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Complementary feeding practices and nutrient adequacy of complementary foods consumed by young children in West Gojam, Ethiopia

By

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ABBREVIATIONS/ACCRONYMS

ASF	Animal Source Food
BF	Breastfeeding
BMI	Body Mass Index
BMVA	Breast Milk Vitamin A
CF	Complementary Feeding
CIAT	International Center for Tropical Agriculture
CSA	Central Statistics Agency
EAR	Estimated Average Requirement
EHNRU	Ethiopian Health and Nutrition Research Unit
ENA	Emergency Nutrition Assessment
ENI	Ethiopian Nutrition Institute
EPHI	Ethiopian Public Health Institute
FAB	Food and Nutrition Board
FAO	Food and Agriculture Organization
FMoH	Federal Ministry of Health
GFDRE	Government of the Federal Democratic Republic of Ethiopia
HEWs	Health Extension Workers
HPLC	High Performance Liquid Chromatography
IMAPP	Intake Monitoring and Planning Programme
IOM	Institute of Medicine
IYCF	Infant and Young Child Feeding
KSE	Knowledge Sharing Effectiveness
LAZ	Length for age Z-score
LMIC	Low and Middle Income Countries
MNP	Micro Nutrient Powder
PAHO	Pan American Health Organization
RE	Retinol Equivalent
RNI	Recommended Nutrient Intake
SHE	Secretary of Health, Ethiopia
SNNP	Southern Nations, Nationalities and Peoples' region
UK	United Kingdom
UL	Upper Level of intake
UN IGME	The UN Inter-Agency Group for Child Mortality Estimation
UNICEF	United Nations Children's Fund
UNU	United Nations University
WAZ	Weight for Age Z-score
WB	World Bank
WHO	World Health Organization
WLZ	Weight for Length Z-score

Complementary feeding practices and nutritional adequacy of complementary foods consumed by young children, in West Gojam, Ethiopia

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ABSTRACT

Stunting is prevalent in Ethiopia and reaches a peak during the complementary feeding period. The present study investigated the complementary feeding practices and nutritional adequacy of complementary foods consumed by young children (12 to 23 months) in Mecha, district, West Gojam, Ethiopia.

One hundred Health Extension Workers (HEWs) and 226 mother-child dyads participated in the study. Data on the socio-demographic characteristics, educational level of the mothers, and mothers' andchildren's anthropometric measurements were collected. Knowledge of mothers and HEWs on optimalInfant and Young Child Feeding (IYCF) was examined using a pre-tested questionnaire. The impact of HEWs knowledge and knowledge sharing effectiveness (KSE) on mothers' knowledge and child nutritional status was studied. IYCF practices were evaluated and child and caregivers' feeding behaviorswere characterized using observation and self-report. The association between child food intake and stunting with caregiver feeding style was studied. The adequacy of energy and selected nutrient intakes from the complementary foods were determined using two 24hour recalls, and the intakes were compared with WHO recommendations. The intake of additional nutrients from MNP was simulated and the risks of inadequate and excessive intakes were estimated. Furthermore, breastmilk vitamin A (BMVA) and concentration of pro-vitamin A carotenoids were measured using HPLC and iCheck, and the agreement between the two measurements studied.

Although nutrition education through the HEP did not reach all the caregivers, those that did receive IYCF training had higher knowledge scores and better child feeding practices than untrained ones ($P< 0.05$). Trained mothers had a lower proportion of stunted children, and KSE of HEWs' was found associated with child linear growth. Although responsive feeding practices were not widely observed, they were associated with accepted mouthful and child linear growth. Not only low food intake, but also low quality complementary foods were observed in this setting. Iron, zinc, but also calcium intakes were found inadequate; while at the same time excessive iron intakes (8 %) were observed. Our simulation of MNP intervention, showed reductions in the prevalence of inadequate intakes, but was accompanied with iron and zinc excessive intakes beyond acceptable limits (< 2.5%). Low BMVA was prevalent and breastmilk provitaminA concentrations were lower than average composition for women in developing countries. BMVA measurements with iCheck are comparable to those obtained using HPLC.

Stunting in West Gojam is associated with poor IYCF knowledge, KSE of HEW, inadequate feeding style, and inadequate micronutrient intakes from complementary foods (iron, zinc, calcium) and breastmilk (vitamin A and carotenoids). Interdisciplinary interventions targeting both behavioral and food aspects of child feeding are needed in this setting. Such interventions would benefit from rigorous monitoring and evaluation that can be facilitated through novel indicators (KSE) and instruments (iCheck).

Keywords: stunting, micronutrients, child feeding, complementary foods, iron, zinc, vitamin A,breastmilk, responsive feeding

Contents

Organization of the thesis

The thesis has six chapters. **Chapter one** presents background information on the study, and indicates gaps to be filled in future studies.

Chapter two presents literature review that covers a brief description of undernutrition, itscauses and forms. It also discusses the conceptual framework of thedeterminants of nutritional status, and outlines the prevalence of global and regional malnutrition. In addition, it presents the importance of caregivers and HEWs nutrition knowledge of optimal infant and young child feeding practices, describes proper complementary feeding, and gives an overview of the complementary feeding practices in Ethiopia. It also covers relevant literatures on risks and benefits associated with use of micronutrient powders. Different feeding styles are outlined, and their association with child food intake is briefly presented, followed by description of BM nutrient composition. BMVA as an indicator of vitamin A status of lactating mother and breastfeeding child and the possibility of using iCheck to measure BMVA are described too. Finally, the importance of carotenoids in child health and development is presented.

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

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

Section 4.1(Paper published in**Food and Nutrition Bulletin**, 2016) presents the result from theevaluation of mothers and HEWs IYCF. It gives an overview of the knowledge of mothers and HEWs on IYCF practices, and the association between HEWs knowledge and knowledge-sharing effectiveness of optimal IYCF with mothers' knowledge and child stunting in rural Ethiopia.

Section 4.2 (Submitted for publication) presents reported caregiver feeding practices andobserved child and caregiver feeding behaviors. It also presents identified behaviors that have association with number of mouthfuls accepted, and comparison of feeding behaviors of

caregivers of stunted and non- stunted children and feeding behaviors that were captured through observation and self-report.

Section 4.3 *(Submitted for publication) presents*the prevailing complementary feeding practicesand estimate the nutrient intakes of young children. It also presents the prevalence of inadequate intake of selected micronutrients, and simulated the effect of fortifying complementary foods with MNP on the risk of inadequate and excessive nutrient intakes.

Section 4.4 (draft article) presents results on the concentration of BMVA and carotenoid, thedifference between actual BMVA concentrations and estimated value, and the agreement between BMVA as measured by HPLC and iCheck.

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

Chapter six presents conclusions, recommendations, and perspectives.

Chapter 1- *Introduction*

Chapter one: Introduction

1.1 Background

Undernutrition is responsible for more than one-third of child deaths globally(Black et al. 2008),and is a major public health concern for most of the developing countries (Petrou & Kupek 2010).About 90% of the world's chronically undernourished children are living in Asia andAfrica (De Onis *et al*., 2011). In Eastern Africa, the proportion of stunted children is around 32% (UNICEF/WHO/WB, 2015), and Ethiopia has one of the highest rates of stunting (40%) and underweight (25%) (CSA, 2014). According to Save the Children UK, 57% of child death in the country is directly or indirectly related to undernutrition(SC-UK, 2009).

According to the UNICEF's conceptual framework (UNICEF, 1990), malnutrition hasimmediate, underlying and basic causes. The immediate causes are inadequate dietary intake and diseases. The underlying causes are household food insecurity, inadequate care of mothers and children and insufficient health services and unhealthy environment. Inadequate dietary intake in infants and young children may refer to improper breastfeeding practices, early and delayed introduction of complementary foods and insufficient nutrient content of the diets.

Undernourished children have substantially lower chances of survival than those who are well nourished. Those who survive are likely to have limited intellectual capacity (Black *et al.*, 2008), which will in turn diminish their working capacity during adulthood. Undernourished children are also more likely to develop chronic illnesses(WHO/FAO, 2004) and disabilities in adult life. Given that undernourished mothers are likely to give low birth weight babies, malnutrition may have intergenerational effects. Such vicious circle of undernutrition has serious health and socio-

economic consequences and thus timely and effective intervention needs to be in place to break the cycle.

In developing countries, stunting prevalence reaches its peak during the complementary feeding period (6-23 months)(WHO/PAHO, 2003). The negative functional impairments associated with stunting are irreversible after 2 years of age(Martorell *et al*., 1994). The first two years in life; therefore, represent a critical window of opportunity for the prevention of undernutrition (Glewwe & King, 2001), and thus proper complementary feeding practices needs to be assured.

Available evidences support that adequate knowledge of health workers on nutrition can improve complementary feeding practices for children less than two years of age(Sunguya *et al.*, 2013; Pelto *et al*., 2004). However, (Baye *et al*., 2013) reported that, inappropriate feeding practices were observed, in northern part of the country, even when 76% of the caregivers reported having received complementary feeding trainings provided by the HEWs. This finding may indicate that either the nutrition messages provided are inappropriate or that the messages are not integrated or adoptable. Therefore, evaluation of the nutritional knowledge of the caregivers and training providers (HEWs) is needed so as to increase the effectiveness of nutrition education programs.

Also, inappropriate feeding practices such as laissez-faire and controlling feeding style, instead of the recommended responsive/active feeding, are prevailing in many developing countries (Adejuyigbe *et al*., 2008; Buffin, 1994). This may partly explain reported lack of appetite and low energy intakes in some contexts (Baye *et al*., 2013; Gibson *et al*., 2009). Therefore, for

assuring appropriate complementary feeding, not only what the child is fed but also how and when the child is fed is important (Pelto *et al*., 2004). A cross-sectional study carried out by (Wondafrash *et al.*, 2012), in southern part of Ethiopia, reported that, unlike most developing countries, responsive feeding was the commonest style practiced by the caregivers in the region. But this result contradicted with the previous report by (Gibson *et al*., 2009), in the same region, which states that, responsive feeding was not practiced in the region at all. Such discrepancy may be due to difference in the methods used to study the feeding style (self-report vs observation). The method of self-report used in the study of (Wondafrash *et al*., 2012)may be more susceptible to self-report bias, and thus further validation of the findings through the use of observational studies is needed.

Besides, most complementary foods in developing countries are predominantly based on cereals, tubers and legumes with little diversity (Arimond & Ruel, 2004). Such complementary diets are of low energy and nutrient density. Hence, infants and young children in developing countries have often been reported to have inadequate energy and nutrient intakes (Baye *et al*., 2013; Anderson *et al*., 2008). Recent quantitative studies on complementary feeding practices in Ethiopia indicated that energy and nutrient intakes were below WHO recommendations and that several feeding practices were not in-line with (WHO/PAHO, 2003) recommendations(Baye *etal*., 2013; Gibson *et al*., 2009). However, intake differences were observed between Southern and Northern part of the country. The difference was also observed between the highlands and lowlands of the same region (North Wollo) (Baye *et al*., 2013). This indicates the need to do more work in other parts of the country.

To increase adequacy of energy and nutrient intake, diversifying the complementary diet by inclusion of animal source foods, fruits and vegetables has been recommended (Baye *et al*., 2013; Gibson *et al*., 2009). But these food groups are often not affordable and thus are less accessible for most rural families. This raises doubts as to whether recommendations will be practical and thus alternatives such as multiple nutrient powders could be used in the short-term, but studies modeling the impact of such interventions are scarce and thus needed.

Furthermore, in these countries, breastfeeding children possibly will take a large portion of some nutrients such as vitamin A through BM(Canfield *et al*., 2003), even beyond six months of age. However, the feeding practices, dietary intakes and nutritional status of lactating women in the country were short of the national and international recommendations (Haileslassie *et al*., 2013), which will leave them to have inadequate nutrient stores(Valentine & Wagner, 2013; Canfield *et al*. 2003; Underwood, 1994). Yet, there is no adequate information about the BMVA, and carotenoid concentration among mothers, particularly at the later stage of lactation; therefore, it is important to study to what extent BM composition varies among lactating mothers.

Vitamin A analysis requires adequate technical and financial resources such as ability to transport and store biological samples, laboratory facilities, and technical expertise. Therefore, iCheck FlUORO, which is a simple, low-cost, rapid point-of-contact analytical method, would be a good alternative. However, BMVA measurements taken using the standard technique (HPLC) and those measured by iCheck FlUORO should be evaluated and validated.

The Ethiopian government has been involved in multiple efforts that aim to improve the nutritional status of infants and young children(FMoH, 2012; FMoH, 2006). Some progresses have been witnessed (i.e. stunting rate in the country has fallen from 57% in 2000 to 40% in 2014 (CSA, 2014). However, given the magnitude of the problem of stunting, much needs to be done in understanding the determinants of stunting to effectively design strategies.

1.2 Objectives

1.2.1 General objectives

The general objective of the thesis was to characterize complementary feeding practices and nutritional adequacies of complementary foods consumed by young children in West Gojam, Amhara region, Ethiopia and investigate strategies to improve them.

1.2.2 Specific objectives

- To evaluate mothers' and HEWs' knowledge of key infant and young child feeding (IYCF) practices.
- To investigate whether mothers' IYCF knowledge and HEW's knowledge-sharingeffectiveness are associated with child stunting.
- To characterize the prevailing complementary feeding practices and caregivers feeding style.
- To estimate the prevalence of inadequate intake of selected micronutrients from the complementary diets.
- To simulate the effect of point of use fortification (MNP) on the risk of inadequate and excessive nutrient intakes.
- To determine adequacy of BM vitamin A (BMVA) and carotenoid concentrations.
- To study the agreement between BMVA as measured by HPLC and iCheck.

Chapter 2 –*Literature review*

Chapter two: Literature review

2.1 Undernutrition

Undernutrition is one of the most significant challenges of the world and it is the underlying cause for ~ 45 percent of all deaths among children (WHO, 2016). Consequences of undernutrition affects one in four of children under age five, globally (UNICEF/WHO/WB, 2015). The problem has several forms, each reflecting a different aspect of this condition (Blössner *et al*., 2005; UNICEF, 2003). However, the effects of undernutrition, in all its forms, impact upon the social, economic and cultural development of societies and nations (Baye, 2016).

2.1.1. Causes of undernutrition

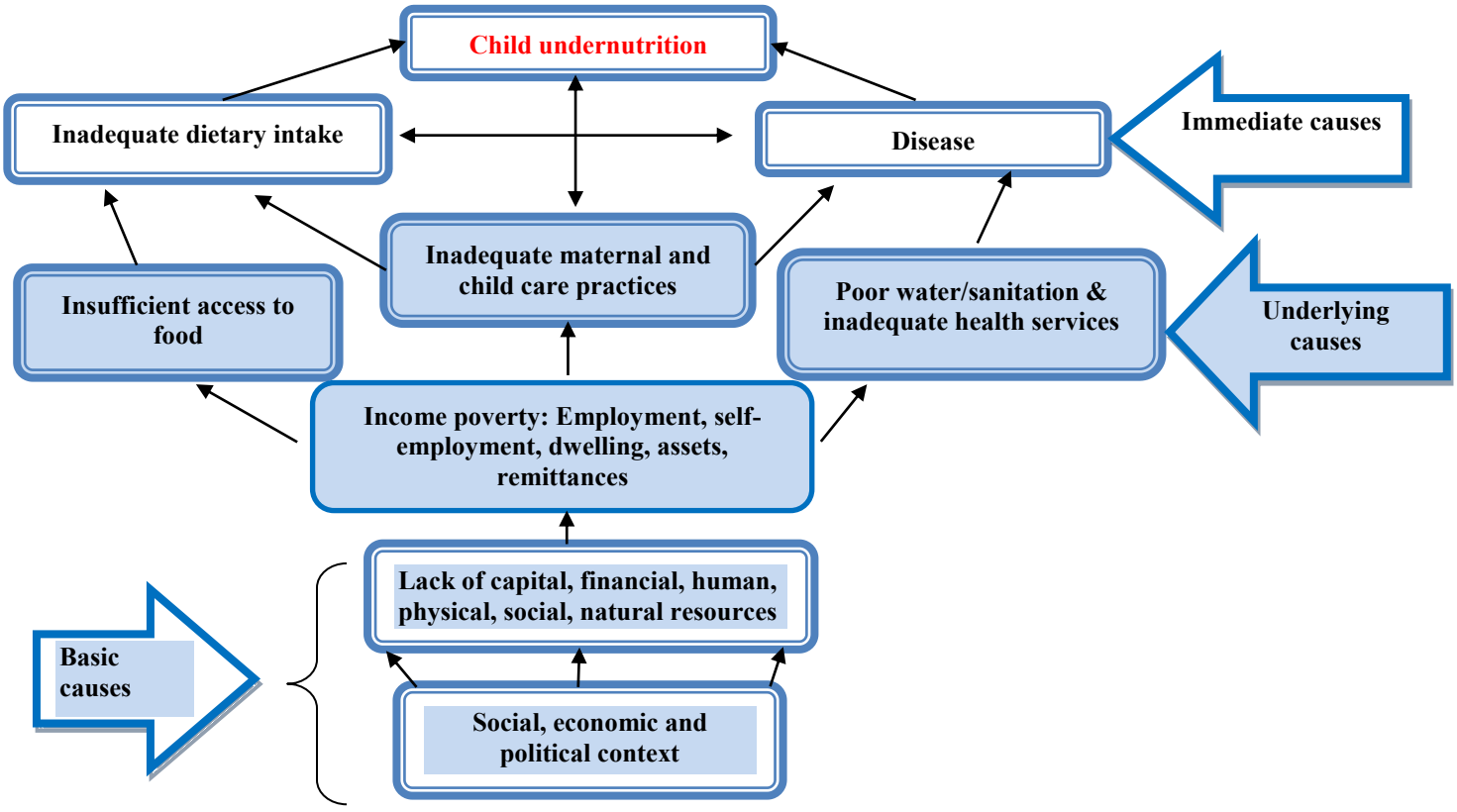

Figure 2. 1 Three levels of causes of undernutrition (UNICEF 1990)

The causes of undernutrition are multidimensional (Onis *et al.*, 2000), and can be categorized into immediate, underlying and basic causes (UNICEF, 1990). The immediate causes are described as insufficient diet and disease. In the case of infants and young children, insufficient dietary intake may refer to poor breastfeeding practices; early weaning; delayed introduction of complementary foods; and insufficient nutrients in the diet (Black *et al.*, 2013). Infectious disease cause under nutrition in children as well (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.

Inadequate levels of household food security, inadequate care of children and women, low education levels and information, insufficient health services (safe water and sanitation) are the underlying causes (UNICEF, 1990). The basic or root causes of undenutrition include, poor availability and control of political, social, ideological and economic resources (UNICEF, 1990). Also, the causes of undernutrition have intergenerational causality (UNICEF, 2003).

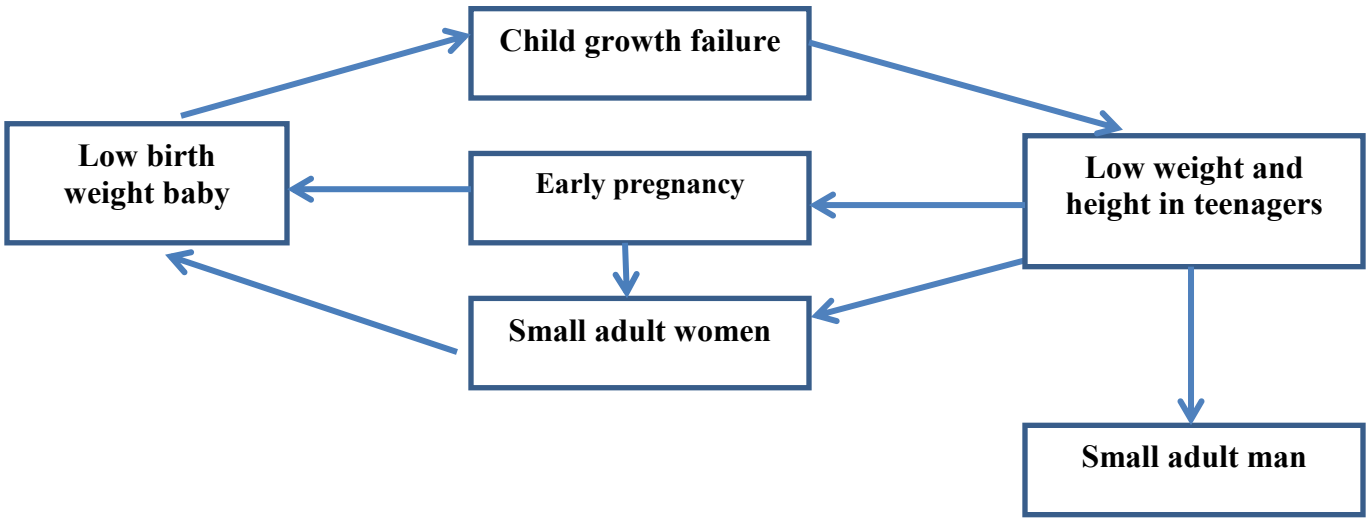

Figure 2.2 Undernutrition throughout the life cycle (UNICEF 2003)

2.1.2 Consequences of undernutrition

2.1.2.1 Stunting, wasting and underweight

Undernutrition is usually characterized by comparing the weights and heights (or lengths) of children at a specific age and sex, with the distribution of observed weights or heights in a reference population, and then calculating z-scores (WHO, 2006). The z-score is the difference between a child's weight or height and the median value at that age and sex in the reference population, divided by the standard deviation (SD) of the reference population. A child whose weight for height, weight for age or height for age is less than -2SD is considered as wasted, underweight, and stunted respectively (UNICEF, 2006; FAO, 2010).

Wasting is a measure of thinness, it often reflects a recent and severe process that has produced substantial weight loss, usually as a consequence of acute shortage of food and/or severe disease (Waterlow, 1994). Wasting significantly increases the risk of mortality and increases the severity of morbidity. Wasted children need urgent medical attention to prevent death (Shrimpton *et al.*, 2001), and it is an indicator in emergency settings. The situation can usually be improved using supplemental feeding (Rivera *et al*., 1991).On the other hand stunting results from chronic undernutrition, which retards linear growth (Martorell *et al*., 1994), and underweight encompasses both stunting and wasting (UNICEF, 2003).However, some of the functional impairments associated with stunting are irreversible past the age of two years (Martorell *et al.*, 1994).

- ***Global stunting prevalence***

Almost 160 million children under age five are stunted (UNICEF/WHO/WB, 2015) worldwide. However, the problem is not evenly distributed throughout the world. Three fourths of the

world's stunted children are living in developing countries. In 2014, more than half of all stunted children under 5 lived in Asia and more than one third lived in Africa (UNICEF/WHO/WB, 2015). The prevalence of stunting in children under 5 years is 32 and 25 per cent in Africa and Asia, respectively. In the past 25 years the global prevalence of under 5 stunting has declined from 39.6 percent to 23.8 percent (UNICEF/WHO/WB, 2015). However, there was unequal progress in stunting reduction among countries. The slowest progress was observed in Africa. Some regions like Asia have achieved nearly 50% reduction. More than half of the sub regions in Africa: Eastern, Middle and Western parts have rising numbers of stunted children (UNICEF/WHO/WB, 2015).

- ***Stunting in Ethiopia***

The prevalence of under 5 stunting is 40% in Ethiopia (CSA, 2014). However, the prevalence has declined on average by 1.2% per year since 2000 (CSA, 2012), but the reduction is below the global average. Disease burden, use of unsafe water, poor sanitation, and low uptake of primary health services and low levels of maternal education are the determinants of stunting in the country (Woodruff *et al*., 2016). Stunting prevalence is above the national average in some regions: Amhara (42%), Tigray (46%), Affar (46%), and SNNP (44%) (CSA, 2014). On the other hand, it is lowest in Addis Ababa and Gambela regions (22.9 and 22.4%, respectively) (CSA, 2014).

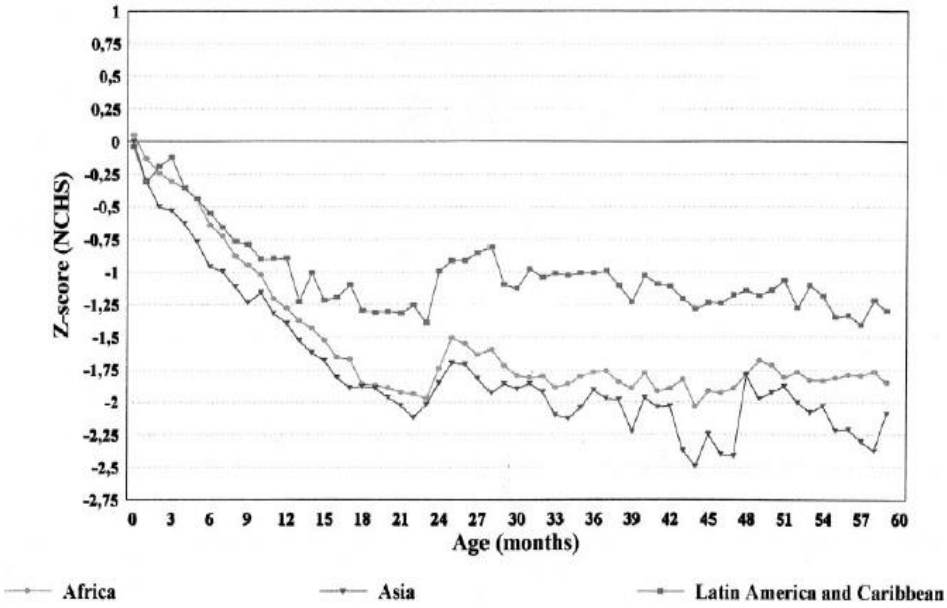

Figure 2.3 Peak period of under-five stunting

The problem often reaches a peak during the complementary feeding period (CSA, 2014; Victora *et al*., 2010) in Ethiopia, similar to other developing countries, Partly due to lack of knowledge (Abebe *et al*., 2016), improper feeding practices and low quality diet (Baye *et al*., 2013; Gibson *et al*., 2009), and poor maternal nutrition (Haskell & Brown, 1999).Hence, appropriate interventions should target the complementary feeding period (Baye & Faber, 2015).

The government of Ethiopia has been showing strong commitment to end undernutrition by 2030, and recognized the role of nutrition to propel sustainable development through Seqota Declaration and the national nutrition programme (GFDRE, 2015). However, considering the size of the problem more needs to be done in the country.

Table 2.1 Estimated cost of hunger in Ethiopia (Cost of hunger, 2013)

	Episodes	Cost in ETB	Percentage of GDP
Heath Costs			
LBW and Underweight	3,139,682	1,256	
Increased Morbidity	1,270,996	566	
Total for Health	4,410,678	1,822	0.54%
Education Cost			
Increased Repetition - Primary	152,488	93	
Increased Repetition - Secondary	-------	------	
Total for Education	152,488	93	0.03%
Productivity Costs			
Lower Productivity - Non-Manual Activities	1,938,632	625	
Lower Productivity - Manual Activities	24,273,274	12,857	
Lower Productivity - Mortality	3,230,218	40,070	
Total for Productivity	29,442,124	53,552	15.97%
TOTAL COSTS FOR ETHIOPIA in 2009		**55,468**	**16.54%**

ETB 55.5 Billion/ USD 4.7 B

- ***Stunting consequences***

Stunting has a long and short term negative consequences. The short-term consequences of stunting are increased risk of infectious diseases, limited intellectual capacity and physical growth(McDonald *et al*., 2013; Abubakar *et al*., 2010; Black *et al*., 2008),which will in turn diminish their working capacity during adulthood(Victora *et al*., 2008).

Obesity, diabetes (Bhargava *et al*., 2004) and disability are the long term consequences. Given that undernourished mothers are likely to give low birth weight babies, stunting may have

intergenerational effects (Kwawukume *et al*., 1993). Such vicious circle of stunting has serious health and socio-economic consequences and thus timely and effective intervention needs to be in place to break the cycle. In developing countries, stunting prevalence reaches its peak during the complementary feeding period (6-23 months) (WHO/PAHO, 2003).

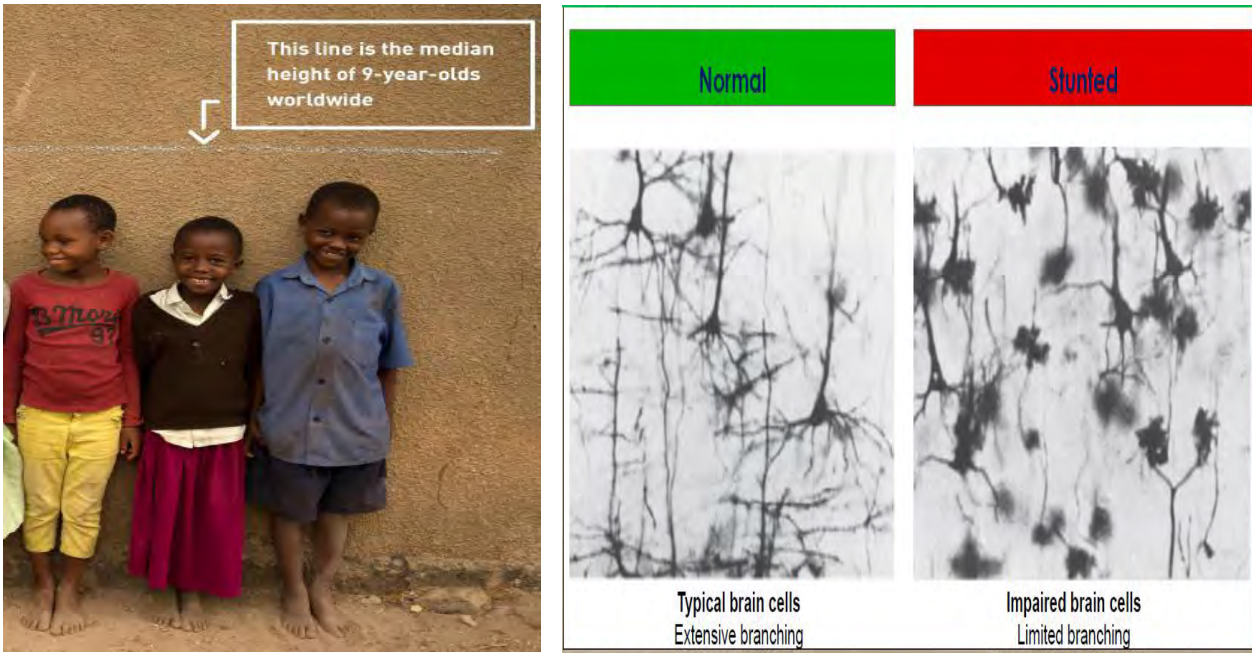

Figure 2.4 Consequences of stunting

The negative functional impairments associated with stunting are difficult to reverse after 2 years of age (Martorell *et al*., 1994). The first two years in life; therefore, represent a critical window of opportunity for the prevention of the problem (Glewwe & King, 2001), and thus proper complementary feeding practices needs to be assured.

2.1.2.2 Micronutrient deficiency

An estimated two billion people are affected by a chronic deficiency of essential vitamins and

minerals / hidden hunger (MI, 2009). Iron, zinc, vitamin A are among the most widespread deficiencies globally(Muthayya *et al*., 2013)**.** Prevalence of anemia is 43 percent among children 6–59 months of age (Stevens *et al*., 2013), while vitamin A deficiency affected close of 30% of the children in the same age group (Stevens *et al*., 2015). Deficiencies of Vitamin A and zinc alone are responsible for 0.6 and 0.4 million child deaths respectively (Muthayya *et al*., 2013).

The problem is more serious in low income countries (Muthayya *et al*., 2013). In these countries, children are frequently affected by multiple micronutrient deficiencies (Allen *et al.*, 2009), which accounts for close to 7% of the global disease burden annually(WHO, 2004). The predominantly plant-based complementary diets with little animal source foods (ASF), fruits and vegetables, as commonly consumed in low income countries, are associated with poor growth and micronutrient deficiencies (Mcevoy *et al*., 2012). Therefore, timely intervention that improve complementary feeding are needed to circumvent the short and long-term adverse effects associated with micronutrient deficiency (De Onis *et al*., 2013).

2.1.3 Strategies to improve Undernutrition

2.1.3.1 Nutrition education

Proper feeding practices of children is affected by caregivers' nutrition knowledge *(*Saloojee *et al.*, 2007). Poor feeding practices, such as low dietary diversity, feeding frequency, and inadequate energy intake (FAO, 2010), may correlate with caregivers' poor knowledge on nutrition and food diversity in their environment (Saloojee *et al*., 2007). Therefore, promoting appropriate feeding practices can reduce child malnutrition and mortality (Butte *et al*., 2002).

Nutrition education can clear cultural and tradition-based misconceptions and improve general nutrition knowledge among caregivers (Shi & Zhang, 2011). Knowledge improvement can be achieved if the caregivers are educated and counseled by knowledgeable health workers and if their progress is monitored closely (Pelto *et al*., 2004). Generally, nutrition training for health workers can improve healthy feeding behaviors (Sripaipan *et al*., 2002), better feeding frequency (Roy *et al*., 2005), dietary diversity (Penny *et al*., 2005), and better protein, and energy intake (Palwala *et al*., 2009; Vitolo *et al*., 2010) during the complementary feeding period (Sunguya *et al*., 2013). However, it was reported that, 76% of caregivers, who have already taken training on proper IYCF practices in Ethiopia, are not observed to practically apply the knowledge (Baye *et al*., 2013). The causes of the problem could be, inadequate training given to the health extension workers, improper delivery of the message or low adoption rate, which need further investigation. Also, the association between health extension workers knowledge sharing effectiveness with caregivers knowledge and child nutritional status is not well studied.

2.1.3.2 Proper caregiver feeding style

Maternal and child care practices has been given due attention in UNICEF's conceptual framework for determinants of nutritional status (UNICEF, 1990). Caregiver-child interaction (feeding style) is one of the components of caring practicesin addition to sufficient food supply at the household level, access to health services, and a clean environment (UNICEF, 1990).

According to Birch & Fisher (1995), feeding style can be defined in to controlling, laissez-faire, and responsive feeding. Caregivers regulate child feeding without responding to hunger and satiety cues in the case of controlling feeding style (Birch & Fisher, 1995). Also, the caregiver

has complete control of when, what, and how much the child eats. In addition, it includes restriction, in which the parent tries to limit intake, even if the child is hungry. The restriction is because of the perception that infants could not recognize their own hunger or satiety (Gross *et al*., 2011). Furthermore, the controlling feeding style includes force feeding the child, in which the parent encourages intake, even if the child is not hungry. Force feeding behavior is usually due to the perception that the baby's appetite is less than other babies (Gross *et al*., 2011). As a result, some children who experience a controlling feeding style regulate their eating based on external feeding cues rather than responding to internal feeding cues (Birch & Fisher, 1995). This makes the children unable to self-regulate their energy intake."Laissez-faire" feeding style is characterized by the caregiver's little effort to encourage eating (Birch & Fisher, 1995). In this feeding style, feeding is not encouraged even when the child may be at nutritional risk.

Table 2.2: Feeding style categories with corresponding caregiver characteristics description(Birch & Fisher, 1995)

	Caregiver's characteristics description
Feeding style	
Controlling	• Regulates child feeding • No response to child hunger and satiety cues • Control when, what, and how much the child eats completely • Force feed & restricts child feeding
Laissez-faire	• Little effort to encourage eating • Expects the child to eat by themselves
Responsive feeding	• Responds to the child's cues in a reasonable time • Synchronous coordinated interaction with child

Rather the caregiver expects the children to eat by themselves, at early age, without assistance. This feeding style reflect the belief that children know how much they should eat and the child's stomach knows its limit (Engle *et al*., 2000). On the other hand, responsive feeding is defined as a condition in which "the caregiver is in close proximity to the young child during the meal and responds to the child's hunger cues in a reasonable time" (Birch & Fisher, 1995). Responsive feeding requires a three-step process, whereby a mother observes the child, interprets the cue or state of the child, and then acts in accordance with the intended meaning of the cue (Eshel *et al*., 2006). It is the result of applying the principle of psychosocial care, drawn from the field of developmental psychology, to the feeding situation (Engle *et al*., 2000; Engle, 1999).

In responsive feeding, the caregiver responds to a child's cues in a contingent and appropriate manner. There will be synchronous coordinated interaction between the caregiver and the child who respond to each other. There is increasing recognition that feeding behaviors and styles could influence acceptance of food and dietary intake on top of the dietary aspect of child feeding (Ruel *et al*., 2003; Gittelsohn *et al*., 1998). This in turn affects the growth of infant and young children. In some developing countries, low levels of responsive feeding, high levels of forceful or controlled feeding is observed (Ha *et al*., 2002; Engle & Zeitlin, 1996). However, these feeding styles are associated with fewer mouthfuls of food taken by the child and more refusals. The refusal is despite the child's malnourished state. In these countries, mothers' feeding style is likely to contribute to the poor appetite and nutritional status of young children.

A 2006 study in Bangladesh (Moore *et al*., 2006) observed a delayed self-feeding beyond 24 months, similar to other developing countries. This is despite children's acquired psychomotor

abilities to feed themselves partly by 9 months (Engle *et al*., 2000). Furthermore, mothers rarely responded to refusals by asking if the child wanted water, another food, or a slower pace of feeding. Rather, they resorted to temporary diversions of the child's attention, promises, threats, following the child around the room, and sometimes forceful feeding. These strategies are unlikely to help the child develop a healthy appetite, recognition of hunger and satiety cues and properly paced self-feeding. Even though, it might lead to short-term fulfillment (Moore *et al*., 2006).

Based on the current available information, researchers and policy makers began incorporating the concept of responsive feeding (RF) into the scientific literature (Engle *et al*., 2000) and nutrition programming (UNICEF, 1997). Study reported that, proper child-caregiver interaction at mealtimes is significantly associated with appetite and eating behavior (Quijada & Gutiérrez, 2012). The two can be modified through proper child-caregiver interaction. Proper child-caregiver interaction proved to bring significant improvements in children's self-feeding and mothers' verbal responsiveness (Aboud, 2009; Aboud *et al*., 2008) and generate a positive impact over the child appetite. This could improve the food consumption and prevent malnutrition. Therefore, appropriate responsive feeding intervention study should be carried out to check the possibility of improving the much lower quantity of food (9g/kg/meal) consumption per meal (Baye *et al*., 2013) of Ethiopian children, than the theoretical gastric capacity (30 g/kg/ meal) through improved appetite.

2.1.3.3 Proper complementary foods

Any non-breast milk foods or nutritive liquids that are given to young children, after six months of exclusive breastfeeding, are defined as complementary foods (WHO/PAHO,

2003).Complementary feeding is the process of introducing the complementary foods. Proper complementary food has adequate energy and micronutrients (WHO, 2000), such as, iron, zinc, calcium, vitamin A, vitamin C and folates. In addition, it is free of pathogens, toxins or harmful chemicals, and is without much salt or spices. It is also, convenient for the child to eat and is easily accepted. Furthermore, it is acceptable by most families and can be prepared easily from family foods with relatively lower cost (WHO, 2000).

Timely introduction of appropriate complementary foods promote good health, nutritional status and growth of infants and young children (Michaelsen *et al*., 2000). Early introduction of complementary foods displace breast milk (Butte *et al*., 2002; Villalpando, 2000). The practice may also result in problem of slow growth, introduce infections into the gastrointestinal tract (Kalanda *et al*., 2006; Hop *et al*., 2000). The problem will occur because the infant's gastrointestinal system is immature before six months (Butte *et al*., 2000). In addition, the micronutrients in the complementary foods are not as well absorbed as those in breast milk (Trowbridge, 2002). Therefore, the transition to complementary foods should be slow and continuous to avoid diarrhea. Similarly, late introduction of complementary foods is disadvantageous too, as it increases the risk of malnutrition and micronutrient deficiency (WHO/PAHO, 2003; WHO, 1998), and stops or slows down growth.

- ***Macronutrient requirements and frequency of complementary feeding***

Estimations of the appropriate energy density of complementary foods are always made under consideration of the feeding frequencies, the child's age, and its intake of breastmilk. The energy requirement estimated for healthy breastfed infants is of approximately 615 kcal/day from 6 to 8 months of life; 686 kcal/day from 9 to 11 months; and 894 kcal/day from 12 to 23 months

(Daelmans *et al*., 2003; Dewey & Brown, 2003). With an average breastmilk intake, complementary foods are estimated to provide close to 200, 300 and 550kcal a day for children aged 6 to 8; 9 to 11 and 12 to 23 months in developing countries (WHO/PAHO, 2003). On the other hand, it provide 130, 310 and 580 kcal a day at 6 to 8; 9 to 11; and 12 to 23 months age respectively in developed countries, because of the difference in the average breastmilk intake and its fat content.

Table 2.3: Age-specific energy requirements for complementary feeding of breastfed children with average breastmilk intake (WHO/FAO, 2004)

Age group [months]	Total energy requirement	Milk energy intake [average]	Energy required from complementary foods
6 to 8	615	413	202
9 to 11	686	379	307
12 to 23	894	346	548

To achieve the recommended intake the feeding frequency and energy density of the complementary foods should be adequate. A complementary food with an energy density of <0.6 kcal/ g is generally considered low (Dewey & Adu-Afarwuah, 2008). In developing countries, complementary foods are usually low in energy density (Baye *et al*., 2013). Therefore, the energy requirement of the children should be satisfied with frequent feedings. With average breastmilk intake and assumed gastric capacity of 30g/Kg body weight/d, and with a minimum energy density of complementary foods (0.8Kcal/g), children aged 6 to 8 and 9 to 24 months

should be provided meals 2-3 and 3-4 times a day respectively. Additional snacks, easily prepared finger food that can be eaten by children alone, should also be provided 1 to 2 times per day (Dewey & Brown, 2003)

With a gastric capacity of 30 g/kg body weight, an assumed functional gastric capacity is calculated as 249 g/meal at 6-8 months of age, 285 g/meal at 9-11 months and 345 g/meal at 12-23 months (FAO/ WHO/ UNU, 2004). If energy density is lower than 0.8 kcal/g, a child consumes a lower amount of food than the assumed gastric capacity, or if the child is no longer breastfed, then more frequent meals would be required. For developing countries, average energy intake from breastmilk intake of healthy children is estimated to be 413, 379 and 346 kcal per day at 6-8, 9-11 and 12-23 months respectively (WHO, 1998). Several studies have been showing that offering higher energy density diets promoted a greater energy intake *(*Darling *et al*., 1995; Mensah *et al*., 1995). Similarly, higher frequency of food offering with the same energy density resulted in the higher total daily energy intake of the children (Brown *et al*., 1995).

Macronutrients, lipids, proteins and carbohydrates are a good source of energy in the stage of rapid growth. They are needed in large proportion for tissue deposition and they form vital components of body functions. In addition, they are crucial to a healthy diet. Protein content (grams of protein per 100 kcal of food) for complementary foods should be 0.7 g/100 kcal, from 5 to 24 months (Dewey *et al*., 1996). In most countries, the protein requirements of infants are met when the energy intake is appropriate, except if there is a predominant intake of low protein foods. The *protein* requirements of infants are, like those of the lipids, about three times higher

than in adults (per kg of bodyweight). The protein needs from complementary foods increases from 21% at 6–8 months to about 50% at 12–24 months (Dewey & Adu-Afarwuah, 2008). For young children, about 20 to 45% of protein should be covered by complementary foods (WHO, 1998).

Lipids provide essential fatty acids and fat soluble vitamins. Also they enhance dietary energy density and sensory qualities. The fat content of complementary foods becomes more important as breastmilk intake declines with age (Dewey & Adu-Afarwuah, 2008). The fat needs at 12-24 months can reach up to 13 g/day. The quality of the fat may be even more important than the quantity (Dewey & Adu-Afarwuah, 2008). For children in developing countries, consuming an average amount of breast milk with a fat concentration of 38 g/l, from complementary foods should cover 0-34% of energy at 6-8 months, 5-38% at 9-11 months and 17- 42% of energy at 12-23 months (FAO/ WHO/ UNU, 2004). Micronutrient needs are also high during the first 2 years of life, to support the rapid rate of growth and development during this period.

- ***Micronutrient composition of complementary foods***

Complementary foods should be chosen carefully to ensure the adequate intake of micronutrients, especially iron, Zinc, Vitamin A, and Vitamin B6 (FAO & WHO, 2004; Butte *et al*., 2002). The quality of the introduced foods defines their amount and bioavailability. Assuming an average intake of breast milk, complementary foods should provide about 5 to 30% of the vitamin A, 50 to 80% of the thiamin, 50 to 65% of the riboflavin, 60% of the calcium, 85% of the zinc and almost 100% of the iron requirements of children six to 23 months (WHO/FAO, 2004; WHO, 1998) . Vitamin C and folate are estimated to be covered by breastmilk; vitamins B6 and B12 depend highly on the mother's status (WHO, 1998). Although,

zinc and iron are essential for a healthy diet and hence normal growth, it is widely recognized that their requirements may be difficult to meet from complementary foods. This problem is common especially in developing countries like Ethiopia, where mostly non-fortified plant based foods are consumed (Baye *et al*., 2013; Gibson *et al*., 2009).

Table 2.4: Recommended Zinc and Iron nutrient intakes (RNI) in mg/day for infants and young children (WHO/FAO, 2004)

Age group	Bioavailability						
(months)	15%	High	12%	Moderate	10%	Low	5%
	Fe	Zn	Fe	Zn	Fe	Zn	Fe
6-12	6.2	0.8	7.7	4.1	9.3	8.4	18.6
13-36	3.9	2.4	4.8	4.1	5.8	8.3	11.6

- ***Micronutrient powders***

Home fortification of foods with powders containing multiple micronutrients has been suggested as an alternative to increase the vitamin and mineral intake in children 6–23 months of age. This intervention consists of the addition of a mixture of micronutrients in powder form to any semi-solid food. The mixture is provided in single-serving sachets, the contents of which are simply sprinkled over the food before consumption (Zlotkin *et al*., 2005). With this intervention, foods can be fortified either in the home or in any other place where meals are to be consumed; thus it is also referred to as "point-of-use fortification" (De-regil *et al*., 2011).

Table 2.5: Recommended content of micronutrient powder per dose for children 6 to 59 months old (HF-TAG, 2015)

Micronutrients	**Amount per dose**
Vitamin A	400 µg RE
Vitamin D	5 µg
Vitamin E	5 mg
Vitamin C	30 mg
Thiamine (vitamin B1)	0.5 mg
Riboflavin (vitamin B2)	0.5 mg
Niacin (vitamin B3)	6 mg
Vitamin B6 (pyridoxine)	0.5 mg
Vitamin B12 (cobalamin)	0.9 µg
Folate	150 µg6
Iron	10 mg
Zinc	4.1 mg
Copper	0.56 mg
Selenium	17 µg
Iodine	90 µg

In recent years, point-of-use fortification of complementary foods with multiple micronutrient powders (MNPs) has received growing attention as a promising approach to tackle micronutrient deficiencies (Dewey *et al*., 2009; WHO/FAO, 2006). The practice has been in use in several countries (Bhutta *et al*., 2008), and following promising results in reversing micronutrient

deficiencies (de Silva *et al*., 2003; Stoltzfus *et al*., 2001), it is now being scaled up in various countries.

Table 2.6: Micronutrients at the core of survival, development and health (MI, 2009)

Micronutrient	**Impact through programmes**
Vitamin A	• 23% reduction in under 5 mortality rate • 70% reduction in childhood blindness
Iodine	• 13% increase in IQ
Iron	• 20% reduction in maternal mortality
Zinc	• 6% reduction in child mortality • 27% reduction of diarrhea incidence in children

However, there are some evidences about the health risk associated with untargeted micronutrient supplementation to preschool children (Ojukwu *et al*., 2010; Sazawal *et al*., 2006). Diarrhea (Zlotkin *et al*., 2001), respiratory morbidities and dysentery *(*Lazzerini*, 2014;* Soofi *et al*., 2013; Sazawal *et al*., 2006; Shankar, 2000) are among the possible health risks associated with excessive intakes. Hence, there is need for good characterization of prevailing complementary feeding practices, knowledge of the nutrient adequacy of complementary foods, and the type and nutrient gaps to be filled with MNPs.

2.1.3. Breastmilk nutrient composition

Breast milk contains multiple nutrients in significant amounts (Picciano, 2001). The protein, fat and carbohydrate as well as vitamin and mineral composition of the human milk support healthy growth and development of children (Oftedal, 2012). Also, other important components in the

breastmilk (Wagner *et al*., 1996) protect the infant from infection and help immune system development.

Breastmilk is the primary source of nutrition for newborn children. For the first six months of life, it is suggested as the only source of nutrient for feeding term infants (WHO/PAHO, 2003). Breastfeeding may be continued for older infants and toddlers either solely or in combination with other foods (WHO/PAHO, 2003), because, it continues to provide substantial amounts of protein, fat, and most vitamins well beyond the first year of life (Dewey, 2001). The sources of nutritional components of human milk are originated from synthesis in the lactocyte, diet, and maternal stores (Sauerwald *et al*., 2001).

The concentrations of nutrients in breast milk are relatively constant. However, some components may vary both within and between individuals (Nommsen *et al*., 1991), as they are affected by many factors. In general, factors affecting breastmilk composition are: the timing of delivery (pre-term vs. term delivery), maternal diet, body weight, period of lactation, time of the day (Allen, 2012; Kang-Yoon *et al*., 1992), and socio-economic status (Qian *et al*., 2010; Al-tamer & Mahmood, 2006). Vitamins A, B1, B2, B3, B6, B12, C, and D, fatty acids, and iodine are at least partially dependent on maternal diet and body stores. Whereas, calories, protein, folate, minerals, and trace element content of breast milk are independent of maternal diet (Allen, 2012; Kang-Yoon *et al*., 1992). After 4 months postpartum, the macronutrient concentrations of human milk is associated with maternal body weight for height, protein intake, return of menstruation, and nursing frequency (Davis *et al*., 1991).

- ***Energy and specific nutrient composition of breastmilk***

The energy composition of breastmilk is dependent on its protein, carbohydrates and lipid content, similar to other foods (Meurant, 1995). Protein and carbohydrate concentration of human milk are relatively invariable between women at any given stage of lactation, although, there is a slight change with duration of lactation. Mothers who deliver prematurely will produce milk with higher protein (Agostoni *et al*., 2010), and it will decrease over weeks after birth (Saarela *et al*., 2005). An increase in energy (65 to 70 kcal/dl), protein (0.9 to 1.2 g/dL), lactose (7.4 to 7.8 g/dL,) and fat (3.2 to 3.6 g/dl) from birth to three months of postpartum was reported *(*Wojcik *et al*., 2009). Breastmilk energy content also varies from the start to the end of a feeding, and allows a diurnal pattern in both term (Khan *et al*., 2013; Kociszewska *et al*., 2012) and pre term milk (Kociszewska *et al*., 2012; Lubetzky *et al*., 2006).

- ***Micronutrient composition of breastmilk***

Breastmilk micronutrients categorized in to group I and group II nutrients (Allen, 1994). Group I nutrients include thiamin, riboflavin, vitamin B-6, vitamin B-12, choline, retinol, vitamin A, vitamin D, selenium, and iodine. And, group II nutrients arefolate, calcium, iron, copper, and zinc. Maternal depletion quickly and significantly reduces secretion of group I nutrients in breastmilk (Allen, 2012). Therefore, they are the most interest in public health nutrition. Breastmilk concentrations of group I nutrients and child status can be improved through maternal supplementation. On the other hand, maternal intake and status does not affect group II nutrients. However, the mother gradually becomes depleted when intake is less than the amount secreted in milk. Hence, maternal supplementation benefits the mother rather than the infant (Allen, 2012).

Table 2.7: Composition of some of the key nutrients found in mature breastmilk (NHMRC, 2003**)**

Component	Mean value for mature breastmilk (per 100mL)
Energy (kJ)	280
Energy (kcal)	67
Protein (g)	1.3
Fat (g)	4.2
Carbohydrate (g)	7
Sodium (mg)	15
Calcium (mg)	35
Phosphorus (mg)	15
Iron (mcg)	76
Vitamin A (mcg)	60
Vitamin C (mg)	3.8
Vitamin D (mcg)	0.01

- ***Calcium Iron and Zinc***

Calcium concentration of human milk is 250-300mg/l. And the concentration does not significantly change during lactation (Meurant, 1995). Also, the calcium concentration is not affected by maternal diet. However, it was reported that poor calcium diet produced milk with lower calcium concentration (Laskey *et al*., 1990) and the concentration did not increase with calcium supplementation (Prentice *et al*., 1997). Unlike calcium, the concentration of iron in human milk declines with time (~0.4–0.8 mg/l in colostrum; ~0.2–0.4 mg/l in mature milk)

(Meurant, 1995), and it is homostatically controlled in the mammary gland (Sigman & BoLonnerdal, 1990). Hence, iron is not affected by maternal iron status or diet. Similarly, the concentration of zinc also declines quickly from 4–5 mg/l in early milk, to 1–2 mg/l at 3 months postpartum, and to ~0.5 mg/l at 6 months (Meurant, 1995). Zinc content of human milk has not been shown to be affect by maternal dietary intake and reported to be resistant to Zinc supplementation (Krebs *et al*., 1995; Moser-Veillon & Reynolds, 1990).

- ***Vitamin A and carotenoids***

Breastmilk is a good source of vitamin A for young children. Preformed vitamin A in breast milk primarily occurs as retinyl esters (mainly retinyl palmitate) (Stoltzfus & Underwood, 1995), and a small fraction occurs as free retinol. Pro-vitamin A carotenoids are also present in breastmilk (Canfield *et al*., 2003). However, there is lack of knowledge on the bioconversion of carotenoids in infants (Panel, 2014), hence it cannot be taken into account in estimating the vitamin A supply to infants. The highest BMVA concentration is found in colostrum in the first week and in transitional milk in the next 2 or 3 weeks (Stoltzfus & Underwood, 1995; Newman, 1994).

Maternal dietary intake determines BMVA concentration after the first three weeks (Valentine & Wagner, 2013; WHO, 2002), and an infant vitamin A status (Haskell & Brown, 1999; Ortega *et al*., 1997). Well-nourished lactating mothers usually have stable vitamin A concentrations (Stoltzfus & Underwood, 1995), while deficient mothers may have lower concentrations (Newman, 1994). A lactating mother should meet the increased vitamin A requirements from diet. Otherwise her body would try to compensate the increased requirement by drawing vitamin A stores in the liver (Allen & Haskell, 2001).

Factors that can affect breastmilk fat concentrations may also affect the BMVA concentration (Stoltzfus & Underwood, 1995). This is because almost the entire Vitamin A in breastmilk is found in fat. Nevertheless, BMVA concentration is not related to breastmilk fat concentration during the first weeks of lactation (Macias & Schweigert, 2001). Fat concentration is higher in mature milk than in colostrum, and it is higher in breastmilk of mothers from developed countries than from developing countries counterparts. Similarly, higher BMVA concentration is found in well-nourished mothers than poorly nourished mother in the same region (Ruel *et al*., 1997). Mean vitamin A concentrations in breastmilk of subjects from developed country is 2.5 µmol/L and it is 1.9µmol/L in subjects in a developing country (Newman, 1994). However the estimated average BMVA concentration of mature milk is 1.75 µmol/L (Underwood, 1994). The recommended daily safe level of vitamin A intake for young children is 1.4 µmol (WHO, 2002).

Carotenoids are pigments produced by photosynthetic organisms, and are structurally related (Olson & Krinsky, 1995). They can be classified in tocarotenes and xanthophylls, based on their chemical composition. Carotenes are hydrocarbons, containing only carbon and hydrogen atoms, while xanthophylls also contain oxygen. High concentration of carotenes found in photosynthetic tissues and play important role in harvesting light and as protective agents (Nagao, 2009; Britton, 1995). They are important substances in human food sources, particularly in fruits, vegetables and plant greens and provide many health benefits. But they are not classified and as essential nutrients (Omoni & Aluko, 2005; Wright *et al*., 2003). Carotenoids play role in child growth and development through their antioxidant property and are known to be associated with child nutritional status (Dancheck *et al*., 2005; Granot & Kohen, 2004). They also protect cells

from free radicals produced during metabolism. β-carotene, α-carotene, and β-cryptoxanthin are carotenoids with provitamin A activity (Eroglu & Harrison, 2013).

Breastmilk is a good source of carotenoid (Song *et al*., 2013; Ballard & Morrow, 2013) and its content is associated with maternal diet (Canfield *et al*., 2003) and plasma carotenoid/vitamin A status (Schweigert *et al*., 2004; de Azeredo & Trugo, 2008). Carotenoid content of breastmilk varies by country/region since it is dependent on dietary pattern. Hence, reference values for individual countries are needed (Canfield *et al*., 2003).

- ***BM VA as a marker of vitamin A status***

Dietary intake assessment can provide evidence of risk of inadequate status of vitamin A. However, quantitative collection of dietary information is fraught with measurement problems. The problems arise from obtaining representative quantitative dietary histories in bio-availability, preparation losses, and variations in food composition data among population groups (Rodriguez-amaya, 1997). The quantitative estimation is especially difficult in populations consuming most of their dietary vitamin a from pro vitamin A carotenoid sources.

Hence, BMVA concentration can be used as a good indicator of vitamin A status in three target populations: lactating women, breast-fed infants, and young children (1-3 years of age) (Stoltzfus & Underwood, 1995).The concentration is related to liver vitamin A store. The relationship between BMVA concentration and liver vitamin A store is stronger when a vitamin A store in the later is low. Therefore, milk vitamin A concentration could be a useful indicator to individuals or populations whose liver stores are low.

In developing countries, complementary foods are low in vitamin A and make minor contribution to total intake (Baye *et al*., 2013; Gibson *et al*., 2009). And, it is estimated that children could get 40 to 60% of their daily vitamin a requirement from breastmilk during the complementary feeding period (Ross & Harvey, 2003). Hence, assessment of BMVA concentration equates to assessment of vitamin A intake of breastfed children (Stoltzfus & Underwood, 1995). Vitamin A in breastmilk is highly bioavailable; therefore, the link between intake of breast milk and child vitamin A status is relatively strong.

- ***Measurement of BMVA using iCheck FLOURO***

It is well established that BMVA is a good indicator of maternal vitamin A status and intake, as well as vitamin A intake of the breastfed infant (Stoltzfus & Underwood, 1995). Nevertheless, the standard method used to determine BMVA, i.e HPLC method requires sophisticated equipment and well-trained technicians and substantial financial and time resources. This will make monitoring of interventions targeting BMVA difficult in developing countries. Thus, simple low-cost, point of contact methods are desirable to reduce the cost and technical burden of sample handling and analysis, and to provide rapid results.

The iCheck FLUORO (Bioanalyt GmbH, Teltow, Germany) is a portable fluorometer, used to perform rapid, quantitative analysis of vitamin A from different matrixs, i.e breastmilk, serum/plasma retinol, VA-fortified foods. These characteristics make the iCheck method an attractive procedure, both for use in epidemiological surveys focusing on measuring the retinol content of breastmilk samples, and for the dairy industry. It enables to do the analysis in a simple laboratory and/or determination can be carried out under field condition (Engle-Stone *et al*., 2014). The device has been observed to be as effective as HPLC method to measure vitamin A

concentration in fortified foods (Laillou *et al*., 2014), cow milk and human milk *(*Schweigert *et al*., 2011). However the device should further be evaluated among different population groups especially where low BMVA concentration is prevalent (Engle-Stone *et al.*, 2014).

2.1.4 Complementary food & feeding practices in Ethiopia

Limited data from some parts of Ethiopia indicated that, complementary feeding practices are suboptimal from several perspectives. In some cases complementary foods are introduced earlier than what is desirable; in other cases, their introduction is inappropriately delayed (Baye *et al*., 2013; Gibson *et al*., 2009). Very few children received solid/semi-solid foods for the recommended minimum number of times, containing the recommended number of food groups (Gibson *et al*., 2009). Responsive feeding is not practiced, and animal products were seldom consumed (Baye *et al.*, 2013; Gibson *et al*., 2009). Intakes of energy and densities of micronutrients from complementary foods are below WHO recommendations (Baye *et al*., 2013; Gibson *et al*., 2009).

Similar to other developing countries, more than half of the children aged 6-23 months in Ethiopia, consume foods made from cereals more often than any other food groups (CSA/ICF, 2012). Cereal based complementary foods (especially unrefined) contain low amount of protein, have problem of starch digestibility and mineral bioavailability; they also contain high antinutritional factors (Gibson *et al*., 2006; Negi *et al*., 2001). The antinutritional factors are known to inhibit the absorption of some major and trace minerals such as iron, zinc, calcium (Manary *et al*., 2002; Gibson *et al*., 1998). The inhibition can have far-reaching adverse consequences on growth, health, and cognitive development during childhood.

It is reported that consumption of iron-rich foods in rural areas is only 12 percent while it is around 22% in urban areas (CSA/ICF, 2012). This problem will be exacerbated by low body stores at birth because of prematurity and/or low birth weight, induced by poor maternal nutritional status during pregnancy (Gibson, 1994). In addition, the dietary quality of lactating mothers is very poor (Haileslassie *et al*., 2013) in the country. However, how much the poor quality diet would affect breastmilk quality is not known.

There is also no adequate data on child feeding behaviors. A previous study indicated that responsive feeding has been a commonest feeding style used caregivers in southern part of Ethiopia (Wondafrash *et al*., 2012). The result is different from what has been determined by other researchers in the country (Baye *et al*., 2013; Gibson *et al*., 1998). The different report might be due to the different methodologies used to study the outcome of interest, in which the study using self-report might be more liable to bias (Wondafrash *et al*., 2012) however, this contradicting result needs to be verified using structured observation.

Chapter 3-*Materials/Subjects & methods*

Chapter three: Materials/subjects and Methods

The present thesis had four major parts. The first part was a nutrition education study where IYCF knowledge of mothers and HEWs was investigated. The second part was an observational study where the feeding practices and behaviors of caregivers of young children were characterized. The third part of the study was a field study where the dietary intake of young children (12 to23 months) was assessed using a cross sectional two in home 24h recalls, and the prevalence of excessive nutrient intakes from MNP was simulated. The fourth part was a study on vitamin A and pro-vitamin A carotenoid composition of BM, and, agreement between the measurement of vitamin A using the HPLC and iCheck.

3.1 Study location

The study was conducted in a food secure district *Mecha* in West Gojam, Amhara region, Ethiopia. Although the district benefits from the HEP, the prevalence of stunting (42 %) is very high and exceeds the national average (40%) (CSA 2014). The inhabitants are predominantly subsistence farmers who grow maize (*Zea mays* L.), millet (*Pennisetum glaucum*), and pulses as food staples. Vegetables such as kale and potato are also grown. Teff (*Eragrostis tef.*) is also grown by many as a cash crop.

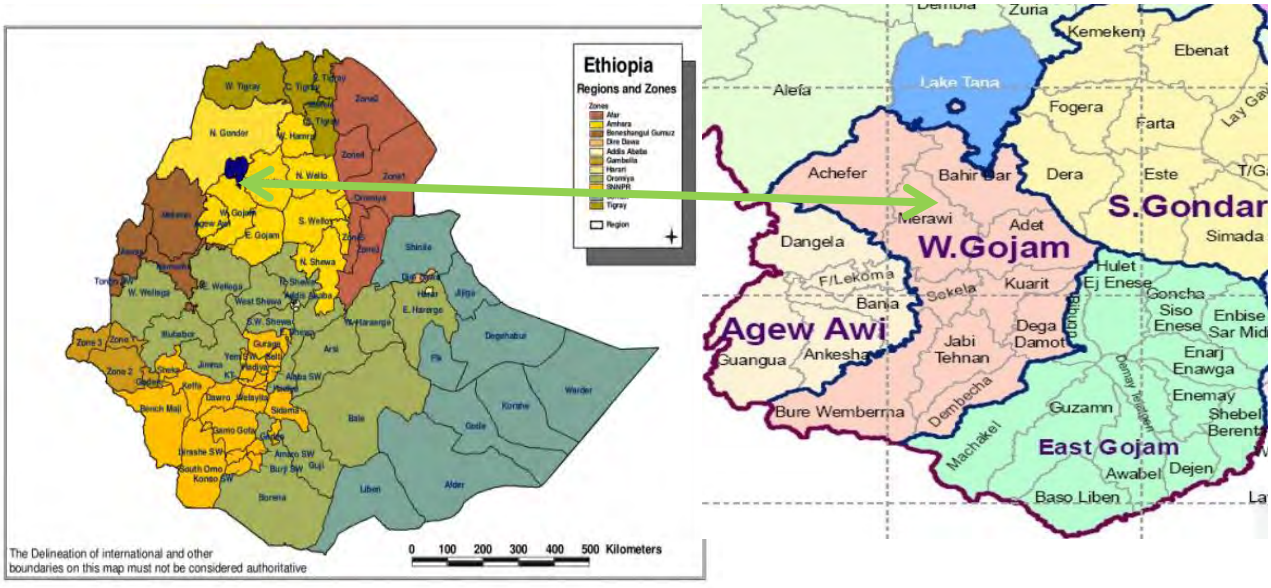

Figure 3.1: Regional map of Ethiopia showing the study site, Mecha district, in West Gojam

3.2 Ethical approval

Ethical approval was obtained from the Human Ethics Committees of the College of Natural Sciences, Addis Ababa University; and the Amhara National Regional State Health Bureau. Verbal informed consent was obtained from the HEWs and mothers of each child after the purpose and methods of the study had been explained in detail and the consent forms read to them in the presence of local health community workers and *kebele* administrators. Also a national health ethical clearance was obtained from the national health research review committee for the samples which were analyzed abroad. All questionnaire and consent forms were translated into Amharic before the survey.

3.3 Socio-demographic and anthropometric measures

The socio-demographic characteristics of the mothers were assessed using a pre-tested questionnaire that included questions on livelihood activities, education level of parents, ownership of livestock, and the size of land owned.

The length/height and weight of the children/mothers were measured in triplicate using standardized techniques, with children and mothers wearing light clothing and no shoes. To measure child length, the measuring board was placed on the ground with the child 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 child's feet. The foot plate was perpendicular to the board when the measurement was read.

Length was measured to the nearest 0.1cm. 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 pantsand 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. If the child was moving, the weight was estimated at the midpoint of the range of oscillations.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 2006b)using the software ENA 2007. Stunting, underweight, and wasting were defined respectively as LAZ, WAZ or WLZ <–2. Maternal body mass index (BMI) was calculated as weight (kg)/height (m) ^{2}.

3.4 Study protocols

3.4.1 Evaluation of IYCF knowledge of HEWs and mothers

3.4.1.1 Study participants

The study participants were HEWs assigned to the district (n = 100) and mothers (n = 122) drawn randomly (through generation of random numbers) from the database of local health centers that was completed by a census conducted before the survey. All the HEWs assigned to the district except four, who were new recruits, were included in the study. The selection criteria for including the mothers were for the mother to have at least one child aged 12-23 months, to be a permanent resident of the study area, and for the 12-23 months aged child to be apparently healthy.

3.4.1.2 Sample size determination

The projected sample size was determined from power analyses calculated to detect a medium effect size (0.5 SD difference between means (one-tailed), with an alpha of 0.05 and power of 0.80 to allow comparison of two groups in one or more of the outcomes considered (LAZ, mean knowledge scores, dietary diversity, etc). The estimated sample size was 102 (51/group), and was increased to 122 to allow ~ 15% non-response rate.

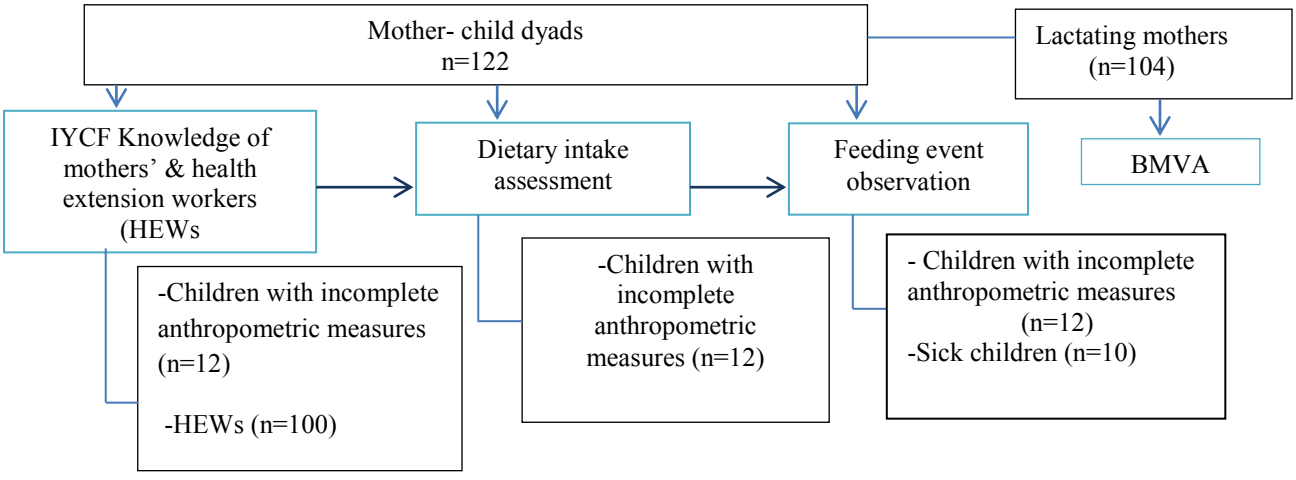

Figure 2: Schematic representation of study participants

3.4.1.3 HEWs' and mothers' knowledge of key IYCF practices

Mothers' and HEWs' knowledge of key IYCF practices was evaluated using a questionnairebased on the PAHO/WHO guiding principles for the complementary feeding of breastfed children (PAHO/WHO 2003) and the WHO's IYCF indicators (WHO/PAHO 2003). Thequestions were divided into three sets: breastfeeding, complementary feeding, and hygiene. The questionnaires were pretested and the data collected in face-to-face interviews with HEWs and

mothers. A score of "1" was assigned for correctly answered questions and "0" for incorrectly answered questions. Mothers' access to IYCF training through the HEP was documented byretrospectively asking whether or not they have received training. For each question, the scores obtained by mothers were compared to access to IYCF training and child stunting.

3.4.1.4 HEWs' knowledge-sharing effectiveness (KSE)

HEWs' knowledge-sharing was assumed to be effective if the knowledge of the trained mothersis concordant with that of the average score of the HEWs. The premise being that, under ideal knowledge-sharing practice by the HEWs, mothers should be able to attain the mean knowledge score of the HEWs. Accordingly, KSE and gap in KSE were calculated as follows:

KSE= Average score of HEW- individual score of trained mothers;
Gap in KSE= -KSE

Gap in KSE had a lower limit of 0 and values close to 0 indicated good knowledge sharing effectiveness. The association between gap in KSE and the child's LAZ was investigated.

3.4.1.5 Quality control

Before the survey, all questionnaire and consent forms were translated into Amharic and were back-translated to English by a certified translator to ensure the consistency and quality of the translation. The study employed three experienced data collectors that were at least diploma holders in nursing. The data collection was closely monitored..In addition, a two-day intensive

training followed by a pre-testing of the questionnaires was conducted before the actual survey. Data collectors were not allowed to interview more than five caregivers per day. The completeness and accuracy of the data collected was checked on a daily-basis. The questionnaires were double-entered.

3.4.2 Characterization of feeding practices and feeding style behaviors

3.4.2.1 Study participants

Hundred caregiver-child pairs participated in the study. The inclusion criteria were having an apparently healthy young child (12-23 months of age), be willing to be observed during two meal occasions, and living in the study area with no intention to leave until the study ends. This study is part of the nutrition education study. The sample size was calculated as described in previous section (section 3.2.2) from power analyses calculated to detect a medium effect size (0.5 standard deviation difference between means [1-tailed], with $\alpha = 0.05$ and power of 0.80 to allow comparison of 2 groups in one or more of the outcomes considered (e.g. stunting). This resultedin a sample size of 102 that was augmentedto 120 to allow 15% non-response rate. However, twenty dropped-out due to various reasons: child sickness that can affect food intake (n=10) and incomplete anthropometric measures (n=10).

3.4.2.2 Child feeding knowledge and practice

During the first visit, before the meal observations, mothers/caregivers were interviewed about their experiences concerning breastfeeding, feeding their child, and other caring practices. Questions were asked to gather information about feeding during and after sickness, childencouragement during feeding, mothers' perception about hunger and satiation cues, strategiesused to overcome child food refusal.

3.4.2.3 Feeding event observation

To assess caregiver child interactions, children and their caregivers were video-taped on two different days, a week apart. The feeding episode was recorded during the day time meal (lunch).

The date of the visit was not announced to the caregivers and the choice of food was free. The videographer had arrived at the participants' home before the feeding event started, and sat in a position that was not intrusive, but still allowing him to videotape the observed behaviors. Before videotaping, caregivers were instructed to feed their child as they would normally do. The families were told that the observer would return the following week again on an unspecified day. On the second visit, observations of the feeding event were repeated. Once the videotaping had completed, the recorded feeding episodes were transferred to a computer for coding and analysis.

3.4.2.3 Coding and analyses of feeding behaviors

The behavioral coding scheme was adapted from a previous study (Moore et al. 2006) with slight modification to fit the Ethiopian context. Behaviors were coded in to five categories: self-feeding, responsiveness, active feeding, social behavior and distraction. Each category had a positive and negative classification: positive meaning that it promoted feeding and negative that it was aversive, intrusive or interrupted feeding. The behaviors of both the caregiver and the child were coded in which the unit of behavior was the smallest meaningful action orword/voice. The caregiver and the child behaviors were coded similarly. The coding was performed twice and any inconsistencies were revised.

The number of times a single behavior occurred was counted. A behavior that occurred at least two times per feeding episode was recorded as present, otherwise not. Each caregiver and child received a behavioral category score reflecting the frequency with which it exhibited that behavior. Number of breast feedings, mouthfuls accepted, and rejected, duration of the feeding episode, and type of offered food were noted. Values for each variable were calculated, summed and averaged for the two visits to the household. The average values were used for the analysis. Also, caregiver and child behaviors were examined in relation to the number of mouthfuls accepted.

If the child refused the last two mouthfuls then the episode was considered to be terminated by the child. If the child accepted at least one of these mouthfuls, but the feeding was stopped, the

episode was considered to be ended by the caregiver. Children who self-fed were judged to terminate feeding themselves.

3.4.2.4 Statistical analysis

The average frequency counts of each observed behaviors were subjected to descriptive analysis. Mann-Whitey non- parametric tests was performed to detect differences in median valuesbetween groups. Categorical data were analyzed using Fisher's exact test. Bivariate correlationtests between predictor variables (caregiver and child behaviors) and outcomes of interest (number of mouthfuls accepted and LAZ) were performed. Differences between two means/medians were considered statistically significant for p-values < 0.05.

3.4.3 Estimation of risk of inadequate and excessive intakes with and without simulation of MNP intake

3.4.3.1 Sample size determination

As part of a larger study that investigated child feeding, sample size calculations were made as described in section 3.2.2 to enable comparison of two groups on various outcomes of interest including stunting. Our data and sample size allowed us to characterize the mean energy intake with a 95% confidence interval of approximately ± 30 kcal.

3.4.3.2 Dietary intake assessment

An interactive quantitative 24 h recall was conducted in-home with the caregiver of each child (n=122), using the multiple-pass technique adapted and validated for use in developing countries (Gibson & Ferguson, 2008). A second day assessment was conducted on n=40 children. All days of the week were equally represented in the final sample. 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.

A day before intake was assessed (two days before the recall). Plates and cups were supplied to the caregivers, who were instructed not to change the dietary pattern of the child on the recall day. A demonstration was given on how weighing of food will be conducted. Portion size of

foods consumed was estimated by direct weighing of salted replicas of actual foods prepared locally. Whenever found appropriate, graduated food models and common household measures were used.

3.4.3.3 Compilation of local food composition database

For the protein, Ca, Fe and Zn contents of the most commonly consumed foods were based on results of biochemical analyses conducted in our laboratory; otherwise data were compiled from the Ethiopian food composition tables (EHNRU 1998; ENI 1981; Agren & Gibson 1968)and published data(Abebe et al., 2007; Umeta et al., 2005).

3.4.3.4 Assessment of nutrient intake adequacy from complementary foods

The median daily intakes from complementary foods of 12–23 month-old children were compared with the estimated energy and nutrient intake from complementary foods (FAO/ WHO/ UNU 2004; FAO & WHO 2004; Butte et al. 2000), assuming average breastmilk intake and composition(WHO 1998; Dewey & Brown 2003).We have assumed an average intake of 549 g/d (533 ml/d), which should provide ~346 kcal energy, 5.8 g protein, 154 mg Ca, 0.2mg Fe, and 0.7 mg Zn. Nutrient densities (per100 kcal) were compared with desired values(Dewey &Brown, 2003). Median dietary diversity scores were calculated based on seven food groups(WHO 2008), and classified as low (0–2), medium (3–4) and high (>4)(WHO 2008).

3.4.3.5 Prevalence of inadequate and excessive nutrient intakes

The prevalence of inadequate and excessive nutrient intakes was estimated after adjusting for within-subject variation using the software Intake Monitoring Assessment and Planning Program (IMAPP). The cut-off point method was used to estimate the prevalence of inadequate orexcessive intakes of zinc and calcium. The approach involves calculating the proportion ofchildren in the target group with usual intakes below the Estimated Average Requirements (EAR) for inadequate intakes, or above the Upper Limit (UL) for excessive intakes (FAO & WHO 2004). For zinc, the EAR set by the International Zinc Nutrition Consultative

Group(Brown et al. 2004), and the UL set by the Institute of Medicine(Institute of Medicine. Food and Nutrition Board 2001) were used. For calcium, the EARs and ULs set by FAO were used(FAO & WHO 2004). Because of skewed intake distribution, the prevalence of inadequate intakes of iron was calculated using the full-probability approach assuming low, moderate and high bioavailability. The nutrient intakes of stunted and non-stunted children were compared.

3.4.3.6 Simulation of home-fortification of complementary foods with MNPs

WHO recommends home-fortification with MNPs in settings where the prevalence of anemia among 6-23months infants and young children is>20%. Mecha, being a candidate for such interventions, we simulated the intake of additional nutrients from MNPs. A 15 element MNP containing 10 mg of elemental iron and 4.1 mg of zinc was used for this purpose. We then estimated the prevalence of inadequate and excessive intakes as described above.

3.4.3.7. Statistical analyses

All continuous variables were checked for normality using Shapiro-Wilk test. Nutrient intakes (per day) and nutrient densities (per 100 kcal) were expressed as medians and inter-quartile range because of non-normal distributions of some nutrients. Differences in the median energy and nutrient intakes between stunted and non-stunted children were examined using the non-parametric Mann–Whitney U test (two-tailed). In all comparisons, differences were considered statistically significant when $P < 0.05$. Statistical analyses were performed using SPSS statistical software package, version 20.

3.4.4 Determination of breastmilk vitamin A and pro-vitamin A carotenoids

3.4.4.1 Sampling procedure

The study subjects were lactating mothers (n= 110) of children aged 6 to 23 months, randomly selected from ten *kebeles*-smallest administrative unit- (n =11 from each kebele). Kebeles' were selected based on their accessibility to allow proper breastmilk sample collection and handling.

The inclusion criteria were for lactating mothers to have an apparently healthy child aged 6-23 month. Six mothers were unable to provide complete sample, therefore, excluded.

3. 4.4.2 Sample collection

The breastmilk samples were collected from standardized portion of the feed. All mothers were provided two bottles. The bottles were marked at 2 mL interval and covered by aluminum foils. The mothers were requested to collect in separate tubes 6 mL of breastmilk during their morning (4-10 AM) and day-time feeding (10AM - 4PM), allowing to control within day variation in breastmilk composition (Kent et al. 2006). The mothers were instructed to collect breastmilk samples as follows: ~ 2mL of the sample before feeding (foremilk), another ~2 mL half-way through the feeding, and the remaining ~2 mL after feeding (hind milk) (Feeley et al. 1983). The samples were obtained from the right breast.

All sample collection materials were sterile. Breastmilk samples were immediately collected in a precooled ice-box and were transported to the laboratory where they were stored under -80^{0}C for five months, until they were shipped to the University of Potsdam, Germany for laboratory analyses.

3.4.4.3 Breastmilk pro-vitamin A carotenoid and retinol analyses using HPLC

Milk samples were thawed to room temperature, and then homogenized at 37^{0}C for 15 minutes (Heidolph UNIMAX 1010). The samples were vortexed (MS2-mimi shaker), and kept on sonication bath for five minutes (BANDELIN, SONOREX, RK 100). Hexane (2 mL) was added to 0.2 mL of breastmilk aliquot, and then diluted with 0.3 mL water. After homogenization with a programmable rotary mix (STARLAB) for 15 minutes, the mixture was centrifuged at 3800 rpm for 10 minutes (Labofuge 200, Heraeus sepatech). Then, the hexane extract was transferred into a separate tube with 1 mL of 0.05% butylated hydroxytoluene (BHT). The extraction was

repeated once, and the extracts were dried under nitrogen at 37^{0}C for 20 minutes. The dried extracts were reconstituted in 0.2 mL isopropanol, vortexed, and kept on a sonication bath (Bandelin, Sonorex, RK100) for 5 minutes. The mixture was then centrifuged at a speed of 5000

rpm, and the supernatant was injected in to the HPLC system (SHIMADZU) for separation and quantification of breastmilk retinol and pro-vitamin A carotenoids.

For separation of compounds a C30 carotenoid column (5μm, 250 × 3mm YMC Wilmington, USA) was used in line with C18. All solvents were HPLC grade, and the following mobile phase concentrations were used: mobile phase A-methanol: ammonium acetate (90:10; v/v) and mobile phase B- methanol: ammonium acetate: tert-butyl methyl ether (8:2:90; v/v). Compounds detected in the absorbance range of 200 to 550 nm wavelength were quantified.

3.4.4.4 Breastmilk retinol analyses using iCheck FLUORO

BMVA values were also measured according to the manufacturer's iCheck FLUORO instructions.To ensure a homogenous fat distribution, the breastmilk (0.5 ml) was swirled and immediately injected into the iExMILA reaction vials using the kit syringe. The vial was thoroughly shaken for 10 seconds, allowed to settle for 5 minutes to separate the organic and water-soluble phases, and was inserted into the portable photometer for reading. Four readings (in μg retinol equivalents (RE)/L) of the same vial from different positions were recorded (CV 1.1%) and the concentration of the breastmilk was within the linear range (50-3000 of the instrument μg RE/L)

3.4.4.5 Determination of breastmilk fat concentration

Breastmilk fat concentration was determined using creamatocrit method (Lucas et al. 1978). Microhematocrit capillary tubes (75 ×15 mm^2, outside diameter) were filled with aliquot of breastmilk samples in triplicate and were centrifuged using microhematocrit centrifuge (Hettich, zentrifugen, Germany) for 15 minutes at 12000 rpm. After centrifugation, the length of the lipid layer and the length of the entire milk "column" were measured to the nearest 0.1 mm usingvernier calipers (CV 5.6%). Lipid concentration in grams per liter was estimated from the lipid concentration by volume using a validated regression equation (Lucas, 1978).

3.4.4.6 Data analyses

All continuous variables were checked for normality using Kolmogorove-Smirnove test.Frequency counts were expressed as percentage and continuous variables' as mean ± SD. Retinol

and pro-vitamin A carotenoid concentrations were expressed as medians and interquartile range because of non-normal distribution. Differences in retinol and pro-vitamin A carotenoid concentration across lactation period were examined using the non-parametric Mann-Whitney U test. In all comparisons, differences were considered statistically significant when $P<0.05$.

The contribution of the BMVA to the daily vitamin A requirement of the children was calculated by assuming average daily breastmilk intake of 641, 598 and 533mL/day at 6 to 8, 9 to 11 and 12 to 23 months respectively (WHO 1998)and daily vitamin A requirement of 1.4 µmol (Butte et al. 2002; FAO & WHO 1998). The percentage gap between the actual BMVA concentration, with the estimated BMVA concentration i.e 1.75 µmol/l (Underwood 1994) was compared at different lactation period. The relation between the measurement of BMVA using HPLC and iCheck was assessed using spearman correlation and the mean difference between the two measurements was explained using Bland and Altman plot. Statistical analyses were performed using SPSS software package version 20.

Chapter 4 - *Results*

4. Chapter four: Results

4.1. Health extension workers' and caregivers' knowledge of IYCFand child growth

4.1.1 Introduction

In Ethiopia stunting is prevalent especially during the complementary feeding period. One of the causes of stunting is inappropriate complementary feeding practices. Previous studies have indicated that nutrition education can improve mothers nutrition related knowledge and child feeding practices. However, the effectiveness of nutrition education programs depends on many factors and complementary feeding is a complex set of behaviors. The understanding of each behavior by front-line health workers may vary and thus affect mothers' IYCF knowledge. However, to what extent HEWs IYCF knowledge affect their knowledge sharing effectiveness,mothers' IYCF knowledge, and its association to child stunting is largely unknown.

In this section (4:1) results from evaluation of IYCF knowledge of mothers and HEWs in West Gojam, Northern Ethiopia is presented in the form of published paper. Accessibility of the IYCF training to mothers was studied. The IYCF knowledge and feeding practices of trained Vs untrained mothers; and mothers of stunted and non-stunted children was evaluated. And the association between HEWs knowledge sharing effectiveness with child nutritional status was examined. Based on the findings of the IYCF knowledge evaluation, the possible influence of HEWs IYCE knowledge on mothers IYCF knowledge and their feeding practices, and the association between HEWs knowledge sharing effectiveness with child nutritional status was highlighted and recommendations that can improve the HEWs IYCF knowledge and understanding level were made.

4.1.2 Heathextension workers' knowledge and knowledge-sharing effectiveness of optimal infant and young child feeding are associated with mothers' knowledge and child stunting in rural Ethiopia

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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

Zeweter Abebe, MSc[1], Gulelat Desse Haki, PhD[2], and Kaleab Baye, PhD[1]

Abstract

Background: Little is known about how the knowledge and the knowledge-sharing effectiveness (KSE) of health extension workers (HEWs) affect maternal knowledge of optimal infant and young child feeding (IYCF) and their child's nutritional status.

Objective: The objective of this study was to evaluate mothers' and HEWs' knowledge of key IYCF practices and to investigate whether mothers' knowledge and HEWs' KSE are associated with stunting in young children (aged 12-23 months).

Methods: This cross-sectional study used face-to-face interviews to assess the IYCF knowledge of HEWs (n ¼ 96) and mothers of 12- to 23-month-old children (n ¼ 122) in Mecha district, West Gojam, Ethiopia. The association between HEWs' KSE and children's length-for-age z scores (LAZ) was investigated.

Results: Stunting (50%), underweight (34%), and wasting (10%) were highly prevalent. Less than half (45%) of the mothers had access to nutrition education through the health extension program, but those who had, had better knowledge of IYCF practices and thus lower rates of stunting ($P < .001$). However, key IYCF practices were not well understood by the HEWs and this affected their KSE. The gap in KSE was negatively associated with LAZ ($r ¼ .475$, $P < .001$) and remained significant even after adjusting for maternal height, socioeconomic status, and maternal education ($r ¼ .423$, $P ¼ .002$). Conclusion: Health extension workers' KSE is associated with child stunting. Future training of HEWs would benefit from emphasis on not only the content of the IYCF messages but also the process of delivery while increasing their counseling skills.

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Keywords
stunting, dietary diversity, complementary feeding, breast-feeding, nutrition education, health workers

Introduction

In developing countries, growth faltering (stunting) is a serious public health concern that remains unacceptably high.[1] Stunting prevalence reaches its peak during the complementary feeding period (6-23 months) mainly because of the difficulty to provide the high energy and nutrient inputs needed to sustain the child's rapid growth during this period.[2] Given the long-lasting negative consequences of early childhood stunting, nutrition interventions tar-geting the first thousand days of life starting from conception are critical.[3]

Inappropriate infant and young child feeding (IYCF) practices are among the major causes of stunting[4] and are determined by the availability of and accessibility to nutrient-dense foods, the mother's knowledge, and prevailing cultural beliefs.[5,6] Previous studies have reported that nutrition education can improve mothers' nutrition-related knowledge,[7] increase dietary diversity,[8] nutrient intake,[9] and hence the nutritional status of infants and young children.[10] Therefore, the provision of appropriately tailored nutrition education on recommended IYCF practices to mothers plays a vital role in the prevention of stunting.

However, the effectiveness of nutrition education programs depends on many factors including the knowledge and knowledge-sharing effectiveness (KSE) of health workers,[11] maternal knowledge acquisition,[12] and the resources available to enable mothers to apply the recommendations. Furthermore, complementary feeding is a com-plex set of behaviors that include the timing of the introduction of complementary food (CF), dietary diversity, feeding frequency, responsive-ness to child cues, and the safe preparation and storage of foods. The understanding of each beha-vior by frontline health workers may vary and thus affect their KSE. To what extent this affects the knowledge of mothers and ultimately the nutritional status of their child is largely unknown.

In recent years, many countries have worked on developing health systems and infrastructure that provide essential services at decentralized level. Ethiopia's health exten-sion program (HEP), which was launched in 2003, is exemplary in this regard. The program provides universal access to primary health care including nutrition through more than 38

0 government-salaried female health extension workers (HEWs). The program operates nationwide and 2 HEWs are placed per health

post to serve each kebele (smallest administrative unit *5000 habitants).

In Ethiopia, from 2005 to 2011 alone, child mortality dropped from 123 to 77 per 1000 live births, and stunting was reduced from 58% to

44%,[13] and these achievements would have been unlikely without the HEP.[14] Nevertheless, stunting rates are still very high and in some regions such as Amhara, they have not gone down as much as expected. This could partly be due to poor access to nutrition education, since nutrition is just 1 of the 16 modules that the HEWs should deliver and thus may not always be prioritized. It could also be due to the ineffective nutrition edu-cation in terms of the knowledge and KSE of the HEWs. However, limited studies are available in

this regard partly because indicators for measuring IYCF were not in place until 2008[15] and also because much of the focus was on the accessibility to the program and little on what makes it effective. This is unfortunate, given that such information could improve the effectiveness of the programs that operate at scale and thereby contribute to the reduction in stunting.

In the present cross-sectional study, data were generated on mothers' access to nutrition education, mothers' and HEWs' knowledge of key IYCF practices, and child anthropometry from Mecha district, West Gojam, Ethiopia, to evalu-ate mothers' and HEWs' knowledge of key IYCF practices and to investigate whether mothers' knowledge and HEWs' KSE are associated with stunting in young children (aged 12-23 months).

Materials and Methods

Study Site and Participants

The study was conducted in a food-secure district Mecha in West Gojam, Amhara region, Ethiopia. Although the district benefits from the HEP, the prevalence of stunting (52%) is very high and exceeds the national average (44%).[13] The inhabitants are predominantly subsistence farmers who grow maize (Zea mays L.), millet (Pennisetum glaucum), and pulses as food staples. Vegetables such as kale and potato are also grown. Teff (Eragrostis tef.) is also grown by many as a cash crop.

The participants of the present study were HEWs assigned to the district (n ¼ 100) and mother–child pairs (n ¼ 122) drawn randomly (through generation of random numbers) from the database of local health centers that was com-pleted by a census conducted before the survey. All the HEWs assigned to the district except 4, who were new recruits, were included in the study. The selection criteria for including the mother–child pairs were the mother should have at least 1 child aged 12 to 23 months, has to be a permanent resident of the study area, and the child should be apparently healthy and aged 12 to 23 months.

The projected sample size was determined from power analyses calculated to detect a medium effect size (0.5 standard deviation differ-ence between means [1-tailed], with an a of .05 and power of .80 to allow comparison of 2 groups in 1 or more of the outcomes considered [length-for-age z scores, LAZ; mean knowledge scores; dietary diversity; etc]). The estimated sample size was 102 (51 per group) and was increased to 120 to allow *15% nonresponse rate. The study was conducted from October to December 2013.

Sociodemographic and Anthropometric Measures

The sociodemographic characteristics of the mothers were assessed using a pretested questionnaire that included questions on livelihood activities, education level of parents, ownership of livestock, and the size of land owned. The socioeconomic status of the households was estimated by creating an asset index that was constructed by converting the available household resources such as livestock, size of land owned, and available household items (radio, chairs, etc) into their monetary value.

The length/height and weight of the children/mothers were measured in triplicate using standardized techniques, with children and mothers wearing light clothing and no shoes. All anthropometric measurements were made by the same person to avoid interexaminer errors. For the chil-dren, z scores for LAZ, weight-for-age z score (WAZ), and weight-for-length z score (WLZ) were calculated using World Health Organization (WHO) multicenter growth reference data[16] using the software ENA 2007. Stunting, under-weight, and wasting were defined, respectively, as LAZ, WAZ, or WLZ < 2. Maternal body mass index (BMI) was calculated as weight (kg)/height (m^2).

Health Extension Workers' and Mothers' Knowledge of Key IYCF Practices

Mothers' and HEWs' knowledge of key IYCF practices were evaluated using a questionnaire based on the Pan American Health Organization/World Health Organization (PAHO/WHO) guiding principles for the complementary feeding of breast-fed children (PAHO/WHO 2003) and the WHO's IYCF indicators.[17] The questions were divided into 3 sets: breast-feeding, complementary feeding, and hygiene. The questionnaires were pretested and the data were collected in face-to-face interviews with HEWs and mothers. A score of ''1'' was assigned for correctly answered questions and ''0'' for incorrectly answered questions. Mothers' access to IYCF training through the HEP was documented by retrospectively asking whether or not they have received training. For each question, the scores obtained by mothers were compared to access to IYCF training and child stunting.

Health Extension Workers' KSE

Health extension workers' knowledge sharing was assumed to be effective if the knowledge of the trained mothers is concordant with that of the

average score of the HEWs. The premise being that, under ideal knowledge-sharing practice by the HEWs, mothers should be able to attain the mean knowledge score of the HEWs. Accordingly, KSE and gap in KSE were calculated as follows:

KSE ¼ Average score of HEW
individual score of trained mothers;

Gap in KSE ¼ KSE.

Gap in KSE had a lower limit of 0, and values close to 0 indicated good KSE. The association between gap in KSE and the child's LAZ was investigated.

Ethical Approval

Ethical approval was obtained from the human ethics committees of the College of Natural Sciences, Addis Ababa University, and the Amhara National Regional State Health Bureau. Verbal informed consent was obtained from the HEWs and mothers of each child after the purpose and methods of the study had been explained in detail and the consent forms read to them in the presence of local health community workers and kebele administrators. All questionnaire and con-sent forms were translated into Amharic before the survey.

Quality Control

Before the survey, all questionnaire and consent forms were translated into Amharic and were back translated to English by a certified translator to ensure the consistency and quality of the translation. The study employed 3 experienced data collectors who were at least diploma holders in nursing. The data collection was closely monitored by Z.A. who was the in-field supervisor of the survey. In addition, a 2-day intensive training followed by a pretesting of the questionnaires was conducted before the actual survey. Data collectors were not allowed to interview more than 5 caregivers per day. The completeness and accuracy of the data collected were checked on a daily basis. The questionnaires were double entered. All anthropometric measures were conducted by the same person.

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 + standard deviation and in frequency counts or percentages, respectively. Differences in the proportion of HEWs and moth-ers who answered questions correctly were tested using w^2 and independent sample t test (1-tailed) for categorical and continuous variables, respec-tively. Bivariate correlation was run to investi-gate the association between LAZ and KSE. Partial correlation between LAZ and KSE, adjust-ing for maternal height, maternal education, and socioeconomic status, was also run. Parity and maternal age were not found associated with LAZ and thus were not adjusted for in the partial cor-relation. Differences were considered statistically significant for P values < .05. Statistical analyses were performed using SPSS statistical software package version 20.

Results

Sociodemographic Characteristics and Anthropometric Status

The sociodemographic characteristics of the mothers and the anthropometric measures of the mother–child pairs are presented in Table 1. The households' profile is typical of a rural population whose main occupation is agriculture (93.4%). The mean age of the mothers was 26 years and the majority (63.1%) could not read and write. The average number of children per household was 3.8. Among the children, 50% were stunted, 34% were underweight, and 10% were wasted.

Health Extension Workers' and Mothers' Knowledge of Optimal IYCF Practices

Although 66.4% of the mothers received antena-tal care 3 times, less than 20% delivered their babies in a health facility. Only 45% of the moth-ers declared they had received IYCF training and

Table 1. Sociodemographic Characteristics and Nutritional Status: Pairs (n ¼ 122) of Mothers and Young Children Aged 12 to 23 Months in Mecha District, West Gojam, Northern Ethiopia, October to December 2013.

Household head/mother characteristics	Mean + SD/ Frequency (%)
Male head of household	114 (93.4)
Household head has some formal education	51 (41.8)
Livelihood strategy—farming	114 (93.4)
Mother	
Age of mothers, years	26 + 6.1
Mothers with some formal education	37 (30.3)
Can read and write	45 (36.9)
BMI < 18.5 kg/m^2	15 (12.3)
Mothers' height 145 cm	13 (10.6)
Height of mothers, cm	152 + .06
Number of children	3.8 + 2.1
Received 3 ANC visits	81 (66.4)
Gave birth in a health facility	23 (18.8)
Already received IYCN training	55 (45)
Child characteristics	
Proportion of male children	63 (51.6)
Age of children, months	16.2 + 3.5
LAZ	2.01 +0.9
WAZ	1.63 + 0.9
WLZ	0.33 + 1.4
Stunted	61 (50)
Underweight	28 (34)
Wasted	12 (10)

Abbreviations: ANC, antenatal care; BMI, body mass index; IYCN, infant and young child nutrition; LAZ, length-for-age z score; SD, standard deviation; WAZ, weight-for-age z score; WLZ, weight-for-length z score.

about 12% had a BMI of <18.5 (Table 1). Not surprisingly, a higher proportion of HEWs than mothers answered questions concerning optimal breast-feeding, complementary feeding, and hygiene correctly (P < .001; Table 2). Almost all HEWs (>95%) and 67% of the mothers answered questions related to optimal breast-feeding cor-rectly, but fewer (50% of the HEWs and *40% of the mothers) knew the optimal duration of con-tinued breast-feeding.

Questions related to complementary feeding including questions on the optimal timing for introduction of CF, feeding strategies when a child refuses food, and feeding during and after illnesses were correctly answered by most HEWs, whereas those were correctly answered only by *70% of the mothers.

Concerning questions on optimal IYCF practices during the complementary feeding period (6-23 months), only 50% of the HEWs correctly answered questions on the optimal duration of continued breast-feeding, the minimum number of meals per day (68%), minimum dietary diversity (53%), and beverages not recommended for IYC such as sugary drinks and tea (70%). An even smaller number of mothers answered these questions correctly. On the other hand, the major-ity of the HEWs (88%) and mothers (65%) cor-rectly answered most of the questions related to hygiene (Table 2).

Knowledge of Trained and Untrained Mothers

The average score of the trained mothers was significantly higher than that of the untrained mothers (Table 2; P < .001). However, training had more effect on the ability of mothers to cor-rectly answer questions related to complementary feeding than those on breast-feeding or hygiene. Regarding questions on optimal breast-feeding, only the question on ''feeding colostrum'' was answered correctly by more trained than untrained mothers (P ¼ .02). Questions on com-plementary feeding such as ''minimum number of meals per day'' (P ¼ .04) and ''age-appropriate consistency of CFs'' (P ¼ .002) were correctly answered by a significantly higher number of trained than untrained mothers (Table 2). In con-trast, the proportion of mothers who correctly answered hygiene-related questions was similar irrespective of the mothers' access to training.

Child Feeding Practices and Nutritional Characteristics in Relation to Mothers' Training and Knowledge of Optimal IYCF

A significantly higher proportion of children of trained mothers met the minimum (3) dietary diversity requirements (P ¼ .03). Although the dietary diversity of the children was low (0-2), a significantly higher proportion of children of

Table 2. Proportion of HEWs and Mothers (Trained vs Untrained) Who Answered Questions on Optimal Infant and Young Child Feeding Correctly, and Child Feeding Practices in Mecha District, West Gojam, Northern Ethiopia, October to December 2013.

		Mothers			
		All (n ¼ 122)	Trained (n ¼ 55)	Untrained (n ¼ 67)	
	HEWs (n ¼ 96)	Frequency (%)/mean + SD			P Value
Child nutritional status					
Proportion of stunted children			20 (36)	35 (52)	.06
Breast-feeding					
BF initiation	92 (96)	66 (54)	30 (55)	36 (54)	.53
Feeding colostrum	96 (100)	78 (64)	41 (75)	37 (55)	.02[a]
Duration of exclusive BF	96 (100)	102 (84)	48 (87)	54 (81)	.23
Duration of continued BF	48 (50)	48 (39)	23 (42)	25 (37)	.40
Complementary feeding					
CF introduction	96 (100)	88 (72)	38 (69)	50 (75)	.31
Minimum number of meals per day	65 (68)	36 (30)	21 (38)	15 (22)	.04[a]
Feeding during and after illnesses	89 (93)	69 (57)	33 (60)	36 (54)	.31
Minimum dietary diversity	51 (53)	51 (42)	24 (44)	27 (40)	.42
Drinks with low nutrient density					
Consumption of coffee, tea, and soda	67 (70)	39 (32)	23 (42)	16 (24)	.03[a]
CF consistency					
Age-appropriate consistency	81 (84)	85 (70)	46 (84)	39 (58)	.002[a]
Responsive feeding					
Strategies for food refusal	95 (99)	80 (66)	41 (75)	39 (58)	.04[a]
Hygiene					
Safe CF storage	89 (93)	104 (85)	47 (85)	57 (85)	.58
Heating before serving CF	89 (93)	112 (92)	51 (93)	61 (91)	.50
Bottle-feeding	75 (78)	22 (18)	13 (24)	9 (13)	.11
Overall score (mean + SD)[b]	12 + 1.4	9.1 + 1.5	9.6 + 1.2	8.5 + 1.9	<.001[a]
Feeding practices					
Dietary diversity score (out of 7)[c]			2.3+.51	2.2+ .43	.09
0-2			34 (62)	53 (79)	.03[a]
3-4			21 (38)	14 (21)	.03[a]
5			0	0	–[a]
Minimum acceptable diet			18 (33)	11 (16)	.03

Abbreviations: BF, breast-feeding; CF, complementary food; HEWs, health extension workers; SD, standard deviation; WHO, World Health Organization.

[a]Difference between trained and untrained mothers was statistically significant according to Fisher exact test (1 tailed).
[b]Comparison of means are from independent student t test.
[c]Dietary diversity scores were calculated based on 7 food groups as described in WHO[15].

untrained mothers had low dietary diversity (P .03). Untrained mothers had a higher proportion of stunted children (52%) than trained mothers (36%; P ¼ .06).

Overall, mothers with a not stunted child had a significantly higher mean score than those with a stunted child (P < .001; Table 3). A significantly higher proportion of mothers with a not stunted child than mothers with stunted child correctly answered questions related to optimal duration of exclusive breast feeding (P ¼ .03), minimum number of meals per day (P ¼ .02), strategies when food is refused (P ¼ .007), feeding during and after illnesses (P ¼ .04), minimum dietary diversity (P ¼ 009), age-appropriate consistency of CFs (P ¼ .03), and proper storage of CFs (P ¼ .02).

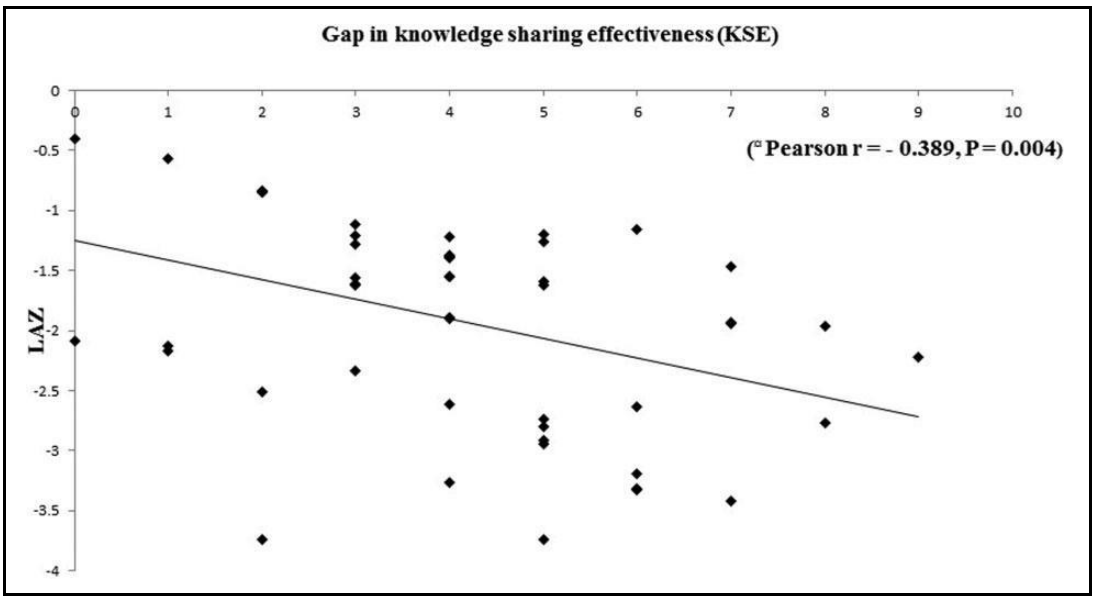

Figure 1. Association between the gaps in the health extension workers' (HEWs) knowledge-sharing effec-tiveness (KSE) and the linear growth of children of mothers trained by the health extension program (HEP). Pearson correlation coefficients and P values are those from a bivariate (unadjusted) correlation. Correlations remained significant after adjusting for maternal height, maternal education, and socioeconomic status (r ¼ .396, P ¼ .005).

Health Workers' KSE

The gap in KSE was inversely associated with the child's LAZ (r^2¼ .475, P < .001) and remained significant after adjusting for vari-ables like maternal height, maternal education, and socioeconomic status that were not affected by the HEP training but were signif-icantly associated with LAZ (r^2¼ .423, P ¼ .002; Figure 1).

Discussion

The present study investigated mothers' and HEWs' knowledge of key IYCF practices and its association with child anthropometric outcomes, in Mecha district, West Gojam, Ethiopia. The study showed that a significant proportion of the mothers have not had access to training on IYCF, but those who had, had better IYCF knowledge and practice. The study also revealed that certain key IYCF recommendations such as minimum duration of continued breast-feeding, minimum dietary diversity, and minimum acceptable meal frequency were not well understood by the HEWs. This affected the effectiveness of the knowledge transfer to mothers and was associated with child stunting.

Stunting remains highly prevalent in most developing countries like Ethiopia and poor IYCF practices are partly the cause.[18,19] Thus, the provision of nutrition education on optimal IYCF practices through the HEP could improve IYCF practices, especially in rural communities like Mecha where the HEWs are often the only source of nutrition information. Trained mothers had higher knowledge scores and better child feeding practices (Dietary diversity score and minimum acceptable diet) than untrained mothers. They also tended to have a lower proportion of stunted children. There is growing evidence that improv-ing the accessibility to and quality of nutrition education through health services can increase both mothers' knowledge and child's dietary intake, both of which can reduce the prevalence of child stunting.[9,11] Unfortunately, more than half of the mothers did not have access to IYCF training through the HEP. This highlights the

Table 3. Proportion of Mothers (%) With Stunted and Not Stunted Children Who Correctly Answered Questions on Optimal Infant and Young Child Feeding and Their Feeding Practices in Mecha District, West Gojam, Northern Ethiopia, October to December 2013.

	Mothers With Children		
	Stunted (n ¼ 55)	Not stunted (n ¼ 55)[a]	
	Frequency (%)/mean + SD		P Value
Breast feeding			
BF initiation	29(53)	30 (55)	.50
Feeding colostrum	34(62)	35 (64)	.50
Duration of exclusive BF	43(78)	51 (93)	.03[b]
Duration of continued BF	21(38)	24 (44)	.35
Complementary feeding			
CF introduction	39(71)	40 (73)	.50
Minimum number of meals per day	11(20)	22 (40)	.02[b]
Feeding during and after illnesses	27(49)	37 (67)	.04[b]
Minimum diet diversity	19(35)	33 (60)	.006[b]
Drinks with low nutrient density			
Consumption of coffee, tea, and soda	17(31)	18 (33)	.50
CF consistency			
Age-appropriate consistency	33(60)	43 (78)	.03[b]
Responsive feeding			
Strategies when food refusal	31(56)	44 (80)	.007[b]
Hygiene			
Proper CF storage	44(80)	52 (95)	.02[b]
Heating before serving CF	51(93)	51 (93)	.64
Bottle-feeding	8(15)	14 (25)	.11
Overall score[c]	8.9 + 1.6	10.1 + 1.2	<.001[b]
Feeding practices			
Dietary diversity score (out of 7)[c,d]	2.1 + 0.45	2.3 + 0.44	.009[b]
0-2	46(84)	33 (60)	.01[b]
3-4	9(16)	22 (40)	.005[b]
5	0	0	– [b]
Minimum acceptable diet	5(9)	20 (36)	.001

Abbreviations: BF, breast-feeding; CF, complementary food; SD, standard deviation; WHO, World Health Organization.
[a]Anthropometric data were incomplete for 12 participants.
[b]Difference between mothers with stunted and not stunted children was statistically significant according to Fisher exact test (1-tailed).
[c]Comparison of means are from independent student t test.
[d]Dietary diversity scores were calculated based on 7 food groups as described in WHO[15]

need to increase the accessibility to nutrition edu-cation provided through the HEP.

One key to improving the quality of the IYCF training is to increase the HEWs' knowledge. Across the globe, the quality of health workers' knowledge of nutrition has been a concern.[20,21] Similarly, the present study showed that not all aspects of the IYCF were equally understood. A significant proportion of the HEWs had difficulty correctly answering questions related to the minimum duration of continued breast-feeding, minimum dietary diversity, and minimum meal frequency. This is unfortunate because these feeding practices are intimately linked to linear growth. For instance, Jones et al[22] showed that dietary diversity and overall diet quality were positively associated with height-for-age z-scores (HAZ) in many countries, including Ethiopia. Although the knowledge of key IYCF practices was higher in trained than in untrained mothers,

this depended on the knowledge of the HEWs. Therefore, improving the HEWs' understanding of such recommended IYCF practices should be a priority in future refreshment training courses.

A considerable knowledge gap between the HEWs and the trained mothers was observed concerning some key IYCF recommendations, even those recommendations about which the HEWs were found to be knowledgeable. The way IYCF messages are conveyed, the education level of mothers, the cultural acceptability/adoptability of the messages, the frequency of the training provided are all factors that can affect the out-come of the targeted behavioral change.[23,24]

One remarkable finding is that this gap in KSE was negatively associated with linear growth. Our findings are in line with recent findings in Haiti, showing that the outcomes of behavioral change communications depend on both the knowledge and the knowledge-sharing efficacy of frontline health workers.[11] This suggests that besides working to increase the knowledge of the HEWs, it is equally important to strengthen their knowledge-sharing efficiency through process training. This is especially needed for aspects of IYCF such as optimal timing for breast-feeding initiation, optimal timing for introduction to com-plementary feeding, feeding during and after ill-nesses, and avoiding beverages not recommended for IYC, about which a large knowledge gap was observed between HEWs and mothers.

Given that mothers with a stunted child had a significantly lower knowledge score than those whose child was not stunted ($P < .001$), increasing the effectiveness of the IYCF training provided through the HEP could also be an effective strategy to prevent stunting.

The cross-sectional nature of the present study does not allow causal inferences regarding the association reported in this study. The study focused on HEWs' and mothers' knowledge and their association with child stunting, but we recognize that knowledge does not always lead to practice. However, we included a few practice-related questions that, considering the age of the child at the time of the survey, were found to be relevant. We recognize that the IYCF knowledge of trained mothers can be affected by the HEWs' knowledge; this can be a potential confounding factor in some of our comparisons between trained and untrained mothers. The pres-ent results came from a single district in West Gojam and thus cannot be extrapolated to all mothers and HEWs in West Gojam, Ethiopia. Nevertheless, the findings of this study may apply to other similar settings, both in Ethiopia and elsewhere, and contribute to the limited body of literature on implementation and utilization of nutrition education interventions.

Conclusion

The present study highlighted that access to train-ing on IYCF through the HEP was associated with higher mothers' knowledge score. Unfortu-nately, IYCF training was not accessible to all mothers and when accessible, its effectiveness depended on the HEWs' level of knowledge and understanding of key IYCF recommendations. Maternal knowledge of optimal child feeding was positively associated with dietary diversity and linear growth. Improving mothers' access to IYCF training along with efforts to increase the HEWs' knowledge and strengthening their KSE may improve feeding practices and prevent stunting.

Authors' Note

All authors were involved in developing the study design. Z.A. and K.B. looked for funding. Z.A. coordi-nated and supervised the fieldwork. Z.A. and K.B. ana-lyzed and interpreted the data. Z.A. wrote the first draft of the manuscript. All the authors contributed to manu-script preparation.

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4.2 Child feeding style, food intake, and linear growth

4.2.1 Introduction

Examination of the IYCF knowledge of mothers and HEWs identified that mothers IYCF knowledge and child feeding practices are dependent on HEWs knowledge and their ability to transfer the knowledge. However the HEWs were unable to transfer knowledge, even those which they were knowledgeable, such as those about optimal breastfeeding initiation and complementary food introduction and about responsive feeding. The lack of knowledge about this key infant and young child feeding practices will affect the mothers' child feeding practices and feeding behaviors which determines child food intake and nutritional status.

In this section (section 4.2) caregivers feeding practices were characterized, difference between feeding practices of caregivers of stunted and non-children were investigated. Caregiver and child feeding behaviors were also characterized. The difference between feeding behaviors of caregivers of stunted and non-stunted children were evaluated, behaviors which were associated with child food intake were identified, and the association between child food intake and LAZ was evaluated.

The results are presented in the form of a draft article.

4.2.2 Child feeding style is associated with food intake and linear growth in rural Ethiopia

(Draft paper submitted to the journal***Appetite***)

Child feeding style is associated with food intake and linear growth in rural Ethiopia

Abstract

Background: Little is known about mother-child feeding interactions and how this is associatedwith food intake and linear growth.

Objective: To characterize mother-child feeding styles and investigate their associations withaccepted mouthful and linear growth in west Gojam, rural Ethiopia.

Subjects/design: Two, in-home, meal observations of children aged 12-23 months (n= 100)were video-taped. The number of mouthful accepted was counted and the caregiver/child feeding styles were coded into positive/negative categories of self-feeding, responsive-feeding, active-feeding, social-behavior and distraction. Data on socio-demographic characteristics, child feeding practices, perception about child's overall appetite, and strategies adopted to overcome food refusal were collected through questionnaire-based interviews. Child and mothers' anthropometric measurements were also taken.

Results: Stunting was highly prevalent (48%) and the number of mouthful accepted was verylow. Offering breastmilk and threatening to harm were the main strategies adopted to overcome food refusal. Although all forms of feeding style were present, active positive feeding style was dominant (90%) and was positively associated with mouthful accepted. Talking with non-feeding partner (64%), and domestic animals (24%) surrounding the feeding place were common distractions of feeding. Feeding was mostly terminated by caregivers (75 %), often prematurely. Overall, caregivers of stunted children had poorer complementary- and breast-feeding practices and were less responsive to child's hunger and satiation cues ($P<0.05$). Positive responsive feeding behaviors were associated with child's number of mouthful accepted ($r= 0.27$; $P =0.007$) and stunting ($r=0.4$; $P<0.001$).

Conclusion: Low complementary food intake in this setting is associated with caregivers'feeding style and stunting. Nutrition interventions that reinforce messages of optimal infant and young child feeding and integrate the promotion of responsive feeding behaviors are needed.

Keywords: feeding style, stunting, responsive-feeding, complementary-feeding, appetite, foodintake

Background

Close to six million children under the age of five died in 2015 and ~45% of these deaths are believed to be linked with malnutrition (WHO 2016). Children that survive this scourge are often undernourished and suffer from the consequences of stunting that includes diminished cognitive and physical development and reduced productivity later in life (Adair et al. 2013; Martorell et al. 2010). Stunted girls grow-up to become mothers with short stature, increasing the risk of delivering low-birth weight babies (Victora et al. 2008) and thus contributing to the inter-generational cycle of malnutrition. Reversing the consequences of stunting is often difficult past the age of two. Consequently, stunting prevention strategies are giving emphasis to the first 1000 days from conception to the child's second birthday (Baye & Faber, 2015).

Stunting often reaches a peak during the complementary feeding period (Victora, de Onis, Hallal, Blössner, & Shrimpton, 2010), partly because of inappropriate complementary feeding. In most developing countries, the amount of food consumed by infants and young children is lower than their theoretical gastric capacity; hence, making intake requirements even more difficult to meet (Baye, Guyot, Icard-Vernière, & Mouquet-Rivier, 2013; Gibson et al., 2009). For example, earlier studies in Ethiopia have consistently reported low energy and nutrient intake from complementary foods (Baye et al. 2013; Gibson et al. 2009).

Efforts to improve complementary feeding have often relied in increasing the energy density through for example the addition of α-amylase and, or increasing the nutrient-density byfortifying the complementary foods with vitamins and minerals (Berger et al. 2013). However, very few studies investigated how feeding style behaviors adopted by caregivers can affect food/energy intake and thereby influence child growth in low income countries (Mouquet-Rivier et al. 2016; Vazir et al. 2013; Bentley et al. 2011). Only a couple of studies specifically looked at the feeding style behaviors of Ethiopian mothers (Aboud & Alemu 1995; Wondafrash et al. 2012). The first is an observational study that recorded feeding styles of mothers with children aged 16-42 months. The second was a questionnaire-based assessment of feeding styles of caregivers of children aged 6-23 months of age. Both studies had a very wide age range, especially when considering the dynamic nature of feeding behaviors in early childhood (Hodges et al. 2013).

To what extent feeding styles are associated with food intake and child growth remains unknown. This is unfortunate, as knowledge on current feeding practices and their association with food intake and child growth may inform policies and programs that aim to improve complementary feeding practices and thereby prevent stunting. Therefore, the objective of the present study was to characterize caregivers' feeding behaviors and examine their associationwith food intake and growth faltering.

Subjects and Methods

Study area and participants

The study took place in rural Ethiopia. Hundred caregiver-child pairs participated in the study. The inclusion criteria were having an apparently healthy young child (12-23 months of age), be willing to be observed during two meal occasions, and living in the study area with no intention to leave until the study ends. This study is part of a series of studies that aimed to investigate complementary feeding in West Gojam. The sample size was calculated from power analyses calculated to detect a medium effect size (0.5 standard deviation difference between means [1-tailed], with $\alpha = 0.05$ and power of 0.80 to allow comparison of 2 groups in one or more of theoutcomes considered (e.g. stunting). This resulted in a sample size of 102 that was augmented to 120 to allow 15% non-response rate. However, twenty dropped-out due to various reasons: child sickness that can affect food intake (n=10) and incomplete anthropometric measures (n=10).

Ethics

Ethical approval was granted by the Human Ethics Committees of the College of Natural Sciences, Addis Ababa University, and the Amhara National Regional State Health Bureau.Verbal informed consent was obtained from families prior to their participation. All questionnaires and consent forms were translated to Amharic prior the survey.

Socio-demographic characteristics

Questions on the socio-demographic characteristics of the caregivers were asked through face-to-face interviews. The questionnaire included questions on land ownership, livelihood, andparents' education.

Anthropometric measurements

The length/height and weight of the children/mothers were measured in triplicate using standardized techniques, with children and mothers wearing light clothing and no shoes. 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 2006) using the software ENA 2007. Stunting, underweight, and wasting were defined respectively as LAZ, WAZ or WLZ <–2. Maternal body mass index (BMI) was calculated as weight (kg)/height (m) ^{2}.

Child feeding knowledge and practice

During the first visit, before the meal observations, mothers/caregivers were interviewed about their experiences concerning breastfeeding, feeding their child, and other caring practices. Questions were asked to gather information about feeding during and after sickness, childencouragement during feeding, mothers' perception about hunger and satiation cues, strategies used to overcome child food refusal.

Feeding event observation

To assess caregiver child interactions, children and their caregivers were video-taped on two different days, a week apart. The feeding episode was recorded during the day time meal (lunch). The date of the visit was not announced to the caregivers and the choice of food was free. The videographer had arrived at the participants' home before the feeding event started, and sat in a position that was not intrusive, but still allowing him to videotape the observed behaviors. Before videotaping, caregivers were instructed to feed their child as they would normally do. The families were told that the observer would return the following week again on an unspecified day. On the second visit, observations of the feeding event were repeated. Once the videotaping was completed, the recorded feeding episodes were transferred to a computer for coding and analysis.

Coding and analyses of feeding behaviors

The behavioral coding scheme was adapted from a previous study (Moore et al. 2006) with slight modification to fit the Ethiopian context. Behaviors were coded in to five categories: self-

feeding, responsiveness, active feeding, social behavior and distraction. Each category had a positive and negative classification: positive meaning that it promoted feeding and negative that it was aversive, intrusive or interrupted feeding. The behaviors of both the caregiver and the child were coded in which the unit of behavior was the smallest meaningful action or word/voice. The caregiver and the child behaviors were coded similarly. The coding was performed twice and any inconsistencies were revised.

The number of times a single behavior occurred was counted. A behavior that occurred at least two times per feeding episode was recorded as present, otherwise not. Each caregiver and child received a behavioral category score reflecting the frequency with which it exhibited that behavior. Number of breast feedings, mouthfuls accepted, and rejected, duration of the feeding episode, and type of offered food were noted. Values for each variable were calculated, summed and averaged for the two visits to the household. The average values were used for the analysis. Also, caregiver and child behaviors were examined in relation to the number of mouthfuls accepted.

If the child refused the last two mouthfuls then the episode was considered to be terminated by the child. If the child accepted at least one of these mouthfuls, but the feeding was stopped, the episode was considered to be ended by the caregiver. Children who self-fed were judged to terminate feeding themselves.

Statistical analysis

The average frequency counts of each observed behaviors were subjected to descriptive analysis. Mann-Whitey non- parametric tests was performed to detect differences in median valuesbetween groups. Categorical data were analyzed using Fisher's exact test. Bivariate correlationtests between predictor variables (caregiver and child behaviors) and outcomes of interest (number of mouthfuls accepted and LAZ) were performed. Differences between two means/medians were considered statistically significant for p-values < 0.05.

Result

Socio-demographic characteristics

Most of the study participants were subsistent farmers (>90%), with only few having >1ha of land for production (table 1). The proportion of educated mothers (36 %) and fathers (59 %) was low. The average years of mothers' schooling were four years. Undernutrition among mothers(9%) and children (48% stunted) was relatively high.

Table 1: Socio-demographic characteristics and nutritional status of mother-child dyads (n=100) in Mecha district, West Gojam, Ethiopia

Variables	Mean ±SD/ (%)
Livelihood strategy (farming)	95
Land size ≥1ha	24
Educated fathers	41
Educated mothers	36
Mothers' year of schooling	4 ±1.8
Mother can read and write	37
Mother age (years)	26± 5.9
BMI < 18.5	9
Child age (Months)	17 ± 3.5
Proportion of first born children	17
Female children	47
Stunted children	48
LAZ	-1.97 ± 0.9

SD, standard deviation; LAZ, length-for-age Z- score, BMI, body mass index

Reported feeding practices

All of the children were breastfed and greater than 90% were still breastfed at the time of the survey (table 2). Timely introduction of complementary foods (6 months) was 69 % and 95% of the children had started consuming family foods at the time of the study. Sixty mothers (60%) had a routine and consistent feeding time. Most children (> 90%) were fed at least three times a day and about 80% of the mothers reported feeding time as pleasant. Significant differences between stunted and non-stunted children were observed in breastfeeding practices, timing of introduction to complementary food, identifying hunger and satiety cues, and perception of child's appetite ($P< 0.05$). Overall, mothers of non-stunted children reported complementaryfeeding practices that were better than their non-stunted counter-parts.

Table 2 Reported feeding practices of caregivers of stunted and non-stunted children aged 12-23 months (n=100) in Mecha district, West Gojam, Ethiopia

	Mother			
	All	With stunted	With non-stunted	
Feeding practices	n = 100	n = 48	n= 52	P-value
Breastfeeding				
Ever BF	100	48 (100)	52 (100)	-
BF within one hour of birth	61	23 (48)	38 (73)	0.009*
Exclusively BF for 6 months	69	28 (58)	41 (79)	0.023*
Currently breastfeeding	96	47 (98)	49 (94)	0.34
Continuous BF ≥ 2 years	96	47 (98)	49 (94)	0.34
Complementary feeding				
Timely introduction to CF (6 months)	69	28 (58)	41 (79)	0.023*
Children consuming family food	95	43 (90)	52 (100)	<0.001*
Fed with consistent feeding time	60	26 (54)	34 (65)	0.174
Fed ≥ 3 times a day	92	42 (88)	50 (96)	0.11
Identified crying as a hunger cue	71	29 (60)	42 (81)	0.021*
Identified pushing food as a satiety cue	45	14 (29)	31 (60)	0.002*
Reported feeding time as pleasant	81	40 (83)	41 (79)	0.38
Perceive child's appetite as low	39	24 (50)	15 (29)	.025*
Other caring practices				
Follow frequency and amount of food the child eats/ day	87	35 (73)	52 (100)	<0.001*
Offer special food during sickness	63	20 (42)	43 (83)	<0.001*
Adapt feeding practice during child's sickness	60	18 (38)	42 (81)	<0.001*
Praise child upon taking food	90	39 (81)	51 (98)	0.005*

BF, breast feeding; CF, complementary food.*difference between caregivers of stunted and non-stunted children was statistically significant **according to Fisher's exact test.; Injera: flat bread usually made from blends of millet and maize flour;** stew, a sauce made mainly from pea flour, water, and sometimes oil and onion will be added.

Over a third of the mothers perceived their child's appetite as low, but about half of the mothers reported of doing nothing to improve their child's appetite (Fig 1). The remaining half reportedincreasing the selection of food (48 %) and force-feeding (2 %) as strategies to make their child eat more. Compared to mothers of stunted children, those with non-stunted children paid more attention to the frequency and amount of food their child consumed (P< 0.001), adapted their feeding during sickness (P< 0.001), and verbally encouraged their children to eat (P= 0.005).

Seventy one (71%) mothers recognized that playing children were a major source of distraction during the feeding event.

Observed feeding practices and behaviors

The average feeding episode was 8 minutes. The children were offered a limited range of foods. Almost three fourth (73%) of the children were given *injera, a* flat fermented bread, made of maize and millet, with a legume-based stew (*shiro*). A small number of children were given bread (6%) or a cup of milk (8%). More than half of the children were fed by the caregivers and about a third were self-fed.

Table 3 Observed feeding styles of mother-child dyads (n=100) in Mecha district, West Gojam, Ethiopia

		Caregiver		Child	
Feeding styles		(%)	Median (Q1,Q3)	(%)	Median (Q1,Q3)
Self-feeding	Positive	53	1.5(0.4, 3.0)	49	1.0 (0.0, 2.5)
	Negative	8	0.0(0.0,0.0)	6	0.0(0.0, 0.0)
Responsive	Positive	51	1.5(0.5 , 3.0)	87	4.5(3.0, 6.5)
	Negative	9	0.0(0.0, 0.5)	78	5.5(1.5, 11.0)
Active	Positive	96	8.8(5.4, 14.6)	54	1.5(0.0, 3.0)
	Negative	41	1.0(0.0, 2.5)	20	0.0(0.0, 1.0)
Social behavior	Positive	71	3.0(1.0, 5.0)	74	2.8(1.0, 5.0)
	Negative	2	0.0(0.0, 0.0)	0	0.0(0.0, 0.0)
Distraction	Positive	8	0.0(0.0, 0.0)	9	0.0(0.0, 0.0)
	Negative	69	2.0(1.0, 5.1)	47	1.0(0.0, 3.5)

Average of two meal observations; values are median and 1st and 3rd quartiles

However, 75% of the feeding episodes were immaturely terminated by the caregiver. More strategies used to overcome food refusal were observed than reported (Fig 1). These strategies included: force-feeding (17%), breast-feeding (50%), threatening to harm (28%), and asking the child why he/she is not eating (19%). Less than half of the caregivers (53%) engaged in behaviors that promoted self-feeding, such as giving the child his/her own plate, and/or verbally

encouraging child to self-feed (Table 3). However, only few (7%) restricted self-feeding behaviors initiated by the child. Almost half (51%) of the caregivers engaged in responsive positive behaviors like providing water and food when a child asked. Significantly higher proportion of caregivers of non-stunted children had responsive feeding behaviors than stunted ones (P< 0.001) (Table 4).More than 90% of the caregivers had an active positive feeding style that adopted strategies to encourage eating by temporarily diverting the child's attention, and demonstrating to the childhow to eat. More than one third (41%) of the caregivers engaged in active negative behaviors such as: force-feeding and threatening to harm if the child does not accept the mouthful provided.

Table 4 Frequency of observed caregivers' feeding style categorized by child stunting

		Caregiver (%)		
		Stunted (n= 48)	**Non-stunted** (n= 52)	**p-value**
Feeding style				
Self-feeding	Positive	24(50)	29(56)	0.35
	Negative	3(6)	5(10)	0.4
Responsive	Positive	14(29)	37(71)	<0.001*
	Negative	3(6)	6(12)	0.29
Active	Positive	47(98)	49(94)	0.34
	Negative	19(40)	22(42)	0.94
Social behavior	Positive	37(77)	34(65)	0.2
	Negative	0 (0)	2(4)	0.27
Distraction	Positive	4(8)	4(8)	0.6
	Negative	36(75)	33(63)	0.21
Mouthful accepted				
Median (Q1,Q3)		2.3 (0.9, 4)	4.0 (2,4, 7.0)	0.02¥

*Statistically significant difference between the proportion of caregivers of stunted and non-stunted children under the corresponding behavioral category according to Pearson chi- square test. ¥ Statistically significant difference in the number of mouthful accepted between stunted and non-stunted children according to Mann-Whitney U test.

Social behaviors such as cleaning the child's mouth, hugging, and kissing were also widelyobserved (Table 4). Activities that distracted the child away from feeding (distractive negative

feeding behavior) like having a conversation with another adult, feeding chickens, chasing animals, and leaving the feeding child for household chores were observed in 69% of the caregivers.

Generally, children tended to be behaviorally more responsive positive than their caregivers (P<0.001). But, both responsive-positive (87 %) and -negative (78%) behaviors like accepting offered food/water following verbal encouragement or turning face when food was offered were observed. Positive responsive feeding behaviors among non-stunted children were significantly higher than in stunted ones (P< 0.05). Both caregivers and children's responsive positive feedingbehaviors were positively associated with accepted mouthful (Table 5).

Table 5: Correlation of mouthful accepted with feeding behavior of young children (12 to 23 months) and their caregivers, Mecha district, West Gojam, Ethiopia

		Caregiver		Child	
Feeding syle		**Correlation coeff.**	**P-value**	**Correlation coeff.**	**p-value**
Self-feeding	Positive	0.16	0.11	-0.04	0.66
	Negative	-0.01	0.91	0.02	0.87
Responsive	Positive	0.27	0.007*	0.33	<0.001*
	Negative	0.12	0.22	0.18	0.07
Active	Positive	0.29	0.004*	0.11	0.28
	Negative	0.29	0.003*	0.19	0.07
Social behavior	Positive	0.15	0.14	0.21	0.03*
	Negative	0.2	0.05	0.008	0.94
Distraction	Positive	-0.02	0.88	0.03	0.74
	Negative	0.09	0.39	0.28	0.006*

*Statistically significant correlation between number of mouthful accepted and caregiver and child observed feeding behaviorfrequencies according to spearman correlation.

Caregivers active feeding, whether positive or negative was associated with mouthful accepted.Mouthful accepted was also positively associated with child's positive social behaviors.Surprisingly, negative distraction behaviors for the child were associated with mouthful accepted

(r=0.28, p= 0.006). Significant numbers of children were distracted from feeding 47% (Table, 3).The major sources of distractions were: mothers' not paying attention to the feeding (talking or laughing; 64%), domestic animals (24%) and playing children (21%) (Fig 1).

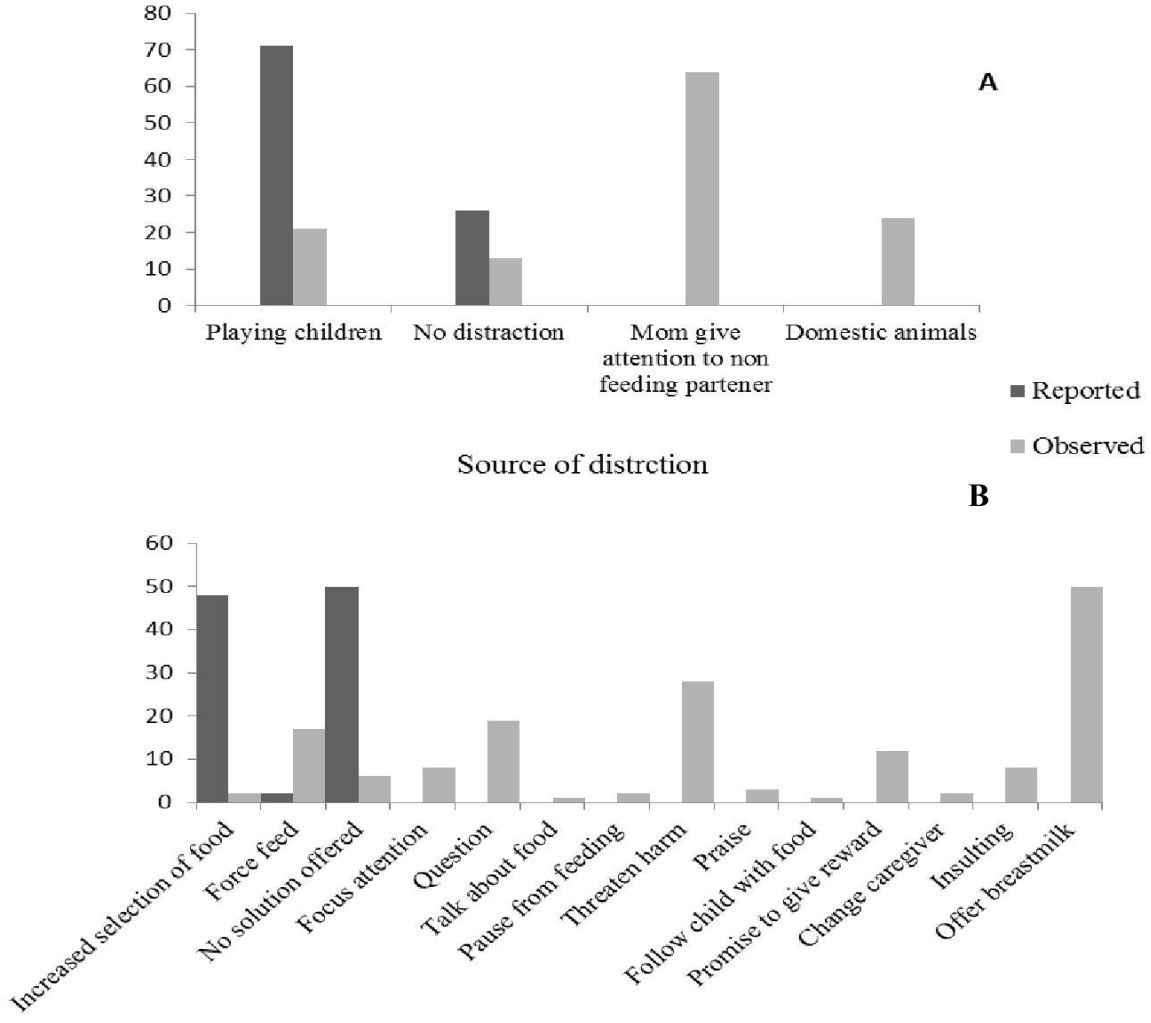

Fig 1: Observed and reported sources of distraction (A) during child feeding and strategies adopted to overcome food refusal (B), in Mecha district, West Gojam, Ethiopia

Discussion

This study characterized child feeding practices and feeding style behaviors in a rural district of Ethiopia. Although continued breastfeeding was the norm, the proportion of caregivers who exclusively breastfeed their child was low. The children had a very low appetite as reflected by the very low number of mouthful accepted. The caregivers were active in feeding their children, but force-feeding instead of responsive feeding was dominant. Overall, caregivers of stunted children had poorer complementary- and breast-feeding practices and were less responsive to the

child's hunger and satiation cues. Positive responsive feeding behaviors were associated with children's number of mouthful accepted and linear growth.

In line with previous reports, the prevalence of stunting and inadequate breast- and complementary-feeding practices is high and can be, at least in part, due to inadequate knowledge about optimal infant and young child feeding (Abebe et al. 2016). Reinforcing nutrition education provided by the health extension system may improve breastfeeding and IYCF practices already addressed in their nutrition package, but will do little to increase the very small number of mouthful accepted. Such a low food intake is certainly related to previous reports of inadequate energy and nutrient intakes among young children living in rural Ethiopia (Baye et al. 2013). Although the low food intake could be related to the caregiver-child interaction during meal time, this remained unexplored.

Our feeding observation revealed that caregivers show active positive feeding behavior, but the low number of mouthful accepted suggest that the active behavior was a compensatory act in response to the child's disinterest in food (Engle & Zeitlin 1996). Caregivers used intermittentbreastfeeding during feeding as a strategy to overcome child food refusal. However, this is not likely to be very useful as children preferred the taste of breastmilk over the complementary food
and thus reduced their food intake. Indeed, in this study, child's negative distraction feeding behavior was found associated with caregiver's positive active feeding behaviors ($r= 0.38, P<0.001$) and the number of mouthfuls accepted ($r= 0.28$, $P= 0.006$). In contrast, the positiveassociation of caregiver's positive responsive behaviors with the numbers of mouthfuls accepted($r=0.27$, $P< 0.001$) and child LAZ ($r= 0.40$, $P< 0.001$) indicates the need to promote responsive feeding behavior. Community-based trials have previously shown that promotion of responsivefeeding, in addition to improved complementary feeding practices, improves food intake and child growth (Vazir et al. 2013).

Despite children's psychomotor abilities to feed themselves by about nine months (Engle et al.2000), the proportion of children aged 12-23 months who practiced self-feeing was low. The low proportion of self-feeding can affect child's feeding skill and healthy appetite in the long term (Aboud et al. 2009). Among the possible reasons that discourage mothers to allow self-feeding is the time the child takes to feed him- or her-self. Mothers in this rural setting are overburdened by

the day-to-day chores, which include fetching fuel wood and drinking water, participating in agricultural works, cooking, and looking after their children. Thus, mothers executed some household chores while feeding to save time; but, this negatively distracted the feeding. Besides, most caregivers felt the urge to force-feed (active negative) and finish the feeding prematurely, before the child is full. Although temporarily effective, such active negative feeding behaviors may lead to fewer mouthful intake and more refusals in the long-term (Ha et al. 2002; Engle & Zeitlin 1996)

The present study has several strengths and limitations. The cross-sectional design of this study does not allow causal inferences to be made. Besides, given that feeding styles are age-dependent, a longitudinal study design would have been ideal. Nevertheless, the study indicated that responsive feeding is associated with food intake and child growth. This fills the gap of recent studies on determinants of stunting in Ethiopia, that due to lack of available data on child feeding did not consider the role of feeding style in their framework (Wirth et al. 2016; Woodruff et al. 2016) The video-taping of two meal occasions among children of a specified age-group (12-23 months) has allowed a more accurate coding and characterization of adopted feeding styles. This is further highlighted by the fact that the observations allowed to capture more feeding behaviors than mothers' self-reports. Only one daily meal (lunch) was observed,and the type and amount of food to be served was not standardized. As a result, the number of mouthful accepted might have been affected by the taste and characteristics of the food served. However, the fact that the majority of the children were served the same type of food (injera with a legume-based stew) makes this a less serious limitation.

Conclusion

To our knowledge, this is the first study that investigated how child feeding styles relates to food intake and linear growth of young children in a rural setting of Ethiopia. Our findings indicate that mothers with stunted children had poorer breast-and complementary-feeding practices and were less responsive than caregivers with a non-stunted child. Positive responsive feeding practices were associated with increased accepted mouthful and linear growth. Population-based controlled trials are needed to investigate how culturally-adapted responsive feeding messages integrated with improved complementary feeding can contribute to stunting prevention in this rural setting.

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Contributions

All authors were involved in developing the study design. ZA and KB looked for funding. ZA coordinated and supervised the fieldwork. ZA and KB analyzed and interpreted the data. ZA wrote the first draft of the manuscript. All the authors contributed to manuscript preparation.

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4.3 Energy and nutrient intakes from complementary foods

4.3.1. Introduction

In the previous sections (section 4.1 & 4.2) the behavioral component of complementary feeding (how, who, when and where the child is fed) were studied. These studies have illustrated that knowledge and practice of complementary feeding are related with child stunting. Section 4.2 demonstrated that the children's food intake was very low. The extent of which this will affect energy and nutrient intakes remains unknown. Besides, to improve child nutritional status, the biological component of complementary feeding (i.e. what is being fed) should also be studied. Therefore, it is important to examine the nutritional adequacy of the complementary foods and evaluate strategies that can improve their nutritional adequacy.

In the present section (4.3), results from food consumption survey conducted in West Gojam, Northern Ethiopia are presented in the form of a draft article. The feeding practices according to nutritional status were characterized, the nutrient density of the complementary diets were compared with the desired value, percentage contribution of different food groups to child energy and nutrient intake was evaluated, estimated daily median nutrient intakes were compared with the estimated need. Finally, the risks of inadequate and excessive intakes were estimatedbefore and after simulation of an MNP intervention.

4.3.2 Risk of inadequate and excessive nutrient intakes is associated with home-fortification of complementary foods with multiple micronutrient powders in West Gojam, Ethiopia

(Submitted to the ***JournalMaternal and Child Nutrition*****)**

Risk of inadequate and excessive nutrient intakes is associated with home-fortification of complementary foods with multiple micronutrient powders in West Gojam, Ethiopia

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Running title: *inadequate and excessive nutrient intakes*

Abstract

Home-fortification of complementary foods with multiple micronutrient powders (MNPs) is being scaled up in various countries, but there is little information on the prevailing complementary feeding practices and the type and nutrient gaps to be filled with MNPs.The present study evaluated the complementary feeding practices of young children and simulated the risk of inadequate and excessive intakes associated with home-fortification with MNP. Using a cross-sectional study, we have assessed the socio-demographic status, anthropometry, and complementary feeding practices of young children (n=122) in Mecha district, rural Ethiopia. Using a two-day, quantitative 24 h recall, usual intakes of energy, protein, iron, zinc and calcium were estimated. The risks of inadequate and excessive iron and zinc intakes with and without home-fortification scenarios were assessed. Stunting was highly prevalent (50%) and was associated with a lower dietary diversity (P =0.009) and nutrient intakes. Median energy, zinc, and calcium intakes were below the estimated needs from complementary foods, but iron and protein needs were met. Assuming low bioavailability, inadequate intake of iron (76%; 95% CI: 68-84%) and zinc (100%) were highly prevalent, but excessive iron intakes (8%; 95% CI: 3-13%) were unacceptably high (> 2.5%). Simulation of a daily fortification with MNP decreased the prevalence of inadequate iron and zinc intake, but significantly increased the risk of excessive intakes. Alternative day's fortification decreased this risk, but the prevalence ofexcessive ironintakes remained unacceptably high. This indicates that untargeted MNP interventions may lead to excessive intakes, even in settings where poor complementary feeding practices are prevalent.

Keywords: Complementary foods, nutrient intakes, home-fortification, micronutrient powders, iron,calcium, zinc

Background

Globally, 161 million children under-five years of age are undernourished(UNICEF/WHO/WB 2015), and about two billion people are micronutrient deficient(WHO 2007). Undernutrition and the associated micronutrient deficiencies disproportionately affect children in low- and middle-income countries (LMIC)(UNICEF/WHO/WB 2014). The period of complementary feeding is a particularly vulnerable time because energy and micronutrient requirements are very high relative to the amount of food consumed by the child(Dewey & Brown 2003).The predominantly plant-based complementary diets with little animal source foods (ASF), fruits and vegetables, as commonly consumed in LMIC are associated with poor growth and micronutrient deficiencies (Mcevoy et al., 2012). Therefore, timely interventions that improve complementary feeding are needed to circumvent the short and long-term adverse effects associated with undernutrition (De Onis et al. 2013).

In recent years, point-of-use fortification of complementary foods with multiple micronutrientpowders (MNPs) has received growing attention as a promising approach to tackle micronutrient (Dewey et al., 2009; WHO/FAO, 2006). The practice has been in use in several countries (Bhutta et al. 2008), and following promising results in reversing micronutrient deficiencies(de Silva et al., 2003; Stoltzfus et al., 2001), it is now being scaled up in various countries. What has not followed pace is good characterization of prevailing complementary feeding practices, knowledge of the nutrient adequacy of complementary foods, and the type and nutrient gaps to be filled with MNPs. In light of the scarce data on nutrient intake, data on dietary diversity scores and prevalence of stunting and anemia have been used as proxies to inform the need for MNP interventions. Particularly, WHO recommends intervention with MNPs in settings with >20 % of anemia prevalence(WHO 2011).

However, anemia can have other causes than micronutrient deficiencies (e.g. infections). Dietary quality indicators like dietary diversity scores do not always give information on the type and amount of nutrients to be supplemented with the MNPs. Thus, considering the possible adverse effects of supplying excessive amounts of nutrients through supplementation or fortification, efforts to monitor nutrient intakes with the aim of designing effective and safe micronutrient interventions are urgently needed. Indeed, this need was highlighted by a recent commentary (Bruins et al., 2016).

In the present cross-sectional study, we have used a two-day quantitative 24-h recalls collected on non-consecutive days to characterize the prevailing complementary feeding practices and estimate the nutrient intakes of young children in a rural farming district, Mecha, Ethiopia. We have estimated the prevalence of inadequate intake of selected micronutrients (iron, zinc and calcium), and simulated the effect of MNP supplementation on the risk of inadequate and excessive nutrient intakes.

Materials and Subjects

Study site and participants

This cross-sectional study was conducted from October 2013 to January 2014 in a food secure, Malaria endemic district, *Mecha,* West Gojam, Amhara region, Ethiopia. In the region, the prevalence of stunting (42 %) is very high and exceeds that of the national average (39 %) (CSA 2014). The inhabitants are predominantly subsistence farmers producing maize (*Zea mays* L.), millet (*Pennisetumglaucum*), and pulses as food staples. In addition, Teff (*Eragrostistef.*) is grown by many as a cash crop. Vegetables such as kale and potato are also grown. Traditional rearing of animals, mainly cattle and chicken are practiced, although rarely used for household consumption.

Sample size determination

As part of a larger study that investigated child feeding, sample size calculations were made to enable comparison of two groups on various outcomes of interest including stunting, knowledge about infants and young children, child feeding practices, etc. The sample size was determined from power analyses calculated to detect a medium effect size (0.5 SD difference between means, with an alpha of 0.05 and power of 0.80). The estimated sample size was adjusted and rounded to 122 to allow for an approximately 15% non-response rate. Our data and sample size allowed us to characterize the mean energy intake with a 95% confidence interval of approximately ± 30 kcal.

Sampling

Ten *kebeles* (smallest administrative unit) that are accessible enough for the collection of perishable samples were randomly selected. All households with children 12-23 months were identified by a census carried out before the survey. From each *kebele*, 12 children were randomly selected to participate in the study. The inclusion criteria were for the mother and childto reside permanently in the study area and for the child to be apparently healthy. In the rare cases, when several children in the same household fulfilled the inclusion criteria, one child was randomly selected.

Ethics

Ethical approval was obtained from the Human Ethics Committees of the College of NaturalSciences, Addis Ababa University,and the Amhara National Regional State Health Bureau.Verbal informed consent was obtained from the mother or guardian of each child after the purpose and methods of the study had been explained in detail to them in the presence of local

health community workers and kebele (smallest administrative unit) administrators. All parents asked to participate in the study accepted. All questionnaires were translated into Amharic before the survey.

Socio-demographic and anthropometric status

The socio-demographic characteristics of the participants were assessed using a pre-tested questionnaire that included questions on livelihood activities, education level of parents, ownership of livestock and the size of land owned. All anthropometric measurements were made by the same person to avoid inter-examiner errors. The length and weight of the children were measured in triplicate using standardized techniques, with the children wearing light clothing and no shoes. Z-scores for length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (WLZ) were calculated using the WHO multicentre growth reference data(WHO 2006a)using the software ENA 2007. Stunting, underweight and wasting were defined as LAZ, WAZ or WLZ, <-2, respectively. Complete anthropometric measurements were collected for 110 of the 122 studied children.

Dietary intake assessment

An interactive quantitative 24 h recall was conducted in-home with the caregiver of each child (n=122), using the multiple-pass technique adapted and validated for use in developing countries(Gibson & Ferguson, 2008). A second day assessment was conducted on n=40 children. All days of the week were equally represented in the final sample. 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.

A day before intake was assessed (two days before the recall). Plates and cups were supplied to the caregivers, who were instructed not to change the dietary pattern of the child on the recallday. A demonstration was given on how weighing of food will be conducted.Portion size of foods consumed was estimated by direct weighing of salted replicas of actual foods prepared locally. Whenever found appropriate, graduated food models and common household measures were used.

Compilation of local food composition database

For the protein, Ca, Fe and Zn contents of the most commonly consumed foods were based on results of biochemical analyses conducted in our laboratory; otherwise data were compiled from the Ethiopian food composition tables (EHNRU 1998; ENI 1981; Agren & Gibson 1968)and published data(Abebe et al., 2007; Umeta et al., 2005).

Assessment of nutrient intake adequacy from complementary foods

The median daily intakes from complementary foods of 12–23 month-old children were compared with the estimated energy and nutrient intake from complementary foods (FAO/ WHO/ UNU 2004; FAO & WHO 2004; Butte et al. 2000), assuming average breastmilk intake and composition(WHO 1998; Dewey & Brown 2003).We have assumed an average intake of 549 g/d (533 ml/d), which should provide ~346 kcal energy, 5.8 g protein, 154 mg Ca, 0.2mg Fe, and 0.7 mg Zn. Nutrient densities (per100 kcal) were compared with desired values(Dewey & Brown 2003). Median dietary diversity scores were calculated based on seven food groups(WHO 2008), and classified as low (0–2), medium (3–4) and high (>4)(WHO 2008).

Prevalence of inadequate and excessive nutrient intakes

The prevalence of inadequate and excessive nutrient intakes was estimated after adjusting for within-subject variation using the software Intake Monitoring Assessment and Planning Program

(IMAPP). The cut-off point method was used to estimate the prevalence of inadequate orexcessive intakes of zinc and calcium. The approach involves calculating the proportion of

children in the target group with usual intakes below the Estimated Average Requirements (EAR) for inadequate intakes, or above the Upper Limit (UL) for excessive intakes (FAO & WHO 2004). For zinc, the EAR set by the International Zinc Nutrition Consultative Group(Brown et al. 2004), and the UL set by the Institute of Medicine(Institute of Medicine. Food and Nutrition Board 2001) were used. For calcium, the EARs and ULs set by FAO were used(FAO & WHO 2004). Because of skewed intake distribution, the prevalence of inadequate intakes of iron was calculated using the full-probability approach assuming low, moderate and high bioavailability. The nutrient intakes of stunted and non-stunted children were compared.

Simulation of home-fortification of complementary foods with MNPs

WHO recommends home-fortification with MNPs in settings where the prevalence of anemia among 6-23months infants and young children is>20%. Mecha, being a candidate for such interventions, we simulated the intake of additional nutrients from MNPs. A 15 element MNP containing 10 mg of elemental iron and 4.1 mg of zinc was used for this purpose. We then estimated the prevalence of inadequate and excessive intakes as described above.

Statistical analyses

All continuous variables were checked for normality using Shapiro-Wilk test. Nutrient intakes (per day) and nutrient densities (per 100 kcal) were expressed as medians and inter-quartile range because of non-normal distributions of some nutrients. Differences in the median energy and nutrient intakes between stunted and non-stunted children were examined using the non-

parametric Mann–Whitney U test (two-tailed). In all comparisons, differences were considered statistically significant when $P < 0.05$. Statistical analyses were performed using SPSS statistical software package, version 20.

Result

Socio-demographic and anthropometric status

More than 90% of the study participants were from subsistence farming household, but owned <1ha of land. Nearly half of caregivers had ≥4 children. More than 70% of the households owned cows and chickens. Less than half of the mothers had at least a primary education (Table 1). The prevalence of stunting, underweight, and wasting were 50%, 34%, and 10%, respectively.

Table 1: Socio-demographic and anthropometric characteristics of mothers and young children (n=122) aged 12 to 23 months form Mecha district, West Gojam, Ethiopia, Oct 2013-Jan 2014

Socio-demographic characteristics	Mean ± SD/frequency (%)
Age of children (months)	16.2 ± 3.5
Proportion of male children	51.6
Age of mothers (Years)	26 ± 6.1
Mothers educated ≥ primary school	30.3
Four or more children	45
Livelihood activity	
Farming	93.4
Small trading activity	4
Farming and small trading activity	2
Land size ≥1ha	23.8
Own cows	85.2
Own chicken	73.8
Child nutritional status	
Stunted	50
Underweight	34
Wasted	10

Complementary feeding practices

The children were on average fed three times a day, with a significantly higher feedingfrequency observed in non-stunted than in stunted children(P< 0.001; Table 2).

Table 2: Feeding practices by stunting categories: young children (n=122) aged 12 to 23 months from Mecha district, Ethiopia, Oct 2013-Jan 2014

	All		Stunted		Non stunted		
	122		55		55		P-value
	n/ mean	**% / SD**	**n/ mean**	**% / SD**	**n/ mean**	**% / SD**	
Number of meals per day*	3	0.71	2.8	0.68	3.4	0.53	< 0.001
Cereal products	120	98.3	53	96	55	100	0.25
Legumes and nuts*	114	93	48	87	55	100	0.006
Animal source foods							
Dairy	37	30	12	23	20	36	0.07
Eggs	2	1.6	0	0	2	3.6	0.15
Meat & poultry	0	0	0	0	0	0	
Vitamin A rich fruits & vegetables	0	0	0	0	0	0	
Other fruits	5	4	3	5	2	3.6	0.5
Tea	21	17	10	18	8	14.5	0.4
Coffee	81	66	33	60	42	76	0.05
Mean number of food groups (out of 7)§*	2.2	0.49	2.1	0.45	2.3	0.44	0.009
0-2*	87	70	46	84	33	60	0.01
3-4*	35	29	9	16	22	40	0.005
≥ 5	0	0	0	0	0	0	
Fed minimum number of solid/semi-solid foods‡*	108	89	43	80	54	98	0.001
Fed minimum number of food groups or more†*	35	29	7	13	24	44	0.005
Fed according to IYCF practices¢§*	33	27	5	9	24	44	0.001

IYCF, infant and young child feeding; *statistically significant difference between stunted and non-stunted children according to Fisher's exact test (one-tailed). §*Difference between stunted and non-stunted was statistically significant according to Student's t test (two-tailed),equality of variances assumed. †Minimum number of food groups: at least three daily; ‡Minimum number of solid/semi-solid foods: three times daily; ¢ Need to be fed solids/semi-solids at least three times daily and be fed a minimum of three food groups (WHO 2008).

Less than 30% of the children consumed the minimum number (≥ 3) of food groups and asa result, the dietary diversity scores of most children (70%) were in the low (0–2) range. Significantly more children in the non-stunted group had a medium (3-4) dietary diversity score (P = 0.005).

The diets of the young children were predominantly cereal- and legume-based (Table 2). Although >70% of the households owned cows and chickens, consumption of animal source foods was very low (Table 2).Fruit and vegetable consumption including those that are vitamin-A rich were also very low (~4 %). Consumption of coffee was frequent (Table 2), and less than one-third of the studied children were fed according to IYCF practices.

Energy and nutrient intakes

Despite a low energy intake, iron and protein intakes met the estimated needs from complementary foods (Table 3).

Table 3: Estimated daily intakes (Median, M) (Q1, Q3) of energy and selected nutrients from complementary foods relative to estimated needs among young children (n=122) aged 12 to 23 months, from Mecha district, Ethiopia Oct 2013-Jan 2014

Nutrients	All (n= 122)	Stunted (n= 55)	Non-stunted (n= 55)	Estimated needs§
Energy (Kcal)*	402 (284, 541)	348 (244,479)	433 (330, 567)	548
Protein (g)	26 (16,50)	24 (13, 43)	29 (17,47)	5
Ca (mg)	78 (37, 267)	66 (32, 227)	84 (41, 241)	346
Fe (mg)*	19 (12, 26)	16 (6.9, 23)	21 (16,30)	11.4 (L) 5.6 (M)
Zn (mg)*	2.1 (1.3, 2.8)	1.6 (0.98, 2.6)	2.2 (1.6, 3)	7.6 (L) 3.8 (M)

M, median; Q1, 1st quartile; Q3, 3rd quartile; §Estimated needs from complementary foods are determined assuming average breast milk intake and composition as proposed by (WHO 1998) and (Dewey & Brown 2003); L, low bioavailability; M, medium bioavailability; *statistically significant difference between stunted and non-stunted children according to the Mann-Whitney Utest: P = 0.01 for energy; P < 0.001 for iron; P = 0.002 for zinc

Iron intake was met even when assuming low bioavailability. In contrast, zinc and calcium intakes were below the estimated needs. Stunted children had lower energy (P= 0.01) and zinc (P= 0.002) intakes than non-stunted counterparts (Table 3).

Nutrient density of the complementary diet

Protein and iron density of the complementary diets met the desired value (Table 4), but lower iron density values were observed among stunted children (P= 0.02). In contrast, zinc and calcium were below the desired densities, and were not affected by stunting. Nevertheless, assuming medium instead of low bioavailability more than doubled the nutrient density of the complementary diet.

Table 4 Median (Q1, Q3) nutrient densities of complementary foods consumed by young children (n=122) aged 12 to 23 months in Mecha district, west Goam, Ethiopia, Oct 2013-Jan 2014

Nutrient density (/100 kcal)	All (n= 122)	Stunted (n= 55)	Non-stunted (n= 55)	Desired values
Protein (g)	8.1 (5.9, 10)	8.1 (5.9, 10.1)	7.8 (5.5, 9.1)	0.9
Ca (mg)*	20.7(12, 4.3)	17.9 (11.5, 51.9)	23.1(12.5,57.8)	63
Fe (mg)*	4.7(3.9,5.6)	4.2 (3.2,5)	4.9 (4.3, 5.7)	2.1(L), 1.0 (M)
Zn (mg)	0.5 (0.4,0.6)	0.5 (0.4, 0.6)	0.5 (0.4, 0.6)	1.4 (L), 0.6 (M)

Q1, 1st quartile; Q3, 3rd quartile; BF, breast-fed; L, low bioavailability; M, medium bioavailability;*Statistically significant difference between stunted and non-stunted children according to the Mann–Whitney U test: p=0.02 for iron -Desired values were those calculated by Dewey and Brown (2003)

Prevalence of inadequate and excessive iron, zinc, and calcium intakes

The prevalence of excessive and inadequate iron, zinc and calcium intakes estimated byassuming average breast milk intakes are presented in table 5. Assuming low bioavailability, theprevalence of inadequate intakes were 76% for iron and 100% for zinc, respectively.

Table 5: The risk of inadequate and excessive intakes of selected nutrients from complementary foods supplemented with (simulated or without MNPs), among children in Mecha district, Amhara region, Ethiopia, Oct 2013-Jan 2014

Nutrients (EAR)	Usual intake (Without MNP)		Usual intake + MNP (10 mg Fe+4.1mg Zn)§ Daily		Usual intake + MNP (10 mg Fe+4.1mg Zn)§ Every other day	
	Inadequate % [95% CI]	Excessive % [95% CI]	Inadequate % [95% CI]	Excessive % [95% CI]	Inadequate % [95% CI]	Excessive % [95% CI]
Iron						
L-11.6	76 [68, 84]	8 [3, 13]	45 [36, 54]	20 [13, 27]	62 [53, 71]	13 [7, 19]
M-5.8	31 [23, 39]	8 [3, 13]	3.9 [0.46, 7]	20 [13,27]	13 [7, 19]	13 [7, 19]
H-3.9	13 [7.0, 19]	8 [3, 13]	<1	20 [13, 27]	2.4	13 [7, 19]
Zinc						
L-6.9	100	0	43 [34, 52]	52 [43, 61]	97 [94, 100]	2.8
M-3.4	70 [62,78]	0	0	52 [43, 61]	<1	2.8
H-2	6.3 [2, 11]	0	0	52 [43, 61]	0	2.8
Calcium	69 [61, 77]	<1		NA		

§Assuming standard MNP composition containing doses of Fe and Zn recommended by HF-TAG (2013). *EARs cannot be calculated from RNIs for these age groups because of the skewed distribution of requirements for iron for young children and menstruating women. Instead, the corresponding RNI values are given; The following ULwere used: 40 mg/day for iron, 7 mg/day for zinc, and 2500 mg/day for calcium (WHO/FAO 2006; FNB/IOM 2001); L ,low bioavailability; M, moderate bioavailability; H, high bioavailability; NA, calcium is not included in the formulation of standard MNPs. CI, 95% confidence interval.

Surprisingly, 8% of our population had excessive iron intakes. While the prevalence of excessive intakes is independent of the bioavailability and thus remains the same, assumingmedium and high bioavailability significantly reduces the prevalence of inadequate intake. Similar to iron and zinc, inadequate calcium intakes were also highly prevalent, but excessive intakes were less than 1%.

Simulation of the riskof inadequate and excessive intakes after home-fortification

Fortification of the complementary diet with 10 mg of iron and 4.1mg of zinc (standard formula) significantly decreased the prevalence of inadequate intake (Table 5). However, this has substantially increased the risk of excessive iron and zinc intakes. Fortification on alternative days decreased the risk of excessive intakes, but the risks remained unacceptably high.

Discussion

The present study evaluated the feeding practice and nutrient intakes of young children in Mecha District, West Gojam, Ethiopia. Half of the children surveyed were stunted. Inadequate feeding practices including low dietary diversity, suboptimal energy and nutrient intakes like zinc and calcium were widespread and were particularly worse for stunted children. In contrast, iron and protein intakes met estimated needs despite a predominantly plant-based complementary diet. Simulation of a home-fortification with MNP showed a decrease in the prevalence of inadequate intake of iron and zinc, but was associated with a concomitant increase in the prevalence of excessive intakes.

The children in this area, like in other parts of Ethiopia, relied predominantly on a monotonous plant-based diets with little consumption of animal-source foods, fruits and vegetables(Baye et

al., 2013; Gibson et al., 2009). Such low dietary diversity has been consistently found to be associated with stunting and micronutrient deficiencies(Kaib et al., 2016; Rah et al., 2010).Similarly, non-stunted children in this study had significantly higher dietary diversityscore, energy and nutrient intakes (i.e. zinc and iron). This further highlights the importance of interventions that improve feeding practices during this critical period of complementary

feeding. We have previously reported that knowledge of caregivers about IYCF remain suboptimal and that delivery of nutrition education through the use of the health extension system can be instrumental, provided that the knowledge-sharing effectiveness and counseling skills of health workers are adequate(Abebe et al., 2016; Gebremedhin et al., 2016).

Besides educational interventions, efforts to improve the zinc- and calcium-density of the complementary foods are needed. However, despite the reliance on plant-based diet, which are not a rich source of bioavailable iron(Hurrell & Egli 2010), the intakes/density of iron met estimated/desired values even when assuming low bioavailability. This is not unheard, as previous studies from Ethiopia consistently reported high iron intakes and low prevalence of iron deficiency despite reliance on plant-based diets(Gashu et al. 2016; Baye et al. 2013). A large proportion of this iron is believed to be from extrinsic sources, possibly due to soil contamination during the processing of cereals(Baye et al., 2014). Little is known on the extent of which this iron is bioavailable. Some earlier studies have suggested that 3-35% of iron from soil could be bioavailable depending on the soil type(Leif Hallberg 1981). Recent studies in Malawi and

Ethiopia have also found low prevalence of iron deficiency despite the reliance of plant-based diets(Gashu et al., 2016; Gibson, et al., 2015). A recent report indicated that contamination with acidic soils may have a small, but non-negligible contribution to human iron nutrition(Gibson, et al., 2015). Systematic evaluation of the bioavailability of such iron from extrinsic sources warrants further in-depth investigation.

In settings like Mecha, program implementers often guided by the high stunting prevalence, the poor infant feeding practices, and the low dietary diversity, and in the absence of data on dietary intake, often recommend intervention with MNP to improve the quality of complementary foods. However, with a closer look at our estimation of the prevalence of excessive intakes of 8%, which was above the 2.5% considered acceptable(WHO/FAO 2006), regular MNPs containing iron cannot be safely provided without some form of targeting. Indeed, our simulation of untargeted daily fortification with MNPs significantly reduced the prevalence of inadequate intakes, but has also resulted in unacceptably high excessive iron intakes (20%). Fortification on alternative days reduced this figure to 13%, which was still unacceptably high. Considering that intake of excessive iron has been found associated with increased susceptibility to infections(Cross et al. 2015; Soofi et al. 2013),reduced growth(Majumdar et al., 2003), and a more pathogenic gut microbiota(Jaeggi et al. 2015; Zimmermann et al. 2010), untargeted provision of MNPs in this setting may not be recommended.

Even more surprising was the high prevalence of excessive zinc intakes associated with the home-fortification with a standard MNP containing 4.1 mg of zinc. Fortification on alternative days alone does not seem to resolve the problem, unless zinc forms of higher bioavailability are

used. This has offset the benefit of decreasing the prevalence of inadequate zinc intake. Such excessive zinc intakes were previously reported for toddlers in the US who have had high consumption of supplements or fortified foods(Butte et al., 2010; Sacco et al., 2013). However, studies on the possible adverse effects are rare, making it difficult to differentiate whether the problem is the use of inappropriate ULs or the presence of excessive intakes. Future studies would benefit from looking at possible adverse effects of such a high consumption of zinc on copper and iron metabolism.

Several limitations need to be considered when interpreting our findings. First, the cross-sectional nature of the present study does not account for possible seasonal variations nor does it allow causal inferences to be made. However, the focus on "problem nutrients" little affected byseasonality like iron, zinc and calcium makes this limitation less serious. The present findings came from a single district and thus cannot be extrapolated to all districts of West Gojam, Ethiopia. Nevertheless, our intake data was in close agreement with those of the national food consumption survey(EPHI 2013), suggesting that our findings may also apply to other similar settingsin Ethiopia and elsewhere. Further limitation is that breast milk intake was not quantified, but this will have little implication to the intakes of iron and zinc considering that breast milk is a relatively poor source of these nutrients after the age of six months (provides ≤2% of the requirements)(Gibson et al., 1998). The intakes of all nutrients contained in the MNPs were not simulated because of lack of a complete and accurate food composition data and resource constraints that did not permit analyses of all nutrients.

Notwithstanding the above limitations, the study revealed that the complementary feeding in the study area is suboptimal and is characterized by a low dietary diversity. Inadequate energy and nutrient intakes are widespread and were associated with stunting. Despite the reliance on such a predominantly plant-based diet, excessive iron intakes were present, and were likely to be exacerbated by home-fortification with MNPs. Although interventions with a standard MNP containing 4.1 mg of zinc led to a substantial decrease in the prevalence of inadequate zinc intakes, it has also led to unacceptably high proportions of children reaching the ULs.

These findings remind the need for a more careful weighing of the potential risks and benefits of untargeted MNP interventions in Ethiopia and in similar settings around the world. Decisions to intervene should be guided by accurate data on nutrient intake possibly supported by biochemical data, and whenever possible by appropriate targeting.

Key messages

- Inadequate complementary feeding was prevalent in the study area and was worst among stunted children.
- While inadequate intakes of iron and zinc were prevalent, high prevalence of excessive intakes were also present.
- Prevalence of excessive intakes was exacerbated by untargeted home-fortification with MNPs.

- MNP interventions should be guided by accurate data on nutrient intake possibly supported by biochemical data.

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The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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4.4. Contribution of breastmilk to Vitamin A and pro-vitamin A carotenoids intake

4.4.1 Introduction

In the previous section (section 4.3) the nutritional adequacy of the complementary foods were studied through a dietary survey. However, dietary surveys are usually not enough in determining the vitamin A status of groups. This is because of multiple factors that affect the absorption and utilization of the pro-vitamin A carotenoids and pre-formed vitamin A. Quantitatively measuring dietary intake of vitamin A could also be challenging because of variation in the seasonal availability of the diets. Therefore**,** BMVA has been recommended as an indicator for assessing the vitamin A status of lactating women and their breastfed infants which equates to assessing the vitamin A intake of the children and their mothers. However, assessing BMVA composition using the standard method is time taking and requires a good laboratory skill. Therefore, evaluating alternative BMVA assessment methods is needed.

In the present section (section 4.4) breast milk vitamin A composition was analyzed; the prevalence of low breast milk vitamin A composition was estimated, the actual BMVA value was compared to the estimated average breast milk value. Finally the agreement between the result of the measurement of the BMVA using HPLC, the standard method, and the measurement using the iCheck were compared.

4.4.2 Both HPLC and iCheck identify low breastmilk vitamin A concentration as prevalent in rural Ethiopia

(Draft paper to be submitted)

Both HPLC and iCheck identify low breastmilk vitamin A concentration as prevalent in rural Ethiopia

Abstract

Breastmilk vitamin A (BMVA) is a useful indicator of vitamin A status of lactating women and their infants. Data on the BMVA and pro-vitamin A carotenoid concentration of lactating women in developing countries is scant, partly due to lack of methods applicable in-field. The objective of this study was to assess the BMVA and pro-vitamin A carotenoid concentrations of samples collected from lactating women (n= 104) of children aged 6-23 months, in Mecha district, rural Ethiopia. Data on socio-demographic and anthropometric characteristics were collected from randomly selected lactating women (n= 104). Breast milk samples were collected and vitamin A and pro-vitamin A carotenoid concentrations were analyzed using HPLC. BMVA concentrations were also measured using iCheck FLUORO and compared with HPLC measurements. The prevalence of underweight (BMI <18.5 kg/m^2) among lactating womenwas 17%. The breastmilk pro-vitamin A carotenoid concentration (μg/gfat) was: β-cryptoxanthin (1.0), β-carotene (0.4) and α-carotene (0.16). Low BMVA concentration was prevalent: 76% of the values were <1.05 μmol/L and 81% were <8 μg/g fat. The mean BMVA concentration only accounted to 41% of the estimated average BMVA composition for mothers in developing countries. The BMVA values from HPLC and iCheck were highly correlated (r =0.59, p = < 0.001). The present findings indicate the possibly low vitamin A status of the lactating women and their children and further suggest that intake assessments should not use average BMVA composition. iCheck can be useful for monitoring interventions designed to improve the vitamin A status of lactating women and their children.

Keywords: breastmilk vitamin A, retinaol, carotenoids,

Background

Vitamin A deficiency remains a public health concern in many low and middle income countries (LMIC) (Stevens et al. 2015). The low consumption of animal source foods (ASF) and vitamin A rich-fruits and vegetables, along with the increased demand for growth among infants and young children, increases the risk of deficiency. In Ethiopia, intake of vitamin A among young children was found to be very low (Baye et al. 2013). Such low vitamin A intake from complementary foods may lead to vitamin A deficiency and thus increase the risk of morbidity and mortality from the already prevalent infectious diseases (Stevens et al. 2015). However, given that infants and young children in rural Ethiopia are frequently breastfed, breastmilk can be a major source of vitamin A (Ross & Harvey 2003).

BMVA is dependent on maternal vitamin A stores and recent dietary intake (Ross et al. 2004; Haskell & Brown 1999) and thus can be an ideal indicator of vitamin A status of lactating women and their children (Stoltzfus & Underwood 1995). However, very few surveys included BMVA assessments and instead relied in assuming average BMVA composition when estimating vitamin A intakes of infants and young children (Lander et al. 2009; Anderson et al. 2008), which may over or underestimate the risk of vitamin A deficiency. One reason for not including BMVA assessment is the substantial time-, technical- and financial-demand of standard methods of vitamin A analyses that requires relatively expensive equipment like High Performance Liquid Chromatography (HPLC). However, more recently a portable fluorometer that is rapid, low-cost, and user-friendly was developed (iCheck FLUORO).

Recent studies have compared vitamin A measurements made by iCheck with standard measurements by HPLC from various matrices including fortified food (Laillou et al. 2014), breastmilk , and cow milk (Reina Engle-Stone et al. 2014; Schweigert et al. 2011). The BMVA analyses using iCheck were found to be comparable with that of HPLC, but given that the number of cases with low BMVA was inadequate for examining the reliability of the method, the authors suggested for more studies in a population with a greater risk of vitamin A deficiency (R. Engle-Stone et al. 2014). In light of the high prevalence of inadequate vitamin A deficiency reported in a recent national micronutrient survey, we aimed to:

1. Determine the pro-vitamin A pro-vitamin A carotenoid and BMVA concentrations of breastmilk samples collected from lactating women living in rural Ethiopia

2. Compare BMVA concentrations measured by iCheck FLUORO with standard measurement by HPLC

Study subjects, materials and Methods

Study site and sampling

The study was conducted in Mecha district, Northern Ethiopia. The study subjects were lactating mothers (n= 110) of children aged 6 to 23 months, randomly selected from ten *kebeles*-smallest administrative unit- (n=11 from each kebele). Kebeles' were selected based on their accessibility to allow proper breastmilk sample collection and handling. The inclusion criteria were for lactating mothers to have an apparently healthy child aged 6-23 month. Six mothers were unable to provide complete sample, therefore, excluded.

Ethics

Ethical approval was obtained from the Human Ethics Committees of the College of Natural Sciences (Addis Ababa University) and the National Ethical Clearance (Ministry of Science and Technology), Ethiopia. The purpose and methods of the study had been explained in detail to thelactating women. Verbal informed consent was obtained in the presence of local health community workers. All questionnaires were translated to Amharic (local language) prior the survey.

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. The length/height and weight of the children/mothers were measured in triplicate using standardized techniques, with children and mothers wearing light clothing and no shoes. 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, 2006) using the

software ENA 2007. Stunting, underweight, and wasting were defined respectively as LAZ, WAZ or WLZ <–2. Maternal body mass index (BMI) was calculated as weight (kg)/height (m) ^{2}.

Sample collection

The breastmilk samples were collected from standardized portion of the feed. All mothers were provided two bottles. The bottles were marked at 2 mL interval and covered by aluminum foils. The mothers were requested to collect in separate tubes 6 mL of breastmilk during their morning (4-10 AM) and day-time feeding (10AM - 4PM), allowing to control within day variation in breastmilk composition (Kent et al. 2006). The mothers were instructed to collect breastmilk samples as follows: ~ 2mL of the sample before feeding (foremilk), another ~2 mL half-way through the feeding, and the remaining ~2 mL after feeding (hind milk) (Feeley et al. 1983). The samples were obtained from the right breast.

All sample collection materials were sterile. Breastmilk samples were immediately collected in a precooled ice-box and were transported to the laboratory where they were stored under -80^{0}C for five months, until they were shipped to the University of Potsdam, Germany for laboratory analyses.

Breastmilk pro-vitamin A carotenoid and retinol analyses using HPLC

Milk samples were thawed to room temperature, and then homogenized at 37^{0}C for 15 minutes (Heidolph UNIMAX 1010). The samples were vortexed (MS2-mimi shaker), and kept on sonication bath for five minutes (BANDELIN, SONOREX, RK 100). Hexane (2 mL) was added to 0.2 mL of breastmilk aliquot, and then diluted with 0.3 mL water. After homogenization with a programmable rotary mix (STARLAB) for 15 minutes, the mixture was centrifuged at 3800 rpm for 10 minutes (Labofuge 200, Heraeus sepatech). Then, the hexane extract was transferred into a separate tube with 1 mL of 0.05% butylated hydroxytoluene (BHT). The extraction was repeated once, and the extracts were dried under nitrogen at 37^{0}C for 20 minutes. The dried extracts were reconstituted in 0.2 mL isopropanol, vortexed, and kept on a sonication bath (Bandelin, Sonorex, RK100) for 5 minutes. The mixture was then centrifuged at a speed of 5000

rpm, and the supernatant was injected in to the HPLC system (SHIMADZU) for separation and quantification of breastmilk retinol and pro-vitamin A carotenoids.

For separation of compounds a C30 carotenoid column (5μm, 250 × 3mm YMC Wilmington, USA) was used in line with C18. All solvents were HPLC grade, and the following mobile phase concentrations were used: mobile phase A-methanol: ammonium acetate (90:10; v/v) and mobile phase B- methanol: ammonium acetate: tert-butyl methyl ether (8:2:90; v/v). Compounds detected in the absorbance range of 200 to 550 nm wavelength were quantified.

Breastmilk retinol analyses using iCheck FLUORO

BMVA values were also measured according to the manufacturer's iCheck FLUORO

instructions.To ensure a homogenous fat distribution, the breastmilk (0.5 ml) was swirled and immediately injected into the iExMILA reaction vials using the kit syringe. The vial was thoroughly shaken for 10 seconds, allowed to settle for 5 minutes to separate the organic and water-soluble phases, and was inserted into the portable photometer for reading. Four readings (in μg retinol equivalents (RE)/L) of the same vial from different positions were recorded (CV 1.1%) and the concentration of the breastmilk was within the linear range (50-3000 of the instrument μg RE/L)

Determination of breastmilk fat concentration

Breastmilk fat concentration was determined using creamatocrit method (Lucas et al. 1978). Microhematocrit capillary tubes (75 ×15 mm^2, outside diameter) were filled with aliquot of breastmilk samples in triplicate and were centrifuged using microhematocrit centrifuge (Hettich, zentrifugen, Germany) for 15 minutes at 12000 rpm. After centrifugation, the length of the lipid layer and the length of the entire milk "column" were measured to the nearest 0.1 mm usingvernier calipers (CV 5.6%). Lipid concentration in grams per liter was estimated from the lipid concentration by volume using a validated regression equation (Lucas, 1978).

Data analyses

All continuous variables were checked for normality using Kolmogorove-Smirnove test.Frequency counts were expressed as percentage and continuous variables' as mean ± SD. Retinol
and pro-vitamin A carotenoid concentrations were expressed as medians and interquartile range because of non-normal distribution. Differences in retinol and pro-vitamin A carotenoidconcentration across lactation period were examined using the non-parametric Mann-Whitney U test. In all comparisons, differences were considered statistically significant when $P<0.05$.

The contribution of the BMVA to the daily vitamin A requirement of the children was calculated by assuming average daily breastmilk intake of 641, 598 and 533mL/day at 6 to 8, 9 to 11 and 12 to 23 months respectively (WHO 1998)and daily vitamin A requirement of 1.4 µmol (Butte et al. 2002; FAO & WHO 1998). The percentage gap between the actual BMVA concentrations, with the estimated BMVA concentration i.e 1.75 µmol/l (Underwood 1994) was compared at different lactation period. The relation between the measurement of BMVA using HPLC and iCheck was assessed using spearman correlation and the mean difference between the two measurements was explained using Bland and Altman plot. Statistical analyses were performed using SPSS software package version 20.

Results

Socio-demographic characteristics

The socio-demographic characteristics of the study subjects are presented in **table1**. Nearly all of the lactating women in the sample were from male-headed subsistent farming households. Less than 1/3 had land sizes ≥ 1ha, while more than 50% had a family size of five or more. More than 80% of the households owned livestock that consisted of cows and chickens. On average, the lactating women were in their twenties and had four children. Only 15% had some formal education. The average BMI of the mothers was 20.6 with 17 of them having a BMI of <18.5 kg/m^2. The average lactation period was 13 months, and 22% of their children were stunted.

Table 1: Socio-demographic characteristics of mothers of children aged 6 to 23 months, Mecha district,West Gojam, Ehiopia

Characteristics	Percent/Mean ± SD
Household	
Livelihood strategy (farming)	100
Male head of household	99
Household head with formal education	33
Land size ≥ 1ha	27
Family size ≥ 5	57
Own cows	87
Own chickens	82
Lactating women	
Age	28.3 ± 5.3
Mean height	154.4 ± 5.0
Mean BMI (kg/m^2)	20.6 ± 2.1
BMI < 18.5 kg/m^2	17
with some formal education	15
Period of lactation (Months)	13.2 ± 4.8
Parity	3.8 ± 1.9
Child	
Proportion of male	50
WAZ	-0.95 ± 1.12
LAZ	-1.03 ± 1.38
WLZ	-0.55 ± 1.05

Breastmilk provitamin A carotenoid concentrations

Three pro-vitamin A carotenoids, namely: β-carotene, α-carotene, and β-cryptoxanthin,, were identified (**Table 2**).

Table 2: Provitamin A carotenoid concentrations in breastmilk collected from mothers of 6 to 23 month old children, Mecha district, West Gojam, Ethiopia

Lactation period	Provitamin A carotenoids		
	β-carotene	α-carotene	β-cryptoxanthin
6 to 8	0.40 (0.30, 0.50)m	0.10(0.10, 0.20)j	0.80 (0.50, 1.20)g
9 to 11	0.700(0.50, 0.80)l	0.20(0.20, 0.30)i	1.50 (1.10, 2.00)f
12 to 23	0.40(0.26, 0.50)m	0.10(0.10,0.20)j	0.70(0.50, 1.20)g
All	0.40(0.30, 0.60)	0.16(0.10, 0.20)	1.00(0.60, 1.40)

Different letters across the columns represent statistically significant differences according to Mann-Whitney U test.

Prevalence of low BMVA concentrations

The median retinol concentration as measured by the standard HPLC method was 0.71 μmol/L and 4.4 μg/g fat (Table 3). Overall, > 75% of the samples had low BMVA concentrations, which is considered as severe based on WHO's classifications (WHO 1996). However, the prevalence of low BMVA varied by duration of lactation (Spearman's correlation, r = -.47; P < 0.001) and fat content (Spearman's correlation, r = -.40; P < 0.001). The breastmilk fat concentration wasnot statistically significant across the lactation period.

BMVA contribution to required nutrient intakes (RNI)

Assuming average intake of breastmilk, BMVA can contribute up to 30% of the required nutrient intakes (**Figure 1**). The contribution of BMVA to the RNI was higher for infants in their first year of life (6-11 months). The BMVA concentrations were only 45% (6-8 mo), 54% (9-11 mo), and 35% (12-23 mo) of the estimated composition of BMVA for mothers in developing countries.

Table 3: BMVA and fat concentration of samples collected from lactating mothers of 6 – 23 months old children in Mecha district, West Gojam, Ethiopia

	Retinol concentration		Prevalence of low BMVA concentration		Fat concentration
	µg/g fat	µmol/l			(g/l)
Lactation period	Median (Q1, Q3)	Median (Q1, Q3)	<1.05µmol/l	<8µg/g fat	Median (Q1, Q3)
6 to 8 months	5.6 (4.6, 7.0)a	0.78 (0.64, 1.2)b	71	86	45.1(34.8, 56.8)a
9 to 11 months	7.8 (5.6, 9.6)a	0.95 (0.69, 1.2)b	54	50	36.5(33.1, 43.0)a
12 to 23 months	3.7(2.5, 4.4)d	0.61 (0.41, 0.77)h	86	96	43.9 (35.9, 64.5)a
All	4.4 (3.4, 6.4)	0.71 (0.54, 1.0)	76	81	42.2(34.8, 56.5)

Different letters across the columns represent statistically significant differences according to Mann-Whitney U test.

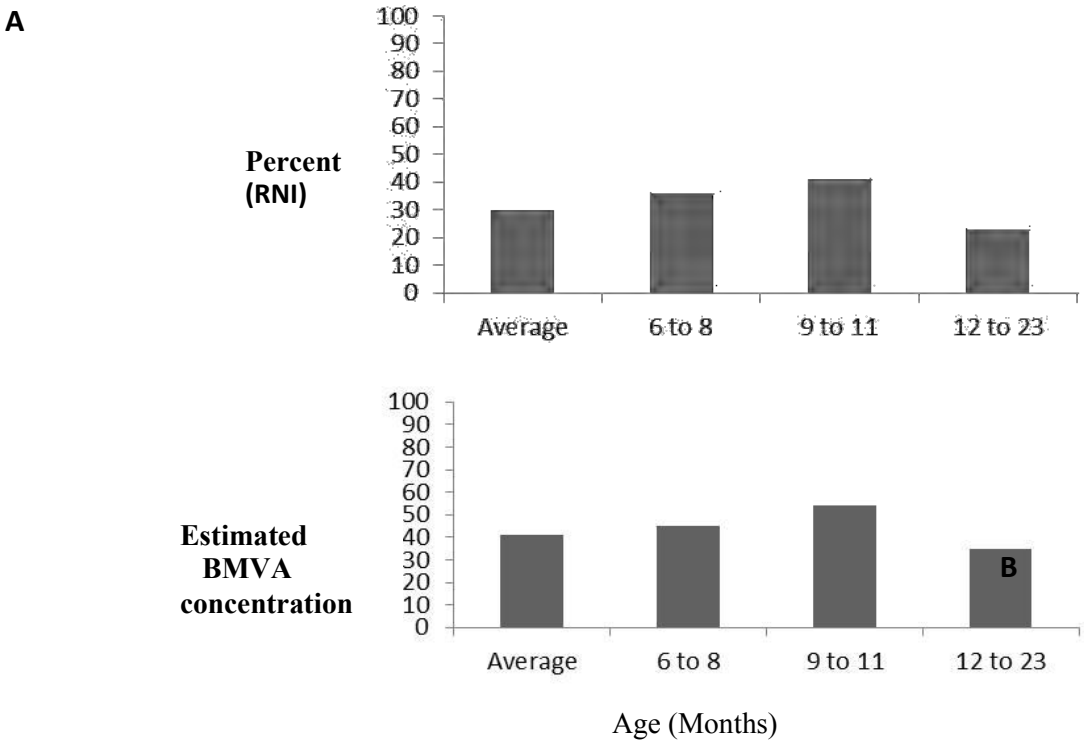

Figure 1: BMVA concentrations relative to required nutrient intakes (A)* and estimated average composition (B) for lactating women in developing countries†

†The estimated average composition (B) for lactating women in developing countries are those reported in Underwood (1994); *the required nutrient intake is for 6 to 23 month old children reported by FAO & WHO (1998)&Butte (2002).

Comparisons of BMVA measurements fromHPLC with iCheck FLUORO

The median value of BMVA using HPLC and the iCheck was the same (0.7 µmol/l), but when concentrations were adjusted for fat content, the iCheck value (4.7 µg/g fat) was slightly higher (P <0.05) than that of the HPLC (4.3 µg/g fat) (**Table 4**).

Table 4 Comparison of BMVA concentration and estimated prevalence of low BMVA as measured by HPLC and iCheck FLUORO

BMVA	HPLC	iCheck	corr. Coeff.	P-value
µg/gfat	4.4 (3.4,6.4)	4.7 (3.8,6.0)	0.59	<0.001
µmol/l	0.71 (0.54,1.0)	0.7 (0.6, 0.9)	0.57	
Low BMVA%				
< 1.05 µmol/l	76	87		
< 8µg/gfat	81	89		

The measurements from the two methods correlated well (r = 0.6, p = < 0.001), but the estimated prevalence of low BMVA using the iCheck was consistently higher than that from HPLC. Nevertheless, the mean difference between the two measurements, were not statistically different from zero (P= 0.08; **Figure 2**).

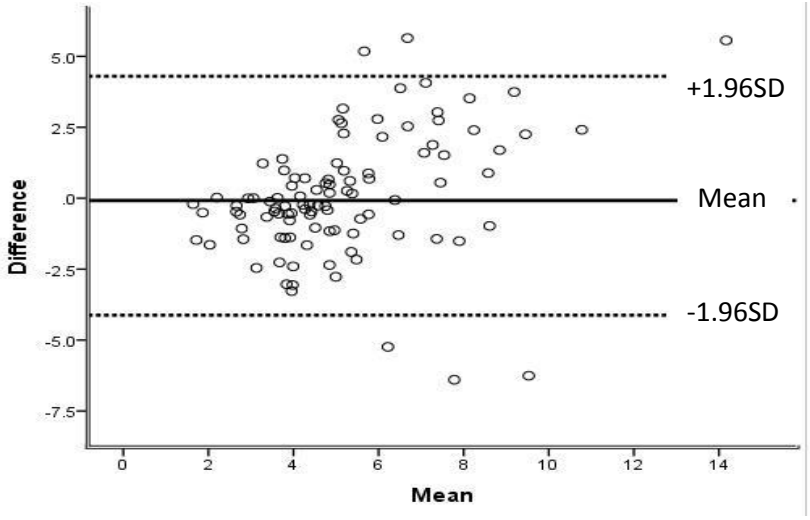

Figure 2: Bland and Altman plot ofdifference in mean BMVA (µg/g fat) of HPLC and iCheck measurements

Difference = Measurement (HPLC-iCheck); Mean = Measurement (HPLC + iCheck)/2; solid black line indicates the mean difference between HPLC and iCheck measurements; the dotted black lines represent mean difference ± s.d

Discussion

The present study evaluated the vitamin A and pro-vitamin A carotenoid concentration of breastmilk samples collected from lactating mother of children aged 6-23 months in rural Ethiopia. Low BMVA concentrations were highly prevalent. The BMVA concentrations were far lower than the estimated average composition for lactating women in developing countries. β-carotene, α-carotene, and β-cryptoxanthin, were the available pro-vitamin A carotenoids. BMVA concentrations were affected by the duration of lactation. Despite the low BMVA concentration in the samples, measurements made with HPLC and iCheck FLUORO correlated. Nonsense

Exclusive and continued breastfeeding is associated with reduced risk of vitamin A deficiency, mainly because breastmilk can contribute a significant proportion of the daily requirements (Ross & Harvey 2003). However, the concentration of BMVA is known to be affected by several factors, notably by maternal vitamin A stores and dietary intake (Haskell & Brown 1999). The very low BMVA concentrations found in this study is in line with earlier reports from Ethiopia indicating very low consumption of ASF and vitamin A-rich fruits and vegetables (Zerfu et al. 2016; Baye et al. 2013). The median BMVA concentrations were even lower that the estimated average breastmilk composition for lactating women from developing countries (WHO 1998). As a result, the breastmilk in this study contributed to only 20- 40% of daily requirements, which is lower than the previously estimated values of 40-60% (Ross & Harvey 2003). This difference suggests that studies that assess vitamin A intakes among infants and young children cannot always assume average breastmilk composition, and thus whenever possible collect samples for BMVA analyses.

In line with earlier studies, β-carotene, α-carotene, and β-cryptoxanthin were available pro-vitamin A carotenoids (Lipkie et al. 2015; Khachik et al. 1997; Canfield et al. 2003) in the breastmilk samples. However, their concentrations were lower than earlier reports (Lipkie et al. 2015). Although cut-off values indicating deficiencies do not exist yet for these pro-vitamin A carotenoids, the low levels of pro-vitamin A carotenoids in our samples may not be desirable. Especially, considering the key role that these pro-vitamin A carotenoids play in child growth and development (Dancheck et al. 2005; Melikian et al. 2001; Sedgh et al. 2000), reducing oxidative stress (Granot & Kohen 2004; Hughes 2004; Han & Maydani 2004), supplying vitamin

A, and promoting immune function (Xu & Kopp 2012; Ahmad et al. 2008). The high prevalence of low BMVA and pro-vitamin A carotenoid concentration are thus likely to have serious implications for the vitamin A status, growth and wellbeing of the infants and young children.

The extent to which the low BMVA concentrations could be improved through vitamin A interventions including dietary diversification, biofortification, and supplementation remains to be investigated in this setting. While many studies have provided evidence that BMVA concentrations increased in response to supplementation with vitamin A-rich plant and animal source foods (Stuijvenberg et al. 2015; Turner et al. 2013), fortification (Jus'at et al. 2015), and medicinal supplementation (Oliveira et al. 2016), recent studies have also noted null effects for similar interventions (Palmer et al. 2016; Klevor et al. 2016). This suggests that the effectiveness of interventions is not always straightforward and may depend on the context, which can be affected by various factors including maternal vitamin A stores. Therefore, interventions in such settings may benefit from effective monitoring of BMVA and pro-vitamin A carotenoid concentrations before and after interventions. Such monitoring is also needed to prevent the risk of excessive vitamin A intakes that may arise as a result of high dosage or concurrent interventions (Tanumihardjo et al. 2016). In this regard, field-adapted, portable instruments like the iCheck FLUORO may be useful, provided that the measurements are reliable across a range of BMVA concentrations.

A recent study reported good agreement between iCheck and HPLC BMVA measurements, but highlighted the need to replicate such comparison in a sample where cases of low BMVA are prevalent (Engle-Stone et al., 2014). Our study is thus unique in filling this gap and found that the mean BMVA values obtained using the two methods correlated very well. However, the estimated prevalence of low BMVA using the iCheck was 8-9 percentage points higher than what was estimated based on HPLC measurements. Notwithstanding this potential limitation, the iCheck proved to be a useful instrument for rapid, in-field, BMVA assessments.

The present study has several limitations that need to be considered while interpreting our findings. First, the cross-sectional nature of the study does not allow a good characterization of the evolution of breast milk composition across lactation stages. Thus, comparisons across lactation stages should only be taken as indicative. We did not directly assess the dietary intake

of lactating women and thus had to rely on reported food consumption data. Nevertheless, our study has for the first time indicated that the prevalence of low BMVA in this rural setting of Ethiopia is a serious public health concern. The strength of our study include the sampling of breastmilk samples from different lactation stages, the careful sampling of early morning and day-time samples representing fore-milk, milk during feeding, and hind-milk, and the use of both HPLC and iCheck for BMVA analyses.

Conclusion

Overall, the present study has highlighted the high prevalence of low BMVA in this ruralcommunity of Ethiopia. Thus, highlighting the urgent need for interventions that improveBMVA concentrations, and whose effectiveness could be closely monitored in-field usingiCheck. Longitudinal studies that include various lactation stages with women adopting different
dietary patterns are needed.

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Chapter 5- *General discussion*

Chapter five: General discussion

Undernutrition is one of the major causes for the death of under 5 children (UN IGME 2015), and disproportionately affects LMIC (De Onis et al. 2011). Close to 60% of Eastern African children are stunted (UNICEF/WHO/WB 2015). The causes of stunting are many, including intra-uterine and postnatal malnutrition (Waterlow 1994). The problem has many negative consequences (Black et al. 2008), some like cognitive impairments are considered largely irreversible after two years of age (Martorell et al. 1994). In developing countries, the prevalence of the problem reaches its peak usually during the complementary feeding period (Shrimpton et al. 2001; Dewey & Adu-Afarwuah 2008); hence, appropriate intervention should target this age range. However, in these countries, inappropriate complementary feeding practices (Baye et al. 2013; Gibson et al. 2009) and feeding styles (Moore et al. 2006), poor quality diets (Baye et al. 2013; Gibson et al. 2009)and low concentrations of BMVA (Haskell & Brown 1999) are common.

Similar to other developing countries, stunting is highly prevalent in Ethiopia (CSA 2014), and reaches a peak during the complementary feeding period. Given the multidimensional causes of stunting, multidisciplinary studies that identify the level and determinants of the problem and the appropriate interventions required are needed. The present series of studies responded to this need through in-depth investigation of the complementary feeding practices of young children in Mecha district, West Gojam, Ethiopia. The studies looked at: the nutrition-related knowledge and practice of caregivers and how they can be influenced by nutrition education; investigated how feeding style is associated with the amount of food consumed and linear growth; investigated iron and zinc intakes from complementary foods and evaluated the risk and benefit associated with a possible MNP intervention. Vitamin A status was also assessed using BMVA analyses, and the possible application of iCheck to measure BMVA in-field was examined.

Although nutrition education through the HEP did not reach all the caregivers, those that did receive IYCF training had higher knowledge scores and better child feeding practices than untrained ones (article 1). Trained mothers also tended to have a lower proportion of

stuntedchildren, and KSE of HEWs' was found associated with child linear growth. Indeed, there isgrowing evidence that improving the accessibility and quality of nutrition education throughhealth services can improve the prevalence of child stunting (Mbuya et al. 2013; Penny et al. 2005). This suggests that improving the counseling skills of HEWs would contribute to stunting prevention strategies in Ethiopia, but improving HEWs' counseling skills is a pre-requisite for aneffective nutrition education intervention. However, this demands that HEWs understand well the messages that they are supposed to deliver to caregivers. Unfortunately, certain key IYCFrecommendations were not well understood by the HEWs, which affected mothers' knowledgeand HEWs' KSE. Among messages that were not well understood are those on responsivefeeding. As a result, responsive feeding practices were not widely practiced (article 2).

Such low levels of responsive feeding practices is unfortunate, when considering that this was associated with accepted mouthful and child linear growth (article 2). This is in line with previous studies in Peru (Penny et al. 2005). Most mothers practiced active positive feeding style, and distraction during feeding was common. Intensive nutrition education on not only what, but also how to feed a child requires attention in future revisions of the HEP. However, to be effective, responsive feeding messages would need to be adapted to the local culture to which they are designed for. This would require adaptive management skills, which in turn requires increased nutrition capacity at the district and kebele level. Thus, policies that create posts for well-trained nutritionist at the district and kebele level are needed.

Not only low food intake, but also low quality complementary foods were observed in this setting (article 3). Such low quality complementary foods lead to micronutrient deficiencies, particularly for nutrients like iron and zinc, for whom the contribution of breastmilk is very low (~2%). Indeed, iron and zinc, but also calcium intakes were found to be inadequate. However, in this same community, excessive iron intakes (8 %) were also observed. Our simulation of an MNP intervention currently under consideration in the country, showed reductions in the prevalence of inadequate intakes of iron and zinc. However, this was associated with simultaneous increases in excessive intakes of iron and zinc beyond proportions considered acceptable (< 2.5%).These findings remind the need for a more careful weighing of the potential

risks and benefits of untargeted MNP interventions in the country and in similar settings around the world. Decisions

to intervene should be guided by proper insight into micronutrient intake and evaluation of too high or too low population intakes. This will help to decide whether there are potential problems regarding inadequacy or excessive intakes. Looking in to intake distribution may help policy makers and program implementers to decide on how to fill the gap while protecting populations from adverse health effects. Nutritionists should assist this process by rigorously monitoring and evaluating micronutrient programs. However, monitoring would benefit from field-adapted instruments (e.g. iCheck) that check whether fortification is properly done, complemented by appropriate biomarkers that enables appropriate targeting of deficient segments of the population. Existing biomarkers are yet not fit for this purpose. Besides, whether current ULs and biomarker cut-off values that are extrapolated from studies on adults are accurate remains unknown and thus futures studies in this regard are highly needed.

One such biomarker is BMVA that could serve to determine the vitamin A status of the mother and the breastfeeding infant. BMVA measurements are not affected by inflammation and thus could be considered superior than serum vitamin A measurements. The very low BMVA and provitamin A concentrations (article 4) found in this study is in line with earlier reports from Ethiopia, indicating very low consumption of ASF and vitamin A-rich fruits and vegetables (Zerfu et al. 2016; Baye et al. 2013). The concentration of BMVA, known to be affected by maternal vitamin A stores and dietary intake (Haskell & Brown 1999), impact the breastfed child. This, along with other reports (Black et al. 2013), underline the need to improve maternal nutrition. However, the extent to which BMVA concentrations could be improved through vitamin A interventions remains to be investigated in this setting. This is particularly important in light of inconsistent results in the effectiveness of various vitamin A interventions including maternal supplementation and fortification (Oliveira et al. 2016; Palmer et al. 2016; Klevor et al. 2016; Stuijvenberg et al. 2015; Jus'at et al. 2015 ;Turner et al. 2013).

Interventions in such contexts of uncertainties will thus benefit from effective monitoring, that can be facilitated through the use of field-adapted, rapid, and accurate BMVA measurements. Such monitoring is also needed to prevent the risk of excessive vitamin A intakes that may arise as a result of high dosage or concurrent interventions (Tanumihardjo et al. 2016). Our finding that BMVA measurements with iCheck are comparable to that obtained using skill- andresource-demanding standard HPLC method, illustrate the potential of this instrument for in-field monitoring, and evaluation of Vitamin A interventions.

Stunting and the associated micronutrient deficiencies are complex nutritional problems that are of multiple causes; hence, interdisciplinary interventions targeting both behavioral and food aspects of child feeding are needed. A combination of strategies such as, appropriately tailored education on proper IYCF and feeding behavior are needed; improvement of dietary quality; and BMVA concentration, are required. However, to prevent stunting and nutrient deficiencies, a focus on long-term solution like dietary diversification, while executing carefully targeted short-term solutions like fortification are needed. Complementary feeding interventions by themselves cannot change the underlying conditions of poverty and poor sanitation that contribute to child malnutrition. This suggests the need to implement such interventions in conjunction with a larger strategy that includes a coherent and coordinated multispectral approach that includes health, agriculture, education, etc. The National Nutrition Program of the Government of Ethiopia has created such an enabling multisectoral policy, which could be more effective by carefully assessing the needs of target population, increasing nutrition-related competencies and skills at grass-root level enabling the implementation of culturally adapted and targeted interventions based on informed decision making.

Several limitations need to be considered when interpreting the findings of the reported studies. The major limitation affecting all the reported studies is their cross-sectional nature. This suggests that any finding should be considered as indicating association and thus not to be interpreted as suggesting a causal relationship. The IYCF knowledge acquisition may be affected by maternal education. Although knowledge-sharing effectiveness was found associated with linear growth, this could be cofounded by other factors related to stunting. The feeding observations were also conducted on one major meal (lunch), and thus might have been affected

by food intakes taken before lunch(Engle & Zeitlin 1996) and the type of food offered. The presence of the camera might affect the mothers feeding style, even though, they were told to follow the usual feeding style.

The food intake and breastmilk vitamin A sample collection did not consider seasonality. A further limitation is the use of average breastmilk composition of iron, zinc and calcium intakeestimations, but this might not have significant effect as the child is expected to get more than 98% of these nutrients from the complementary foods. However, for vitamin A that is more affected by maternal dietary pattern, we quantified the BMVA. Although our study looked at many factors that are related with stunting, some like WASH, maternal nutrition, intrauterine growth restriction did not receive enough attention.

Chapter 6- *Conclusion&Perspectives*

Chapter six: Conclusion and perspectives

6.1 Conclusion

In the present thesis IYCF knowledge of mothers and HEWs was examined in Mecha district, West Gojam, Ethiopia. The association between the IYCF knowledge and KSE of the HEWswith mothers' knowledge and child LAZ was examined. Also, feeding behaviors of caregiverand child dyads were studied both through observation and self-report. The association between caregiver feeding behaviors with number of mouthful accepted and child LAZ was assessed. The feeding practices of the caregivers were characterized, the adequacy of nutrient intakes from the complementary foods was estimated, and the prevalence of inadequate and excessive nutrient intake from the simulated intake of MNP was investigated. Finally, the vitamin A and provitaminA carotenoid concentration of breastmilk was analyzed and the prevalence of low BMVA assessed. In addition, the agreement between the measurement of BMVA using HPLC and iCheck was studied.

Access to IYCF training through the HEP was associated with higher mothers' knowledge score.Unfortunately, the training was not accessible to more than half of the mothers. When it is accessible its effectiveness was dependent on the HEWs' level of knowledge and understandingof key IYCF recommendations.Maternal knowledge of optimal child feeding was positively associated with dietary diversity and linear growth. The HEWs KSE was negatively associated with child LAZ. Improving mother's access to IYCF training along with efforts to increase the HEWs' knowledge and strengthening their knowledge-sharing effectiveness may improvefeeding practices and child linear growth.

In general, breastfeeding and complementary feeding behaviors were inadequate but proper feeding practices were associated with child stunting. There were many distractions during child feeding event. Mothers were active feeder but it is usually to compensate for child food refusal. Maternal positive active and responsive behaviors were associated with child food acceptance and LAZ. Hence, further promoting proper breastfeeding and complementary feeding practices in combination with proper child feeding styles would help to improve child stunting. Additional interventions such as: helping caregivers identify children's hunger signals, and encouraging

them to become feeders from an enhancement rather than a compensatory perspective, would also be important to improve child food intake. Also avoiding distraction and force feeding would be necessary. Greater number of behaviors could be captured and coded through observation than self-report. Hence, it is important to use at least one structured observation to study feeding behaviors. The ability of the caregivers to perform optimal feeding behaviors was compromised by the many responsibilities in the household that they have. Hence, there is a need for program planners to determine an intervention which can reduce caregivers competing responsibilities and increase mothers' ability to satisfy the needs of her child.

Except for iron and protein, energy and nutrient intakes from complementary foods was below WHO recommendations. Particularly, the dietary diversity of the majority of the children was low and lower for stunted children. This may indicate the importance of promoting consumption of diverse food groups such as fruits, vegetables and animal source foods to prevent stunting. Improving nutrient bioavailability would also increase the nutrient adequacy of the complementary diets. Although, the use of MNP reduces the prevalence of inadequate intakes with can also lead to undesirable levels of excessive intakes, unless the dosage is adjusted to the local needs.

The prevalence of low BMVA concentration was severe and as a result the contribution of theBM to the child's daily vitamin A intake was lower than previous estimates. Similarly, theprovitamin A concentration of the breastmilk was lower than samples from other low-income countries, further suggesting the urgent need to improve the vitamin A and provitaminA carotenoid concentration of BM. This also underlines that, whenever possible, the BMVA concentration should be analyzed and not assumed to be of average composition. To this end, iCheck could be reliably used in-field. The iCheck would also allow frequent monitoring of interventions aimed at improving breastmilk vitamin A status of lactating mothers.

Improving complementary food and feeding practices of young children in the study area requires programs to consider both the behavioral and biological aspect of complementary feeding.

6.2 Perspectives

- The nutrition education was not accessible to more than half of the caregivers. Therefore, the possible factors that determine accessibility of nutrition education to mothers should be studied.
- There was a knowledge gap between the health extension workers and the caregivers even for those which the HEWs were knowledgeable, hence, the barrier that keep the HEWs from transferring the existing knowledge to the mothers should be examined.
- The risk of excessive nutrient intakes through point of use fortification should be examined further through biochemical indicators. Further work in defining the upper limit in young children is required.
- Longitudinal studies that investigate feeding behaviors across the complementary feeding period and beyond are required.
- The reasons of the low BMVA concentrations and how they can be improved through interventions warrants further investigation.

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8. Annexes

Annex A: Consent form

Date

Subject: Informed consent form

Dear Sir/Madame

We belong to the Center for Food Science and Nutrition of the Addis Ababa University. We work on the complementary feeding practices of young children. Our goal is to improve the complementary feeding practices and nutritional adequacy of the complementary foods. You are selected to take part of the study. If you are willing to take part of our study, you will be asked questions regarding your child food consumption, about the age at which she/he was first offered breast milk and foods other than breast milk, how many times a day you give food to your child, how you prepare the foods for your child, the type of food you give to your child and about the way you feed your child and the like. In addition, your height and weight will be recorded. You will also be asked to give 12 ml breast milk as well. The breast milk sample will be taken once, 6ml during the morning time and the additional 6ml during the afternoon time of the same day. We are, therefore, here to ask for your consent to take part of the study. You are totally free to accept or refuse to Participate in the study. If you decide to refuse, for whatever reason, there will not be any repercussions. If you accept, we guarantee you that confidentiality of all information collected will be assured. You are free to quit at any moment of the survey, without any prior notice or justification.

Household identification N°: |__||__||__||__||__|
Name of the mother or the guardian of the child:----------
Name of the household head:----------
Name of the person for which consent is asked:----------

Signature:

Subject: Assent form

Dear Sir/Madame

We belong to the Center for Food Science and Nutrition of the Addis Ababa University. We work on the complementary feeding practices of young children. Our goal is to improve the complementary feeding practices and nutritional adequacy of the complementary foods. Your child has been selected to take part of our study. If your child participates in the study, only his/her height and weight will be measured. The measurement will bring no harm to your child. We are, therefore, here to ask for your assent, so that, your child will take part of the study. You are totally free to accept or refuse. If you decide to refuse, for whatever reason, there will not be any repercussions. If you accept, we guarantee you that confidentiality of all information collected will be assured. You are free to quit at any moment of the survey, without any prior notice or justification.

Household identification N°: |__||__||__||__||__|
Name of the mother or the guardian of the child:----------
Name of the household head:----------
Name of the person for which assent is asked:----------

Signature:

Annex B- Questionnaires

I. Background characteristics

Name of the kebele:---------- ID: |__||__||__|| Name of the interviewer: _____
Name of the child:----------
The child's birth date: |__||__|/|__||__|/|__||__||__||__|
Sex: 1=male 2=female |__|
Start of the interview: _____ end of the interview: _____

1	**The child's birth weight:**	----------
2	Marital status of the mother:	1) Married 2) Unmarried 3) Widowed 4) Other, specify
3	Age of the mother:	
4	Who is the household head	1) Mother 2) Father 3) Grand mother 4) Other, specify
5	What is the educational status of the household head?	1) Primary school 2) Secondary school 3) Higher education 4) Other, specify
6	Livelihood strategy of the household head?	1) farmer 2) pastoralist 3) civil servant 4) business man 5) Other, specify
7	Total number of household members:	_____
8	Does the child have brothers and sisters?	1) yes 2) No
9	If your answer for the previous question is "yes", how many in total?	_____
10	If your answer for question number 8 is "yes" write the number of brothers and sisters in the following age groups	1) < 12months|__| 2) 12-23 months |__| 3) 24-59 months |__|

		4) 5-18 years|__|
		5) + 18 years |__|
11	Position of the child:	|__||__|
12	Religion:	1)Orthodox
		2) Catholic
		3) Muslim
		4) Protestant
		5) other (specify) |__|
13	How much land do you have? _____	
14	Do you have radio?	
15	Do you have TV set?	
16	What is the material from which the roof of the house is made of?	1) Corrugated sheet
		2) Dried grass
		3) Other (specify)
17	Please tell us the number of the following animals you have:	1) Oxes:_____
		2) Cows:_____
		3) Sheep:_____
		4) Goat: _____
		k) Chicken:_____
18	Do you have Eucalyptus tree? If yes how many?	
19	If you have stock, for how long it will last?	
Characteristics of the caregiver		
1.	What is your Relation to the child	1) Mother
		2) Father
		3) Grandmother
		4) Other, specify
2	Can you read and write?	1)yes
		2) No
3	What is your level of education?	1)Primary school
		2) secondary school
		3) higher education
		4) other, specify
4	Caregivers' activities: _____	

5	How many times did you visit a health care center for a prenatal visit during your pregnancy with the child in the study?	
6	Where did you give birth to the child?	1) home 2) health centre 3) other, *specify*

Mother and child weight and height/length

Weight of the mother

1st measure	**2nd measure**		
|__|__| . |__| kg	|__|__| . |__| kg		

Height of the mother

1st measure	2nd measure		
|__|__| . |__| m	|__|__| . |__| m		

Weight of the child

1st measure	**2nd measure**	
|__|__| . |__| kg	|__|__| . |__| kg	

length of the child

1st measure	**2nd measure**	
|__|__| . |__| m	|__|__| . |__| m	

Assessment of knowledge of caregivers (CG) and health extension workers (HEW) on
Proper complementary feeding practices.

Interviewer name:----------
Kebele name: ----------
Educational level (HEW):----------
Have you ever attended training on optimal complementary feeding practices 1. Yes 2. No

1	What is the right time to initiate breastfeeding after birth?	1) with in 1 hour
		2) 2 to 3 hours
		3) 4 to 6 hours
		4) Other, specify
2	What should be done to the first milk that comes out after birth?	1) should be discarded
		2) should be given to the child
		3) Other, specify
3	In your opinion, what conditions will keep a mother from giving breast milk to her child?	1) illness
		2) workload
		3) child refusal
		4) pregnancy
		5) she can give in all circumstances
		6) other, specify
4	What is the meaning of exclusive breastfeeding?	1) giving breast milk & water
		2) giving breast milk only
		3) giving breast milk and cow's milk
		4) giving breast milk and additional food
		5) other, specify
5	For how long should a child be exclusively breastfeed?	1) 4 months
		2) 6 months
		3) 8 months

		4) other, specify
6	In your opinion, what is the optimal time of complementary food introduction?	1) 4 months
		2) 6 months
		3) 8 months
		4) other, specify
7	What is the optimal time of continued breastfeeding?	1) 4 to 6 months
		2) up to 1 year
		3) 1 to 2 years
		4) 2 to 3 years
		5) other, specify
8	Who should feed the child when he/she starts to take semi solid/solid food?	1) anybody
		2) the mother
		3) a person who has positive relation with the child
		4) other, specify
9	When should a child (6 to 2 years) given food to eat?	1) when the child shows a feeling of hunger
		2) whenever food is available
		3) whenever the child ask for it
		4) following scheduled Mealtime
10	When a child is mature enough to eat by him/herself how should the child be fed?	1) on a separate bowel
		2) together with other family members

		3) with his/her friends
		4) other, specify
11	When should we wash our hands?	----------
12	How should we wash our hands?	1)only with water
		2) with water and soap
		3) other, specify
13	What is the proper complementary food handling procedure?	1) Uncovering the food then Keeping it at the Cool and clean side of the house.
		2) Covering the food then keeping it at the cool and clean side of the house
		3) keeping anywhere covered or uncovered
		4) other, specify
14	If the complementary food is prepared once a day and given for the child again and again what should be done before serving the food?	1) it should be given as it is
		2) it should be warmed
		3) other, specify
15	When should the materials used to prepare child food be washed?	1) sometimes before using
		2) sometimes after using
		3) always before and after using
		4) other, specify
16	What should be the thickness of the complementary food as the age of the child increases?	1) It should be thick like porridge
		2) It should be thin like gruel

		3) No need of change in thickness
		4) Other, specify
17	How many times a day should a child (1 to 2 years) be fed?	1) 3 times
		2) 2 times
		3) 5 times
		4) other, specify
18	What should be the frequency of food and breastmilk given for a child during and after illness?	1) Give more food and breastfeed more frequently
		2) Give less food and breast feed less frequently
		3) It should always be the same
		4). other, specify
19	What should be done when the child becomes mature enough to feed her/himself?	1) encouraging the child to eat by her/himself
		2) discouraging the child not to eat by him/herself
		3) nothing should be done
		4) other, specify
20	What should be done when a child (6 to 2yrs) refuses to eat without taking adequate amount of food?	1) Stop feeding
		2) Try other foods
		3) Avoid any destruction
		4) Encourage the child to eat more using different strategies
		5) Trying all of the above except number "1"
		6) other, specify
21	What is your opinion about bottle feeding?	1) It is good
		2) It is not good

		3) it is neither good nor bad
		4) other, specify
22	What type of food should children get to ensure that their nutrient needs are met?	1) Meat and meat products
		2) Milk and milk products
		3) Fruits and vegetables
		4) All type
		5) Other, specify
23	Is it recommended to give coffee, tea and sugary drinks such as soda for children?	1) yes
		2) No
		3) I don't know
		4) other, specify
24	Do you think that mineral/vitamin supplements are useful for children?	1) yes
		2) No
		3) I don't know
		4) other, specify
25	Which one of the following food or preparation has better nutrient content?	1)gruel
		2) porridge
		3) other, specify

Questionnaire prepared for caregivers on optimal infant and young and child feeding practices

Name of the child: _____
Sex: _____

1	How many hours/days/ after birth did you start breastfeeding your child for the first time?	1) Within1hour 2) After ___ hours 3) After----------days 4) I do not remember 6) Other, specify
2	If your answer for the previous question is other than number "1", what was the reason?	
3	If your answer for question number "1" is other than "1", what did you give to the child in place of breastmilk?	
4	Have you fed the colostrum to the child?	1) yes 2) No
5	If your answer is "no" for the previous question, what was the reason?	
6	When the age of the child was below six months, how did you determine whether the child was in need of breast milk/hungry?	
7	For how long did you exclusively breastfeed your child?	1) ___month 2)I do not remember 3) Other, specify
8	Are you currently breastfeeding?	1) Yes 2) No
9	If your answer is "yes" for the previous question, for how long are you planning to breastfed your child?	
10	If your answer for question number 8 is "no", when did you stop giving breastmilk to the child?	
11	When did you introduce complementary food to the child for the first time?	1) ___month 2)I do not remember 3) Other, specify
12	If the answer given for the previous question is "before or after 6 months" what was the reason?	
13	Who usually feeds the child	1) Mother 2) Father 3) Siblings 4) Other, specify

14	What was the food or preparation that you first gave to Your child?	1) Injera 2) Bread 3) Porridge 4) Gruel 5) Other, specify
15	Has the child started to eat family foods?	1) yes 2) No
16	If your answer is "no" for the previous question, which of the following looks like the thickness of the food you prepare for your child?	1) Porridge 2) Gruel 3) Other, specify
17	How many times a day do you prepare food for the child?	1) Once 2)Twice 3) Other, specify
18	When do you wash your hands?	
19	Do you put an effort to make the child's food palatble?	
20	If your answer is "yes" for the previous question, what do you exactly do to make the food palatable?	
21	Where do you put the prepared complementary food?	1) At the Cool and clean side of the house uncovered 2) At the Cool and clean side of the house covered 3) Anywhere covered or uncovered 4) Other, specify
22	How many times a day do you feed your child?	
23	Have you prepared scheduled meal time for the child?	1) yes 2) No
24	How do you know whether your child is in need of food or not?	
25	Do you follow what and how much the child eats per day?	1) yes 2) No
26	How do you determine whether your child is full?	
27	Does the child feed him or herself?	1) yes 2) No
28	If your answer for the previous question is yes, do you support your child while self-feeding?	

29	Which sign of illness was observed on the child in the last two weeks	1) Diarrhea 2) Cough 3) Fever 4) Other, specify
30	Do you prepare special meal when the child gets sick? if so what kind of food?	1) yes 2) No
31	Do you feed the child in a different way when he/she gets sick? If yes how?	
32	If your child stops eating, and if you think she/he is still hungry or did not eat enough, what do you do?	1) Try other foods 2) Force feed the child 3) Stop feeding 4) Other, specify
33	If the child eats well, do you praise the child?	1) yes 2)No
34	Which of the following will be in the house during the feeding event/feeding place?	1) Guests 2) Playing children 3) Domestic animals m 4) Other, specify
35	Is there any destruction in the house during the child mealtime? if so please mention them.	
36	Do you use any strategy to avoid distraction during the feeding event?	1) yes 2) No
37	If your answer is yes for the previous question, what strategies do you use?	
38	How do you describe the time that you feed your child?	1) Happiness 2) Frustrating 3)Fighting 4) Other, specify
39	Is there any food that your child does not like to eat?	1) yes 2) No
40	If your answer for the previous question is "yes" is there any strategy you use to make him like the food?	1) yes 2) No
41	If your answer is "yes" for the previous question, what strategy do you use?	

		1) Accept
		2) Reject
42	How does your child respond to a new food?	3) Other, specify
	If the child refuses to accept new food is there any strategy that	1) yes
43	you use to make him like it?	2) No
44	If your answer is "yes" for the previous question, what strategy do you use?	
45	Have you ever bottle fed your child? if so, when?	
		1) yes
46	Do you allow your child to eat whatever he/she wants to eat?	2) No
	Do you give coffee, tea and sugary drinks like soda to your	1) yes
47	child?	2) No
		1) yes
48	Has your child ever received mineral/vitamin supplements?	2) No
49	How long does it take you to reach the nearest health center?	
	Have you ever received messages about how you should fed your	1) yes
50	child?	2) No
51	If your answer for the previous question is "yes" what was the message?	
	Does the daily activity you are engaged in keep you from	1) yes
52	properly feeding your child?	2) No
		1) High
		2)Low
		3)Medium
53	How do you rate your child’s appetite?	4) Other, specify

Feeding behavior	Description					
	Mother		Child			
	Positive	Negative	Positive		Negative	
			Active	Responsive	Active	Responsive
Self-feeding: Directed toward or indicative of child putting food into own mouth	Allows, promotes self-feeding, e.g. gives child food to eat herself, verbally encourages or praises	Discourages, disallows, interrupts, e.g. ; pushes child s hand away, tells the child that mother will feed	Self-feeding attempt, e.g. holds utensils/cup, puts food into mouth			Rejects self-feeding, e.g. says o o th o s food that was given
Responsive: sensitive, synchrony, responds in accordance with the cue	Synchronous response promotes continued feeding, e.g. interprets child feeding cues, responds to child s needs.	Synchronous response interrupts feeding, e.g. responds to child 's reluctance cues by ending feeding episode prematurely.	accepts food when it is offered		interrupting feeding, e.g. walks away, refusal	
Active: encourages, keeps interested	mother-initiated attempt to arouse child' s interest, e.g. talks about food, models, food games, verbal encouragement, distraction if intent is to feed, refocusing attention such as taking on knee.	Aversive, intrusive attempts to direct feeding, e.g. force-feed, holds child s head, threats, shakes child	Child-initiated attempt to get food, e.g. looks at food, says food words, requests food/drink, touches food, opens mouth, cries for food.		Shows disinterest, discouragement	
Social behavior: toward feeding partner only but not directly related to feeding	Talking (but not about food), touching, smiling, looking, laughing.		Talking (but not about food), touching, smiling, looking, laughing, cries but not in response to food.			
Distracting feeding behavior	Encourages attention away from feeding, e.g. by talking to someone other than child		Child is distracted from eating, child can be active party or reactive (include social interaction directed towards non-feeder)			

24 hour recall questionnaire

"I would like you to tell me what your child had to eat or drink after he/she woke up yesterdaymorning. Did he/she eat that food at home?

_____

What did you have next and at what time?" _____

Proceed through the day, repeating these questions as necessary, and record each food or drink (including drinking water) consumed in column 3 of the 24-hour recall form (Table 5.1). Remember to probe for any snacks and drinks consumed between meals.

Interviewer:_____ Id no._____ Interview date:_____ Mon Tue Wed Thu Fri Sat Sun			Location:_____ Sex:_____ Subject Id:_____ Age:_____ Subject name:_____ Weight:_____ Height:_____			
Time	Place eaten	Food/drink	Description, cooking method	Amount	Weight equivalent(g)	Food code
Probe for sickness: Yes No If yes, did sickness affect appetite? Yes No If yes, how? Increase decrease						
Was food intake unusual? Yes No If yes, how was it unusual?			Probe for tablets: Yes No Iron vitamins other supplements anti-malarial			
Was it a feast day? Yes No Was it a market day? Yes No Was it a fasting day? Yes No						

Subjects name: sex:
Subject Id:
Interview date:
Day of the week food eaten:
Name of interviewer: **Name of mixed dish 1 - 5**

Amount of eaten by respondent (g or mL)

Wt empty pot Wt cooked mixed dish+pot:

Wt mixed dish Volume of cooked mixed dish: (mL)

Proportion of mixed dish consumed by respondent:

Ingredient	Description of ingredient and cooking method	Amount of raw ingredient in recipe	Weight of raw ingredient in recipe	Weight of raw ingredient consumed	Weight of cooked ingredient in recipe	Weight of cooked ingredient consumed

Feeding behavior	Description					
	Mother		Child			
	Positive	Negative	Positive		Negative	
			Active	Responsive	Active	Responsive
Self feeding: Directed toward or indicative of child putting food into own mouth	Allows, promotes self-feeding, e.g. gives child food to eat herself, verbally encourages or praises	Discourages, disallows, interrupts, e.g. o ; pushes hild s ha d away, tells the child that mother will feed	Self-feeding attempt, e.g. holds utensils/cup, puts food into mouth			Rejects self-feeding, e.g. says o o th o s food that was given
Responsive: sensitive, synchrony, responds in accordance ith othe s ue	Synchronous response promotes continued feeding, e.g. interprets child feeding cues, responds to hild s eeds.	Synchronous response interrupts feeding, e.g. responds to hild s reluctance cues by ending feeding episode prematurely.	accepts food when it is offered		'espo ds to othe s ue y interrupting feeding, e.g. walks away, refusal	
Active: encourages, keeps interested	mother-initiated attempt to a ouse hild s interest, e.g. talks about food, models, food games, verbal encouragement, distraction if intent is to feed, refocusing attention such as taking on knee.	Aversive, intrusive attempts to direct feeding, e.g. force-feed, holds hild s head, threats, shakes child	Child-initiated attempt to get food, e.g. looks at food, says food words, requests food/drink, touches food, opens mouth, cries for food.		Shows disinterest, discouragement	
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Distracting feeding behavior	Encourages attention away from feeding, e.g. by talking to someone other than child		Child is distracted from eating, child can be active party or reactive (include social interaction directed towards non-feeder)			

Additional codes for mother active positive

- **Verbal directions (e.g. ''eat, eat'').**
- **Talks about food, labels food (e.g. ''this is rice, rice is nice'').**
- Asks the child a question about food.
- **Diverts momentarily (e.g. ''Look at the ants'').**
- **Focuses the child's attention (e.g. Puts child on lap, Brings child back to feeding place).**
- Modeling (e.g. shows child how to eat, swallow).
- Makes a positive statement about the **child during eating (''you are so good'').**

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149/1028. Building the evidence-base for effective communication strategies to improve child feeding in rural Ethiopia

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