108 from each exposed and unexposed infants were randomly selected from the three kebele.

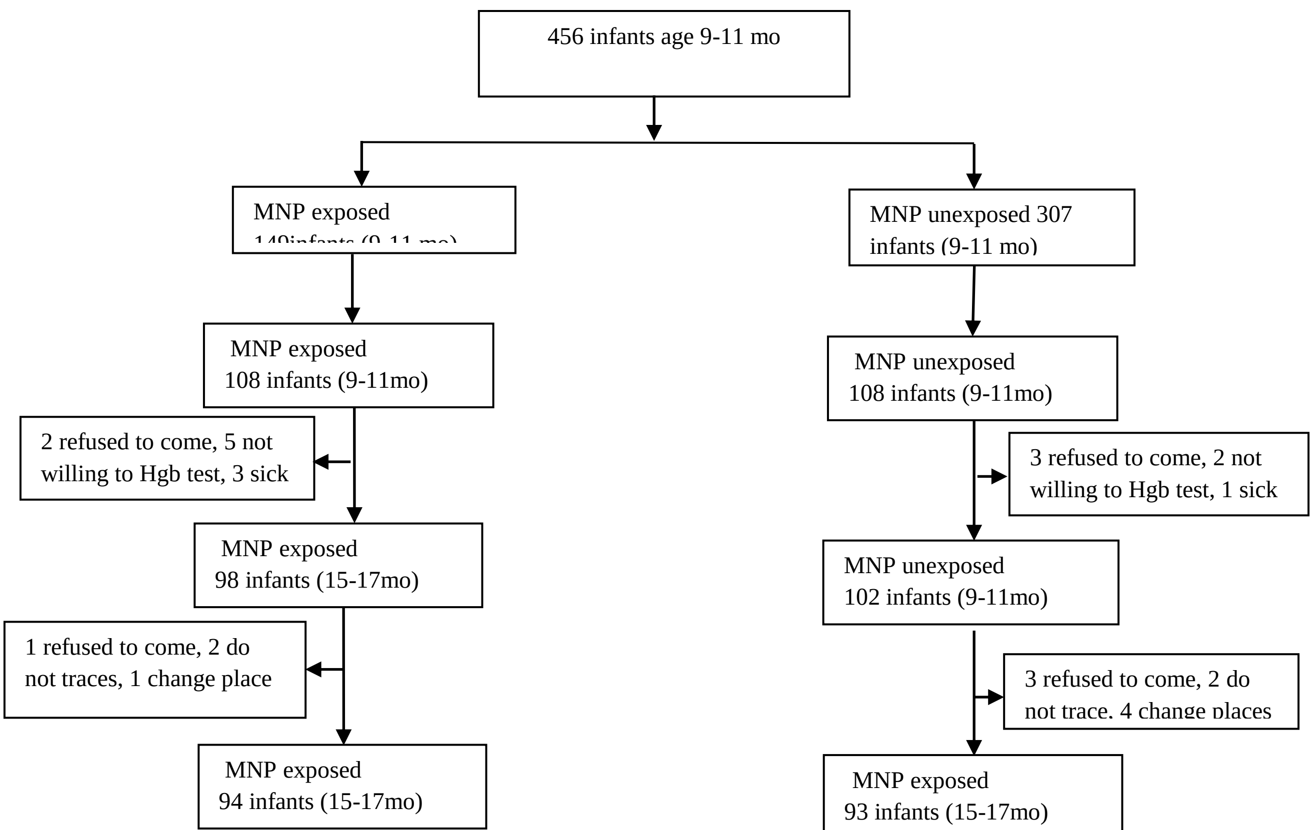


Figure 3.3 Study participant’s flow of the cohort in SNNPR, Ethiopia

The inclusion criteria for this study were to be apparently healthy with no signs of severe malnutrition and with no disabilities that prevent accurate measurement of motor development. A total of 98 exposed and 102 unexposed were involved in the study. This sample size allowed us to detect a medium effect size difference between two means (Cohen's *d*= 0.5) assuming α= 0.05, and a power of 90%. The age of the infants was confirmed by checking the health post records and through maternal recall.

3.5 Socioeconomic and demographic characteristics

The socio-demographic characteristic of the mothers was assessed using a pre-tested questionnaire. The questionnaire were adapted and modified from the Ethiopian Demographic and Health Survey (EDHS 2016) and included questions on livelihood activities, parents education level, ownership of livestock, and the size of land owned.

3.6 Infant feeding practices

Infant feeding practices was assessed using the WHO infant and young child feeding indicators (WHO, 2010b). Experienced data collectors were locally recruited and trained in a classroom setting. This was followed by a pilot test on a group comparable to that of the actual study. Infant dietary diversity, food frequency data were collected using the IYCF guidelines (WHO, 2010b). Dietary diversity score was calculated using the following seven food groupings: 1) grains, roots, and tubers; 2) legumes and nuts; 3) dairy products (milk, yogurt, cheese); 4) eggs; 5) flesh foods (meat, fish, poultry, and liver/organ meats); 6) vitamin A–rich fruits and vegetables; and 7) other fruits and vegetables (Kennedy, Ballard, & Dop, 2011). The proportion of breastfed infants meeting the minimum meal frequency ($\geq$4) and the minimum dietary diversity score ($\geq$4) was calculated.

3.7 Hemoglobin measurements

Blood specimens for anaemia testing were collected from whom consent was obtained from their parents. Blood samples were drawn from a drop of blood taken from a finger prick and collected in a microcuvette. After cleaning the infants' middle finger with a disinfectant wipe, the side of the finger was pricked using a lancet. After wiping the first two drops, light pressure was applied and the third drop was collected using a microcuvette. Haemoglobin analysis was carried out on-site using a battery-operated portable HemoCue (HB 301) analyser. Measurements were adjusted for altitude (WHO, 2011b). Anaemia was defined as Haemoglobin concentrations <11.0 g/dl. Infants' with severe anaemia (≤ 7 g/dl) were referred to the healthy facility for follow-up.

3.8 Anthropometric measurement

The length and weight of the infant and young children were measured in triplicate using standardized techniques, with infant and young children wearing light clothing and no shoes. Each infant weight was measure on a solar digital scale balance (Uniscale, UNICEF, NY). A salter scale with a weighing capacity of 25 kg was used to measure weight of the child. Weighing pant was suspended from the lower hook and the scale was adjusted to zero. The child's clothes were removed and the child placed in the weighing pants and then hung freely from the hook. When the child was still, the weight was recorded to the nearest 0.1kg with the scale at eye level. When the child was swing the weight was estimated at the midpoint of the range of oscillations.

To measure infant and young children length, the measuring board was placed on the ground with the infant and young children lying in the middle. An assistant held the child's head and positions it to touch the headboard. The measure placed her hands on the child's legs and gently stretched the child, keeping one hand on the infants and young children's feet. The foot plate was perpendicular to the board when the measurement was read (Adult Board, Schorr Productions, Olney, MD). Length was measured to the nearest 0.1cm. Mid upper arm circumference (MUAC) and head circumference (HC) were also measured using a plastic measuring tape (Institute, 2013). All anthropometric measurements were made by the same person to avoid inter-examiner errors. For the children, Z-scores for length for- age (LAZ), weight-for-age (WAZ) and weight-for-length (WLZ) were calculated using WHO multicenter growth reference data (WHO, 2006a) using the software ENA 2007. Stunting, underweight, and wasting were defined respectively as LAZ, WAZ or WLZ < –2.

3.9 Assessing infant development milestone acquisition

Two methods were implemented to measure infant development which was adopted from "WHO multicenter growth reference study motor development" and "Malawian development assessment tool".

i. ***Method of the WHO multicenter growth reference study***

Motor development was assessed using the World Health Organization (WHO) Multicenter Growth Reference Study methodology(Wijnhoven et al., 2004). Six distinct gross motor milestones were assessed for this study (Table3.1). These are simple measurements to take and are considered to be universal and fundamental to the acquisition of self-sufficient erect locomotion.

Table 3.1 MGRS performance criteria for six gross motor milestones

Gross motor milestone	MGRS performance criteria
Sitting without support	Child sits up straight with the head erect for at least 10 seconds. Child does not use arms or hands to balance body or support position
Hands-and-knee crawling	Child alternately moves forward or backward on hands and knees. The stomach does not touch the supporting surface. There are continuous and consecutive movements, at least three in a row
Standing with assistance	Child stands in upright position on both feet, holding onto a stable object (e.g., furniture) with both hands without leaning on it. The body does not touch the stable object, and the legs support most of the body weight. Child thus stands with assistance for at least 10 seconds
Walking with assistance	Child is in upright position with the back straight. Child makes sideways or forward steps by holding onto a stable object (e.g., furniture) with one or both hands. One leg moves forward while the other supports part of the body weight. Child takes at least five steps in this manner
Standing alone	Child stands in upright position on both feet (not on the toes) with the back straight. The legs support 100% of the child's weight. There is no contact with a person or object. Child stands alone for at least 10 seconds
Walking alone	Child takes at least five steps independently in upright position with the back straight. One leg moves forward while the other supports most of the body weight. There is no contact with a person or object

Sources: Wijnhoven et al. (2004)

ii. *Malawian development assessment tool*

This tool is a culturally appropriate developmental assessment tool validated for use in rural sub-Saharan African, and is likely to be applicable in other similar settings. It includes four domain of development that is gross motor, fine motor, language and social. Each domain in the tool had thirty four items (Gladstone et al., 2010).

- ✓ ***Gross motor*** *(large muscle)* ***development*** *refers to improvement of skills and control of the large muscles of the legs, arms, back and shoulders which are used in walking, sitting, running, jumping, climbing, and riding a bike (Ruffin, 2009; Sabanathan, Wills, & Gladstone, 2015)..*
- ✓ ***Fine motor*** *(small muscle)* ***development*** *refers to use of the small muscles of the fingers and hands for activities such as grasping objects, holding, cutting, drawing, buttoning, or writing (Ruffin, 2009; Sabanathan et al., 2015).*
- ✓ ***Language skills*** *include understanding others and putting words together to communicate ideas (receptive language), whereas speech skills are the ability to produce speech sounds correctly or fluently (expressive language) (Sabanathan et al., 2015).*
- ✓ ***Social-emotional*** *development are often described and grouped together because they are closely interrelated growth patterns. Feelings of trust, fear, confidence, pride, friendship, and humor are all part of social-emotional development (Ruffin, 2009).*

For this study out of the 34 item, 8 for gross motor 9 for fine motor 6 for language and 10 for social skill were selected as relevant for the age range 15-18mo, totally 33 item were selected for testing. The MDAT were translated to the local language and were back-translated to English. Items were scored based on pass or fail technique. Item were consistently and repeatedly performed by the children a pass mark (usually 1) was given but if it were not repeatedly and consistently performed a fail mark (usually 0) was given.

3.10 Analysis of fat-soluble vitamins in MNP fortified complementary foods

The main complementary food used as a vehicle for fortification was identified. Volunteer mothers were asked to prepare a fortified complementary food the way they usually prepare for their child. Complementary foods (porridge) were collected from three kebels (n=30) with polyethylene plastic bag and was immediately covered using aluminium foil. The weight of each sample was measured using digital portable balance, and the sample was transported to Addis Abeba University Center for Food Science and Nutrition using a precooled Ice box. Each sample was dried using freeze dryer (Beta 1-8 LSCbasic- Lyophilizer) and homogenized using a homogenizer. Then, the powder was placed in polyethylene bags and was covered with aluminum foil and stored at -18^{o}c until the analysis was conducted. For fat soluble vitamins analyses, sample were transported to the Department of Chemistry, Washburn University, Topika, USA.

Standard reagent preparation, sample preparation and extraction of the analyte were done following standard methods. The HPLC system consists of a quaternary pump, degasser, a column thermostat and a diode array detector. A C-18 column (4.6 x 250 mm, 3.5 µm particles, Agilent Pursuit XRS, column temperature of 25^{o}C) was used. The mobile phase was a mixture of commonly used solvents 98% Methanol/ 2% water, by volume. Detection was conducted with diode array detector at 325 nm, 292 nm and 266 nm for vitamin A, E and D, respectively. Separation was carried out with a flow rate of 1.00 mL/min. Injection volume was 20µL and total run time for each injection was 11min.

3.11 Data collection procedures

At phase one data were collected by trained nurses' health extension workers. Training was provided on methods, procedures presenting the questionnaire, anthropometric measurements and other activities for three days prior to data collection. After the training, the data collectors got the consent from mother of infant and started to collect all the information according to the questionnaire. After which hemoglobin measurement, anthropometric measurements, which include height, weight, mid upper arm circumference measurement were conducted. Then, developmental status of the infant and young children was conducted by trained nurse. In line with this sample of complementary food sample was collected for fat soluble vitamins analysis. Then, the experimental analysis was conducted using validated method and instrument. These data collection steps were repeated after six months.

3.12 Ethical issues

The protocol was approved by the Institutional Review Board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University. Permission and support letters were also obtained from the Sidama zone health bureau, SNNPR, Ethiopia. Informed consent was obtained orally after the purpose and methods of the study had been explained in detail and the consent forms read to them in the presence of the health extension workers and the MNP project coordinator. All the questionnaires were translated to the local language and data collection was interview-based.

3.13 Data analyses

Data were entered using Epi-data statistical software (version 20). The data were double entered, cleaned and exported to SPPS (IBM SPSS Statistics 20.0.0 (SPSS Inc., Chicago, IL, USA) version 20.0)) for statistical analyses.

All continuous variables were checked for normality using Shapiro-Wilk test. Descriptive statistics of continuous and categorical values were presented as mean ± SD and in frequency counts/ percentages, respectively. An independent student's t test was used to compare means of variables in the MNP exposed and unexposed groups for parametric test. Mann-Whitney U test was used for comparing exposed and unexposed groups for non parametric test. Chi-square test was used for categorical variables to test differences between MNP exposed and unexposed. In all comparisons, differences were considered statistically significant at $P < 0.05$.

The associations between continuous variables were computed using multivariate linear regression analyses. The associations between categorical variables were computed using bivariate and multivariate logistic regression analyses. Variables with P value ≤0.2 were entered in multivariate logistic regression which controlled for the undesirable effects of confounding variables. The result was presented with adjusted odds ratio (AOR) and 95% confidence interval (CI) and variables with P value of less than 0.05 were considered as significant predictors.

Mediation analysis was conducted to estimate the direct and indirect effect of MNP exposure on motor development using PROCESS macro for SPSS version 3. Regression coefficients and their 95% CIs were presented, and P< 0.05 was considered as statitistically significant. The difference in difference (DID) was used to assess changes over time between the two groups (exposed/unexposed) six-month post the interruption of the MNP intervention. Relative risks (RRs) at 95% CI of stunting, anemia, motor development were reported to assess risk of unexposed verses exposed to MNP

Partial correlation (age-adjusted) of the developmental domain with nutritional status, feeding practice, hemoglobin level, health and mother’s education were done. The association between the four developmental domains (gross motor, fine motor, language and social skill) with various predictor variables was done using multivariate linear regression analyses. Variables with *P* value ≤ 0.2 in a partial correlation were entered in multivariate linear regression which controlled for confounding variables.

Chapter 4

Results

4. Result

4.1 Association of low-iron MNP and infant motor development, stunting and anemia

4.1.1 Introduction

Inappropriate complementary feeding contributes to developmental delay, growth faltering, and anaemia. Particularly in countries like Ethiopia where child stunting (38%) and anaemia (56%) prevalence is very high due to in adequate nutritional intake. Nevertheless, several studies in Ethiopia also show that excessive iron intake exists. Under such circumstance a low-iron MNP intervention was provided on alternative days. However, limited programmatic evidence exists on the effectiveness of low-iron MNPs on stunting, anaemia and motor development when provided every other day in the first year of life.

In this section, results from evaluation of exposure of low-iron MNP in the first year of life on motor development, stunting and anemia among infants 9-11 months of age using a retrospective cohort study.

4.1.2. Provision of low-iron micronutrient powders on alternate days is associated with lower prevalence of anaemia, stunting, and improved motor milestone acquisition in the first year of life

(Submitted to ***Maternal and Child Nutrition*****)**

Provision of low-iron micronutrient powders on alternate days is associated with lower prevalence of anaemia, stunting, and improved motor milestone acquisition in the first year of life: A retrospective cohort study

Ashenafi Geletu[1, 2]; Azeb Lelisa[3]; Kaleab Baye[1]

1. Center for Food Science and Nutrition, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia
2. Department of Chemistry , College of Natural and computational Sciences, Kotebe Metropolitan University, Addis Ababa, Ethiopia
3. Nutrition International, Addis Ababa, Ethiopia

Abstract

Inappropriate complementary feeding contributes to growth faltering, anaemia, and cognitive impairments. Limited programmatic evidence exists on the effectiveness of low-iron micronutrient powders (MNPs) on anaemia and motor development when provided every other day in the first year of life.

This study used an existing demonstration project to evaluate associations between exposure of low-iron MNPs, anaemia, and motor development of infants in Southern Ethiopia.

Using a retrospective cohort design, 200 infants aged 9-11 months (98 MNP exposed; 102 unexposed) were recruited and data on socio-economic characteristics, anthropometric measures, dietary diversity scores (DDS), hemoglobin concentrations, and motor development acquisition was collected and MNP exposed and unexposed groups were compared. Logistic regressions were run to identify predictors of acquisition of motor development milestones.

Socio-demographic characteristics and dietary patterns were similar between MNP exposed and unexposed groups. Provision of low-iron (6 mg) MNP every other day, for three months, was associated with reduced risk of anaemia and stunting, and increased achievement of motor development. After adjusting for age, infants exposed to MNPs had a higher likelihood of standing alone (AOR=3.1; 95% CI: 1.53, 6.46) and walking alone (4.9; 95% CI 2.12, 11.37) than unexposed ones. Exposure to MNPs, DDS, stunting, and mothers education were associated with acquisition of motor development milestones.

Provision of low-iron dose MNPs on alternate days is associated with lower prevalence of anaemia, stunting, and increased motor development achievements. Integrating routine monitoring of motor development milestones with growth monitoring and anaemia screening can inform nutrition interventions to support optimal brain development.

Keywords: anaemia, motor development, iron, home fortification,

INTRODUCTION

Worldwide it is estimated that more than two billion people are deficient in key vitamins and minerals (Tulchinsky, 2010). Infants and children in low and middle income countries (LMIC) are disproportionately affected (R. E. Black et al., 2013). This is particularly explained by the high energy and nutrient requirements needed to support the rapid growth that occurs during the complementary feeding period (6-23 months of age). Unfortunately, inappropriate complementary feeding, characterized by late introduction and inadequate intake of nutrient-dense complementary foods is widespread and contributes significantly to growth faltering (Krasevec, An, Kumapley, Bégin, & Frongillo, 2017). Growth faltering in young children is associated with serious short- and long-term consequences including impaired physical and cognitive development (K. G. Dewey & Begum, 2011). Growth faltering and nutrient deficiencies are often experienced during the first years of life, posing a particular concern as this is when the basis for life time brain function is established (Cesar Gomes Victora et al., 2010).

Many of the brain's anatomical regions and processes (e.g. myelination) have development trajectories that begin in utero and accelerate in the first two to three years of life (Cusick & Georgieff, 2016). However, different regions of the brain have different pick rates of development that should not be missed. Ensuring time-coordinated development is also critical for healthy brain development as different brain regions and functions need to work together as circuits. The timing of a nutritional deficit and the brain region that requires it will thus determine the vulnerability of the developing brain process, region and circuit (Georgieff, 2007b). This suggests that interventions can be more effective if started within the first 1000 days (e.g. 6-11 months), but earlier than later. For example, the earlier the brain is protected from suboptimal iron status, the better it is for cognitive function (Cusick & Georgieff, 2012). Similar findings exist for zinc, particularly when provided in combination with iron (Colombo et al., 2014b). However, interventions with iron among iron-replete infants have also shown long-term negative cognitive outcomes (Lönnerdal, 2017; Lozoff, Castillo, Clark, & Smith, 2012). This suggests that close monitoring of children's nutritional status and cognition within the continuum of care is critical for timely and effective interventions. Besides alternative intervention modalities that maximize benefit while minimizing adverse outcomes are needed.

Monthly growth monitoring and anaemia screening is promoted in many LMIC. While these are very important for monitoring overall health and wellbeing of children, stunting and anaemia are late findings (endpoints) that indicate sustained poor nutritional environment. Such late findings can delay the timing of interventions that aim to prevent cognitive deficits and may reduce their effectiveness (Murray-Kolb et al., 2012), particularly in countries like Ethiopia where child stunting (38%) and anaemia (56%) prevalence is very high (Central Statistical Agency - CSA/Ethiopia & ICF, 2017). Thus, monitoring tools like motor development measures that can predict early onset of cognitive and nutritional deficits, but yet simple enough to administer within the continuum of care in the community or health facilities are needed. However, to what extent the effect of timely-provision of multiple micronutrient intervention on motor development is direct, or is mediated by anaemia reduction remains unknown. Besides, limited programmatic evidence exists on the effectiveness of MNPs containing low iron dose (6 mg) when provided every other day.

Therefore, the present study used the opportunity of an existing demonstration project to evaluate associations between exposure of micronutrient powder in the first year of life, anaemia, and motor development millstones in Southern Ethiopia using a retrospective cohort design.

METHODS

Study site

The study was conducted in Aleta Chuko district, Sidama Zone, Southern Nations, Nationalities, and People's region (SNNPR) of Ethiopia. The prevalence of stunting (aged 6–59 months) in SNNPR is equal to the national average (Central Statistical Agency - CSA/Ethiopia & ICF, 2017). The population heavily depends on staples like 'Enset' (*Enset ventricosum*) and maize (*Zea mays L.*). The study designed is depicted in Figure 1.

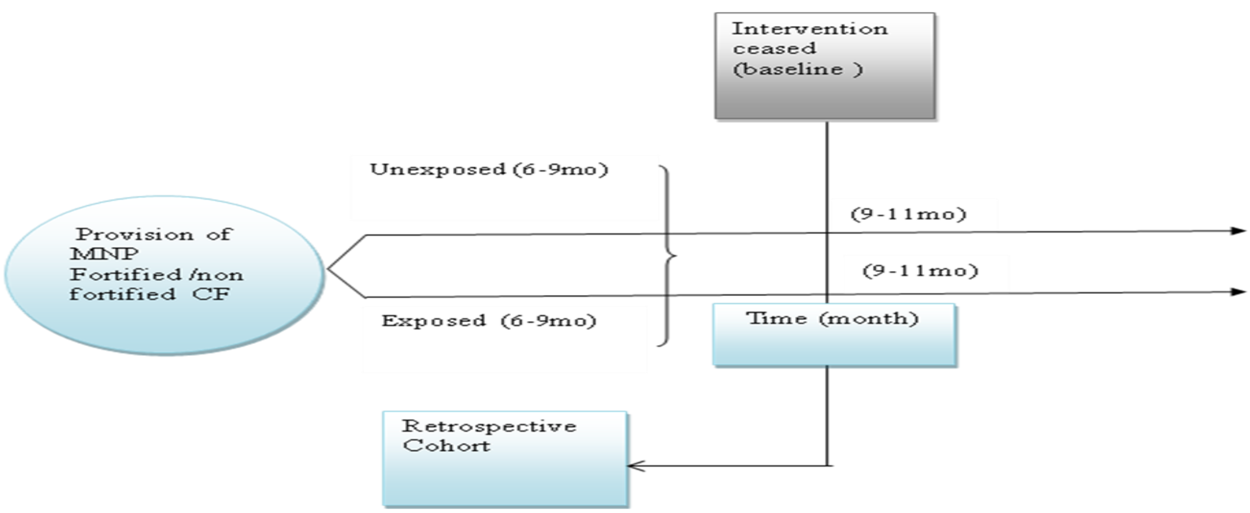


Figure 1 Study design

Programme description

Home fortification with multiple micronutrients powders was implemented by Terebeza Development Association (TDA) with the support of Micronutrient Initiatives, now known as Nutrition International. The project was implemented in five districts, namely East Badewacho, Shebedino, Aleta Chuko, Geta, and Damot Pulasa. From these five districts, Aleta Chuko (three kebele namely Korke, Chuko lemela, and Rufo waeno) were purposively selected for this study.

Sampling of study participants

From 456 infants in 9-11 months of age, 149 were exposed to intermittent (every other day) low-iron (6 mg) micronutrient powders in the three months preceding the survey. A sub-sample (n= 216) of eligible infants, 108 from each exposed and unexposed infants were randomly selected from the three kebele. The age of the infants was confirmed by checking the health post records and through maternal recall. The inclusion criteria for this study were to be apparently healthy with no signs of severe malnutrition and with no disabilities that prevent accurate measurement of motor development. A total of 98 exposed and 102 unexposed completed the study. This sample size allowed us to detect a medium effect size difference between two means (cohen *d*= 0.5) assuming $\alpha = 0.05$, and a power of 90%.

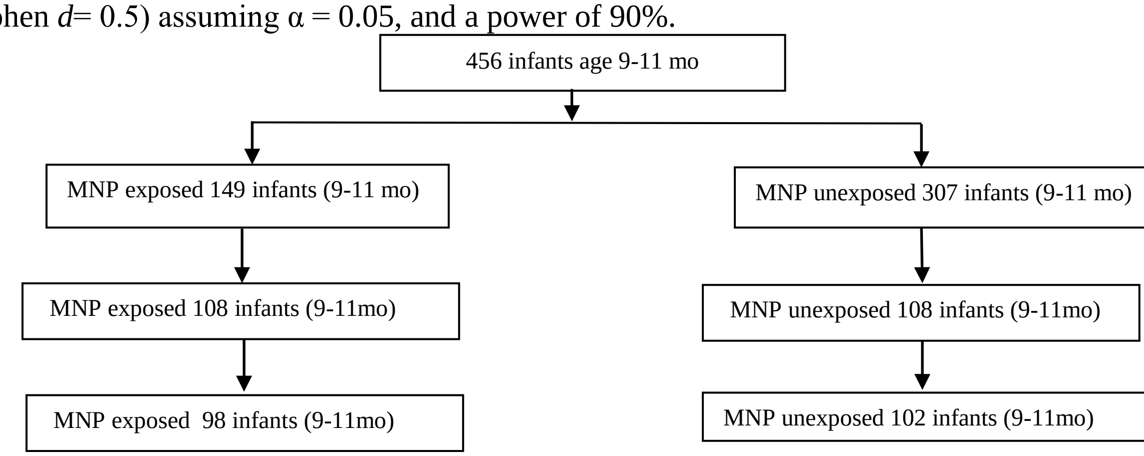


Figure 2 Study participants flow of the retrospective cohort in SNNPR, Ethiopia

Ethics

The protocol was approved by the Institutional Review Board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University. Permission and support letters were also obtained from the Sidama zone health bureau, SNNPR, Ethiopia. Informed consent was obtained orally in the presence of the health extension workers and the MNP project coordinator. All the questionnaires were translated to the local language and data collection was interview-based.

Socio-demographic and anthropometric characteristics

Information about the socio-demographic characteristics including livelihood activities, education level, ownership of land and livestock were collected using a pre-tested questionnaire.

Triplicate measures of weight and length were taken using standardized techniques. Measurements were taken at the health post with infants wearing light clothing and no shoes. All anthropometric measurements were made by the same persons to avoid inter-examiner errors. Z-scores length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (LHZ) were calculated using WHO multicenter growth reference data (WHO, 2006b) using the software ENA 2007. Stunting, underweight, and wasting were defined as LAZ, WAZ or WLZ <–2, respectively.

Infant feeding practices

Infant feeding practices was assessed using the WHO infant and young child feeding indicators (WHO, 2010a). Information about food groups consumed in the 24 hours prior the survey was collected using open ended questionnaire. Ingredients used in mixed foods were captured. Dietary diversity score was calculated using the following seven food groupings: 1) grains, roots, and tubers; 2) legumes and nuts; 3) dairy products (milk, yogurt, cheese); 4) eggs; 5) flesh foods (meat, fish, poultry, and liver/organ meats); 6) vitamin A–rich fruits and vegetables; and 7) other fruits and vegetables. The proportion of breastfed infants meeting the minimum meal frequency (≥3) and the minimum dietary diversity score (≥4) was calculated.

Hemoglobin measurements

Hemoglobin was measured using a portable photometer (Hemocue HB 301). After cleaning the infants' middle finger with a disinfectant wipe, the side of the finger was pricked using a lancet. After wiping the first two drops, light pressure was applied and the third drop was collected using a microcuvette and hemoglobin concentrations were recorded. Measurements were adjusted for altitude (WHO, 2011b). Anaemia was defined as hemoglobin concentrations <11.0 g/dl. Infants' with severe anaemia (≤ 7 g/dl) were referred to the healthy facility for follow-up.

Motor development

Motor development was assessed using the World Health Organization (WHO) Multicenter Growth Reference Study guidelines (Wijnhoven et al., 2004). Six distinct gross motor milestones were assessed for this study: 1) sitting without support; 2) hands-knees crawling; 3) standing with assistance; 4) walking with assistance; 5) standing alone; and 6) walking alone. These are simple measurements to take and are considered to be universal and fundamental to the acquisition of self-sufficient erect locomotion.

Quality control

All data collectors were experienced nurses and health extension workers. Data collectors have received three days training on anthropometric, motor milestone development measurements and hemoglobin measurements. The training was followed by a pretest on subjects not included in the actual study. The field supervision was supported by three experienced supervisors (one/site), and the first author checked the completeness and quality of the data on a daily basis. All anthropometric measurements were taken by the first author using calibrated measuring board and weighting scales.

Statistical analysis

The data was double entered in Epi-data statistical software (version 20), was cleaned and exported to SPPS (IBM SPSS Statistics 20.0, Chicago, IL, USA) for statistical analyses. All continuous variables were checked for normality using the Shapiro-Wilk test. Descriptive statistics of continuous and categorical values were presented as mean ± SD or in frequency/percentages, respectively. An independent student's t test was used to compare means of variables in the MNP exposed and unexposed groups for parametric test. Mann-Whitney U test was used for comparing exposed and unexposed groups for non parametric test. Chi-square test was used for categorical variables to test differences between MNP exposed and unexposed groups. In all comparisons, differences were considered statistically significant at $P < 0.05$.

The association between motor milestone development (standing alone and walking alone) with MNP exposure, feeding practice, nutritional status, hemoglobin and some socioeconomic and demographic characteristics were investigated using bivariate and multivariate logistic regression analyses. Variables associated with motor development milestones at P values ≤ 0.2 in a bivariate analysis were entered in multivariate logistic regression that controlled for confounding variables. The odds ratio (OR) and 95% confidence interval (CI) are presented. A mediation analysis was then conducted to estimate the direct and indirect effect of MNP exposure on motor development using PROCESS for SPSS version 3. Regressions were run with and without hemoglobin concentrations as a mediator. For all statistical comparisons, p-values less than 0.05 were considered to be statistically significant.

RESULTS

From the 216 infant (108 per arm) meeting the eligibility criteria, a total of 200 (98 MNP exposed/ 102 unexposed) completed the study. The average caregiver/mother was in her twenties and was married (Table 1). About a third of the mothers (29.6% MNP exposed/ 39.2% unexposed) did not have any formal education. The average household family size was higher in the MNP exposed (5.1) than in the unexposed (4.6) group ($p<0.05$). The average age of the children in this study was similar between the two groups (~11 months). Most of the study participants were from male-headed farming households, with over half of owning less than one hectare of land. Most of the socio-economic variables were similar between the MNP exposed and unexposed groups, except for chicken ownership that was significantly higher in the unexposed group. More than 90% of the study participants had access to potable water (public tap/stand pipe) and improved toilet facilities (pit latrines).

All children were breastfed and about 73% were fed the minimum number of times (≥ 3) per day, but small number of children met the minimum (≥ 4) dietary diversity score (Table 2). There was no statistically significant difference in meal frequency and dietary diversity between MNP exposed and unexposed groups. Consumption of animal source foods (ASF) was very low (< 5%). All the children had received Vitamin A supplements in the last six months.

Table 1 Selected socio-demographic characteristics by exposure to micronutrient powder (MNP)

	All *N= 200*	**MNP exposed** *n = 98*	**MNP unexposed** *n =102*	**P-values**
Child's age (mo)	10.7 ±1.1	10.9±1.0	10.5±1.1	0.08
Child's sex (male : female)	1.0	1.0	1.0	0.89
Mothers' age (years)				
<20	3 (1.5)	3 (3.2)	-	
20-24	54 (27.0)	25 (25.5)	29 (28.4)	
25-30	123 (61.5)	58 (59.2)	65 (63.7)	0.22
>30	20 (10.0)	12 (12.2)	8 (7.8)	
Mother's education				
No formal	69 (34.5)	29 (29.6)	40 (39.2)	0.52
Primary	95 (47.5)	49 (50.0)	46 (45.1)	
Secondary	36 (18.0)	20 (20.4)	16 (15.7)	
Married	200 (100)	98 (100)	102 (100)	
Household size	4.9 ± 1.6	5.1 ± 1.7	4.6 ± 1.5	0.03*
Livelihood activity				
Farming	188 (94.0)	91(92.9)	97 (95.1)	
Trading	8 (4.0)	5 (5.1)	3 (2.9)	0.74
Other	4 (2.0)	2 (2.0)	2 (2.0)	
Monthly income (ETB)	336.5 ± 479.4	291.8 ± 414.1	379.4 ±533.3	0.20
Land size (≤1 ha)	123 (61.5)	62 (63.3)	61(59.8)	0.62
Owns cow/oxen	122 (61.0)	65 (66.3)	57 (55.9)	0.13
Owns chicken	98 (49.0)	41 (41.8)	57 (55.9)	0.047*
Access to potable drinking water	196 (98)	96 (98)	100 (98)	0.97
Improved toilet facility (pit latrines)	74 (37)	43 (43.9)	31 (30.4)	0.10

Values aremean ± SD or *n* (%); ETB, Ethiopian birr; * indicates significant differences in mean /proportion between MNP exposed/unexposed groups at P<0.05 levels using independent t-test (two-tailed) or chi-square test.

Stunting was highly prevalent (30%) and was significantly lower in the MNP exposed than in the unexposed group (P< 0.05). MUAC, head circumference, and hemoglobin concentrations were significantly higher in the MNP exposed than in the unexposed ones. Anaemia prevalence in the MNP exposed group was twenty percentage points lower than in the unexposed group.

Table 2 Infant (9-12 months) feeding practices, anthropometry, hemoglobin and morbidity measures by micronutrient powder (MNP) exposure

	All *N= 200*	**MNP exposed** *n = 98*	**MNP unexposed** *n =102*	**P-values**
Feeding practices				
Currently breastfed	200 (100)	98 (100)	102 (100)	-
Fed minimum number of meals/day (≥ 3)	147 (73.5)	72 (73.5)	75 (73.5)	0.99
Dietary diversity score (DDS)	2.3 ± 0.8	2.2 ± 0.8	2.4 ± 0.8	0.23
Met minimum DDS (≥ 4 food groups)	7 (3.5)	3 (3.1)	4(3.9)	0.74
Vitamin A supplement within the last 6mo	200 (100)	98 (100)	102 (100)	-
Anthropometric characteristics				
Stunting (LAZ< -2)	60 (30.0)	22 (22.4)	38 (37.3)	0.02*
Underweight (WAZ < -2)	8 (4.0)	3 (3.1)	5 (4.9)	0.51
Wasting (WHZ < -2)	6 (3.0)	3 (3.1)	3 (2.9)	0.96
¶MUAC (cm)	13.2 ± 1.0	13.3 ± 1.1	13.0 ± 0.8	0.02*
¶Head circumference (cm)	46.9 ± 2.1	47.4 ± 1.8	46.4 ± 2.3	<0.001*
Hemoglobin and morbidity				
Hemoglobin level (g/dL)£	11.8 ± 3.7	12.4 ± 3.6	11.3 ± 3.8	0.03*
Anemic status	82(41.0)	30(30.6)	52(51.0)	0.003*
Child sick in the past one week	23 (11.5)	15 (15.3)	8 (7.8)	0.10

Values aremean ± SD or *n* (%);£ Hemoglobin values were adjusted for altitude; * indicate significant mean differences/proportion between unexposed and exposed groups at P<0.05 levels using two-tailed independent t-test (continuous variables) or chi-square (frequencies) test; ¶Mann-Whitney U test was used for comparing exposed and unexposed groups.

Although a higher proportion of children in the MNP exposed group were reported to be sick in the one week prior the survey, the difference between the exposed and unexposed groups was not statistically significant (P> 0.05).

Differences in the acquisition of motor development milestones were observed between the MNP exposed and unexposed groups (Table 3). The proportion of infants standing and walking alone was higher in the MNP exposed than in the unexposed group (P<0.05).

Table 3 Motor development milestones of infants (9-12 months) by MNP exposure

	All *N= 200*	**MNP exposed** *n = 98*	**MNP unexposed** *n =102*	**P-values**
Motor development milestones				
Sitting without support	200(100)	98(100)	102(100)	
Hand and knee crawling	200(100)	98(100)	102(100)	
Stand with assistance	189(94.5)	94(95.9)	93(91.2)	0.174
Walking with assistance	130(65.0)	67(68.4)	71(69.6)	0.850
Standing alone	60(30.0)	38(38.8)	22(21.6)	0.008*
Walking alone	48(24.0)	35(35.7)	13(12.7)	<0.001*

Values are mean ± SD or *n* (%)

* indicate significant differences between unexposed and exposed groups at P<0.05 levels using chi-square test (two-tailed).

¥ The scale of consciousness was rated either as drowsy or as awake and alert, and child's irritability was rated as being calm, fussy or upset

These associations between MNP exposure and standing- and walking- alone remained significant in the multivariate logistic regression that adjusted for age and other covariates (Table 4). Infants exposed to MNP had a three-fold (AOR=3.18; 95% CI=1.55, 6.53) likelihood of standing alone and a four-fold likelihood (AOR=4.94; CI 2.15, 11.36) of walking alone. Dietary diversity score (AOR 8.0; CI 1.50, 42.82) and child sickness (AOR=0.16; CI 0.03, 0.81) in the past week were significant predictors of standing alone, whereas stunting (AOR=0.33; CI 0.13, 0.87) and mothers 'education level (AOR=0.33; CI 0.12, 0.88) were significant predictors of walking alone (P< 0.05).

Table 4 Factors predicting standing and walking alone

	Standing alone				Walking alone			
	Able N (%)	Unable N (%)	COR (95%CI)	AOR (95%CI)	Able	Unable	COR (95%CI)	AOR (95%CI)
MNP Exposed								
No	22 (36.7)	80 (57.1)	**1**	**1**	13 (27.1)	89 (58.6)	**1**	**1**
Yes	38 (63.3)	60 (42.9)	**2.30* (1.24, 4.30)**	**3.18* (1.55, 6.53)**	35 (72.9)	63 (41.4)	**3.80* (1.86, 7.77)**	**4.94*(2.15, 11.36)**
Dietary diversity score								
Not adequate (≤4)	56 (93.3)	137 (97.9)	**1**	**1**	44(92)	149 (98)	1	1
Adequate	4 (6.7)	3 (2.1)	**0.31 (0.07, 1.41)**	**8.00*(1.50, 42.82)**	4(8)	3(2)	1.28 (0.24, 6.81)	4.27 (0.64, 28.59)
Fed min. number of meals/day							**1**	1
Not adequate	10 (16.7)	43 (30.7)	**1**	1	7 (14.6)	46 (30.3)	**2.50* (1.06, 6.09)**	2.10 (0.81, 5.43)
Adequate (≥ 3)	50 (83.3)	97 (69.3)	**2.20* (1.03, 4.78)**	1.91 (0.84,4.36)	41 (85.4)	106 (69.7)		
Hemoglobin level								
<11(g/dL)	31 (51.7)	52 (37.1)	1	1	23 (47.9)	58 (38.2)	1	1
≥11(g/dL)	29 (48.3)	88 (62.9)	0.70 (0.35, 1.20)	0.50 (0.25, 1.01)	25 (52.1)	94 (61.8)	0.70 (0.36, 1.33)	0.51 (0.23, 1.14)
Stunting								
LAZ≥-2	11 (18.3)	61 (43.6)	1	1	7 (14.6)	65 (42.8)	**1**	**1**
LAZ<-2	49 (81.7)	79 (56.4)	0.70 (0.36, 1.40)	0.70 (0.33, 1.50)	41(85.4)	87 (57.2)	**0.30* (0.13, 0.76)**	**0.33* (0.13, 0.87)**
Child sickness								
No	57 (95.0)	120 (85.7)	1	**1**	3 (6.2)	20 (13.2)	1	1
Yes	3 (95.0)	20 (14.3)	0.30 (0.09, 1.11)	**0.16* (0.03, 0.81)**	45 (93.8)	132 (86.8)	0.40 (0.13, 1.55)	0.24 (0.05, 1.20)
Mother education								
Secondary education	26 (43.3)	46 (32.9)	1	1	19 (39.6)	53 (34.9)	**1**	**1**
Primary education	22 (36.7)	76 (54.3)	0.40 (0.18, 1.04)	2.31 (1.05, 5.05)	16 (33.3)	82 (53.9)	0.50 (0.19, 1.14)	2.27 (0.92, 5.56)
No education	12 (20.0)	18 (12.9)	0.90 (0.35, 2.03)	0.65 (0.26, 1.59)	13 (27.1)	17 (11.2)	**0.30* (0.10, 0.63)**	**0.33* (0.12, 0.88)**

COR: crude odds ratio; AOR, adjusted odds ratio; CI: confidence interval; Logistic regression adjusted for age; *Associations are significant at P< 0.05

Our exploratory mediation analyses suggested that improved motor development milestone is directly explained by MNP exposure, but a smaller but significant share of the associations is mediated by an increase in hemoglobin concentration (Fig. 2).

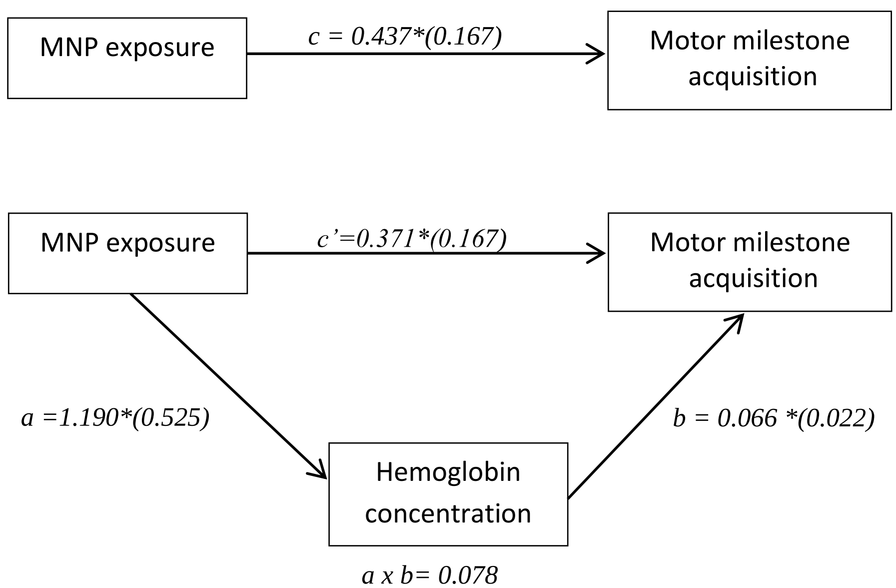


Figure 2 Path diagram presenting mediation effect of MNP exposure on motor milestone acquisition through increased hemoglobin concentration

a and *b* coefficients define the indirect effect of MNP exposure on motor milestone acquisition through hemoglobin concentrations; *c*' coefficient denotes the direct effect of MNP exposure on motor milestone acquisition

DISCUSSION

The present retrospective cohort study used the opportunity of an existing demonstration project to show that a low-iron (6 mg) MNP provided every other day for infants in first year of life is associated with increased achievement of motor milestone development and a lower risk of anaemia. MNP exposure in the first year of life is directly and indirectly (through increased hemoglobin concentration) associated with improved motor development outcome. Dietary diversity predicted ability to stand alone, whereas stunting and mothers' education predicted ability to walk alone.

Inadequate complementary feeding is widespread as illustrated by the very low proportion of infants meeting the minimum dietary diversity score. This finding is in line with findings from previous studies in LMIC including Ethiopia (Baye, Guyot, Icard-Verniere, & Mouquet-Rivier, 2013; Mayén, Marques-Vidal, Paccaud, Bovet, & Stringhini, 2014; Mengistu, Moges, Samuel, & Baye, 2017). Diets of infants in this and other Ethiopian settings are predominantly plant-based with little or no consumption of nutritious food groups like animal source foods (Baye et al., 2013). Such a low dietary diversity score has consistently been found associated with higher risk of anaemia and stunting among infants and young children (Gashu et al., 2015; Krasevec et al., 2017). The finding that 30% of our study participants were stunted and 40% were anemic suggests that these infants are already at an increased risk of cognitive deficit that, without timely intervention, may be irreversible. Given that achieved motor development milestones are consistently found to mediate cognitive function (Marrus et al., 2017; Oudgenoeg-Paz, Leseman, & Volman, 2015), the significant association between dietary diversity score, stunting, and motor development milestones (standing- and walking-alone) further supports the need for urgent interventions that save infants' developing brains from nutritional insults.

Among such interventions, home fortification of complementary foods with MNPs has been promoted in various LMICs (De-Regil et al., 2013). Although found highly effective in preventing/treating anaemia, recent studies have shown concern over the use of MNPs with standard iron concentrations of 12.5 mg (Bruins et al., 2016; Paganini & Zimmermann, 2017). This concern arises from the increased risk of diarrhea and gut inflammation, particularly when MNPs are provided to iron-replete children (Lönnerdal, 2017; Paganini & Zimmermann, 2017). The provision of iron to iron-replete infants was also associated with increased stunting and lower long-term cognitive function (Lönnerdal, 2017; Lozoff et al., 2012). These findings pose serious challenge to interventions that aim to prevent anaemia/micronutrient deficiencies and the associated adverse effects.

In an effort to increase benefit and minimize adverse effects of MNPs containing iron, the present demonstration project used a low iron (6 mg) dose provided on alternative days. This was informed by consumption and epidemiologic surveys that showed mild to moderate prevalence of anaemia despite a high (excessive) iron intake, even among young children (Abebe, Haki, & Baye, 2018; Central Statistical Agency - CSA/Ethiopia & ICF, 2017). In spite of this modified regimen, MNP exposure was associated with lower rates of anaemia and stunting ($P< 0.05$). This finding, if confirmed through community-based clinical trials, can present an alternative for programs in countries like Ethiopia, where both the risk of inadequate and excessive iron exists.

While MNP interventions are often designed as a strategy to prevent anaemia, they may also confer additional advantages in supporting cognitive and motor development (Adu-Afarwuah et al., 2007b; Larson & Yousafzai, 2017; Matias et al., 2017). Indeed, the present study showed that MNP exposure was significantly associated with increased motor milestone achievement like standing and walking alone. Our mediation analyses revealed that the effect of MNP exposure on motor development milestone achievements was in part explained by increased hemoglobin concentration. However, a far more substantial share of the association was not related to an increase in hemoglobin concentrations. This finding is of interest and may have important programmatic implications. First, in an effort to minimize possible adverse effects, screening and targeting only anemic infants for MNP interventions may not be an option as some of the positive effects on motor development are not related to hemoglobin concentrations (anaemia). Second, micronutrient deficiencies without anaemia can affect motor milestone achievements and possibly have implication on cognitive development (Krebs, Lozoff, & Georgieff, 2017).

The present study has several limitations that need to be considered when interpreting our findings. First, this is an opportunistic study that was tied to an existing project and as a result we had no control over the enrollment of infants or on the implementation of intervention. Consequently, the comparisons between the exposed and the unexposed may be affected by possible pre-intervention differences. However, the comparability in socio-demographic characteristics between the exposed and the unexposed is reassuring and suggests minimal bias, in any. Given that this is a retrospective cohort study, causal inferences cannot be made and differences between the exposed and unexposed group should be interpreted as associations. Although we have tried to capture morbidity, our sample size might have not been adequate enough to detect differences between the two groups (exposed/unexposed).

Notwithstanding the above limitations, the present study has shown that provision of a low-iron dose MNP on alternate days, in the first year of life, is associated with lower prevalence of anaemia, stunting, and increased motor development achievements. Given that a share of the associations between MNP and motor development milestones is independent of hemoglobin concentrations, targeting only anemic children for MNP interventions may not be effective. Interventions that improve complementary diets in the first year of life are needed not only to prevent anaemia/micronutrient deficiencies, but also to support the developing brain. A community-based clinical trial is needed to establish the effectiveness of this modified regimen.

Nevertheless, integrating simple, but useful monitoring tools like acquisition of motor development milestones (proxy for cognitive development) into the health system, along with anaemia screening and growth monitoring can inform intervention that aim to improve child nutrition and promote optimal brain development.

Running head: *timely low- iron micronutrient interventions*

Acknowledgements The authors thank all of the children and families who took part in this survey. Nutrition international (formerly Micronutrient initiative) is acknowledged for allowing us to use their demonstrating project for this study. Dr. Tadesse Alemu's in-field and statistical support is acknowledged.

Source of funding: The graduate programme of Addis Ababa University

Conflict of interest statement: The authors declare that they have no conflict of interest

Contributor statement All authors were involved in developing the study design. AG and KB designed the study; AG coordinated and supervised the fieldwork. AL provided critical information about the demonstration project. AG and KB analyzed and interpreted the data. AG wrote the first draft of the manuscript. All the authors contributed to manuscript preparation.

References

Abebe Z., Haki G.D. & Baye K. (2018) Simulated effects of home fortification of complementary foods with micronutrient powders on risk of inadequate and excessive intakes in West Gojjam, Ethiopia. *Maternal & child nutrition* **14**.

Adu-Afarwuah S., Lartey A., Brown K.H., Zlotkin S., Briend A. & Dewey K.G. (2007) Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on growth and motor development–. *The American journal of clinical nutrition* **86,** 412-420.

Baye K., Guyot J.-P., Icard-Verniere C. & Mouquet-Rivier C. (2013) Nutrient intakes from complementary foods consumed by young children (aged 12–23 months) from North Wollo, northern Ethiopia: the need for agro-ecologically adapted interventions. *Public health nutrition* **16,** 1741-1750.

Black R.E., Victora C.G., Walker S.P., Bhutta Z.A., Christian P., De Onis M., et al. (2013) Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet* **382,** 427-451.

Bruins M.J., Kupka R., Zimmermann M.B., Lietz G., Engle-Stone R. & Kraemer K. (2016) Maximizing the benefits and minimizing the risks of intervention programs to address micronutrient malnutrition: Symposium report. *Maternal & child nutrition* **12,** 940-948.

Central Statistical Agency - CSA/Ethiopia & ICF (2017) Ethiopia Demographic and Health Sruvey 2016. CSA and ICF, Addis Ababa, Ethiopia.

Colombo J., Zavaleta N., Kannass K.N., Lazarte F., Albornoz C., Kapa L.L., et al. (2014) Zinc Supplementation Sustained Normative Neurodevelopment in a Randomized, Controlled Trial of Peruvian Infants Aged 6–18 Months, 2. *The Journal of nutrition* **144,** 1298-1305.

Cusick S.E. & Georgieff M.K. (2012) Nutrient supplementation and neurodevelopment: timing is the key. *Archives of pediatrics & adolescent medicine* **166,** 481-482.

Cusick S.E. & Georgieff M.K. (2016) The role of nutrition in brain development: the golden opportunity of the "first 1000 days". *The Journal of pediatrics* **175,** 16-21.

De-Regil L.M., Suchdev P.S., Vist G.E., Walleser S. & Peña-Rosas J.P. (2013) Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Evidence-Based Child Health: A Cochrane Review Journal* **8,** 112-201.

Dewey K.G. & Begum K. (2011) Long-term consequences of stunting in early life. *Maternal & child nutrition* **7,** 5-18.

Gashu D., Stoecker B.J., Bougma K., Adish A., Haki G.D. & Marquis G.S. (2015) Stunting, selenium deficiency and anaemia are associated with poor cognitive performance in preschool children from rural Ethiopia. *Nutrition journal* **15,** 38.

Georgieff M.K. (2007) Nutrition and the developing brain: nutrient priorities and measurement–. *The American journal of clinical nutrition* **85,** 614S-620S.

Krasevec J., An X., Kumapley R., Bégin F. & Frongillo E.A. (2017) Diet quality and risk of stunting among infants and young children in low-and middle-income countries. *Maternal & child nutrition* **13**.

Krebs N.F., Lozoff B. & Georgieff M.K. (2017) Neurodevelopment: the impact of nutrition and inflammation during infancy in low-resource settings. *Pediatrics* **139,** S50-S58.

Larson L.M. & Yousafzai A.K. (2017) A meta-analysis of nutrition interventions on mental development of children under-two in low-and middle-income countries. *Maternal & child nutrition* **13**.

Lönnerdal B. (2017) Excess iron intake as a factor in growth, infections, and development of infants and young children. *The American journal of clinical nutrition* **106,** 1681S-1687S.

Lozoff B., Castillo M., Clark K.M. & Smith J.B. (2012) Iron-fortified vs low-iron infant formula: developmental outcome at 10 years. *Archives of pediatrics & adolescent medicine* **166,** 208-215.

Marrus N., Eggebrecht A.T., Todorov A., Elison J.T., Wolff J.J., Cole L., et al. (2017) Walking, Gross Motor Development, and Brain Functional Connectivity in Infants and Toddlers. *Cerebral Cortex* **28,** 750-763.

Matias S.L., Mridha M.K., Tofail F., Arnold C.D., Khan M.S.A., Siddiqui Z., et al. (2017) Home fortification during the first 1000 d improves child development in Bangladesh: a cluster-randomized effectiveness trial–3. *The American journal of clinical nutrition* **105,** 958-969.

Mayén A.-L., Marques-Vidal P., Paccaud F., Bovet P. & Stringhini S. (2014) Socioeconomic determinants of dietary patterns in low-and middle-income countries: a systematic review–. *The American journal of clinical nutrition* **100,** 1520-1531.

Mengistu G., Moges T., Samuel A. & Baye K. (2017) Energy and nutrient intake of infants and young children in pastoralist communities of Ethiopia. *Nutrition* **41,** 1-6.

Murray-Kolb L.E., Khatry S.K., Katz J., Schaefer B.A., Cole P.M., LeClerq S.C., et al. (2012) Preschool micronutrient supplementation effects on intellectual and motor function in school-aged Nepalese children. *Archives of pediatrics & adolescent medicine* **166,** 404-410.

Oudgenoeg-Paz O., Leseman P.P. & Volman M. (2015) Exploration as a mediator of the relation between the attainment of motor milestones and the development of spatial cognition and spatial language. *Developmental psychology* **51,** 1241.

Paganini D. & Zimmermann M.B. (2017) The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: a review. *The American journal of clinical nutrition* **106,** 1688S-1693S.

Tulchinsky T.H. (2010) Micronutrient deficiency conditions: global health issues. *Public Health Reviews* **32,** 243.

Victora C.G., de Onis M., Hallal P.C., Blössner M. & Shrimpton R. (2010) Worldwide timing of growth faltering: revisiting implications for interventions. *Pediatrics***,** peds. 2009-1519.

WHO (2006) WHO Child Growth Standards: Length/height-for-age, weightfor-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. *Geneva: World Health Organization* **157**.

WHO (2010) Indicators for assessing infant and young child feeding practices part 3: country profiles.

WHO (2011) Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. *Download from: http://www. who. int/vmnis/indicators/haemoglobin. pdf*.

Wijnhoven T.M., de Onis M., Onyango A.W., Wang T., Bjoerneboe G.-E.A., Bhandari N., et al. (2004) Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food and nutrition bulletin* **25,** S37-S45.

4.2 Evaluation of the sustainability of the effects of low-iron dose MNP home fortified complementary foods

4.2.1. Introduction

Infants and young children (IYC) are vulnerable to malnutrition because of their high nutritional requirements for growth and development. Particularly during the transition period when complementary feeding begins, at six months. The estimated national prevalence of stunting among children under five in Ethiopia was 38%. Therefore, globally, home fortification of complementary foods using MNP coupled with BCC to ensure appropriate IYCF practices are well accepted as an effective strategy for reducing anemia and growth faltering and improve the motor and mental development among children. However, whether benefits persist when interventions are stopped remains unknown.

In this **section (4.2)**, we report the results of a prospective cohort study that followed MNP exposed/unexposed children for six months after the intervention was stopped. Using a difference-in- difference (DID) methodology, changes between and within groups are presented.

4.2.2 Consuming low-iron micronutrient powder home fortified complementary food in the first year of life reduce the risk of anemia, stunting and motor millstone delay of young children (15-18mo) in rural Ethiopia

(Draft paper to be submitted)

Consuming MNP fortified complementary food in the first year of life reduce the risk of anemia, stunting and motor millstone acquisition delay of young children (15-18mo): Prospective cohort study in rural Ethiopia

Abstract

Infants and young children during the nutritionally demanding periods of their life, (the first 1000 days) are exposed to inadequate nutrition which is increased risk of physical growth retardation and neurodevelopmental delay.

This study used an existing demonstration project to evaluate the sustainability of benefit that gained during the provision of low-iron micronutrient powders (MNPs) every other day in the first year of life on nutritional and developmental outcomes six month later of post-intervention of young children in Southern Ethiopia.

Using a prospective cohort design, at baseline 200 infants aged 9-11 months (98 MNP exposed; 102 unexposed) were recruited and data on socio-economic characteristics, anthropometric measures, dietary diversity scores (DDS), hemoglobin concentrations, and motor development acquisition was collected and MNP exposed and unexposed groups were compared. A difference in difference (DID) was used to assess differences in changes over time between MNP-exposed and –unexposed groups six-month post-intervention. Relative risks (RRs) at 95% CI of stunting, anemia, motor development were reported to assess risk of unexposed verses exposed to MNP.

Except for household size, there was no statistically significant difference in socio-economic indicators between MNP exposed and un-exposed groups. The average dietary diversity score was 2.5, and with only 6.4% of children meeting their minimum dietary diversity score. The differences in hemoglobin concentration, prevalence of anemia, LAZ, WAZ, and WLZ between the two groups were widened at the base line. Six-month post MNP intervention, children in the exposed group had reduced risk of being stunted lower risk of being anemic and reduced risk of not being able to stand and walk alone, respectively.

Therefore this finding underline the importance of the first 1000 days as a window of opportunity and provide further evidence that nutritional deprivation in early life, if left untreated, may have long-lasting effects.

Keywords: anemia, stunting, motor development, micronutrients

INTRODUCTION

The complementary feeding period is characterized as a period when infants and young children are at a high risk of malnutrition (Taghizade Moghaddam et al., 2015) . This is related to the high energy and nutrient requirement to support the rapid physical and mental growth. Despite such high needs of energy and nutrients, complementary foods in many low and middle income countries (LMIC) are predominantly plant-based with little inclusion of nutrient-dense foods like animal source foods (ASF), fruits and vegetables (Abebe, Haki, & Baye, 2016; Baye et al., 2013). Consequently, malnutrition is directly or indirectly associated with 45% of childhood mortality (R. E. Black et al., 2013). Those who survive are often affected by stunting, micronutrient deficiencies, and related cognitive deficits (Baye, 2017). About 43% of children less than five years of age in LMIC are estimated to be at risk of not reaching their full developmental potential (M. M. Black & Hurley, 2016).

By age two, the child's brain reaches about 80% of its adult size; hence, the first 1000 days from conception to the second birthday is considered as a critical window of opportunity that should not be missed (Tau & Peterson, 2010). Consequently, many nutrition interventions have been designed to target this critical age group. Recent studies have shown that interventions that start early within the first 1000 days are likely to be more effective than those that start later (Schwarzenberg & Georgieff, 2018). However, because of budget limitations, problems of consistent supply (e.g. micronutrient powders), and short-lived projects, many interventions do not accompany infants and young children through age two (Berti et al., 2014b; R. E. Black, 2014; Jefferds et al., 2015). Little is known as to whether any of the benefits sustain or attenuate when micronutrient interventions are stopped remains unknown.

In our previous retrospective cohort study, we have shown that a low-iron micronutrient powder, when provided in the first year of life (starting from 6-11 months) is associated with increased nutritional and developmental outcomes (e.g. motor development) in rural Southern Ethiopia (**section 4.1).** This intervention was however immediately interrupted after our survey because of supply-related issues. We have visited the children six months later with the objective of investigating any changes in child growth, anemia, and gross motor development between MNP exposed and unexposed children post-intervention.

SUBJECTS AND METHODS

Study site and subjects

The study was conducted Aleta Chuko district, Sidama Zone, Southern region of Ethiopia. Children that were part of a retrospective cohort study were followed six-month post intervention. The study designed is depicted in Figure 1.

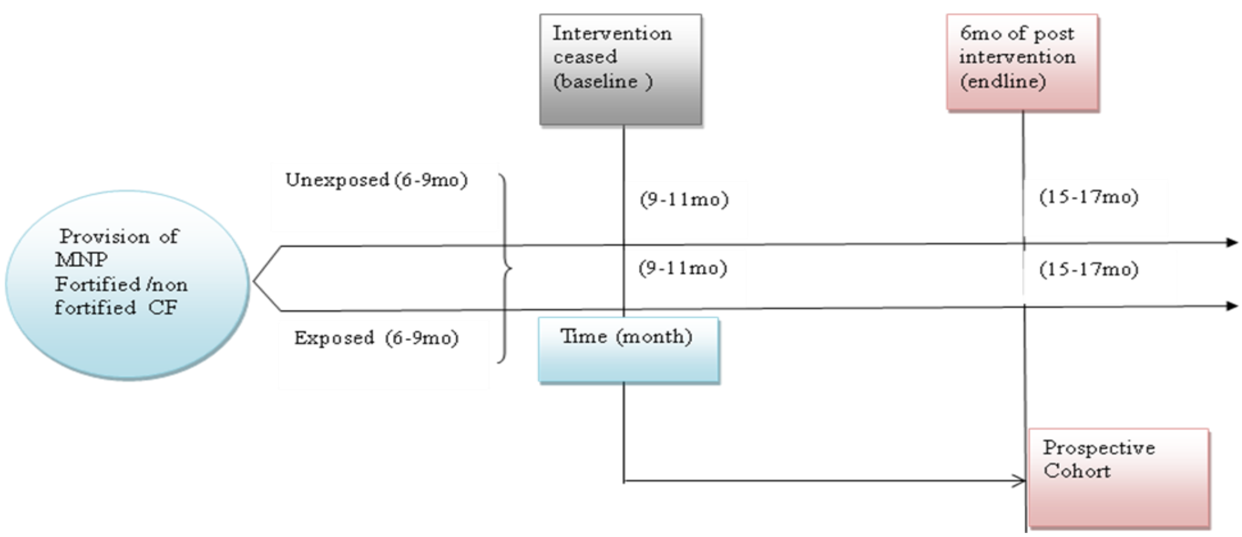


Figure 1 Study design

Information about recruitment and sample size is described in section 4.1.2. However, 13 children were lost to follow-up in this round of survey as indicated in the flow digram of the prospective cohort study Figure 2.

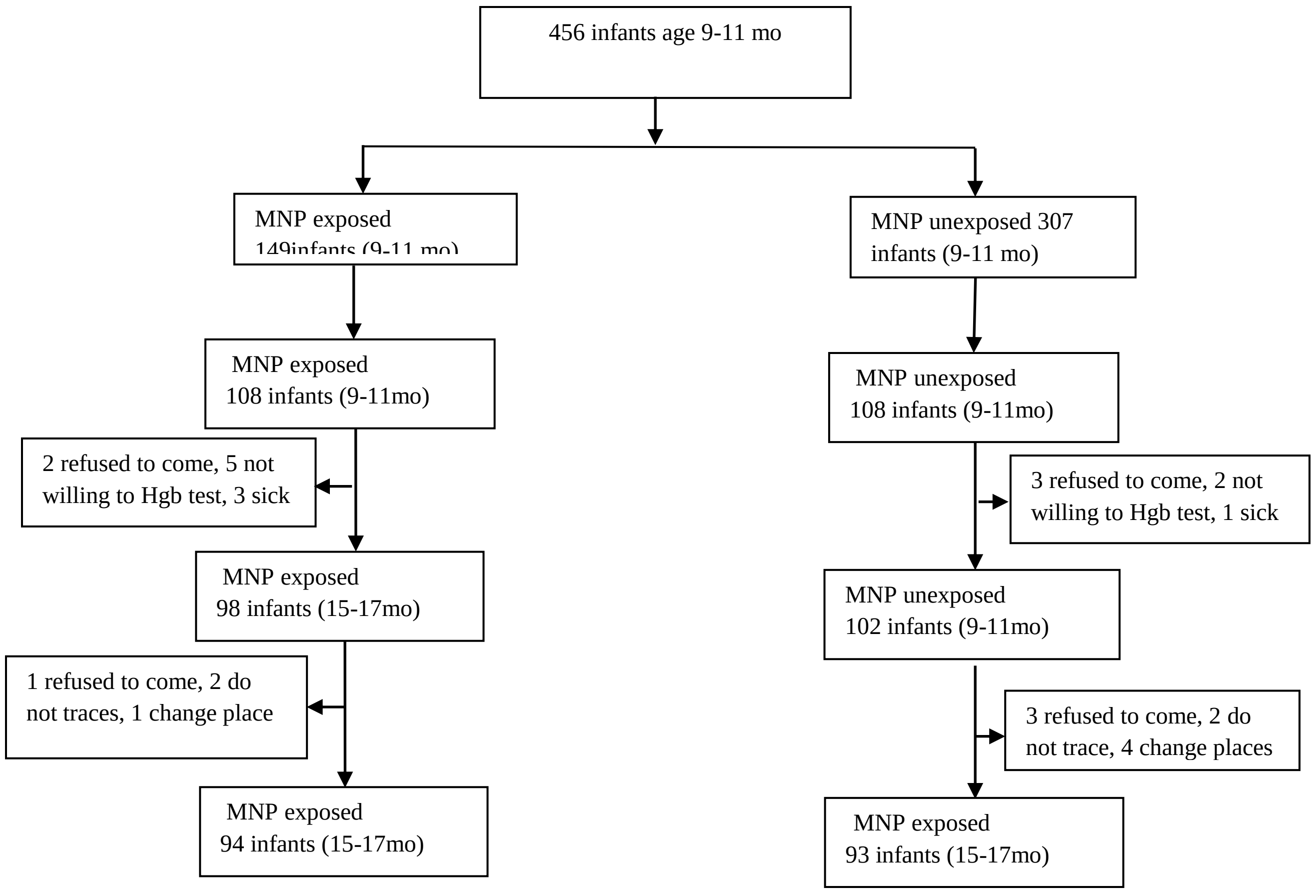


Figure 2 study participant's flow of cohort in SNNPR, Ethiopia

Socio-demographic and anthropometric characteristics

Information about the socio-demographic characteristics including livelihood activities, education level, ownership of land and livestock were collected using a pre-tested questionnaire.

Triplicate measures of weight and length were taken using standardized techniques. Measurements were taken at the health post with children wearing light clothing and no shoes. All anthropometric measurements were made by the same persons to avoid inter-examiner errors. Z-scores length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (LHZ) were calculated using WHO multicenter growth reference data (WHO, 2006b) using the software ENA 2007. Stunting, underweight, and wasting were defined as LAZ, WAZ or WLZ <–2, respectively.

Young children feeding practices

The different food groups consumed in the 24 hours prior the survey was collected using open ended questionnaire and the seven food groups identified in the WHO infant and young child feeding indicators (WHO, 2010a), namely: 1) grains, roots, and tubers; 2) legumes and nuts; 3) dairy products (milk, yogurt, cheese); 4) eggs; 5) flesh foods (meat, fish, poultry, and liver/organ meats); 6) vitamin A–rich fruits and vegetables; and 7) other fruits and vegetables. The proportion of breastfed infants meeting the minimum meal frequency (≥3) and the minimum dietary diversity score (≥4) was calculated.

Hemoglobin measurements

Hemoglobin was measured using a portable photometer (Hemocue HB 301). Measurements were adjusted for altitude (WHO, 2011b). Any anaemia was defined as hemoglobin concentrations <11.0 g/dl, whereas severe anemia was defined as Hb concentrations ≤ 7 g/dl. In the latter cases, children were referred to the nearby healthy facility for follow-up.

Motor development

Motor development was assessed using the World Health Organization (WHO) Multicenter Growth Reference Study guidelines (Wijnhoven et al., 2004). Six distinct gross motor milestones were assessed for this study: 1) sitting without support; 2) hands-knees crawling; 3) standing with assistance; 4) walking with assistance; 5) standing alone; and 6) walking alone.

Ethics

The protocol was approved by the Institutional Review Board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University. Permission and support letters were also obtained from the Sidama zone health bureau, SNNPR, Ethiopia. Informed consent was obtained orally in the presence of the health extension workers and the MNP project coordinator. All the questionnaires were translated to the local language and data collection was interview-based.

Statistical analysis

Data were double-entered in Epi-data statistical software (version 20), and was exported to SPPS after clean-up (IBM SPSS Statistics 20.0, Chicago, IL, USA) for statistical analyses. All continuous variables were checked for normality using the Shapiro-Wilk test. An independent student's t test was used to compare means of variables in the MNP exposed and unexposed groups. A Chi-square test was used to test for independence in distribution of categorical variables between the two study groups.

A difference in difference (DID) methodology was used to assess differences in changes over time between MNP-exposed and –unexposed groups six-month post-intervention. To test the significance for continuous data two sample t-test were used and for proportion two sample z-tests were used with online software "EpiTools epidemiological calculators" ("EpiTools epidemiological calculators,"). Relative Risks (RRs) were find at 95% CI and values are reported. In all comparisons, differences were considered statistically significant for $P < 0.05$.

RESULT

The socio-demographic and anthropometric characteristic of study subjects is presented in **Table 1**. Except for household size, there was no statistically significant difference in socio-economic indicators between MNP exposed and un-exposed groups. However, MNP exposed infants had significantly better anthropometric measures than unexposed ones ($P < 0.05$).

Table1 Baseline socio-demographic characteristics of households with children exposed/ unexposed to MNP in the first year of life (9-11 months)

	All *N= 200*	**MNP exposed** *N = 98*	**MNP unexposed** *N =102*	**P-values**
Household characteristics				
Household Size	4.86±1.60	5.11±1.72	4.62±1.45	0.029*
Land size (hectare)	0.67±0.26	0.68±0.26	0.67±0.26	0.927
Own livestock	151(80.7)	76 (80.9)	75 (80.6)	0.972
Source of drinking water				
Public tap/stand pipe	183 (97.9)	92 (97.9)	91 (97.8)	0.991
Toilet facility with latrine	199 (99.5)	98 (100.0)	101 (99.0)	0.326
Maternal characteristics				
Age (years)	26.43±4.36	26.69±4.73	26.19±3.99	0.412
Able to read and write	131 (65.5)	69 (70.4)	62(60.8)	0.152
Children characteristics				
Age (months)	10.70±1.08	10.87±1.00	10.53±1.13	0.075
Male: female ratio	1.0	1.0	1.0	0.890
Weight (Kg)	9.13±1.20	9.56±1.13	8.72±1.13	<0.001*
Height (cm)	70.62±5.39	72.76±4.84	68.57±5.09	<0.001*
MUAC(cm)	13.16±0.96	13.31±1.05	13.01±0.84	0.024*
Head circumference (cm)	46.86±2.12	47.38±1.76	46.35±2.32	0.001*
Hemoglobin Level¥	11.81 ± 3.74	12.39 ± 3.57	11.25 ± 3.83	0.031*
Sick with in the last two week	33(17.6)	20(21.3)	13(14.0)	0.191

Values aremean ± SD or *n* (%)

* indicate significant mean differences between unexposed and exposed groups at P<0.05 levels using independent t-test (two-tailed) and chi-square test.

¥Hemoglobin concentration was adjusted for altitude.

Continued breastfeeding was a norm, children consumed on average three meals a day (**table 2**). However, only about half of the children met the minimum meal frequency (≥3). The average dietary diversity score was 2.5, and with only 6.4% of children meeting their minimum dietary diversity score.

Table 2 Endline (six month post-intervention) feeding practices of children aged 15-18 months

	All *N= 187*	**MNP exposed** *n= 94*	**MNP unexposed** *n=93*	**P-values**
Breast feeding	187 (100)	94 (100)	93 (100)	
Number of meals per day	3.02±0.72	2.98±0.69	3.05±0.74	0.541
Fed minimum no. of meals/day (≥ 3)	99 (52.9)	55 (59.1)	44 (46.8)	0.091
Dietary diversity score	2.50±0.71	2.41±0.79	2.59±0.59	0.087
Met minimum dietary diversity score (≥ 4)	12 (6.4)	7 (7.4)	5 (5.4)	0.5.63
Minimum acceptable diet	12 (6.4)	7 (7.4)	5 (5.4)	0.563

Values aremean ± SD or *n* (%)
* indicate significant mean differences between unexposed and exposed groups at P<0.05 levels using independent t-test (two-tailed) and chi-square test.

There was an increasing trend in mean hemoglobin concentration and motor milestone scores with age **(Table 3)**. The percentage of stunted and underweight children increased with age, whereas it decreased for anemia. The differences in hemoglobin concentration, prevalence of anemia, LAZ, WAZ, and WLZ between MNP exposed and unexposed at baseline (end of intervention) was widened, whereas differences in motor milestone achievements remained stable (P< 0.05).

Table 3 Endline (6-month post exposure) hemoglobin, anthropometric and motor development outcomes in children (15-18 months)

	All *N= 187*	**MNP exposed** *N = 94*	**MNP unexposed** *N =93*	**DID**[¥] **(95%CI)**	**P-value**
Hemoglobin conc. (mg/dL)					
Baseline[¶]	11.84±3.75	12.44 ± 3.58	11.25 ± 3.83	0.74	0.04*
6mo post-exposure	11.89±1.20	12.11±0.97	11.66±1.37	(0.02,1.46)	
Anemia					
Baseline[¶]	82(41.0)	30 (30.6)	52(51.0)	-5.7	0.157
6mo post-exposure	60(32.1)	18(19.1)	42(45.2)	(-0.02,0.14)	
LAZ					
Baseline	-1.03±2.12	-0.33±1.96	-1.71±2.05	0.20	<0.001*
6mo post-exposure	-1.36±1.86	-0.77±1.79	-1.95±1.74	(0.13, 0.27)	
WAZ					
Baseline	0.10±1.12	0.43±1.03	-0.21±1.11	0.92	<0.001*
6mo post-exposure	-0.35±1.15	0.02±1.12	-0.72±1.06	(0.89,0.94)	
WLZ					
Baseline	0.97±1.33	0.87±1.32	1.07±1.33	0.43	<0.001*
6mo post-exposure	0.42±1.19	0.53±1.36	0.30±0.99	(0.36,0.49)	
Stunting (LAZ <-2)					
End of exposure	60(30.0)	22(22.4)	38(37.3)	2.6	0.495
6mo post of exposure	70(37.4)	27(28.7)	43(46.2)	(-0.05,0.10)	
Underweight(WAZ<-2)					
Baseline	8(4.0)	3(3.1)	5(4.9)	2.6	0.325
6mo post of exposure	14(7.5)	5(5.3)	9(9.7)	(-0.03,0.08)	
Wasting (WLZ <-2)					
Baseline	6(3.0)	3(3.1)	3(2.9)	0.06	0.510
6mo post of exposure	5(2.7)	3(3.2)	2(2.2)	(-0.01,0.02)	
Motor milestone score					
Baseline	4.16±1.20	4.39±1.31	3.93±1.06	0.06	0.524
6mo post-exposure	5.64±0.68	5.84±0.40	5.44±0.84	(-0.13,0.25)	
Standing alone					
Baseline	60(30.0)	38(38.8)	22(21.6)	2.1	0.766
6mo post of exposure	167(89.3)	93(98.9)	74(79.6)	(-0.12,0.16)	
Walking alone					
Baseline	48(24.0)	35(35.7)	13(12.7)	2.4	0.738
6mo post of exposure	142(75.9)	81(86.2)	61(65.6)	(-0.12,0.16)	

[¶]end of intervention;*Statistically significant, p<0.05;[¥] DID, Difference in difference

Six-month post MNP intervention, children in the exposed group had a 32.6 % (RR: 0.674; 95% CI: 0.482, 0.941) reduced risk of being stunted at age 15-18 months **(Table 4)**. Similarly, the risk of being anemic in the exposed group was 42.6% (RR: 0.574; 95% CI: 0.438, 0.751) lower than in the unexposed group. Children in the exposed group had a 43.7 % and 39.5% reduced risk of not being able to stand and walk alone, respectively.

Table 4 Risk of stunting, anemia, and deficits in motor milestone achievements in children (15-18 months) six-month post MNP exposure

Outcome	**N (%)**	**RR(95%CI)**[2]
Stunted		
MNP exposed	27(28.7)	0.674 (0.482, 0.941)
MNP unexposed	43(46.2)	1
Total	70(37.4)	
Anemic		
MNP exposed	18(30.0)	0.574 (0.438, 0.751)
MNP unexposed	42(45.2)	1
Total	60(32.1	
Unable standalone		
MNP exposed	1(1.1)	0.466 (0.383,0.568)
MNP unexposed	19(20.4)	1
Total	20(10.7)	
Unable to walk alone		
MNP exposed	13(13.8)	
MNP unexposed	32(34.4)	0.604 (0.463, 0.788)
Total	45(24.1)	1

[1]Total N = 187: exposed group, n = 94; unexposed group, n = 93; MNP, micronutrient powder.
[2]Values were estimated by using log-binomial regression adjusted for baseline differences: all anthropometric characteristic, household size hemoglobin.

DISCUSSION

The present prospective cohort study, followed infants that were MNP exposed/unexposed in their first year of life (9-12 months) six-month post intervention to evaluate changes in anthropometric, hemoglobin and motor milestone achievement measures. Six months post-MNP exposure, children in the MNP exposed group had a lower risk of stunting, anemia, and delays in motor development than their unexposed counterparts. Except for motor milestone achievements, baseline differences in hemoglobin concentration, prevalence of anemia, LAZ, WAZ, and WLZ was significantly widened.

While breastfeeding continued, the diets of the children six-month post MNP intervention remained low in diversity, with little consumption of nutrient-dense foods like ASFs. While continued breastfeeding is likely to be advantageous for cognitive function (Jedrychowski et al., 2012), as well as to reduce the risk of stunting and morbidities from infectious diseases (Richard et al., 2018), the poor dietary diversity on the other hand suggests a suboptimal nutrient intake from complementary foods, which during this critical period of complementary feeding is associated with growth faltering (Onyango, Borghi, de Onis, del Carmen Casanovas, & Garza, 2014). Indeed, during the six months of follow-up increases in the prevalence of stunting and underweight were observed. This is in line with the general trend in growth faltering in LMIC (Humphrey & Prendergast, 2017).

The widening of baseline differences in the prevalence of stunting, underweight, and anemia between the MNP exposed and unexposed group six-month post-intervention is an alarming finding. This suggests that early adverse nutritional experiences, when untreated, predispose children at a greater nutritional risk that increases with the age of the child. On the other hand, the baseline differences in motor milestone achievements remained stable six-month post exposure, suggesting that there is some level of tracking. These findings underline the importance of the first 1000 days as a window of opportunity and provide further evidence that nutritional deprivation in early life, if left untreated, may have long-lasting effects.

Several limitations need to be considered when interpreting our findings. First, this study is a follow-up of a retrospective cohort study that did not have control over the allocation of children into MNP exposed or unexposed group. As children were not randomly allocated into MNP exposed and unexposed groups, the baseline differences observed might be confounded by factors other than MNP exposure. Consequently, causal inferences cannot be made. Unfortunately, our study did not have a group that continued receiving MNP and thus cannot provide evidence on possible outcomes if the intervention was continued. Nevertheless, our study is useful in indicating that differences in anthropometric measures, anemia, and motor milestones in infancy (9-12 months) do not only track, but also widen (e.g. stunting and anemia) six-month post intervention. Therefore, improving complementary diets as early as possible may have long-lasting effects. Follow-up of these children over the long-term and evaluating cognitive function outcomes like fine/gross motor, but also social and language skills is needed.

Acknowledgements The authors thank all of the children and families who took part in this survey. Nutrition international (formerly Micronutrient initiative) is acknowledged for allowing us to use their demonstrating project for this study.

Source of funding: The graduate programme of Addis Ababa University

Conflict of interest statement: The authors declare that they have no conflict of interest

Contributor statement Both authors were involved in developing the study design. AG coordinated and supervised the fieldwork. AG and KB analyzed and interpreted the data. AG wrote the first draft of the manuscript. All the authors contributed to manuscript preparation

Reference

Abebe, Z., Haki, G. D., & Baye, K. (2016). Health extension workers' knowledge and knowledge-sharing effectiveness of optimal infant and young child feeding are associated with mothers' knowledge and child stunting in rural Ethiopia. *Food and nutrition bulletin, 37*(3), 353-363.

Baye, K. (2017). The Sustainable Development Goals cannot be achieved without improving maternal and child nutrition. *Journal of public health policy, 38*(1), 137-145.

Baye, K., Guyot, J.-P., Icard-Verniere, C., & Mouquet-Rivier, C. (2013). Nutrient intakes from complementary foods consumed by young children (aged 12–23 months) from North Wollo, northern Ethiopia: the need for agro-ecologically adapted interventions. *Public health nutrition, 16*(10), 1741-1750.

Black, M. M., & Hurley, K. M. (2016). Early child development programmes: further evidence for action. *The Lancet Global Health, 4*(8), e505-e506.

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., De Onis, M., . . . Martorell, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet, 382*(9890), 427-451.

EpiTools epidemiological calculators. http://epitools.ausvet.com.au/content.php?page=home date1/23/2018.

Humphrey, J. H., & Prendergast, A. J. (2017). Population-level linear growth faltering in low-income and middle-income countries. *The Lancet Global Health, 5*(12), e1168-e1169.

Onyango, A. W., Borghi, E., de Onis, M., del Carmen Casanovas, M., & Garza, C. (2014). Complementary feeding and attained linear growth among 6–23-month-old children. *Public health nutrition, 17*(9), 1975-1983.

Schwarzenberg, S. J., & Georgieff, M. K. (2018). Advocacy for Improving Nutrition in the First 1000 Days To Support Childhood Development and Adult Health. *Pediatrics*, e20173716.

Taghizade Moghaddam, H., Khodaee, G. H., Ajilian Abbasi, M., & Saeidi, M. (2015). Infant and Young Child Feeding: a Key area to Improve Child Health. *International Journal of Pediatrics, 3*(6.1), 1083-1092.

Tau, G. Z., & Peterson, B. S. (2010). Normal development of brain circuits. *Neuropsychopharmacology, 35*(1), 147.

WHO. (2006). WHO Child Growth Standards: Length/height-for-age, weightfor-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. *Geneva: World Health Organization, 157*.

WHO. (2010). Indicators for assessing infant and young child feeding practices part 3: country profiles.

WHO. (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. *Download from: http://www. who. int/vmnis/indicators/haemoglobin. pdf*.

Wijnhoven, T. M., de Onis, M., Onyango, A. W., Wang, T., Bjoerneboe, G.-E. A., Bhandari, N., . . . Al Rashidi, B. (2004). Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food and nutrition bulletin, 25*(1_suppl1), S37-S45.

4.3 Early child nutrition and associated developmental milestone of young children

4.3.1 Introduction

The early years of life is conceptualized as a period of experience-based brain development and as a critical stage for the programming of neurobiological pathways that can affect health, learning and behaviour during the entire life cycle. In section 4.1 and 4.2, we have evaluated the relationship of exposure of MNP with motor development and found strong relationships. In this section **(4.3)**, we expand our analyses to evaluate predictors of developmental milestones on cognitive domains like language, social, fine and motor skills.

4.3.2 Early child nutrition is associated with achievement of development milestones in young children aged 15-17 months in rural Ethiopia

(Draft paper to be submitted)

Early child nutrition is associated with achievement of development milestones in young children aged 15-17 months in rural Ethiopia

Abstract

Inadequate breast- and complementary-feeding are associated with growth faltering, putting children at risk of developmental delays that are largely irreversible. Consequently, tools that are culturally-sensitive and accurate to help monitor children's achievements of development milestones are needed to introduce timely interventions when needed.

We aimed to measure acquisition of child development milestones and identify their predictors.

Using a cross-sectional study (n= 187), data on socio-demographic characteristics, anthropometry, hemoglobin concentration, head circumference, breastfeeding and complementary feeding practices were collected from caregivers/children aged 15-18 months in rural Southern Ethiopia. The Malawian Developmental Assessment Tool (MDAT) was used to measure acquisition of motor (fine/gross), language, and social skills. Correlation and regression were run to identify associated factors.

All children were breastfed, but complementary feeding was suboptimal with a mean dietary diversity score of 2.5 ± 0.68. The prevalence of stunting (37%), anemia (32%), and morbidity (18%) was high. Age-appropriate developmental milestones were acquired except for some delays observed in "walking alone". Length-for-age (LAZ), weight-for-age (WAZ), head circumference, and exposure to fortified foods were positively associated with higher scores of motor, language and social skill scores ($P<0.01$). Mid-upper arm circumference (MUAC) was positively associated with social and language skills, whereas hemoglobin concentration was only associated with social skills ($P<0.05$).

The MDAT could be useful to predict subtle developmental delays associated with undernutrition. The use of this tool in this and other similar settings in Africa could help monitor early child development and provide an opportunity to intervene timely before endpoints like stunting are reached.

Keywords: complementary feeding, stunting, anemia, cognitive function, child development

INTRODUCTION

The early years of life, from conception to the second birthday of a child, is a critical period when the foundations of cognitive development are laid though programming of neurobiological pathways that can affect lifelong health, learning, and behavior (Tinajero & Loizillon, 2012; (Cesar G Victora et al., 2008). Socio-cultural context including poverty and maternal depression increases exposure to biological and psychosocial risks that can affect cognitive development by eliciting changes in brain structure and function. Malnutrition is a biological, social, and environmental factor is widely recognized to affect cognitive development. In fact, stunting (growth faltering) along with poverty was used as a proxy to estimate the proportion of children younger than five years of age at risk of not reaching their developmental potential (M. M. Black & Hurley, 2016).

Among children less than five years of age living in low- and middle-income countries, some 250 million (43%) are estimated to be at risk of not reaching their full developmental potential (M. M. Black & Hurley, 2016). In the absence of uniform measurement of cognitive function and in light of the scarcity of data, these estimates used stunting and poverty as proxies and called for an urgent need for population-level indicators of early child development. However, more direct indicators of cognitive outcomes are needed. Such indicators would not only be useful to monitor the state of child development in different countries, but can also inform the design of effective interventions that promote children to grow and lead a healthy and productive life as adults.

The Malawian Developmental Assessment Tool (MDAT) is such a tool that was developed to measure cognitive outcomes of children in Sub-Saharan Africa (resource-poor settings). The

MDAT is culturally adapted to the African setting, has been validated, and is relatively simple and fast to use (Gladstone et al., 2010). Such a tool could be useful to monitor and identify predictors of poor cognitive outcomes to inform interventions that are urgently needed in countries like Ethiopia, which are at high risk of cognitive development impairments associated with the high child stunting rates (38%) and micronutrient deficiencies.

Therefore, the objectives of this study was to measure child development and identify factors associated with motor (fine/gross), language, and social skills using MDAT in rural Southern Ethiopia.

SUBJECTS AND METHODS

Study site and participants

The study was conducted in Aleta Chuko district, Sidama Zone, Southern region of Ethiopia. The stunting rate of this region is of similar values than the national average (40 %), which is quite high (Central Statistical Agency - CSA/Ethiopia & ICF, 2017). Most households in this district are subsistent farmers, growing staple crops like maize (*Zea mays L.*) or root and tubers like *kocho* (*Enset ventricosum*).

Sampling

This study is part of a larger study that followed children from 9-11 months onwards to evaluate the association between dietary patterns, micronutrient supplementation, and motor milestone achievements. Given the various outcomes of interest, sample size was calculated to detect a medium effect size difference (Cohen's d= 0.5) between two means assuming $\alpha = 0.05$, and a power of 90%. After allowing 20% attrition, the total sample size required was 202. The inclusion criteria for this study were to be apparently healthy with no signs of severe malnutrition, with no disabilities that prevent accurate measurement of development milestones.

Ethics

The study protocol was approved by the institutional review board of the college of natural and computational sciences of Addis Ababa University. Permission and support letters were also obtained from the Sidama zone health bureau, SNNPR, Ethiopia. Oral informed consent was obtained in the presence of health extension workers. All questionnaires were translated to the local language and data collection was interview-based.

Data collection

Socio-demographic characteristics and anthropometric measures

Using pretested questionnaires, data on socio-demographic parameters including livelihood, household size, and employment, level of education, land ownership and size was collected from child caregivers. Young children's anthropometric measures (weight and height) were collected using standardized techniques, with children wearing light clothing and no shoes. All anthropometric measures were collected by the same investigator to avoid inter-examiner errors. Z-scores of length-for-age (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ) were calculated using the software ENA 2007(WHO, 2006a). Children with LAZ, WAZ, and WLZ z-scores <-2 were considered as stunted, underweight and wasted, respectively.

Morbidity and hemoglobin screening

Morbidity was assessed by asking the caregiver whether the index child had fever, diarrhea, and or cough in the last two weeks. Hemoglobin was measured using a portable photometer (Hemocue HB 301), and was adjusted for altitude. Measurements were adjusted for altitude (Sullivan, Mei, Grummer-Strawn, & Parvanta, 2008; V. WHO, 2011). Anemia was defined as hemoglobin concentration values of <11.0 g/dl , and children with severe anemia (<7 g/dl) were referred to the nearby health facility for follow-up.

Complementary feeding practices

Information about continued breastfeeding, type of complementary foods provided, consumption of fortified foods, and food groups consumed was over the 24h preceding the survey was collected. Dietary diversity score was calculated using the standard seven food group categories, as follows: 1) grains, roots, and tubers; 2) legumes and nuts; 3) dairy; 4) eggs; 5) flesh foods; 6) vitamin A-rich fruits and vegetables; and 7) other fruits and vegetables. Consumption ≥ 3 food groups for breastfed and ≥ 4 for non-breastfed children was considered as adequate dietary diversity.

Assessment of developmental milestone achievements

The Malawi Developmental Assessment Tool (MDAT) was used to assess developmental outcomes. The tool has been validated for use in rural settings of Sub-Saharan Africa (Gladstone et al., 2010) to assess gross motor, fine motor, language and social skills. Each developmental domain is assessed guided by a 34-item questions/observation guide. However, given the narrow age range of our sample (15-18 months), out of the 34 items only eight for gross motor, nine for fine motor, six for language, and ten for social skills were relevant. A score of 1 was given if the child was able to perform the task and 0 otherwise. The proportion of children able to perform a given activity was calculated and was compared to the proportion of a reference population that is able to perform that same activity.

Statistical analyses

Data were double-entered using Epi-data statistical software (version 20), were cleaned, and then exported to SPSS (SPPS version 20.0). All continuous variables were checked for normality using the Shapiro-Wilk test. Descriptive statistics of continuous parameters were presented as mean ± standard deviation, whereas frequency/percentages were used for categorical parameters. Partial correlations adjusting for age were run to evaluate associations between development milestone achievements and socio-demographic characteristics (mothers' education), nutritional status, complementary feeding practices, and anemia. Multivariate logistic regression was run with the scores of the different development milestones as dependent and stunting, underweight, hemoglobin concentrations, MNP exposure, head circumference, and MUAC as predictors.

RESULTS

From the total of 200 households that we envisaged to collect data, complete data was collected in 187 of them; this represents a 9% non-response rate. The mean (± SD) household size was 5.0 ± 1.6, all the households were male-headed, and more than 90% were subsistent farming households (**Table 1**). About one-third of the farming households had farming land of >1 hectare. Education of mother above a primary level was low (17%). More than 50% of the households had some form of livestock. The prevalence of stunting (35%), anemia (32%), and morbidity (18%)

was high. The commonest illnesses reported were diarrhea (46.4%), cough (28.6%), and fever (14.3%). Other illnesses (10.7 %) reported were skin rash, sore throat and eye infections (data not shown).

Table 1 Socio-demographic and anthropometric characteristic of young children (N=187) aged 15-18 months from Aleta Chuko district Sidama Zone, Ethiopia

	All (*N= 187)*	
	n/mean	%/SD
Socio-demographic characteristics		
Household Size	4.96	1.61
Four or more children	65	34.8
Farming households	176	94.1
Monthly income (Birr)	343.85	492.09
Land size ≥ 1ha	62	33.2
Own cow/oxen	109	58.3
Own chicken	92	49.2
Age of mother (years)	26.5	4.4
Mother's education ≥primary school	32	17.1
Married	187	100
Male: female ratio	1.0	
Anthropometric characteristics		
LAZ	-1.36	1.86
WAZ	-0.35	1.15
WLZ	0.42	1.19
Stunting	70	37.4
Underweight	14	7.5
Wasting	5	2.5
Hemoglobin and morbidity		
Hemoglobin level	11.89	1.20
Anemia	60	32.1
Sick with in the last two week	33	17.6

Values are mean ± SD or *n* (%); WAZ, weight-for-age z score; LAZ, Length-for-age z score; WLZ, weight-for- length z score

All the studied children were breastfed **(Table 2)**, and half of them had on average consumed three meals a day. Very small proportion children had minimum acceptable diet (6.4%). The average dietary diversity was 2.5±0.68 and 50.26% of the children were exposed for MNP in their first year of their age. Except for dairy that was consumed by 40 % of the children, consumption of animal source foods like meat (4.8%) and eggs (11.8%) was very low.

Table 2 Complementary feeding practices of young children (n=187) aged 15 to 18 months from Aleta Chuko district Sidama Zone, Ethiopia

	All(N=187)	
Feeding practice	n/mean	%/SD
Breast feeding	187	100
Food commonly fed (porridge)	187	100
Number of meals per day	3.02	0.72
Cereal product	187	100
Legumes and nuts	29	15.5
Dairy	74	39.6
Eggs	22	11.8
Meat and poultry	9	4.8
Vitamin A-rich fruits and vegetables	116	62.7
Other fruits	28	15.0
Mean number of food group (out of 7)	2.50	0.71
Fed minimum number of food groups or more	12	6.4
Minimum acceptable diet	12	6.4
MNP exposure in the last 6 month	0	-
MNP exposure in the last 1 year	94	50.27

MNP, Micronutrient powder

Although most items were performed, delays in the achievement of some gross and social development milestones were observed (**Table 3**). Particularly delays in the items “walking well” and “indicating that they need to go for a poo/pee” were observed.

Table 3 Age-related score of gross motor, fine motor, language, and social skills of children using MDAT (N=187) from Aleta Chuko district Sidama Zone, Ethiopia

Age-relevant developmental assessment items	% (reference) of children able to perform the activities			
	15 months	16 months	17 months	18 months
Gross motor activity				
1.Pull self to stand	100 (75-90)	100(75-90)	100(75-90)	100(75-90)
2. Able to stand well if holding on to things	97.1 (75-90)	100 (75-90)	100 (75-90)	100 (75-90)
3. Walks using both hands of somebody	100 (75-90)	100 (75-90)	100 (75-90)	100 (75-90)
4. Walks with help using someone's hand or furniture	100 (75-90)	100 (75-90)	97.8 (75-90)	100 (75-90)
5. Walks, but falls over at times	91.2 (75-90)	93.5 (75-90)	91.3 (75-90)	100 (75-90)
6. Stoops over and gets back up	79.4 (50-75)	82.6 (50-75)	89.1(75-90)	98.4 (75-90)
7. Walks well	**44.1 (50-75)**	69.6 (50-75)	89.1 (75-90)	91.8 (75-90)
8. Runs, but basic running – may fall over at times	35.3 (25-50)	60.9 (50-75)	69.6(50-75)	82.0 (75-90)
Mean (SD) score	**7.3 (1.2)**			
Fine motor activity				
1. Finds object under the piece of cloth	97.1 (75-90)	97.8(75-90)	93.5(75-90)	95.1(75-90)
2. Picks up maize or bean with thumb and one finger	100(75-90)	100(75-90)	97.8(75-90)	100(75-90)
3. Puts blocks in and out of cup in imitation	100(75-90)	100(75-90)	95.7(75-90)	100(75-90)
4. Pushes a little car along	100(75-90)	97.8(75-90)	95.7(75-90)	100(75-90)
5. Puts blocks into bottle with screw on lid in imitation	73.5 (50-75)	82.6 (75-90)	78.3(75-90)	96.7(75-90)
6. Dumps blocks out of bottle with lid purposefully	61.8 (25-50)	73.9 (25-50)	76.1(25-50)	93.4(50-75)
7. Scribbles on paper (straight scribble)	50(25-50)	60.9 (25-50)	73.9 (25-50)	75.4 (50-75)
8. Scribbles on paper (circular scribble)	2.9 (<25)	2.2 (<25)	4.3 (<25)	9.8(<25)
9. Tower of 2 blocks	5.9 (<25)	4.3 (<25)	2.2 (<25)	14.8 (25-50)
Mean (SD) score	**6.4 (1.1)**			
Language				
1. Indicates by gesture to say "No"	100 (75-90)	100 (75-90)	100 (75-90)	100 (75-90)
2. Follows simple commands eg. "give me the cup"	79.4 (50-75)	84.8 (50-75)	89.1(75-90)	91.8(75-90)
3. Unclear talk/jabber in sentences - pretends to talk	82.4 (50-75)	84.8 (50-75)	95.7(75-90)	96.7(75-90)
4. Says 2 words, but words other than mama/dada	64.7 (50-75)	71.7(50-75)	87.0(50-75)	93.4(75-90)
5. Says 2 words together	23.5 (<25)	41.3 (<25)	56.5(25-50)	78.7(25-50)
6. Says 6 words (words other than mama/dada)	14.7 (<25)	17.4 (<25)	21.7(<25)	50.8(25-50)
Mean (SD) score	**4.4 (1.4)**			
Social activity				
1. Drinks form a cup well without spilling	85.3 (75-90)	84.8 (75-90)	93.5(75-90)	96.7(75-90)
2. Able to indicate, by pointing, something in need	97.1 (75-90)	87.0(75-90)	97.8(75-90)	100 (75-90)
3. Made Eat by picking from the plate	91.2(75-90)	80.4(75-90)	91.3(75-90)	95.1(75-90)
4. Puts hands out to have them washed by mum	85.3 (50-75)	84.8 (75-90)	91.3(75-90)	96.7(75-90)
5. Can hold a spoon and take food by self, but spills some	79.4 (50-75)	78.3 (50-75)	87.0(75-90)	98.4(75-90)
6. Indicates they need to go for a poo/pee (e.g. cries)	**23.5 (50-75)**	**34.8(50-75)**	**28.3(75-90)**	**45.9(75-90)**
7. Wants to join in with singing games	38.2 (<25)	52.2 (<25)	63.0(25-50)	60.7(25-50)
8. Able to greet either by extending hand or verbally	58.8 (25-50)	54.3 (25-50)	69.6(25-50)	78.7(25-50)
9 Sharing things, including food with others	41.2 (<25)	56.5 (<25)	60.9(25-50)	80.3(25-50)
10. Does a poo/ pees by themselves without wetting their pants	5.9(<25)	15.2 (<25)	10.9 (<25)	27.9(25-50)
Mean (SD) score	**6.9 (2.2)**			

Stunting, wasting and lower head circumference measurements were significantly associated with lower scores of gross- and fine-motor, language, and social skills (P<0.01). MUAC was positively associated with language and social skills, whereas hemoglobin concentrations was positively associated social skills (P<0.05). MNP exposure in the first year of life was positively associated with better scores in all the measured domains of development milestones (P<0.05), but stronger associations were observed for gross motor milestone achievement scores.

Table 4 Factors associated with developmental outcomes from Aleta Chuko district Sidama Zone, Ethiopia

	Developmental Outcomes			
	Gross motor	Fine Motor	Language	Social skill
LAZ	0.347* (<0.001)	0.236*(0.001)	0.284*(<0.001)	0.373*(<0.001)
WAZ	0.218*(0.003)	0.149*(0.043)	0.235*(0.001)	0.306*(<0.001)
WLZ	-0.082	-0.055	0.029	0.037
MUAC	0.123	0.042	0.149*(0.043)	0.168*(0.022)
Head circumference	0.269*(<0.001)	0.153*(0.037)	0.141* (0.040)	0.157* (0.032)
Dietary Diversity score	0.014	0.033	0.128	0.078
MNP exposure	0.338*(<0.001)	0.272*(<0.001)	0.270*(0.001)	0.474*(<0.001)
Sickness in the past two week	-0.122	-0.086	0.002	-0.004
Hemoglobin level (g/dL)	0.075	0.039	0.119	0.221*(0.002)
Mothers Education (y)	0.066	0.132	0.078	0.138

* Results are from partial correlation adjusting for age; P< 0.05 is considered statistically significant; Length-for-age (LAZ); Weight-for-Age (WAZ); Weight-for-Length (WLZ) z-scores.

The age-adjusted multiple regression analyses showed that LAZ, MNP exposure, head circumference, and morbidity predicted most development milestone achievements **(Table 5)**.

Table 5 Multivariate linear regression analyses adjusted for covariates from Aleta Chuko district Sidama Zone, Ethiopia

	Gross Motor (β; 95%CI)	Fine motor (β; 95%CI)	Language (β; 95%CI)	Social (β; 95%CI)
R^2	0.327	0.214	0.273	0.367
LAZ	0.202*(0.088, 0.316)	0.111*(0.003, 0.220)	0.142*(0.010, 0.274)	0.272*(0.068, 0.477)
WAZ	-0.105(-0.323, 0.113)	-0.071(-0.241, 0.100)	-0.001(-0.252, 0.249)	-0.021(-0.408, 0.366)
MUAC	-0.010(-0.220, 0.200)	-	0.047(-0.193, 0.286)	0.033(-0.339, 0.405)
HC(cm)	0.096*(0.009, 0.182)	0.035(-0.048, 0.119)	0.024(-0.076, 0.125)	0.003(-0.151, 0.158)
MNP exposure	0.590**(0.268, 0.912)	0.482*(0.173, 0.792)	0.476*(0.104, 0.849)	1.664**(1.091, 2.237)
Sickness	-0.410*(-0.803, -0.017)	-0.280 (-0.654, 0.093)	-	-
Hemoglobin (g/dL)	-	-	0.030(-0.118, 0.179)	0.162 (-0.067, 0.390)
Maternal education (y)	-	0.029(-0.013, 0.070)	-	0.046 (-0.032, 0.123)

Values are β unstandardized regression coefficients (95% CI); MNP exposure minimum acceptable diet and sickness (categorical variable were converted to a dummy variable (0-unexposed; 1- exposed and1-yes;0-No). The rest of the variables were continuous; R^2, unadjusted R-squared/coefficient of determination; **P<0.001 *p<0.05

DISCUSSION

This study, to our knowledge, is the first to use the MDAT in Ethiopia to assess developmental milestone acquisition and identify associated factors. Breastfeeding was a norm; complementary foods were predominantly plant-based with little consumption of fruits and vegetables. Except for some motor developmental milestones like "walking well", most of the developmental milestones were achieved. LAZ, WAZ, head circumference, hemoglobin concentration and exposure to micronutrient fortified foods were associated with higher scores in various developmental domains.

Continued breastfeeding was universal in this setting and this can confer several advantages including protection against child infections, increased intelligence, and probable reductions in overweight and diabetes (Cesar G Victora et al., 2016). On the other hand, suboptimal complementary feeding characterized by low dietary diversity is consistently associated with stunting, which is associated with both short and long-tern cognitive impairments (Gashu et al., 2015; Hirvonen & Hoddinott, 2017; Sudfeld et al., 2015). In line with various reports from different parts of Ethiopia, complementary feeding in this setting was suboptimal, and missed key nutrient-dense food groups like fruits, vegetables, and animal source foods (Abebe et al., 2016; Baye et al., 2013). Such poor dietary diversity of complementary foods in Ethiopia has been associated with stunting and inadequate micronutrient intakes (Abebe et al., 2016, 2018).

In this study stunting, but not dietary diversity score was associated with scores of developmental milestone achievements. Perhaps the lack of association between dietary diversity score and the development milestone scores could be because of the overall poor dietary diversity score in this setting and may not suggest the irrelevance of micronutrient adequacy of complementary diets. In fact, the strong association between exposure to micronutrient powder in the first year of life and scores of developmental milestone achievements confirm this finding, which is supported by recent studies (Prado, Abbeddou, Adu-Afarwuah, et al., 2016). Furthermore, hemoglobin concentration was associated with social skills.

The present study has several limitations that need to be considered when interpreting the present findings. Because of the cross-sectional nature of the study, we are unable to demonstrate whether the MDAT can be used as a tool for early prediction of malnutrition before endpoints like anemia and stunting are reached. Furthermore, causal inferences cannot be made, but the MDAT was able to detect associations between nutritional parameters like stunting, underweight, head-circumference, hemoglobin concentrations, and exposure to fortified foods in early life with various developmental domains.

Notwithstanding the above limitations, our finding indicates that the MDAT can be a useful tool to predict subtle developmental delays that can be associated with undernutrition. The use of the tool in this and other similar settings in Africa could help monitor early child development and provide an opportunity to intervene timely before endpoints like stunting are reached. However, the ability of the tool to predict early signs of malnutrition should be evaluated using longitudinal studies.

Acknowledgement

The authors thank all of the children and families who took part in this survey.

Authorship contribution

Both authors were involved in developing the study design. AG coordinated and supervised the fieldwork. AL. AG and KB analyzed and interpreted the data. AG wrote the first draft of the manuscript. Both the authors contributed to manuscript preparation

Disclosure of conflict of interest: The authors declare that they have no conflict of interest

Reference

Abebe, Z., Haki, G. D., & Baye, K. (2016). Health extension workers' knowledge and knowledge-sharing effectiveness of optimal infant and young child feeding are associated with mothers' knowledge and child stunting in rural Ethiopia. *Food and nutrition bulletin, 37*(3), 353-363.

Abebe, Z., Haki, G. D., & Baye, K. (2018). Simulated effects of home fortification of complementary foods with micronutrient powders on risk of inadequate and excessive intakes in West Gojjam, Ethiopia. *Maternal & child nutrition, 14*(1).

Baye, K., Guyot, J.-P., Icard-Verniere, C., & Mouquet-Rivier, C. (2013). Nutrient intakes from complementary foods consumed by young children (aged 12–23 months) from North Wollo, northern Ethiopia: the need for agro-ecologically adapted interventions. *Public health nutrition, 16*(10), 1741-1750.

Black, M. M., & Hurley, K. M. (2016). Early child development programmes: further evidence for action. *The Lancet Global Health, 4*(8), e505-e506.

Central Statistical Agency - CSA/Ethiopia, & ICF. (2017). Ethiopia Demographic and Health Sruvey 2016. Addis Ababa, Ethiopia: CSA and ICF.

Gashu, D., Stoecker, B. J., Bougma, K., Adish, A., Haki, G. D., & Marquis, G. S. (2015). Stunting, selenium deficiency and anemia are associated with poor cognitive performance in preschool children from rural Ethiopia. *Nutrition journal, 15*(1), 38.

Gladstone, M., Lancaster, G. A., Umar, E., Nyirenda, M., Kayira, E., van den Broek, N. R., & Smyth, R. L. (2010). The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. *PLoS medicine, 7*(5), e1000273.

Hirvonen, K., & Hoddinott, J. (2017). Agricultural production and children's diets: Evidence from rural Ethiopia. *Agricultural Economics, 48*(4), 469-480.

Prado, E. L., Abbeddou, S., Adu-Afarwuah, S., Arimond, M., Ashorn, P., Ashorn, U., . . . Maleta, K. (2016). Linear growth and child development in Burkina Faso, Ghana, and Malawi. *Pediatrics, 138*(2), e20154698.

Sudfeld, C. R., McCoy, D. C., Danaei, G., Fink, G., Ezzati, M., Andrews, K. G., & Fawzi, W. W. (2015). Linear growth and child development in low-and middle-income countries: a meta-analysis. *Pediatrics, 135*(5), e1266-e1275.

Sullivan, K. M., Mei, Z., Grummer-Strawn, L., & Parvanta, I. (2008). Haemoglobin adjustments to define anaemia. *Tropical Medicine & International Health, 13*(10), 1267-1271.

Victora, C. G., Adair, L., Fall, C., Hallal, P. C., Martorell, R., Richter, L., . . . Group, C. U. S. (2008). Maternal and child undernutrition: consequences for adult health and human capital. *The lancet, 371*(9609), 340-357.

WHO. (2006). WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. *Methods and development. WHO (nonserial publication). Geneva: WHO, 2007*.

WHO, V. (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. *Geneva: Vitamin and Mineral Nutrition Information System, WHO*.

4.4 Retention of fat soluble vitamins in a home fortified complementary food

4.4.1 Introduction

In section 4.1, 4.2, and 4.3, we have seen that exposure to low-Fe MNP in early life is associated with better cognitive outcomes. If confirmed through community trials, these findings may have implications in the design of future MNP interventions. To maximize benefits related to MNP exposure, the nutrients may reach the end-consumer. This is not guaranteed for fat-soluble vitamins like vitamin A, D, and E that are susceptible to heat, light, and oxidation.

In this **section (4.4)**, the vitamin A, D, and E concentrations were determined from representative samples of home-fortified complementary foods using HPLC-DAD.

4.4.2 Retention of fat-soluble vitamins (A, D and E) in home-fortified complementary foods with micronutrient powder

(Draft paper to be submitted)

Retention of fat-soluble vitamins (A, D and E) in home-fortified complementary foods with micronutrient powder

Abstract

Micronutrient malnutrition is estimated to affect about a third of the world population. Infants and young children during the complementary feeding period are the most affected. Home fortification with micronutrient powders (MNP) has been promoted as a cost-effective strategy, however, little is known about the stability of sensitive vitamins like vitamin A, D, and E in home-fortified complementary foods.

The objective of the study was to determine concentrations of vitamin A, D_3, and E in home-fortified complementary foods and evaluate whether they meet the fortification target.

Caregivers' knowledge, attitude, and practice related to the use of MNPs were assessed. Home-fortified complementary foods were collected (n=30) and vitamin A (retinol), D_3 (cholecalciferol) and E (α –tocopherol) concentrations were determined using HPLC-DAD in a composite sample. Inappropriate use of MNPs like introducing MNPs during cooking, and fortifiying a meal prepared for the whole day was common. Concentrations of retinol 2.5-3.9 µg/g, vitamin E 3.7-4.8 µg/g, and vitamin D_3 (0.01µg/g) were low. Retinol (63-97%), cholecalciferol (2-20%), and α –tocopherol (6-10 %) were lower than the fortification targets.

The benefits of MNP interventions can be maximized by ensuring closer monitoring of delivery mechanisms. Routine monitoring of the use and nutrient concentrations in the MNPs as well as the fortified complementary foods is needed to increase benefits while reducing potential harms related to consumption.

Keywords: fat soluble vitamins, method validation, HPLC.

INTRODUCTION

Vitamins and mineral deficiencies affect approximately half of the infants and young children worldwide (De-Regil et al., 2013). Recognizing that complementary foods in many low- and middle-income countries (LMIC) are nutrient-poor (Kathryn G Dewey, 2013), home-fortification with multiple micronutrients has been recommended (De-Regil et al., 2013). Consequently, many countries have started programs that distribute micronutrient powders (MNPs), and such interventions are being rapidly scaled-up.

MNPs are composed of different micronutrient with various chemical characteristics are packed into one sachet. For example, iron, vitamin A, E, and C are all sensitive to oxidation. Vitamin A and E, although stable under inert atmospheric conditions, they are less stable when exposed to light and heat (Eitenmiller, Landen Jr, & Ye, 2016). Similarly, vitamin D is unstable in the presence of reducing agents and common oxidizing agents such as peroxides. It is also sensitive to light, and can be degraded during heat treatments like boiling and roasting (Lešková et al., 2006).

In most LMIC, ambient temperatures could be very high, exposure to sunlight is also high, and storage conditions are not always optimal, thus putting at stake the stability of such sensitive vitamins. Besides, the low adherence to guidelines by caregivers (e.g. adding MNP before cooking) and poor distribution channels may compromise the effectiveness of MNP interventions (de Barros & Cardoso, 2016). Indeed, recently unfavorable storage conditions in LMIC were found to affect the stability of vitamins in fortified oil (Hemery et al., 2018). However, whether cereal-based complementary foods fortified with MNPs are susceptible to degradation is unknown. Therefore, the objective of this study was to determine the concentration of vitamin A, D_3 and E in home-fortified complementary foods and compare to fortification targets.

MATERIALS AND METHODS

Knowledge, attitude and practice of caregivers

Information on how caregivers used MNPs, at which time point during cooking did they add MNPs, and their perceived benefits were collected through interviews.

Sample collection and preparation

Volunteer mothers (n=30) were asked to prepare fortified complementary food the way they habitually prepare the food for mixing with MNPs. Complementary foods (porridges) were collected with polyethylene plastic bag that was immediately sealed and covered with aluminium foil. The sample was kept in a pre-cooled ice box and was transported to Addis Ababa University Center for Food Science and Nutrition. The samples were freeze-dried, and were transported to the department of Chemistry, Washburn University, Kansas, USA for vitamin analyses.

Chemicals and reagents

All the solvent (Methanol, Ethanol absolute, Petroleum ether, n-hexane) and other chemicals (Potassium hydroxide, Pyrogallol, Nitrogen gas) were of analytical grade. Millpore Milli-Q water (18.2 Mo) was used in all experiments in this report. Standards: Vitamin A (Retinol), Vitamin E (α-Tocopherol), Vitamin D3 (Cholecalciferol).

Sample extraction procedure

Three composite samples (maize, wheat and mixed cereals) collected from the three *kebeles* were prepared by mixing equal proportion from each. Then a subsample of 20 g from each composite was weighed and transfer to a separate round bottom flask in triplicate. Ethanol (40mL) and 50% KOH (10 mL) and few drops of of pyrogallol (as an antioxidant) were added and mixed well. The mixture was refluxed in an oil bath at 95ºc for 45 minute, with agitations every 10 minutes. Then, the reflux-analyte-sample mixtures were cooled immediately using cold water. Then, 40ml deionized water was added and the mixture was transfered to a separatory funnel. The different layers were extracted according to the modified method described in AOAC (1990).

Retinol (vitamin A) , α-Tocopherol (vitamin E), and Cholecalciferol (Vitamin D_3) standard preparation

Retinol (1 g) standard was dissolved with methanol, and was transferred to a 100 mL volumetric flask. After several rounds of rinsing, the volume was made up to the mark with methanol. The retinol concentration of the standard was 9300 µg/mL. A 1000 µg/ mL stock vitamin E standard solution was prepared by dissolving 100 mg of vitamin E standard with methanol. Cholecalciferol (vitamin D_3) standard was dissolved with ethanol, methanol, and acetone (10 ml each). The mixture was transferred to 100 mL volumetric flask and was filled-up to the volume with ethanol. All standards were stored at 2-8ºC.

Vitamins mixture standard

Vitamins mixture standard was prepared in 10mL volumetric flask with a concentration of 100 µg/ mL, 200 µg/mL and 9 µg/mL of vitamin A, E and D, respectively. Taking 1 mL vitamin A

(1000 µg/mL), 2 mL vitamin E (1000 µg/mL), and 0.6 mL (150 µg/mL) vitamin D_3, the mixture was completed with methanol to 10 mL and was stored at < 20ºC.

Quality controls and instrumental conditions

Analysis was done in triplicate. Matrix spike was prepared by spiking 0.5 mL from vitamins mixture standard. Direct spike was prepared by spiking same amount of vitamins mixture like the matrix spike on a 10ml volumetric flask and diluted with methanol to the mark without following the extraction process.

The HPLC system consisted of a quaternary pump, degasser, a column thermostat and a diode array detector (DAD). A C-18 column (4.6 x 250 mm, 3.5 µm particles, Agilent Pursuit XRS, column temperature of 25ºC) was used. The mobile phase was a mixture of commonly used solvents (98% Methanol/2% water). Detection was conducted with DAD at 325 nm, 292 nm and 266 nm for vitamin A, E and D, respectively. Separation was carried out at a flow rate of 1.00 mL/min. Injection volume was 20µL and total run time for each injection was 11min.

Method validation

The precision and accuracy of the proposed method was evaluated in terms of linearity, limit of detection (LOD) and limit of quantification (LOQ), precision (repeatability and repeatability limit) accuracy (spiked recovery) (Hill & Reynolds, 1999). More detail is provided in the appendix.

RESULTS

Knowledge, attitude, and practice

The knowledge attitude and practice (KAP) assessment of mother/caregiver about home fortification highlighted several knowledge and practice gaps (Table 1). About 13% of mothers/caregivers reported to have added the MNPs during cooking, whereas about a third (28%) mixed MNPs with foods to be consumed for the whole day.

Table 1 MNP home fortification Knowledge, Attitude and Practice (KAP) assessment

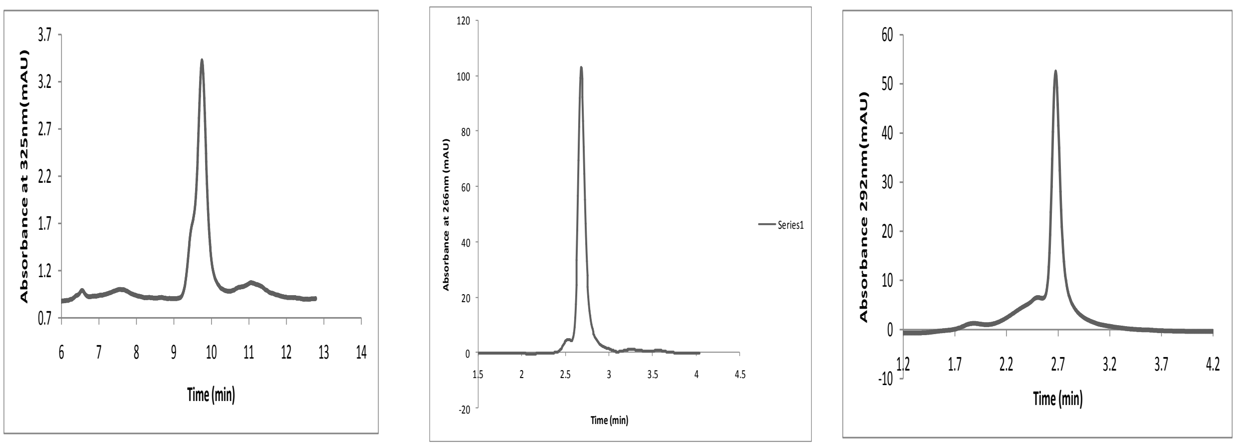
	All N(98)	*%*
Fed the infant MNP fortified Complementary food at least for the last 3mo	98	100
MNP fortified Complementary food benefit their children	98	100
MNP fortifying practice at home;		
Mix the MNP when the food is cooking	13	13.3
Mix the MNP when the food is ready to eat	85	86.7
Estimated amount of food mix with one sachet of MNP;		
With one serving size	73	74.5
With consuming for one day	27	27.6
Change (health, activity and growth) observed on child after using MNP	47	49.0

Method validation

All the parameters that were used for method validation are with in the standard range.That is, the correlation coefficient were >0.997. The values of limit of detection were below the lowest calibration level. Repetability of the method was below 20% and recovery range was with in78.13 to114.29.

Vitamin analyses

A standard solution of each single vitamin was injected separately to determine the individual retention times. The maximum absorbance (highest peak) of the standard vitamin A (λ=325 nm) vitamin E (λ = 292 nm) and vitamin D (λ = 266 nm) appeared at 10.20 min, 2.66 min and 2.67min respectively (**Figure 1**). The identity of the vitamins was confirmed by spiking.



a) b) c)

Figure 1 Representative chromatogram of standard vitamins, a) Vitamin A b) Vitamin D and c) Vitamin E

Vitamin A, D_3 and E concentrations in fortified complemetnary foods

Vitamin concentrations in the three composite samples and the concentration of the vitamins added to the porridge are presented in **table 2**. Retention of vitamin A was much higher than vitamin E and D.

Table 2: Concentrations of vitamin A, D, and E in home-fortified complementary foods relative to fortification targets

Vitamins	MNP µg/100g	Maize-CF µg/100g	Wheat-CF µg/100g	Mixed-CF µg/100g	% retained
Vitamin A	400	386	342	254	63-97 %
Vitamin D	5	1	1	0.1	2-20%
Vitamin E	5000	307	481	458	6-10 %

DISCUSSION

The present study highlighted that a significant proportion of mothers/caregivers used MNPs inappropriately. Besides, the MNP used at the time of the survey was approaching its best-before date. Consequently, the concentrations of vitamins A, D_3, and E in the home-fortified complementary foods were far below fortification targets.

The finding that a significant proportion of caregivers used MNPs inappropriately despite the fact that they reported receiving training is alarming. This suggests that either the training did not contain accurate information, or the information were not well translated to the communities. Similar findings were observed in previous studies for IYCF trainings by HEWs (Abebe et al., 2016). With the increased set of nutritional interventions with varying level of complexity that are relying on the HEWs for their provision, highlight the need to reconsid other delivery platforms or the need for providing clear guidance with job-aids. Otherwise, proven interventions may fail to meet their goal or could even be deleterious.

For example, the addition of one sachet into a food that is to be served for the whole day could lead to oxidation of several of the vitamins. Such an oxidation could be catalyzed by various metals like iron, copper, etc (Hemery et al., 2018) . This can reduce the palatability of the fortified; hence, children may not benefit from the intervention. More importantly, if consumed, such a food containing oxidized minerals and vitamins can lead to cellular damage, futher putting children at risk of poor health(Lobo, Patil, Phatak, & Chandra, 2010; Pisoschi & Pop, 2015). Besides, a significant proportion fortified the complementary food during cooking, which would lead to significant losses of the analysed vitamins (Jakobsen and Knuthsen (2014).

The other reasons for the reduction of the amount of the vitamins in the fortified food might be the storage condition and its exposure to heat and light. The MNP product delivery may take long time from production sit to the end user. Within this period it might be susceptible to different unfavorable environmental conditions. Indeed more recently Hemery et al (2018), has reported that more than 45% of vitamin A is lost within three months environmental conditions like the the mildest, whereas over 85% could be lost within three months in more severe environmental conditions. Most of this loss was reported to be due to oxidation that happens during storage, highlighting the need for more effective packaging.

The cross-sectional nature of the study and the fact that the MNPs were not analyzed are key limitations. Notwithstanding these limitations, the present study highlighted for the first time the need for closer monitoring of nutrients in MNPs and how they are used to maximize benefit and reduce potential harm related to oxidation. Such monitoring could be facilitated by introducing easy and rapid point of use instruments like iCheck, but their use and accuracy should be validated.

Acknowledgements

This research was financially supported by Addis Ababa University. Mr. Geletu is thankful to Washburn University, Department of chemistry for allowing him to work in the labs and use chemical and instruments.

Conflicts of interest

The authors declare that they have no conflict of interest.

Reference

Abebe, Z., Haki, G. D., & Baye, K. (2016). Health extension workers' knowledge and knowledge-sharing effectiveness of optimal infant and young child feeding are associated with mothers' knowledge and child stunting in rural Ethiopia. *Food and nutrition bulletin, 37*(3), 353-363.

AOAC, A. o. O. A. l. C. (1990). Official Methods of Analysis: Washington, DC.

Bliesner, D. M. (2006). *Validating chromatographic methods: a practical guide*: John Wiley & Sons.

De-Regil, L. M., Suchdev, P. S., Vist, G. E., Walleser, S., & Peña-Rosas, J. P. (2013). Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Evidence‐Based Child Health: A Cochrane Review Journal, 8*(1), 112-201.

de Barros, S. F., & Cardoso, M. A. (2016). Adherence to and acceptability of home fortification with vitamins and minerals in children aged 6 to 23 months: A systematic review. *BMC public health, 16*(1), 299.

Dewey, K. G. (2013). The Challenge of Meeting Nutrient Needs of Infants and Young Children during the Period of Complementary Feeding: An Evolutionary Perspective–3. *The Journal of nutrition, 143*(12), 2050-2054.

Eitenmiller, R. R., Landen Jr, W., & Ye, L. (2016). *Vitamin analysis for the health and food sciences*: CRC press.

Gobo, A. B., Kurz, M. H., Pizzutti, I. R., Adaime, M. B., & Zanella, R. (2004). Development and validation of methodology for the determination of residues of organophosphorus pesticides in tomatoes. *Journal of the Brazilian Chemical Society, 15*(6), 945-950.

Hemery, Y. M., Laillou, A., Fontan, L., Jallier, V., Moench-Pfanner, R., Berger, J., & Avallone, S. (2018). Storage conditions and packaging greatly affects the stability of fortified wheat flour: Influence on vitamin A, iron, zinc, and oxidation. *Food Chemistry, 240*, 43-50.

Hill, A. R., & Reynolds, S. (1999). Guidelines for in-house validation of analytical methods for pesticide residues in food and animal feeds†‡. *Analyst*, *124*(6), 953-958.

Jakobsen, J., & Knuthsen, P. (2014). Stability of vitamin D in foodstuffs during cooking. *Food Chemistry*, *148*, 170-175.

Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J., & Holčíková, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and analysis*, *19*(4), 252-276.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, *4*(8), 118.

Magnusson, B. (2014). *The fitness for purpose of analytical methods: a laboratory guide to method validation and related topics (2014)*: Eurachem.

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry*, *97*, 55-74.

Chapter 5

General Discussion

5. General discussion

The present thesis used mixed methods including cohort (retrospective and prospective), observation (cognitive measurements) and laboratory analyses of food samples. The main findings are that exposure to low-iron MNP on alternate days is associated with lower risk of stunting, anemia, and delays in motor development. Many of these observe benefits persisted six-month after the intervention was stopped, and for some parameters the difference between MNP exposed and unexposed children widened with age of the child. Micronutrient intake was found associated with various cognitive domains including language, social, fine and gross motor skills. Nevertheless, the benefits of the intervention could be maximized by making sure that complementary foods are appropriately fortified.

Children less than two-years of age are at a higher risk of malnutrition because of the high nutrient needs to support the rapid growth and development. This is further exacerbated by the small gastric capacity (30 g/kg body weight/meal) that is even not met in many countries (Baye et al., 2018; Kathryn G. Dewey, 2013). Various strategies have been put in place to improve complementary feeding in low and middle income countries (LMIC). These include dietary diversification, biofortification, fortification and supplementation (Berti et al., 2014b). Although dietary diversity is considered the most sustainable interventions, home-fortification is often considered because it is easier and quicker to implement and is considered to be cost-effective (Allen et al., 2009).

Nevertheless, more recent studies are indicating the need to adapt interventions to avoid the risk of excessive intakes (Abebe et al., 2018; Bruins et al., 2016; Paganini & Zimmermann, 2017). This concern arises from the increased risk of diarrhea and gut inflammation, particularly when MNPs are provided to iron-replete children (Lönnerdal, 2017; Paganini & Zimmermann, 2017). The provision of iron to iron-replete infants was also associated with increased stunting and lower long-term cognitive function (Lönnerdal, 2017; Lozoff et al., 2012). These findings pose serious challenge to interventions that aim to prevent anaemia/micronutrient deficiencies and the associated adverse effects. In an effort to increase benefit and minimize adverse effects of MNPs containing iron, the demonstration project used in this study used a low iron (6 mg) dose provided on alternative days. This was informed by consumption and epidemiologic surveys that showed mild to moderate prevalence of anaemia despite a high (excessive) iron intake, even among young children (Abebe et al., 2018; Central Statistical Agency - CSA/Ethiopia & ICF, 2017).

Using a retrospective cohort study used the opportunity of an existing demonstration project we showed that a low-iron (6 mg) MNP when provided every other day for infants in first year of life is associated with increased achievement of motor milestone development and a lower risk of anaemia (***Manuscript I***). MNP exposure in the first year of life is directly and indirectly (through increased hemoglobin concentration) associated with improved motor development outcome. These findings would need to be confirmed through community-based clinical trials, and if found effective can present an alternative for programs in countries like Ethiopia, where both the risk of inadequate and excessive iron exists.

Following the same children in a prospective cohort study (***manuscript II***), six-month post the interruption of the intervention, children in the MNP exposed group had a lower risk of stunting, anemia, and delays in motor development than their unexposed counterparts. More importantly, except for motor milestone achievements, baseline differences in hemoglobin concentration, prevalence of anemia, LAZ, WAZ, and WLZ were significantly widened, suggesting the need to intervene as early as possible to prevent inequalities. These findings underline the importance of the first 1000 days as a window of opportunity and provide further evidence that nutritional deprivation in early life, if left untreated, may have long-lasting effects.

Nesting a case-control study within our cohort, we expanded the repertoire of developemental domains to include language, social and fine motor skills in addition to gross motor, which was assessed using the MDAT tool for the first time in Ethiopia (***manuscript III***). This study confirmed that MNP exposure in early life can have effect on various cognitive domains measured in the second year of life (15-18 months). The MDAT was also found to predict delays in growth, anemia, and correlated well with head circumference, indicating that its use to monitor cognitive outcomes is justified.

Despite such a consistent benefit of the exposure of MNPs, the finding that many of the fat-soluble vitamins (A, D_3 and E) were not retained to meet fortification targets (***manuscript IV***) suggests that impact could be maximized by giving enough attention to delivery mechanisms. Ensuring constant supply, screening for timely intervention, and monitoring the appropriate storage and use of the MNPs are critical. This could be supported by integrating easy and validated cognitive measurement tools (e.g. MDAT) with the routine growth monitoring, anemia screening, and random check of fortified complementary foods with point of use equipment's like iCheck.

Chapter 6

Conclusions &

Perspectives

6. Conclusions and perspective

6.1 Conclusion

In the present study low-iron MNP provision in the first year of life of infant through home fortification of complementary food was evaluated using the opportunity of an existing demonstration project in Aleta Chuko district, Sidam Zone, SNNP Ethiopia. This study disclosed that provision of a low-iron dose MNP on alternate days, in the first year of life, is associated with lower prevalence of anaemia, stunting, and increased motor development achievements retrospectively. However, a community-based clinical trial is needed to establish the effectiveness of this modified regimen.

Post intervention follow-up (after MNP provision was interrupted) showed that some of the benefits were sustained, while difference between MNP exposed and unexposed widened for some nutritional parameters. Intervening early in life, in the first year of age, may be beneficial to prevent the long-term effects of undernutrition.

Integrating simple, but useful monitoring tools like acquisition of motor development milestones (proxy for cognitive development) into the health system, along with anaemia screening and growth monitoring can inform such intervention that aim to improve child nutrition and promote optimal brain development.

This study showed that the MDAT can be a useful tool to predict subtle developmental delays that can be associated with undernutrition. The use of the tool in this and other similar settings in Africa could help monitor early child development and provide an opportunity to intervene timely before endpoints like stunting are reached. However, the ability of the tool to predict early signs of malnutrition should be evaluated using longitudinal studies.

The suboptimal levels of vitamins found in the home-fortified complementary foods suggests the need for closer monitoring of nutrients in MNPs and how they are used to maximize benefit and reduce potential harm. Such monitoring could be facilitated by introducing easy and rapid point of use instruments like iCheck, but their use and accuracy should be validated.

6.2 Perspectives

- Home fortification of MNP knowledge attitude and practice was not satisfactory. Therefore, the possible factors that determine accessibility of nutrition education particularly focusing on home fortification of complementary food with MNP should be studied.

- The risk of excessive/low nutrient intakes through home fortification should be examined further through biochemical marker.

- Longitudinal studies that investigate effect of dietary diversity on development, nutritional status and anemia of infant and young children during their complementary feeding period and beyond are required.

- Which micronutrients predict the specific developmental domain of infant and young children should be studied using biochemical indicator on developmental domain.

- Infant developmental assessment tool which could free from confounding factors (culture, language etc.) such as the IOWA technique should be develop for LMICs.

- Modern extraction method (solid-phase extraction) should be employed in the extraction of fat soluble vitamin from food matrix and simultaneous determination using a single injection should implemented. In addition , for the analysis easy and rapid point of use instruments like iCheck

Reference

Abebe, Z., Haki, G. D., & Baye, K. (2016). Health extension workers' knowledge and knowledge-sharing effectiveness of optimal infant and young child feeding are associated with mothers' knowledge and child stunting in rural Ethiopia. *Food and nutrition bulletin, 37*(3), 353-363.

Abebe, Z., Haki, G. D., & Baye, K. (2018). Simulated effects of home fortification of complementary foods with micronutrient powders on risk of inadequate and excessive intakes in West Gojjam, Ethiopia. *Maternal & child nutrition, 14*(1).

Aboud, F. E., & Yousafzai, A. K. (2015). Global health and development in early childhood. *Annual Review of Psychology, 66*, 433-457.

Aburto, N., Ramirez-Zea, M., Neufeld, L., & Flores-Ayala, R. (2010). The effect of nutritional supplementation on physical activity and exploratory behavior of Mexican infants aged 8–12 months. *European journal of clinical nutrition, 64*(6), 644-651.

Adu-Afarwuah, S., Lartey, A., Brown, K. H., Zlotkin, S., Briend, A., & Dewey, K. G. (2007a). Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on growth and motor development. *The American journal of clinical nutrition, 86*(2), 412-420.

Adu-Afarwuah, S., Lartey, A., Brown, K. H., Zlotkin, S., Briend, A., & Dewey, K. G. (2007b). Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on growth and motor development–. *The American journal of clinical nutrition, 86*(2), 412-420.

Allen, L. H., Peerson, J. M., & Olney, D. K. (2009). Provision of multiple rather than two or fewer micronutrients more effectively improves growth and other outcomes in micronutrient-deficient children and adults. *The Journal of nutrition, 139*(5), 1022-1030.

Andersson, M., Karumbunathan, V., & Zimmermann, M. B. (2012). Global Iodine Status in 2011 and Trends over the Past Decade–3. *The Journal of nutrition, 142*(4), 744-750.

Anjos, T., Altmäe, S., Emmett, P., Tiemeier, H., Closa-Monasterolo, R., Luque, V., . . . Demmelmair, H. (2013). Nutrition and neurodevelopment in children: focus on NUTRIMENTHE project. *European journal of nutrition, 52*(8), 1825-1842.

AOAC, A. o. O. A. l. C. (1990). Official Methods of Analysis: Washington, DC.

Arimond, M., & Ruel, M. T. (2004). Dietary diversity is associated with child nutritional status: evidence from 11 demographic and health surveys. *The Journal of nutrition, 134*(10), 2579-2585.

Ashworth, A., Morris, S. S., Lira, P., & Grantham-McGregor, S. (1998). Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. *European journal of clinical nutrition, 52*(3), 223-227.

Atamna, H., Walter, P. B., & Ames, B. N. (2002). The role of heme and iron-sulfur clusters in mitochondrial biogenesis, maintenance, and decay with age. *Archives of biochemistry and biophysics, 397*(2), 345-353.

Bailey, R. L., West Jr, K. P., & Black, R. E. (2015). The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism, 66*(Suppl. 2), 22-33.

Balarajan, Y., Ramakrishnan, U., Özaltin, E., Shankar, A. H., & Subramanian, S. (2011). Anaemia in low-income and middle-income countries. *The lancet, 378*(9809), 2123-2135.

Barros, F. M. (2007). Normal vs. Malnourished brain scan Retrieved 04/27/2018, from https://www.pinterest.com/pin/237072367857471727/

Baye, K. (2017). The Sustainable Development Goals cannot be achieved without improving maternal and child nutrition. *Journal of public health policy, 38*(1), 137-145.

Baye, K., Guyot, J.-P., Icard-Verniere, C., & Mouquet-Rivier, C. (2013). Nutrient intakes from complementary foods consumed by young children (aged 12–23 months) from North Wollo, northern Ethiopia: the need for agro-ecologically adapted interventions. *Public health nutrition, 16*(10), 1741-1750.

Baye, K., Tariku, A., & Mouquet-Rivier, C. (2018). Caregiver–infant's feeding behaviours are associated with energy intake of 9-11 month-old infants in rural Ethiopia. *Maternal & child nutrition, 14*(1).

Benton, D. (2001). Micro-nutrient supplementation and the intelligence of children. *Neuroscience & Biobehavioral Reviews, 25*(4), 297-309.

Benton, D. (2012). Vitamins and neural and cognitive developmental outcomes in children. *Proceedings of the nutrition society, 71*(1), 14-26.

Berti, C., Faber, M., & Smuts, C. (2014a). Prevention and control of micronutrient deficiencies in developing countries: current perspectives. *Nutrition and Dietary Supplements, 6*, 41-57.

Berti, C., Faber, M., & Smuts, C. M. (2014b). Prevention and control of micronutrient deficiencies in developing countries: Current perspectives. *Nutrition and Dietary Supplements, 6*, 41-57.

Bhandari, S., & Banjara, M. R. (2015). Micronutrients deficiency, a hidden hunger in Nepal: prevalence, causes, consequences, and solutions. *International Scholarly Research Notices, 2015*.

Bhatnagar, S., & Taneja, S. (2001). Zinc and cognitive development. *British Journal of Nutrition, 85*(S2), S139-S145.

Bhutta, Z. A., Das, J. K., Rizvi, A., Gaffey, M. F., Walker, N., Horton, S., . . . Black, R. E. (2013). Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? *The lancet, 382*(9890), 452-477.

Black, M. M. (2003). Micronutrient deficiencies and cognitive functioning. *The Journal of nutrition, 133*(11), 3927S-3931S.

Black, M. M. (2008a). Effects of vitamin B12 and folate deficiency on brain development in children. *Food and nutrition bulletin, 29*(2 Suppl), S126.

Black, M. M. (2008b). Effects of vitamin B12 and folate deficiency on brain development in children. *Food and nutrition bulletin, 29*(2_suppl1), S126-S131.

Black, M. M., Baqui, A. H., Zaman, K., Persson, L. A., El Arifeen, S., Le, K., . . . Black, R. E. (2004). Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. *The American journal of clinical nutrition, 80*(4), 903-910.

Black, M. M., & Hurley, K. M. (2016). Early child development programmes: further evidence for action. *The Lancet Global Health, 4*(8), e505-e506.

Black, M. M., Sazawal, S., Black, R. E., Khosla, S., Kumar, J., & Menon, V. (2004). Cognitive and motor development among small-for-gestational-age infants: impact of zinc supplementation, birth weight, and caregiving practices. *Pediatrics, 113*(5), 1297-1305.

Black, R. (2003). Micronutrient deficiency: an underlying cause of morbidity and mortality: SciELO Public Health.

Black, R. E. (2014). Global distribution and disease burden related to micronutrient deficiencies *International Nutrition: Achieving Millennium Goals and Beyond* (Vol. 78, pp. 21-28): Karger Publishers

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., De Onis, M., Ezzati, M., . . . Group, C. U. S. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *The lancet, 371*(9608), 243-260.

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., De Onis, M., . . . Martorell, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet, 382*(9890), 427-451.

Bliesner, D. M. (2006). *Validating chromatographic methods: a practical guide*: John Wiley & Sons.

Bogale, A., Stoecker, B. J., Kennedy, T., Hubbs-Tait, L., Thomas, D., Abebe, Y., & Hambidge, K. M. (2013). Nutritional status and cognitive performance of mother–child pairs in Sidama, Southern Ethiopia. *Maternal & child nutrition, 9*(2), 274-284.

Brown, K. H., Rivera, J. A., Bhutta, Z., Gibson, R. S., King, J. C., Lönnerdal, B., . . . Hotz, C. (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document# 1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and nutrition bulletin, 25*(1 Suppl 2).

Bruins, M. J., Kupka, R., Zimmermann, M. B., Lietz, G., Engle-Stone, R., & Kraemer, K. (2016). Maximizing the benefits and minimizing the risks of intervention programs to address micronutrient malnutrition: Symposium report. *Maternal & child nutrition, 12*(4), 940-948.

Castillo-Durán, C., Perales, C. G., Hertrampf, E. D., Marín, V. B., Rivera, F. A., & Icaza, G. (2001). Effect of zinc supplementation on development and growth of Chilean infants. *The Journal of pediatrics, 138*(2), 229-235.

Caulfield, L. E., Richard, S. A., & Black, R. E. (2004). Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old. *The American journal of tropical medicine and hygiene, 71*(2_suppl), 55-63.

Caulfield, L. E., Richard, S. A., Rivera, J. A., Musgrove, P., & Black, R. E. (2006). Stunting, wasting, and micronutrient deficiency disorders.

Ceglia, L., & Harris, S. S. (2013). Vitamin D and its role in skeletal muscle. *Calcified tissue international, 92*(2), 151-162.

Central Statistical Agency - CSA/Ethiopia, & ICF. (2017). Ethiopia Demographic and Health Sruvey 2016. Addis Ababa, Ethiopia: CSA and ICF.

Christian, P., Kim, J., Mehra, S., Shaikh, S., Ali, H., Shamim, A. A., . . . West Jr, K. P. (2016). Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: the JiVitA-3 Trial, 2. *The American journal of clinical nutrition, 104*(4), 1175-1182.

Christian, P., Kim, J., Mehra, S., Shaikh, S., Ali, H., Shamim, A. A., . . . West, K. P. (2016). Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: the JiVitA-3 Trial. *The American journal of clinical nutrition, 104*(4), 1175-1182.

Colombo, J., Zavaleta, N., Kannass, K. N., Lazarte, F., Albornoz, C., Kapa, L. L., & Caulfield, L. E. (2014a). Zinc supplementation sustained normative neurodevelopment in a randomized, controlled trial of peruvian infants aged 6–18 months. *The Journal of nutrition*, *144*(8), 1298-1305.

Colombo, J., Zavaleta, N., Kannass, K. N., Lazarte, F., Albornoz, C., Kapa, L. L., & Caulfield, L. E. (2014b). Zinc Supplementation Sustained Normative Neurodevelopment in a Randomized, Controlled Trial of Peruvian Infants Aged 6–18 Months, 2. *The Journal of nutrition, 144*(8), 1298-1305.

CSA. (2016). Ethiopia Demographic and Health Survey 2016: Key Indicators Report. *Addis Ababa, Ethiopia, and Rockville, Maryland, USA. CSA and ICF.*

Cusick, S. E., & Georgieff, M. K. (2012). Nutrient supplementation and neurodevelopment: timing is the key. *Archives of pediatrics & adolescent medicine, 166*(5), 481-482.

Cusick, S. E., & Georgieff, M. K. (2016). The role of nutrition in brain development: the golden opportunity of the "first 1000 days". *The Journal of pediatrics*, *175*, 16-21.

De-Regil, L. M., Suchdev, P. S., Vist, G. E., Walleser, S., & Peña-Rosas, J. P. (2013). Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Evidence-Based Child Health: A Cochrane Review Journal*, *8*(1), 112-201.

de Barros, S. F., & Cardoso, M. A. (2016). Adherence to and acceptability of home fortification with vitamins and minerals in children aged 6 to 23 months: A systematic review. *BMC public health*, *16*(1), 299.

de Benoist, B. (2008). Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. *Food and nutrition bulletin, 29*(2_suppl1), S238-S244.

De Escobar, G. M., Obregón, M. J., & Del Rey, F. E. (2004). Role of thyroid hormone during early brain development. *European Journal of Endocrinology*, *151*(Suppl 3), U25-U37.

Delange, F. (2000). The role of iodine in brain development. *Proceedings of the nutrition society, 59*(1), 75-79.

Dewey, K. (2002). Guiding principles for complementary feeding of the breastfed child.

Dewey, K. G. (2013). The Challenge of Meeting Nutrient Needs of Infants and Young Children during the Period of Complementary Feeding: An Evolutionary Perspective–3. *The Journal of nutrition, 143*(12), 2050-2054.

Dewey, K. G. (2013). The Challenge of Meeting Nutrient Needs of Infants and Young Children during the Period of Complementary Feeding: An Evolutionary Perspective. *The Journal of nutrition, 143,* 2050–2054.

Dewey, K. G. (2016). Reducing stunting by improving maternal, infant and young child nutrition in regions such as South Asia: evidence, challenges and opportunities. *Maternal & child nutrition, 12*(S1), 27-38.

Dewey, K. G., & Adu-Afarwuah, S. (2008). Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. *Maternal & child nutrition, 4*(s1), 24-85.

Dewey, K. G., & Begum, K. (2011). Long-term consequences of stunting in early life. *Maternal & child nutrition, 7*(s3), 5-18.

Dewey, K. G., & Vitta, B. S. (2013). Strategies for ensuring adequate nutrient intake for infants and young children during the period of complementary feeding. *Washington: Alive & Thrive, 7*.

DIOUF, J., & SHEERAN, J. (2010). The State of Food Insecurity in the World: Addressing food insecurity in protracted crises. *Rome: Food and Agriculture Oranization of the United Nations (FAO) and World Food Programme (WFP)*.

Dong, J., Yin, H., Liu, W., Wang, P., Jiang, Y., & Chen, J. (2005). Congenital iodine deficiency and hypothyroidism impair LTP and decrease C-fos and C-jun expression in rat hippocampus. *Neurotoxicology, 26*(3), 417-426.

Dora, C., Haines, A., Balbus, J., Fletcher, E., Adair-Rohani, H., Alabaster, G., . . . Neira, M. (2015). Indicators linking health and sustainability in the post-2015 development agenda. *The lancet, 385*(9965), 380-391.

Dror, D. K., & Allen, L. H. (2011). Vitamin E deficiency in developing countries. *Food and nutrition bulletin, 32*(2), 124-143.

Egger, M., Smith, G. D., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *Bmj, 315*(7109), 629-634.

Eilander, A., Gera, T., Sachdev, H. S., Transler, C., van der Knaap, H. C., Kok, F. J., & Osendarp, S. J. (2009). Multiple micronutrient supplementation for improving cognitive performance in children: systematic review of randomized controlled trials–. *The American journal of clinical nutrition, 91*(1), 115-130.

Eitenmiller, R. R., Landen Jr, W., & Ye, L. (2016). *Vitamin analysis for the health and food sciences*: CRC press.

EpiTools epidemiological calculators.

Eyles, D., Brown, J., Mackay-Sim, A., McGrath, J., & Feron, F. (2003). Vitamin D 3 and brain development. *Neuroscience, 118*(3), 641-653.

Faber, M. (2005). Complementary foods consumed by 6–12-month-old rural infants in South Africa are inadequate in micronutrients. *Public health nutrition, 8*(4), 373-381.

Faber, M., Kvalsvig, J. D., Lombard, C. J., & Benadé, A. S. (2005a). Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. *The American journal of clinical nutrition, 82*(5), 1032-1039.

Faber, M., Kvalsvig, J. D., Lombard, C. J., & Benadé, A. S. (2005b). Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants–. *The American journal of clinical nutrition, 82*(5), 1032-1039.

Fekete, K., Berti, C., Trovato, M., Lohner, S., Dullemeijer, C., Souverein, O. W., . . . Decsi, T. (2012). Effect of folate intake on health outcomes in pregnancy: a systematic review and meta-analysis on birth weight, placental weight and length of gestation. *Nutr J, 11*(1), 75.

Gashu, D., Stoecker, B. J., Bougma, K., Adish, A., Haki, G. D., & Marquis, G. S. (2015). Stunting, selenium deficiency and anemia are associated with poor cognitive performance in preschool children from rural Ethiopia. *Nutrition journal, 15*(1), 38.

Gashu, D., Stoecker, B. J., Bougma, K., Adish, A., Haki, G. D., & Marquis, G. S. (2016). Stunting, selenium deficiency and anemia are associated with poor cognitive performance in preschool children from rural Ethiopia. *Nutrition journal, 15*(1), 38.

Gathwala, G., Yadav, S., & Lal, G. (2013). Vitamin E Level in Normal Indian Children.

Georgieff, M. K. (2007a). Nutrition and the developing brain: nutrient priorities and measurement. *The American journal of clinical nutrition, 85*(2), 614S-620S.

Georgieff, M. K. (2007b). Nutrition and the developing brain: nutrient priorities and measurement–. *The American journal of clinical nutrition, 85*(2), 614S-620S.

Georgieff, M. K. (2011). Long-term brain and behavioral consequences of early iron deficiency. *Nutrition reviews, 69*(s1).

Gibson, R., Ferguson, E., & Lehrfeld, J. (1998). Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. *European journal of clinical nutrition, 52*(10), 764.

Gibson, R. S., Abebe, Y., Hambidge, K. M., Arbide, I., Teshome, A., & Stoecker, B. J. (2009). Inadequate feeding practices and impaired growth among children from subsistence farming households in Sidama, Southern Ethiopia. *Maternal & child nutrition, 5*(3), 260-275.

Gibson, R. S., & Hotz, C. (2001). Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *British Journal of Nutrition, 85*(S2), S159-S166.

Gimeno, E., Castellote, A., Lamuela-Raventos, R., De La Torre, M., & Lopez-Sabater, M. (2000). Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography. *Journal of chromatography A, 881*(1-2), 251-254.

Gladstone, M., Lancaster, G. A., Umar, E., Nyirenda, M., Kayira, E., van den Broek, N. R., & Smyth, R. L. (2010). The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. *PLoS medicine, 7*(5), e1000273.

Gobo, A. B., Kurz, M. H., Pizzutti, I. R., Adaime, M. B., & Zanella, R. (2004). Development and validation of methodology for the determination of residues of organophosphorus pesticides in tomatoes. *Journal of the Brazilian Chemical Society, 15*(6), 945-950.

Gómez-Galera, S., Rojas, E., Sudhakar, D., Zhu, C., Pelacho, A. M., Capell, T., & Christou, P. (2010). Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic research, 19*(2), 165-180.

Grantham-McGregor, S., & Ani, C. (2001). A review of studies on the effect of iron deficiency on cognitive development in children. *The Journal of nutrition, 131*(2), 649S-668S.

Grantham-McGregor, S., Cheung, Y., Cueto, S., Glewwe, P., Richter, L., & Strupp, B. (2007). Child development in developing countries. 1. Developmental potential inthe first five years for children in devloping countries. *Lancet, 69*, 6-7.

Grantham-McGregor, S., Cheung, Y. B., Cueto, S., Glewwe, P., Richter, L., Strupp, B., & Group, I. C. D. S. (2007). Developmental potential in the first 5 years for children in developing countries. *The lancet, 369*(9555), 60-70.

Haddad, L., Hawkes, C., Udomkesmalee, E., Achadi, E., Ag Bendech, M., & Ahuja, A. (2016). Global Nutrition Report 2016: From Promise to Impact: Ending Malnutrition by 2030. *Washington, DC.: International Food Policy Research Institute*.

Haile, D., Belachew, T., Berhanu, G., Setegn, T., & Biadgilign, S. (2015). Complementary feeding practices and associated factors among HIV positive mothers in Southern Ethiopia. *Journal of Health, Population and Nutrition, 34*(1), 5.

Hamadani, J. D., Fuchs, G. J., Osendarp, S. J., Khatun, F., Huda, S. N., & Grantham-McGregor, S. M. (2001). Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. *The American journal of clinical nutrition, 74*(3), 381-386.

Haug, A., Graham, R. D., Christophersen, O. A., & Lyons, G. H. (2007). How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. *Microbial ecology in health and disease, 19*(4), 209-228.
Hemery, Y. M., Laillou, A., Fontan, L., Jallier, V., Moench-Pfanner, R., Berger, J., & Avallone, S. (2018). Storage conditions and packaging greatly affects the stability of fortified wheat flour: Influence on vitamin A, iron, zinc, and oxidation. *Food Chemistry, 240*, 43-50.
Henry, C. J., Whiting, S. J., & Regassa, N. (2015). Complementary feeding practices among infant and young children in Southern Ethiopia: Review of the findings from a Canada-Ethiopia project. *Journal of Agricultural Science, 7*(10), 29.
Hill, A. R., & Reynolds, S. (1999). Guidelines for in-house validation of analytical methods for pesticide residues in food and animal feeds†‡. *Analyst, 124*(6), 953-958.
Hirvonen, K., & Hoddinott, J. (2017). Agricultural production and children's diets: Evidence from rural Ethiopia. *Agricultural Economics, 48*(4), 469-480.
Horton, S., Alderman, H., & Rivera, J. A. (2008). Hunger and malnutrition [Copenhagen Consensus 2008 challenge paper]: Copenhagen: Copenhagen Consensus Center.
Hotz, C., & McClafferty, B. (2007). From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food and nutrition bulletin, 28*(2_suppl2), S271-S279.
Hulthén, L. (2003). Iron deficiency and cognition. *Scandinavian Journal of Nutrition, 47*(3), 152-156.
Humphrey, J. H., & Prendergast, A. J. (2017). Population-level linear growth faltering in low-income and middle-income countries. *The Lancet Global Health, 5*(12), e1168-e1169.
Iannotti, L. L., Tielsch, J. M., Black, M. M., & Black, R. E. (2006). Iron supplementation in early childhood: health benefits and risks–. *The American journal of clinical nutrition, 84*(6), 1261-1276.
Indyk, H., & Woollard, D. (1984). The determination of vitamin D by high performance liquid chromatography. *New Zealand Journal of Dairy Science and Technology (New Zealand)*.
Institute, E. P. H. (2013). Ethiopia National Food Consumption Survey Addis Ababa, Ethiopia.
Jakobsen, J., & Knuthsen, P. (2014). Stability of vitamin D in foodstuffs during cooking. *Food Chemistry, 148*, 170-175.
Jedrychowski, W., Perera, F., Jankowski, J., Butscher, M., Mroz, E., Flak, E., . . . Sowa, A. (2012). Effect of exclusive breastfeeding on the development of children’s cognitive function in the Krakow prospective birth cohort study. *European journal of pediatrics, 171*(1), 151-158.
Jefferds, M. E. D., Mirkovic, K. R., Subedi, G. R., Mebrahtu, S., Dahal, P., & Perrine, C. G. (2015). Predictors of micronutrient powder sachet coverage in Nepal. *Maternal & child nutrition, 11*(S4), 77-89.
Jones, A. D., Ickes, S. B., Smith, L. E., Mbuya, M. N., Chasekwa, B., Heidkamp, R. A., . . . Stoltzfus, R. J. (2014). World Health Organization infant and young child feeding indicators and their associations with child anthropometry: a synthesis of recent findings. *Maternal & child nutrition, 10*(1), 1-17.
Jones, G., Steketee, R. W., Black, R. E., Bhutta, Z. A., Morris, S. S., & Group, B. C. S. S. (2003). How many child deaths can we prevent this year? *The lancet, 362*(9377), 65-71.
Kennedy, G., Ballard, T., & Dop, M. C. (2011). *Guidelines for measuring household and individual dietary diversity*: Food and Agriculture Organization of the United Nations.

Keunen, K., van Elburg, R. M., van Bel, F., & Benders, M. J. (2014). Impact of nutrition on brain development and its neuroprotective implications following preterm birth. *Pediatric research, 77*(1-2), 148-155.

Khor, G. L., & Misra, S. (2012). Micronutrient interventions on cognitive performance of children aged 5-15 years in developing countries. *Asia Pacific journal of clinical nutrition, 21*(4), 476-486.

Krasevec, J., An, X., Kumapley, R., Bégin, F., & Frongillo, E. A. (2017). Diet quality and risk of stunting among infants and young children in low-and middle-income countries. *Maternal & child nutrition, 13*(S2).

Krebs, N. F., Lozoff, B., & Georgieff, M. K. (2017). Neurodevelopment: the impact of nutrition and inflammation during infancy in low-resource settings. *Pediatrics, 139*(Supplement 1), S50-S58.

Krebs, N. F., Miller, L. V., & Michael Hambidge, K. (2014). Zinc deficiency in infants and children: a review of its complex and synergistic interactions. *Paediatrics and international child health, 34*(4), 279-288.

Lanzkowsky, P. (2016). Iron-deficiency anemia *Lanzkowsky's Manual of Pediatric Hematology and Oncology (Sixth Edition)* (pp. 69-83): Elsevier

Larson, L. M., & Yousafzai, A. K. (2017). A meta-analysis of nutrition interventions on mental development of children under-two in low-and middle-income countries. *Maternal & child nutrition, 13*(1).

Leroy, J. L., Ruel, M., Habicht, J.-P., & Frongillo, E. A. (2014). Linear Growth Deficit Continues to Accumulate beyond the First 1000 Days in Low-and Middle-Income Countries: Global Evidence from 51 National Surveys, 2. *The Journal of nutrition, 144*(9), 1460-1466.

Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J., & Holčíková, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and analysis, 19*(4), 252-276.

Levitt, P. (2003). Structural and functional maturation of the developing primate brain. *The Journal of pediatrics, 143*(4), 35-45.

Li, Q., Yan, H., Zeng, L., Cheng, Y., Liang, W., Dang, S., . . . Tsuji, I. (2009). Effects of maternal multimicronutrient supplementation on the mental development of infants in rural western China: follow-up evaluation of a double-blind, randomized, controlled trial. *Pediatrics, 123*(4), e685-e692.

Lind, T., Lönnerdal, B., Stenlund, H., Gamayanti, I. L., Ismail, D., Seswandhana, R., & Persson, L.-Å. (2004). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *The American journal of clinical nutrition, 80*(3), 729-736.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews, 4*(8), 118.

Locks, L. M., Manji, K. P., McDonald, C. M., Kupka, R., Kisenge, R., Aboud, S., . . . Duggan, C. P. (2016). The effect of daily zinc and/or multivitamin supplements on early childhood development in Tanzania: results from a randomized controlled trial. *Maternal & child nutrition*.

Locks, L. M., Manji, K. P., McDonald, C. M., Kupka, R., Kisenge, R., Aboud, S., . . . Duggan, C. P. (2017). The effect of daily zinc and/or multivitamin supplements on early childhood

development in Tanzania: results from a randomized controlled trial. *Maternal & child nutrition, 13*(2).
Lönnerdal, B. (2017). Excess iron intake as a factor in growth, infections, and development of infants and young children. *The American journal of clinical nutrition, 106*(Supplement 6), 1681S-1687S.
Lozoff, B., Beard, J., Connor, J., Felt, B., Georgieff, M., & Schallert, T. (2006). Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutrition reviews, 64*(suppl_2), S34-S43.
Lozoff, B., Castillo, M., Clark, K. M., & Smith, J. B. (2012). Iron-fortified vs low-iron infant formula: developmental outcome at 10 years. *Archives of pediatrics & adolescent medicine, 166*(3), 208-215.
Lozoff, B., & Georgieff, M. K. (2006). *Iron deficiency and brain development.* Paper presented at the Seminars in pediatric neurology.
Magnusson, B. (2014). *The fitness for purpose of analytical methods: a laboratory guide to method validation and related topics (2014)*: Eurachem.
Manno, D., Kowa, P. K., Bwalya, H. K., Siame, J., Grantham-McGregor, S., Baisley, K., . . . Filteau, S. (2012). Rich micronutrient fortification of locally produced infant food does not improve mental and motor development of Zambian infants: a randomised controlled trial. *British Journal of Nutrition, 107*(4), 556-566.
Marriott, B. P., White, A., Hadden, L., Davies, J. C., & Wallingford, J. C. (2012). World Health Organization (WHO) infant and young child feeding indicators: associations with growth measures in 14 low-income countries. *Maternal & child nutrition, 8*(3), 354-370.
Marrus, N., Eggebrecht, A. T., Todorov, A., Elison, J. T., Wolff, J. J., Cole, L., . . . Swanson, M. R. (2017). Walking, Gross Motor Development, and Brain Functional Connectivity in Infants and Toddlers. *Cerebral Cortex, 28*(2), 750-763.
Martínez, A., Knappskog, P. M., & Haavik, J. (2001). A structural approach into human tryptophan hydroxylase and its implications for the regulation of serotonin biosynthesis. *Current medicinal chemistry, 8*(9), 1077-1091.
Matias, S. L., Mridha, M. K., Tofail, F., Arnold, C. D., Khan, M. S. A., Siddiqui, Z., . . . Dewey, K. G. (2017). Home fortification during the first 1000 d improves child development in Bangladesh: a cluster-randomized effectiveness trial–3. *The American journal of clinical nutrition, 105*(4), 958-969.
Mayén, A.-L., Marques-Vidal, P., Paccaud, F., Bovet, P., & Stringhini, S. (2014). Socioeconomic determinants of dietary patterns in low-and middle-income countries: a systematic review–. *The American journal of clinical nutrition, 100*(6), 1520-1531.
Mayer, J. E., Pfeiffer, W. H., & Beyer, P. (2008). Biofortified crops to alleviate micronutrient malnutrition. *Current opinion in plant biology, 11*(2), 166-170.
McCann, J. C., & Ames, B. N. (2007). An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function–. *The American journal of clinical nutrition, 85*(4), 931-945.
McDonald, C. M., Olofin, I., Flaxman, S., Fawzi, W. W., Spiegelman, D., Caulfield, L. E., . . . Study, N. I. M. (2013). The effect of multiple anthropometric deficits on child mortality: meta-analysis of individual data in 10 prospective studies from developing countries–. *The American journal of clinical nutrition, 97*(4), 896-901.

McEvoy, C. T., Temple, N., & Woodside, J. V. (2012). Vegetarian diets, low-meat diets and health: a review. *Public health nutrition, 15*(12), 2287-2294.

McGuire, S. (2015). FAO, IFAD, and WFP. The state of food insecurity in the World 2015: Meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO, 2015: Oxford University Press.

Melse-Boonstra, A., & Jaiswal, N. (2010). Iodine deficiency in pregnancy, infancy and childhood and its consequences for brain development. *Best Practice & Research Clinical Endocrinology & Metabolism, 24*(1), 29-38.

Mengistu, G., Moges, T., Samuel, A., & Baye, K. (2017). Energy and nutrient intake of infants and young children in pastoralist communities of Ethiopia. *Nutrition, 41*, 1-6.

Menon, P. (2012). The crisis of poor complementary feeding in South Asia: where next? *Maternal & child nutrition, 8*(s1), 1-4.

Merson, M. H., Black, R. E., & Mills, A. J. (2012). *Global health*: Jones & Bartlett Publishers.

Mitchodigni, I. M., Hounkpatin, W. A., Ntandou-Bouzitou, G., Avohou, H., Termote, C., Kennedy, G., & Hounhouigan, D. J. (2017). Complementary feeding practices: determinants of dietary diversity and meal frequency among children aged 6–23 months in Southern Benin. *Food Security, 9*(5), 1117-1130.

Morse, N. L. (2012). Benefits of docosahexaenoic acid, folic acid, vitamin D and iodine on foetal and infant brain development and function following maternal supplementation during pregnancy and lactation. *Nutrients, 4*(7), 799-840.

Müller, O., & Krawinkel, M. (2005). Malnutrition and health in developing countries. *Canadian Medical Association Journal, 173*(3), 279-286.

Murray-Kolb, L. E., Khatry, S. K., Katz, J., Schaefer, B. A., Cole, P. M., LeClerq, S. C., . . . Christian, P. (2012). Preschool micronutrient supplementation effects on intellectual and motor function in school-aged Nepalese children. *Archives of pediatrics & adolescent medicine, 166*(5), 404-410.

Nagatsu, T. (1995). Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays in biochemistry, 30*, 15-35.

Nahar, B., Hossain, M., Hamadani, J., Ahmed, T., Huda, S., Grantham-McGregor, S., & Persson, L. (2012). Effects of a community-based approach of food and psychosocial stimulation on growth and development of severely malnourished children in Bangladesh: a randomised trial. *European journal of clinical nutrition, 66*(6), 701.

Nestel, P., Bouis, H. E., Meenakshi, J., & Pfeiffer, W. (2006). Biofortification of staple food crops. *The Journal of nutrition, 136*(4), 1064-1067.

Olson, C. R., & Mello, C. V. (2010). Significance of vitamin A to brain function, behavior and learning. *Molecular nutrition & food research, 54*(4), 489-495.

Onyango, A. W. (2003). Dietary diversity, child nutrition and health in contemporary African communities. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 136*(1), 61-69.

Onyango, A. W., Borghi, E., de Onis, M., del Carmen Casanovas, M., & Garza, C. (2014). Complementary feeding and attained linear growth among 6–23-month-old children. *Public health nutrition, 17*(9), 1975-1983.

Ortiz-Monasterio, J. I., Palacios-Rojas, N., Meng, E., Pixley, K., Trethowan, R., & Pena, R. (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science, 46*(3), 293-307.

Osredkar, J., & Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *J. Clinic. Toxicol. S, 3*, 2161-0495.

Oudgenoeg-Paz, O., Leseman, P. P., & Volman, M. (2015). Exploration as a mediator of the relation between the attainment of motor milestones and the development of spatial cognition and spatial language. *Developmental psychology, 51*(9), 1241.

Paganini, D., & Zimmermann, M. B. (2017). The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: a review. *The American journal of clinical nutrition, 106*(Supplement 6), 1688S-1693S.

Palacios, C., & Gonzalez, L. (2014). Is vitamin D deficiency a major global public health problem? *The Journal of steroid biochemistry and molecular biology, 144*, 138-145.

Pasricha, S.-R., Hayes, E., Kalumba, K., & Biggs, B.-A. (2013). Effect of daily iron supplementation on health in children aged 4–23 months: a systematic review and meta-analysis of randomised controlled trials. *The Lancet Global Health, 1*(2), e77-e86.

Pathak, P., Kapil, U., Dwivedi, S. N., & Singh, R. (2008). Serum zinc levels amongst pregnant women in a rural block of Haryana state, India. *Asia Pacific journal of clinical nutrition, 17*(2), 276-279.

Penny, M. E., Creed-Kanashiro, H. M., Robert, R. C., Narro, M. R., Caulfield, L. E., & Black, R. E. (2005). Effectiveness of an educational intervention delivered through the health services to improve nutrition in young children: a cluster-randomised controlled trial. *The lancet, 365*(9474), 1863-1872.

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry, 97*, 55-74.

Prado, E. L., Abbeddou, S., Adu-Afarwuah, S., Arimond, M., Ashorn, P., Ashorn, U., . . . Maleta, K. (2016). Linear growth and child development in Burkina Faso, Ghana, and Malawi. *Pediatrics, 138*(2), e20154698.

Prado, E. L., Abbeddou, S., Jimenez, E. Y., Somé, J. W., Ouédraogo, Z. P., Vosti, S. A., . . . Ouédraogo, J.-B. (2016). Lipid-Based Nutrient Supplements Plus Malaria and Diarrhea Treatment Increase Infant Development Scores in a Cluster-Randomized Trial in Burkina Faso. *The Journal of nutrition, 146*(4), 814-822.

Prado, E. L., Abbeddou, S., Yakes Jimenez, E., Somé, J. W., Ouédraogo, Z. P., Vosti, S. A., . . . Ouédraogo, J.-B. (2015). Lipid-Based Nutrient Supplements Plus Malaria and Diarrhea Treatment Increase Infant Development Scores in a Cluster-Randomized Trial in Burkina Faso–3. *The Journal of nutrition, 146*(4), 814-822.

Prado, E. L., & Dewey, K. G. (2014). Nutrition and brain development in early life. *Nutrition reviews, 72*(4), 267-284.

Prado, E. L., Maleta, K., Ashorn, P., Ashorn, U., Vosti, S. A., Sadalaki, J., & Dewey, K. G. (2016a). Effects of maternal and child lipid-based nutrient supplements on infant development: a randomized trial in Malawi. *The American journal of clinical nutrition, 103*(3), 784-793.

Prado, E. L., Maleta, K., Ashorn, P., Ashorn, U., Vosti, S. A., Sadalaki, J., & Dewey, K. G. (2016b). Effects of maternal and child lipid-based nutrient supplements on infant development: a randomized trial in Malawi, 2. *The American journal of clinical nutrition, 103*(3), 784-793.

Ramakrishnan, U. (2002a). Prevalence of micronutrient malnutrition worldwide. *Nutrition reviews, 60*(5), S46.

Ramakrishnan, U. (2002b). Prevalence of micronutrient malnutrition worldwide. *Nutrition reviews, 60*(suppl_5), S46-S52.

Ramakrishnan, U., Goldenberg, T., & Allen, L. (2011). Do Multiple Micronutrient Interventions Improve Child Health. *Growth, and Development*, 2066-2075.

Regassa, N. (2014). Infant and child feeding practices among farming communities in Southern Ethiopia. *Kontakt, 16*(4), e215-e222.

Reynolds, E. (2006). Vitamin B12, folic acid, and the nervous system. *The lancet neurology, 5*(11), 949-960.

Richard, S. A., McCormick, B. J., Seidman, J. C., Rasmussen, Z., Kosek, M. N., Rogawski, E. T., . . . Maciel, B. L. (2018). Relationships among Common Illness Symptoms and the Protective Effect of Breastfeeding in Early Childhood in MAL-ED: An Eight-Country Cohort Study.

Rosado, J. L., López, P., García, O. P., Alatorre, J., & Alvarado, C. (2011). Effectiveness of the nutritional supplement used in the Mexican Oportunidades programme on growth, anaemia, morbidity and cognitive development in children aged 12–24 months. *Public health nutrition, 14*(05), 931-937.

Ruffin, N. J. (2009). Understanding growth and development Patterns of infants.

Sabanathan, S., Wills, B., & Gladstone, M. (2015). Child development assessment tools in low-income and middle-income countries: how can we use them more appropriately? *Archives of disease in childhood*, archdischild-2014-308114.

Salam, R. A., Das, J. K., & Bhutta, Z. A. (2014). Impact of intrauterine growth restriction on long-term health. *Current Opinion in Clinical Nutrition & Metabolic Care, 17*(3), 249-254.

Salgueiro, M. a. J., Zubillaga, M. B., Lysionek, A. E., Caro, R. A., Weill, R., & Boccio, J. R. (2002). The role of zinc in the growth and development of children. *Nutrition, 18*(6), 510-519.

Saloojee, H., De Maayer, T., Garenne, M. L., & Kahn, K. (2007). What's new? Investigating risk factors for severe childhood malnutrition in a high HIV prevalence South African setting1. *Scandinavian Journal of Public Health, 35*(69_suppl), 96-106.

Schwarzenberg, S. J., & Georgieff, M. K. (2018). Advocacy for Improving Nutrition in the First 1000 Days To Support Childhood Development and Adult Health. *Pediatrics*, e20173716.

Singla, D. R., Shafique, S., Zlotkin, S. H., & Aboud, F. E. (2014). A 22-element micronutrient powder benefits language but not cognition in Bangladeshi full-term low-birth-weight children. *The Journal of nutrition, 144*(11), 1803-1810.

Smith, L. C., & Haddad, L. (2015). Reducing child undernutrition: past drivers and priorities for the post-MDG era. *World Development, 68*, 180-204.

Spann, M. N., Smerling, J., Gustafsson, H., Foss, S., Altemus, M., & Monk, C. (2015). Deficient maternal zinc intake—but not folate—is associated with lower fetal heart rate variability. *Early human development, 91*(3), 169-172.

Sripaipan, T., Schroeder, D. G., Marsh, D. R., Pachón, H., Dearden, K. A., Ha, T. T., & Lang, T. T. (2002). Effect of an integrated nutrition program on child morbidity due to respiratory infection and diarrhea in northern Viet Nam. *Food and nutrition bulletin, 23*(4_suppl2), 67-74.

Stewart, C. P., Iannotti, L., Dewey, K. G., Michaelsen, K. F., & Onyango, A. W. (2013). Contextualising complementary feeding in a broader framework for stunting prevention. *Maternal & child nutrition, 9*(S2), 27-45.

Stone-Jimenez, M. (2014). Preventing Moderate Acute Malnutrition (MAM) Through Nutrition-Specific Interventions.

Sudfeld, C. R., McCoy, D. C., Danaei, G., Fink, G., Ezzati, M., Andrews, K. G., & Fawzi, W. W. (2015). Linear growth and child development in low-and middle-income countries: a meta-analysis. *Pediatrics, 135*(5), e1266-e1275.

Sullivan, K. M., Mei, Z., Grummer-Strawn, L., & Parvanta, I. (2008). Haemoglobin adjustments to define anaemia. *Tropical Medicine & International Health, 13*(10), 1267-1271.

Sunguya, B. F., Poudel, K. C., Mlunde, L. B., Shakya, P., Urassa, D. P., Jimba, M., & Yasuoka, J. (2013). Effectiveness of nutrition training of health workers toward improving caregivers' feeding practices for children aged six months to two years: a systematic review. *Nutrition journal, 12*(1), 66.

Surkan, P. J., Siegel, E. H., Patel, S. A., Katz, J., Khatry, S. K., Stoltzfus, R. J., . . . Tielsch, J. M. (2013). Effects of zinc and iron supplementation fail to improve motor and language milestone scores of infants and toddlers. *Nutrition, 29*(3), 542-548.

Szajewska, H., Ruszczynski, M., & Chmielewska, A. (2010). Effects of iron supplementation in nonanemic pregnant women, infants, and young children on the mental performance and psychomotor development of children: a systematic review of randomized controlled trials–. *The American journal of clinical nutrition, 91*(6), 1684-1690.

Taghizade Moghaddam, H., Khodaee, G. H., Ajilian Abbasi, M., & Saeidi, M. (2015). Infant and Young Child Feeding: a Key area to Improve Child Health. *International Journal of Pediatrics, 3*(6.1), 1083-1092.

Taneja, S., Bhandari, N., Bahl, R., & Bhan, M. K. (2005). Impact of zinc supplementation on mental and psychomotor scores of children aged 12 to 18 months: a randomized, double-blind trial. *The Journal of pediatrics, 146*(4), 506-511.

Tau, G. Z., & Peterson, B. S. (2010). Normal development of brain circuits. *Neuropsychopharmacology, 35*(1), 147.

Tofail, F., Persson, L. Å., El Arifeen, S., Hamadani, J. D., Mehrin, F., Ridout, D., . . . Grantham-McGregor, S. M. (2008). Effects of prenatal food and micronutrient supplementation on infant development: a randomized trial from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study. *The American journal of clinical nutrition, 87*(3), 704-711.

Tontisirin, K., Nantel, G., & Bhattacharjee, L. (2002). Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proceedings of the nutrition society, 61*(2), 243-250.

Torsvik, I., Ueland, P. M., Markestad, T., & Bjørke-Monsen, A.-L. (2013). Cobalamin supplementation improves motor development and regurgitations in infants: results from a randomized intervention study–. *The American journal of clinical nutrition, 98*(5), 1233-1240.

Tulchinsky, T. H. (2010). Micronutrient deficiency conditions: global health issues. *Public Health Reviews, 32*(1), 243.

Uauy, R., Olivares, M., & Gonzalez, M. (1998). Essentiality of copper in humans. *The American journal of clinical nutrition, 67*(5), 952S-959S.

Unicef. (1990). *Strategy for improved nutrition of children and women in developing countries*: Unicef.

UNICEF. (2003). Strategy to reduce maternal and child undernutrition. *Health and Nutrition Working Paper. Bangkok: East Asia and Pacific Regional Office, UNICEF*.

UNICEF. (2008). WHO: Indicators for assessing infant and young child feeding practices. Part 1 Definitions: Geneva: WHO.

Unicef. (2013). *Improving child nutrition: the achievable imperative for global progress*.

UNICEF. (2015). Improving Child Nutrition: The achievable imperative for global progress. 2013. ISBN: 978-92-806-4686-3.

Unicef. (2016). Improving child nutrition. The achievable imperative for global progress. 2013. *Google Scholar*.

UNICEF. (2017). WHO, World Bank Group. Levels And Trends in Child Malnutrition in UNICEF/WHO/World Bank Group Joint Child Malnutrition Estimates Key Findings of the 2016 edition. New York: UNICEF; Geneva: WHO; Washington DC: World Bank.

UNICEF, W. (2012). WHO, World Bank. *Levels and trends in child malnutrition: UNICEF-WHO-World Bank joint child malnutrition estimates. UNICEF, New York*.

Veena, S. R., Krishnaveni, G. V., Srinivasan, K., Wills, A. K., Muthayya, S., Kurpad, A. V., . . . Fall, C. H. (2010). Higher maternal plasma folate but not vitamin B-12 concentrations during pregnancy are associated with better cognitive function scores in 9-to 10-year-old children in South India. *The Journal of nutrition, 140*(5), 1014-1022.

Victora, C. G., Adair, L., Fall, C., Hallal, P. C., Martorell, R., Richter, L., . . . Group, C. U. S. (2008). Maternal and child undernutrition: consequences for adult health and human capital. *The lancet, 371*(9609), 340-357.

Victora, C. G., Bahl, R., Barros, A. J., França, G. V., Horton, S., Krasevec, J., . . . Rollins, N. C. (2016). Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *The lancet, 387*(10017), 475-490.

Victora, C. G., de Onis, M., Hallal, P. C., Blössner, M., & Shrimpton, R. (2010). Worldwide timing of growth faltering: revisiting implications for interventions. *Pediatrics*, peds. 2009-1519.

Vitta, B., & Dewey, K. (2012). Identifying Micronutrient Gaps in the Diets of Breastfed 6-11-Month-Old Infants in Bangladesh, Ethiopia and Viet Nam Using Linear Programming. *Alive & Thrive: Washington, DC*.

Walker, S. P., Wachs, T. D., Gardner, J. M., Lozoff, B., Wasserman, G. A., Pollitt, E., . . . Group, I. C. D. S. (2007). Child development: risk factors for adverse outcomes in developing countries. *The lancet, 369*(9556), 145-157.

Warthon-Medina, M., Qualter, P., Zavaleta, N., Dillon, S., Lazarte, F., & Lowe, N. M. (2015). The Long Term Impact of Micronutrient Supplementation during Infancy on Cognition and Executive Function Performance in Pre-School Children. *Nutrients, 7*(8), 6606-6627.

Waterlow, J. (1973). Note on the assessment and classification of protein-energy malnutrition in children. *The lancet, 302*(7820), 87-89.

Welch, R. M. (2002). Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. *The Journal of nutrition, 132*(3), 495S-499S.

Wessells, K. R., Singh, G. M., & Brown, K. H. (2012). Estimating the global prevalence of inadequate zinc intake from national food balance sheets: effects of methodological assumptions. *PloS one, 7*(11), e50565.

WHO. (2005). Guiding principles for feeding non-breastfed children 6-24 months of age.
WHO. (2006a). WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. *Methods and development. WHO (nonserial publication). Geneva: WHO, 2007*.
WHO. (2006b). WHO Child Growth Standards: Length/height-for-age, weightfor-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. *Geneva: World Health Organization, 157*.
WHO. (2007a). Joint statement by the World Health organization, the World Food Programme and the united nations children's Fund: Preventing and controlling micronutrient deficiencies in populations affected by an emergency. *WHO, Geneva*.
WHO. (2007b). Preventing and controlling micronutrient deficiencies in populations affected by an emergency. *Joint Statement by the World Health Organization, the World Food Programme and the United Nations Children's Fund. Internet: http://www. who. int/nutrition/publications/nut_emergencies/en/index. html (accessed September 15, 2007)*.
WHO. (2008). Indicators for assessing infant and young children feeding practices-part 1: definitions. *Geneva: WHO*.
WHO. (2010a). Indicators for assessing infant and young child feeding practices part 3: country profiles.
WHO. (2010b). Indicators for assessing infant and young child feeding practices: part 2: measurement.
WHO. (2011a). Guideline: use of multiple micronutrient powders for home fortification of foods consumed by pregnant women.
WHO. (2011b). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. *Download from: http://www. who. int/vmnis/indicators/haemoglobin. pdf*.
WHO. (2014). Childhood stunting: challenges and opportunities: report of a webcast colloquium on the operational issues around setting and implementing national stunting reduction agendas, 14 October 2013-WHO Geneva.
WHO, V. (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. *Geneva: Vitamin and Mineral Nutrition Information System, WHO*.
Wijnhoven, T. M., de Onis, M., Onyango, A. W., Wang, T., Bjoerneboe, G.-E. A., Bhandari, N., . . . Al Rashidi, B. (2004). Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food and nutrition bulletin, 25*(1_suppl1), S37-S45.
WorldBank. (2012). Levels and trends in child malnutrition: UNICEF-WHO-the world bank joint child malnutrition estimates. *Washington DC*.
Yousafzai, A. K., Rasheed, M. A., Rizvi, A., Armstrong, R., & Bhutta, Z. A. (2014). Effect of integrated responsive stimulation and nutrition interventions in the Lady Health Worker programme in Pakistan on child development, growth, and health outcomes: a cluster-randomised factorial effectiveness trial. *The lancet, 384*(9950), 1282-1293.
Zeisel, S. H. (2009). Epigenetic mechanisms for nutrition determinants of later health outcomes–. *The American journal of clinical nutrition, 89*(5), 1488S-1493S.
Zeng, L., Yan, H., Cheng, Y., & Dibley, M. J. (2011). Modifying effects of wealth on the response to nutrient supplementation in pregnancy on birth weight, duration of gestation and perinatal mortality in rural western China: double-blind cluster randomized controlled trial. *International journal of epidemiology, 40*(2), 350-362.

Zhang, Y., Wu, Q., Wang, W., van Velthoven, M. H., Chang, S., Han, H., . . . Scherpbier, R. W. (2016). Effectiveness of complementary food supplements and dietary counselling on anaemia and stunting in children aged 6–23 months in poor areas of Qinghai Province, China: a controlled interventional study. *BMJ open, 6*(10), e011234.

Zimmermann, M. B. (2009). Iodine deficiency. *Endocrine reviews, 30*(4), 376-408.

Zimmermann, M. B. (2011). *The role of iodine in human growth and development.* Paper presented at the Seminars in cell & developmental biology.

Annexes

Annex: 1 Consent form

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

CENTER OF FOOD SCIENCE AND NUTRITION

This informed consent form is for the parents of infants ages of 6 month who receive micronutrient powder and standard of care, and who we are asking to participate in research in investigation of multiple micronutrient supplements (MNPs) on growth, cognitive development, serum micronutrient concentration of infants aged 6-12 months in rural Ethiopia

Principal Investigator: **Ashenafi Geletu**

Advisor **: Dr. Kaleab Baye (Asso.Prof)**

Co-advisor **Dr. Seid M.Adem (Asso.Prof)**

Organization- **Addis Ababa University Center for Food Science and Nutrition**

Name of Sponsor- **Addis Ababa University Center for Food Science and Nutrition**

Name of Proposal and version- **A PhD proposal;**

Investigation of the exposure to low-iron multiple micronutrient powders (MNPs) on development, anaemia and growth of infants and young children in Sidama Zone, Southern Ethiopia

PART I: Information Sheet

I am ______________________________, working as researcher/data collector in a research conducted by PhD student of Center for Food Science and Nutrition, Natural Science faculty, of Addis Ababa University. We are doing research on investigation of multiple micronutrient supplements (MNPs) on growth, cognitive development, serum micronutrient concentration of infants aged 6-12 months in rural Ethiopia.

I would very much appreciate your participation in this study. I am going to give you information and invite you to have your child participate in this research. You do not have to decide today whether or not you agree that your child may participate in the research. Before you decide, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me.

For this study we will be presented a questionnaire for you, which is about your living style your child feeding practice, type of food you feed your child and your child health condition. In this research the cognitive development of your child will be measured twice in six months difference by experienced and professional psychologist with the help of pediatrics nurse. Also a small amount of blood, equal to about a teaspoon will be taken from your child's arm and urine samples will also be taken at 6 and 12 months of age.

We are inviting you to take part in this research because it is important that we investigate the effect of multiple micronutrients on cognitive development, growth and micronutrient status. Because you and your child live in this area and your child is one of the beneficiary of MNPs.

Your decision to have your child participate in this study is entirely voluntary. It is your choice whether to have your child participate or not. If you choose not to consent, all the services your child receives at this program will continue and nothing will change. You may also choose to change your mind later and stop participating, even if you agreed earlier, and the services for your child receives at the program will continue.

The risk induced by the conduct of the research if any, would be minimal. The only foreseeable risk would be related to blood drawing, which would be nullified by experienced pediatric phlebotomist and nurses. In addition, anonymity of participants will be guaranteed by making use of codes that will be kept in a safe during and after the study.

Your child participates in this research; he/she will have the following benefits: If we identify any interim illnesses will be recommended to get treatment. There may not be any other benefit for your child but his/her participation is likely to help us find the answer to the research question, which is very important for future generations are likely to benefit. But you will not be provided any incentive to take part in this research.

The information that we collect from this research will be kept confidential. Information about your child that will be collected from the research will be put away and no-one but the researchers will be able to see it. Any information about your child will have a number on it instead of his/her name. Only the researchers will know what his/her number. It will not be shared with or given to anyone.

PART II: Certificate of Consent

I have been invited to have my child participate in this research. I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate as a participant in this study.

Print name of participant child__________________ Thumb print of parent

Print name of parent or guardian_______________

Signature of Parent or guardian ___________________

Date ___________________________

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the parent of the potential participant, and to the best of my ability made sure that the person understands that the following will be done:

1. Cognitive development of the participant will be measured
2. Blood and urine sample will be taken from the participant
3. Anthropometric measurement will performed

I confirm that the parent was given an opportunity to ask questions about the study, and all the questions asked by the parent have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Print Name of Researcher/person taking the consent________________________

Signature of Researcher /person taking the consent__________________________

Date ___________________________

Day/month/year

Annex 3: Questionnaire

Section I: Information

001 Questionnaires identification No__________________

002 Region____________________

003 Wereda____________________

004 Kebele_____________________

005 Village name________________

006 Household number____________

Interviewer Visit

	Visit 1	Visit 2	Visit 3
Date			
Interviewer			
Result			

Result Codes:

Completed 1

Not at Home 2

Refused 3

Partially Completed 4

Other (specify)_____ 5

Checked by Supervisor Name___________________Signature _______

Date__________

008 Interviewer`s: Code__________ Name________________________________

009 Date of interview:________/________/_________

DD MM YYYY

010Time of start of the interview___________:___________

Hour Minute

Questionnaires for Mother/ Caregiver of the infant

Section II: Socioeconomic and demographic information

No	**Question**	**Coding categories**	**Skip to**
201	What is your relation with the child?		
202	How old are you? Maternal age in years	___________year	
203	Family size (Total number of family member)		
204	House hold head		
205	What is the highest education level you completed?	Unable to read & write……………11 Able to read & write………………12 Grade completed _____________	
206	What is your current marital status?	Married……………………………11 Divorced…………………………..12 Widowed………………………….13 Separated………………………….14 Other________________ Specify (96)	
207	What is your religion?	Orthodox…………………………..11 Protestant………………………….12 Catholic…………………………….13 Muslim……………………………..14 Other_________________ Specify (96)	
208	What ethnic or linguistic group do you belong to?	Oromo……………………………..11 Amhara……………………………12 Sidama…………………………….13 Gurage…………………………….14 Other_________________ Specify (96)	
209	What is your current occupation	Housewife………………………...11	

		Daily laborer……………………….12 Merchant………………………….13 Government employee…………….14 Other________________ Specify (96)	
210	Do you have monthly family income?	No income………………………….11 Don't know………………………..12 Other________________ Specify (96)	
211	If yes , how much is it? (Approximately)?	_____________Eth. Birr	
212	What is the main source of drinking water for members of your household?	Piped to yard/plot...........................11 Public tap/standpipe........................12 Water from spring………..……13 Rainwater……………………..….14 Surface water………………..…15 Other________________ Specify (96)	
213	What kind of toilet facility do members of your household usually use?	Flush or pour flush toilet…………11 Pit latrine with sla………………..12 Pit latrine without slab/open pit….13 No facility/bush/field…………….14 Other________________ Specify (96)	
214	Do you share this toilet facility with other households?	Yes. . . .. .1 No. 2	
215	How many households use this toilet facility?	____________________ .Don't know. 98	
216	Does your household have	An electricity……………………….11 A radio………………………….12 A television……………………….13 A mobile telephone ………………14 A non-mobile telephone………..…15 Other________________ Specify (96)	

217	Does any member of this household own any agricultural land?	Yes. . . . . 1 No. 2	
218	How many (LOCAL UNITS) of agricultural land do members of this household own?	Local units Don't know. 98	
219	Does this household own any livestock,?	Yes. . . .. 1 No. 2	
220	How many of the following animals does this household own? *If none, enter '00'* *If unknown, enter '98'*	Cows/Bulls/Oxen............. Horses Donkeys/Mules...... Camels................................ Goats.................................. Sheep................................. Chickens............................ Beehives	
221	Is your household receiving cash or food from the 1Safety Net Program?	Yes. . .. . .1 No. 2	

Section III: Health/supplementary questions

301	Is your child currently breastfeeding?	Yes…………………………….....1 No……………………………….2 Don't know. 98	
302	Was your child sick in the past day? (2 weeks ahead)	Yes……………………………......1 No……………………………….2 Don't know. 98	

303	If yes what was the sickness?	Cough...11 Diarrhoea...................................12 Fever..13 Malaria.......................................14 Other..15 Other________________ Specify (96)	
304	Is what YOUR CHILD ate yesterday similar to what HE/SHE normally eats?	Yes…………………………….....1 No……………………………..2	
305	If no what was the reason?	Little food available……………..11 No food available………………..12 Fasting…………………………..13 Extra food available……………..14 Feast/ holiday……………………15 Sick day…………………………16 Don't know. . . . .98 Other________________ specify (96)	
306	In the past 2 weeks, has your child taken any vitamin or mineral supplements or PlumpyNut?	Yes…………………………….....1 No……………………………...2	
307	If yes, which vitamins and minerals were taken?	Vitamin A.................................11 Iron..12 Zinc...13 PlumpyNut………….................14 Other________________ specify (96)	
308	In the past 6 months, did your child take a vitamin A capsule? (show the blue capsule)	Yes…………………………….....1 No……………………………..2	

Section IV: Feeding practice of the infant using 24-hour Dietary Intake

Name of Child:___________________________________ ID No______-

Sex of Child(1=Male; 2=Female): |___|

Now I would like to ask you about everything about YOUR CHILD consumed from the time he/she awoke yesterday until the awaking today. If you are not sure of the time, you can describe from sunrise yesterday to sunrise today

	Questions	Coding categories	Skip
401	In what month and year was ***born***? How many months old is your child? *Or* *Rerecord the date of birth as documented on the* health/vaccination *card.*	Day.....................................|___|___| *If day is not known, enter '98'* Month.............................. |___|___| Year.................... |___|___||___|___| Age in completed months ...... |___|___|	
402	Are you still breastfeeding your young child?	Yes... 1 No.. 2 Don't know 98	
403	How many times do you breastfeed during the day? How many times do you breastfeed during the night?	Number of day times............... |___|___| Number of day times............... |___|___| Don't know 98	
404	Sometimes babies are fed breast milk in by spoon, cup or bottle from the mother or another women. Did ***your child*** consume breast milk in any of these ways yesterday during the day or at night?	Yes.. 1 No.. 2 Don't know 98	
405	Do you give your baby any feedings besides breastfeeding?	Yes... 1 No.. 2	

		Don't know 98	
406	If yes How many times do you feed your baby each day?	________________________	
407	Do you give for your baby an infant formula (Formula milk)?	Yes.. 1 No.. 2 Don't know 98	
408	If yes , when did you start to use this formula milk? (At what month of the baby)	__________________________	
409	Was ***your child*** given any vitamin drops or other medicines as drops yesterday during the day or at night?	Yes.. 1 No.. 2 Don't know 8	

410. Can you please tell me what food you normally feed your child at each meal during a day? For example, what did you feed your child when he/ she weak up yesterday morning ? If any of the meals include porridge or soup, *ask*: How thick is the porridge? Or, if soup was given, *ask*: Do you feed just the broth or both broth and the solids in the soup? If you take a spoonful and turn the spoon sideways, will the porridge or soup run off or will it fall in clumps?

Time	**Place**	**Food/ drink**	**Food preparation and ingredient**	**Amount weight (g)**	**Food code**

Section V: MNP supplement question

501	At least in the past 3 months, did your child take MNP supplement?	Yes.. 1 No... 2	
502	For each month how many MNP you were taken from the distributer?	______________________	
503	For a single day how many sachet MNP you were use for your child?	______________________	
504	How did you mix the MNP with your child food?	I add the powder when the food is cooking………..……………11 I add the powder when the food is ready to fid the child……….12 Other____________ specify (96)	
505	With how much child food that you were mix one sachet of MNP?	With The food consumed for one serving size…………………..11 With the food which could consume for the whole day….12 Other____________ specify (96)	

Section VI: Anthropometry

601 Weight

1st measure	2nd measure	3rd measure
|__||__| . |__| kg	|__||__| . |__| kg	|__||__| . |__| kg

602 Height

1st measure	2nd measure	3rd measure
|__||__| . |__| cm	|__||__| . |__| cm	|__||__| . |__| cm

603 MUAC and HC

MUAC (cm)	**HC (cm)**

Section VII: Motor development assessment

	Multicentre Growth Reference Study Motor development assessment	
Test items	Examiner report (a) Observed *1 = No (inability)* *2 = No (refusal)* *3 = Yes* *9 = Unable to test*	
Sitting without support	|__|	
Hands-and-knees crawling	|__|	
Standing with assistance	|__|	
Walking with assistance	|__|	
Standing alone	|__|	
Walking alone	|__|	
Child's emotional state *Rate the child's emotional state during the testing of all the milestones.* *Enter a code for each of the two scales.*	(a) First scale *1= Drowsy* *2= Awake and alert* |__|	(b) Second scale *1= Calm* *2= Fussy* *3= Crying* |__|

Section VIII: hemoglobin level

ID No	Name of the child	Sex	Hemoglobin level g/L	Is Anemic or Non anemic?
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Annex 2: concent and quesionnaire (amharic version)

አዲስ አበባ ዩኒቨርስቲ

የምረቃ ጥናት ትምህርት ቤት

የምግብ ሳይንስና ስነ ምግብ ማዕከል

ይህ ህጋዊ ስምምነት ቅጽ የአልሚ ምግብ/ንጥረ ምግብ ዱቄትና የእንክብካቤ ደንብ እንዲሁም በተለያዩ የአልሚ ምግብ ማሟያ በአካላዊ እድገት፣ በአእምሯዊ እድገት በአጠቃላ በደም ውስጥ የሚገኝ የአልሚ ምግብ መጠን ፍተሻ ምርምር ላይ እንዲሳተፉ ለጠየቅናቸው ከ6 ወር እስከ 12 ወራት ለሆኑ የህፃናት ወላጆች ነው፡፡

ዋና ተመራማሪ፡ አሸናፊ ገለቱ

አማካሪ፡- ዶ/ር ቃልአብ ባዬ

ዶ/ር ሰኢድ ሙሄ አደም

ድርጅት፡- አዲስ አበባ ዩኒቨርስቲ የምግብ ሳይንስና ስነ ምግብ ማዕከል

የገንዘብ ወይም ሌላ ደጋፊ ስም፡- አዲስ አበባ ዩኒቨርስቲ የምግብ ጥናትና ስነ ምግብ ማዕከል

የምርምሩ እቅድና እትም ስም፡- ፒ ኤች ዲ የምርምር እቅድ

እድሜያቸው ከ6 ወራት አስከ 12 ወራት በሆኑ በገጠሪቷ ኢትዮጵያ በሚገኙ ህፃናት ላይ የተለያየ የአልሚ ምግብ ማሟያዎች በአካላዊ እድገት፣ በአዕምሮአዊ እድገት ላይ ስላላቸው ዉጤትና በአጠቃላይ በደማቸው ውስጥ የሚገኝ የአልሚ ምግብ መጠን ላይ የተሰራ ምርምር፡፡

ክፍል አንድ: የመረጃ

እኔ ____ በአዲስ አበባ ዩኒቨርስቲ በተፈጥሮ ሳይንስ ፋኩልቲ በምግብ ሳይንስና ስነ ምግብ ማዕከል በፒ ኤች ዲ ተማሪ ለሚከናወነው ምርምር በመረጃ ሰብሳቢነት እየሰራሁ ያለሁ ነኝ፡፡እድሜያቸው ከ6 ወራት እስከ 23 ወራት ባሉ በገጠሪቷ ኢትዮጵያ በሚኖሩ ህፃናት ላይ የተለያዩ የአልሚ ምግብ ማሟያዎች በህፃናቱ አካላዊ እድገት፣ አእምሮአዊ እድገትና የእነዚህ ንጥረ ምግቦች በደም/በሴረም ውስጥ ምን ያህል እንዳለ ምርም እየሰራን ነው፡፡

በዚህ ጥናት ላይ ለምታደርጉተ ተሳትፎ በጣም አመሰግናለሁ፡፡ በዚህ ምርምር ላይ አንዳንድ መረጃዎችን ሰጥቼ ልጃችሁ በምርምሩ እንዲሳተፍ/እንድትሳተፍ እጋብዛለሁ፡፡ ልጃችሁ በምርምሩ ላይ ይሳተፍ ወይም አይሳተፍ የሚለውን ሀሳብ ዛሬ መወሰን የለባችሁም፡፡ ከመወሰናችሁ በፊት ይስማማናል ብላችሁ የምታስቡትን ሰው ማነጋገር ትችላላችሁ፡፡ እባክዎ መረጃውን ሳነብላቹ ያልገባችሁ ነገር ካለ ለማብራራት ግዜ አወስዳለሁ፡፡ የምናቀርብላችሁ መጠይቅ የሚያካትተው ስለ የአኗኗር ዘይቤያችሁ፣ ስለ ልጃችሁ የአመጋገብ ልማድ፣ ልጃችሁን ስለምትመግቡት የምግብ አይነት እና ስለ ልጃችሁ የጤና ሁኔታ ነው፡፡ በዚህ ምርምር ልምድና ብቃት ያለው የስነ ልቦና ባለሙያ ከህፃናት ነርስ ጋር በመሆን የልጅዎን የአካላዊና የአእምሮ እድገት በስድስት ወር ልዩነት ውስጥ ሁለቴ ይለካል፡፡ የሽንትና የደም ናሙናም 2mL (የሻይ ማንኪያ አካባቢ የሚሆን) ሁለት ጊዜ በ6 ወራት ልዩነት ከህጻኑ ላይ ይወሰዳል፡፡

በዚህ ምርምር ላይ እንዲሳተፉ የጋበዝንዎት ዋነኛ ምክንናያት አናንተና የልጆቻችሁ የዚህ አካባቢ ነዋሪ ስለሆናችሁና (እንዲሁም ህጻናቱ ተጨማሪ አልሚ ምግብ ተጠቃሚዎች ስለሆኑ ነው፡፡) ልጃችሁ በዚህ ጥናት ላይ እንዲሳተፍ/እንድትሳተፍ የምታደርጉትን ውሳኔ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው፡፡ በኃላ ሀሳብዎትን በመቀየር ተሳትፎዎን ማቆም ይችላሉ፡፡ ከልጅዎ ደም በመወሰዱ የልጅዎ የአእምሮ እድገት በመለካቱ ምክንያት እና በዚህ ምርምር ላይ በማሳተፎ በልጅዎ ላይ የሚደርስ ምንም አይነት ጉዳት የለም፡፡ ሁሉም ስራዎች የሚከናወኑት ልምድናት ብቃት ባላቸው ሰራተኞች ነው፡፡

ልጅዎ በዚህ ምርምር ላይ ቢሳተፍ/ብትሳተፍ የሚያገኙት ጥቅምለምሳሌ ጊዜያዊ ህመም/የጤና መታወክ ቢገኝበት/ቢገኝባት ተገቢውን ህክምና እንዲያኝ/እንድታገኝ እንመክራለን አገልግሎቱንም እንዲያኝ/እንድታገኝ እንጥራልን፡፡ ከዚህ በተረፈ ለልጅዎ ሌሎች ጥቅሞች ላይኖሩ ይችላሉ ነገር ግን በምርምራችን ላይ የልጁዎ ተሳትፎ ለምርምራችን ጥያቄዎች መልስ ለማግኘት ይረዳናል እንዲሁም ከዚህም ምርምር ቀጣዩ ትውልድ ተጠቃሚ ይሆናል፡፡

ከዚህ ምርምር ስለ ልጅዎ የሰበሰብነው መረጃ ሚስጥራዊ ይሆናል፡፡ ከተመራማሪው በስተቀር ማንም ሊያየው አይችልም፡፡ ማንኛውም አይነት ስለ ልጅዎ የተሰበሰበ መረጃ በላዩ ላይ ቁጥር እንጂ የልጅዎ ስም አይኖርበትም፡፡ የልጅዎን ቁጥር የሚያውቁት ተመራማሪዎቹ ብቻ ናቸው፡፡

ለበለጠ ማብራሪያ ተመራማሪዎቹን ይጠይቁ:-

ዋና ተመራማሪ፡ አሸናፊ ገለቱ **አማካሪ:** ዶ/ር ቃልአብ ባዬ

ስልክ ቁጥር:- 0911 430346 0911 890489

ክፍል ሁለት:ስምምነት

ልጄ በዚህ ምርምር ላይ እንዲሳተፍ/እንድትሳተፍ ተጋብዣለሁ፡፡ ከላይ ያለውን መረጃ አምብቤዋለሁ/ተነቦልኛል፡፡ ሰለ መረጃውም ጥያቄዎች ለመጠየቅ እድል አግኝቻለሁ፡፡ የጠየቅኃቸው ጥያቄዎችም በሚገባ ተመልሰውልኛል፡፡ ልጄ በዚህ ጥናት ላይ ተሳታፊ እንዲሆን ወይም እንድትሆን በፈቃደኝነት ተስማምቻለሁ፡፡

የተሳታፊው/ የህፃኑ/የህፃኗ ስም ______________________________

የወላጅ ወይም ያሳዳጊ ስም______________________________

የወላጅ ወይም ያሳዳጊ ፊርማ______________________________

ቀን: ______________ ______________ ________

/ቀን/ /ወር/ /ዓ.ም/

የወላጅ የአውራ ጣት ፊርማ ______________________________

ስምምነት የሚወስደው/ የሚያደርገው ተመራማሪ መግለጫ:-

የመረጃ ወረቀቱን ወደፊት ተሳታፊ ለሚሆነው ህፃን ወላጅ በትክክል አንብቤያለሁ፡፡ የተቻለውን ሁሉ በማድረግ ወላጅ እንዲረዳኝ ከታች የተዘረዘሩትን ስራዎች እንደማከናውን በግልፅ አስረድቼአለሁ፡፡

1. የተሳታፊው የአእምሮ እድገት ይለካል
2. የደምና የሽንት ናሙና ከተሳታፊው ይወሰዳል
3. የአካላዊ መጠን ልኬት ይከናወናል

የህፃኑ/የህፃኗ ወላጅ ስለ ጥናቱ ጥያቄዎችን እንዲጠይቅ እድል መስጠቱንና የተጠየቁትንም ጥያቄዎች በተቻለ መጠን በትክክል መመለሴን አረጋግጣለሁ፡፡

የህፃኑ/የህፃኗ ወላጅም ሳይገደድ ወይም ሳትገደድ በፈቃዱ/በፈቃዷ መስማማቱን/ መስማማቷን አረጋግጣለሁ፡፡ የዚህ ስምምነት ቅጂ ለተሳታፊው ቀርቧል፡፡ ስምምነቱን የሚወስደው የተመራማሪ ስም

____

ስምምነቱን የሚወስደው የተመራማሪ ፊርማ ______________________________

ቀን ______________________________

ክፍል I፡ መጠይቆች

01 የመጠይቆች መለያ መረጃ ____

02 ክልል ____________________________________

03 ወረዳ ____________________________________

04 ቀበሌ ____________________________________

05 የመንደር ስም____________________________________

06 የቤት ቁጥር ______________________________________

የጥያቄ አቅራቢው/ የጠያቂው ጉብኝት

	ጉብኝት I	ጉብኝት II	ጉብኝት III
ቀን			
ጠያቂ			
ውጤት			

የተጠናቀቀ/የተሟላ 1 ቤት ውስጥ የሌለ 2 እምቢ ያለ 3 በከፊል የተሞላ 4 ሌሎች (ካለ ይግለፁ)5

ያፀደቀው/ያረጋገጠው ተቆጣጠሪ ስም ________________________ፊርማ ________________ቀን ________

008 የጠያቂው ስም __________________________________ ፊርማ ________________ቀን ________

009 ጥያቄው የተጠየቀበትን ቀን ______________/____________________/______________________

ቀን ወር ዓ.ም.

010 ቃለ መጠይቅ የተጀመረበት ሰዓት _____________: _________________

ሰዓት ደቂቃ

ለህፃኑ/ኗ እናት ወይም ሞግዚት የሚቀርቡ መጠይቆች

ክፍል II: የማህበራዊ ኢኮኖሚያዊና የስነ ህዝብ መረጃ

ተ.ቁ	ጥያቄ	የሚስጥራዊ ፅሑፍ ምድቦች	እለፍ
201	ከህጻኑ/ኗ ጋር ያለዎት ዝምድና ምንድን ነው ?		
202	እድሜሽ ስንት ነው ?	____________________ ዓመት	
203	የቤተሰብ ብዛት	____________________	
204	የቤተሰብ ሐላፊ		
205	ያለዎት የትምህርት ደረጃ	ማንበብና መፃፍ አልችልም....................11 ማንበብና መፃፍ እችላለሁ....................12 የክፍል ደረጃ______________________	
206	ባሁኑ ሰዓት ያለዎት የጋብቻ ሁኔታ	ያገባች...11 የተፋታች..12 በሞት የተለዩ...................................13 የተለያዩ..14	
207	ሀይማኖት	ኦርቶዶክስ.......................................11 ፕሮቴስታንት....................................12 ካቶሊክ..13 ሙስሊም..14 ሌላ ____________________ይግለፅ (96)	
208	ከየትኛው ብሔረሰብ ነዎት	ኦሮሞ...11 አማራ...12 ሲዳማ...13 ጉራጌ……………………..…………14 ሌላ ____________________ይግለፅ (96)	
209	ስራ	የቤት እመቤት..................................11 የቀን ሰራተኛ.....................................12 ነጋዴ...13 የመንግስት ሰራተኛ.............................14 ሌላ ___________________ይግለፅ (96)	

210	ወርሃዊ የቤተሰብ ገቢዎ አለዎት?	ገቢ የለኝም......................................11 አላውቀውም....................................12 ሌላ ____________________ይግለፅ (96)	
211	የወር ገቢ ካለዎት ምን ያህል ነው?	____________________ የኢትዮጵያ ብር	
212	የቤተሰብዎ ዋና የመጠጥ ውሃ ምንጭ ምንድነው ?	የግል ቧንቧ......................................11 የህዝብ ቧንቧ....................................12 የ ምንጭ ውሃ....................................13 የዝናብ ውሃ......................................14 የገፀ ምድር ውሀ…..…………………15 ሌላ ___________________ይግለፅ (96)	
213	እርስዎና የቤተሰብዎ አባላት ምን አይነት ሽንት ቤት ይጠቀማሉ?	ውሃ የሚለቀቅበት ሽንት ቤት………..…11 ክዳን ያለው የጉድጓድ ሽንት ቤት...............12 ክዳን የሌለው የጉድጓድ ሽንት ቤት............13 ጢሽ፣ ቁጥቋጥ፣ መስክ…......................14 ሌላ ____________________ይግለፅ (96)	
214	ይህን ሽንት ቤት ከሌላ ቤተሰብ ጋር ይጠቀሙታል ?	አዎ ....1 አይ 2	
215	በአጠቃላይ ስንት ቤተሰቦች ይህን ሽንት ቤት ይጠቀማሉ ?	______________________________	
216	ቀጥሎ ከተዘረዘሩት ውስጥ እቤትዎ የትኞቹ ይገኛሉ	የኤሌክትሪክ ሀይል…………..........12 መብራት.....................................13 ሬድዮ..14 ቴሌቪዥን ....15 ተንቀሳቃሽ ስልክ...........................16 መደበኛ ስልክ...............................17	
217	ቤተሰብዎ የእርሻ መሬት አለው	አዎ...1 የለውም.......................................2	
218	መልሱ አዎ ከሆነ ምን ያህል የእርሻ መሬት አለዎት?	………………………(በጥማድ/በሄክታ)	
219	በቤትዎ ውስጥ የቤት እነሰሳት አለዎት?	አዎ………………………………..…1 የለኝም……………………………..2	

220	መልሱ አዎ ከሆነ ምን ያህል አለዎት? ምንም ከሌለው "00"	ላሞች/ኮርማዎች/በሬዎች			
		ፈረሶች/አህዮች/በቅሎዎች			
		ግመሎች			
		ፍየሎች			
		በጐች			
		ዶሮዎች			
		ቀፎዎች			
221	ቤተሰብዎ ከሴፍቲኔት መርሃ ግብር የገንዘብ ወይም የምግብ እርዳታ ያገኛል	አዎ...1 አያገኝም..2			

ክፍል III: የጤና/የማሟያ ጥያቄዎች

301	ልጅዎን ጡት እያጠቡ ነው?	አዎን..1 አይጠባም/አትጠባም……………………...2 አይታወቅም---------------------------------98	
302	ልጅዎት ባለፉት ቀናት አሞት ነበር ? (ባለፉት 2 ሳምንታት)	አዎ...1 አይ…………………………………..….2	
303	መልሱ አዎ ከሆነ ልጅዎት የታመመዉ/ችዉ ምን ነበር?	ሳል..11 ተቅማጥ...12 ትኩሳት..13 ወባ...14 ሌላ ______________________ይግለፅ (96)	
304	ልጅዎ ትላንትና የተመገበው/ችው ምግብ አብዛኛውን ግዜ ከሚመገቡት ምግብ መጠኑ ተመሳሳይ ነዉ?	አዎ..1 አይ…………………………………...2	
305	መልሱ አይ (መጠኑ አይመሳሰልም) ከሆነ ምክንያቱ ምን ነበር?	ትንሽ ምግብ ስለነበር………….……….11 ምግብ ስላልነበረ …………….……….12 ጾም ነበር………………………………13 ትርፍ ምግብ ስለነበረ…………………..…14 አመት በዓል ስለነበረ…………….……...15	

		አሞት ስለነበር……………….………16 አይታወቅም__________________98	
306	ባለፉት 2 ሳምንታት ህፃንዎ የቫይታሚን ወይም የማእድን ማሟያ ንጥረ ነገር ወይም የለውዝ ድልህ ወስዶ/ዷ ነበር?	አዎ..1 አይ…………………………..……....2	
307	መልሱ አዎ ከሆነ የትኞቹን ንጥረ ነገር ወስዶ/ዳ ነበር?	ቫይታሚን A..11 ብረት...12 ዚንክ...13 የለውዝ ድልህ......................................14 ሌላ ______________________ይግለፅ (96)	
308	ባለፉት 6 ወራት ህፃንዎ የቫይታሚን A ጠብታ ወስዶ/ዳ ነበር? (ሰማያዊ እንክብልን ያሳዩአቸው)	አዎ..11 አይ…………………………………..12	

ክፍል IV: የህፃኑ/ኗ በ24 ሰአት ውስጥ የተመገበውን/የተመገበ ውን በማየት የአመጋገብ ልማድ

የህፃኑ/ኗ ስም ____ መለያ ቁጥር___________________________

የህፃኑ/ኗ ጾታ (1፡ ወንድ 2፡ ሴት) የህፃኑ/ኗ ክብደት________________________(g) ቁመት__________________(cm)

እናት /የሞግዚት ስም ____

401	የህፃኑ/ኗ የትውልድ ዘመን መቼ ነዉ?	ቀን /ወር /ዓ.ም /__________/_________/___________/ እድሜ (በወር)_______________________	
402	ልጅዎን ጡት እያጠቡ ነው?	አዎን..1 አይ………………………………2 አይታወቅም……………………….. 98	
403	በግምት በቀን ስንት ግዜ ጡት ያጠባሉ? በግምት ማታ ስንት ግዜ ጡት ያጠባሉ?	ቀን _________ማታ______________ አይታወቅም ………………… 98	
404	አንዳንድ ግዜ ህፃናት የእናት ጡት ወተት ወይም የሌላ	አዎን..1	

	ሰው ጡት ወተት በማንኪያ፣ በኩባያ ወይም በጡጦ ይጠባሉ ከእነዚህ ከተጠቀሱት መንገዶች ትናንትና ቀን ወይም ማታ ልጅዎ የጡት ወተት መግበዋል?	አይ…...2 አይታወቅም...98	
405	ከእናት ጡት ወተት በተጨማሪ ለልጅዎ ተጨማሪ መግብ ይመግባሉ ?	አዎን..1 የለም...2 አይታወቅም...8	
406	መልስዎ አዎ ከሆነ በቀን ስንት ግዜ ተጨማሪ ምግብ ይመግባሉ?	___________________________________	
407	ለልጅዎ በቆርቆሮ ታሽጎ የሚሸጥ ወተት (Infant Formula) ይጠቀሙለታል? (ያጠጡታል?)	አዎን..1 አይ…..2 አይታወቅም...98	
408	መልስዎ አዎ ከሆነ ለልጅዎ በቆርቆሮ ታሽጎ የሚሸጥ ወተት መጠቀም መቼ ጀመሩ?	__________________________________	
409	ልጅዎን ትላንትና ቀን ወይም ማታ የቫይታሚን ጠብታ ወይም ሌላ መድሀኒት ተሰጥቶት ነበር?	አዎን..1 አይ…..2 አይታወቅም...98	

410. አብዛኛውን ግዜ በቀን ውስጥ ህፃንዎን ምን አይነት ምግብ እንደሚመግቡት ሊነግሩን ይችላሉ? ለምሳሌ

- ✓ ትላንትና ጠዋት ልጅዎን ከእንቅልፉ ሲነሳ ምን አይነት ምግብ መገቡት/ቧት ወይም የሚጠጣ ነገር አጠጡት/ አጠጧት? ጊዜው መቼ ነው?
- ✓ ምገቡን ሲመግቡት ሌላ ተጨማሪ ነገርነበር? ምን ነበር?
- ✓ ከዚህ ከጥሎ ለልጅዎ የመገቡት ምግብ ምን ነበር? ጊዜው መቼ ነው?
- ✓ ምገቡን ሲመግቡት ሌላ ተጨማሪ ነገርነበር? ምን ነበር?

(ማስታወh ከትናነተ ጠዋት እሰከ ዛሬ ጠዋት በመቀጠለ ህነ፡ የተመገበዉን ማንኛውንም ምግብ ሆነ መጠጥ (ውሃንም ጨምሮ) ከታች በተመለከተዉ ሰንጠረ» በረደፍ 3 ውስጥ መዝግብ/ቢ፡፡ መጠይቁን ሰታቀርብ/ቢ ህፃኑ ትመገበዉን መከሰስም ሆነ መዳረh መጠየቅና መመዝገብ እንዳትተዉ/ይ)

ሰ®ƒ	ቦታ	ምግብ/መጠጥ	የምግቡ አዘገጃጀት	መጠን	ክብደት(ግራም)	የምግቡ መለያ

ክፍል V: የህፃኑ/ኗ አየልሚ ምግብ አጠቃቀም ጥያቄዎች

501	ባለፉት ስድሰት ወራት ልጅዎ ያአለሚ ንጥረነገሮች ተጠቃሚ ነበረ/ች?	አዎ..1 አይ………………………..……………2	
502	ለእያንዳንዱ ወር ምን ህል ያአለሚ ንጥረነገሮች የወስዱ ነበር?	------------------------- (በቁጥር ይጠቀስ)	
503	ለአነድ ቀን ምን ያህሉን ያአለሚ ንጥረነገ ለልጅዎ ይጠቀማሉ?	___________________________________	
504	ያአለሚ ንጥረነገሩን ከልጅዎ ምግብ ጋር ሲያዋህዱት አዘገጃጀቱ እንዴት ነበር?	ምግቡን ሳበስል እጨምረዋለሁ------------------11 ምግቡ በስሎ ለመመገብ ዝግጁ ሲሆን እጨምረዋለሁ---12 ሌላ ካለ ይገለ	
505	አንዱን ጥቅል ያአለሚ ንጥረነገር ከምን የህል የልጅዎ ምግብ ጋር ይቀላቅሉታለ?	ለአንድ ጊዜ የሚዘጋጀው ምግብ ውስ………..11 ቀኑን ሙሉ ሊመገበው የሚችለው ምግብ ውስጥ…..12 ሌላ ካለ ይጠቀስ_________________________	

ክፍል VI: አንትሮፖሜትሪ

የህፃኑ/ኗ ስም ____ መለያ

ቁጥር__________________________ የህፃኑ/ኗ ጾታ (1፡ ወንድ 2፡ ሴት)

601 ክብደት

የመጀመሪያ ልኬት	ሁለተኛ ልኬት	ሶስተኛ ልኬት
|__||__| . |__| kg	|__||__| . |__| kg	|__||__| . |__| kg

602ቁመት

የመጀመሪያ ልኬት	ሁለተኛ ልኬት	ሶስተኛ ልኬት
|__||__| . |__| cm	|__||__| . |__| cm	|__||__| . |__| cm

603ሙአክ እና

ክፍል VII: ሞተር እድገት መመርመሪያ

<table>
<tr><td rowspan="2"></td><td rowspan="2">መጠይቅ (Test items)</td><td colspan="2">Multicentre Growth Reference Study
ሞተር እድገት መለኪያ (Motor development assessment)</td></tr>
<tr><td colspan="2">የጠያቂው ውጤት (Examiner report)
<u>ምልከታ</u>
1 = አይ (ስለማይችል/ትችል)
2 = አይ (ስላልፈለገ/ች)
3 = አዎ
9 = መመዘን አልተቻለም</td></tr>
<tr><td>701</td><td>ህጻኑ/ኗ ያለምንም ድጋፍ ይቀመጣል</td><td colspan="2">|__|</td></tr>
<tr><td>702</td><td>ህጻኑ/ኗ በእጁ እና በእግሩ ዳዴ ይላል</td><td colspan="2">|__|</td></tr>
<tr><td>703</td><td>ህጻኑ/ኗ እርስዎን ይዞ ወይም እቃ ተደግፎ (በድጋፍ) መቆም ይችላል</td><td colspan="2">|__|</td></tr>
<tr><td>704</td><td>ህጻኑ/ኗ እርስዎን ይዞ ወይም እቃ ተደግፎ (በድጋፍ) መራመድ ይችላል</td><td colspan="2">|__|</td></tr>
<tr><td>705</td><td>ህጻኑ/ኗ ያለምንም ድጋፍ መቆም ይችላል</td><td colspan="2">|__|</td></tr>
<tr><td>706</td><td>ህጻኑ/ኗ ያለምንም ድጋፍ መራመድ ይችላል</td><td colspan="2">|__|</td></tr>
<tr><td>707</td><td>ህጻኑ አሁን ያለበት የስሜታዊነት ደረጃ
Rate the child’s emotional state during the testing of all the milestones.
Enter a code for each of the two scales.</td><td>(ሀ) የመጀመሪያ ምዘና
1= ፈዛዛ (የተጫጫነው)
2= ንቁ (ፈጣን)t
|__|</td><td>(ለ) ሁለተኛ ምዘና
1= የተረጋጋ
2= ያልተረጋጋ
3= አልቃሻ
|__|</td></tr>
</table>

ክፍል VIII: ሄሞግሎቢን መጠን

መለያ ቁጥር	የህፃኑ/ኗ ስም	ጾታ	Hemoglobin level g/L	Is Anemic or Non anemic?
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Annex 3- Ethical Clearance

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

የተፈጥሮና ኮምፒቴሽናል ሣይንስ ኮሌጅ
አዲስ አበባ ዩኒቨርሲቲ

OFFICE OF THE DEAN
የዲን ጽ/ቤት

Ref. No. / ቁጥር CNSDO/403/16
Date / ቀን April 25 2016

To Whom It may Concern

College of Natural Science Institutional Review Board (CNS-IRB) in its meeting held on 10/04/2016 Minute No. IRB/021/2016 has reviewed Phd thesis project proposal entitled **"Investigation of multiple micronutrient supplements (MNPs) on growth, cognitive development, serum micronutrient concentration of infants aged 6-12 months in rural Ethiopia),** Addis Ababa by Ashenafi Geletu from the Department of Food Science and Nutrition.

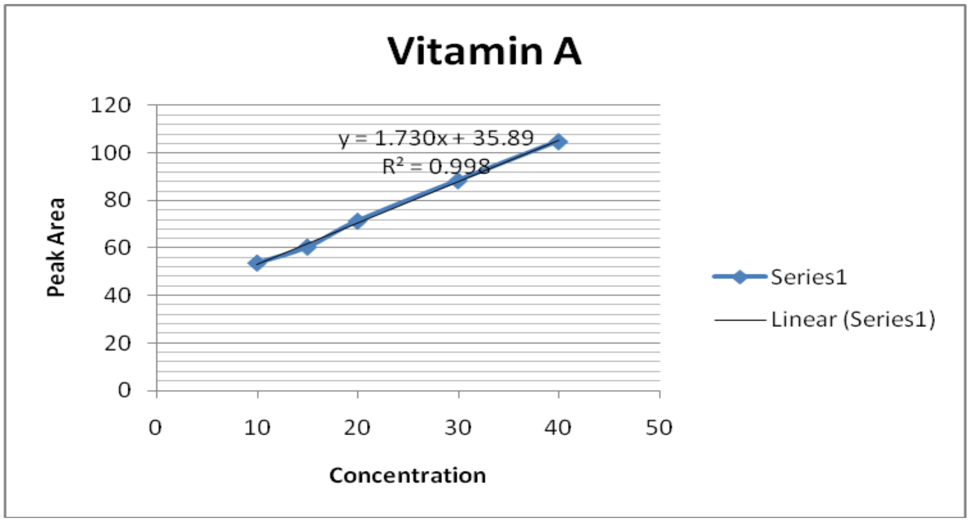
The proposal was approved for implementation.

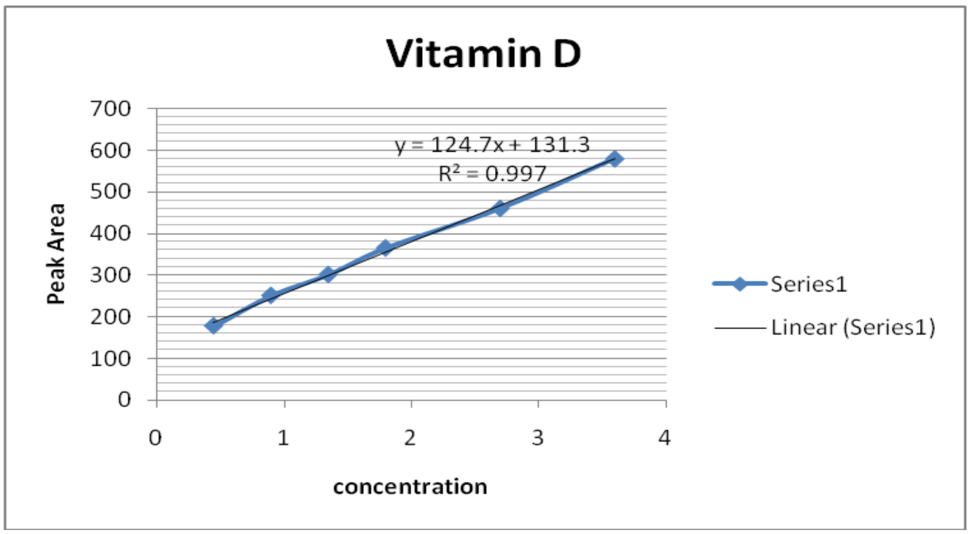
With regards,

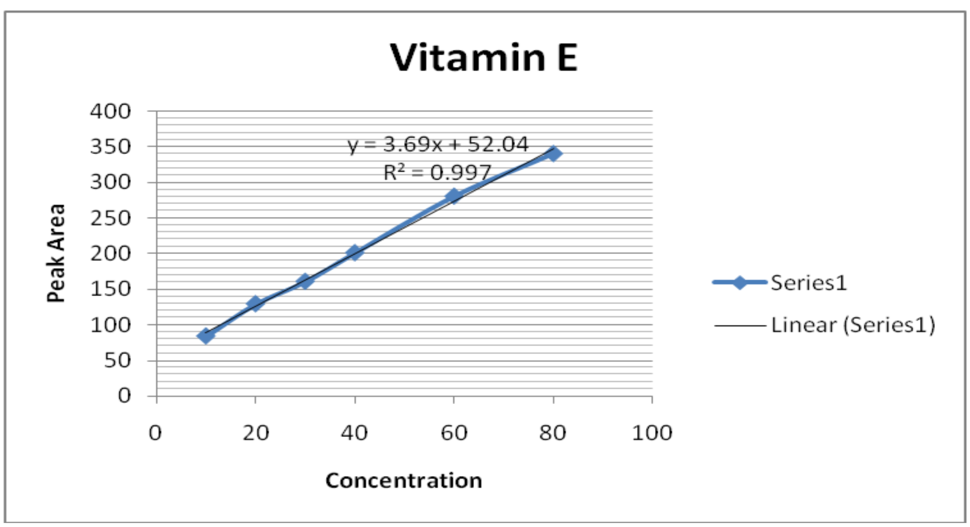
Shibru Temesgen /Dr./
Dean College of Natural & Computational Science

ስልክ/Tel.+251-11-123-94-72
ፋክስ/Fax: +251-11-123-94-69
ፖ.ሣ.ቁ/POBox 1176 Addis Ababa, Ethiopia
ኢ.ሜይል/E-mail: dean_cns@aau.edu.et
Please Quote our reference number in you correspondence
"Examine all things; hold fast that which is good"
"ሁሉን መርምሩ መልካሙን ያዙ"

Annex 4- Calibration curve of vitamins standard







Annex 5- Representative chromatogram of the vitamins

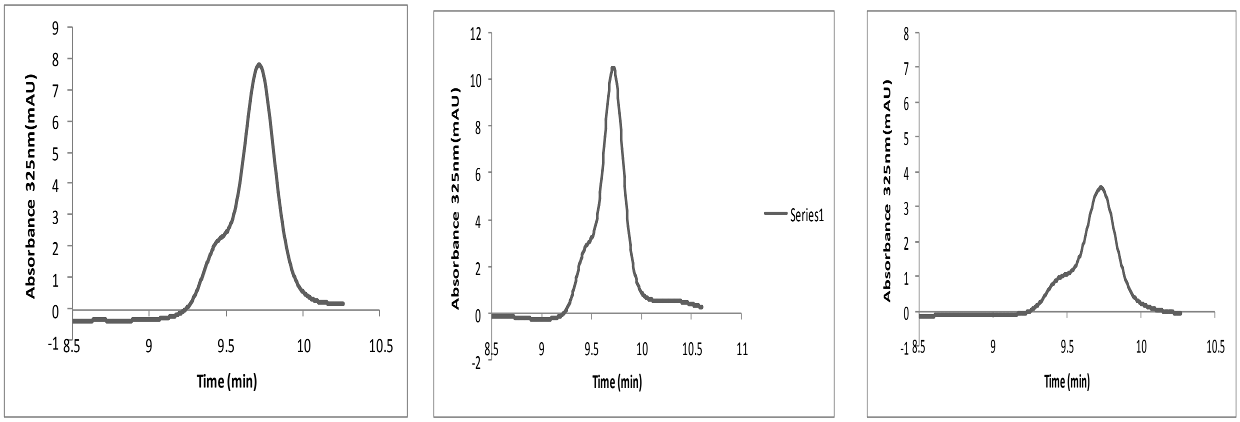


Figure A1: Chromatogram of Vitamin A a) maize b) wheat and c) mixed cereals

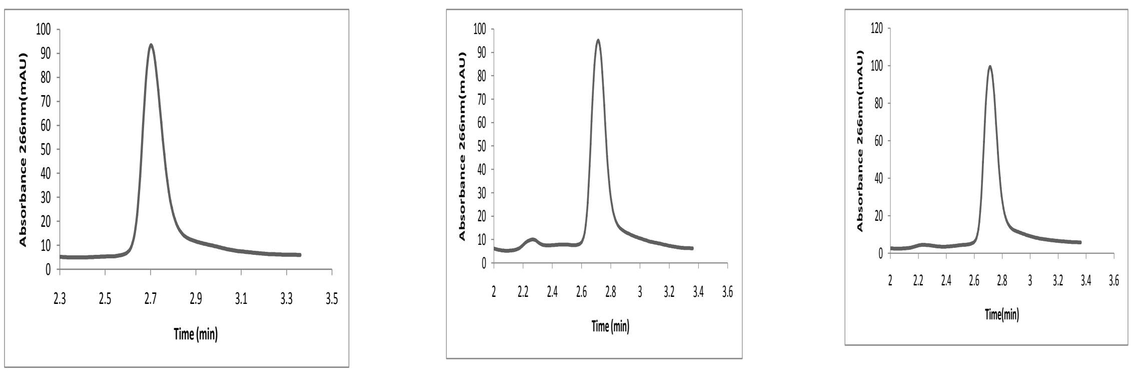


Figure A2: Chromatogram of standard Vitamin D a) maize b) wheat and c) mixed cereals

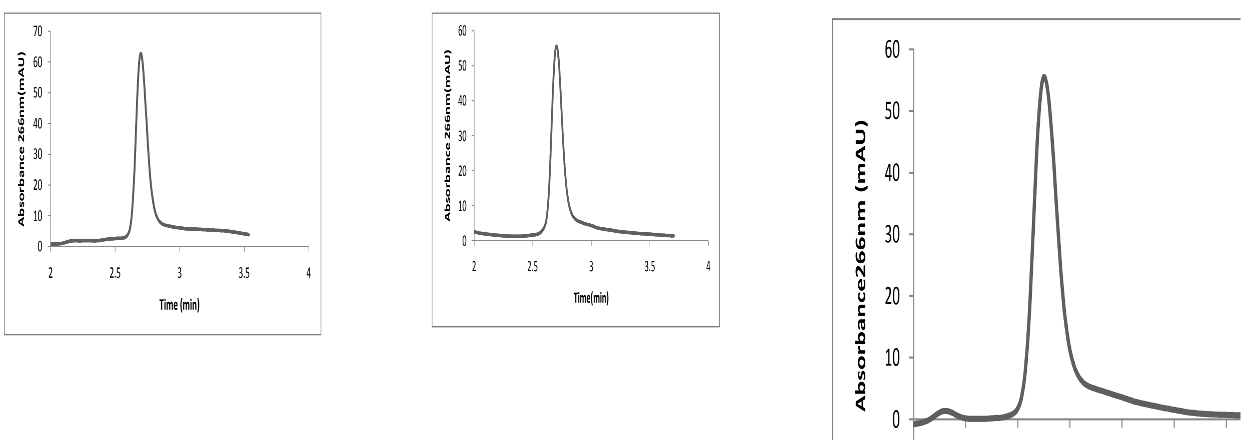


Figure A3: Chromatogram of standard Vitamin E a) maize b) wheat and c) mixed cereals

Annex 6-Method Validation Parameter

Limit of Detection and limit of quantitation

The limit of detection of vitamins were calculated using Blank + 3s' method (Magnusson, 2014) 1.74, 0.04 and 0.99 μg/mL for retinol, cholecalciferol, and α -tocopherol respectively for maize sample, 4.12, 0.04 and 0.31 μg/mL of retinol, cholecalciferol and α -tocopherol respectively for wheat sample and 0.22, 0.05 and 0.20 μg/mL of retinol cholecalciferol and α-tocopherol respectively of mixed cereal (Table 1A). Similarly the limit of quantification was calculated using Blank + 10s' (Magnusson, 2014) of the vitamins were 4.87, 0.09 and 1.21 μg/mL of retinol, cholecalciferol and α-tocopherol respectively for maize sample, 7.65, 0.17 and 0.94 μg/mL of retinol, cholecalciferol and α-tocopherol respectively for wheat sample and 1.93, 0.23 and 0.64 μg/mL of retinol, cholecalciferol and α-tocopherol, respectively for mixed cereals.

Table 1A: Calibration parameters, LOD and LOQ

Vitamins	r^2	Linear range, (μg/mL)	Maize sample		Wheat sample		Mixed cereal sample	
			LOD (μg/mL)	LOQ (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
Vitamin A	0.998	5-40	1.74	4.87	4.12	7.65	0.22	1.93
Vitamin D	0.997	10-80	0.04	0.09	0.04	0.17	0.05	0.23
Vitamin E	0.997	0.45-3.6	0.39	1.21	0.31	0.94	0.20	0.64

Linearity

The calibration curves were obtained using analytical solutions of standard vitamin mixture were prepared in pure solvent (Bliesner, 2006). Acceptability of linearity data is often judged by examining the correlation coefficient (r^2) and the y intercept of the linear regression line for the response versus concentration. The y- intercept should be less than a few percent of the response obtained from the target level (Gobo, Kurz, Pizzutti, Adaime, & Zanella, 2004). The responses were linear in the following ranges: 5–40 μg/mL (*n=6*) with a slope of 1.73 mV mL/mg, an intercept of 35.89 mV, and a correlation coefficient of 0.998 for retinol, 10– 80 μg/mL (*n*=6) with a slope of 124.7 mV ml/mg, an intercept of 131.3 mV and a correlation coefficient of 0.997 for cholecalciferol and 0.45–3.6 μg/mL (*n=6*) with a slope of 3.69 mV mL/mg, an intercept of 52.04 mV, and a correlation coefficient of 0.997, for α-tocopherol. Linearity was evaluated by the calculation of six- point linear plots with three replicate based on linear regression and squared correlation coefficient, r^2, which were > 0.997 (Table 2).

Precision: Repeatability and intermediate precision repeatability limit

The precision of the method were tested by repeatability study with relative standard deviation and repeatability limit from six replicate of each of the three spiked sample (Table 2A). Relative standard deviation more useful because concentration has been factored out (Magnusson, 2014). Also from the standard deviation *s* a ‘precision limit’ were calculated to decide whether there was a significant difference, at a specified level of confidence (CI:95%), between results from replicate analyses of a sample obtained under specified conditions (Magnusson, 2014). The RSD values of retinol were 13.31% 4.45% and 16.44% for maize, wheat and mixed cereal respectively. For cholecalciferol the RSD value were 5.56%, 6.49% and 4.71% for maize, wheat and mixed cereal sample respectively. Furthermore the RSD of α-tocopherol were 1.87%, 0.92% and 0.67% for maize, wheat and mixed cereal respectively.

Table 2A: Repeatability and repeatability limit of the studied vitamins in fortified CF samples

	Maize		Wheat		Mixed	
	RSD=(s/conc)%	$r = \sqrt{2} \times t \times s_r$	RSD=(s/conc)%	$r = \sqrt{2} \times t \times s_r$	RSD=(s/conc)%	$r = \sqrt{2} \times t \times s_r$
Vitamin A	13.31	10.97	4.45	3.51	16.44	11.00
Vitamin D	5.56	2.41	6.49	2.84	4.71	2.03
Vitamin E	1.87	0.79	0.91	0.61	0.67	0.42

(s/conc) relative standard deviation/

Accuracy: Recovery

Accuracy was estimated by means of recovery assays. In a composite sample known amounts (0.5mL) of standard retinol, cholecalciferol and α- tocopherol (10, 20 and 0.9 µg/mL, respectively) had been added (spiked).

The recovery percentages of vitamins were calculated 108.59 %, 78.13 and 107.66% of retinol, cholecalciferol and α-tocopherol respectively for maize sample, 102.81 %, 78.76 and 106.95% of retinol, cholecalciferol and α-tocopherol respectively in wheat sample and 114.29 %, 77.49 and 112.75% of retinol, cholecalciferol and a-tocopherol respectively in mixed cereal sample.

Table 3A Recoveries of the studied Vitamins in fortified CF samples

Vitamins	Spiked (µg/g)	Spiked Mean Conc. (µg/g)			Blank Mean Conc. (µg/g)			Mean Recovery%		
		Maize	Wheat	Mixed	Maize	Wheat	Mixed	Maize	Wheat	Mixed
Vitamin A	10	5.95	5.69	4.83	3.86	3.63	2.54	108.59	102.81	114.29
Vitamin D	20	3.13	3.16	3.10	0.01	0.01	0.01	78.13	78.76	77.49
Vitamin E	0.9	3.26	4.99	4.78	3.07	4.81	4.58	107.66	106.95	112.75

DECLARATION

I, the undersigned, declare that the thesis is my original work, has not been presented for degrees in any other university and all sources of material used for the thesis have been duly acknowledged.

Name: Ashenafi Geletu Hayi

Signature:____

Place: College of Natural Sciences, Addis Ababa University

Date:

This thesis has been submitted for examination with my approval as a university advisor.

Name of advisor: Dr. Kaleab Baye

Signature:____