ZINC, IRON, IODINE AND COGNITIVE FUNCTION

OF WOMEN FROM SIDAMA ZONE,

SOUTHERN ETHIOPIA

By

TAFERE G/EGZIABHER BELAY

Bachelor of Science in Rural Development and Family

Sciences

Debub University

Awassa, Ethiopia

2006

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July 30, 2010

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SIDAMA ZONE,

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Thesis Approved:

Dr. Barbara J Stoecker
_____
Thesis Adviser

Dr. Tay Kennedy
_____

Dr. David Thomas
_____

Dr. Laura Hubbs-Tait
_____

Dr. Mark Payton
_____
Dean of the Graduate College

ACKNOWLEDGMENTS

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CHAPTER I

INTRODUCTION

Various disciplines define cognition in many different ways but generally it refers
to processes such as attention, language, perception, action, association, memory, concept
formation, problem solving, and mental imagery (Coren *et al.*, 1999). Cognitive function
refers to the processing of information taken in from the environment and reflected in the
form of behavior (Isaacs & Oates, 2008).

Cognition is influenced by various environmental factors at different stages of
life; however, the effect of nutrition is thought to be crucial throughout life. The role of
nutrition on cognition is getting attention, because people came to realize that the
development of brain occurs at more stages of the life-span than previously understood
(Toga *et al.*, 2006). Nutrition is one of the major environmental variables that can be
manipulated relatively easily with regard to brain function. Not only does nutrition affect
brain development but it also plays a significant role in maintaining function of brain
(Isaacs & Oates, 2008).

Diet affects neurochemistry in three ways. First, the availability of precursors that
are needed for the synthesis of neurotransmitters is affected by the food ingested. Second,
neurotransmitters are synthesized by enzymes which require co-factors such as minerals
and vitamins obtained from food. Third, neuronal functions are influenced by the
composition of myelin sheath and cell membranes which are also affected by dietary fats
(Greenwood & Craig, 1987). Additionally, cognitive function is enhanced by glucose,

because brain uses glucose as its main metabolic fuel indicating that neuronal function and consequently cognition can be affected by variations in the normal diet other than malnutrition (Benton *et al.*, 2003).

Cognitive level as a whole might be affected through nutritional interventions especially in early life and be reflected in IQ scores; whereas, in later life during adolescence, adulthood, and aging, nutritional interventions might affect performance of attention and memory which are specific cognitive domains. For example, eating breakfast can have a short-term effect on cognitive performance and might effect a specific function such as attention (Isaacs & Oates, 2008).

A study conducted in Guatemala showed that slower reaction time on neuropsychological tests such as perception, attention, memory and reasoning were observed in vitamin B_{12} deficient school-aged children than those who were not deficient (Black, 2003a). Several studies suggest that micronutrient deficiencies such as iron, zinc, vitamin A, and iodine bring about impairments in growth, immune competence and cognitive function among school-age children (Viteri & Gonzalez, 2002; Benton, 2008). Such adverse health consequences can lead to reduction in both reproductive and intellectual potential in adulthood (Horton, 2000). All domains of cognitive functioning are impaired by micronutrient deficiencies (Sungthong *et al.*, 2002) and are sensitive to change due to micronutrient status among school children (Hughes & Bryan, 2003).

Zimmerman and colleagues indicated that schoolchildren who have increased iodine status by iodine supplementation showed better performance in rapidity of information transfer into working memory than those who were not supplemented (Zimmermann *et al.*, 2006a).

Iodine deficiency may lead to fetal hypothyroidism and irreversible neurological and cognitive impairment manifested as cretinism. Neurological cretinism is a general term which includes visual problems, facial deformities, abnormal neuromuscular response, developmental disability (mental retardation), stunted growth and paralysis (diplegia) (Black, 2003a).

In 1993, a situational analysis was carried out by United Nations Children's Fund (UNICEF) and Ministry of Health (MoH) in Ethiopia. During that time, it was reported that 42 million people (78% of the total population of Ethiopia) exhibited signs of iodine deficiency disorder: 35 million (62%) were iodine deficient, 14 million (26%) had goiter, at least one in 1000 people was a cretin, and about 50,000 prenatal deaths were attributed to iodine deficiency (Ministry of Health & UNICEF, 1995). In 2007, Cherinet Abuye and Yemane Berhane surveyed goiter nationally and found that goiter rate among 15 to 49 year old women was 35.8%, which included 24.3% palpable and 11.5% visible goiter. They estimated that about 6 million women in this age category were affected by goiter in Ethiopia. In the SNNP Region, out of 1702 women examined for goiter, 43.2% had palpable and 17.7% had visible goiter, which is a total goiter rate of 60.9% (Cherinet & Yemane, 2007).

Improvements in cognitive function due to iron supplementation have also been observed in older children and adults (Falkingham *et al.*, 2010). One of the most important causes of impaired memory skills and lowered concentration abilities is found to be iron deficiency anemia (IDA) (Kumar & Rajagopalan, 2008).

In developing countries multiple micronutrient deficiencies occur in the poorer segments of the population (WHO, 2000). In sub-Saharan Africa malnutrition is highly

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prevalent, particularly in the rural areas where most of the people have limited resources (Uthman & Aremu, 2008). One recent survey in Ethiopia found that $> 20\%$ of rural women were underweight (Uthman & Aremu, 2008). According to the Ethiopian Demographic and Health Survey (EDHS), in Ethiopia the level of chronic energy deficiency among women is relatively high. Twenty-seven percent of women fall below the cutoff point for chronic malnutrition (BMI < 18.5). Furthermore, 27 % of women age 15-49 are anemic, with 17 % mildly anemic, 8 % moderately anemic, and over 1 % severely anemic. Anemia also is higher among rural than urban women. In the Southern Nations, Nationalities and Peoples Region (SNNPR) of Ethiopia, the percentage of anemia among women of reproductive age is 23.5 %, 14.8 %, 7.7 % and 1.0 % , which is classified as any anemia, mild anemia, moderate anemia and severe anemia respectively (Central Statistics Agency, 2005).

In addition to iron and iodine deficiency, zinc deficiency may be prevalent in most developing countries. Due to lack of biochemical indicators and /or specific clinical signs related to mild zinc deficiency, its extent is not known (King, 1990). However, according to Hotz and Brown in 2004, 21.7% of the population in Ethiopia were at risk of inadequate zinc intake (Hotz & Brown, 2004)

The effect of zinc on cognitive function is less clear and the results reported so far have been inconsistent (Black, 2003a). However a study conducted in Southern Ethiopia indicated that zinc deficiency is a factor related to cognition in pregnant women (Stoecker *et al.*, 2009). Although evidence for zinc is more limited, Maylor and colleagues showed younger adults performed better in cognitive tests following zinc supplementation than older adults (Maylor *et al.*, 2006).

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Various reports with regard to the effects of iron, iodine and zinc on cognitive functioning at different stages of life are available (Gewa *et al.*, 2009); however, to the best of our knowledge information related to the effects of the aforementioned micronutrients among women of reproductive age is very limited. Any information related to adult women mostly focuses on pregnant and/or lactating mothers.

Pursuant to the above stated scenario, this study is primarily designed to assess the status of iron, iodine, and zinc and their relation to cognitive functioning of non-pregnant women age 18 and above in three kebeles (the smallest administrative unit of Ethiopia) situated near Awassa town. The study also examined relations of some selected demographic and socioeconomic variables to cognitive functioning of study participants.

General objective

- The major objective of this study was to examine zinc, iron, and iodine status and their relation to cognitive function of women age 18 and above.

Specific objective

The specific objectives include

i) To assess cognitive performance by Raven's CPM and selected KABC-II Tests

ii) To examine biomarkers for zinc, iron and, iodine status

iii) To investigate household level socioeconomic and demographic variables.

Hypothesis

- Iron, zinc and iodine status will be related to cognitive function of women.

The thesis consists of 4 chapters following this introduction. Chapter 2 is literature review which includes research findings on cognition and its association with micronutrients specifically with zinc, iron and iodine. Chapter 3 is the methodology which includes subject selection, design of the study, administration of questionnaire, cognitive tests and assessment of biomarkers. In chapter 4 research findings such as characteristics of study participants, status of micronutrients (zinc, iron and iodine), scores of cognitive performance, association of cognition and micronutrients and differences of variables in the three study areas are presented and discussed. Chapter 5 is the recommendations section. In this section, solutions to identified problems of the study participants and suggestions for future research are presented.

CHAPTER II

LITERATURE REVIEW

Overview

The purpose of this study is to investigate possible associations of zinc, iron and

iodine with cognitive performance of adult women. Although the effects of these

micronutrients on cognitive development and functioning have been clearly described in

infants and children, information is scarce in adults. Therefore, this review will show the

importance of these micronutrients to brain development and function in general and

cognition in particular in human beings.

Cognition and micronutrient deficiency

Cognition

The most metabolically active and complex organ of the human body is the brain,

which is responsible for cognition (Benton, 2001). Cognition includes visual and

somatosensory perception, thinking, memory and learning and is considered to be an

outcome of millions of metabolic processes where its main task is registering, encoding,

selecting, maintaining, transforming, storing and retrieving information (Ruff &

Rothbart, 1996). Cognition, as well as its elements which include reasoning, attention,

memory, and psychomotor coordination, is complex. It is not difficult to understand how

cognition is complex when we see how intricate even the sub components are. Memory

for instance, as part of cognition includes visual, long term, short term, verbal, spatial,

declarative, strategic, and semantic and requires several tools for assessment (Bellisle,

2004). Another example, attention is widely considered to be a single unit involving several separable systems that are mediated by their own pathways (Colombo, 2001).

Cognitive function, as defined by Wainwright and Colombo, is the neural process that is necessary to support the flexible use of information to carry out adaptive and goal-directed behavior. The ability to execute adaptive behavioral responses depends on the capacity to focus and maintain attention and these processes also depend on the holding capacity of information in working memory (Wainwright & Colombo, 2006) . Other information processing functions that are known to be more complex are also involved. One example could be the ability to use conceptual information and organize it in a meaningful way for appropriate responses in different contexts and to hold back other competing behavioral responses (Wainwright & Colombo, 2006).

The decision making processes and information processing of mammalian brain are mediated by neural circuits comprising a series of reciprocal cortical-subcortical loops. The cortex is the place from which these circuits originate and then project to various sub-cortical structures. They project to structures including basal ganglia and nucleus accumbens. They then return to their origin, region of cortex, via the thalamus (Alexander *et al.*, 1986). These structures and the pathways are influenced by the inputs from many other regions of the brain (Chambers *et al.*, 2003) and the presence of such functional interactions between these structures is essential to both behavioral and physiologic regulation (Everitt & Robbins, 2005). The hippocampus, an important source of input to the nucleus accumbens, in addition to the prefrontal cortex (Everitt & Robbins, 2005), is necessary for the formation of long-term memory (O'reilly & Rudy, 2001). The hippocampus, by representing the relations between distinct stimuli, also

plays a role in higher-level decision making processes (O'reilly & Rudy, 2001). The prefrontal cortex, during the decision making process, generates possible behavioral alternatives in response to the specific nature and emotional categorization of sensory stimuli which can later be transferred to the nucleus accumbens (O'Donnell & Grace, 1995). The neural transmission involving the hippocampus, prefrontal cortex, and nucleus accumbens is vital in enabling behavioral flexibility (Atallah *et al.*, 2004).

The amygdala, the other source of input to the nucleus accumbens is involved in processing the affective emotion of sensory stimuli and passing on this information to the prefrontal cortex (Everitt & Robbins, 2005; Phelps & LeDoux, 2005). In addition, the amygdala also plays a vital role in stress responses during occurrence of perceived dangers (Phelps & LeDoux, 2005). According to McGaugh, the stress response includes the release of glucocorticoid hormones. Appropriate concentrations of glucocorticoids are necessary for mnemonic functions to be accomplished effectively (McGaugh, 2004) and the hippocampus together with the prefrontal cortex, are involved in the feedback regulation of these glucocorticoid concentrations (Kloet *et al.*, 2005).

Cognition is affected by multiple factors such as previous learning, fatigue, motivation, individual skill, general arousal and time of day. Although less attention has been given previously, nutrition is found to be one of the major factors to affect cognition (Bellisle, 2004). Due to the fact that about 70% of the major development of the brain takes place during the prenatal period and most of the remaining 30% is during the first three years postpartum, optimal nutrition is most fundamental during pregnancy and the first 3 years of life (Singh, 2004).

Micronutrient deficiency

The term micronutrient includes both vitamins and essential trace minerals. Inadequate intake of these nutrients increases rates of illness and death from infectious diseases, and of disability such as mental impairment. Although deficiencies of any of the essential micronutrients can result in health problems, there are a few that are particularly important and their deficiencies are highly prevalent in low- and middle- income countries (Black, 2003b). Despite all the efforts to prevent and control micronutrient deficiencies, worldwide more than two billion people are at risk of vitamin A, iodine, and/or iron deficiency and the prevalence is particularly high in sub-Saharan Africa and Southeast Asia (Ramakrishnan, 2002). Even if the actual prevalence is not known, deficiencies of zinc, folate and the B vitamins also are of public health concern. Most of the time, a single micronutrient deficiency doesn't occur independently. Therefore, approaches to assess and evaluate multiple micronutrient deficiencies are necessary (Ramakrishnan, 2002). In developing countries, deficiencies of iron, iodine, vitamin A and zinc are common and are the main indicators of malnutrition (Muller & Krawinkel, 2005).

As mentioned above micronutrient deficiencies commonly overlap each other in low- and middle-income settings. However, the effects of some micronutrients such as vitamin A, iron and zinc deficiencies might be independent but zinc and iron may interfere with each other in absorption. Therefore it is vital to understand the situation prior to supplementation (Black, 2003b).

Several factors contribute to the widespread prevalence of micronutrient deficiencies. These factors include a low dietary intake, low bioavailability as in the case

of iron and zinc, poor utilization due to environmental factors, such as poor hygiene that lead to increased infections and infestations, adverse nutrient-nutrient interactions and genetic causes (Sandström, 2001).

Although all nutrients are required for bodily functioning, specific nutrients are required for brain development and cognition. Deficiency of some micronutrients influences the cognition and behavior of children (Benton, 2008).

The effect of selected micronutrients on cognitive function

Micronutrients are required in small concentrations as essential components of biological enzyme systems or of the structural portion of biologically active constituents. The effects of trace elements are related to their concentration and recorded observations of micronutrients range from a deficiency state, to biologically sufficient state, to function as biologically essential components, to an unbalance when excess of one element interferes with the function of another, to pharmacologically active concentrations, and finally to toxic and even life-threatening concentrations (Andrasi *et al.*, 2007).

Micronutrients are required for production of several enzymes and co-factors for a number of metabolic pathways. Among the micronutrients, iron is required for production of gamma-aminobutyric acid (GABA), serotonin and dopamine which are essential for neurotransmission. Iodine is required for production of thyroxin and tri-iodothyronine. The concentration of zinc in the brain, particularly in the hippocampus, is high, and zinc is a component of metalloenzymes (Singh, 2004; Yuanxun *et al.*, 2006).

Zinc

Zinc and its importance

Zinc is one of the essential trace elements that is most important in human
nutrition and health (Hambidge, 2000). Its significance for both humans and animals has
been known since the 1930's. Zinc is required for more than 300 enzymatic reactions
and has a role in a large number of macromolecules. Deprivation of zinc reduces growth
and development and produces system dysfunction in both humans and animals. Zinc
ions have roles in the action, synthesis, and storage of peptide hormones and structural
maintenance, metabolism, transmission and regulation of the expression of genetic
information. Zinc is also needed for protein and DNA synthesis, cell mediated immunity,
thyroid and bone metabolism, growth and development, and neuro-sensory functions
(Meunier *et al.*, 2005). The absence of redox properties in the zinc atom allows it to have
a better incorporation in the body, unlike iron and copper which exert the risk of
oxidative damage during their metabolism. Zinc has several chemical properties that
account for its various biological roles. Among others, the strained status of the zinc atom
geometry in some zinc metalloenzymes has an enhanced catalytic activity due to
closeness of the extensive protein β–sheets to the catalytic sites. These structural roles of
zinc in enzyme molecules as well as proteins are very important in cellular and sub-
cellular metabolism (Hambidge, 2000). Physiochemical methods resulted in less insight
into the biology of zinc than iron and copper as a result of its properties (Maret, 2001).

Zinc is found dispersed among thousands of proteins in the human body unlike
iron, where about 80% of a total of 3 g is situated in the heme group only. The role and

presence of zinc in some proteins that are less abundant is difficult to establish due to its scattered appearance (Maret, 2001).

Except acrodermatits enteropathica, a genetic disease of zinc metabolism, so far there is no disease directly related to zinc deficiency. Although the significance of zinc in health is not yet clear, mild zinc deficiency can cause immunological dysfunction, impair defense mechanisms and increase infectivity. On the contrary, excess accumulation of zinc in neurons leads to neurodegeneration. Therefore, zinc may be a major etiological factor for disease and an important nutrient for human health (Maret, 2001).

The advancing knowledge of tissue specific expressions and functions of zinc through metal-responsive transcription factor or cytokines or growth factors complement each other. The current insight on the role of Zn^{2+} as mediator and control of secondary signaling via cellular and vascular transport is providing clues to functional effects of zinc trafficking. Specific diseases are linked to abnormalities in pathways of zinc transport. The Zn^{2+}, as it exists in metallothionein, is considered labile as demonstrated by the effect of reactive oxidative species in mobilizing Zn^{2+} back to cellular pools (Fig 2.1) (Cousins *et al.*, 2006).

Due to its antioxidant properties and impact on different body activities, zinc is getting attention (Lonnerdal, 2000). Very good sources of zinc are red meats (especially organ meats) and seafood (especially oysters and mollusks) (Hunt *et al.*, 1998; Lonnerdal, 2000; Hughes & Bryan, 2003). Zinc is also available in cereals, corn and vegetables, but people who depend on these diets are susceptible to zinc deficiency. This is because these types of food contain a substantial amount of inositol phosphate, also called phytate, which are known compounds that inhibit zinc absorption (Hunt, 2003).

Fig 2.1 Signaling pathways that regulate some ZnT & Zip genes & influence Zn^{2+} traffiking.
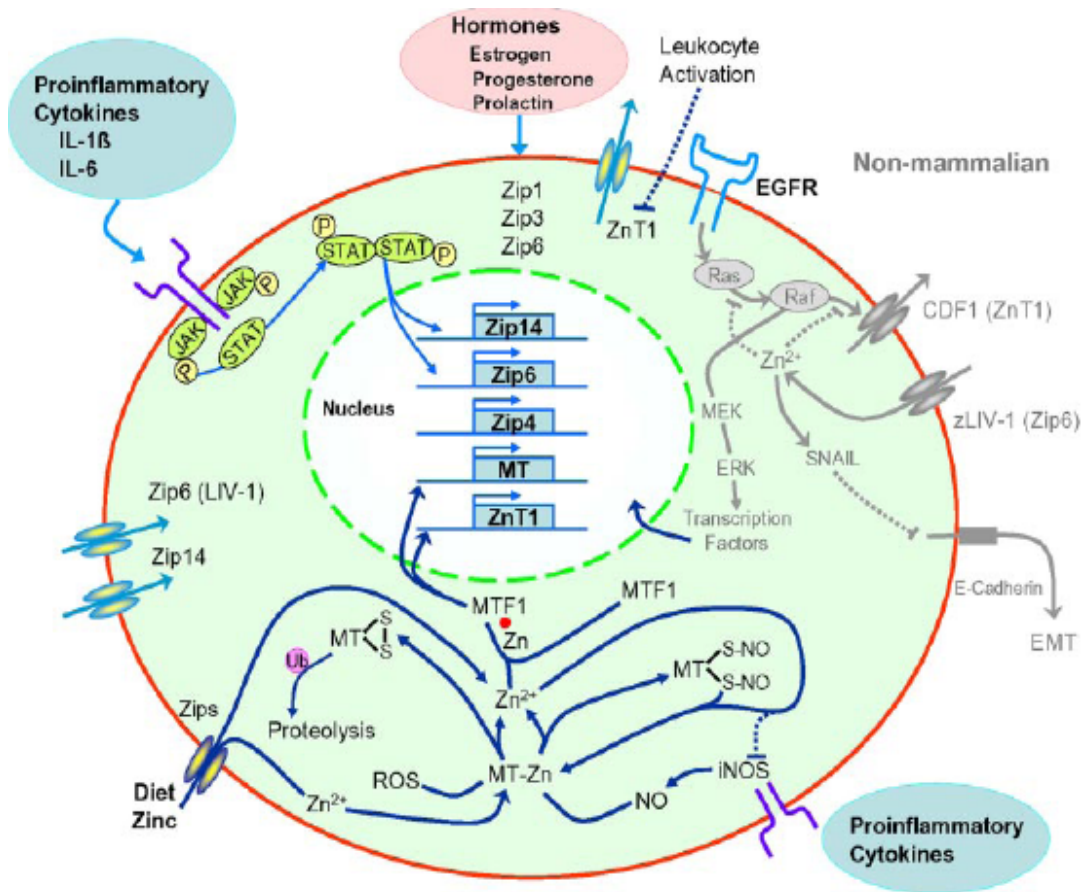
Zinc absorption can be impaired when meat consumption is less and intake of phytate-rich grains and legumes is high. Hence, absorption of zinc and iron is lower in vegetarian than in non-vegetarian diets (Hunt, 2003).

Bioavailability of zinc however can be improved by dietary protein. Although plant sources of protein are generally high in phytic acid, its effect can be modified by the sources and amount of dietary proteins consumed (Shils *et al.*, 1999). Animal proteins such as cheese, milk and beef have amino acids that have a mechanism to withstand or breakthrough the binding effect of phytate on zinc (Sandström & Cederblad, 1980).

The World Health Organization (WHO) report issued in 2003 estimated absorption of dietary zinc to be 50%, 30% and 15% for diets having zinc: phytate molar ratio less than 5, 5-15 and >15 respectively (WHO *et al.*, 2002). A study in Sidama (Southern Ethiopia) of pregnant women, where their major sources of zinc came from cereals and enset (E. *ventricosum*) showed that the median phytate: zinc molar ratio of their diets was 18.6, which is moderately high to contribute for the low zinc intakes (Abebe *et al.*, 2006).

Studies on humans indicate that fractional zinc absorption increases with increasing protein content. Emphasis should be given to the concept that protein is a major source of dietary zinc that brings about an increased intake of zinc when protein content of the meal is increased. Therefore, a higher bioavailability and increased intake of zinc can be obtained as an outcome of increased dietary protein (Lonnerdal, 2000).

Lonnerdal has reviewed different methods to reduce phytate content of various foods. These are fermentation, leavening of bread, germination and milling, and treatment of foods by addition of phytase to the diet. Moreover, producing low phytate cultivars of legumes and cereals to increase mineral bioavailability is possible by plant breeding and genetic engineering (Lonnerdal, 2000).

Alferez and colleagues in their study in rats found that zinc and selenium bioavailability was greater from a diet based on goat's milk than in the cow's milk diet. This may be because goat's milk is richer in cysteine which contributes to high absorption and metabolism of zinc and selenium (Alferez *et al.*, 2003). Zinc solubility and absorbability increase when zinc forms a complex between a low-molecular-weight

ligand or chelator. Zinc absorption can also be improved by an amino acid called histidine (Ishihara *et al.*, 2008).

A study conducted in Malawi showed that improving the bioavailability of zinc had different effects on zinc absorption in different groups of children. Dietary phytate reduction resulted in greater fractional absorption and total absorbed zinc, for the children who were on the way to recover from tuberculosis. On the contrary, dietary phytate had no effect on zinc absorption among the healthy children (Manary *et al.*, 2000).

A conducted in China showed that cereal grains are rich in zinc and contribute to dietary zinc around 27.4 – 63.2 %, whereas animal food sources contribute 16.8 – 54.8%. The contribution from fruits and vegetables is 8.2 – 13.8%, while 1.3 – 3.4% comes from legumes (Ma *et al.*, 2007). However, zinc absorption is influenced by several factors other than its bioavailability. These factors include the physiologic state of the individual, the duration of low zinc intake, and the amount consumed (Lukaski, 2005).

A study conducted on men during exercise indicated that low dietary zinc resulted in considerably lower serum and RBC zinc concentration, lower zinc retention, and decreased activity of carbonic anhydrase in RBC indicating marginal zinc deficiency (Lukaski, 2005). Another study examining long-term zinc consumption of healthy adult men concluded that current dietary zinc intake directly affects the total amount of zinc that was absorbed and inversely influenced the efficiency of zinc absorption. The extent of these effects was remarkably greater than any presumed effect of long term zinc intake. Having a very low zinc intake (<4mg/d) for longer periods of time increased zinc absorption, however consumption of extremely low zinc for long periods is not common in free – living adults (Chung *et al.*, 2008).

Because zinc bioavailability and absorption are affected by various factors, recommendations other than gender should consider age and metabolic need such as lactation and pregnancy. For instance younger individuals may require lower values. According to Gibson because zinc is not readily available in vegetarian diets, the requirement should be at least 50% higher. Besides, pregnant and nursing women need an extra 4 and 3 mg per day, respectively. When recommending dietary intakes of zinc, consideration is needed for the type of diet, physiologic factors and effects of diseases (Gibson, 1994).

Zinc is essential for development of brain and its function (Sandstead, 2003). Zinc deficiency contributes to reduced brain zinc and cognitive abnormalities (Noseworthy & Bray, 2000; Sandstead *et al.*, 2000). Zinc is necessary for the hippocampal formation and cerebral cortex and is stored in the synaptic vesicles of these parts of brain. Therefore, a proper zinc supply to immature brain is necessary for improvement of potential learning ability. As a study on rats showed, when 8-week-old rats were fed a diet deficient in zinc for about 4 weeks, the learning behavior was significantly impaired, and it was recovered to almost normal level by feeding with control zinc-adequate diet for 5 weeks. This result leads to a conclusion that proper zinc supply to mature brain is necessary for maintenance of the learning ability. It was also indicated that hippocampal zinc level may be susceptible to zinc deprivation (Takeda *et al.*, 2000).

According to a study by Sandstead, when mothers consume more animal protein and zinc, their infants had higher scores on the Brazelton Neonatal Development Assessment Scales. When the infants were assessed using Bayley Scale of Infant Development after six months, the infants showed better motor development. When the

infants were provided with formula containing 11 mg Zn/L, their growth and motor

development (Griffiths Development Assessment Test) were greater than when provided

with formula containing 6.7 mg Zn/L (Sandstead, 2003).

Zinc was found to improve neuropsychologic performance in Chinese children

(Sandstead *et al.*, 1998). However, the influence of zinc is not limited to brain function

but also affects the central nervous systems in different ways. For instance, less

sensitivity to taste and smell was observed in zinc deficient humans (Arnold &

Disilvestro, 2005).

There is no question about the importance of zinc for brain structure, but more

research is needed to investigate the mechanisms by which zinc affects cognitive

development (Bhatnagar & Taneja, 2001). Zinc is suspected to be a key modulator of

neuronal excitability due to its presence in the synaptic vesicles of the specific zinc

containing neurons in the forebrain along with its function in biochemical processes like

myelination and release of neurotransmitters like gamma amino butyric acid (GABA) and

glutamate, in high concentration (Frederickson *et al.*, 2000).

Furthermore, zinc is a component in glutamatergic neurons, and these zinc

containing glutamatergic neurons are situated particularly in the cerebral cortex and

amygdala. Thus, the presence of zinc in glutamatergic synapses may be related to

cognitive and mnemonic operations unique to the cerebrum (Frederickson & Danscher,

1990). Zinc containing somata are located almost exclusively in the cerebral cortex and in

the amygdalar nuclei. Efferent zinc-containing fibers from these regions are in turn

directed almost exclusively to striatum or limbic targets such as septum, nucleus of the

diagonal band and medial hypothalamus, amygdala and cerebral cortex (Christensen & Frederickson, 1998).

The neuronal networks that contain zinc are places for junction of the hippocampal formation and amygdalar complex. All amygdalar nuclei receive some zinc containing input, and most of the nuclei also send zinc containing efferents to both remote and local targets (Christensen & Frederickson, 1998). Vesicular zinc is highly present in the telencephalon, in areas such as the basal ganglia, and in the septum. Several studies strongly relate vesicular zinc and excitatory amino acid neurotransmission. Zinc is co-localized with glutamate within synaptic boutons where it contributes much in the spinal cord and amygdalae sensory information (Perez-Clausell, 1996; Takeda *et al.*, 1999).

In the visual system of monkeys, cats, and humans, cortico-cortical connections between visual areas are organized in a hierarchy according to the laminar distribution of the neurons and terminal fields (Rockland, 1997). These zinc-rich systems may have a direct influence in processing visual information. By comparison with the somatosensory cortex, they may even be involved in the stimulus-dependent modeling of the visual cortex during postnatal development (Casanovas-Aguilar *et al.*, 2002). A study on juvenile monkeys showed that zinc-deficient animals were emotionally less mature and a cognitive deficit was associated with severe zinc deprivation (Sandstead *et al.*, 1996). As mentioned above, although zinc has a major role in cognitive structure and functions, understanding the period when zinc is most crucial in those activities is essential. Not only that, but also the biological mechanisms of zinc in cognitive function and development also require in depth and specific research (Bhatnagar & Taneja, 2001).

Zinc deficiency

Physiologic functions required for optimal work performance can be affected by low zinc status. There is an association between reduced serum zinc concentrations and low zinc intakes with impaired muscle function, including reduced strength and increased susceptibility to fatigue (Loan *et al.*, 1999).

Deficiencies of zinc and iron are among the deficiencies that commonly occur simultaneously. Having mild-to-moderate deficiencies can cause neuropsychologic dysfunction (Sandstead, 2000). There is convincing evidence from animal research that zinc deficiency affects cognitive development by increasing emotional behavior, impairing the capacity to learn, impairing memory and decreasing motor activity (Bhatnagar & Taneja, 2001). Zinc deficiency affects cognitive development by alterations in attention, activity, and other features of neuropsychological behavior and motor development (Black, 1998).

Maternal zinc deficiency in early pregnancy, a period of fetal organogenesis, results in impaired implantation, fetal resorption, abortion and fetal brain malformations (Dreosti & Smith, 1983). A study in humans indicated that when maternal zinc intake was low the neonates had less attention and poor motor activity (Bhatnagar & Taneja, 2001). The effects of zinc deficiency on cognitive development vary also by the behavior of the mother, by her age and by other social factors (Black, 1998).

Zinc deficiency is related to extreme clinical morbidities such as cognitive dysfunction, hypogonadism in males, growth retardation, and cell mediated immune disorders. A study in the Middle East also showed that most of the zinc-deficient dwarfs did not live beyond the age of 25 years (Meguid, 2001).

Zinc deficiency can be severe. A genetic disorder called acrodermatitis enteropathica is caused by zinc deficiency other than by excessive use of alcohol and penicillamine therapy (Prasad, 2008). Acrodermatitis enteropathica is characterized by inflammation of the skin mostly around the body orifices, on the head, hands and feet. It occurs in early childhood and also causes severe gastrointestinal and cutaneous disease followed by diarrhea and true steatorrhea (Agnew *et al.*, 1965). Severe zinc deficiency in humans can also cause alopecia, bullous pustular, dermatitis, emotional disorders, diarrhea, intercurrent infections, weight loss and ulcers that fail to heal. If these complications are not treated promptly, they can be fatal (Prasad, 2008).

The health problems related to zinc deficiency also include reproductive difficulties and impaired cognition (Sandstead, 2000). Prevalence of zinc deficiency in developing countries in children is becoming so severe that it requires universal attention (Black, 2003c). The effect of zinc deficiency is not limited to morbidity but also increases mortality rate. If oral zinc supplementation is not provided earlier, it is fatal in later infancy for those patients who have acrodermatitis enteropathica (Hambidge, 2000). At this point, hence, it is paramount to recommend that increased dietary zinc intake is considerably important as a preventive measure (Hambidge & Krebs, 2007).

Women who have low plasma zinc concentration and those who have acrodermatitis enteropathica were found to have poor pregnancy outcomes. This

condition clearly emphasizes the role of zinc in human pregnancy. The results in humans were very similar to those of pregnant animals that were deficient in zinc. These animals showed growth retardation of the fetus and congenital anomalies. Even if acrodermatitis enteropathica is very rare, the effect of marginal maternal zinc is an important issue on pregnancy outcome (King, 2000).

Health problems related to pregnancy such as postpartum hemorrhage, congenital malformation, hypertension, prolonged labor and spontaneous abortion have correlations with low plasma zinc concentrations (Gibson, 1994). Milder zinc deficiency has been related to preterm delivery, low birth weight and intrauterine growth retardation (Jameson, 1993). Nevertheless, the results of studies on zinc absorption in pregnant women are not straightforward (King, 2000).

Zinc deficiency and reduction of appetite also are associated which is likely to contribute to other nutrient deficiencies. In animal studies, decreased food intake was observed early in zinc depletion (Gibson *et al.*, 2000); similarly, anorexia is considered as a symptom of clinical zinc deficiency in humans (Birmingham & Gritzner, 2006).

Severe zinc deficiency in animals has been associated with structural malformations of the brain, such as anencephaly, microcephaly, and hydrocephaly with behavioral problems, such as reduced activity and deficits in short term memory and spatial learning (Prasad, 1981). Moderate deprivation in prepubertal monkeys also resulted in reduced motor activity and less accurate performance on measures of attention and short-term memory (Golub *et al.*, 1994). In humans, severe zinc deficiency can cause abnormal cerebellar functions and impair behavioral and emotional responses (Prasad, 1981).

The limited access to zinc-rich foods, such as animal products, and the presence of zinc inhibitors, such as phytates, have caused millions of people to have inadequate levels of zinc in their diet throughout the world (Sandstead, 1991). The estimated global prevalence of zinc deficiency is 31%, and ranges from 4% to 73%. The prevalence of zinc deficiency is low (4-7%) in USA and Europe; however high prevalence is found throughout South and Central Africa (37-62%), North Africa and the Eastern Mediterranean (25-52%), and South and South-East Asia (34-73%) (Caulfield & Black, 2004).

Zinc deficiency contributes significantly to death and disability throughout the world, and particularly in Africa, the Eastern Mediterranean, and South-East Asia (Caulfield & Black, 2004). In sub-Saharan Africa, 68% of the population are at risk of low dietary intake of zinc (Brown *et al.*, 2001). Although there are no national data, a study conducted in pregnant women in rural Southern Ethiopia indicated low dietary zinc intake and plasma zinc levels (Hambidge *et al.*, 2006).

Assessment of zinc status

Zinc is not easily detectable and diagnosis of deficiency by zinc concentration in plasma or serum and other tissues is challenging because it is distributed relatively evenly throughout the body, and it is a component of thousands of zinc metalloproteins or zinc-binding proteins and also of nucleic acids (Hambidge & Krebs, 2007). Zinc can be assessed through various methods although there is not yet a universally accepted method that could measure accurately, particularly for individuals. In a large population however, serum is often used to assess zinc status (Wood, 2000).

According to Seshadri, there is no single indicator that adequately defines zinc status. Some time ago, a battery that measured the amount of zinc in the diet, zinc concentration in blood, 24 hr urinary excretion of zinc and determination of a zinc-dependent enzyme like alkaline phospahtase was suggested, but recently serum or plasma zinc concentrations have been found to be better and are the most used measures (Seshadri, 2001). Fiorella and colleagues in their ZINCAGE study used plasma zinc to assess zinc level (Marcellilni *et al.*, 2006).

Risk of zinc deficiency in a population can be measured by assessment of dietary intake. When assessing population dietary zinc adequacy, it is vital to compare against proper dietary requirements. The following points should be considered when setting dietary requirements: 1) an estimate of absorbable dietary zinc, 2) absorbed zinc physiological requirement; and 3) an estimate of the coefficient of variation (CV) of usual intakes of zinc in the population. Absorbable dietary zinc is important and gives accurate information to form zinc dietary requirements from the physiological requirements. This is because zinc absorption can be affected by factors such as physico-chemical interactions in the intestine in addition to factors mentioned earlier. However, zinc absorption can also be enhanced by dietary protein (Hotz *et al.*, 2003a).

Although zinc is found intracellularly in the body, a small and important amount of zinc is found in the circulation, mostly bound to plasma protein. Even if the processes and signals involved in homeostatic zinc regulation are not understood well, plasma zinc levels are homeostatically regulated. Plasma zinc level is also affected by factors such as stress, starvation, plasma protein level, diurnal rhythm and infection. Due to these reasons, and other related factors, plasma zinc level is not considered as an accurate tool

to assess zinc status or intake. However, in a large – scale study this assessment method can help (Wood, 2000).

Meals can be labeled with either stable or radio isotopes of zinc and the feces monitored, for total diet studies for zinc absorption data. The method requires a correction factor for intestinal zinc endogenous loss to estimate true absorption for each individual (Hotz *et al.*, 2003a). In contrast, radioisotope tracers and whole body counting methods have been used by most single meal studies, which consider an estimated figure as a correction factor for intestinal losses of endogenous zinc. However, there is evidence from iron research that single meal studies may exaggerate the inhibiting (such as phytate) and enhancing (such as ascorbic acid) effects of dietary factors (Hotz *et al.*, 2003a).

In order to estimate the prevalence of inadequate zinc intakes worldwide, different techniques have been used. Those techniques included per capita food availability based on country level information on food production, imports and exports, per capita availability of energy, population estimates for each country, per capita zinc availability estimated based on the zinc: energy ratio, bioavailability tests, the proportion of absorbable zinc, and other related methods (Brown *et al.*, 2001).

As mentioned earlier, because there is no single indicator to determine zinc status, the prevalence of marginal or sub-optimal zinc status is not known with certainty. However, the likelihood of widespread marginal zinc deficiency in pregnant women is indicated in several studies although definite data on the prevalence of zinc deficiency are hard to produce (Seshadri, 2001).

Iron

Iron and its importance

Iron is an essential nutrient for humans and has a considerable impact on several physiological processes such as production of oxidative energy, transport and storage of oxygen (Pynaert *et al.*, 2005). Iron is also vital for other metabolic processes such as DNA synthesis and electron transport (Crichton, 1991). Additionally it is an indispensable mineral needed for the production of hemoglobin, as a cofactor for several enzymes necessary for proper functioning of brain, and for the immune system and muscle (Beard, 2001).

If iron is removed from tissue culture media, cellular metabolic functions and cell division will cease rapidly (Halloran *et al.*, 1997). However, iron must be either bound to proteins or kept in the trivalent redox state in order to prevent tissue damage from free radical formation (McCord, 2004). Thus absorption, concentrations in body organs, and the redox states of iron must be carefully regulated; too little iron produces iron deficiency anemia (IDA), and excess iron causes siderosis and organ damage (Conrad & Umbreit, 2000).

The proximal small intestine is the site where body iron concentrations are maintained primarily by regulation of absorption of dietary iron (Conrad *et al.*, 1999). Quantitatively, body loss of iron must be equally important as uptake, but it plays a more passive role than iron absorption in body iron regulation (Conrad & Umbreit, 2000). For instance, a man with a 4 g body store of iron will lose only 1 mg daily (Conrad *et al.*, 1999); whereas a woman of childbearing age average loses about twice that amount due to menstruation and childbirth. The fact that hemoglobin contains 3.46 mg of iron per

gram of hemoglobin means that each milliliter of blood loss (Hb 15g/dl) results in depletion of half a milligram of iron. Intestinal cells, intestinal secretion and desquamated epithelium of skin are some additional losses of iron from the body (Conrad & Umbreit, 2000).

The difference between iron requirement and iron retention represents iron balance. The absorbed iron, mostly called the retention of iron, is the result of bioavailability of supplemental, contaminant and dietary iron. The excess iron beyond the daily requirement is stored within the ferritin molecule core, which is available for cellular needs. When there exists a persistent negative iron balance for a longer period of time, exhaustion of the iron store will be followed by diminishing of the body's essential iron pool. Hence, some iron dependent body functions such as nuclear metabolism, oxidative metabolism, gene transcription and oxygen transport will be impaired due to insufficient iron. This poor iron status will later lead to anemia, poor immune function and decreased work performance. If this occurs during pregnancy, particularly in the first trimester, it can cause poor fetal outcomes (Beard, 2000).

The amount of iron absorbed from a meal is determined by iron status, the content of heme and nonheme iron, and the bioavailability of the two kinds of iron, which in turn is determined by the balance between dietary factors enhancing and inhibiting the absorption of iron, especially nonheme iron. The bioavailability of iron can heavily affect the variation in dietary iron absorption from a meal (Hallberg & Hulthen, 2000). An inverse association between serum ferritin, an indicator of iron stores, and both heme- and nonheme-iron absorption suggests that humans biologically adapt their iron absorption in relation to iron stores. The adaptive response seems greater for nonheme

iron than for heme iron. For instance, nonheme-iron absorption from a meal with high iron bioavailability varied 10-15 fold ($\approx$ 1-15% absorbed) whereas heme-iron absorption varies only 2-3 fold ($\approx$ 15-45% absorbed) as serum ferritin varied from $\approx$ 10 to 200 µg/L (Hunt & Roughead, 2000). Iron absorption is increased in iron deficiency and in hereditary hemochromatosis, and the absorption of both heme and non-heme iron is inversely associated with body iron stores. Nonheme iron and heme iron are generally believed to enter a common cytosolic pool in the mucosal cells (Roughead *et al.*, 2002).

Heme iron is the type of iron that is not influenced by diet ligands and is more absorbable than non-heme iron. It is primarily found in meat. Moreover, unlike non-heme iron, it is directly taken up into enterocytes by an absorption pathway. Heme-iron can also withstand the high pH of the upper small bowel, which renders some forms of inorganic iron insoluble. However, the absorption of both heme and non-heme iron is affected by extraluminal factors such as rate of erythropoiesis and iron stores (Pizarro *et al.*, 2003).

Iron from animal sources is more bioavailable than iron from vegetable sources. For instance, the absorption mean value for corn, wheat, black bean, lettuce and spinach were relatively low with ranges between 1.7 to 7.9%, whereas for soybeans, fish, veal and hemoglobin, absorption was between 15.6% to 20.3% (Layrisse *et al.*, 1969). In general, the absorbability of iron from animal products ranges from 20% to 30%, whereas in plants, it is only about 5%. Animal source foods not only provide well absorbed iron but also counter the effects of iron inhibitors in plant products. Animal products are also the only source of vitamin B-12, an important micronutrient for preventing anemia (Population Health and Nutrition Information Project, 2003).

Iron absorption is affected by dietary factors as well. Poultry, fish, meat and ascorbic acid enhance iron absorption, whereas calcium, phytate and polyphenols inhibit iron absorption (Hallberg & Hulthen, 2000). The absorbed iron or the physiological iron requirement represents the amount of iron needed to be absorbed in healthy subjects to meet certain functional needs. For instance, the amount of iron required for adult men and menstruating women is different. The blood loss due to menstruation varies among individuals and this loss is the main source of variation in the requirement of iron for menstruating women and pregnant women. The amounts of blood lost can be translated into iron losses (Hallberg & Rossander-Hulten, 1991). Moreover, the requirements for iron and other micronutrients during adolescence increase. This is due to the rapid expansion of the total blood volume and increase in lean body mass during the growth spurt, and following the onset of menstruation (Brabin & Brabin, 1992). Hence, the recommended dietary allowance of iron for different age groups and different sexes is 8 mg/day for age 19 and above for men, 18 mg/day for women age 19 to 50 and 8 mg/day for women age 51 and above (Institute of Medicine, 2001).

Due to the fact that there is body iron loss through the skin, in the urine and in the stool, dietary intake of iron is required for men as well as women. These basal losses represent approximately 14 µg per kg of body weight per day, or approximately 0.9 mg of iron for an adult male and 0.8 mg for an adult female (DeMaeyer *et al.*, 1989). (See table 2.1).

Table 2.1 Iron requirements of 97.5% of individuals (Mean + 2 S.D.) in terms of absorbed iron by age group and sex. (Source, DeMaeyer *et al*., 1989)

Age/Sex	in µg/Kg/day	in mg/day
4-12 months	120	0.96
13-24 months	56	0.61
2-5 years	44	0.70
6-11 years	40	1.17
12-16 years (girls)	40	2.02
12-16 years (boys)	34	1.82
Adult male	18	1.14
Pregnant women	*	*
Lactating women	24	1.31
Menstruating women	43	2.38
Post-menopausal women	18	0.96

*Requirement during pregnancy depends on the woman's iron status prior to pregnancy

Although the volume of menstrual blood lost from month to month varies greatly among women, it is relatively constant for a given woman. An average woman's blood loss ranges between 25 and 30 ml per month during menstruation. This is equivalent to an iron loss of 0.4 to 0.5 mg per day or 12.5 to 15 mg per month (DeMaeyer *et al.*, 1989).

The amount of iron absorbed from diet is influenced by two main physiological factors: the iron status of the subject, which noticeably influences the amount of iron absorbed from a diet, and the diet composition. There is a significant difference in absorption between a subject with iron deficiency anemia and in someone having considerable stores even from same meal (Hallberg & Rossander-Hulten, 1991).

Iron is the most abundant metal in the human body, and like the liver, the brain also contains a substantially higher concentration of iron unlike other metals. Iron is unevenly distributed within the brain where the highest concentration is situated in the red nucleus, basal ganglia and dentate nucleus. Iron storage in the brain is mainly in

organic form such as ferritin, but not hemosiderin, with relatively little in a free and reactive form (Gerlach *et al.*, 1994).

As studies on adults and aged rats show, the oligodendrocyte is the predominant cell type containing ferritin, transferrin, and iron throughout the brain at all ages. Neurons in most brain regions contain granular iron deposits which become more apparent with age. Iron and ferritin are also present in microglial cells in all brain regions, but are particularly abundant in the hippocampus (Benkovic & Connor, 1993).

Iron is crucial for the synthesis of the monoamines, dopamine, norepinephrine, and serotonin (Lozoff *et al.*, 1991). Its influence on dopamine transporters has been shown by introducing the iron chelator desferrioxamine (DFO) to the cells. When iron is removed from these cells by DFO the concentration of dopamine transporter proteins and uptake of dopamine decreases (Wiesinger *et al.*, 2007). Dopaminergic neurons have been found to influence response inhibition and fine, gross and sequential movements, spatial learning, attention and hyperactivity (Lozoff *et al.*, 2006). Among parts of the brain that have impact on cognitive functioning that are dependent on dopamine function, the frontal lobe is the one that controls attention and response inhibition. The cognitive deficits due to effects of iron deficiency on the dopaminergic system are most easily seen in children who were iron deficient during infancy (Chudasama & Robbins, 2006).

According to Ballin and colleagues, iron supplements in adolescent girls decreased fatigue, increased ability to concentrate in school and improved mood (Ballin *et al.*, 1992). As determined by electrophysiological measurements, neurological malfunction in young children, adolescents, and adults have an association with iron deficiency (Basta *et al.*, 1979).

Iron deficiency

Iron deficiency (ID) is a state in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain, and muscle. ID without the occurrence of anemia is possible before the concentration of hemoglobin falls below the threshold for the specific sex and age group (WHO *et al.*, 2001).

The development of ID has three stages:

1. Iron depletion is marked by a reduction in the serum ferritin level, with no evidence of functional consequences,

2. Iron deficient erythropoisis occurs when the needs of the erythroid marrow for iron are no longer met with a subsequent rise in erythrocyte protoporphyrin and serum transferrin receptor levels; and finally,

3. Iron deficiency anemia, the most severe form associated with functional consequences (WHO/UNICEF/UNU, 1996).

ID is the most common micronutrient deficiency in the world. Unlike most nutrients, ID is the most prevalent disorder in both developing and developed nations and it affects all age groups, particularly children and women (Pynaert *et al.*, 2005). ID is the most widespread hematologic disorder in childhood (Dallman *et al.*, 1984) and is more severe during rapid growth, especially age 6-24 months, adolescence, pregnancy and during other periods of high nutritional demand (Black, 2003a).

As bone marrow stores of iron are depleted during a period of accelerated growth, infants from age 9 to 24 months may develop dietary iron deficiency. Due to poor dietary intake and high iron requirement, adolescent girls also are susceptible to dietary iron deficiency related to menstrual blood loss and rapid growth (Looker *et al.*, 1997).

According to Oski, ID is a systematic condition that includes various consequences such as impaired exercise and functional alterations of the small bowel other than anemia (Oski, 1993). Children's behavior and cognitive performance is also altered as the result of ID.

The association between ID and lower mental or lower motor developmental test scores in early childhood has been well described (Booth & Aukett, 1997). Grantham-McGregor and Ani also have identified a relation between iron deficiency and risk in cognitive, physical and behavioral development in children (Grantham-McGregor & Ani, 2001). Although the relation between iron status and cognitive functioning for older children and adolescents is less clear (Burner *et al.*, 1996), there is evidence that ID can impair cognitive performance at all stages of life, and affect cognitive development and school performance (Stoltzfus, 2001; WHO, 2002). The effect of ID on cognitive impairment in both infants and children is clearly identified even after controlling possible confounding factors such as maternal education and socioeconomic variables (Hubbs-Tait *et al.*, 2007).

Anemia, including IDA, mostly affects preschool children and pregnant women and remains one of the most pervasive public health problems in developing countries (WHO, 2001). Due to the rapid expansion of total blood volume and increase in lean body mass during the growth spurt and the onset of menstruation, requirements of iron and other micronutrients also increase during adolescence (Brabin & Brabin, 1992).

Some animal studies showed that ID reduced metabolic activity in certain brain areas such as hippocampal activity in rodents (Ranade *et al.*, 2008). Similar results were obtained in humans, particularly in the hippocampal cortex. These metabolic deficiencies

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are particularly concentrated in areas involved with memory (deUngria *et al.*, 2000). Due to the effect of iron on hippocampal development and function, IDA would be expected to alter attention and recognition memory (Burden *et al.*, 2007).

Iron deficiency in early infancy causes childhood social and attention problems such as anxiety, impairment and depression (Lozoff *et al.*, 2000). Differences between iron-deficient young children and non-iron deficient young children were also seen in the amount of crying, time spent sleeping, levels of irritability, and time spent in interactive play (Lozoff *et al.*, 1987). Other studies showed that anemic children under the age of 2 years scored lower in the psychomotor development indices of the Bayley scales than the non-anemic ones (Idjradinata & Pollitt, 1993).

Similarly, results from Chile indicated that visual-motor integration, and fine and gross motor skills were negatively affected at the age of 5.5 years due to ID occurrence in the early years of life. This suggests that ID in the early years of life that affects motor development can persist throughout childhood (Andraca *et al.*, 1990).

According to Lozoff and colleagues, preschool children who had IDA showed lower levels of social looking toward their mothers and demonstrated hesitant behavior by moving more quickly to mothers and being slower to touch a novelty toy for the first time. She suggested that preschoolers with IDA have temperaments reflecting hesitancy, wariness, and inhibition, which is typically conceptualized as the slow-to-warm-up temperament style (Lozoff *et al.*, 2007).

Regardless of advances in the reduction of several nutrient deficiencies worldwide, ID remains the most widespread single nutrient deficiency. According to WHO estimates, there are 2 billion anemic people and twice as many are iron deficient

worldwide (United Nations - Administrative Committee on Coordination/Sub -
Committee on Nutrition (ACC/SCA), 2000). Infants, children and women of reproductive
age are at high risk of developing ID and IDA due to their greater physiologic
requirements, in addition to increased losses and poor dietary intake (Murray-Kolb &
Beard, 2007). Moreover, adolescents are at higher risk of having anemia, with or without
ID in places where other risk factors for anemia, such as malaria and inadequate food
intake exist (United Nations - Administrative Committee on Coordination/Sub -
Committee on Nutrition (ACC/SCA), 1997). The prevalence of IDA in postpartum
women even in countries such as the United States is as high as 22% among low-income
pregnant women. This might be due to inadequate dietary iron intake (Bodnar *et al.*,
2002).

According to a WHO report, anemia is a problem, at least a mild public health
problem all over the world in children, pregnant women and women of reproductive age.
The regional estimates for preschool-age children and women of child-bearing age (both
pregnant and non pregnant women) indicate that the highest proportion of individuals
affected by anemia are in Africa, that is 47.5 – 67.6% (WHO, 2008).

Ethiopia is one of the countries where prevalence of anemia is serious in all age
groups. One report estimated anemia prevalence (the proportion of the population with
Hb < 120 g/L) in reproductive age women to be 52.3% which is classified as severe
(WHO, 2008). According to the Ethiopian Demographic and Health Survey (EDHS),
however, prevalence of anemia in Ethiopia among women of different age categories, is
as reported in table 2.2 (Central Statistics Agency, 2005).

Table. 2. 2 Percentage of Ethiopian women with anemia

| | Anemia status | | | | |
Age	Any anemia	Mild anemia	Moderate anemia	Severe anemia	Number of women measured
15 – 19	24.8	16.6	7.4	0.9	1,489
20 – 29	24.5	15.9	7.4	1.2	2,163
30 – 39	30.6	19.9	8.8	1.9	1,489
40 – 49	27.7	18.2	8.3	1.3	1,000

In the SNNPR prevalence of any anemia was 23.5% with 14.8% mild anemia, 7.7% moderate anemia, and 1.0% severe anemia, which is close to the national prevalence (26.9%, 17.7%, 8.0% and 1.3% respectively) (Central Statistics Agency, 2005).

Assessment of iron status

There is no consensus on assessment methods of iron deficiency, however most data on ID prevalence are based on hemoglobin level as an indicator of anemia which might be caused by other factors as well (WHO, 2007a). Ferritin, which stores iron, and transferrin receptors which control the entry of iron bearing transferrin into cells, are the major iron-related proteins in the body. Posttranscriptional regulation leads to reciprocal production of these proteins. The mechanism involves an iron-responsive element binding protein which interacts with iron-responsive elements in the mRNA of ferritin and transferrin receptor with a contrary effect. The total tissue concentration of these proteins is directly proportional to the amount detected in the serum. In this regard higher levels of serum ferritin indicate the availability of corresponding amounts of storage iron (Baynes, 1996).

Besides the aforementioned advantages of the measurements, they also provide an accurate result in measuring population iron status. However, serum ferritin is not reliable

as an indicator in places where infectious diseases are common due to the fact that serum ferritin concentration increases with inflammation as a result of the acute phase response to disease (WHO, 2004a).

Table 2.3 Success of indicators to detect changes in iron status in 10 controlled trials of treatments, estimated as the number of indicators showing a change of ≥ 0.2 standard deviation units (SDUs)

Indicator of iron status	Success of indicator based on mean change of ≥ 0.2 SDUs for all subjects	Success of indicator based on mean change of 0.2 SDUs for top or bottom 10%[a]
Hemoglobin	60%, 6 of 10 studies	80%, 8 of 10 studies
Mean cell volume	50%, 2 of 4 studies	75%, 3 of 4 studies
Serum ferritin[b]	90%, 9 of 10 studies	60%, 6 of 10 studies
Transferrin receptor[c]	56%, 5 of 9 studies	56%, 5 of 9 studies
Body iron stores	78%, 7 of 9 studies	78%, 7 of 9 studies
Zinc protoporphyrin	50%, 3 of 6 studies	67%, 4 of 6 studies

[a] Depends on whether the indicator was expected to rise or fall
[b] Transformed to logarithms
[c] Results were the same with or without transforming values to logarithms
Source: (WHO, 2004)

Hemoglobin (Hb) values, one of the indicators of iron status, vary with age, sex, and state of pregnancy, and they are also affected by ethnicity, altitude, and smoking. For these reasons, adjustment should be done in population-based surveys when interpreting Hb values (Nestel, 2002). Hence, the following formula is suggested to estimate altitude specific Hb cutoff values (Nestel, 2002).

$$Hb = -0.032 \times (\text{altitude in meters} \times 0.0033) + 0.022 \times (\text{altitude in meters} \times 0.0033)^2$$

The World Health Organization defines anemia as a hemoglobin value below the age-specific 2.5[th] percentile value in a non anemic distribution. The accepted cutoff to define anemia in non pregnant females, age above 15 years living at sea level is 120 g/L (WHO *et al.*, 2001). IDA is diagnosed when the Hb concentration is lower than the level considered normal for the person's age, sex and physiological status (i.e. below a

statistically defined threshold of 2 S.D. from the mean for a healthy population)

(WHO/UNICEF/UNU, 1996). The restriction in Hb production causes distortion of

erythrocytes with microcytosis and hypochromia (Monarrez-Espino *et al.*, 2001).

Determination of Hb provides more accurate diagnosis of anemia compared to

assessment based on clinical signs like pallor (Bhaskaram *et al.*, 2003).

Based on the estimation from Hb concentration, IDA would be considered a

public health problem if the prevalence exceeds 5% of the population

(UNICEF/UNU/WHO, 2001). Internationally accepted Hb values for defining anemia in

different population groups are shown in table 2.4.

Table 2.4 Hemoglobin values defining anemia for population groups

Age or Sex Group	Hemoglobin Value Defining Anemia (g/dL)
Children 6 – 59 months	< 11.0
Children 5 – 11 years	<11.5
Children 12 – 14 years	< 12.0
Non pregnant women > 15 years	< 12.0
Pregnant women	< 11.0
Men > 15 years	< 13.0

Source: WHO/UNICEF/UNU (2001)

Anemia is sub classified into mild, moderate, and severe levels as Hb values

decline (Population Health and Nutrition Information Project, 2003). Hence, Hb value in

g/dL defining anemia at sea level for non pregnant women over 15 years of age is <12.0

as all anemia, <10 - 11.9 as mild anemia, <7.0 - 9.9 as moderate anemia and <7.0 as

severe anemia (WHO *et al.*, 2001). Anemia can also be defined in terms of the hematocrit

content of packed blood cell volume (Population Health and Nutrition Information

Project, 2003). Hematocrit value <36% at sea level is classified as all anemia (WHO *et*

al., 2001).

Iodine

 Iodine and its importance

Iodine is a chemical element found in trace amounts in the human body. Its primary function is in the synthesis of thyroid hormones. Iodine is primarily obtained through the diet but is also a component of some medications, such as radiology contrast agents, iodophor cleansers, and amiodarone (deBenoist *et al.*, 2008; Zimmerman *et al.*, 2008).

As mentioned above iodine is required for the synthesis of thyroid hormone and that thyroid hormone in turn acts by regulating the metabolic pattern of most cells of the organism. Iodine also plays a crucial part in the process of early growth and development of most organs, especially of the brain (Chan & Kilby, 2000). A constant supply of thyroid hormone is necessary for proper development of the brain and for body growth as well as to maintain basal metabolism and functional activity of most organs (Andrasi *et al.*, 2007).

Mental retardation and endemic cretinism result from an insufficient supply of thyroid hormone to the developing brain. Thyroid hormone action is exerted through the binding of triiodothyronine to nuclear receptors, which regulate the expression of specific genes in different brain regions following a precise development schedule (Koibuchi & Chin, 2000).

Many lines of evidence demonstrated that hypothyroidism may affect cognitive functions by impairing synaptic plasticity in the hippocampus (Dong *et al.*, 2005). The maturation and function of the hippocampus are dependent upon thyroid hormones (Gerges & Alkadhi, 2004).

In developing countries where iodine deficiency and goiter are severe, oral or parenteral administration of iodized oil was demonstrated to be an effective method of iodine prophylaxis, leading to eradication of goiter and cretinism in the new generations (Ermans, 1994). Moreover, after the administration of iodized oil, a significant decrease in goiter size was obtained in goitrous patients (Tonglet *et al.*, 1992).

As a study in Albanian children showed, iodine treatment improved information processing rates, as measured by the symbol search, rapid naming, and rapid target marking tests. The improvement on Raven's colored progressive matrices (Raven's CPM) suggests iodine repletion was associated with a small but significant increase in intelligence (Zimmermann *et al.*, 2006a).

The public health importance of an adequate iodine supply for the physical and mental well-being of humankind has been well described (Hetzel & Pandav, 1994). Most studies on the relation between iodine status and mental performance of children have concentrated on effects of iodine supply in utero and shortly after birth on mental and psychomotor development (Delong, 1996). The most commonly held argument is that the mental capacity of children, once affected by iodine deficiency in early life, is impaired permanently (Briel *et al.*, 2000). Nevertheless, it was found that correction of iodine deficiency improved mental performance (Bautista *et al.*, 1982).

Marginal iodine intake can cause goiter, which is characterized by an enlarged thyroid gland (Buchinger *et al.*, 1997). Experiments on rats showed that environmental goitrogens and thiocyanates might be significant determinants in the etiology/prevalence of endemic goiter (Sarkar *et al.*, 1988). Apart from or along with iodine deficiency, the goitrogens present in staple foods may be important contributory factors (Nagtilak *et al.*,

1994). As a review by Kotwal and colleagues indicated, not only goitrogens in food, but also goitrogens in synthetic chemicals and polluted water contribute to prevalence of iodine deficiency disorder (IDD). However, with all the benefits of universal salt iodization programs in preventing IDD, attention should be given to the fact that excess iodine interferes with iron metabolism, and consequently enhances anemia. Excess iodine also can cause an increase in goiter (Kotwal *et al.*, 2007). Thyroid hormone production and metabolism is not only affected by iodine deficiency but also by deficiency of vitamin A, iron and selenium (Dunn, 2002).

In addition to affecting thyroid size, iodine intake can influence the concentration of thyroid hormones and thyroglobulin in the blood. Chronic iodine deficiency, including marginal iodine deficiency, increases serum thyroglobulin concentration (Buchinger *et al.*, 1997). The recommended dietary allowance of iodine is 150 µg/day during adolescence and adulthood, and 200-300 µg/day during pregnancy and lactation (WHO, 1996).

Iodine deficiency

Iodine deficiency is one of the most common nutritional problems of the world. More than one billion people, especially in the developing countries, suffer the inadequate intake of iodine (Ravanshad *et al.*, 2003). Populations living in areas where the soil has been depleted of iodine are at a great risk of iodine deficiency. Some of the causes of soil depletion are: glaciations, leaching, water, and heavy rain. Iodine deficiency in the soil affects all forms of plant life and thus the crops grown on the soil will have low iodine content. Thus, populations who rely on subsistence agriculture for a

living are likely to be exposed to iodine deficiency if the soil iodine content is low (Koutras *et al.*, 1980).

IDD refers to the ill effects of iodine deficiency that can be prevented through an adequate intake of iodine (WHO/UNICEF/ICCIDD, 2001a). Some of the ill effects of iodine deficiency include goiter with its complications, hypothyroidism, impaired mental function, iodine-induced hyperthyroidism and increased susceptibility of the thyroid gland to nuclear radiation (Stanbury *et al.*, 1998).

IDDs have become a major public health problem in many parts of the world. Developing prevention and control methods can impact the quality of life, educability and productivity of millions and would make a significant contribution to the development of countries where risk of developing IDD is high (Mostafavi, 2005).

Severe iodine deficiency can cause serious consequences on brain and physical development called endemic cretinism. Endemic cretinism is characterized by mental retardation, severe and irreversible alterations in brain development, and multiple neurological signs including deaf mutism, squint, spastic diplegia, motor rigidity, shuffling gait, and signs of severe thyroid insufficiency with dwarfism, myxoedma, and sexual immaturity (Delange, 2001).

Neurological cretinism, the most marked central nervous system (CNS) damage, leads to neurological abnormalities such as hearing and speech defects, mental deficiency, and motor defects in people who are born and raised in areas with severe iodine deficiency (Halpern *et al.*, 1991). This damage in the nervous system is related to the mother's IDD and her inability to increase circulating thyroxine during pregnancy (Morreale *et al.*, 1997).

People living in areas affected by severe IDD were estimated to have an intelligence quotient (IQ) of as much as 13.5 points below those of the comparable communities in areas where there was no IDD (Bleichrodt & Born, 1994). Having lower intelligence affects children's learning capacity, women's health, the quality of life of communities, and economic productivity (WHO/UNICEF/ICCIDD, 2001a).

Children with iodine deficiency are slow learners and have low motivation to achieve goals (Tiwari *et al.*, 1996). Hypothyroidism can also cause mood disorders including depression, social withdrawal, and paucity of speech (Esposito *et al.*, 1997). The effects of maternal hypothyroxinemia on the cytoarchitecture of the cortex and hippocampus are permanent (Zoeller, 2003).

In addition to being the cause for mental deficiencies, IDD also presents reproductive risks. Hypothyroidism causes anovulation, infertility, gestational hypertension, increased first trimester abortion, and still births, all of which are common in iodine deficiency. Additionally, iodine deficiency causes cultural and socioeconomic problems for the mother. A mother who is infertile and loses a fetus might also be stigmatized. Furthermore, a mother who gives birth to a defective child will be responsible for caring for the child throughout her life (Dunn & Delange, 2001).

One of the most common causes of hypothyroidism in adult women is chronic autoimmune thyroiditis (Glinoer, 2003). When hypothyroid women become pregnant, they have an increased risk for obstetrical complications, intrauterine fetal demise, gestational hypertension, placental abruption and a poorer perinatal outcome (Glinoer, 2003). However, the postnatal effects of iodine deficiency on cognitive functions are much less clear (Zimmermann *et al.*, 2006a). Nevertheless, it is the single most

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preventable cause of brain damage or mental retardation worldwide (WHO/UNICEF/ICCIDD, 2001a).

Over 600 million people around the world have goiter and 20 million have a certain level of brain damage caused by the effects of iodine deficiency in pregnancy (WHO, 1993). A report by UNICEF estimates that over 1.6 billion people around the world are at risk of IDD. Among those, 760 million have goiter, 43 million suffer from brain damage, and 11 million suffer from overt cretinism (United Nations, 1998).

Despite the significant progress against IDD, it is estimated that 125 million people in Africa, or 20% of the population, remain iodine deficient (WHO/UNICEF/ICCIDD, 2001b). However, according to the 2004 survey report by WHO, there were about 350 million Africans who were at risk of iodine deficiency and goiter. Twenty eight percent of the African population suffers from iodine deficiency and goiter (WHO, 2004b).

In Ethiopia, IDD affects a large number of the population. A national survey showed that goiter prevalence rate in school children and their household members ranged from 0.4 to 66.3%, with a mean value of 35% (Woldegebriel *et al.*, 1993). The Ethiopian Health and Nutrition Institute (EHNRI) and UNICEF have reported that goiter prevalence in Ethiopia increased from 26% in 1981 to 40% in 2005. The rate in children is estimated to be 63% in some areas (EHNRI & UNICEF, 2005). The reason for the increase in goiter prevalence might be due to the loss of salt iodization plants after the dispute with Eritrea in 1998 (Ministry of Health, Unpublished document 2001).

Assessment of iodine status

Thyroid size and urinary iodine excretion (UIE) are the two most widely used measures of iodine status (Delange, 1994). Similarly, the prevalence of goiter and the median urinary iodine (UI) concentration are the most important indicators for assessing IDD and for defining the severity of any associated public health problem (WHO/UNICEF/ICCIDD, 1994).

Most iodine absorbed in the body eventually appears in the urine. Therefore, UIE is a good marker of very recent dietary iodine intake (WHO/UNICEF/ICCIDD, 2001a). However, UI concentration is not a direct measure of thyroid function, but reflects recent iodine intake and thyroid hormone catabolism. Thus, population groups, even if currently found to be in the mildly deficient to normal range of UI concentration, may still have experienced serious functional consequences of iodine deficiency in preceding periods (Briel *et al.*, 2001).

In population studies, iodine intake is usually assessed by measuring iodine excretion in spot urine samples and is expressed relative to creatinine excretion or as a concentration. These methods have showed reliable results for groups, but because of the large day-to-day variation, they cannot definitively establish iodine intake for individuals (Rasmussen *et al.*, 1999). Based on this fact, median UI concentrations of 100µg/l and above define a population without significant iodine deficiency, i.e. at least 50% of the sample should be above 100µg/l. In addition, not more than 20% of samples should be below 50µg/l (WHO/UNICEF/ICCIDD, 2001a).

According to WHO/UNICEF/ICCIDD, the public health problem cut-off point for the prevalence of goiter is 5% (WHO/UNICEF/ICCIDD, 1994). In other words the

reduction of goiter rate to < 5% in school-aged children indicates the disappearance of IDD as a significant public health problem (WHO/UNICEF/ICCIDD, 2001a). However, information on the contribution of iodine by food groups is also necessary for making recommendations to improve the dietary iodine intake of individuals (Haldimann *et al.*, 2005).

According to Rasmussen and colleagues, serum thyroglobulin (Tg) concentration is a good marker of iodine status and serum Tg could be used as an objective measure of iodine status in a population (Rasmussen *et al.*, 2002). According to Zimmermann and colleagues, Tg used in conjunction with UI to measure recent iodine intake and thyroid volume to assess long-term anatomic response, may be useful biological indictors for monitoring thyroid function in children after introduction of iodized salt. The dried blood spot- thyroglobulin (DBS – Tg) assay makes sampling practical even in remote areas (Zimmermann *et al.*, 2006b).

Iodine intake can also be estimated by using various dietary assessment methods such as the dietary record, food-frequency questionnaire (FFQ), and diet history interview. The FFQ is often preferred because it is more cost-effective than the diet history interview (Rasmussen *et al.*, 2002).The knowledge of the previous status of iodine intake in a population is mandatory to understand, for example, the apparent discrepancy between a high goiter prevalence and a border line low/normal iodine intake (Aghini-Lombardi *et al.*, 1997).

Based on the WHO/UNICEF/ICCID classification, physical examination of thyroid gland can be done to assess goiter rate as follows (WHO/UNICEF/ICCIDD, 2001a):

Grade 0: None or no goiter (palpable or visible)

Grade 1: A goiter that is palpable but not visible when the neck is in the normal position, (i.e. the thyroid is not visibly enlarged).

Grade 2: A swelling in the neck that is clearly visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated.

According to WHO the epidemiological criteria for IDD are as follows (median urinary iodine values): severe, $<20\,\mu g/L$ ($0.16\mu mol/L$); moderate, $20-49\,\mu g/L$ ($0.16 - 0.38\,\mu mol/L$); mild, $50 - 99\,\mu g/L$ ($0.39 - 0.78\,\mu mol/L$); and no deficiency, $> 100\,\mu g/L$ ($0.79\,\mu mol/L$) (WHO, 1992).

Iodine intake relative to body weight might be a good indicator of iodine status. Rasmussen and colleagues reported that this measure is predictive of thyroid volume and serum Tg concentration, and it was the measure that showed the most consistent relation to prevalence of thyroid nodules (Rasmussen *et al.*, 2002). Measurement of thyroid volume by ultrasound is also possible; even preferable, due to the fact that thyroid ultrasound is noninvasive, quickly done (2-3 min per subject), and feasible even in remote areas by using portable equipment (Zimmermann *et al.*, 2004).

Summary of literature

Micronutrient deficiencies including zinc, iron and iodine are very common in the world. But the effect of micronutrients on cognition has been controversial due to the inconsistent results found by different researchers. Because micronutrients have overlapping and interacting effects, studying the effect of a single micronutrient on cognition could be one of the reasons for the inconsistent results. Therefore,

consideration should be given to approach the problem in multivariate ways (Grantham-McGregor & Ani, 1999).

However, having the above scenario in mind, there are some reports that indicated the effect of the aforementioned nutrients on adult cognition. For instance, Maylor and colleagues showed younger adults improved in cognitive tests with zinc supplementation (Maylor *et al.*, 2006). Improvements in cognitive function by iron supplementation have also been observed in older children and adults (Falkingham *et al.*, 2010). Although information on the effect of iodine supplementation on adults is scarce, it is clearly described that iodine deficiency disorders cause mental impairment in adults (WHO/UNICEF/ICCIDD, 2007).

CHAPTER III

METHODOLOGY

Data source and the study population

The data for this study were collected from a convenience sample of non-pregnant women age 18 and older living in three adjacent rural communities in Ethiopia: Alamura, Tullo and Finchawa, which are located near Awassa town. On selecting the aforementioned sites among others, three important factors were considered. First and foremost, the three kebeles (village areas) are known to have malnutrition, indicating that micronutrients such as zinc, iodine and iron deficiencies could exist. Secondly, one of their staple foods, maize, is likely to have nutrient inhibitors, and hence decreased bioavailability of micronutrients. Thirdly, the selected study areas are accessible for transportation and are an outreach area of Bushulo Health Center which facilitates communication.

Study Design

The study employed a survey based-cross-sectional research design. A cross-sectional survey design is one of the commonly used study designs in which data are collected from a larger number of subjects at a point in time. This design is commonly used in social and applied sciences where the interest is understanding relationships among various variables through the use of large databases.

Subject selection methodology

After ethical clearances were obtained from Oklahoma State University, Hawassa University, South Nations, Nationalities and Peoples Region Health Bureau, and the Ministry of Science and Technology, Ethiopia, a detailed explanation about the research was given to the health workers at the woreda level. The health workers are responsible to monitor the health condition of the rural communities. After a thorough discussion was made at each community with the health workers, a convenience sample of 202 women, 68 women from Finchawa, 65 women from Tullo and 69 women from Alamura, who volunteered to participate in the study were selected and registered. The same explanation was given to the study participants, and after all their questions were answered the data collection period was set according to the following schedule. In Finchawa from 07/13/2009 to 07/15/2009, in Tullo from 07/16/2009 to 07/20/2009 and in Alamura from 07/21/2009 to 07/23/2009. On the scheduled date a written consent was read for each woman and signed by finger print before data collection was started. All study participants were women age 18 and above, non-pregnant and inhabitants of one of the study areas. The criteria for exclusion were pregnancy, history of bleeding disorder, and/or recent feeling of fever.

Data collection

A one week intensive training prior to data collection was given to research assistants on the questionnaire, cognitive tests and anthropometric measurements. The questionnaire and the cognitive tests were pretested in 10 women that were not involved in the study.

Women's history and demographic data

Baseline data were collected as part of a general history including community of residence, name, age (best estimate), breastfeeding status, child deaths, family size, age at marriage, number of live births. Demographic questions were adapted from the Ethiopian Demographic and Health Survey 2005 report (Central Statistics Agency, 2005).

Socioeconomic Status (SES)

SES was assessed by a combination of parental education and family wealth. Wealth criteria included maternal possession of radio, television, bicycle, torch, cart, phone, livestock, matured enset plants, type of roof, number of rooms, type of window, and type of floor. The SES questions were adapted from the Ethiopia Demographic and Health Survey 2005 report (Central Statistics Agency, 2005).

Cognitive tests

Cognitive functioning was assessed by Raven's Colored Progressive Matrices (CPM) and by components of the Kaufman Assessment Battery for Children (KABC-II). The Raven's CPM consists of 36 figures, each of which is missing one piece. The examiner explained about the procedure to the participants by saying, there is a figure here but one pattern is missing in a series, so your duty is to choose the missing pattern from the six alternatives given to complete the figure. For each test item the same question was asked. Each set of items gets progressively harder, requiring greater cognitive capacity to encode and analyze. The Raven's CPM is a non-verbal intelligence test (Raven, 2000).

The KABC-II is composed of different scales and each scale assesses different aspects of cognition such as sequential processing (short-term memory), simultaneous

processing (visualization), learning (long-term retrieval), planning (fluid ability), and verbal knowledge (crystallized ability). Each scale contains different subtests (Kaufman *et al.*, 2004). In this study selected subtests were employed as measures of cognition. The subtests included were word order and number recall for the sequential processing, Rover and triangles for the simultaneous processing and pattern reasoning for the planning measures.

Administration of each subtest used for the KABC-II is explained below:

- Word order: - Some sets of pictures in a certain order, that are commonly known to the study population, were displayed. The examiner named each picture first and let the subject repeat the name until s/he was sure that the subject knew all of the pictures by name. Following that the subtests were done in a fashion that the examiner named a series of words and the examinee pointed at the pictures of those words in the same order. Later, the examiner covered the pictures while naming but then uncovered them for the examinee to name and point to the pictures.

- Number recall: - In this test, the examiner said a series of single digit numbers and the examinee repeated what was said. The first set started with three numbers and then went on increasing as the examinee proceeded to say them until she made errors and reached a discontinue point (3 consecutive errors). The numbers were not consecutive.

- Rover: - For this test a Rover stimulus booklet, the Rover plastic dog, the easel pages, and the record form to administer Rover were used. The procedures were: the booklet was placed before the examinee, Rover was placed on the dot and the test administrator demonstrated how Rover moves, how to count when Rover is moving, and where Rover can and cannot go. The examinee was given an instruction to get Rover to the bone in the

smallest number of moves. Correction was given when she broke a rule, and she was supposed to count out loud. This test was not timed.

- Triangles: - For administration of this subtest the easel, record form, and plastic and rubber shapes were used. The pieces the examiner used for each item were properly pictured in the lower right-hand corner of the examiner's pages. The examiner displayed pieces needed to make the picture before opening the stimulus page for the examinee and then asked the examinee to do exactly as shown on the picture using the plastic or rubber shapes. This subtest was not timed.

- Pattern reasoning: - This subtest was administered with the easel and record form. A row of images with one image missing was displayed to the examinee, and the examinee's task was to identify the missing image that fits the pattern. This test was not timed.

Anthropometric measurement

Each woman's weight was measured on a solar digital scale (Uniscale, UNICEF, NY) and recorded to the nearest 100 grams. Women wore light clothing, but they removed shoes and heavy outer wear (e.g. sweaters) before obtaining weight. Height was measured to the nearest 0.1 cm using a single calibrated instrument (Adult Board, Schorr Productions, Olney, MD). Participants stood bare footed on a flat surface with weight distributed evenly on both feet, heels together, and the head positioned so that the line of vision was perpendicular to the body. Arms were hanging freely by the sides, and head back, buttocks and heels were in contact with the vertical board. Mid upper arm circumference was also measured using a plastic measuring tape. Anthropometric measurement instructions were adopted from Gibson (Gibson, 2005).

Sample collection for measurement of biomarkers

A fasting morning (10:00 AM to 12:00 AM) venipuncture blood sample was collected from each participant in a sitting position using a disposable 7.5 cc syringe coated lithium heparin with a 21 gauge needle (Sarstedt, Inc., Newton, N.C.). Blood collection took place using hygienic techniques by an experienced lab technician who came to the health post with the research team. The blood was centrifuged and plasma was separated immediately at the health post where the data collection took place. Plasma was transferred into trace-element-free vials with disposable plastic pipettes. The plasma was transported from the data collection site to Hawassa University in an ice box and was kept in a freezer until packed for transport to Oklahoma State University (OSU), USA. The plasma was transported from Ethiopia to OSU in an ice box. It arrived thawed but was cool.

Urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition of the community when carried out with appropriate technology and sampling. This approach assesses iodine nutrition only at the time of measurement (WHO/UNICEF/ICCIDD, 2001a). Urine was collected in a cup out of which urine samples were taken to fill 2ml tightly sealed vials in duplicate. In order to avoid unpleasant odor, samples were kept in a refrigerator at Hawassa University until they were transported to OSU for analysis. Urinary iodine analysis was in duplicate for all samples.

Total goiter was determined by a well-trained health officer based on the following grades: grade 0, no palpable or visible goiter; grade 1, goiter palpable but not

visible when neck is in the normal position; grade 2, goiter visible when neck is in the normal position (WHO/UNICEF/ICCIDD, 2001a).

Assessment of iron, zinc, and iodine status

- Hemoglobin

Two drops of blood were taken from the collected blood to measure hemoglobin concentration on site at the health center. Hemoglobin level was measured with a HemoCue. The Hemocue system consists of a portable, battery-operated photometer and a supply of treated, disposable cuvettes in which blood is collected. The Hemocue system is a reliable quantitative method for determining hemoglobin concentrations in field surveys (WHO/UNICEF/UNU, 2001). Correction for altitude was calculated based on the following formula:

$Hb = -0.032$ x (altitude in meters x 0.0033) + 0.022 x (altitude in meters x $0.0033)^2$

(UNICEF/UNU/WHO, 2001; Nestel, 2002). Altitude of the study area is 1708 m.

- Ferritin

Plasma ferritin was analyzed using an immunoradiometric procedure (Ramco Laboratories, Stafford, TX). Serial replications of quality control sera for plasma ferritin were used to check the precision and accuracy of the assay.

- Plasma zinc

Plasma zinc was analyzed by inductively coupled plasma mass spectrometer (ICP-MS Elan 9000, Perkin Elmer, Norwalk, CT). All plasma samples were diluted 20 fold (200 μL diluted to 4 mL) with 0.1% HNO3 (GFS Chemicals, Powell, OH) in Millipore water. Standard solutions of zinc were prepared by dilution of certified standard solutions (Perkin Elmer, Norwalk, CT). Dilute working standards were prepared by dilution of an intermediate stock standard solution.. The calibration standards were

prepared in 0.1% nitric acid solution at 0, 50 and 100 μg/L. All samples and standards were spiked with 10 μg/L gallium as an internal standard (Perkin Elmer, Norwalk, CT). Polypropylene plasticware was used for reagent and sample preparation (Sarstedt, Inc., Newton, NC) to avoid zinc contamination. Quality control samples (Utak Laboratories, Inc., Valencia, CA) were utilized to verify method performance was within recommended ranges.

- Acute phase proteins

Due to the fact that iron status indicators are influenced by inflammatory processes it was important to measure acute-phase proteins (WHO/USCDC, 2005). Hence, high sensitivity human C-reactive protein (hsCRP) and Alpha-1-Acid Glycoprotein (AGP) were assessed in this study. However, these biomarkers have their own limitations. For instance CRP rises quickly after onset of an infection and declines 24 – 48 hours after its onset. AGP reaches its maximum concentration 48 hours after the onset of inflammatory processes and remains elevated for 120 to 144 hours (Feelders *et al.*, 1998). Therefore, in order to minimize the uncertainties, simultaneous assessments of the biomarkers are used.

CRP was assessed by the Helica[TM] C-reactive protein ELISA assay (Helica Biosystems Inc., Fullerton, CA) and AGP was assessed by using an ELISA quantification kit (GenWay Biotech, Inc., San Diego, CA). Plasma samples were diluted in test tubes prior to analysis. Both CRP and AGP were measured by plate reader (Bio Tek Instruments, Inc. Winooski, VT).

Urinary iodine excretion

Urinary iodine was analyzed using the Sandel-Kolthoff reaction. Urine was allowed to reach room temperature and mixed to suspend sediment; 250 μL of each urine sample, working standards from 0 to 300 μg/L and internal urine controls were pipetted into 13 x 100 mm test tubes; a set of internal urine controls were included in each batch. One mL of ammonium persulfate was added to each tube. All tubes were heated for 60 minutes at 91 - 95 °C and allowed to cool to room temperature; 2.5 mL arsenium acid solution was added, mixed by vortex and let stand for 15 minutes. Then 300 μL of ceric ammonium sulfate solution was added to each tube at 15 to 30 second intervals between successive tubes. Each tube was mixed with a vortex after addition and allowed to sit at room temperature. Absorbance was read at 405 nm in a spectrophotometer (Beckman Courter, Inc. Fullerton, CA) 30 minutes after addition of ceric ammonium sulfate to the first tube; successive tubes were read at the same time interval as when the ceric ammonium sulfate was added. In order to calculate results, a standard curve was constructed by plotting the log of the absorbance at 405 nm on the X-axis versus the standard iodine concentration in μg/L on the Y-axis with a scatter plot, using Excel. The iodine concentration in μg/L of each specimen was calculated by using the equation of the linear trend line of the chart (WHO/UNICEF/ICCIDD, 2007).

Statistical analysis

Once the data collection was completed, the collected data were organized, entered into the EXCEL computer package and cleaned for wrongly entered data by running to find minimum and maximum values. After cleaning, data were exported to SAS (V. 9.2). Data were analyzed using selected descriptive and analytical statistical

measures. In the descriptive section percentages, frequency distributions, means and standard deviations were used in describing the socio-economic and demographic characteristics of the respondents and status of zinc, ferritin and iodine. In the analytical section Pearson's correlation coefficient was used to examine relations between variables and multivariate analysis of variance was used to see the combined effects of several variables on cognition. The Proc GLM procedure was used for analysis of variance to determine differences among Kebeles. All the analyses were performed with SAS 9.2 Software (SAS Institute Inc, Cary, NC, USA).

CHAPTER IV

RESULTS AND DISCUSSION

Results

Demographic, socioeconomic and anthropometric characteristics of women

Characteristics of the study participants are shown in Table 4.1. The self –

reported age (Mean ± SD) of the participants was 30.8 (7.8) years. About 50% of the

participants were in the age category between 18 and 30 years old and the rest were

between 30 and 49 with only 2 participants above 49 years. Most of the women (92.1%)

were married whereas 6.4%, 1% and 0.5% were widowed, single and divorced

respectively. More than 90% of the participants responded that the husband is the

household head. The household size and number of children were 6.1(2.4) and 4.2 (2.2)

respectively. However in 9.1% of the households there were 10-12 people and in 15% of

the households there were 7 – 11 children living. Of the participants, 63.5% had no

formal education and most of the remaining (36.5%) had limited education. Out of those

who had education, there was only one who completed 2 years of college after high

school and one other who completed high school.

Of the participants, 26% owned mature enset (one of their staple foods other than

corn) and 85% owned livestock. There were 4 households who had all the kinds of

livestock that are commonly owned in the area such as cows, oxen, sheep and chickens.

Of those who owned mature enset plants, the number varied from 1 to 500 and most of

them owned less than 50 plants. The average size of land was 0.4 (0.2) hectare and there

was only one participant who had as much as 1.5 hectare of land.

A majority of the participants (63.7%) had a house with roof made of grass or straw whereas the rest had houses with roofs made of corrugated iron. The walls of the house were built either with wood and mud, dry mud blocks or wood and grass. Most had floors made of mud or cow dung smeared, but 10% had cemented floors.

As shown in fig 4.1, 2.5% of the participants were severely thin (BMI < 16), 3% were moderately thin (BMI between 16 and 16.9), 19.3% had mild thinness (BMI between 17.0 and 18.4), 73.3% were in the normal range (BMI between 18.5 and 24.9) and 2% were overweight (BMI ≥ 25) (WHO, 2006). The mean (SD) height and weight were 157.3 (6.0) cm and 50 (6.5) kg respectively.

The mid upper arm circumference (MUAC) presented in Fig 4.2 indicates 2 (1%) women had MUAC below 19 cm which is classified as severe wasting, 8% had MUAC below 22 cm which is classified as undernourished and 91% had MUAC above 22 cm which is classified as normal (Ferro-Luzzi & James, 1996).

Table 4.1 Demographic and socioeconomic characteristics of

women (n = 202)

	Frequency	Percent	Mean (SD)
Age			30.8(7.8)
Household size			6.1(2.4)
Number of children			4.2(2.2)
Marital status			
- Single	2	1.0	
- Married	186	92.1	
- Divorced	1	0.5	
- Widowed	13	6.4	
Education level			
- No education	127	63.5	
- Some education	73	36.5	
Size of land (ha)			0.4 (0.2)
Livestock ownership			
- Yes	172	85.2	
- No	30	14.8	
Owning mature enset			
- Yes	52	25.7	
- No	150	74.3	
Type of roof of house			
- Grass/straw	123	63.7	
- Corrugated iron	70	36.3	

Fig. 4.1 Body mass index (BMI) of women (n = 202)
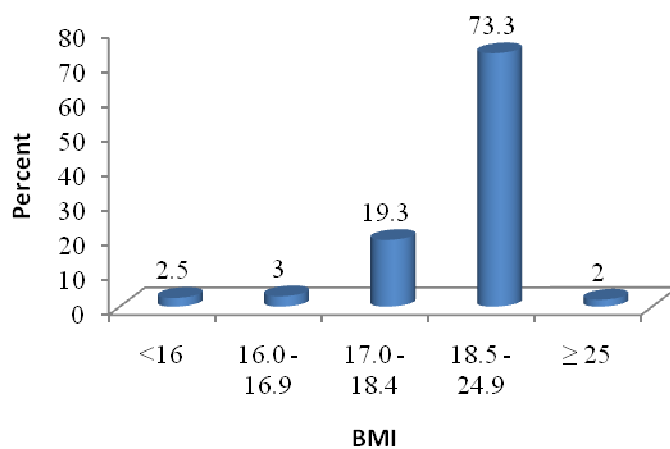
Figure 4.2 Mid upper arm circumference (MUAC) of women (n = 202)
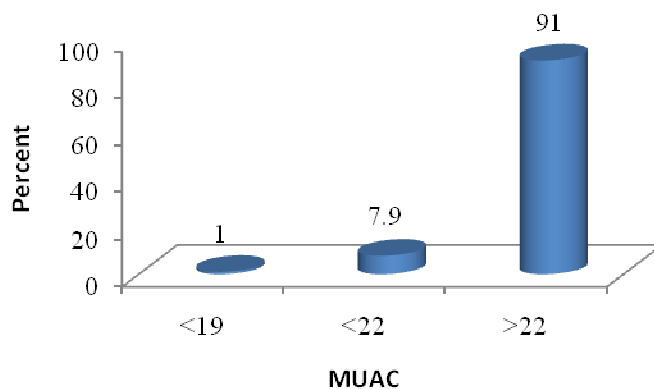
Assessment of biomarkers

Table 4.2 presents values for plasma zinc, ferritin, hsCRP, and AGP, for hemoglobin, and for urinary iodine excretion and goiter rate. Of the 194 participants with useable blood samples, 102 (52.6%) had plasma zinc below 70 µg/dL classified as zinc deficient. Among these, 11 (5.7%) had zinc level below 50 µg/dL. The remaining 47.4% were above 70 µg/dL classified as normal zinc concentration according to the cutoff recommended by Hotz and colleagues (Hotz *et al.*, 2003b).

Assessment of urinary iodine excretion (UIE) indicated that 96.5% of participants had urinary iodine concentration below 100 µg/L which is an indicator of iodine deficiency. Among these, 22.8% were below 20 µg/L classified as severely iodine deficient, 46.5% were between 20 and 50 µg/L classified as moderately iodine deficient, and 27.2% were between 50 to 100 µg/L classified as mildly iodine deficient (WHO, 2007b). Women who had normal iodine concentration (UIE >100 µg/L) were only 3.5%. Median UIE was only 37.7µg/L. Moreover, the maximum UIE obtained among the participants was 146.5 µg/L.

The palpation-based goiter assessment showed that 15.9% had goiter, with 14.4% palpable and 1.5% visible goiter. Dietary assessment indicated that no women had ever consumed iodized salt at all (data not shown). Interestingly zinc was negatively correlated with UIE (r = -0.14, p ≤ 0.05) and goiter (r = -0.15, p ≤ 0.05).

With respect to the biochemical iron indices, 22% of the participants had depleted iron stores, that is plasma ferritin concentrations below the cutoff of 12 µg/L (Worwood, 1982) and the majority 152 (78%) had ferritin level above the cutoff point.

Table 4.2 Hemoglobin, plasma zinc, ferritin, and inflammation indicators (hsCRP &

AGP), urinary iodine concentrations and goiter rate of women (n = 194)

	n	Percent	Mean (SD), median
Zinc (μg/dL)			
- < 70	91	52.6	
- > 70	92	47.4	
Mean (SD)			69 (11.6)
Ferritin (μg/L)			
- < 12	42	21.6	
- > 12	152	78.4	
Mean (SD)			26.7 (20.4)
hsCRP (mg/L)			
- < 1.0	179	88.6	
- 1.0 to 3.0	15	7.4	
- > 3.0	8	4.0	
AGP (g/L)			
- < 1.0	201	99.5	
- > 1.0	1	0.5	
Hemoglobin (g/dL)			
- < 12.5	44	22	
- ≥ 12.5	150	78	
Mean (SD)			13.7(2.1)
UIE (μg/L)			
- < 20	46	22.8	
- 20 to < 50	94	46.5	
- 50 to100	55	27.2	
- > 100	7	3.5	
Median			37.2 μg/L
Goiter rate			
- No goiter	170	84.1	
- Palpable goiter	29	14.4	
- Visible goiter	3	1.5	
Total Goiter Rate (TGR) (%)			16

The altitude specific hemoglobin level indicated that 22.2 % of the women were anemic with hemoglobin level below 12.5 µg/L (adjusted cutoff for altitude of 1708 m) and median hemoglobin level was 13.7 µg/L.

Analysis of the inflammation indicators hsCRP and AGP showed that 88.6 % of the women had hsCRP level below the cutoff level for low risk, 7.4% had average risk and 4% had high risk for having inflammation (American Association for Clinical Chemistry, 2008). Their AGP level indicated that 99.5% of the women were below the cutoff level for low risk of inflammation (Sankaranarayanan *et al.*, 2004).

Performance of cognitive tests

Table 4.3 indicates scores of participants on each cognitive test. The mean (SD) scores on tests measuring non-verbal intelligence (Raven's CPM), visualization (Simultaneous), short-term memory (Sequential) and fluid ability (Pattern reasoning) were 16.3 (4.5), 21.6 (5.7), 18.6 (3) and 5.1 (2.4) respectively; none of the participants reached the ceiling performance except in Raven's A and AB. All of the four cognitive tests were strongly correlated to each other ($p \leq 0.001$).

Table 4.3 Cognitive performance of women (n = 202)

Cognitive tests	Mean (SD)	Minimum score	Maximum score	Possible maximum score
Raven's total	16.3 (4.5)	7	31	36
- Raven's A	7.8 (1.8)	3	12	12
- Raven's AB	4.5 (1.8)	3	12	12
- Raven's B	4 (1.8)	1	9	12
Sequential	18.6 (3)	11	27	53
- Word order	11.5 (2.1)	5	18	31
- Number recall	7.1 (1.5)	4	12	22
Simultaneous	21.6 (5.7)	11	46	89
- Rover	12.8 (4.0)	7	32	44
- Triangle	8.8 (3.3)	0	21	29
Pattern reasoning	5.1 (2.4)	1	17	36

Relationship between micronutrients, selected variables and cognition

As shown in table 4.4 when the relations between zinc, iron, iodine and cognition were assessed, there was no positive association between plasma zinc, or ferritin and cognition, rather zinc was negatively correlated with Raven's CPM and planning measures ($r = -0.15$, $p \leq .05$). Urinary iodine excretion was significantly correlated with sequential ($r = 0.15$, $p \leq .05$), simultaneous ($r = 0.14$, $p \leq .05$) and Raven's CPM ($r = 0.14$, $p \leq .05$). Ferritin and hemoglobin level did not correlate with any of the cognitive tests.

A significant correlation was observed between education of the women and sequential ($r = 0.37$, $p \leq 0.001$), simultaneous ($r = 0.33$, $p \leq 0.001$), and planning ($r = 0.34$, $p \leq 0.001$) indices from the KABC-II and with the Raven's CPM ($r = 0.33$, $p \leq 0.001$). Economic level based on a wealth index was significantly associated with sequential ($r = 0.18$, $p \leq 0.01$) and planning measures ($r = 0.16$, $p \leq 0.01$) and with Raven's CPM ($r = 0.19$, $p \leq .01$). One of the indicators of nutritional status, body mass index was significantly correlated with sequential ($r = 0.16$, $p \leq 0.05$), simultaneous ($r = 0.17$, $p \leq 0.01$) and Raven's CPM ($r = 0.14$, $p \leq 0.05$). But mid upper arm circumference did not correlate with any of the cognitive tests.

Table 4.4 Pearson correlation coefficient for selected micronutrients, cognitive, nutritional status, demographic and socioeconomic variables of women (n = 194).

	Sequen[1]	Simul[2]	Planning	RCPM[3]	Age	Zinc	Ferritin	UIE[4]	Goiter	Wealth	Education	BMI[5]
Sequen[1]												
Simul[2]	.43#											
Planning	.42#	.42#										
RCPM[3]	.32#	.22#	.35#									
Age	-.14†	-.22Φ	-.09	-.01								
Zinc	-.04	.01	-.15†	-.15†	-.01							
Ferritin	.12	-.04	-.00	-.02	0.13†	-.05						
UIE[4]	.15†	.14†	.05	.14†	-.03	-.14†	-.03					
Goiter	-.04	-.03	-.02	.13	.05	-.15†	.15†	.03				
Wealth	.18Φ	.10	.16Φ	.19Φ	.24#	-.07	.04	.00	.04			
Education	.37#	.33#	.34#	.33#	-.22#	.02	-.01	.06	.02	.24#		
BMI[5]	.16Φ	.17Φ	-.05	.14†	-.08	.05	-.02	-.08	-.01	.19Φ	.16†	
MUAC[6]	-.06	.04	-.03	-.06	.01	.02	-.01	-.08	-.05	.22#	.09	.84#

1 = Sequential processing   2 = Simultaneous processing   3 = Raven's colored progressive matrices
4 = Urinary iodine excretion   5 = Body mass index   6 = Mid upper arm circumference
     † p≤.05   Φ p≤.01   # p≤.001

Table 4. 5 Multiple regression analysis predicting sequential, simultaneous, pattern reasoning and Raven's CPM scores (n = 202)

	Intercept	Education	Age	UIE	Ferritin	Zinc
1. Sequential						
- β	17.065	0.346	-0.056	0.016	0.024	
- *S.E*	2.571	0.086	0.028	0.008	0.010	
- *p - value*	<0.0001	<0.0001	0.049	0.047	0.017	NS
2. Simultaneous						
- β	20.439	0.574	-0.122			
- *S.E*	4.745	0.159	0.052			
- *p - value*	<0.0001	0.0004	0.021	NS	NS	NS
3. Raven's CPM (total)						
- β	14.771	0.530		0.021		-0.006
- *S.E*	3.835	0.123		0.012		0.003
- *p - value*	0.0002	<0.0001	NS	0.074	NS	0.037
4. Pattern reasoning						
- β	10.351	0.305				-0.003
- *S.E*	2.110	0.071				0.001
- *p - value*	<0.0001	<0.0001	NS	NS	NS	0.042

- Other variables such as wealth, MUAC and BMI were tested but showed no significance in the regression models.
- NS = Not significant
1. Adjusted R^2 = 0.173, *P < 0.0001*
2. Adjusted R^2 = 0.128, *P < 0.0001*
3. Adjusted R^2 = 0.141, *P < 0.0001*
4. Adjusted R^2 = 0.140, *P < 0.0001*

Age of the participants was an important factor associated with several variables. Age was positively associated with economic status ($r = 0.24$; $p \leq 0.001$) and negatively associated with education of the participants as expected ($r = -0.22$; $p \leq 0.001$). It was also negatively associated with sequential ($r = -0.14$; $p \leq 0.05$) and simultaneous ($r = -0.22$; $p \leq 0.01$). Moreover, a significant association was observed between age and ferritin ($r = 0.13$; $p \leq 0.05$).

Except for BMI these results were also confirmed by the multiple regression model. In this model; education, age, UIE and ferritin combined together predicted 17% of the variation in sequential indices; education and age together predicted 13% of the variation in simultaneous indices; education, UIE and zinc together predicted 14% of the variation in Raven's CPM; education and zinc together predicted 14% of the variation in pattern reasoning (Table 4.5).

Differences in variables by Kebele

As shown in table 4.6, the three adjacent kebeles (study areas) were different in several variables. The difference didn't have an overall trend but each of the kebeles had some variables that made them different from the others. For instance women from Finchawa had significantly higher scores than those from Tullo and Alamura on cognitive tests such as in Rover, Raven's CPM_A, and the total Raven's CPM but they also had a higher goiter rate. Women from Tullo were younger, had higher plasma zinc concentrations and scored higher on the number recall test (sequential) than women from Finchawa and Alamura. Women from Alamura had better iron status than women from from Finchawa and Tullo based on hemoglobin and plasma ferritin concentrations, but their urinary iodine excretion was lower.

Table 4. 6 Differences in variables by Kebele

Variables*	Finchawa	Tullo	Alamura
Rover	14.3^a	12.6^b	11.5^b
Number recall	5.1^b	5.3^a	4.8^b
Raven's CPM_A	4.8^a	4.5^b	4.1^b
Raven's CPM_total	17.7^a	16.1^b	15.2^b
Age (years)	31.2^a	27.9^b	33^a
Hemoglobin (g/dL)	13.1^b	13.1^b	14.8^a
Ferritin (µg/L)	25.3^b	19.7^b	34.1^a
Goiter (%)	0.3^a	0.06^b	0.1^b
UIE (µg/L)	48.6^a	48.8^a	29.1^b
Zinc (µg/dL)	66.6^b	71.6^a	69.3^b

*Only variables that are significantly different are included

- Values in a row that share a superscript are not significantly different from each other

<center>**Discussion**</center>

Micronutrient deficiencies often occur in the poorest segment of the population in developing countries (WHO, 2000). Although micronutrients are required in small concentrations, they are essential components of biological enzyme systems or are biologically active constituents (Andrasi *et al.*, 2007). Micronutrient deficiency could increase rates of illness, death from infectious disease and even mental impairment (Black, 2003b). Among the micronutrients vitamin A, zinc, iron and iodine are the major deficiencies that mostly occur in developing nations (Muller & Krawinkel, 2005).

The study population was drawn from subsistence farming households. According to a report by Yewelsew and colleagues, only 2% of the women respondents who participated in their study always had enough food to eat and less than 10% ate animal products such as meat or fish in the four months prior to the study period in July, 2004. Their major food sources were enset (*E. ventricosum*) and unrefined maize, which is high in phytate, known to inhibit zinc and non-heme iron absorption (Gibson & Huddle, 1998; Abebe *et al.*, 2006).

Concentration of plasma zinc, ferritin and urinary iodine of women

There have been no studies published on zinc, iron, and iodine status and cognitive function of non-pregnant women in Sidama, Southern Ethiopia. Analyses of plasma indicated that 52.6% of the participants had low plasma zinc concentrations indicative of zinc deficiency. Such high prevalence of low plasma zinc concentrations is not surprising because of their limited dietary sources of zinc and high consumption of foods that are rich in inhibitors as mentioned earlier (Abebe *et al.*, 2006). Zinc can only

be obtained through the diet and is most available from red meat and sea foods (Hunt *et al.*, 1998).

In several studies, it has been reported that plasma zinc during pregnancy is decreased (Gibson & Huddle, 1998; King, 2000). Although our study participants were non-pregnant, their previous pregnancies might have affected their plasma zinc level. Another study has indicated that geographic conditions such as humid hot climate induce endogenous losses through perspiration and exfoliation of the skin (Heaver & Hunt, 1995). Low soil zinc and iron content was also reported to aggravate maternal zinc and iron deficiency (Cakmuk & Erdal, 1996). According to Yewelsew and colleagues, the zinc content of their staple foods, maize and enset, is low (Abebe *et al.*, 2006) which reflects low levels of zinc of the local soil (Alloway, 2004).

Analysis of status of iron in this study reflected that the majority of the participants (78.4%) had plasma ferritin concentrations above the cutoff point of 12 μg/L. The same percentage of people (78%) also had hemoglobin concentrations above the cutoff point 12.5 gm/dL. However, ferritin and hemoglobin level were not significantly correlated ($r = 0.09$, $p = 0.23$). Therefore, the cause of some of the anemia in those 22% of the study participants might be as a result of one or more other essential nutrient deficiencies rather than iron (DeMaeyer & Adiels-Tegman, 1985).

Based on literature reports serum ferritin might not be a valid biochemical marker of iron stores in developing countries due to high prevalence of infection (Hallberg & Asp, 1994; Huddle *et al.*, 1999) however, in the current study only a small number (4%) of the participants had elevated CRP and AGP (inflammation indicators) indicating that the relatively adequate plasma levels of ferritin were not caused by infection. The low

level of infection could also help narrow the broad suspects for cause of anemia mentioned above.

The prevalence of anemia in the study population was 22% which is lower compared to prevalence of anemia in non-pregnant women in other countries in Africa and globally which is 47.5% and 30.2% respectively (WHO, 2008). Yewelsew and colleagues have found similar results in communities near Awassa that indicate 23.5% of the pregnant women were anemic (Abebe *et al.*, 2006).

Even though the levels of anemia are lower than global rates, Haidar and Pobocik stressed that iron deficiency anemia is not a rare problem in Ethiopia (Haidar & Pobocik, 2009). Their estimates of iron deficiency anemia, 18.0%, based on hemoglobin and serum ferritin, indicated that it is a moderate public health problem according to WHO standards (WHO, 2008; Haidar & Pobocik, 2009). Their conclusion is in contrast to earlier studies which reported iron deficiency to be a rare problem in Ethiopia (Gebremedhin, 1981; Zewdie *et al.*, 1993).

Some researchers have reported that the Ethiopian diet is high in iron. Although the food consumption behavior varies from region to region, the diet is mostly cereals (Gebremedhin *et al.*, 1976). Hofvander suggested that the high iron intake is not due to the food but to the contaminant iron from the soil (Hofvander, 1968). Yigzaw and colleagues have indicated that fermentation enhances nutritive value of cereals by increasing availability of proteins and improving amino acid profiles. Fermentation also reduces nutrient inhibitor compounds such as phytates (Yigzaw *et al.*, 2001),

Urinary iodine excretion (UIE) is an important biomarker to assess current iodine intake in the diet due to the fact that most iodine absorbed in the body eventually appears

in the urine. However UIE helps assess iodine status only at the time of measurement, not longer than days, whereas thyroid size reflects iodine nutrition over months and years (WHO/UNICEF/ICCIDD, 2007) and also reflects a chronic situation of iodine deficiency (Parded *et al.*, 1998).

In the current study, we assessed urinary iodine excretion and thyroid size by palpation. According to the WHO standard for UIE, 22.8% of the study participants were severely iodine deficient, 46.5% were moderately deficient, 27.2% were mildly deficient and 3.5% had adequate iodine nutrition. None of the participants had ever consumed iodized salt. Based on the criteria for monitoring progress towards sustainable elimination of IDD as a public health problem, the proportion of households using adequately iodized salts should be > 90% and median urinary iodine in the general population should be 100 to 199 µg/L (WHO/UNICEF/ICCIDD, 2007); thus IDD is a serious public health problem in the present study population. Our palpation-based goiter assessment also indicated that 16% had palpable and visible goiter. Generally, based on the UIE, 97% of the women were iodine deficient. Although there is no standard set for adults, for school age children a goiter rate of 5% or more indicates the presence of a public health problem (WHO/UNICEF/ICCIDD, 2007).

The cause for the high prevalence of IDD may be not only as a result of lack of iodized salt but also may be due to the cereal-based diet. The iodine content of plants depends on the iodine content of the soil, and iodine in soil also affects the content of the water. This indicates that not only does iodine content vary from crop to crop, but it also varies geographically (Cherinet & Kelbessa, 2000). In the present study we did not investigate associations between IDD prevalence and drinking water. But, Cherinet and

Kelbessa (2000) found that goiter prevalence was higher among subjects whose major drinking water source was polluted. Thus goiter prevalence was correlated with bacterial contamination of drinking water (Cherinet & Kelbessa, 2000). The majority of the present study participants used tap water. We have not assessed the level of contamination, but it is likely that the water could be contaminated from the tap itself, or the container they used to collect water or handling at home.

Deficiencies of other micronutrients such as vitamin A, selenium, and iron can have effects on thyroid hormone production and metabolism leading consequently to iodine deficiency. Other dietary factors, such as thiocyanate from cassava and goitrogenic compounds from millet, can also block thyroid hormone synthesis and make hypothyoroidism and goiter worse (Dunn, 2002). However, this study showed their plasma iron and selenium were normal; all of the participants had plasma selenium concentration above the cutoff point 7 µg/dL (data not shown) (Food and Nutrition Board Institute of Medicine, 2000). Plasma vitamin A was not measured in this study. Moreover, their food habit was different from the goitrogenic thiocyanatic food substances mentioned above. The negative correlation between plasma zinc and urinary iodine and goiter rate requires further investigation. It was also suggested that the interaction and/or interference of multiple nutrient deficiencies requires an in depth investigation (Ruz *et al.*, 1999). Although there is still a need to do some further analysis, the above scenario would take us to a conclusion that lack of iodine is the major cause for the IDD in the study population.

Relation between zinc, iron, iodine and cognition

Deficiency of the micronutrients so far discussed would cause impairments in growth, immune competence and cognitive function among school-age children (Viteri & Gonzalez, 2002). Such adverse health consequences also lead to reduction in both reproductive and intellectual potential in adulthood (Horton, 2000) along with all of the clearly known clinical deficiency symptoms.

Zinc is one of the most important micronutrients essential for brain development and central nervous system function (Wallwork, 1987). Zinc deficiency has been associated with neurological dysfunction and human brain pathology (Cuajungco & Lees, 1997). Measures of various psychological variables such as mood, perceived stress and cognitive status were related to lower plasma zinc concentrations, especially in areas with low zinc intakes and a limited variety of foods containing zinc (Marcellini *et al.*, 2008).

However, the present study, which showed nearly 50% of the study participants to have plasma zinc deficient Zn < 70 µg/dL, found that zinc was negatively correlated with Raven's CPM and with pattern reasoning (p ≤ 0.05). A study in Bangladeshi infants has shown the placebo group had significantly higher Bayley mental development index (MDI) scores than the zinc supplemented group (Hamandani *et al.*, 2001). Another study found no association between zinc status and mood in an aging European sample and suggested that the potential influence of zinc on mood may be small and undetectable when zinc status is within normal limits (McConville *et al.*, 2005). In community studies of older children and adolescents, zinc supplementation did not show any significant and consistent improvements in psychosocial or behavioral functioning (Kordas *et al.*, 2005; Penland *et al.*, 2005).

On the contrary, Stoecker and colleagues in their study on pregnant women found that Raven's CPM scale A score was positively correlated with plasma zinc. However, the participants of this study were severely zinc deficient and 76% had values below 7.6 µmol/L (< 50 µg/dL) which is the cutoff for zinc deficiency in the third trimester of pregnancy (Stoecker *et al.*, 2009). This is supported by the suggestion that "the threshold of severity for zinc deficiency that would influence cognitive performance is essential and would have major implications in developing country settings where mild to moderate zinc deficiency is common" (Bhatnagar & Taneja, 2001).

According to a study by Sandstead, when mothers consume more animal protein and zinc, their infants had higher scores on the Brazelton Neonatal Development Assessment Scales. When the infants were assessed using Bayley Scale of Infant Development after six months, the infants showed better motor development. When the infants were provided with formula contained 11 mg Zn/L, their growth and motor development (Griffiths Development Assessment Test) were greater than when provided with formula containing 6.7 mg Zn/L (Sandstead, 2003).

Inconsistent results with regard to zinc and cognition might be due to the reason that the reservoir for zinc in the body is not easily measurable and the measure based on plasma zinc is less sensitive. Moreover, there is no single indicator that adequately shows zinc status (Wood, 2000; Seshadri, 2001). Maylor and colleagues in their ZENITH study in healthy middle aged and older adults indicated that there was no consistent association between zinc intake or urine and blood levels of zinc and cognition at baseline (Maylor *et al.*, 2006).

Iron deficiency is a state in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain, and muscle (WHO *et al.*, 2001). ID and IDA mostly affect infants, children and women of reproductive age due to physiological requirements, poor dietary intake and increased losses (Murray-Kolb & Beard, 2007). Iron deficiency contributes to reduced physical activity, poor immune function, unstable emotion, and behavior (Lozoff *et al.*, 1998). The effect of iron deficiency on cognition is well investigated in infants and children but the mechanism by which cognition is altered by iron deficiency is largely unexplored in adults (Beard, 1995; Corwin *et al.*, 2003).

In the current study, ferritin level and hemoglobin did not show significant association in any of the cognitive tests. In this study, 22% of the participants had low ferritin and hemoglobin level below the cutoff point but no correlation was observed in each other. This finding is consistent with the study conducted by Beard and colleagues which showed no difference between iron-deficient anemic mothers and non-anemic mothers at baseline in the cognitive and behavioral variables despite significant improvements found after iron treatment (Beard *et al.*, 2005).

A systematic review and meta-analysis on the effects of oral iron supplementation indicated that iron supplementation had a statistically significant beneficial effect on attention and concentration. The improvement was observed in children aged 6 -18 but not in pre-menopausal women. No significant effect of iron supplementation was observed on intelligence, memory, psychomotor function, and scholastic achievement tests despite heterogeneous results shown on scholastic achievement tests (Falkingham *et al.*, 2010).

On the other hand, several treatment trials showed an association between measures of lethargy, fatigue, ability to concentrate, memory, and iron treatment indicating that better iron status improves cognitive and behavioral domains (Ballin *et al.*, 1992; Burner *et al.*, 1996; Beard *et al.*, 2005). Moreover, the severity of iron deficiency was found to be an important factor in affecting the level of cognitive function and in seeing an effect of iron supplementation (Murray-Kolb & Beard, 2007). Ferritin level was found to be associated with goiter rate and age of the present study participants (p ≤ 0.05). Hess and colleagues in their study indicated that iron and iodine deficiencies overlap each other (Hess *et al.*, 2002) . Iron deficiency negatively affects the physiology of thyroid, and iron supplementation improves the efficiency of iodine supplementation in children who have both iron deficiency anemia and goiter (Zimmermann *et al.*, 2000).

IDD causes a most serious disorder called cretinism, characterized by mental impairment and physical abnormalities (Boyages, 1993). People living in areas affected by severe IDD were estimated to have an intelligence quotient (IQ) of as much as 13.5 points below those of the comparable communities in areas where there was no ID (Bleichrodt & Born, 1994). This mental deficiency has an immediate effect on child learning capacity, women's health, the quality of life of communities, and economic productivity (WHO/UNICEF/ICCIDD, 2001a).

A meta-analysis study in China reported a difference of approximately 10 IQ points between moderate to severely iodine deficient and iodine sufficient populations. Spanish school children who were mildly iodine deficient had a better IQ than those who had worse iodine status (Santiago-Fernandez *et al.*, 2004; Qian *et al.*, 2005). However, different iodine supplementation studies in children have shown inconsistent results with

regard to cognition (Bautista *et al.*, 1982; Shrestha *et al.*, 1994; Huda *et al.*, 2001). Huda and colleagues reported that low urinary iodine and high goiter prevalence in the presence of normal thyroid function did not affect cognition and no significant difference in cognitive and motor scores as a result of a single dose of 400 mg iodized poppy seed oil, were observed between treatment and placebo groups (Huda *et al.*, 2001).

Unlike zinc and iron, a significant association was obtained between iodine status and cognition in the present study. Among the four different types of cognitive tests, UIE was positively correlated with sequential processing, simultaneous processing and Raven's CPM. In other words, those who had higher urinary iodine excretion had better short-term memory and visualization and performed better in a non-verbal intelligence test ($p \leq 0.05$). The correlation found with UIE was even significant with all of the three subunits of the Raven's CPM (Raven's A, AB and B) separately ($p \leq 0.05$). Consistent with this, urinary iodine status has been positively related to cognitive performance in other studies (Parded *et al.*, 1998; Briel *et al.*, 2000).

A randomized controlled study in iodine-deficient schoolchildren in Albania showed that iodine supplementation improved significantly their Raven's CPM, rapid target marketing, symbol search, and rapid object naming tests (Zimmermann *et al.*, 2006a). In another randomized controlled study, iodine supplementation was found to improve cognition in mildly iodine deficient children (Gordon *et al.*, 2009). On the other hand, iodine supplementation did not cause cognitive differences between treatment and control groups of Malaysian children. The same was true in iodine-deficient Bolivian children (Bautista *et al.*, 1982; Isa *et al.*, 2000).

Our regression model indicated that in addition to a positive effect of education, iron and iodine deficiencies have contributed to the impairment of cognition based on the performance on sequential indices but zinc did not. Among all the variables tested in the model, education was a strong predictor for the four cognitive tests.

In conclusion, it is difficult to evaluate the effect of micronutrients on cognition in cross-sectional and/or observational studies due to the fact that such approaches are often confounded by SES, education, health and other factors that may affect cognition and are difficult to eliminate (Sameroff *et al.*, 1993; Gordon *et al.*, 2009). Murray-Kolb and Beard in their study of young women noted that cognition is affected by many factors mostly by SES. Those who had better socioeconomic background had better cognitive performance than those who had poor socioeconomic background (Murray-Kolb & Beard, 2007). Beard and colleagues showed that mothers who had higher levels of education performed better in the Raven's CPM test than those who had less education (Beard *et al.*, 2005). Alemtsehay and colleagues also found similar results in the study conducted in Sidama, Southern Ethiopia. Education of the mother was significantly correlated with sequential processing, simultaneous processing and Raven's CPM (Bogale *et al.*, 2010).

In the present study, selected socioeconomic variables and education were tested. Education was positively correlated with all of the four cognitive tests (p ≤0.001 in all tests) and wealth was significantly correlated with Sequential processing, planning measures and Raven's CPM (p ≤ 0.01 in all cases). Age was also an important factor to affect cognition. Age was negatively correlated with sequential processing (p ≤ 0.05) and simultaneous processing (p ≤ 0.01). In other words, as age increased in these adult

women, their scores were lower. This might be due to the fact that participant's education was significantly lower as age increased ($p \leq 0.001$). But it should be noted that this does not mean the level of education decreases as women's age increases. Instead it means that younger women have more education. This is because firstly, the availability of education is higher in the present than the past times and secondly, the rate of sending women to school is increased in the young generation compared to former times (Central Statistics Agency, 2005). Additionally, BMI was positively and significantly associated with three out of four of the cognitive tests suggesting that nutritional status is an important factor to determine cognitive function.

The impact of community difference on variables

From our observation we thought the study populations were homogenous. However, during the process of the analysis we unexpectedly found interesting differences in the three adjacent rural communities (the study areas) in several variables. Because the three kebeles are very close to each other we have considered them almost as one community but the data clearly show community differences. The results observed gave us a clue on how some undetermined variation can make a difference.

As indicated in table 4.6, hemoglobin levels of the women in Alamura were significantly higher than the other two Kebeles. Although we didn't measure elevation of the three Kebeles, from our observation, Alamura is more elevated than Tullo and Finchawa therefore a small amount of the difference in hemoglobin might be due to the altitude difference these communities (Nestel, 2002). Ferritin concentration was also higher in Alamura but evidence is lacking about whether ferritin concentration is affected by altitude (Cook *et al.*, 2005).

Moreover, Alamura is situated near the biggest mountain in the area called Mount Alamura. The mountain could cause heavy run-off which could wash away the iodine (Cao *et al.*, 1994) and selenium content of the soil (Dhillon *et al.*, 2008), which might be a reason for the significantly lower level of UIE observed in Alamura. However, further investigation on altitude, type of soil, nutrient content of soil, source of water, nutrient content of water, water contaminants and all other variables that can possibly affect micronutrient status is required.

In summary, this study was conducted to assess for the first time biomarkers of micronutrient status and their relations to cognitive function of non-pregnant women in the study population. Although we found some significant associations, assessment of cognitive function is affected by several confounding factors such as SES as mentioned earlier. Hence, in order to see the extent of the effects of these micronutrients on cognition, large randomized controlled trials, at different ages and physiological conditions are required.

CHAPTER V

RECOMMENDATION

In our cross-sectional study, participants had low socioeconomic status and education. Hence they could not afford to feed themselves and also their families with nutrient (micronutrient) rich diets such as animal products that include meat, poultry, dairy products and fish. The participants have multiple micronutrient deficiencies that exposed them to mild and severe health complications.

In order to alleviate this well rooted problem therefore, government and non-government organizations need to intervene at different levels. IDD is one of the serious problems in the study area that can easily be solved by availing iodized salt in the local market. However, certain mechanisms should be devised on how to make sure that the iodized salt reaches each and every household. Although implementation of universal salt iodization may not always be feasible, salt has to be iodized at the source before it is distributed in the market. This can only be done by the government. In addition, local level salt iodization programs can also be implemented by both governmental and non-governmental organizations.

The community depends on enset and cereals, mostly maize, as their staple foods. Cereals are rich in micronutrients such as zinc and iron, but also rich in phytates that inhibit the bioavailability of the nutrients. Therefore, in order to increase bioavailability of nutrients, household level food processing methods can be implemented. These methods include fermentation, soaking, and germination which help to minimize the

phytate content of the grain and hence increase the bioavailability of nutrients in the body (Gibson *et al.*, 2006).

Nutrition education programs are vital and should be given to the rural community on a regular basis. The nutrition education should include creating awareness on the concept of balanced diet and cooking methods to reduce over cooking so as to minimize nutrient losses. Devising a way to generate income at the household level using appropriate technology can also help increase household income.

During data collection shortage of time was one of the constraints we had. Thus, in order to alleviate this problem we let the participants stay longer and do the cognitive tests and the questionnaire at the same time. We administered the questionnaire first and then did six different cognitive tests one after the other. Keeping participants for this longer time could make them bored and tired and that causes loss of attention during the interview and during the cognitive tests. Therefore, cognitive tests should be done separately from the interview. It is also important to make sure that the surrounding should be quiet enough when administering cognitive tests.

Moreover, it is important to make sure that participants eat their breakfast or lunch (depending on the time) before starting data collection, particularly for the cognitive tests, because a hungry person cannot give adequate attention. There is also a need to find a way for the plasma samples to stay frozen if transportation to a different place has to be done. Finally, as long as data are taken from adjoining kebeles, it is important not to assume they are the same.

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APPPENDICES

Table 1. Socioeconomic and demographic characteristics of women

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
1	**Age of study subjects (years)**				
	- 18 – 24	48	23.76	48	23.76
	- 25 – 29	52	25.74	100	49.50
	- 30 – 34	39	19.31	139	68.81
	- 35 – 39	30	14.85	169	83.66
	- 40 – 44	16	7.92	185	91.58
	- 45 - 49	15	7.43	200	99.01
	- > 49	2	0.99	202	100.00
2	**Household head**				
	- Woman (wife)	15	7.50	15	7.50
	- Husband	183	91.50	198	99.0
	- Parent of woman	1	0.50	199	99.50
	- Other	1	0.50	200	100.00
	Frequency missing = 2				
3	**Marital status**				
	- Single	2	0.99	2	0.99
	- Married	186	92.08	188	93.07
	- Separated	1	0.50	189	93.56
	- Widowed	13	6.44	202	100.00
4	**Marriage years**				
	- 0 – 4	33	16.34	33	16.34
	- 5 – 9	43	21.29	76	37.62
	- 10 – 14	41	20.30	117	57.92
	- 15 – 19	36	17.82	153	75.74
	- 20 – 24	26	12.87	179	88.61
	- 25 – 29	16	7.92	195	96.53
	- 30 – 35	7	3.47	202	100.00
5	**Religion**				
	- Muslim	28	14.36	28	14.36
	- Protestant	154	78.97	182	93.33
	- Catholic	10	5.13	192	98.46
	- Other	3	1.54	195	100.00
	Frequency missing = 7				
6	**Husband have another wife**				
	- No	170	85.43	170	85.43
	- Yes	29	14.57	199	100.00
	Frequency missing = 3				

7	**No. of people in house hold**				
	- 0 – 4	49	24.62	49	24.62
	- 5 – 9	132	66.34	181	90.96
	- 10 – 12	18	9.05	199	100.00
	Frequency missing = 3				

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
8	**School years attended**				
	- No education	125	62.81	125	62.81
	- 1 year	5	2.51	130	65.33
	- 2 years	14	7.04	144	72.36
	- 3 years	12	6.03	156	78.39
	- 4 years	17	8.54	173	86.93
	- 5 years	11	5.53	184	92.46
	- 6 years	7	3.52	191	95.98
	- 7 years	2	1.01	193	96.98
	- 8 years	1	0.50	194	97.49
	- 9 years	1	0.50	195	97.99
	- 10 years	2	1.01	197	98.99
	- 12 years	1	0.50	198	99.50
	- 14 years	1	0.50	199	100.00
	Frequency missing = 3				
9	**Education level of respondent**				
	- No education	127	63.50	127	63.50
	- Some reading/writing	24	12.00	151	75.50
	- Some elementary school	34	17.00	185	92.50
	- Elementary school complete	12	6.0	197	98.50
	- Some high school	2	1.00	199	99.50
	- High school complete	1	0.50	200	100.00
	Frequency missing = 2				
10	**Education level of husband**				
	- No education	80	41.03	80	41.03
	- Some reading/writing	23	11.79	103	52.82
	- Some elementary school	41	21.03	144	73.85
	- Elementary school complete	31	15.90	175	89.74
	- Some high school	11	5.64	186	95.38
	- High school complete	9	4.62	195	100.00
	Frequency missing = 7				

11	**Husband occupation**				
	- Farmer	105	54.97	105	54.97
	- Civil servant	2	1.05	107	56.02
	- Daily labor	6	3.14	113	59.16
	- Self-employed	4	2.09	117	61.26
	- Paid employment	3	1.57	120	62.83
	- Farmer and Civil servant	12	6.28	132	69.11
	- Farmer and daily laborer	27	14.14	159	83.25
	- Farmer and self employed	19	9.95	178	93.19
	- Farmer and other	3	1.57	181	94.76
	- Farmer and paid employee	10	5.24	191	100.00
	Frequency missing = 11				

Table 2. Health and reproductive characteristics of women

N0.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
1	**Number of pregnancy**				
	- No pregnancy	3	1.49	3	1.49
	- 1 – 3	70	34.65	73	36.14
	- 4 – 6	83	41.09	156	77.23
	- 7 – 9	37	18.32	193	95.54
	- 10 – 12	8	3.96	201	99.50
	- 14	1	0.50	202	100.00
2	**Number of children**				
	- No children	3	1.49	3	1.49
	- 1 – 3	80	39.60	83	41.09
	- 4 – 6	89	44.06	172	85.15
	- 7 – 9	27	13.37	199	98.51
	- 10 – 11	3	1.49	202	100.00
3	**Ever had miscarriage**				
	- Yes	31	15.35	31	15.35
	- No	171	84.65	202	100.00
4	**Number of miscarriage**				
	- One times	21	67.74	21	67.74
	- Two times	8	25.81	29	93.55
	- Three times	2	6.45	31	100.00
5	**Ever lost a child**				
	- Yes	55	27.23	55	27.23
	- No	147	72.77	202	100.00

6	Age of first child when died/Month				
	- 0.03	6	10.91	6	10.91
	- 0.09	1	1.82	7	12.73
	- 1	2	3.64	9	16.36
	- 2	1	1.82	10	18.18
	- 3	1	1.82	11	20.00
	- 4	2	3.64	13	23.64
	- 5	2	3.64	15	27.27
	- 6	3	5.45	18	32.73
	- 7	3	5.45	21	38.18
	- 8	2	3.64	23	41.82
	- 9	3	5.45	26	47.27
	- 12	6	10.91	32	58.18
	- 24	5	9.09	37	67.27
	- 36	7	12.73	44	80.00
	- 48	3	5.45	47	85.45
	- 60	1	1.82	48	87.27
	- 72	1	1.82	49	89.09
	- 120	1	1.82	50	90.91
	- 132	2	3.64	52	94.55
	- 192	1	1.82	53	96.36
	- 228	1	1.82	54	98.18
	- 240	1	1.82	55	100.00
	Frequency missing = 147				
7	Cause of first child death				
	- Sudden	6	10.91	6	10.91
	- Unknown	10	18.18	16	29.09
	- Evil spirit	2	3.64	18	32.73
	- Malaria	13	23.64	31	56.36
	- Bleeding	1	1.82	32	58.18
	- Headache	2	3.64	34	61.82
	- Tb	2	3.64	36	65.45
	- Measles	2	3.64	38	69.09
	- Fever & cough	3	5.45	41	74.55
	- Chocking	4	7.27	45	81.82
	- Swollen face	1	1.82	46	83.64
	- Accident	2	3.64	48	87.27
	- HIV	1	1.82	49	89.09
	- Drunk butter	1	1.82	50	90.91
	- Kwashiorkor	1	1.82	51	92.73
	- Cough	1	1.82	52	94.55
	- Influenza	1	1.82	53	96.36
	- Sickness	1	1.82	54	98.18

	- Cholera	1	1.82	55	100.00
	Frequency missing = 147				
8	**Age of second child when died/months**				
	- 0.4	1	5.88	1	5.8
	- 1	1	5.88	2	11.76
	- 2	1	5.88	3	17.65
	- 4	1	5.88	4	23.53
	- 6	1	5.88	5	29.41
	- 10	1	5.88	6	35.29
	- 12	4	23.53	10	58.82
	- 36	1	5.88	11	64.71
	- 60	1	5.88	12	70.59
	- 84	1	5.88	13	76.47
	- 120	2	11.76	15	88.24
	- 180	1	5.88	16	94.12
	- 192	1	5.88	17	100.00
	Frequency missing = 185				
9	**Cause of second child death**				
	- Sudden	1	5.88	1	5.88
	- Unknown	6	35.29	7	41.18
	- Evil spirit	1	5.88	8	47.06
	- Malaria	4	23.53	12	70.59
	- Accident	1	5.88	13	76.47
	- Cough	1	5.88	14	82.35
	- Sickness	2	11.76	16	94.12
	- Diarrhea	1	5.88	17	100.00
	Frequency missing = 185				
10	**Age of third child when died/month**				
	- 0.03	1	50.00	1	50.00
	- 12	1	50.00	2	100.00
	Frequency missing = 200				
11	**Cause of third child death**				
	- Unknown	1	50.00	1	50.00
	- Sickness	1	50.00	2	100.00
	Frequency missing = 200				
12	**Ever used contraceptive**				
	- Yes	125	61.88	125	61.88
	- No	77	38.12	202	100.00
13	**Contraceptive method**				
	- Pill	11	8.66	12	9.45
	- Injection	109	85.83	121	95.28

	- Loup or Norplant	4	3.15	125	98.43
	- Both pill & injection	2	1.57	127	100.00
	Frequency missing = 76				
14	**Place of medical treatment**				
	- Health center	193	96.50	193	96.50
	- Health center & prayer	7	3.50	200	100.00
	Frequency missing = 2	2			
15	**Ever taken iron supplement**				
	- Yes	23	11.39	23	11.39
	- No	179	88.61	202	100.00
16	**Supplement taken for the last time**				
	- 2008-2009	10	45.45	10	45.45
	- 2006-2007	7	31.82	17	77.27
	- Before 2006	5	22.73	22	100.00
	Frequency missing = 180				

Table 3. Household socioeconomic indicators

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
1	**Land ownership**				
	- Yes	199	98.51	199	98.51
	- No	3	1.49	202	100.00
2	**Size of land/Hectare**				
	- 0.25	85	42.93	85	42.93
	- 0.5	84	42.42	169	85.35
	- 0.75	14	7.07	183	92.42
	- 1	14	7.07	197	99.50
	- 1.5	1	0.50	198	100.00
	Frequency missing = 4	4			
3	**Livestock ownership**				
	- Yes	172	85.15	172	85.15
	- No	30	14.85	202	100.00
4	**Kind of livestock**				
	- Cows & oxen	54	31.40	54	31.40
	- Goat	7	4.07	61	35.47
	- Sheep	1	0.58	62	36.05
	- Poultry	8	4.65	70	40.70
	- Donkey	1	0.58	71	41.28
	- Cow, oxen & goat	25	14.53	96	55.81
	- Cow, oxen & sheep	2	1.16	98	56.98

	- Cow, oxen & poultry	37	21.51	135	78.49
	- Cow, oxen & donkey	2	1.16	137	79.65
	- Goat & poultry	2	1.16	139	80.81
	- Sheep & poultry	1	0.58	140	81.40
	- Cow, oxen, goat & sheep	5	2.91	145	84.30
	- Cows, oxen, goat & poultry	19	11.05	164	95.35
	- Cows, oxen, goat, sheep & poultry	2	1.16	166	96.51
	- Goat, sheep & poultry	2	1.16	168	97.67
	- All of the above	4	2.33	172	100.00
	Frequency missing = 30				
5	**Number of cows/oxen owned**				
	- One	47	31.76	47	31.76
	- Two	61	41.22	108	72.97
	- Three	21	14.19	129	87.16
	- Four	8	5.41	137	92.57
	- Five	7	4.73	144	97.30
	- Six	4	2.70	148	100.00
	Frequency missing = 54				
6	**No. of goats owned**				
	- One	33	50.77	33	50.77
	- Two	20	30.77	53	81.54
	- Three	5	7.69	58	89.23
	- Four	5	7.69	63	96.92
	- Five	1	1.54	64	98.46
	- Seven	1	1.54	65	100.00
	Frequency missing = 137				
7	**No. of sheep owned**				
	- One	9	52.94	9	52.94
	- Two	5	29.41	14	82.35
	- Three	1	5.88	15	88.24
	- Four	2	11.76	17	100.00
	Frequency missing = 185				
8	**No. of poultry owned**				
	- One	12	15.79	12	15.79
	- Two	19	25.00	31	40.79
	- Three	12	15.79	43	56.58
	- Four	11	14.47	54	71.05
	- Five	6	7.89	60	78.95
	- Six	5	6.58	65	85.53

	- Seven	2	2.63	67	88.16
	- Eight	2	2.63	69	90.79
	- Nine	1	1.32	70	92.11
	- Ten	4	5.26	74	97.37
	- Fifteen	1	1.32	75	98.68
	- Twenty	1	1.32	76	100.00
	Frequency missing = 126				
9	**Functioning items**				
	- Radio/tape player	7	4.64	7	4.64
	- Bicycle	10	6.62	17	11.26
	- Hand torch	49	32.45	66	43.71
	- Mobile phone	5	3.31	71	47.02
	- Horse/donkey cart	1	0.66	72	47.68
	- Radio & Bicycle	6	3.97	78	51.66
	- Radio & hand torch	12	7.95	90	59.60
	- Radio & mobile	2	1.32	92	60.93
	- Bicycle & hand torch	6	3.97	98	64.90
	- Bicycle & mobile	3	1.99	101	66.89
	- Hand torch & mobile	3	1.99	104	68.87
	- Three of the above items	31	20.53	135	89.40
	- Four of the above items	12	7.95	147	97.35
	- Five of the above items	3	1.99	150	99.34
	- All of the above	1	0.66	151	100.00
	Frequency missing = 51	51			
10	**Utensil use for cooking**				
	- Clay material	31	15.42	31	15.42
	- Iron	54	26.87	85	42.29
	- Both	116	57.71	201	100.00
	Frequency missing = 1	1			

Table 4. Food security indicators and living condition

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
1	**Owning mature enset**				
	- Yes	52	25.74	52	25.74
	- No	150	74.26	202	100.00
2	**Number of mature enset**				
	- 1	1	1.89	1	1.89
	- 2	1	1.89	2	3.78
	- 3	2	3.77	4	7.55

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	- 4	2	3.77	6	11.32
	- 10	10	18.87	16	30.19
	- 15	3	5.66	19	35.85
	- 20	5	9.43	24	45.28
	- 30	4	7.55	28	52.83
	- 35	1	1.89	29	54.72
	- 40	6	11.32	35	66.04
	- 50	6	11.32	41	77.36
	- 100	7	13.21	48	90.57
	- 130	1	1.89	49	92.46
	- 200	2	3.77	51	96.23
	- 300	1	1.89	52	98.11
	- 500	1	1.89	53	100.00
	Frequency missing = 4				
3	**Age of enset when consumed**				
	- 2 years	1	0.50	1	0.50
	- 3 years	7	3.48	8	3.98
	- 4 years	36	17.91	44	21.89
	- 5 years	85	42.29	129	64.18
	- 6 years	47	23.38	176	87.56
	- 7 years	19	9.45	195	97.02
	- 8 years	6	2.98	201	100.00
	Frequency missing = 1				
4	**Ever sell produces**				
	- Yes	49	24.38	49	24.38
	- No	152	75.62	201	100.00
	Frequency missing = 1				
5	**Food store lasts after harvest**				
	- Less than 2 months	32	15.92	32	15.92
	- 2 to 4 months	84	41.79	116	57.71
	- 5 to 8 months	73	36.32	189	94.03
	- 9 to 12 months	12	5.97	201	100.00
	Frequency missing = 1				
6	**Number of meals per day during harvest season**				
	- Once	1	0.50	1	0.50
	- Twice	6	2.99	7	3.48
	- Three times	184	91.54	191	95.02
	- More than three	10	4.98	201	100.00
	Frequency missing = 1				

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
7	**Number of meals per day during dry season**				
	- Twice	20	9.90	20	9.90
	- Three times	177	87.62	197	97.52
	- More than three	5	2.48	202	100.00
8	**Number of meals ate yesterday**				
	- Once	1	0.50	1	0.50
	- Twice	44	21.78	45	22.28
	- Three times	155	76.73	200	99.01
	- More than three	2	0.99	202	100.00
9	**Run out of staple food in the last seven days**				
	- No	73	36.14	73	36.14
	- Yes	129	63.86	202	100.00

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
10	**Reduce own consumption in the last seven days**				
	- Never	41	21.03	41	21.03
	- Once	39	20.00	80	41.03
	- 2 or 3 times	95	48.72	175	89.74
	- 5 & more times	20	10.26	195	100.00
11	**Reduce children consumption in the past seven days**				
	- Never	67	33.33	67	33.33
	- Once	34	16.92	101	50.25
	- 2 or 3 times	82	40.80	183	91.04
	- 5 & more times	18	8.96	201	100.00
	Frequency Missing = 1				
12	**Worry of not having enough food in the past 4 wks**				
	- Yes	171	84.65	171	15.35
	- No	31	15.35	202	100.00
13	**No. of worries' for not having enough food**				
	- Rarely (Once)	19	10.80	19	10.80
	- 2 or 3 times	71	40.34	90	51.14
	- 5 & more times	86	48.86	176	100.00
	Frequency missing = 26				

14	**Not able to eat preferred food in the past 4 wks**				
	- Yes	160	79.21	160	79.21
	- No	42	20.79	202	100.00
15	**No. of times not able to eat preferred food in the past 4 wks**				
	- Rarely (Once)	14	8.81	14	8.81
	- 2 or 3 times	87	54.72	101	63.52
	- 5 & more times	58	36.48	159	100.00
	Frequency missing = 43				
16	**Ate limited variety of food in the past 4 wks**				
	- Yes	164	81.19	164	81.19
	- No	38	18.81	202	100.00
17	**No. of times ate limited variety of food**				
	- Rarely (Once)	20	12.35	20	12.35
	- 2 or 3 times	78	48.15	98	60.50
	- 5 & more times	64	39.50	162	100.00
	Frequency missing = 40				
18	**Ate disliked food in the past 4 wks**				
	- Yes	114	56.72	114	56.72
	- No	87	43.28	201	100.00
	Frequency missing = 1				
19	**No. times ate disliked foods**				
	- Rarely (Once)	20	17.54	20	17.54
	- 2 or 3 times	57	50.00	77	67.54
	- 5 & more times	37	32.46	114	100.00
	Frequency missing = 88				
20	**Ate smaller food than usual in the past 4 wks**				
	- Yes	157	77.72	157	77.72
	- No	44	21.78	201	100.00
	Frequency missing = 1				
21	**No. of times ate smaller food**				
	- Rarely (Once)	21	13.55	22	14.19
	- 2 or 3 times	78	50.32	100	64.52
	- 5 & more times	55	35.48	155	100.00
	Frequency missing = 48				

No	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
22	**Ate fewer meals per day in the past 4 wks**				
	- Yes	133	66.17	133	66.17
	- No	68	33.83	201	100.00
	Frequency missing = 1				
23	**No. times ate fewer meals**				
	- Rarely (Once)	26	19.70	26	19.70
	- 2 or 3 times	73	55.30	99	75
	- 5 & more times	33	25	132	100.00
	Frequency missing = 70				
24	**No food at all in the past 4 wks**				
	- Yes	85	42.08	85	42.08
	- No	117	57.92	202	100.00
25	**No. of times with no food**				
	- Rarely (Once)	21	24.71	21	24.71
	- 2 or 3 times	46	54.12	67	78.82
	- 5 & more times	18	21.18	85	100.00
	Frequency missing = 117				
26	**Sleep hungry in the past 4 wks**				
	- Yes	45	22.28	45	22.28
	- No	157	77.72	202	100.00
27	**No. of times sleep hungry**				
	- Rarely (Once)	20	45.45	20	45.45
	- 2 or 3 times	19	43.18	39	88.64
	- 5 & more times	5	11.36	44	100.00
	Frequency missing = 158				

No	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
28	**Go whole day & night without eating**				
	- Yes	26	12.87	26	12.87
	- No	176	87.13	202	100.00
29	**No. of times go whole day & night without eating**				
	- Rarely (Once)	14	53.85	14	53.85
	- 2 or 3 times	11	42.31	25	96.16
	- 5 & more times	1	3.84	26	100.00
	Frequency missing = 176				

30	**Means of cultivation**				
	- Rain based	193	95.54	193	95.54
	- Rain & traditional irrigation	2	0.99	195	96.53
	- Rain & motor	1	0.50	196	97.03
	- Rain & harvested water	1	0.50	197	97.53
	- Rain & hand dug well	4	1.98	201	99.51
	- Rain, hand dug well & motor	1	0.50	202	100
31	**Main water source**				
	- Tap water	194	96.04	194	96.04
	- Water from protected well	8	3.96	202	100.00
32	**Treat drinking water**				
	- Yes	26	12.87	26	12.87
	- No	176	87.13	202	100.00
33	**Use for treatment**				
	- Chlorine	23	92.00	23	92.00
	- Filter using cloth	1	4.00	24	96.00
	- Wuha agar (chemical)	1	4.00	25	100.00
	Frequency missing = 177				
34	**Type of toilet**				
	- Don't have toilet	6	2.97	6	2.97
	- Pit latrine	182	90.10	188	93.07
	- Pit latrine with walls	14	6.93	202	100.00
35	**Type of walls of house**				
	- Build from wood & mud	138	69.35	138	69.35
	- Build from dry mud blocks	1	0.5	139	69.85
	- Build from wood & grass	60	30.15	199	100.00
36	**Type of roof of house**				
	- Grass/straw	123	63.73	123	63.73
	- Corrugated iron	70	36.27	193	100.00
	Frequency missing = 9				
37	**Type of floor**				
	- Mud/soil	177	88.94	177	88.94
	- Cow dung smeared	3	1.51	180	90.45
	- Cement	19	9.55	199	100.00
38	**Have windows**				
	- Yes	85	42.08	85	42.08
	- No	117	57.92	202	100.00

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
39	**Type of window**				
	- Open window	2	2.30	2	2.30
	- Made of mesh	3	3.45	5	5.75
	- Made of glass	4	4.60	9	10.34
	- Made of wood	78	89.66	87	100.00
	Frequency missing = 115				
40	**Number of rooms**				
	- No rooms	3	1.49	3	1.49
	- One	86	42.79	89	44.28
	- Two	57	28.36	146	72.64
	- Three	51	25.37	197	98.01
	- Four	3	1.49	200	99.5
	- Six	1	0.50	201	100.00
	Frequency missing = 1				
41	**Use mosquito net**				
	- Yes	173	85.64	173	85.64
	- No	29	14.36	202	100.00
42	**No. of mosquito net in the household**				
	- No net	26	13.07	26	13.07
	- One	87	43.72	113	56.78
	- Two	75	37.69	188	94.47
	- Three	10	5.03	198	99.50
	- Four	1	0.50	199	100.00
	Frequency missing = 3				
43	**Use mosquito net -for whom**				
	- Family	96	56.14	96	56.14
	- Parents	63	36.84	159	92.98
	- Mother	7	4.10	166	97.08
	- Father	1	0.58	167	97.66
	- Children	4	2.34	171	100.00
	Frequency missing = 31				

Table 5. Food consumption behavior and method of preparation

N0.	Food item consumed	Frequency	Percent	Cumulative frequency	Cumulative percent
1	**Beef**				
	- Never	8	3.96	8	3.96
	- During holiday	82	40.59	90	44.55
	- Very rarely	54	26.73	144	71.29
	- Twice per month	38	18.81	182	90.10
	- Once per week	18	8.91	200	99.01
	- 3 – 6 times per week	2	0.99	202	100.00
2	**Chicken**				
	- Never	82	40.59	82	40.59
	- During holiday	65	32.18	147	72.77
	- Very rarely	45	22.28	192	95.05
	- Twice per month	6	2.97	198	98.02
	- Once per week	3	1.49	201	99.50
	- 3 – 6 times per week	1	0.50	202	100.00
3	**Sheep**				
	- Never	146	72.28	146	72.28
	- During holiday	17	8.42	163	80.69
	- Very rarely	38	18.81	201	99.50
	- Twice per month	1	0.50	202	100.00
4	**Goat**				
	- Never	117	57.92	117	57.92
	- During holiday	20	9.90	137	67.82
	- Very rarely	62	30.69	199	98.51
	- Twice per month	3	1.49	202	100.00
5	**Catfish**				
	- Never	119	58.91	119	58.91
	- During holiday	1	0.50	120	59.41
	- Very rarely	24	11.88	144	71.29
	- Twice per month	11	5.45	155	76.73
	- Once per week	23	11.39	178	88.12
	- 3 – 6 times per week	12	5.94	190	94.06
	- Once per day	7	3.47	197	97.52
	- More than once per day	5	2.48	202	100.00
6	**Tilapia**				
	- Never	49	24.26	49	24.26
	- During holiday	1	0.50	50	24.75
	- Very rarely	23	11.39	73	36.14
	- Twice per month	32	15.84	105	51.98
	- Once per week	48	23.76	153	75.74
	- 3 – 6 times per week	33	16.34	186	92.08

	- Once per day	9	4.46	195	96.53
	- More than once per day	7	3.47	202	100.00
7	**Egg**				
	- Never	89	44.06	89	44.06
	- During holiday	18	8.91	107	52.97
	- Very rarely	51	25.25	158	78.22
	- Twice per month	14	6.93	172	85.15
	- Once per week	24	11.88	196	97.03
	- 3 – 6 times per week	6	2.97	202	100.00
8	**Lentil**				
	- Never	41	20.30	41	20.30
	- Very rarely	54	26.73	95	47.03
	- Twice per month	20	9.90	115	56.93
	- Once per week	56	27.72	171	84.65
	- 3 – 6 times per week	27	12.87	197	97.52
	- Once per day	4	1.98	201	99.50
	- More than once per day	1	0.50	202	100.00
9	**Pea**				
	- Never	36	17.82	36	17.82
	- During holiday	1	0.50	37	18.32
	- Very rarely	55	27.23	92	45.54
	- Twice per month	22	10.89	114	56.44
	- Once per week	45	22.28	159	78.71
	- 3 – 6 times per week	33	16.34	192	95.05
	- Once per day	7	3.47	199	98.51
	- More than once per day	3	1.49	202	100.00
10	**Kidney bean**				
	- Never	6	2.97	6	2.97
	- During holiday	1	0.50	7	3.47
	- Very rarely	15	7.43	22	10.89
	- Twice per month	7	3.47	29	14.36
	- Once per week	17	8.42	46	22.77
	- 3 – 6 times per week	84	41.58	130	64.36
	- Once per day	41	20.30	171	84.65
	- More than once per day	31	15.35	202	100.00
11	**Peanut**				
	- Never	194	96.04	194	96.04
	- Very rarely	7	3.47	201	99.50
	- Once per week	1	0.50	202	100.00

12	**Soy**				
	- Never	155	76.73	155	76.73
	- Very rarely	24	11.88	179	88.61
	- Twice per week	3	1.49	182	90.10
	- Once per week	11	5.45	193	95.54
	- 3 – 4 times per week	9	4.46	202	100.00
13	**Corn**				
	- Twice per month	2	0.99	2	0.99
	- Once per week	3	1.49	5	2.48
	- 3 – 6 times per week	17	8.42	22	10.89
	- Once per day	49	24.26	71	35.15
	- More than once per day	131	64.85	202	100.00
14	**Wheat**				
	- Never	55	27.23	55	27.23
	- Very rarely	82	40.59	137	67.82
	- Twice per month	22	10.89	159	78.71
	- Once per week	28	13.86	187	92.57
	- 3 – 6 times per week	8	3.96	195	96.53
	- Once per day	3	1.49	198	98.02
	- More than once per day	4	1.98	202	100.00
15	**Teff**				
	- Never	106	52.48	106	52.48
	- During holiday	42	20.79	148	73.27
	- Very rarely	34	16.83	182	90.10
	- Twice per month	12	5.94	194	96.04
	- Once per week	7	3.47	201	99.50
	- Once per day	1	0.50	202	100.00
16	**Sweet potato**				
	- Never	30	14.85	30	14.85
	- Very rarely	76	37.62	106	52.48
	- Twice per month	36	17.82	142	70.30
	- Once per week	44	21.78	186	92.08
	- 3 – 6 times per week	14	6.93	200	99.01
	- Once per day	2	0.99	202	100.00
17	**Potato**				
	- Never	17	8.42	17	8.42
	- Very rarely	28	13.86	45	22.28
	- Twice per month	38	18.81	83	41.09
	- Once per week	60	29.70	143	70.79
	- 3 – 6 times per week	44	21.78	187	92.57
	- Once per day	14	6.93	201	99.50
	- More than once / day	1	0.50	202	100.00

18	**Yam**				
	- Never	187	92.57	187	92.57
	- Very rarely	12	5.94	199	98.51
	- Twice per month	2	0.99	201	99.50
	- 3 – 6 times per week	1	0.50	202	100.00
19	**Enset**				
	- Never	40	19.80	40	19.80
	- During holiday	1	0.50	41	20.30
	- Very rarely	63	31.19	104	51.49
	- Twice per month	12	5.94	116	57.43
	- Once per week	40	19.80	156	77.73
	- 3 – 6 times per week	25	12.38	181	89.60
	- Once per day	13	6.44	194	96.04
	- More than once per day	8	3.96	202	100.00
20	**Kocho**				
	- Never	5	2.48	5	2.48
	- Twice per month	1	0.50	6	2.97
	- Once per week	6	2.97	12	5.94
	- 3 – 6 times per week	32	15.84	44	21.78
	- Once per day	51	25.25	95	47.03
	- More than once per day	107	52.97	202	100.00
	-				
21	**Cow milk**				
	- Never	57	28.22	57	28.22
	- During holiday	2	0.99	59	29.21
	- Very rarely	38	18.81	97	48.02
	- Twice per month	20	9.90	117	57.92
	- Once per week	38	18.81	155	76.73
	- 3 – 6 times per week	12	5.94	167	82.67
	- Once per day	18	8.91	185	91.58
	- More than once per day	17	8.42	202	100.00
22	**Goat milk**				
	- Never	118	58.42	118	58.42
	- During holiday	2	0.99	122	59.41
	- Very rarely	20	9.90	140	69.31
	- Twice per month	5	2.48	145	71.78
	- Once per week	11	5.45	156	77.23
	- 3 – 6 times per week	15	7.43	171	84.65
	- Once per day	19	9.41	190	94.06
	- More than once per day	12	5.94	202	100.0

23	**Cheese**				
	- Never	195	96.53	195	96.53
	- During holiday	2	0.99	197	97.52
	- Very rarely	4	1.98	201	99.50
	- 3 – 6 times per week	1	0.50	202	100.00
24	**Yoghurt**				
	- Never	165	81.68	165	81.68
	- During holiday	1	0.50	166	82.18
	- Very rarely	23	11.39	189	93.56
	- Twice per month	1	0.50	190	94.06
	- Once per week	5	2.48	195	96.53
	- 3 – 6 times per week	4	1.98	199	98.51
	- Once per day	1	0.50	200	99.01
	- More than once per day	2	0.99	202	100.00
25	**Butter**				
	- Never	35	17.33	35	17.33
	- During holiday	1	0.50	36	17.82
	- Very rarely	46	22.77	82	40.59
	- Twice per month	39	19.31	121	59.90
	- Once per week	53	26.24	174	86.14
	- 3 – 6 times per week	15	7.43	189	93.56
	- Once per day	8	3.96	197	97.52
	- More than once per day	5	2.48	202	100.00
26	**Whey**				
	- Never	177	87.62	177	87.62
	- During holiday	1	0.50	178	88.12
	- Very rarely	15	7.43	193	95.54
	- Once per week	3	1.49	196	97.03
	- Once per day	4	1.98	200	99.01
	- More than once per day	2	0.99	202	100.00
27	**Kale**				
	- Never	4	1.98	4	1.98
	- Very rarely	4	1.98	8	3.96
	- Once per week	2	0.99	10	4.95
	- 3 – 6 times per week	18	8.91	28	13.86
	- Once per day	62	30.9	90	44.55
	- More than once per day	112	55.45	202	100.00

28	**Cabbage**				
	- Never	35	17.33	35	17.33
	- Very rarely	58	28.71	93	46.04
	- Twice per month	22	10.89	115	56.93
	- Once per week	47	23.27	162	80.20
	- 3 – 6 times per week	35	17.33	197	97.52
	- Once per day	3	1.49	200	99.01
	- More than once per day	2	0.99	202	100.00
29	**Carrots**				
	- Never	117	57.92	117	57.92
	- Very rarely	48	23.76	165	81.68
	- Twice per week	13	6.44	178	88.12
	- Once per week	22	10.89	200	99.01
	- 3 – 6 times per week	2	0.99	202	100.00
30	**Tomato**				
	- Never	51	25.25	51	25.25
	- Very rarely	47	23.27	98	48.51
	- Twice per week	32	15.84	130	64.36
	- Once per week	41	20.30	171	85.65
	- 3 – 6 times per week	28	13.86	199	98.51
	- Once per day	3	1.49	202	100.00
31	**Mango**				
	- Never	151	74.75	151	74.75
	- Very rarely	28	13.86	179	88.61
	- Twice per week	13	6.44	192	95.05
	- Once per week	7	3.47	199	98.51
	- 3 – 6 times per week	3	1.49	202	100.00
32	**Avocado**				
	- Never	16	7.92	16	7.92
	- Very rarely	34	16.83	50	24.75
	- Twice per month	9	4.46	59	29.21
	- Once per week	60	29.70	119	58.91
	- 3 – 6 times per week	55	27.23	174	86.14
	- Once per day	21	10.40	195	96.53
	- More than once per day	7	3.47	202	100.00
33	**Papaya**				
	- Never	92	45.54	92	45.54
	- During holiday	1	0.50	93	46.04
	- Very rarely	38	18.81	131	64.85
	- Twice per month	22	10.89	153	75.74
	- Once per week	21	10.40	174	86.14
	- 3 – 6 times per week	22	10.89	196	97.03

		2	0.99	198	98.02
	- Once per day				
	- More than once per day	4	1.98	202	100.00
34	**Guava**				
	- Never	76	37.62	76	37.62
	- During holiday	2	0.99	78	38.61
	- Very rarely	60	29.70	138	68.32
	- Twice per month	21	10.40	159	78.71
	- Once per week	27	13.37	186	92.08
	- 3 – 6 times per week	11	5.45	197	97.52
	- Once per day	1	0.50	198	98.02
	- More than once per day	4	1.98	202	100.00
35	**Banana**				
	- Never	29	14.36	29	14.36
	- During holiday	1	0.50	30	14.85
	- Very rarely	37	18.32	67	33.17
	- Twice per month	24	11.88	91	44.05
	- Once per week	57	28.22	148	73.27
	- 3 – 6 times per week	38	18.81	186	92.08
	- Once per day	13	6.44	199	98.51
	- More than once per day	3	1.49	202	100.00
36	**Ferment dough before baking**				
	- No	37	18.32	37	18.32
	- Sometimes	144	71.29	181	89.60
	- Always	21	10.40	202	100.00
37	**Type of salt used**				
	- Rock salt	202	100.00	202	100.00

Table 6. Biomarkers, BMI and MUAC

No.	Characteristics	Frequency	Percent	Cumulative frequency	Cumulative percent
1	**Zinc µg/dL**				
	- < 70	174	86.14	174	86.14
	- > 70	28	13.86	202	100.0
2	**Ferritin µg/dL**				
	- < 12	42	21.6		
	- > 12	152	78.4		
3	**Hemoglobin (g/dL)**				
	- < 12.5	44	22		
	- ≥ 12.5	150	78		

4	**hsCRP (mg/L)**				
	- < 1.0	179	88.6		
	- 1.0 to 3.0	15	7.4		
	- > 3.0	8	4.0		
5	**AGP (g/L)**				
	- < 1.0	201	99.5		
	- > 1.0	1	0.5		
6	**UIE (µg/L)**				
	- < 20	46	22.8		
	- 20 to < 50	94	46.5		
	- 50 to100	55	27.2		
	- > 100	7	3.5		
7	**Goiter rate**				
	- No goiter	170	84.1		
	- Palpable goiter	29	14.4		
	- Visible goiter	3	1.5		
8	**BMI**				
	- < 16.0 (severe)	5	2.48	5	2.48
	- 16.0 – 16.9 (Moderate)	6	2.97	11	5.45
	- 17.0 – 18.4 (Mild)	39	19.31	50	24.75
	- 18.5 – 24.9 (Normal)	148	73.27	198	98.02
	- ≥25 (Overweight)	4	1.98	202	100.00
9	**MUAC**				
	- 16 – 18.4 (Moderate)	1	0.50	1	0.50
	- 18.5 – 21 (Mild)	17	8.42	8	8.91
	- > 21 (Normal)	184	91.09	202	100.00

Oklahoma State University Institutional Review Board

Date: Friday, May 08, 2009

IRB Application No   HE0922

Proposal Title: Zinc, Iron, Iodine Status and Cognitive Function in Women from Sidama Zone, Southern Ethiopia with Evaluation of New Techniques for Zinc Status Assessment in a Subsample of Women

Reviewed and   Expedited
Processed as:

Status Recommended by Reviewer(s): Approved   Protocol Expires: 5/7/2010

Principal
Investigator(s):

Tafere G. Belay	Yewelsew Abebe Kibret	Barbara Stoecker
301 HES	Hawassa University Ethiopia	421 HES
Stillwater, OK 74078	Stillwater, OK 74078	Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.
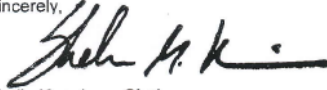
☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Beth McTernan in 219 Cordell North (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Shelia Kennison, Chair
Institutional Review Board

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The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

ቁጥር RDHE/86-87/2009
Ref. No. **1 2 JUN 2009**
ቀን................
Date

Hawassa University

Awassa, SNNPR

Re   Zinc, iron, iodine status and cognitive function in women from Sidama zone, Southern Ethiopia with evaluation of new techniques for zinc assessment in a sub sample of women

Dear Sir/Mr/s/Dr.

The National Health Research Ethics Review Committee (NERC) has reviewed the aforementioned project proposal with special emphasis on the following points

1.   Are all ethical principles considered?
       1.1 Respect for persons   Yes ☑ No ☐
       1.2 Beneficence   Yes ☑ No ☐
       1.3 Justice   Yes ☑ No ☐
2.   Are the objectives of the study ethically achievable? Yes ☑ No ☐

       Are/Is methods ethically sound?   Yes ☑ No ☐

Based on the above mentioned ethical assessment NERC has

a)   **Approved** the proposal for implementation ☑
       Expiry date of the review
                   11   Jun   2010
                   Date   Month   Year
b)   Conditionally approved   ☐
c)   Not approved   ☐

Finally we would like to take this opportunity to request your good office to maintain the highest ethical standards in the execution of the program and to monitor the ethical implementation of the project as stipulated in the project document.

With best regards,

Feleke Kibret
Secretary of NERC

cc: **Tafere G/Egziabher**
      Hawassa University
      Awassa,SNNPR

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You may Contact

ፖ.ሳ.ቁ   አዲስ አበባ ኢትዮጵያ   ስልክ   ፋክስ
B.O BOX 2490

VITA

Tafere G/Egziabher Belay

Candidate for the Degree of

Master of Science

Thesis: ZINC, IRON, IODINE AND COGNITIVE FUNCTION OF WOMEN FROM
          SIDAMA ZONE, SOUTHERN ETHIOPIA

Major Field: Nutritional Sciences

Biographical:

    Personal Data:

- Birth date: November 23/1969
- Place of birth: Wukro, Tigray, Ethiopia

    Education:

    Completed the requirements for the Master of Science in Nutritional Sciences at
    Oklahoma State University, Stillwater, Oklahoma in July, 2010.

    Completed the requirements for the Bachelor of Science in Rural Development
    and Family Sciences at Debub University, Awassa, SNNPR/Ethiopia in 2006.

    Experience:
- September 2005 to December 2007: Hawassa University, Faculty of
   Agriculture, Department of Rural Development and Family Sciences: as an
   academic staff.
- September 1999 to August 2003: Debub University, Faculty of Agriculture,
   Department of Rural Development and Family Sciences: as technical assistant.
    Professional Memberships:

- Member of the Association of College Honor Societies: KAPPA OMICRON NU
   NATIONAL HONOR SOCIETY.
- Member of the Family Guidance Association of Ethiopia.
- Member and committee member of the Professional Association of Rural
   Development and Family Science