

MULTIPLE MICRONUTRIENT DEFICIENCIES IN
ADOLESCENT SCHOOL GIRLS FROM TIGRAY,
NORTHERN ETHIOPIA

By

AFEWORK MULUGETA BEZABIH

Bachelor of Science in Chemistry

Addis Ababa University

Addis Ababa, Ethiopia

1991

Master of Science in Chemistry

Addis Ababa University

Addis Ababa, Ethiopia

1995

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Oklahoma State University

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NORTHERN ETHIOPIA

Dissertation Approved:

Dr. Barbara J. Stoecker

Dissertation Adviser

Dr. Tay S. Kennedy

Dr. Edralin A. Lucas

Dr. Mark E. Payton

Dr. A. Gordon Emslie

Dean of the Graduate College

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ABBREVIATIONS

AGP- α -1 Acid Glycoprotein

BAZ – Body Mass Index for Age Z score

BMI – Body Mass Index

CPS – Complete Primary School

EDHS – Ethiopian Demographic and Health Survey

ELISA – Enzyme Linked Immuno Sorbent Assay

FDRE – Federal Democratic Republic of Ethiopia

GDP – Gross Domestic Product

HAZ – Height-for-Age Z score

Hgb – Hemoglobin

Hct – Hematocrit

HPLC- High Pressure Liquid Chromatography

HRP – Horseradish Peroxidase

hsCRP – High Sensitivity C Reactive Protein

ICCIDD – International Council for the Control of Iodine Deficiency Disorders

ICPMS – Inductively Couple Plasma Mass Spectrometer

ID – Iron Deficiency

IDA – Iron Deficiency Anemia

IDD- Iodine Deficiency Disorders

IMR – Infant Mortality Rate

IQ – Intelligence Quotient

IRB – Institutional Review Board

IRMA – Immunoradiometric Assay

IZiNCG – International Zinc Nutrition Consultative Group

masl – meters above sea level

MMND – Multiple Micronutrient Deficiencies

MMR – Maternal Mortality Ratio
MoH – Ministry of Health (Ethiopia)
MRDR – Modified Relative Dose Response
MUAC – Mid Upper Arm Circumference
NSB – Non Specific Binding
PEM – Protein Energy Malnutrition
PCM – Protein Calorie Malnutrition
PSAC – Preschool Age Children
RAE – Retinol Activity Equivalent
RBCs- Red Blood Cells
RBP- Retinol Binding Protein
RDR – Relative Dose Response
sTfR – Soluble Transferrin Receptors
TFR - Total Fertility Rate
TGR - Total Goiter Rate
TIBC – Total Iron Binding Capacity
TPO – Thyroperoxidase
TSH – Thyroid Stimulating Hormone
UFMR – Under-five Mortality Rate
UIE – Urinary Iodine Excretion
UNICEF – United Nations Children’s Fund
VAD - Vitamin A deficiency
VADD – Vitamin A Deficiency Diseases
WEO – Wereda Education Office
WHO- World Health Organization

CHAPTER I

INTRODUCTION

Ethiopia is a tropical developing country of about 82 million with an annual growth rate of 2.7% and average family size of 4.8 (FDRE 2009). About 85% of the population lives in the rural areas and about 47% live below the poverty line (FDRE 2009). Ethiopia, with a total expenditure on health as percent of GDP of 11.6% (FDRE 2009) is characterized by low life expectancy at birth of 48 years (FDRE 2009), high infant (IMR) mortality rate of 77/1000 live births (EDHS 2006; FDRE 2009), under-five mortality rate (UFMR) of 123/1000 live births (EDHS 2006; FDRE 2009) and high maternal mortality ratio (MMR) of 871/100,000 live births (FDRE 2009) with a total fertility rate (TFR) of 5.4 births (EDHS 2006).

According to the 2005 Ethiopian Demographic and Health Survey (EDHS), the rates of stunting, underweight and wasting were 47%, 38% and 11%, respectively, in under-five Ethiopian children (EDHS 2006). The prevalence rate of anemia in under-five children and mothers was 53.5% and 26.5%, respectively (EDHS 2006). Tigray, the northern most part of Ethiopia and where this study has been conducted, has a similar trend to most of the indicators at the national level except for its highest prevalence rate of obstetric fistula (EDHS 2006), perhaps because of the common occurrence of early marriage in the area.

Protein energy malnutrition (Umeta, West et al. 2003; Haidar, Abate et al. 2005; EDHS 2006; Zerfu and Mekasha 2006; Hall, Kassa et al. 2008; Umeta, Haidar et al. 2008; Mulugeta, Hagos et al. 2009; Worku, Erko et al. 2009) and micronutrient deficiencies, particularly iodine (Woldegebriel, Demeke et al. 1993; Woldegebriel, West et al. 1993; Abuye and Urga 2000; Kidane and Woldegebriel 2006; Abuye and Berhane 2007; Abuye, Berhane et al. 2007; Bezabih, Assefa et al. 2007; Abuye, Berhane et al. 2008), iron (Woldegebriel, West et al. 1993; Adish, Esrey et al. 1999; Adish, Esrey et al. 1999; Haidar, Nekatibeb et al. 1999; Haidar, Muroki et al. 2003; Haidar and Pobocik

2009) and vitamin A (Woldegebriel, Demeke et al. 1991; Woldegebriel, Gebru et al. 1993; Woldegebriel, West et al. 1993; Haidar, Demisse et al. 1999; Haidar and Demissie 1999; Kassaye, Receveur et al. 2001; Haidar, Tsegaye et al. 2003) have long been identified as public health problems in both Ethiopian children under-five and mothers. Quite recently, zinc (Umeta, West et al. 2000; Haidar, Umeta et al. 2005; Hambidge, Abebe et al. 2006; Abebe, Bogale et al. 2008; Gibson, Abebe et al. 2008; Kassu, Yabutani et al. 2008; Stoecker, Abebe et al. 2009) has received attention. Undernutrition affects all segments of the population, especially those who are under an intense rate of growth such as children and adolescents. However, there are no reports regarding the micronutrient nutrition situation of adolescents from Ethiopia.

Adolescence, the time period between 10 and 19 years of age, is characterized by rapid growth and development next to the period of infancy (Tanner 1972; Giuseppina 2000). Such remarkable physical growth and development significantly increases needs for both macro and micronutrients (Abalkhail and Shawky 2002; Soekarjo, Pee et al. 2004). The nutrients of particular concern are vitamins A and folate and the minerals calcium, zinc, iron and iodine. Calcium needs are greater because bone mass is acquired at much higher rates during adolescence (Bonjour, Theintz et al. 1991; Slemenda, Reister et al. 1994), making adolescence a crucial time for osteoporosis prevention (Golden 2000). The need for vitamin A also increases during adolescence because of its role in gene expression (Petkovich 1992), reproduction (vanPelt and deRooij 1991; vanPelt, Morena et al. 1996) and bone growth (Djakoure, Guibourdeuche et al. 1996; Crandall 2004). Because of its role in *de novo* biosynthesis of purines and thymidylate and thus of DNA (Duthie 1999; Lin, Lin et al. 1999; Fang and Xiao 2003), folate requirements increase during adolescence.

The need for increased iron during adolescence is based on the rapid rate of linear growth, increase in blood volume, increase in lean body mass and the onset of menarche in girls (Beard, Dawson et al. 1996; Beard 2000). During adolescence, iron deficiency can also impair cognitive functioning and short term memory (Halterman, Kaczorowski et al. 2001).

Given its role in growth, particularly growth retardation (Hambidge 2000; MacDonald 2000), deficiency of zinc can pose serious physiologic challenges during

adolescence. Iodine is an essential component of thyroid hormones (Zimmermann 2009) and the thyroid gland plays a very important role in normal growth and development during adolescence (Kimball 1923). Without thyroid function, normal development during adolescence is impaired and the sex organs and breasts remain infantile and atrophic (Kimball 1923).

These concerns make the period of adolescence extremely vulnerable to the consequences of suboptimal nutrition. This state of affairs is further complicated when adolescents are exposed to frequent infections, heavy workload or increased physical activity and in girls, to physiological requirements such as menstruation and early pregnancy (Lopez and Martos 2004). Micronutrient deficiencies potentially have far-reaching consequences as they can affect performance at school and work and increase the risk of poor obstetric outcomes resulting in low birth weight babies and heightened obstetric risk throughout their reproductive life (Camilleri 1981; Harrison 1990; Steketee 2003). Children born to short, thin women are more likely to be stunted and underweight (Camilleri 1981; Harrison 1990). Suboptimal nutrition in adolescent girls leads to the birth of undernourished children thus transmitting undernutrition to future generations and continuing the intergenerational cycle of malnutrition (Naeya 1981; Harrison, Fleming et al. 1985; Brabin and Brabin 1992). Thus, serious attention needs to be given to improving the nutritional status of girls prior to conception to break the intergenerational cycle of undernutrition.

Studies from the developing world (Lwambo, Brookers et al. 2000; Shahabuddin, Talukder et al. 2000; Pawloski 2002; Assis, Prado et al. 2004; Mulugeta, Hagos et al. 2004; Friedman, Phillips-Howard et al. 2005; Leenstra, Petersen et al. 2005; Bose and Bisai 2008; Mulugeta, Hagos et al. 2009) reveal that children end their early childhood with significant nutritional deficits. Despite the availability of scientific reports on the magnitude of undernutrition from the developing world (Lwambo, Brookers et al. 2000; Shahabuddin, Talukder et al. 2000; Pawloski 2002; Assis, Prado et al. 2004; Mulugeta, Hagos et al. 2004; Friedman, Phillips-Howard et al. 2005; Leenstra, Petersen et al. 2005; Bose and Bisai 2008; Mulugeta, Hagos et al. 2009), adolescents have not been considered a high risk group for poor health and nutrition. As a result, limited health and nutrition resources have been channeled to address adolescent nutrition. This approach ignores the

fact that many health problems later in life could be improved by addressing nutrition during adolescence.

Ensuring adequate nutrition for adolescents could provide an opportunity for healthy transition from childhood to adulthood and could break the vicious cycle of intergenerational malnutrition. However, resources have traditionally been aimed at preschool age children (PSAC) and pregnant women. The relative marginalization of adolescent nutrition is astonishing considering the fact that developmental processes of adolescence exert significantly increased demands on both macro- and micronutrients (Abalkhail and Shawky 2002; Soekarjo, Pee et al. 2004).

Information regarding the nutritional status of adolescents from Ethiopia is lacking. Part of the reason for the lack of information could be lack of resources, the difficulty of interpreting anthropometric data in these age groups (deOnis and Habicht 1996) or the tendency for adolescents to be left unnoticed by the services expected to address adolescent nutrition (Kurz 1996). Information on the extent and severity of nutritional deficits of adolescents are urgently needed for prioritizing, designing and initiating intervention programs aimed at improving adolescent nutrition. The process for priority setting should start with the assessment and analysis of the situation that adolescents face in their environment.

General objective:

The general objective of the study was to determine the level, severity and determinants of protein energy malnutrition (PEM) and multiple micronutrient deficiencies (MMND) in adolescent school girls from Tigray, Northern Ethiopia.

Specific objectives:

The specific objectives of the study were to determine the

- Prevalence and severity of stunting and thinness in adolescent school girls
- Prevalence and severity of vitamin A deficiency in adolescent school girls
- Prevalence and severity of iron deficiency in adolescent school girls
- Prevalence and severity of iodine deficiency in adolescent school girls
- Prevalence and severity of zinc deficiency in adolescent school girls

Hypothesis

The null hypothesis (H_0) to achieve the above objectives is that chronic and acute deficiencies of iodine, iron, zinc and vitamin A; and stunting and thinness are not of public health significance in adolescent school girls from Tigray, Northern Ethiopia.

Significance of the study

Despite scientific evidence on both protein energy and micronutrient malnutrition in adolescents from the developing world, they have not been considered a high risk group for poor nutrition. As a result, information regarding adolescents' nutritional status is not available from Ethiopia. Nutrition related efforts in the country put much emphasis on early childhood and on pregnancy and lactation. The lack of interest in adolescent nutrition may be due in part to lack of resources, difficulty in interpreting their anthropometry or the tendency for adolescents to fall between the cracks of other services (such as maternal and child health, and reproductive health) intended for adolescents. As a result, a vital opportunity to address nutrition issues of adolescent girls prior to child bearing is missed. This research will thus play a vital role in providing data for policy makers, program planners, researchers and relevant stakeholders on adolescence, a period during which much greater dividends in terms of improved nutritional status of mothers and children could be attained with appropriate allocation of resources.

Organization of the dissertation

The dissertation is organized in such a way that it briefly introduces adolescent nutrition and the role of iodine, iron, vitamin A and zinc during adolescence followed by a review of key literature for each nutrient on adolescent nutrition and physiological functions, dietary sources, health consequences of inadequate intakes, assessment methods and the epidemiology of the micronutrient deficiency. Following the literature review is the detailed methodology section. Next are four chapters prepared as manuscripts for journal submission. In the four manuscripts, the status of iodine, iron, zinc and vitamin A nutrition in school age girls are discussed. The manuscript chapters are followed by a chapter on summary of findings and conclusions. This chapter is followed by the bibliography and the appendices. References are indicated in the text by name and date and listed at the end of the dissertation in alphabetical order of first author.

CHAPTER II

REVIEW OF LITERATURE

IRON

Iron is one of the essential trace elements for life. Its nutritional essentiality was recognized nearly a century ago (Fairbanks 1998). Iron is found in the body in two different forms, namely the functional or essential and storage forms. The functional iron serves metabolic or enzymatic function and the storage iron, primarily as ferritin and hemosiderin, is responsible for the maintenance of iron homeostasis (Dallman, Siimes et al. 1980). The functional iron mediates its physiological function through iron containing proteins including iron containing nonenzymatic proteins (hemoglobin and myoglobin), iron-sulfur enzymes, heme containing enzymes and iron containing enzymes (Beard 2001).

Physiological functions

Iron plays a vital role in binding and transport of oxygen, electron transfer reactions, gene regulation, regulation of cell growth and differentiation, immune function, energy metabolism (Beard 2001) and cognitive function (Walter, Kovalskys et al. 1983; Soemantri, Pollitt et al. 1985; Pollitt, Hathirat et al. 1989; Seshadri and Gopaldas 1989; Lozoff, Jimenez et al. 1991; Beard, Connor et al. 1993; Krebs 2000; Beard 2001; Beard 2003; Hubbs-Tait, Kennedy et al. 2007).

Dietary sources of iron

Despite its abundance in the earth's crust, iron deficiency is a common occurrence in both the developing and developed world (WHO 2001). Iron in the diet comes from contaminant iron (Bogale, Abebe et al. 2007), plants and animal sources. Much of the iron from animal products is heme iron and the iron from plant products is non-heme iron. Meat, poultry and fish are good sources of heme iron, whereas leafy green vegetables, meat, egg, legumes, and whole and enriched grains are good sources of non-heme iron.

Interaction with other micronutrients

Absorption and bioavailability of iron can be influenced by a number of factors. Trace minerals with chemical similarities can compete for uptake mechanisms or transport proteins (Sandstrom 2001). Negative effects on zinc indices have been reported after iron supplementation with doses above 18 mg/d (Breskin, Worthington-Roberts et al. 1983; Hambidge, Krebs et al. 1983; Hambidge, Krebs et al. 1987; Dawson, Albers et al. 1989; O'Brien, Zavaleta et al. 1999). Ascorbic acid has been shown to have a strong non-heme iron absorption-promoting effect by preventing the formation of insoluble and unabsorbable iron compounds and reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron, requirements for the uptake of iron into the mucosal cells (Hallberg, Brune et al. 1989). Interactions have also been recognized with retinol. Vitamin A supplementation has been shown to reduce anemia due to increased hepatic mobilization of iron (Semba and Bloem 2002).

Health consequences of iron deficiency

The main causes for failure to meet iron needs could be dietary or non dietary. Among the dietary causes for iron deficiency are inadequate intake of both heme and non-heme iron rich diets, regular consumption of high phytate plant-based meals (Craig 1994; Shaw, Chin et al. 1995), inadequate intake of iron absorption enhancers, increased physiological requirements such as menstruation (Leenstra, Kariuki et al. 2004) and frequent parasitic infections including malaria (Stoltzfus, Chwaya et al. 2000), hookworm (Stoltzfus, Chwaya et al. 1997; Stoltzfus, Dreyfuss et al. 1997; Stoltzfus, Chwaya et al. 2000), trichuriasis (Robertson, Crompton et al. 1992; Aini, Al-Mekhlafi et al. 2007) and schistosomiasis (Tjalling, Luz et al. 2006).

The iron status of individuals and populations is a function of intake, absorption efficiency, and loss (Beard, Dawson et al. 1996). When the body's requirement for iron is not met, the body iron balance is disturbed (Beard 2000). Exhaustion of iron stores results in functional consequences (Beard 2000). Anemia is among the serious health consequences due to iron deficiency (Brooker, Peshu et al. 1999). Iron deficiency anemia leads to microcytic RBCs due to a defect in hemoglobin synthesis and hypochromic RBCs due to a decreased amount of hemoglobin. As a result, transport of oxygen by blood to different cells and tissues is impaired (Provan 1999). Furthermore, iron

deficiency causes impairment in motor and cognitive development in children (Webb and Oski 1973; Walter, Kovalskys et al. 1983; Soemantri, Pollitt et al. 1985; Lozoff 1989; Pollitt, Hathiral et al. 1989; Seshadri and Gopladas 1989; Soemantri 1989; Lozoff, Jimenez et al. 1991; Andraca, Castillo et al. 1997; Halterman, Kaczorowski et al. 2001), declines in reproductive performance (Schorr and Hediger 1994; Christian, Khattry et al. 2003; Cogswell, Parvanta et al. 2003), work performance and productivity in adults (Davies, Chukwuemeka et al. 1973; Viteri and Torun 1974; Gardner, Edgerton et al. 1977; Wolgemuth, Latham et al. 1982; Cook and Lynch 1986; Marx 1997) and depressed thyroid function (Dillman, Gale et al. 1980; Martinez-Torres, Cubeddu et al. 1984). Excess beyond the daily requirement is stored as ferritin or hemosiderin.

Assessment methods

Laboratory assessments are essential for iron deficiency as it is difficult to judge iron deficiency conditions on the basis of clinical signs and symptoms. However, in areas where laboratory testing is not feasible, clinical assessments could be used to assess severe iron deficiency. Pallor of skin, conjunctiva, tongue and palms are used to formulate an impression during clinical screening for iron deficiency anemia. A number of hematologic and biochemical tests including hemoglobin, hematocrit, plasma or serum iron, total iron binding capacity (TIBC), transferrin saturation, serum ferritin, erythrocyte protoporphyrins and soluble serum transferrin receptors (sTfR) are used to characterize iron status. Hemoglobin and hematocrit levels are relatively insensitive indices as they detect only the more severe states of iron deficiency. Plasma/serum iron levels or transferrin saturation levels are useful for screening purposes, but their usefulness is compromised by the large number of factors which induce changes in plasma iron transport (Hawkins 2007). Among the different markers for iron status, plasma or serum ferritin has been the most commonly used and well accepted marker of iron body stores. One of the problems with serum ferritin, however, is that various conditions including infection, inflammation, tissue damage, malignancy or liver disease can falsely elevate its levels (Hawkins 2007). The inclusion of a marker of infection such as C-reactive protein (CRP) has been recommended to address this concern. Measurement of sTfR has also been recognized to be a sensitive index of tissue iron availability (functional iron) as they are unaffected by underlying acute or chronic infection (Ahluwalia 1998) and unlike most

other methods of iron assessment, sTfR concentration can be used to distinguish between iron deficiency anemia and other anemias (Ferguson, Skikne et al. 1992).

Table 2.1: Relative extent of iron stores on the basis of serum ferritin concentrations (WHO 2001).

Iron stores	Serum ferritin, $\mu\text{g/L}$			
	< 5 years of age		More than 5 years of age	
	Male	Female	Male	Female
Depleted iron stores	< 12	< 12	< 15	< 15
Depleted iron stores in the presence of infection	< 30	< 30	-	-
Severe risk of iron overload	-	-	>200 (adult male)	> 150 (adult female)

Methods of preventing iron deficiency

Iron deficiency represents a spectrum ranging from iron depletion to iron deficiency anemia. Among the accepted methods for preventing iron deficiency in the general public are food-based approaches (diet improvement and food fortification) and iron supplementation (WHO 2001). The food-based approach, designed to increase micronutrient intake through diet, is the preferred prevention strategy for adolescent girls except in areas where the prevalence of anemia is severe (>40%) in which additional preventive iron supplementation of 60 mg/d with 400 μg folic acid for three months is recommended (WHO 2001). Improving year round availability of micronutrient rich foods, ensuring the household access to micronutrient rich foods and changing of feeding practices with respect to micronutrient rich foods are the pillars of the food-based approach to preventing iron deficiency (WHO 2001). The food-based approaches should also focus on foods which enhance absorption or utilization of iron, simple but effective alterations in meal patterns that enhance iron absorption and home based methods of food preparation and processing which enhance the bioavailability of iron (WHO 2001; Gibson, Yeudall et al. 2003). Non-nutrition programs like environmental sanitation may also improve iron absorption through the reduction of the frequent parasitic infections (Stoltzfus, Dreyfuss et al. 1997; Brooker, Peshu et al. 1999).

Epidemiology of iron deficiency

Iron deficiency is one of the most common and widespread nutritional disorders across the globe. There are no current global figures for iron deficiency but using anemia

as an indirect indicator, the WHO (de Benoist, McLean et al. 2008) estimated that anemia affects almost a quarter (24.8%) of the world's population. The highest prevalence is in preschool age children (47.4%), the lowest prevalence is in men (12.7%) and the largest affected population group is nonpregnant women with 468 million (Table 2.2).

Table 2.2: Global anemia prevalence and number of individuals affected (de Benoist, McLean et al. 2008).

Population group	Prevalence of anemia	
	Percent	Number (million)
Preschool age children	47.4	29.3
School age children	25.4	305
Pregnant women	41.8	56
Nonpregnant women	30.2	468
Men	12.7	260
Elderly	23.9	164

WHO has classified the countries all over the world by the degree of public health significance of anemia (Table 2.3). According to this classification, anemia is not of public health significance in only three countries. According to WHO (de Benoist, McLean et al. 2008) for countries where estimates are available, there is no country in the world where anemia is not at least a mild public health problem in preschool age children, pregnant women and nonpregnant women (Table 2.3).

Table 2.3: Number of countries categorized by public health significance of anemia (de Benoist, McLean et al. 2008).

Public health problem ^a	PSAC ^b (# of countries)	Pregnant women (# of countries)	Nonpregnant women (# of countries)
None	2	0	1
Mild	40	33	59
Moderate	81	91	78
Severe	69	68	54

a: The prevalence of anemia as a public health significance is categorized as follows: < 5%, no public health problem; 5 – 19%, mild public health problem; 20 – 39%, moderate public health problem; ≥40%, severe public health problem.

b: PSAC – preschool age children.

According to the recent WHO (de Benoist, McLean et al. 2008) regional estimates for anemia in preschool age children, pregnant women, and nonpregnant women, Africa had the highest proportion of individuals affected (47.5 – 67.6%) and Southeast Asia had the greatest number of individuals (315 million) affected by anemia. This same WHO estimate (de Benoist, McLean et al. 2008) showed anemia to be a severe public health

problem in preschool age children, pregnant women and nonpregnant women from Ethiopia, though the magnitude and importance of anemia as a public health significance has been a controversial issue (Adish, Esrey et al. 1999; Haidar and Pobocik 2009).

Survey of the literature on the magnitude and severity of anemia in Ethiopia revealed mixed reports regarding the public health significance of anemia. According to a 1972 facility-based report from 100 nonpregnant women from five antenatal clinics in Addis Ababa, anemia was not a significant problem with a mean hemoglobin level of 152 ± 5 g/L (Ross 1972). Four years later, Gebre-Medhin and colleagues (Gebre-Medhin, Killander et al. 1976) reported the rarity of anemia in pregnant women in their last month of pregnancy on the basis of hemoglobin, hematocrit, total iron binding capacity, serum folate and serum vitamin B₁₂ values. In the early 1990s, Zein (Zein 1991) for the first time reported a higher prevalence of anemia (47.2%) in 6 months to 6 years old children from Northwestern Ethiopia. Two years later, a facility-based study by Desalegn (Desalegn 1993) conducted in 279 pregnant women reported 41.9% of the pregnant women from Southwestern Ethiopia to be anemic. A multicenter study conducted between 1992 and 1994 (Haidar, Nekatibeb et al. 1999) on 1449 women from 13 randomly selected sites across the country (except the northern part of the country) revealed anemia in 18.4% of 15 – 49 year old pregnant and lactating mothers. Higher rates of anemia were common in the study subjects for whom maize, milk and sorghum were their staple diets (Haidar, Nekatibeb et al. 1999). The same year, a study by Adish and colleagues (Adish, Esrey et al. 1999) on 2080 preschool age children from northern Ethiopia reported a high prevalence of anemia (42%) in under-five children from Tigray. In a subsample of 230 anemic children, 56% had a low red blood cell count and 43% had a serum ferritin less than 12 μ g/L suggesting that the anemia was largely due to iron deficiency (Adish, Esrey et al. 1999). Similarly, a high prevalence of anemia (61%) was reported by Muhe and coworkers (Muhe, Oljira et al. 2000) in their facility-based study conducted in 2540 preschool age children who visited the health center. A study on 383 pregnant women 14-49 years old visiting antenatal clinic in Hawassa, Southern Ethiopia reported 32.2% prevalence of anemia with a mean hemoglobin level of 115 ± 14 g/L (Gies, Brabin et al. 2003). Comparable mean hemoglobin level (114 ± 4 g/L) but lower prevalence of anemia (22.3%) was reported in a study by Haidar and colleagues (Haidar,

Muroki et al. 2003) in 1017 lactating women, 15-49 years old, from six urban slum communities of Addis Ababa.

According to the recent Ethiopian Demographic and Health Survey (EDHS 2006) conducted on 6141 women, anemia was detected in 26.6% of pregnant and nonpregnant women. Moreover, anemia was reported in 52.1% and 55.0% of preschool age female and male children, respectively. The same document revealed anemia in 56.5% and 29.3% of children and pregnant and nonpregnant women from Tigray, respectively. Another recent report by Umeta and colleagues (Umeta, Haidar et al. 2008), conducted in 22,861 women of reproductive age (15-49 years), reported clinical anemia in 11.3%, anemia in 30.4%, iron deficiency in 49.7% and iron deficiency anemia in 17% of women. This multicenter study from 270 clustered villages and 9 administrative regions substantiated the existence of mild to moderate iron deficiency anemia among women of reproductive age with significant geographic variation. The prevalence of anemia and iron deficiency anemia in Tigray was 25.8% and 8.3% compared to the national average of 30.4% and 17.0%, respectively (Umeta, Haidar et al. 2008). A recent work by Haidar and Pobocik (Haidar and Pobocik 2009) conducted on 970 women of reproductive age (15-49 years) revealed prevalence of 32.1% and 29.4% of iron deficiency and anemia, respectively, substantiating the public health significance of anemia in Ethiopia. Though the magnitude of anemia has been a controversial issue in Ethiopia, careful review of the literature confirmed that anemia is still a public health problem in Ethiopia. All the recent reports indicated a high prevalence of anemia in children and pregnant and nonpregnant mothers. Surprisingly, none of the studies on iron deficiency from Ethiopia have addressed the issue of iron deficiency in adolescents.

VITAMIN A AND CAROTENOIDS

Vitamin A is a fat-soluble vitamin. The term vitamin A includes a number of retinoids with different degrees of biological activity compared to all-trans-retinol. Retinoids usually consist of a β -ionone ring with a side chain composed of three isoprenoid units linked at the 6-position of the β -ionone ring (Solomons 2001). It is provided in the diet in two forms, namely preformed vitamin A (retinoids) and provitamin A (carotenoids). Vitamin A exists in the form of an alcohol (retinol), an

aldehyde (retinal), an acid (retinoic acid) and an ester (retinyl palmitate). Over 600 carotenoids have been identified but the principal carotenoids found in human plasma are β -carotene, α -carotene, β -cryptoxanthin, lutein and lycopene (Bendich and Olson 1989).

Physiological functions of vitamin A

Vitamin A is essential for numerous metabolic processes including vision (Wald 1967; WHO 2009), growth (Djakoure, Guibourdeuche et al. 1996; WHO 2009), epithelial integrity (Wolbach and Howe 1925; WHO 2009), red blood cell production (WHO 2009), immunity (Semba 1999; Stephensen 2001; WHO 2009), reproduction (van Pelt and de Rooij 1991; van Pelt, Morena et al. 1996; WHO 2009), regulation of gene expression (Petkovich 1992) and embryonic development (Zile 2001).

Dietary sources

The *de novo* synthesis of vitamin A is not possible in humans and animals (Blomhoff and Blomhoff 2006). Thus, it must be provided from the diet in sufficient quantities to meet physiologic needs. Dietary vitamin A from animal sources or fortified foods is consumed as preformed retinyl esters (Roos, Islam et al. 2003). Excellent sources of preformed vitamin A are animal products such as milk, liver, butter, egg and cod liver oil (Roos, Islam et al. 2003). Good sources of provitamin A include dark green leafy vegetables, deeply colored yellow and orange vegetables and fruits sources (Mangles, Holden et al. 1993; Castenmiller and West 1998). Among the various provitamin A carotenoids, β -carotene is the most common and bioavailable (Bauernfeind 1972; Castenmiller and West 1998). The provitamin A dietary carotenoids such as β -carotene, α -carotene and others are obtained from plant sources (Mangles, Holden et al. 1993; Castenmiller and West 1998). For the vast majority of people from the developing world, the consumption of foods containing preformed vitamin A is limited and provitamin A carotenoids are the major sources of dietary vitamin A (Engleberger, Darnton-Hill et al. 2003). Infants are the exception because breast milk is an excellent source of the highly bioavailable retinyl ester (palmitate) (Haskell and Brown 1999).

Bioconversion of carotenoids to retinol

Apart from their antioxidant roles, the carotenoids are particularly useful nutritionally if they are converted to retinol. But, their bioconversion into retinol is a complex process influenced by numerous factors including the species of the carotenoid

and molecular linkage; amount of carotenoid consumed in a meal; the source food matrix; enhancers or inhibitors of digestion, absorption and bioconversion; nutritional status; genetic makeup and health status of the host; and nutrient interactions (Castenmiller and West 1998; West and Castenmiller 1998; West, Eilander et al. 2002; Blomhoff and Blomhoff 2006).

One β -carotene molecule can theoretically give rise to two molecules of retinol. In α - and γ -carotene as well as cryptoxanthin, one of the rings differs from that of retinol so these carotenes are not as active as β -carotene in the formation of retinol. Such observations have led to the recent equivalency changes on intestinal carotenoid-to-retinol conversion ratio of 6:1 to 12:1 (Trumbo, Yates et al. 2001), a conversion efficiency that is about half of that previously thought. Based on these observations, it is now generally assumed that 1 retinol activity equivalent (1 RAE) is equal to 1 μg of dietary or supplemental preformed vitamin A (i.e. 1 μg retinol), 2 μg of supplemental β -carotene, 12 μg of dietary β -carotene or 24 μg of other dietary provitamin A carotenoids such as α -carotene and β -cryptoxanthin (Trumbo, Yates et al. 2001). Conversion of provitamin A carotenoids to all trans-retinol is postulated to occur through central and eccentric or random cleavage in the intestinal wall. Oxidative central cleavage at the 15-15' double bond by 15-15'dioxygenases yields two moles of retinol per mole of β -carotene and the eccentric or random cleavage provides only one mole of retinol per mole of β -carotene (Parker 1997). Carotenoids are included in the vitamin A family because of their biological activity, which in turn is due to their un-substituted β -ionone ring. The β -ionone ring is essential for activity and when it is absent or altered structurally, the compound loses its biological activity. Thus, carotenoids such as lycopene do not possess vitamin A activity and carotenoids such as β -cryptoxanthin with one hydroxylated β -ionone ring have approximately 50% of the biological activity of β -carotene. Hydroxylation of both β -ionone rings, as in the case of lutein results in a complete loss of vitamin A activity (Eitenmiller and Landen 1999).

Assessment methods

Quite large numbers of procedures have been employed to evaluate vitamin A status in populations, namely serum or plasma retinol or retinol-binding protein (RBP) concentrations (Pitt 1981; Underwood 1990), relative dose response (RDR) measurement

(Amedee-Manesme, Anderson et al. 1984; Amedee-Manesme, Mourey et al. 1987; Olson 1991), modified relative dose response (MRDR) measurement (Tanumihardjo, Koellner et al. 1990; Tanumihardjo and Olson 1991), isotope dilution analysis (Furr, Amedee-Manesme et al. 1989), clinical assessment (WHO 1976), conjunctival impression cytology (Gadomski, Kjolhede et al. 1989; Kjolhede, Gadomski et al. 1989; Reddy, Rao et al. 1989) and liver biopsy (Underwood, Siegel et al. 1970; Olson, Gunning et al. 1984). However, the clinical assessment and biochemical measurement of serum or plasma retinol or RBP have been the commonly used procedures to evaluate the vitamin A status in population groups. Though the uneven distribution of vitamin A in the liver leaves doubt as to the usefulness of liver biopsy (Olson, Gunning et al. 1979), it has been considered as a direct measure of vitamin A stores since the liver is the major storage site of vitamin A in the body (90%) (Underwood, Siegel et al. 1970; Olson, Gunning et al. 1984). However, the use of liver biopsy has been limited due to the invasiveness and impracticality for routine use in population studies.

Methods including the RDR assay and the MRDR assay have been developed (Loerch, Underwood et al. 1979) and validated as indirect techniques of estimating liver vitamin A reserves and evaluating an individual's vitamin A status (Amedee-Manesme, Anderson et al. 1984; Amedee-Manesme, Mourey et al. 1984; Flores, Campos et al. 1984; Amedee-Manesme, Mourey et al. 1987; Amatayakul, Underwood et al. 1989; Tanumihardjo, Koellner et al. 1990; Olson 1991; Tanumihardjo and Olson 1991). The tests are based on the observation that during vitamin A deficiency with diminished stores of vitamin A, apo-retinol-binding protein (RBP) accumulates several fold in the liver. A dose-response test usually involves a baseline measurement, followed by an oral challenge dose of the nutrient in question and a second measurement after a specified time interval (Underwood 1990). If a low plasma retinol concentration is due to lack of retinyl esters reserves in the liver, the relative dose response is high; if the low plasma concentration is due to other factors, giving extra retinol will have little effect. As its name implies, the MRDR is a modification of the RDR and requires no baseline measurement but only the oral challenge dose. However, the RDR and MRDR are prone to potential interferences including severe protein deficiency which could interfere by decreasing liver synthesis of the rapidly turning over apo-retinyl-binding protein

(Morrow, Guerreo et al. 1990; Solomons, Morrow et al. 1990); infection (Campos, Flores et al. 1987) which may decrease retinol-binding protein levels and liver diseases (Mobarhan, Russel et al. 1981) that impair apo-retinol binding protein formation.

Interaction with other nutrients

The absorption, metabolism or excretion of vitamin A can be influenced by interactions with other nutrients such as vitamin E (McLaren and Frigg 2001), iron (Suharno, West et al. 1993; Strube, Beard et al. 2002), zinc (Brown, Chan et al. 1976; Sundaresan, Cope et al. 1977; Boron, Hupert et al. 1988; McLaren and Frigg 2001; Sang and Sung 2003), copper (Rachman, Conjat et al. 1987), lipids (Lietz, Henry et al. 2001; Ribaya-Mercado 2002) and proteins (Reddy, Mohanram et al. 2008). Vitamin E protects vitamin A from oxidation inside the cell and the intestinal lumen (McLaren and Frigg 2001). During iron deficiency, hepatic mobilization of retinol is impaired (Strube, Beard et al. 2002). Zinc deficiency results in a decreased synthesis of retinol binding protein (Boron, Hupert et al. 1988) and elevated levels of hepatic vitamin A due to decreased retinol degradation (Boron, Hupert et al. 1988). Copper deficiency compromises the hepatic mobilization or transport of vitamin A from liver to blood (Rachman, Conjat et al. 1987). Lipids are essential for dissolving and facilitating the absorption of vitamin A and its precursors from the intestine into the enterocytes and their transport to the liver (Ribaya-Mercado 2002). The transport of vitamin A from the liver through the circulation to the target tissues is accomplished with RBP. During protein deficiency, the synthesis of RBP is reduced and this might compromise the hepatic mobilization of vitamin A into the general circulation and to target tissues further aggravating vitamin A deficiency (Reddy, Mohanram et al. 2008).

Health consequences of vitamin A deficiency

Vitamin A deficiency (VAD) occurs when intake of preformed or provitamin A is inadequate to meet the body's needs (WHO 2009). Inadequate intake of vitamin A during the nutritionally demanding periods of life such as infancy, childhood, adolescence, pregnancy and lactation greatly raises the risk of health consequences or vitamin A deficiency disorders (VADD) (Sommer, Tarwotjo et al. 1986; Zachman 1989; Humphrey, West et al. 1992; WHO 2009). A grave consequence of VAD is the development of xerophthalmia (xeros = dryness; ophthalmia = pertaining to the eye), the

leading cause of preventable blindness in children (WHO 1992; Sommer 1995; Sommer 2008; WHO 2009). The term xerophthalmia encompasses “the clinical spectrum of ocular manifestations of VAD, from milder stages of night blindness and Bitot’s spots, to potentially blinding stages of corneal xerosis, ulceration and necrosis (keratomalacia)”(Sommer 1995). Vitamin A deficiency also increases the risk of disease and death from severe infections (Hussey and Klein 1990; Humphrey, West et al. 1992; WHO 2009).

Epidemiology of vitamin A deficiency

Vitamin A deficiency disorders (VADD) are public health consequences attributable to vitamin A deficiency (Sommer and Davidson 2002). Vitamin A deficiency is a leading cause of preventable childhood blindness (WHO 1992; Sommer 1995; Sommer 2008; WHO 2009), and morbidity and mortality (Glasziou and Mackerra 1993) among preschool age children and is an increasingly recognized problem among women of reproductive age in many developing countries (West 2002; Ahmed, Azim et al. 2003; Gorstein, Shrestha et al. 2003; Semba, dePee et al. 2003).

According to a recent report from WHO (WHO 2009), vitamin A deficiency is of public health significance in 122 countries. WHO (WHO 2009) estimates that one third (190 million) of the world’s preschool children and 15.3% (19.1 million) of pregnant women are vitamin A deficient with the highest burden being from Africa and Southeast Asia (Table 2.4).

Table 2.4: Global prevalence of vitamin A deficiency in preschool age children and pregnant women (WHO 2009).

WHO region	PSAC*		Pregnant women	
	Prevalence (%)	# affected (million)	Prevalence (%)	# affected (million)
Africa	44.4	56.4	13.5	4.18
Americas	15.6	8.68	2.0	0.23
Southeast Asia	49.9	91.5	17.3	6.69
Europe	19.7	5.81	11.6	0.72
Eastern Mediterranean	20.4	13.2	16.1	2.42
Western Pacific	12.9	14.3	21.5	4.90
Global	33.3	190	15.3	19.1

*PSAC: Preschool age children.

Ethiopia, the second most populous country in Africa, would have obviously contributed to the high burden of VAD in Africa. Various studies have investigated the vitamin A situation in the fetus (Gebre-Medhin and Vahlquist 1984), children (Lindtjorn 1983; De Sole, Belay et al. 1987; Woldegebriel, Demeke et al. 1991; Woldegebriel, Gebru et al. 1993; Lemma and Mariam 1996; Tafesse, Fisseha et al. 1996; Zerihun and Mabey 1997; Haidar and Demissie 1999; Getaneh, Assefa et al. 2000; Kassaye, Becklake et al. 2001; Kassaye, Receveur et al. 2001; Asrat, Omwega et al. 2002; Haidar, Tsegaye et al. 2003; Kello and Gilbert 2003; Semba, Pee et al. 2008), pregnant women (Wondimkun 2002; Wondimkun 2005) and adults (Kassu, Andualem et al. 2007) from Ethiopia and confirmed its public health significance.

According to the 1980-1981 stratified multistage cluster sampling national survey (Woldegebriel, Demeke et al. 1991), conducted on a total of 6636 children, aged 6 months to 6 years, xerophthalmia was observed in 1.0% of the preschool age children with the highest prevalence (1.6%) in children from the pastoral areas. On the basis of the biochemical indicators, 16% of the children were deficient ($<0.35 \mu\text{mol/L}$) and 60% had low ($< 0.70 \mu\text{mol/L}$) vitamin A serum levels with mean serum retinol value of $0.62 \mu\text{mol/L}$. Two years later, Lindtjorn (Lindtjorn 1983) reported xerophthalmia in 0.02% of 406 (age 2 – 10 years) children from Gardula, Southern Ethiopia. Gebremedhin and Vahlquist (Gebre-Medhin and Vahlquist 1984) compared vitamin A nutrition in human fetuses post mortem and new born infants from Swedish and Ethiopian women and found significant difference between the vitamin A concentration in the liver of the Swedish ($37 \mu\text{g/g}$, $n = 39$) and Ethiopian ($9.1 \mu\text{g/g}$, $n = 49$) fetuses. However, the retinol binding protein levels of the Swedish and healthy Ethiopian newborns were comparable (18.6 mg/L). De Sole and colleagues (De Sole, Belay et al. 1987) surveyed a cluster sample of households including 2647 children, aged 6 months to 6 years, from Southeast Ethiopia and reported hyperendemic VAD linked to mono-crop farming and higher prevalence of mild xerophthalmia in males than females. Moreover, higher incidence of measles and prevalence of diarrhea and respiratory diseases in vitamin A deficient children were reported. A study conducted in 1988 in a rural village from Eastern Ethiopia (Woldegebriel, Gebru et al. 1993) on a total of 240 children reported perhaps the most severe ever recorded xerophthalmia prevalence of 48.3% of the 12 years and younger

children. Based on the biochemical indicators of vitamin A, 30.2% were deficient ($< 0.35 \mu\text{mol/L}$) and 88.2% had low vitamin A levels ($< 0.7 \mu\text{mol/L}$) with low median serum retinol binding protein levels. The extreme levels could be due to the high dependence on food aid because of consistent drought six years prior to the study period.

Between 1996 and 1997, Haidar and colleagues (Haidar, Tsegaye et al. 2003) assessed the impact of vitamin A supplementation on child mortality and nutritional status of 4770 children, aged 6-72 months, from Northern Ethiopia. Vitamin A capsule coverage was 87% in their study communities. Pre and post intervention comparison on selected variables indicated that vitamin A capsule supplementation had resulted in a significant reduction in the prevalence of Bitot's spots, fever, diarrhea, edema, measles, conjunctivitis, stunting, wasting and underweight and a significant increase in mean serum retinol levels (from 36.8 to 56.2 $\mu\text{g/dL}$).

The 1993 community-based cross-sectional study (Lemma and Mariam 1996) from eight kebeles of Southwest Ethiopia in 434 randomly selected children (6 months to 6 year old) reported xerophthalmia in 6.2% of the preschool age children. Clinical examination and biochemical indicators of 147 children (6 months to 6 year old) from Dodota district, Central Ethiopia (Tafesse, Fisseha et al. 1996), revealed night blindness in 17%, Bitot's spot in 26.5%, corneal ulceration in 2.7% and corneal scars in 0.7%. On the basis of biochemical levels of retinol, 31.9% of the children had $< 0.35 \mu\text{mol/L}$ and 80.8% had $< 0.7 \mu\text{mol/L}$. Umeta and coworkers (Umeta, West et al. 2003) conducted a study in 1996 and reported a high rate of night blindness (20%) in 5 to 11 months old infants from the same study area confirming the public health significance of vitamin A deficiency in the community. Clinical assessment by Zerihun and Mabey (Zerihun and Mabey 1997) of 7423 preschool age children from Southwest Ethiopia indicated that 0.9% of the preschool age children had signs of VAD. According to the reports from a community based study by Haidar and Demissie (Haidar and Demissie 1999) on 15087 preschool children, aged 6 to 71 months, the highest burden of vitamin A deficiency was children between 36 to 72 months of age. When their data were further disaggregated by sex, higher prevalence rates of xerophthalmia were observed in males (53%) than females (26%). Getaneh and colleagues (Getaneh, Assefa et al. 2000) studied dietary practices towards vitamin A rich diets and prevalence of xerophthalmia in 831 preschool children

from Southwest Ethiopia and reported inadequate intake of vitamin A rich foods in 92% of the children with a relatively low prevalence of xerophthalmia (0.6%). Another cross-sectional study by Asrat and colleagues (Asrat, Omwega et al. 2002) on preschool and school age children from Southeast Ethiopia reported higher rates of night blindness (7.2%) and xerophthalmia (3.4%). In this study, school age children were more highly affected by xerophthalmia than preschool age children and overall 51% of the children had serum retinol levels below 20 µg/dL.

Vitamin A deficiency was not only a problem of children but also of adolescents. According to the study by Asrat and coworkers (Asrat, Omwega et al. 2002), the prevalence of xerophthalmia was higher in school-aged children than pre-school children. Another study (Woldegebriel, West et al. 1993) on 14740 school age children from seven study sites from Central Ethiopia reported Bitot's spots in 0.91% of the children indicating that vitamin A deficiency was a public health problem in school age children as well. Kassaye and coworkers (Kassaye, Receveur et al. 2001) conducted a cross-sectional study on 1339 school age children (6 – 9 years of age) from Northern Ethiopia and reported a prevalence of xerophthalmia in 5.8% of the children. About 8% and 60% of the children had serum retinol levels below 0.35 µmol/L and 0.7 µmol/L, respectively. The results revealed that 41% of the children had low liver vitamin A reserves (MRDR ratio ≥ 0.06). According to the ocular examination studies by Kello and Gilbert (Kello and Gilbert 2003) on 360 blind school children from schools for the blind in Ethiopia, VAD and measles were the major causes of severe visual impairment /blindness in these schools.

A facility-based study by Wondimkun (Wondimkun 2005), designed to evaluate serum retinol status of 324 pregnant women in their third trimester from Northwest Ethiopia, reported serum retinol levels lower than 0.52 and 1.05 µmol/L in 2.7% and 38.5% of the pregnant women, respectively. Their mean serum retinol level was 1.23 µmol/L. Similarly, Wondimkun (Wondimkun 2002) investigated the dark adaptation pattern of 96 (48 cases and 48 controls) pregnant women in his case control study and found a strong association between dark adaptation and serum retinol levels where vitamin A deficient pregnant women had impaired dark adaptation. The mean serum retinol concentrations of cases and controls were 23.35 and 40.47 µg/dL, respectively.

A study conducted to investigate vitamin A deficiency in adult patients with diarrhea and HIV infection from Northwest Ethiopia (Kassu, Andualem et al. 2007) revealed the common occurrence of VAD in adults. VAD was prevalent in 13% of healthy adults (mean = 1.52 $\mu\text{mol/L}$), 29.3% of asymptomatic HIV infected subjects (mean = 0.96 $\mu\text{mol/L}$), 52.7% of diarrheic HIV positive patients (mean = 0.82 $\mu\text{mol/L}$) and 45.5% of diarrheic HIV negative patients (mean = 0.84 $\mu\text{mol/L}$).

The public health significance of vitamin A deficiency has been confirmed by the recent nationally representative survey (EDHS 2006) on 14,500 households. Night blindness and adjusted night blindness (adjusted for daytime visual problem) prevalence in 15 to 49 years old women was 22.1% and 6.1%, respectively. The highest prevalence of both xerophthalmia and adjusted xerophthalmia was reported from women in Tigray and the lowest from women in Addis Ababa (8.30 vs. 0.90%).

IODINE

Iodine was second to iron to be recognized as an essential trace element for health (Saubert 1999). It was discovered by a saltpeter manufacturer in 1811 in France (Zimmermann 2009). Iodine has been used in the treatment of goiter since 1820 (Kimball 1923), but its deficiency was shown to be the causative agent for thyroid enlargement in 1917 (Zimmermann 2009). Iodine was recognized to be an essential component of the thyroid in 1895 for the first time (Kimball 1923), and nowadays it is well accepted that iodine is an integral constituent of the thyroid hormones, namely 3,5,3',5'-tetraiodothyronine (thyroxine, T_4) and 3,5,3'-triiodothyronine (T_3) (Zimmermann 2009). Public health measures for the prevention of thyroid enlargement (goiter) began among school girls in Akron, Ohio, USA between 1916 and 1920 (Kimball 1923). Three decades later, studies conducted in Papua, New Guinea helped goiter and cretinism to gain renewed attention (Semba and Delange 2008). To encompass the wide spectrum of the effect of suboptimal iodine nutrition on health, including physical impairment and mental retardation, the term Iodine Deficiency Disorders (IDDs) was introduced by Hetzel in 1983 (Hetzel 1983).

Physiological functions of iodine

Iodine is an essential micronutrient for the biosynthesis of thyroid hormones produced by the thyroid gland (Zimmermann 2009). Thyroid hormones are essential for maintaining the body's metabolic rate by controlling cellular energy production and oxygen consumption, for normal growth and for neural and sexual development (Ristic-Medic, Piskackova et al. 2009).

Dietary sources of iodine

The richest dietary sources of iodine are seafood, seaweed and iodized salt (Zimmermann 2009). Foods of animal origin including meat and milk can also constitute a significant source of iodine if animals have grazed on iodine sufficient soils. Similarly, crops from iodine sufficient soils may supply some dietary iodine. Iodine fortified foods like bread and milk are also significant sources of dietary iodine.

In countries like Ethiopia, where the staple diets are of plant origin and marine foods are rarely consumed, the use of iodized salt for cooking and at the table constitutes a viable source of iodine. Most of the Ethiopian cereals grow in iodine deficient soils. Soils in the highlands are believed to be low in iodine as it is leached out of the soil due to its solubility in water. If soil is deficient in iodine, so are the plants grown in it, including the grains and vegetables consumed by people and animals. So, inadequate intake of foods of marine origin and residence in places where soil and water are poor in iodine, exacerbated by other micronutrient deficiencies, might be the most important risk factors for IDD in Ethiopia.

Interaction with other nutrients

Deficiencies of selenium (Zimmermann and Kohrle 2002; Schomburg and Kohrle 2008), iron (Zimmermann, Adou et al. 2000; Zimmermann 2002; Zimmermann 2006) and vitamin A (Zimmermann, Jooste et al. 2007; Zimmermann 2007) have been implicated in exacerbating iodine deficiency. Accumulated peroxides may damage the thyroid gland during selenium deficiency due to impaired production of the selenium-dependent glutathione peroxidase (Zimmermann and Kohrle 2002; Schomburg and Kohrle 2008). Moreover, selenium deficiency impairs thyroid hormone metabolism as the monodeiodination of T_4 into T_3 is catalyzed by selenium-dependent enzymes called

iodothyronine deiodinases (Kohrle 1999; Zimmermann and Kohrle 2002; Kohrle, Jakob et al. 2005).

Iron deficiency reduces the iron-dependent hemoprotein thyroperoxidase (TPO), an enzyme which catalyzes the oxidation of iodine and its substitution for hydrogen in the tyrosine residues and the H₂O₂ dependent generation of the iodothyronines (Song, Driessens et al. 2007; Schomburg and Kohrle 2008). Vitamin A deficiency activates thyroid stimulating hormone (TSH) and increases risk for goiter through decreased vitamin A mediated suppression of the pituitary TSH β gene (Zimmermann, Wegmuller et al. 2004; Zimmermann, Jooste et al. 2007).

Foods of plant origin contain goitrogens which interfere with the thyroidal uptake of iodine. Glucosinolates and their metabolites from cruciferous vegetables and thiocyanates which are the metabolites of cyanogenic glucosides from cassava, sorghum and others can aggravate iodine deficiency by competing with iodine for thyroidal uptake (Ermans, Delange et al. 1972; Laurberg, Nohr et al. 2004; Messina and Redmond 2006; Zimmermann 2009). Moreover, humic substances from unclean drinking water are reported to interfere with thyroidal iodination (Gaitan 1990).

Assessment methods

Various methods are available to assess iodine deficiency but TSH, total goiter rate (TGR), thyroglobulin (Tg) and urinary iodine excretion (UIE) are generally recommended (Zimmermann 2009). TSH provides an indirect measure of iodine status. Goiter rate reflects chronic (months to years) suboptimal iodine nutrition and Tg is a good indicator for an intermediate response (weeks to months) (Zimmermann 2009). UIE is a sensitive marker of recent iodine intake (days) as more than 90% of dietary iodine is excreted through urine (Vought and London 1967). UIE can be measured in spot urine samples of children or adults provided that a sufficient number of specimens are collected (WHO 2007). Diurnal intakes of iodine and thus spot UIE are highly variable (Andersen, Karmisholt et al. 2008). Therefore, to estimate iodine intakes in individuals, 24 h urine collections are preferable. When collections of 24 h samples are operationally difficult, an alternative is to use the age and sex adjusted iodine:creatinine ratio in adults but this also has limitations (Knudsen, Christiansen et al. 2000). Creatinine may be unreliable for

estimating daily iodine excretion from spot samples especially in malnourished subjects where creatinine concentration is low.

Health consequences of iodine deficiency

Inadequate intake of iodine leads to iodine deficiency disorders (IDD). The term IDD encompasses all consequences of IDD which can be prevented by optimal iodine nutrition (ICCIDD 2001). The most damaging effect of inadequate intake of iodine is on the developing brain (Pharoah, Buttfield et al. 1971; Escobar, Obrego'n et al. 2007; Glinioer 2007; Zimmermann 2007). Cretinism is an extreme form of neurological damage due to severe iodine deficiency or fetal hypothyroidism (Chaouki, Maoui et al. 1988; Zimmermann 2009). A meta analysis of 18 studies by Bleichrodt and Born (Bleichrodt and Born 1994) has shown that iodine deficiency alone lowered mean IQ scores by 0.9 SD or 13.5 IQ points. In addition to its impact on brain and intellectual development, iodine deficiency can induce thyroid enlargement at any period in life. Goiter reflects an attempt of the thyroid gland to adapt to increased need to produce thyroid hormones (Semba and Delange 2008) and is said to be endemic when more than 5% of the 6 – 12 years old school children have an enlarged thyroid as assessed by the WHO criteria (ICCIDD 2001). Moreover, it can cause impaired reproductive outcomes (Pharoah, Buttfield et al. 1971; Pharoah, Ellis et al. 1976; Chaouki and Benmiloud 1994; DeLong, Leslie et al. 1997), child mortality (Pharoah, Buttfield et al. 1971; Pharoah, Ellis et al. 1976; DeLong, Leslie et al. 1997) and a high degree of apathy and reduced work productivity (Hetzl 1983) in the adult population living in severely iodine deficient areas, leading to economic stagnation of communities.

Epidemiology of iodine deficiency disorders

Globally, iodine deficiency still remains a public health problem in many countries (de Benoist, McLean et al. 2008). Two billion people are estimated to be at risk of iodine deficiency disorders due to suboptimal iodine nutrition (de Benoist, McLean et al. 2008). Based on the survey data from 192 WHO member states, 36.5% (285.4 million) of school age children are at risk of iodine deficiency (Table 2.5). The largest numbers of school age children with low iodine intake are from Southeast Asia (96 million) and Africa (50 million). The highest proportions of school age children with

inadequate iodine intake are from Europe (60%) and the Eastern Mediterranean (55%) (Table 2.5).

Table 2.5: Proportion of population and number of individuals with insufficient iodine intake in school age children (6-12 years) and in the general population (all age groups) by WHO region, 2003 (de Benoist, McLean et al. 2008).

WHO region ^a	Insufficient iodine intake (UIE < 100 µg/L)			
	School age children		General population	
	Proportion (%)	Total number (Millions) ^b	Proportion (%)	Total number (millions) ^b
Africa	42.3	49.5	42.6	260.3
Americas	10.1	10.0	9.8	75.1
Southeast Asia	39.9	95.6	39.8	624.0
Europe	59.9	42.2	56.9	435.5
Eastern Mediterranean	55.4	40.2	54.1	228.5
Western Pacific	26.2	48.0	24.0	365.3
Total	36.5	285.4	35.2	1988.7

a: 192 WHO Member states.

b: Based on population estimates in the year 2002.

Various epidemiological studies have shown the public health significance of IDD in Ethiopia (Woldegebriel, West et al. 1993; Abuye and Urga 2000; Cherinet and Kelbessa 2000; Takele, Belachew et al. 2003; Abuye and Berhane 2007; Bezabih, Assefa et al. 2007). In Ethiopia, surveys on iodine deficiency have been carried out since 1903 (Nekatebeb 1993) but the earliest report about IDD was published in 1976 (NSEID 2005). According to this report, area specific goiter was indicated to be as high as 71% (NSEID 2005). The country is still one of the most severely iodine deficient countries in the world. About three quarters of the population was estimated to be at risk for IDD in 1995 (MOH 1995). According to the joint report by the Ministry of Health (MOH) of the Federal Democratic Republic of Ethiopia (FDRE) and United Nations Children's Fund (UNICEF) (MOH 1995), 78% (42 million) of the total population were at risk of iodine deficiency. Moreover, 62% (35 million) were iodine deficient and 26% (14 million) had goiter. At least one in 1000 people had cretinism and iodine deficiency contributed to about 50,000 prenatal deaths annually (MOH 1995).

Iodine deficiency has been increasing and getting worse in Ethiopia (Abuye, Berhane et al. 2008) despite the fact that an effective prevention strategy is known. According to the recent press release from the Ethiopian Ministry of Health (MOH 2009),

less than 5% of Ethiopian households utilize adequately iodized salt. It was further pointed out in this press release that the estimated productivity loss due to the negative impact on health, poor physical growth, compromised intellectual capacity and lower educational attainment associated with IDD in Ethiopia will be 64 billion ETB (Ethiopian currency) between 2006 and 2015 (MOH 2009). An accompanying press release from the Micronutrient Initiative (MI), Ethiopia, indicated that severe iodine deficiency in Ethiopian women leads to 50,000 stillbirths annually and that the country's goiter rate has worsened from 26% in the 1980s to about 40% today (MI 2009).

The first nationally representative IDD survey from Ethiopia was conducted in 1981-82 (Woldegebriel, Demeke et al. 1993). This household and school-based survey from 38 provinces (including 19,158 households and 35,635 school children) found a total goiter rate (TGR) of 36.1% in females and 25.1% in males (Woldegebriel, Demeke et al. 1993).

A cross-sectional study carried out in the late 1990s on 2485 school children from 10 villages across four regions reported that 53.3% of the children had goiter while based on UIE, 70% had moderate and 30% had mild iodine deficiency (Abuye and Urga 2000). Goiter was more common in girls (56.1%) than in boys (50.8%) and children from Kodowono had the highest (91%) and those from Abossara had the lowest (31%) goiter rate (Abuye and Urga 2000). In a school-based cross-sectional study of 1044 students, aged 6 – 15 years, from Southwestern Ethiopia, 27.4% of the children had goiter (Berhanu, Woldemichael et al. 2004). According to a report by Kidane and Woldegebriel (Kidane and Woldegebriel 2006) from Tigray, Northern Ethiopia, goiter was rampant and epidemiological estimates of other IDD manifestations were unacceptably high. Of the 755 subjects examined for goiter, 59.5% of the males and 80.2% of the females had goiter. The authors have estimated a 71.4% community prevalence of goiter from an epidemiological model (Kidane and Woldegebriel 2006).

Recently, Abuye and colleagues (Abuye, Berhane et al. 2007) reported a weighted total goiter prevalence rate of 39.9% in children 6 to 12 yrs of age. Similarly a recent community-based cross-sectional study among 15-49 years old Ethiopian women revealed a total goiter prevalence of 35.8% with 24.3% palpable and 11.5% visible goiter (Abuye and Berhane 2007).

ZINC

Zinc is the second most ubiquitous metal in biological systems after iron and, because of its abundance, nutritional deficiencies were considered improbable (Walravens 1979). Evidence of the essentiality of zinc in rats was demonstrated in 1934 (King and Keen 1999) and its significance in human nutrition and public health was recognized in the 1960s, when the consumption of diets with low zinc bioavailability was associated with "adolescent hypogonadism and nutritional dwarfism" from the Middle East (Prasad, Halsted et al. 1961; Prasad, Schalert et al. 1963; Sandstead, Prasad et al. 1967). The first recognition of its role in biochemistry was the discovery in 1940 (Walravens 1979) that it was an essential component of carbonic anhydrase, a key enzyme that catalyzes the formation of bicarbonate from carbon dioxide and water. The next zinc requiring enzyme to be discovered in 1955 was carboxypeptidase A and several hundred zinc-requiring enzymes have been found since then (Coleman 1992). Zinc is an essential component of the catalytic site of at least one enzyme from each of the six classes of enzymes (Rink and Gabriel 2000). Zinc is required by the largest number of proteins in biological systems and its binding proteins make up nearly 10% of the proteome of eukaryotic organisms (Andreini, Banci et al. 2006; Bertini and Rosata 2007).

Physiological functions

Zinc is an essential trace element for all forms of life. It is involved in a number of metabolic actions in biological systems including growth (Gibson 1993; Brandao-Neto, Stefan et al. 1995; Nishi 1996; Gibson, Manger et al. 2007), immunity (Sazawal, Jalla et al. 1997; Lesourd, Mazari et al. 1998; Shankar and Prasad 1998; Solomons 1998; Salgueiro, Zubillaga et al. 2000), reduction in morbidity (Sazawal, Black et al. 1996; Rosado, Lopez et al. 1997; Bhutta, Black et al. 1999; Bhutta, Bird et al. 2000), pregnancy (Hambidge, Krebs et al. 1983; Hambidge, Krebs et al. 1987), reproduction (Prasad, Schalert et al. 1963; Favier 1992), appetite (Safai-Kutti 1990; Lee, Rains et al. 1998), protection of structural and functional integrity of biological membranes (Conte, Narindrasorasak et al. 1996; Kelly, Quaife et al. 1996), behavior and brain function (Sandstead, Penland et al. 1998; Krebs 2000; Sandstead 2000; Sandstead 2003; Hubbs-Tait, Kennedy et al. 2007; Stoecker, Abebe et al. 2009) and gene expression and protein synthesis (Coleman 1992; Andreini, Banci et al. 2006; Bertini and Rosata 2007).

Dietary sources of zinc

The rich sources of zinc are diets of animal origin, particularly those containing lean red meat, and plant-based diets including whole-grain cereals, pulses and legumes (WHO 2004). Among the modest sources of zinc are fish, roots and tubers, green leafy vegetables and fruits (WHO 2004). The absorption of zinc depends on a number of dietary factors as potential enhancers or antagonists (Gibson 1993; WHO 2004). Soluble low molecular weight organic substances such as amino acids and hydroxyl acids facilitate zinc absorption and organic compounds such as phytates, which form stable and poorly soluble complexes with zinc, impair absorption (Gibson 1993; Sandstead and Smith 1996; Gibson and Ferguson 1998; WHO 2004).

Interaction with other nutrients

Competitive interactions between zinc and other ions with similar physicochemical properties affect absorption of zinc. High concentrations of divalent metals like calcium (Ferguson, Gibson et al. 1989; Oberleas, Muhrer et al. 1996), copper (Reinstein, Lonnerdal et al. 1984; Keen, Reinstein et al. 1985) and iron (Sandstrom, Davidsson et al. 1985; Solomons 1986; Sandstrom 2001; Troost, Brummer et al. 2003) have been reported to compromise zinc intestinal absorption.

Assessment of zinc status

The lack of reliable laboratory indices for zinc has remained a challenge for assessing zinc status (King 1990). However, various zinc status indicators such as serum and plasma zinc concentrations (Kant, Moser-Veillon et al. 1989; Neggers, Goldenberg et al. 1997), erythrocyte zinc concentration (Neggers, Goldenberg et al. 1997), hair zinc concentrations (Hambidge, Hambidge et al. 1972; Ferguson, Gibson et al. 1993), zinc dependent enzymes including alkaline phosphatase (Naber, Baadenhuysen et al. 1996), copper zinc superoxide dismutase (Davis, Milne et al. 2000) lymphocyte 5'-nucleotidase (Beck, Kaplan et al. 1997), plasma metallothionein (Sato, Mehra et al. 1984) and erythrocyte metallothionein (Thomas, Bailey et al. 1992) have been proposed as indicators to evaluate the likelihood of zinc deficiency. But, plasma and serum zinc concentrations have been commonly used to evaluate zinc status although they are not sensitive enough to detect zinc deficiency (Lukaski, Bolonchuk et al. 1984).

Health consequences of zinc deficiency

A negative zinc balance can arise with prolonged inadequate dietary intake of zinc. Considering the participation of zinc in many metabolic processes, dietary imbalances of this essential trace element can impede a variety of physiological and developmental processes (Prasad 1983; Prasad 1985; Prasad 1991; Tamura and Goldenberg 1995). Growth retardation (Gibson 1993; Brandao-Neto, Stefan et al. 1995; Nishi 1996; Gibson, Manger et al. 2007), poor immune status and increased susceptibility to infections (Sazawal, Black et al. 1996; Rosado, Lopez et al. 1997; Sazawal, Jalla et al. 1997; Lesourd, Mazari et al. 1998; Shankar and Prasad 1998; Solomons 1998; Bhutta, Black et al. 1999; Bhutta, Bird et al. 2000; Salgueiro, Zubillaga et al. 2000; Fischer Walker, Aboubaker et al. 2007; Gibson, Hess et al. 2008), impaired reproduction (Prasad, Schalert et al. 1963; Favier 1992; Yamaguchi, Miura et al. 2009), loss of appetite (Safai-Kutti 1990; Lee, Rains et al. 1998; Cousins, Blanchard et al. 2003; Shay and Mangian 2003) and cognitive impairment (Sandstead, Penland et al. 1998; Krebs 2000; Sandstead 2000; Sandstead 2003; Hubbs-Tait, Kennedy et al. 2007; Stoecker, Abebe et al. 2009) are some of the health consequences of zinc deficiency.

Epidemiology of zinc deficiency

The lack of sensitive indicators of zinc deficiency, absence of a highly specific deficiency syndrome for marginal zinc deficiency and the inability to recognize severe clinical zinc deficiency in humans have contributed to the lack of a worldwide database on prevalence and incidence of zinc deficiency compared to the other three (iodine, iron and vitamin A) micronutrients (Shrimpton and Shankar 2008) emphasized by WHO. However, various epidemiological and zinc supplementation studies have provided evidence that suboptimal zinc nutrition is a worldwide problem (Castillo-Duran, Heresi et al. 1987; Gibson 1993; Sazawal, Black et al. 1996; Sempertegui, Estrella et al. 1996; Sian, Mingyian et al. 1996; Rosado, Lopez et al. 1997; Ruel, Rivera et al. 1997; Ruz, Castillon-Duran et al. 1997; Gibson and Huddle 1998; Gibson, Manger et al. 2007; Abebe, Bogale et al. 2008; Gibson, Abebe et al. 2008; Stoecker, Abebe et al. 2009). Some have even argued that suboptimal zinc nutrition is one of the ten most important factors contributing to the burden of disease in the developing world (Shrimpton, Gross et al. 2005).

According to a recent report on global and regional child mortality and burden of disease attributable to zinc deficiency, suboptimal zinc nutrition was responsible for 4.4% of childhood deaths and 1.2% of the burden of disease in Latin America, Africa and Asia (Walker, Ezzati et al. 2009). About 58% of the deaths attributable to zinc deficiency were from Africa, 40% from Asia and 2% from Latin America (Walker, Ezzati et al. 2009). Overall child death due to zinc deficiency remains high in Africa. When the African data is further disaggregated, about 32% of the deaths were from eastern Africa, 42% from western, 21% from middle, 4% from northern and 1% were from southern Africa. India (18.6%) from Asia and Nigeria (11.6%) from Africa contribute the largest share to global child death attributable to zinc deficiency. Five countries namely India, Nigeria, Democratic Republic of Congo, Ethiopia and Afghanistan are estimated to contribute 47% of the child deaths due to zinc deficiency (Walker, Ezzati et al. 2009).

Ethiopia is one of the five countries contributing to many of the global child deaths attributable to zinc deficiency (Walker, Ezzati et al. 2009). Though the prevalence of zinc deficiency and its consequences are yet to be established, the limited available reports indicate that zinc deficiency is of public health concern in Ethiopian children (Umeta, West et al. 2000) and mothers (Haidar, Umeta et al. 2005; Hambidge, Abebe et al. 2006; Abebe, Bogale et al. 2008; Gibson, Abebe et al. 2008; Kassu, Yabutani et al. 2008; Stoecker, Abebe et al. 2009). Nearly 48% of the Ethiopian children under-five years old are stunted (EDHS 2006). Stunting in children is considered as an indirect indicator of zinc nutritional status (Hotz and Brown 2004) as zinc has a particular role in physical growth (Gibson 1993; Brandao-Neto, Stefan et al. 1995; Nishi 1996; Gibson, Manger et al. 2007) and stunted children respond to zinc supplementation with rapid increases in growth (Umeta, West et al. 2000; Gibson, Hess et al. 2008). A double blind randomized placebo controlled zinc supplementation trial conducted by Umeta and colleagues (Umeta, West et al. 2000) showed that 10 mg of elemental zinc as syrup supplementation daily for 6 months resulted in a greater increment in linear and ponderal growth in stunted than nonstunted children.

Zinc deficiency is not affecting children only. Pregnant and nonpregnant women are also affected by zinc deficiency. A study by Kassu and colleagues (Kassu, Yabutani et al. 2008) showed that 66.7% of pregnant women from Gonder, Northwest Ethiopia

were zinc deficient and Haidar and colleagues (Haidar, Umata et al. 2005) reported a prevalence rate of 11.3% for marginal zinc deficiency in lactating women from Metropolitan, Addis Ababa. Recently, high prevalence rates of zinc deficiency (74%) among women in their third trimester have also been reported from Southern Ethiopia (Abebe, Bogale et al. 2008; Gibson, Abebe et al. 2008; Stoecker, Abebe et al. 2009).

Careful review of the literature revealed that the deficiencies of iodine, iron, vitamin A and zinc are of public health significance in Ethiopia. Iron deficiency anemia is a severe public health problem in Ethiopian preschool age children, pregnant women and nonpregnant women. The prevalence rates of xerophthalmia in children and night blindness in pregnant women are unacceptably high, despite the biannual vitamin A capsule distribution to children and postpartum supplementation to mothers. About three quarters of the Ethiopia population was estimated to be at risk for IDD in 1995 and the situation is worsening as evidenced by the increase of goiter rate from 26% in the 1980s to about 40% today. Though the extent of zinc deficiency is not fully established yet, the available reports indicate that zinc deficiency is a growing public health concern in Ethiopia. A database on these and other micronutrients in the adolescent population is critical to the formulation of intervention strategies to correct micronutrient deficiencies in Ethiopia.

CHAPTER III

METHODOLOGY

Pre-survey preparation

Prior to the actual data collection, the principal investigator visited the three local Wereda Education and Health Offices to explain the purpose of the study and to randomly select the nine Complete Primary Schools (CPS) for the study. Immediately after the random selection of the schools, support letters were written to the school principals from the Wereda Education Offices (WEO). After the support letters were sent to the schools, the research team visited each school to further explain the purpose and importance of the study to the school principals and teachers and to randomly select the schoolgirls.

Design

The study design was cross-sectional. An interview based on a structured questionnaire (Appendix A) was used to collect sociodemographic information. Blood and urine were collected using standard techniques to assess the nutritional status of the school girls from biochemical indicators. Stool samples were collected to determine the parasite load in the school age girls. The interviewers were trained to standardize the questionnaire administration. Close follow-up was made by the principal investigator of the research project during the data collection. At the end of the data collection in every study unit, a meeting was held among the research team to discuss practical problems and issues of major concern. The study was conducted in the Southeast zone of Tigray, Northern Ethiopia. The zone is one of the five zones of the National State of Tigray. The data was collected between January and February 2008.

Ethical consideration

The study has been approved by the Institutional Ethics Review Committee of the College of Health Sciences at Mekelle University, the Health Research Ethics Committee at Mekelle University, the National Health Research Ethics Review Committee at the

Ethiopian Science and Technology Commission and the Institutional Review Board (IRB) of Oklahoma State University, USA.

Study subjects

The study subjects were 10 – 15 year old adolescent school girls, recruited from the nine CPS. The school roster was used as a frame for randomization of the school girls. The sample size determination was based on an estimated 50% prevalence (p) of micronutrient (vitamin A, iron, iodine and zinc) deficiencies, a 95% confidence interval for the true prevalence and a relative precision (d) of 5%. The formula adopted was $z^2p(1-p)/d^2$ where $z = 1.96$, $p = 0.5$ and $d = 0.05$. The calculated sample size was 385. Assuming a dropout rate of 15%, a total of 50 adolescent school girls were recruited from the nine CPS. Girls who volunteered to participate in the study were requested to come with their parents the next day or given a consent form to take to their parents for signature as evidence that the parents allowed the girls to participate in the study. Three inclusion/exclusion criteria were established. First, adolescents were included if they are girls. Second, study subjects were included if they are 10 – 15 year old. Third, study subjects were included if they are students at the time of the survey. We excluded nonstudents, girls younger than 10 and older than 15 years of age and boys.

Anthropometrics

Weights of the school girls were measured to the nearest 0.1 kg on a battery powered digital scale (SECA, UNICEF, Copenhagen) and heights were measured to the nearest 0.1 cm using a wooden height-measuring board with a sliding head bar following standard anthropometric techniques (WHO 1995). For weight and height measurements, study subjects removed their shoes, emptied their pockets, removed their jackets and wore light clothing. Mid upper arm circumference (MUAC) was also measured using a non-stretching tape. Anthropometric results were calculated using the WHO Anthroplus software (WHO AnthroPlus v1.0.2, Geneva, Switzerland) and presented as z-scores for height-for-age (HAZ), BMI and BMI-for-age z scores (BAZ). School girls below -2 HAZ and BAZ scores were classified as stunted and thin, respectively.

Laboratory analyses

Sample sizes for urine and blood specimens were lower than the sample sizes for sociodemographic information and clinical assessments. These were due to refusals to give biological specimens and to insufficient quantities of serum for the various analyses.

Urine collection and analysis

Each girl was given a screw-capped plastic cup in which she collected 5 – 10 mL of middle urine. The urine samples were then transported to the nearby clinic for refrigeration and later in the day were transported to the Microbiology Laboratory at Mekelle University. The urine samples were aliquoted into 500 μ L eppendorf tubes and stored at -20°C at the Microbiology Department of the College of Health Sciences at Mekelle University until transported to the laboratory of the Department of the Nutritional Sciences at Oklahoma State University, USA for iodine content analysis. The urinary iodine (UI) content was determined by digesting the urine with ammonium persulfate following the Sandel-Kolthoff reaction (WHO 2001).

In this method, iodine is determined by employing its catalytic role in the reduction of ceric ammonium sulfate to cerous ion coupled to the oxidation of arsenous acid (i.e. As^{+3} to As^{+5}). As the reduction proceeds, the intensity of color decreases. The concentration of iodine was determined using a spectrometer at 405nm.

Specifically, the urinary iodine concentration was determined as follows: 250 μ L urine samples and standards or calibrators were added into pyrex glass test tubes (13x100 mm) and digested with 1 mL of 1M ammonium persulfate at 95°C for 60 minutes in a heating block. After cooling the tube contents to room temperature, 2.5 mL of arsenous acid was added to the tubes containing samples and standards and vortexed briefly. After 15 minutes of reaction time, 300 μ L of ceric ammonium sulfate was added, vortexed briefly and the rate of the yellow color disappearance was measured after exactly 30 minutes using a Beckmann spectrometer (DU 800, Fullerton, CA). The color intensity was due to the catalytic reduction of ceric ammonium sulfate in the presence of arsenous acid. WHO cutoff points for urinary iodine levels were used to define iodine status: deficiency $< 100 \mu\text{g/L}$ (severe deficiency $<20\mu\text{g/L}$; moderate deficiency 20-49 $\mu\text{g/L}$; mild deficiency 50-99 $\mu\text{g/L}$), optimal 100 – 199 $\mu\text{g/L}$, more than adequate 200-299 $\mu\text{g/L}$, excessive $\geq 300 \mu\text{g/L}$ (WHO 2007).

Stool collection and examination

Each girl was given a cup with cover in which she collected a 3 – 5 g sample of stool. The stool samples were examined by a medical laboratory technologist for the presence of geo-helminths (hookworm, *Ascaris lumbricoides*, *Trichuris trichuria* and *Strongyloides stercoralis*) and *Schistosoma mansoni*. Stool samples were subjected to the following three techniques.

Wet preparation (Direct saline mount)

Using an applicator stick, about 50 mg of stool was mixed with one or two drops of normal saline (0.85% NaCl) on a clean microscope slide. A uniform thin suspension was made and covered with a 22 mm square cover slip. The entire slide was screened systematically for the presence of helminthes ova and larvae or protozoan cysts and trophozoites under an Olympus microscope using the x100 objective lens (Cheesbrough 1981).

Formalin-Ether concentration method

Using an applicator stick, about 1 g of stool was placed in a clean 15 mL conical centrifuge tube containing 5 mL of 1% formalin and vortexed thoroughly. The resulting suspension was filtered through gauze into a beaker and the debris discarded. The filtrate was poured back to the centrifuge tube, 2 – 3 mL of diethylether added and the contents were centrifuged for 3 minutes at 3000 rpm. The sediment was systematically examined using x10 and x40 objective lenses of an Olympus microscope (Cheesbrough 1981).

Kato-Katz method

The Kato-Katz method was performed as per the WHO bench aids for the diagnosis of intestinal parasites (WHO 1994). In brief, about 0.5 g of stool was placed on a plastic foil and a screen was pressed on top so that some of the stool sieved through the screen (80 mesh) and accumulated on top. The sieved stool was scraped using a disposable plastic spatula. A plastic template was placed on a slide and the sieved stool was added with the spatula so that the hole (6 mm in diameter and 1.5 mm thick) in the template was completely filled thus delivering approximately 42 mg of stool to the microscope slide. Excess stool was removed by passing the plastic spatula over the filled template. The template was removed carefully so that a cylinder of stool was left on the microscope slide. The stool on the slide was covered with a cellophane strip that had been

soaked for a minimum of 24 hours in a 1:1 mixture of glycerol and distilled water. The slide was inverted and the stool sample was pressed firmly against the hydrophilic cellophane strip to spread evenly. Slides were examined immediately under a light microscope (100x) for hookworm eggs. For all helminthes, the slides were kept for one or more hours at room temperature prior to microscopic examination. The number of ova recorded was multiplied by 24 to obtain the number of eggs per gram (epg) of stool. Heavy hookworm and schistosomiasis infections were defined as an egg count ≥ 2000 and ≥ 400 per gram of stool, respectively (Stephenson, Latham et al. 2000).

Blood collection and analysis

Capillary and venous blood samples were collected aseptically by a phlebotomist following standard procedures. Disposable lancets and syringes were used to collect blood specimens. Blood samples were collected and processed inside rooms with lights turned off and serum samples were kept in an ice box after separation and during transport. The capillary blood obtained from a finger prick was used for blood film and for hematocrit determination while the venous blood obtained from the antecubital area of the arm was used for serum retinol, β -carotene, α -carotene, ferritin, zinc, sTfR and CRP. About 5 – 10 mL of venous blood was collected from each school girl and allowed to clot in plain glass vacutainer blood collection tubes. After 15 minutes, serum was separated by centrifugation at 2500 rpm for 10 minutes. Serum was aliquoted into plastic sample tubes and stored at -20°C at the Microbiology Laboratory of Mekelle University, Ethiopia until transported to the laboratory of the Department of Nutritional Sciences at Oklahoma State University, USA. Frozen samples were shipped in an insulated ice box while being air freighted to the US. On arrival samples were thawed but quite cool. Trace mineral free gloves and pipette tips were used during analysis of the serum samples.

Hematocrit

Capillary blood from a finger prick was filled into heparinized hematocrit tubes. After sealing with clay, the hematocrit tubes were then centrifuged for ten minutes. The red zone (red blood cells) of the capillary tube was read with a hematocrit reader. Anemia was defined as hematocrit (at sea level) $\leq 36\%$. For study sites of 1500 meters above sea level (masl), the cutoff was adjusted by adding 1% to the cutoff at sea level; 2% for 2000 masl and 3% for those with an altitude of 2050 masl and above. Living at high altitudes

causes increased hematocrit values as the body's response to decreased atmospheric concentrations of oxygen at such heights. Long term residency at high altitude (greater than 3000 ft or 914 m) causes a generalized upward shift in hemoglobin concentration and hematocrit. Thus, the cut off values were adjusted for this factor. The altitude of the study communities ranges from 1500 to 2646 m above sea level. The cut off was adjusted accordingly by adding 1% to Finarua (1500 masl), 2% to Samre (2000 masl) and Adigudom (2050 masl) and 3% to the rest of the schools (2150 – 2646 masl).

Blood film for malaria parasites

Thick blood smears from finger puncture were stained immediately using Giemsa solution. Blood films were examined for the presence of malaria parasites with a x100 oil-immersion objective during the nights and weekends. Malaria parasitemia was defined as the presence of asexual stage parasites (any species) in the thick smear.

Vitamin A and Carotenoids

All-trans-retinol (external standard), retinol acetate (internal standard), β -carotene, α -carotene, methanol, dichloromethane and acetonitrile, ethanol and hexane chemicals were purchased from Sigma Chemical Company (St. Louis, MO). All were High Pressure Liquid Chromatography (HPLC) grade except the analytical grade butylated hydroxytoluene, which was an analytical reagent grade. All handling operations of the serum were carried out in semi-darkness. Serum samples were analyzed by reversed phase HPLC following the methods of Talwar and colleagues (Talwar, Ha et al. 1998) with slight modification. Stock solutions of each of the calibrators (*all-trans* retinol, β -carotene and α -carotene) and the internal standard (retinol acetate) were dissolved in methanol. For calibration, different concentrations of each of the calibrators were prepared in ethanol - butylated hydroxytoluene. The final concentration of the internal standard was 0.25 $\mu\text{g}/\text{mL}$.

Briefly, serum retinol, α -carotene and β -carotene levels were determined as follows. In a test tube, 200 μl of serum was deproteinized with an equal volume (200 μl) of ethanol- butylated hydroxytoluene. Exactly 50 μl of 2.25 $\mu\text{g}/\text{mL}$ retinol acetate was added to the deproteinized sample and mixed by vortex for 5 seconds. Samples were then extracted by the addition of 1000 μL of hexane and mixed by vortex for 30 seconds followed by centrifugation at 2000 rpm for 3 minutes. About 800 μL of the supernatant

was transferred into another test tube. The extraction was repeated for the second time and 1000 μL of the supernatant was transferred into the test tube containing the hexane extract. Samples were dried under a stream of nitrogen. Then, contents of the tube were reconstituted in 100 μL of ethanol- butylated hydroxytoluene solution and transferred to HPLC cuvetts (inserts) and placed in the autosampler compartment of the HPLC. The injection volume was 20 μL .

For the HPLC system, an autosampler (Waters 717 plus autosampler, Milford, MA), controller (Waters 600 Controller, Milford, MA) and a detector (model 2487 dual wavelength absorbance detector) were used. A guard column (Waters Nova Pak C18, Milford, MA) and reversed phase column, (C18 Waters 250 mm x 3.9 mm (id); 4 μm bead size, Milford, MA) were used. The chromatographic separation was performed by isocratic elution with a mixture of methanol, dichloromethane and acetonitrile (60:20:20 by volume) and at a flow rate of 0.8 mL/min. The concentrations of retinol and the carotenoids (β -carotene and α -carotene) were quantified at 325 and 450 nm, respectively.

Serum ferritin

Serum levels of ferritin were determined by one stage, two-site immunoradiometric (sandwich) assay (Ramco Laboratories, Inc., Stafford, TX). In short, samples were diluted 100 times (1:100) by taking 5 μL of samples and diluting to 500 μL using sample diluting buffer provided with the kit. Prediluted ferritin calibrators or samples (10 μL) were pipetted into 12 x 75 mm tubes followed by 200 μL of radiolabelled antihuman ferritin and vortexed for 10-15 seconds. Blank tubes were included to measure non specific binding (NSB) and only contained the radiolabelled antihuman ferritin and antihuman ferritin coated beads. The tubes were incubated overnight after the addition of one antihuman ferritin bead to each reaction tube for optimum binding. After overnight incubation, the solution was aspirated using a suction system and each bead was washed three times with 2 mL Millipore water. Beads were counted in a Cobra II Auto Gamma Counter (Packard, Downers Grove, IL, USA) for 2 minutes. A calibration curve was constructed and net count of samples determined by subtracting the mean count of the NSB from each tube. Iron deficiency (ID) was defined as a ferritin level of $<15 \mu\text{g/L}$ (WHO 2001) and iron deficiency anemia (IDA) was defined as anemia with ferritin of $<15 \mu\text{g/L}$.

Serum soluble transferrin receptors

Concentrations of serum sTfR on a subsample were determined using an in vitro enzyme immunoassay based upon the double antibody sandwich method (Ramco Laboratories, Inc., Stafford, TX). Using a pipettor, 5 μL of serum samples and controls were diluted 100x (5 μL of serum or control was diluted to 500 μL) using a diluent provided with the kit and vortexed thoroughly for 10-15 seconds. Then 50 μL of prediluted standards, diluted samples or controls were pipetted individually into the flat-bottomed plate wells provided with the kit. Horseradish peroxidase (HRP) conjugate (150 μL) was pipetted into all individual wells containing standards, samples or controls. After mixing the well contents in a laboratory horizontal rotator for 10 minutes, the reaction was allowed to proceed for an additional two hours at room temperature. After the two hours of incubation, the well contents were washed three times with a wash solution provided with the kit using a wash bottle. The wells were tapped dry between each wash and after the final wash, wells were checked for complete removal of well contents and absence of bubbles. If not empty, they were further tapped dry on paper towels. Then 200 μL of substrate solution provided with the kit was added to each individual well, mixed for 1 minute at 190 rpm on the horizontal rotator and incubated in the dark for 30 minutes at room temperature. The reaction was stopped by adding 50 μL of an acid stop solution. Finally, the absorbance was read in an ELISA plate reader (Synergy HT, BioTek, Winooski, VT, USA) within 5 minutes at 450 nm using a background correction wavelength at 570 nm. A calibration curve was constructed from 0 to 200 ng/mL and sTfR levels were determined from the calibration curve.

Serum zinc

Serum zinc was analyzed by Inductively Coupled Plasma Mass Spectrometer (ICP-MS). All serum samples were diluted 20 times (200 μL diluted to 4 mL) with 0.1% HNO_3 (GFS Chemicals, Powell, OH) in Millipore water. The calibration standards at 0, 50 and 100 $\mu\text{g/L}$ were prepared in 0.1% nitric acid solution. Standard solutions of zinc were prepared by dilution of certified standard solutions (Perkin Elmer, Norwalk, CT). Dilute working standards were prepared immediately prior to their use by dilution of an intermediate stock standard solution. All samples and standards were spiked with 10 $\mu\text{g/L}$ gallium as an internal standard (Perkin Elmer Pure Atomic Absorption Standard,

Norwalk, CT). Samples were analyzed by ICP-MS (Elan 9000, Perkin Elmer, Norwalk, CT) with gallium as internal standard. To avoid zinc contamination, only polypropylene plasticwares were used for reagent and sample preparation (Sarstedt, Newton, NC). Quality control samples (Utak Laboratories, Inc., Valencia, CA) utilized to verify method performance was within recommended ranges.

C - reactive protein

CRP, an acute phase protein synthesized in the liver, was quantified using ELISA (Helica Biosystems, Inc., Fullerton, CA) following the procedure provided with the kit. CRP serves as an acute phase marker in conditions of infection, trauma or inflammation. All controls were within recommended ranges. Serum concentrations ≥ 3 mg/L were taken to indicate the presence of inflammation or infection (Beard, Murray-Kolb et al. 2006). Briefly, hsCRP (high sensitivity C – reactive protein) was determined as follows. Serum samples were serially diluted 1000x using diluent provided with the kit. Using a pipette, 100 μ L of diluted serum, standards and controls were added into the microplate wells provided with the kit. After incubation for 30 minutes at ambient temperature, plates were washed with a wash buffer (Tris with 0.05% Tween 20, pH 7.4). Immediately after the wash step, 100 μ L of conjugate (horseradish peroxidase labeled rabbit anti-human serum, CRP-IgG) was added to each well and the plate was incubated for 30 minutes at room temperature. Plates were washed with the wash buffer and 100 μ L of TMB substrate (3,3',5,5'-tetramethylbenzidine) solution was added to each well. The reaction was allowed to proceed for 5 – 10 minutes at room temperature and stopped by adding 100 μ L of stop solution (diluted phosphoric acid). Absorbance was read at 450 nm using a plate reader (Synergy HT, BioTek, Winooski, VT, USA).

Nutritional status

Determination of the nutritional status of the school girls was based on anthropometric (stunting, thinness, MUAC) variables, biochemical indicators and clinical examinations. Biochemical iodine status was based on urinary iodine levels; iron status on ferritin levels as well as sTfR for a subsample of subjects with low ferritin levels; zinc status was based on serum zinc and vitamin A status on serum retinol concentrations.

Clinical assessment

All girls were examined by a health officer experienced in diagnosing clinical manifestations due to vitamin A (for ocular signs such as conjunctival xerosis, Bitot's spots, corneal xerosis, etc), iron (pallor of skin, conjunctiva, tongue and palms) and iodine deficiencies (thyroid size by palpation). Thyroid size was graded according to WHO criteria (WHO 2007) as follows: grade 0 (no palpable or visible goiter), Grade 1 (palpable but not visible goiter), grade 2 (visible goiter with neck in the normal position).

Statistical analyses

Statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC, USA). Student's t tests, chi square tests, correlation analysis, nonparametric tests, ANOVA, multiple and logistic regressions were employed for statistical analyses. The height, weight, age, hematocrit values, height-for-age z scores, BMI, BMI-for-age z scores, serum ferritin, serum sTfR, serum retinol, serum α -carotene, serum β -carotene, serum zinc, CRP and urinary iodine concentrations were the continuous variables. Groups including ages; schools; elevation; stunted and nonstunted; goitrous and nongoitrous; thin and normal; nutrient deficient and sufficient; anemic and nonanemic were treated as categorical variables. Socioeconomic and sociodemographic variables including location (school), home garden, water source, education level, mother's and father's occupation, type of residence, family food source, impact of food shortage, workload/physical activity, type of salt, latrine facilities and shoe wearing practices were treated as independent variables with different levels. Student's t tests were used to compare the means of dependent variables of two groups of an independent variable. Chi square tests were employed to analyze categorical variables. Correlation analysis was employed to indicate the strength of a linear relationship between two continuous variables. A test for normality was done using univariate analysis. Parameters that were not normally distributed including urinary iodine concentration, β -carotene and CRP, were presented as medians and were compared by Wilcoxon-Mann-Whitney and Kruskal-Wallis tests. Analysis of Variance (ANOVA) was used to compare the means of continuous dependent variables of three or more groups of an independent variable. Multiple regression analysis was employed to relate two or more continuous independent variables to a continuous dependent variable. Logistic regression was used to relate

continuous independent variables to dichotomous dependent variables such as nutrient deficient and sufficient; stunted and nonstunted; thin and normal; goitrous and nongoitrous; and anemic and nonanemic groups. Statistical significance was set at $p < 0.05$.

CHAPTER IV
IRON DEFICIENCY IN ADOLESCENT SCHOOL GIRLS FROM TIGRAY,
NORTHERN ETHIOPIA

Abstract

We conducted a cross-sectional study to examine the magnitude and severity of iron deficiency among 413 adolescent randomly selected school girls (10-15 years old) from Tigray, Northern Ethiopia. Mean (sd) hematocrit (hct) and ferritin values were 42.9 (3.3) % and 48.4 (25.1) $\mu\text{g/L}$, respectively. Stunting was common (23.1%) and 26.8% were thin on the basis of their BMI. Serum transferrin receptors (sTfR) were determined for 148 school girls with lower ferritin values. Their sTfR ranged between 4.6 and 18.5 mg/L with a mean of 8.2 (2.6) mg/L. The median (25th, 75th) level of C-reactive protein was 0.4 (0.2, 0.8) mg/L. The prevalence of anemia in the school girls was 7.1% after adjusting hct levels for elevation (1500 – 2646 m). Thirty-two (8.9%) of the school girls were iron depleted (ferritin < 15 $\mu\text{g/L}$) and 15.3% had low iron stores (ferritin 15 - 30 $\mu\text{g/L}$) suggesting that iron deficiency was prevalent in this population. Anthropometric indicators including HAZ ($r = 0.16$, $p = 0.0026$) and MUAC ($r = 0.16$, $p = 0.0036$) were correlated with hct values. Age ($F = 4.01$, $p = 0.0015$), study sites ($F = 9.38$, $P < 0.0001$) and involvement in domestic activities such as fire wood collection ($F = 4.16$, $p = 0.0422$) and agricultural activities ($F = 6.03$, $p = 0.0145$) were significantly related to hct. The most common single and mixed parasites observed were *Entamoeba histolytica* (19.9%) and *Hymenolepis nana* (8.6%). However, the parasites commonly associated with anemia including hookworm, 11 (3.3%), *Schistosoma mansoni*, 1 (0.3%) and malaria infection, 2 (0.6%) were rarely detected. Anemia was a public health problem in the adolescent school girls and large proportions (24%) of the girls were at risk of developing iron deficiency (ferritin < 30 $\mu\text{g/L}$). Carefully designed studies to investigate the multicausal factors for anemia in the school girls and the contribution of contaminant iron to the intrinsic non-heme iron pool from food are recommended.

IRON DEFICIENCY IN ADOLESCENT SCHOOL GIRLS FROM TIGRAY, NORTHERN ETHIOPIA

Introduction

Iron deficiency is one of the most common and widespread nutritional disorders across the globe. There are no current global figures for iron deficiency but using anemia as an indirect indicator, the WHO (de Benoist, McLean et al. 2008) estimated that anemia affects almost a quarter (24.8%) of the world's population. The highest prevalence is in preschool age children (47.4%) and the lowest prevalence is in men (12.7%). The largest affected population group is nonpregnant women with 468 million (de Benoist, McLean et al. 2008).

According to recent WHO (de Benoist, McLean et al. 2008) regional estimates for anemia in preschool age children, pregnant women and nonpregnant women, Africa had the highest proportion of individuals affected (47.5 – 67.6%) and Southeast Asia had the greatest number of individuals (315 million) affected by anemia. This same WHO estimate (de Benoist, McLean et al. 2008) showed anemia to be a severe public health problem in preschool age children, pregnant women and nonpregnant women from Ethiopia, though the magnitude and severity of iron deficiency anemia as a public health significance has been debatable amongst public health professionals from Ethiopia (Adish, Esrey et al. 1999; Haidar and Pobocik 2009).

A survey of the literature on the magnitude and severity of anemia in Ethiopia revealed mixed reports regarding the public health significance of anemia. Earlier reports supported the rarity of anemia in Ethiopia and recent reports emphasized the public health significance of anemia in Ethiopia. According to a 1972 facility-based report from 100 nonpregnant women from five antenatal clinics in Addis Ababa, mean hemoglobin level was 152 ± 5 g/L and anemia was not a significant problem (Ross 1972). On the basis of hemoglobin, hematocrit, total iron binding capacity, serum folate and serum vitamin B₁₂ values, Gebre-Medhin and colleagues (Gebre-Medhin, Killander et al. 1976) reported the rarity of anemia in pregnant women in their last month of pregnancy. In the early 1990s, Zein (Zein 1991) for the first time reported a high prevalence of anemia (47.2%) in 6 months to 6 year old children from Northwestern Ethiopia. A multicenter study conducted between 1992 and 1994 (Haidar, Nekatibeb et al. 1999) on 1449 women from

13 randomly selected sites across the country (except the northern part of the country) revealed anemia in 18.4% of 15 – 49 year old pregnant and lactating mothers. The same year, a study by Adish and colleagues (Adish, Esrey et al. 1999) on 2080 preschool age children reported a high prevalence of anemia (42%) in under-five children from Tigray, Northern Ethiopia. Similarly, a high prevalence of anemia (61%) was reported by Muhe and coworkers (Muhe, Oljira et al. 2000) in their facility-based study conducted in 2540 preschool age children. A study on 383 pregnant women 14-49 years old visiting antenatal clinic in Hawassa, Southern Ethiopia reported a mean hemoglobin level of 115 ± 14 g/L and 32.2% prevalence of anemia (Gies, Brabin et al. 2003). Comparable mean hemoglobin levels (114 ± 4 g/L) but lower prevalence of anemia (22.3%) was reported in a study by Haidar and colleagues (Haidar, Muroki et al. 2003) of 1017 lactating women (15-49 year old) from six urban slum communities of Addis Ababa.

According to the recent Ethiopian Demographic and Health Survey (EDHS 2006) conducted on 6141 women, anemia was detected in 26.6% of pregnant and nonpregnant women. Moreover, anemia was reported in 52.1% and 55.0% of preschool age female and male children, respectively. Another report by Umeta and colleagues (Umeta, Haidar et al. 2008) conducted in 22,861 women of reproductive age (15-49 years) reported clinical anemia in 11.3%, anemia in 30.4%, iron deficiency in 49.7% and iron deficiency anemia in 17% of women. This multicenter study from 270 clustered villages and 9 administrative regions substantiated the existence of mild to moderate iron deficiency anemia among women of reproductive age with significant geographic variation. A recent study by Haidar and Pobocik (Haidar and Pobocik 2009) conducted on 970 women of reproductive age (15-49 years) revealed a prevalence of 32.1% and 29.4% of iron deficiency and anemia, respectively, substantiating the public health significance of anemia in Ethiopia. Though the magnitude of anemia has been a controversial issue in Ethiopia, careful review of the literature confirmed that anemia is still a public health problem. All the recent reports indicated the common occurrence of anemia in children and pregnant and nonpregnant women. Surprisingly, none of the studies on iron deficiency from Ethiopia have addressed the iron status of adolescents. Thus, the objective of this study was to describe the magnitude and severity of iron deficiency in adolescent girls from Tigray, Northern Ethiopia.

Methods

Presurvey preparation: Prior to the actual data collection, the principal investigator visited the three local Wereda Education and Health Offices to explain the purpose of the study and to randomly select the nine Complete Primary Schools (CPS) for the study. Immediately after the random selection of the schools, support letters were written to the school directors from the Wereda Education Offices (WEO). After the support letters were sent to the schools, the research team visited each school to further explain the purpose and importance of the study to the school principals and teachers and to randomly select the schoolgirls.

Design: The study design was cross-sectional. A structured questionnaire was used to collect sociodemographic information. Blood samples were collected using standard techniques to assess the nutritional status of the school girls from biochemical indicators. Stool samples were collected to determine the parasite load in the school age girls. The interviewers were trained to standardize the questionnaire administration. Close follow-up was made by the principal investigator of the research project during the data collection. At the end of the data collection in every study unit, a meeting was held among the research team to discuss practical problems and issues of major concern. The study was conducted in the Southeastern zone of Tigray, Northern Ethiopia. The zone is one of the five zones of the National State of Tigray.

Ethical consideration: The study has been approved by the Institutional Ethics Review Committee of the College of Health Sciences at Mekelle University, the Health Research Ethics Committee at Mekelle University, the National Health Research Ethics Review Committee at the Ethiopian Science and Technology Commission and the Institutional Review Board (IRB) of Oklahoma State University, USA.

Study subjects: The study subjects were 10 – 15 year old adolescent school girls, recruited from the nine CPS. The school roster was used as a frame for randomization of the school girls. The sample size determination was based on an estimated 50% prevalence of iron deficiency, a 95% confidence interval for the true prevalence and a relative precision of 5%. Girls who volunteered to participate in the study were requested to come with their parents the next day or given a consent form to take to their parents for signature as evidence that the parents allowed the girls to participate in the study.

Anthropometrics: Weights of the school girls were measured to the nearest 0.1 kg on a battery powered digital scale (SECA, UNICEF, Copenhagen) and heights were measured to the nearest 0.1 cm using a wooden height-measuring board with a sliding head bar following standard anthropometric techniques (WHO 1995). For weight and height measurements, study subjects removed their shoes, emptied their pockets, removed their jackets and wore light clothing. Mid upper arm circumference (MUAC) was also measured using a non-stretching tape. The mean MUAC of 5 – 15 years old healthy Nigerian girls (17.8 cm) was used as a cutoff (Owa and Adejuyigbe 1997). Anthropometric results were calculated using the WHO Anthroplus software (WHO AnthroPlus v1.0.2, Geneva, Switzerland) and presented as z-scores for height-for-age (HAZ), BMI and BMI-for-age z scores (BAZ). School girls below -2 HAZ or BAZ scores were classified as stunted or thin, respectively.

Stool collection and examination: Each girl was given a cup with cover in which she collected a 3 – 5 g sample of stool. The stool samples were examined for the presence of geo-helminths (hookworm, *Ascaris lumbricoides*, *trichuris trichuria* and *Strongyloides stercoralis*) and *Schistosoma mansoni*. Stool samples were subjected to the following three techniques.

Wet preparation (Direct saline mount): Using an applicator stick, about 50 mg of stool was mixed with one or two drops of normal saline (0.85% NaCl) on a clean microscope slide. A uniform thin suspension was made and covered with a 22 mm square cover slip. The entire slide was screened systematically for the presence of helminthes ova and larvae or protozoan cysts and trophozoites under an Olympus microscope using x100 objective lenses (Cheesbrough 1981).

Formalin-Ether concentration method: Using an applicator stick, about 1 g of stool was placed in a clean 15 mL conical centrifuge tube containing 5 mL of 1% formalin and vortexed thoroughly. The resulting suspension was filtered through gauze into a beaker and the debris discarded. The filtrate was poured back to the centrifuge tube, 2 – 3mL of diethylether added and the contents were centrifuged for 3 minutes at 3000 rpm. The sediment was systematically examined using x10 and x40 objective lenses of an Olympus microscope (Cheesbrough 1981) for schistosoma eggs.

Kato-Katz method: The Kato-Katz method was performed as per the WHO bench aids for the diagnosis of intestinal parasites (WHO 1994). In brief, about 0.5 g of stool was placed on a plastic foil and a screen was pressed on top so that some of the stool sieved through the screen (80 mesh) and accumulated on top. The sieved stool was scraped using a disposable plastic spatula. A plastic template was placed on a slide and the sieved stool was added with the spatula so that the hole (6 mm in diameter and 1.5 mm thick) in the template was completely filled thus delivering approximately 42 mg of stool to the microscope slide. Excess stool was removed by passing the plastic spatula over the filled template. The template was removed carefully so that a cylinder of stool was left on the microscope slide. The stool on the slide was covered with a cellophane strip that had been soaked for a minimum of 24 hours in a 1:1 mixture of glycerol and distilled water. The slide was inverted and the stool sample was pressed firmly against the hydrophilic cellophane strip to spread evenly. Slides were examined immediately under a light microscope (100x) for hookworm eggs. For all helminthes, the slides were kept for one or more hours at room temperature prior to microscopic examination. The number of ova recorded was multiplied by 24 to obtain the number of eggs per gram of stool. Heavy hookworm and schistosomiasis infections were defined as an egg count ≥ 2000 and ≥ 400 per gram of stool, respectively (Stephenson, Latham et al. 2000).

Blood collection and analysis: Nonfasting capillary and venous blood samples were collected aseptically by a phlebotomist following standard procedures. Disposable lancets and syringes were used to collect blood specimens. The capillary blood obtained from a finger prick was used for blood film for parasite examination and for hematocrit determination while the venous blood obtained from the antecubital vein of the arm was used for serum retinol, β -carotene, α -carotene, ferritin, zinc, sTfR and CRP. Approximately 5 – 10 mL of venous blood was collected from each school girl and allowed to clot in plain glass vacutainer blood collection tubes. After 15 minutes, serum was separated by centrifugation at 2500 rpm for 10 minutes. Serum was aliquoted into plastic sample tubes and stored at -20°C at the Microbiology Laboratory of Mekelle University, Ethiopia until transported to the laboratory of the Department of Nutritional Sciences at Oklahoma State University, USA. Frozen samples were shipped in an

insulated ice box while being air freighted to Oklahoma. On arrival, samples were thawed but quite cool and were frozen immediately.

Blood film for malaria parasites: Thick blood smears from finger puncture were stained immediately using Giemsa solution. Blood films were examined for the presence of malaria parasites with a x100 oil-immersion objective during the nights and weekends. Malaria parasitemia was defined as the presence of asexual stage parasites (any species) in the thick smear.

Hematocrit: Capillary blood from a finger prick was filled into heparinized hematocrit tubes. After sealing with clay, the hematocrit tubes were centrifuged and the hematocrit level determined using a hematocrit reader. Anemia was defined as hematocrit (at sea level) $\leq 36\%$. Living at high altitudes causes increased hematocrit values as the body's response to decreased levels of oxygen at such heights. Long term residency at high altitude (greater than 3000 ft or 914 m) causes a generalized upward shift in hemoglobin concentration and hematocrit. Thus, the cut off values were adjusted for this factor. The altitude of the study communities ranges from 1500 to 2646 m above sea level. The cut off was adjusted accordingly by adding 1% to Finarua (1500 masl), 2% to Samre (2000 masl) and Adigudom (2050 masl) and 3% to the rest of the schools (2150 – 2646 masl) (Alton 2005). Iron deficiency (ID) was defined as a ferritin level of $<15 \mu\text{g/L}$ (WHO 2001) and iron deficiency anemia (IDA) was defined as anemia with ferritin level of $<15 \mu\text{g/L}$.

Serum ferritin: Serum levels of ferritin were determined using a one stage, two-site immunoradiometric (sandwich) assay (Ramco Laboratories, Inc., Stafford, TX). In short, samples were diluted 100 times (1:100) by taking 5 μL of samples and diluting to 500 μL using sample diluting buffer provided with the kit. Prediluted ferritin calibrators or samples (10 μL) were pipetted into 12 x 75 mm tubes followed by 200 μL of radiolabelled antihuman ferritin and vortexed for 10-15 seconds. Blank tubes were included to measure non specific binding (NSB) and only contained the radiolabelled antihuman ferritin and antihuman ferritin coated beads. The tubes were incubated overnight for optimum binding after the addition of one antihuman ferritin bead to each reaction tube. After overnight incubation, the solution was aspirated using a suction system and each bead was washed three times with 2 mL Millipore water. Beads were

counted in a Cobra II Auto Gamma Counter (Packard, Downers Grove, IL, USA) for 2 minutes. A calibration curve was constructed and net count of samples determined by subtracting the mean count of the zero calibrator (NSB) from each tube.

The levels of serum soluble transferrin receptors (sTfR) of girls with the lowest ferritin concentration were determined using an in vitro enzyme immunoassay based upon the double antibody sandwich method (Ramco Laboratories, Inc., Stafford, TX). C-reactive protein was analyzed using ELISA (Helica Biosystems, Inc, Fullerton, CA) following the procedure provided with the kit to screen for infection. All controls were within recommended ranges. Serum concentrations ≥ 3 mg/L were taken to indicate the presence of inflammation or infection (Beard, Murray-Kolb et al. 2006).

Nutritional status: Determination of the nutritional status of the school girls was based on anthropometric (stunting, thinness, MUAC) variables, biochemical indicators and clinical examinations. Anemia was determined based on the hematocrit levels and clinical signs, and iron status was determined based on serum ferritin and sTfR levels. All girls were examined by a health officer experienced in diagnosing clinical manifestations due to iron deficiency.

Statistical analysis: Statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC, USA). Student's t tests, chi square tests, correlation analysis, nonparametric tests, ANOVA, multiple and logistic regressions were employed for statistical analyses. The height, weight, age, hematocrit values, height-for-age z scores, BMI, BMI-for-age z scores, serum ferritin, serum sTfR, serum retinol, serum α -carotene, serum β -carotene, serum zinc, CRP and urinary iodine concentrations were the continuous variables. Groups including ages; schools; elevation; stunted and nonstunted; goitrous and nongoitrous; thin and normal; nutrient deficient and sufficient; anemic and nonanemic were treated as categorical variables. Socioeconomic and sociodemographic variables including location (school), home garden, water source, education level, mother's and father's occupation, type of residence, family food source, impact of food shortage, workload/physical activity, type of salt, latrine facilities and shoe wearing practices were treated as independent variables of different levels. Student's t tests were used to compare the means of dependent variables of two groups of an independent variable. Chi square tests were employed to analyze categorical variables. Correlation

analysis was employed to indicate the strength of a linear relationship between two continuous variables. A test for normality was done using univariate analysis. Parameters that were not normally distributed including urinary iodine concentration, β -carotene and CRP, were presented as medians and were compared by Wilcoxon-Mann-Whitney and Kruskal-Wallis tests. Analysis of Variance (ANOVA) was used to compare the means of continuous dependent variables of three or more groups of an independent variable. Multiple regression analysis was employed to relate two or more continuous independent variables to a continuous dependent variable. Logistic regression was used to relate continuous independent variables to dichotomous dependent variables such as nutrient deficient and sufficient; stunted and nonstunted; thin and normal; goitrous and nongoitrous; and anemic and nonanemic groups. Statistical significance was set at $p < 0.05$.

Results

The mothers of 88.6% of the girls had no formal education. Major occupation for 81% of the mothers and 70% of the fathers was farming. Nearly 5.6% of the girls were from lowland areas (< 2000 masl) and 94.4% were from highland areas (≥ 2000 masl). The common source of food to the households was their own agricultural produce suggesting that the study subjects were mostly from farming households. Most households (71%) owned domestic animals but the frequency of the consumption of foods of animal origin was very low (6%) suggesting that the domestic animals were used for other purposes or activities. None of the households had access to sea foods. Anthropometric characteristics and mean biochemical levels of selected indicators are summarized in Table 4.1.

The mean (sd) age, hematocrit, and ferritin concentrations were 12.7 (1.5) years, 42.9 (3.3) % and 48.4 (25.1) $\mu\text{g/L}$, respectively. Their sTfR ranged between 4.6 and 18.5 mg/L with a mean (sd) of 7.7 (2.3) mg/L . Approximately twenty four percent (23.5%) of the school girls were stunted and 27.1% were thin on the basis of their BMI. The mean (sd) MUAC was 18.7 (2.1) cm and median (25th, 75th percentile) MUAC was 18.5 (17.0, 20.0) cm. Nearly 35.9% of the adolescent school girls had MUAC less than 17.8 cm (Owa and Adejuyigbe 1997). The prevalence of anemia in the school girls was 7.1% after adjusting hematocrit for elevation. Twenty six (6.3%) of the school girls had clinical

signs and symptoms related to anemia. The adolescent school girls with clinical signs and symptoms pertinent to anemia had low hematocrit values. Only 13 (3.2%) of the girls reported menarche.

Table 4.1: Anthropometric characteristics and biochemical indicators of the study subjects.

Characteristics	n	Mean (sd)
Age, years	413	12.7 (1.5)
HAZ	411	-1.3 (1.0)
BMI, kg/m ²	411	15.8 (2.0)
MUAC, cm	409	18.7 (2.1)
BAZ	411	-1.5 (1.0)
Hct, %	351	42.9 (3.3)
Ferritin, µg/L	360	48.4 (25.1)
sTfR, µg/mL	148	8.2 (2.6)
Zinc, µg/dL	354	72.3 (14.4)
Retinol, µg/dL	359	35.9 (9.6)
α-Carotene, µg/dL	347	10.5 (3.8)
β-Carotene*, µg/dL	359	46.4 (24.5, 77.9)
CRP*, mg/L	356	0.4 (0.2, 0.8)

*: median (25th, 75th percentile)

Hematocrit values were significantly associated with the anthropometric status of the school girls. Stunted school girls had lower mean hematocrit (42.1%) than nonstunted girls (43.2%) ($p = 0.0064$). Similarly, thin girls had lower hematocrit (42.4%) than those with > -2 BAZ scores (43.2%) ($p = 0.0429$).

ANOVA revealed that age ($F = 4.0$, $p = 0.0015$) and study sites or schools ($F = 9.4$, $P < 0.0001$) of the girls had a significant association with hematocrit values. The 10 year old school girls had the lowest mean hematocrit levels of 41% and the school girls from Finarua had the lowest mean hematocrit levels of 40.2%. However, we observed no statistically significant difference in ferritin concentrations across the age groups ($p = 0.4100$) or study sites ($p = 0.0567$).

Table 4.2: Percent of adolescents with depleted, low and normal ferritin levels by study school.

School	Ferritin			Hematocrit (%)*	
	Depleted < 15µg/L(n)	Low 15–30µg/L (n)	Normal ≥30µg/L(n)	< Cutoff	> Cutoff
Adigudom	8.8(3)	11.8(4)	79.4(27)	5.3(2)	94.6(36)
Debre Haila	7.1(2)	7.1(2)	85.7(24)	17.9(5)	82.1(23)
Debub	14.3(6)	16.7(7)	69.1(29)	2.1(1)	97.9(47)
Finarua	15.8(3)	21.1(4)	63.2(12)	27.8(5)	72.2(13)
Hageresalam	5.7(3)	9.4(5)	84.9(45)	3.8(2)	96.2(50)
Hareqo	8.1(3)	24.3(9)	67.6(25)	19.3(6)	80.7(25)
Qel'ae	7.8(4)	11.8(6)	80.4(32)	5.6(2)	94.4(34)
Samre	9.5(4)	14.3(6)	76.2(32)	0.0(0)	100(46)
Tikul	7.4(4)	22.2(12)	70.4(38)	3.7(2)	96.3(52)
Total	8.9 (32)	15.3 (55)	75.8(273)	7.1(25)	92.3(326)

*: Cutoff = 37% for Finarua, 38% for Samre and Adigudom and 39% for all of the rest.

Thirty two school girls (8.9%) had depleted iron stores (ferritin < 15 µg/L) and 15.3% girls had low iron stores (ferritin 15- 30 µg/L) showing that iron deficiency is prevalent in this population (Table 4.2). The prevalence of iron depletion was higher in Finarua (15.8%) and Debub (14.3%) and lowest in Hageresalam (5.7%) (Table 4.2). Only 1.2% of the anemia was due to iron deficiency (Table 4.3).

Table 4.3: Prevalence of iron deficiency and iron deficiency anemia in 10 – 15 years old adolescent school girls from Tigray, Northern Ethiopia.

Criteria	Cut off	%
Iron deficiency (N = 360)	Serum ferritin < 15 µg/L	8.9
Anemia (N=351)	Hct < 37, 38, 39% (adj for altitude)	7.1
Iron deficiency anemia (N = 322)	Serum ferritin < 15 µg/L and Hct < 37, 38, 39% (adj. for altitude)	1.2

Anthropometric indicators including weight ($r = 0.17$, $p = 0.0012$), height ($r = 0.20$, $p = 0.0002$), HAZ ($r = 0.16$, $p = 0.0026$) and MUAC ($r = 0.16$, $p = 0.0036$) had statistically significant correlations with hematocrit values. Among the sociodemographic variables tested, only the water source for the households (three levels) was associated with iron deficiency in the school girls ($F = 3.9$, $p=0.0214$). School girls from families who consumed water from wells had lower mean ferritin levels (37.6 µg/L) than those from springs (52.0 µg/L) or taps (48.8 µg/L). School girls who reported consuming foods of animal origin had mean ferritin levels of 55.4 µg/L while those who didn't had 52.9

µg/L), but this was not a statistically significant difference ($p = 0.8050$). Fire wood collection and involvement in agricultural activities were statistically associated with hematocrit levels. School girls who got involved in agricultural activities ($F = 6.0$, $p = 0.0145$) had lower mean hematocrits than those who didn't (43.0 vs. 41.2). Similarly, girls involved in wood collection had lower hematocrits (43.5 %) than who weren't (42.7%) ($F = 4.2$, $p = 0.0422$).

Among the school girls for whom a stool test for intestinal parasites was performed, 33% had at least one intestinal parasite based on the direct microscopy method of stool analysis (Tables 4.4). The most common single and mixed parasites observed were *Entamoeba histolytica* (19.9%) and *Hymenolepis nana* (8.6%). *Schistosoma mansoni*, hookworm and malaria are among the parasites commonly associated with anemia. However, frequency of hookworm, 11 (3.3%), *Schistosoma mansoni*, 1 (0.3%) and malaria infection, 2 (0.6%) as determined by Kato, concentration and Giemsa staining methods, respectively, were rarely detected.

Table 4.4: Prevalence and type of intestinal parasites in the school girls.

Parasite	Frequency	Percent
Negative	224	66.7
<i>Ascaris lumbricoides</i>	2	0.6
<i>Entamoeba histolytica</i>	57	17.0
<i>Hymenolepis nana</i>	22	6.6
Hookworm	8	2.4
<i>Giardia lamblia</i>	4	1.2
<i>Schistosoma mansoni</i>	1	0.3
<i>Enterobius vermicularis</i>	5	1.5
Two parasites		
<i>A. lumbricoides</i> and <i>H. nana</i>	1	0.3
Hookworm and <i>E. histolytica</i>	2	0.6
<i>E. histolytica</i> and <i>G. lamblia</i>	4	1.2
Hookworm and <i>H. nana</i>	1	0.3
<i>E. histolytica</i> and <i>H. nana</i>	4	1.2
<i>G. lamblia</i> and <i>H. nana</i>	1	0.3
Total	336	

Results on serum transferrin receptors were available for 148 school girls with serum ferritin less than 20 µg/L. Their sTfR ranged between 4.5 and 20.5 mg/L with mean sTfR levels of 8.2±2.6 µg/mL. The sTfR and ferritin concentrations were

negatively correlated ($r = -0.51$, $p < 0.0001$). We used the reference intervals of sTfR in preschool children from Zimbabwe (3.9 – 9.5 $\mu\text{g/mL}$) as our reference for sTfR (Kasvosve, Gomo et al. 2007) as opposed to the normal range (2.8 – 8.3 $\mu\text{g/mL}$) provided by the kit which were the average serum sTfR results of 239 normal healthy volunteers from Kansas City, USA. The mean sTfR for school girls with depleted, low iron and normal ferritin levels was 10.5, 7.7 and 7.4 $\mu\text{g/mL}$, respectively. Serum sTfR were significantly higher in anemic school girls ($p = 0.0183$). The mean (sd) sTfR levels of the anemic school girls was 10.0 (4.8) compared to 8.0 (2.3) $\mu\text{g/mL}$ of nonanemic school girls. Correlation analysis showed that sTfR concentrations were negatively correlated with ferritin ($r = -0.51$, $p < 0.0001$) and retinol ($r = -0.20$, $p = 0.0136$) levels and positively correlated with zinc ($r = 0.21$, $p = 0.0117$). C-reactive protein (hsCRP) values were also measured to rule out infection in the school girls. Median (25th, 75th percentile) CRP was 0.4 (0.2, 0.8). Median CRP levels of the school girls with depleted iron stores, low iron stores and normal ferritin levels were 0.4, 0.5 and 0.4, respectively.

Discussion

Globally, anemia affects 47% (293 million) of preschool age children, 25% (305 million) of school age children, 42% (56 million) of pregnant women and 30% (468 million) of nonpregnant women (de Benoist, McLean et al. 2008). However, it is not clear how much of the anemia is due to iron deficiency, infection, other micronutrient deficiencies or other factors known to cause anemia. Moreover, the proportion of adolescents affected by anemia is not known despite the increased iron requirements during adolescence. The overlap of increased iron requirement for growth, onset of menses and frequent infections is believed to predispose girls from developing countries to inadequate iron stores. According to the recent WHO estimates (de Benoist, McLean et al. 2008), anemia was a severe public health problem in preschool age children, pregnant women and nonpregnant women from Ethiopia. While adolescents are also at increased risk of developing iron deficiency and iron deficiency anemia, there is a paucity of data on anemia in adolescents from Ethiopia. To the best of our knowledge, this is the first study to report on clinical and biochemical indicators of anemia from adolescent girls from Ethiopia. We had hypothesized that diminished iron stores are likely in adolescent school girls from the study communities. Our study demonstrated that anemia was a mild

public health problem in school age girls from our study communities and a quite large proportion of girls were found to have low iron stores.

Nearly 7.1% of the school girls were anemic (based on altitude adjusted hct cutoffs) and 8.9% had depleted iron stores (ferritin < 15 µg/L). Compared to the 25.4% prevalence of anemia in African 5 – 14 year old school children (WHO 2001), the prevalence of anemia in our study girls was not high (7.1%). Earlier studies have attributed the low prevalence of anemia in Ethiopia to the consumption of an indigenous cereal called *Eragrotis tef* (teff) (Hofvander 1968; Gebre-Medhin, Killander et al. 1976; Woldegebriel, West et al. 1993) which appears to contain relatively high levels of iron, >150 mg Fe/100g teff (Abebe, Bogale et al. 2007). Moreover, Ethiopian vertisols are believed to be high in iron content. Thus, some investigators have linked high iron intake to contaminant iron from the soil more than to iron intrinsic to food (Hallberg and Bjorn-Rasmussen 1981; Hallberg, Bjorn-Rasmussen et al. 1977). However, the bioavailability of contaminant iron from soil compared to the intrinsic iron from food is still debatable (Crichton and Ward 1992).

Another plausible reason for the relatively low prevalence of anemia in the school girls could be the traditional method of food preparation in the study communities. Injera, a thin and soft pancake prepared from fermented dough, is the staple food in the study communities. A study from Southern Ethiopia by Abebe and colleagues (Abebe, Bogale et al. 2007) showed that fermentation significantly reduced the hexa – (IP6) and penta-inositol (IP5) phytate contents and the phytate to iron molar ratio was reduced to < 0.4, a level that will not compromise iron absorption. When the phytate to iron ratio is below one, the inhibitory effect of phytate on iron is less important (Hurrell 2003). Though injera is served with a sauce commonly prepared from tomato and hot pepper, the high cooking temperature of the sauce is believed to destroy enhancers of iron absorption such as the heat labile ascorbic acid. However, the acids formed during the fermentation of injera could facilitate iron absorption by acting as chelating agents and increasing the solubility of contaminant and intrinsic iron (Harvey, Dexter et al. 2000; Teucher, Olivares et al. 2004), thereby boosting the iron absorption in the population.

Studies from the developing world have demonstrated geohelminth infections including hookworm (Stoltzfus, Chwaya et al. 1997; Stoltzfus, Dreyfuss et al. 1997;

Dreyfuss, Stoltzfus et al. 2000; Stoltzfus, Chwaya et al. 2000), malaria (Dreyfuss, Stoltzfus et al. 2000; Stoltzfus, Chwaya et al. 2000) and trichuriasis (Robertson, Crompton et al. 1992; Aini, Al-Mekhlafi et al. 2007) to be responsible for a substantial number of cases of iron deficiency anemia in children. The contribution of parasitic infections to anemia in our study subjects was insignificant. Unlike in other regions, hookworm, 11 (3.3%), *Schistosoma mansoni*, 1 (0.3%) and *Plasmodium vivax* 2 (0.6%) were not common in the school girls corresponding to earlier reports on parasitic infections in preschool children by Adish and colleagues (Adish, Esrey et al. 1999) from communities adjacent to our study communities. The most common parasites harbored by the school girls were *E. histolytica* and *H. nana* (Table 4.4). While it would be difficult to attribute the occurrence of anemia in the girls to these parasites, the low rates of malaria, hookworm infection and *schistosomiasis* in the study communities could partially explain the low rate of anemia in the school girls.

We found no statistically significant association between home gardens and hematocrit ($p=0.4520$) and ferritin concentrations ($p=0.7042$). Plausible reasons for such observation include; first, the vegetables in the households might be displacing other staple iron rich foods from the plates. Secondly, the school girls' involvement in home gardening activities might be depleting their iron stores for extra energy production. Third, girls may be at a disadvantage from exposure to harsh climatic conditions when involved in such energy demanding domestic activities thereby contributing to vulnerability to parasitic infections. And finally, the vegetables from the home gardening might not be used for household consumption but for income generation destined for purposes other than provision of sufficient food to the household members.

Among the sociodemographic variables tested, the water source to the household was statistically associated with ferritin values of the school girls ($F = 3.9$, $p=0.0214$). School girls from households who consumed water from wells had lower mean ferritin levels ($37.6 \mu\text{g/L}$) than those from taps ($48.8 \mu\text{g/L}$) and spring ($52.0 \mu\text{g/L}$). The reasons for such observation are not apparent. Inflammation from contaminated water could raise the ferritin values but we found no statistically significant association between CRP and the water source to the households ($p=0.9479$). So, we suggest that this scenario could be

due to contamination of the spring waters with iron from the top soil as the spring water sources are mostly open and the tap or well waters are protected.

Significant differences in hematocrit levels were observed across the ages ($F = 4.0$, $p = 0.0015$) and the different study sites ($F = 9.4$, $P < 0.0001$). The 10 year old girls had significantly lower hematocrit compared to the girls in the other age categories. This may possibly be due to increased nutrient intake in the older adolescents. School girls from Finarua, a study site with the lowest elevation (1500 masl), had low mean hematocrit levels of 40.2% compared to the other study sites. Such a significant difference in hematocrit between the schools emphasizes the importance of environmental factors other than iron nutrition.

Among the limitations of this study were the failure to collect dietary intake data and use of hematocrit. Hematocrit is an easy and rapid test but is subject to limitations as a screening tool. It is less sensitive compared to hemoglobin. During iron deficiency, the hematocrit falls after hemoglobin formation is impaired. Hematocrit values are influenced by the hydration status of an individual. Dehydration elevates hematocrit levels. However, no tests were done to evaluate the hydration status of study subjects. Therefore, the use of hematocrit rather than hemoglobin might have underestimated the true prevalence of anemia in the adolescent girls.

In conclusion, this study found little evidence to support the clinical belief that severe iron deficiency is common among adolescent school girls. Prevalence of anemia is lower in adolescent school girls compared to previous reports in children and mothers from Ethiopia. However, a large proportion of the adolescent school girls had depleted iron stores (8.9%) and low iron stores (15.3%) suggesting that the girls are at risk of developing clinical manifestations of iron deficiency when the demand for iron is increased as in menstruation or pregnancy. Their bodies don't have time to replenish stores and they may give birth to low birth weight infants thereby perpetuating the intergenerational cycle of malnutrition. Carefully designed studies to investigate the multi-causal factors for anemia in the school girls and the contribution of contaminant iron to the non-heme iron pool from the food are recommended.

CHAPTER V
VITAMIN A STATUS AND SERUM CAROTENOID LEVELS OF
ADOLESCENT SCHOOL GIRLS FROM NORTHERN ETHIOPIA

Abstract

This cross-sectional study was designed to investigate the vitamin A status and serum carotenoid levels of 413 adolescent school girls (10 – 15 year old) from Tigray, Northern Ethiopia. Mean (sd) levels of serum retinol and α -carotene were 35.9 (9.6) $\mu\text{g/dL}$ and 10.5 (3.8) $\mu\text{g/dL}$, respectively and the median (25th, 75th) of β -carotene was 46.4 (24.5, 77.9) $\mu\text{g/dL}$. Two and half percent of the school girls were vitamin A deficient ($< 20 \mu\text{g/dL}$) and 25.6% had serum retinol values indicative of marginal vitamin A status (20 – 30 $\mu\text{g/dL}$) and were thus vulnerable to vitamin A deficiency. On the basis of our clinical assessments, 3.7% had Bitot's spots and 3.2% had night blindness. Moreover, 27.4% of the girls mentioned the word "HIMA", a local term for night blindness suggesting that night blindness was a common occurrence in the communities. Serum retinol levels were significantly correlated with hematocrit ($r = 0.16$, $p = 0.0003$), α - carotene ($r = 0.19$, $p = 0.0003$) and β -carotene ($r = 0.23$, $p < 0.0001$) concentrations. Prevalence of vitamin A deficiency tended to be higher at younger ages. Serum retinol concentrations were found to vary by school ($F = 6.5$, $p < 0.0001$). The lowest mean retinol values were noted in Hareqo ($30.1 \pm 8.0 \mu\text{g/dL}$) and Debrehaila ($30.5 \pm 8.1 \mu\text{g/dL}$) and the highest in Qel'ae ($39.8 \pm 7.9 \mu\text{g/dL}$) and Debub ($39.6 \pm 10.1 \mu\text{g/dL}$). We observed no statistically significant effect of home gardens on vitamin A status of the girls ($p = 0.1208$). Age, hematocrit and β -carotene were the significant predictors of serum retinol concentrations ($r^2 = 0.14$, $F = 9.53$, $p < 0.0001$). Our results demonstrated high levels of marginal vitamin A deficiency in an age group which is not usually considered to be at risk. We therefore suggest that measures to combat vitamin A deficiency should include nutrition education to increase knowledge as well as attitudes and practices concerning the consumption of vitamin A rich foods by adolescents. Because of the diverse cultural differences in food preparation, food consumption and food taboos in different parts of the country, our study is not generalizable to all regions from Ethiopia and thus multicenter studies to substantiate our results and establish the vitamin A status of adolescents are recommended.

VITAMIN A STATUS AND SERUM CAROTENOID LEVELS OF ADOLESCENT SCHOOL GIRLS FROM NORTHERN ETHIOPIA

Introduction

Vitamin A deficiency disorders (VADD) are public health consequences attributable to vitamin A deficiency (Sommer and Davidson 2002). Vitamin A deficiency is a leading cause of preventable childhood blindness (WHO 1992), morbidity and mortality (Glasziou and Mackerra 1993) among preschool age children. VADDs are an increasingly recognized problem among women of reproductive age in many developing countries (West 2002; Ahmed, Azim et al. 2003; Gorstein, Shrestha et al. 2003; Semba, dePee et al. 2003) as well. According to a recent report from WHO (WHO 2009), one third (190 million) of the world's preschool children and 15.3% (19.1 million) of pregnant women are vitamin A deficient with the highest burden being in Africa and Southeast Asia.

Various studies from Ethiopia, have investigated the vitamin A situation in the fetus (Gebre-Medhin and Vahlquist 1984), children (Lindtjorn 1983; De Sole, Belay et al. 1987; Woldegebriel, Demeke et al. 1991; Woldegebriel, Gebru et al. 1993; Lemma and Mariam 1996; Tafesse, Fisseha et al. 1996; Zerihun and Mabey 1997; Haidar and Demissie 1999; Getaneh, Assefa et al. 2000; Kassaye, Becklake et al. 2001; Kassaye, Receveur et al. 2001; Asrat, Omwega et al. 2002; Haidar, Tsegaye et al. 2003; Kello and Gilbert 2003; EDHS 2006; Semba, Pee et al. 2008), pregnant women (Wondimkun 2002; Wondimkun 2005; EDHS 2006) and adults (Kassu, Andualem et al. 2007) and have confirmed its public health significance.

Vitamin A deficiency was not only a problem for children and pregnant women but also for adolescents. According to the study by Asrat and colleagues (Asrat, Omwega et al. 2002), the prevalence of xerophthalmia was higher in school aged children than pre-school children from Ethiopia. Another study (Woldegebriel, West et al. 1993) conducted on 14,740 school age children from seven study sites from Central Ethiopia reported Bitot's spots in 0.91% of the children, confirming the public health significance of vitamin A deficiency in school age children. Kassaye and coworkers (Kassaye, Receveur et al. 2001) conducted a cross-sectional study on 1339 school age children (6 – 9 years of age) from Northern Ethiopia and reported a prevalence of xerophthalmia of 5.8% in the

children. Nearly 8% and 60% of the children had serum retinol levels below 0.35 μ mol/L and 0.7 μ mol/L, respectively. Furthermore, 41% of the children were predicted to have low hepatic vitamin A reserves (MRDR ratio \geq 0.06). The study by Kello and Gilbert (Kello and Gilbert 2003) conducted on 360 blind school children reported that VAD and measles were the major causes of severe visual impairment/blindness in blind school children in Ethiopia.

The main purpose of assessing vitamin A status is to determine the magnitude, severity and distribution of vitamin A deficiency in a given population. However, most surveys assess its prevalence in preschool children, pregnant or lactating women. Although the body's need for vitamin A will dramatically increase during adolescence and VAD is likely to be widespread following the preschool years (Woldegebriel, West et al. 1993; Ahmed, Hasan et al. 1997; Fazio-Tirrozzo, Brabin et al. 1998; Kassaye, Receveur et al. 2001; Asrat, Omwega et al. 2002; Seema, Mishra et al. 2003; Siekmann, Allen et al. 2003; Singh and West 2004; Ahmed, Rahman et al. 2005; Ahmed, Khan et al. 2007), few data exist on the extent of VAD in school age and young adolescent children. In undernourished populations, vitamin A deficiency may extend into the preadolescent years (Singh and West 2004) which in turn may predispose girls to chronic vitamin A deficiency in adult life that could exacerbate deficiency during pregnancy and lactation (Christian, West et al. 1998). Considering the likelihood that prevention of clinical and subclinical vitamin A deficiencies will substantially reduce morbidity and mortality, it is essential to evaluate the extent and severity of vitamin A deficiency in different population groups. In this study, we employed clinical examination to detect the more severe signs of deficiencies and biochemical serum retinol to investigate the vitamin A status of the girls.

Methods

Presurvey preparation: Prior to the actual data collection, the principal investigator visited the three local Wereda Education and Health Offices to explain the purpose of the study and to randomly select the nine Complete Primary Schools (CPS) for the study. Immediately after the random selection of the schools, support letters were written to the school directors from the Wereda Education Offices (WEO). After the support letters were sent to the schools, the research team visited each school to further explain the

purpose and importance of the study to the school principals and teachers and to randomly select the schoolgirls.

Design: The study design was cross-sectional. A structured questionnaire was used to collect sociodemographic information. Blood samples were collected using standard techniques to assess the nutritional status of the school girls from biochemical indicators. The interviewers were trained to standardize the questionnaire administration. Close follow-up was made by the principal investigator of the research project during the data collection. At the end of the data collection in every study unit, a meeting was held among the research team to discuss practical problems and issues of major concern. The study was conducted in the Southeast zone of Tigray, Northern Ethiopia. The zone is one of the five zones of the National State of Tigray.

Ethical consideration: The study has been approved by the Institutional Ethics Review Committee of the College of Health Sciences at Mekelle University, the Health Research Ethics Committee at Mekelle University, the National Health Research Ethics Review Committee at the Ethiopian Science and Technology Commission and the Institutional Review Board (IRB) of Oklahoma State University, USA.

Study subjects: The study subjects were 10 – 15 year old adolescent school girls, recruited from the nine CPS. The school roster was used as a frame for randomization of the school girls. The sample size determination was based on an estimated 50% prevalence of micronutrient (vitamin A, iron, iodine and zinc) deficiencies, a 95% confidence interval for the true prevalence and a relative precision of 5%. Girls who volunteered to participate in the study were requested to come with their parents the next day or given a consent form to take to their parents for signature as evidence that the parents allowed the girls to participate in the study.

Anthropometrics: Weights of the school girls were measured to the nearest 0.1 kg on a battery powered digital scale (SECA, UNICEF, Copenhagen) and heights were measured to the nearest 0.1 cm using a wooden height-measuring board with a sliding head bar following standard anthropometric techniques (WHO 1995). For weight and height measurements, study subjects removed their shoes, emptied their pockets, removed their jackets and wore light clothing. Mid upper arm circumference (MUAC) was also measured using a non-stretching tape. Anthropometric results were calculated using the

WHO Anthroplus software (WHO AnthroPlus v1.0.2, Geneva, Switzerland) and presented as z-scores for height-for-age (HAZ), BMI and BMI-for-age z scores (BAZ). School girls below -2 HAZ and BAZ scores were classified as stunted and thin, respectively.

Blood collection and analysis: Nonfasting venous and capillary blood samples were collected aseptically by a phlebotomist following standard procedures. Blood samples were collected and processed inside rooms with lights turned off and serum samples were kept in an ice box after separation and during transport. The capillary and venous bloods were obtained from finger pricks and antecubital vein of the arm, respectively. About 5 – 10 mL of venous blood was collected from each school girl and allowed to clot in plain glass vacutainer blood collection tubes. After 15 minutes, serum was separated by centrifugation at 2500 rpm for 10 minutes. Serum was aliquoted into plastic sample tubes and stored at -20°C at the Microbiology laboratory of Mekelle University, Ethiopia until transported to the laboratory of the Department of Nutritional Sciences at Oklahoma State University, USA. Frozen samples were shipped in an insulated ice box while being air freighted to the US. On arrival samples were thawed but quite cool. The serum samples were analyzed for retinol, α -carotene, β -carotene, ferritin, zinc, CRP and soluble transferrin receptors (sTfR). Trace mineral free gloves and pipette tips were used during analysis of the serum samples.

Hematocrit: Capillary blood from a finger prick was filled into heparinized hematocrit tubes. After sealing with clay, the hematocrit tubes were then centrifuged and the hematocrit level determined using a hematocrit reader. Anemia was defined as hematocrit (at sea level) $\leq 36\%$.

Vitamin A and Carotenoids: All-trans-retinol (external standard), retinol acetate (internal standard), β -carotene, α -carotene, methanol, dichloromethane and acetonitrile, ethanol and hexane chemicals were purchased from Sigma Chemical Company (St. Louis, MO). All were High Pressure Liquid Chromatography (HPLC) grade except the analytical grade butylated hydroxytoluene (BHT), which was an analytical reagent grade. All handling operations of the serum were carried out in semi-darkness. Serum samples were analyzed by reversed phase HPLC following the methods of Talwar and colleagues (Talwar, Ha et al. 1998) with slight modification. Stock solutions of each of the

calibrators (*all-trans* retinol, β -carotene and α -carotene) and the internal standard (retinol acetate) were dissolved in methanol. For calibration, different concentrations of each of the calibrators were prepared in ethanol-BHT. The final concentration of the internal standard was 0.25 $\mu\text{g/mL}$.

Briefly, serum retinol, α -carotene and β -carotene levels were determined as follows. In a test tube, 200 μL of serum was deproteinized with an equal volume (200 μL) of ethanol-BHT. Exactly 50 μL of retinol acetate was added to the deproteinized sample and mixed by vortex for 5 seconds. Samples were then extracted by the addition of 1000 μL of hexane and mixed by vortex for 30 seconds followed by centrifugation at 2000 rpm for 3 minutes. About 800 μL of the supernatant was transferred into another test tube. The extraction was repeated for the second time and 1000 μL of the supernatant was transferred into the test tube containing the hexane extract. Samples were dried under a stream of nitrogen. Then, contents of the tube were reconstituted in 100 μL of ethanol-BHT solution and transferred to HPLC cuvetts (inserts) and placed in the autosampler compartment of the HPLC (Waters 717, Milford, MA). The injection volume was 20 μL .

For the HPLC system, an autosampler, controller and a model 2487 dual wavelength detector were used. Waters C18 guard column and Symmetric C18 column (250 x 3.9 mm (id); 4 μm bead size) was used. The chromatographic separation was performed by isocratic elution with a mixture of methanol, dichloromethane and acetonitrile (60:20:20 by volume) and at a flow rate of 0.8 mL/min. The concentrations of retinol and the carotenoids (β -carotene and α -carotene) were quantified at 325 and 450 nm, respectively.

Serum ferritin: Serum ferritin concentrations were determined by one stage, two-site immunoradiometric (sandwich) assay (Ramco Laboratories, Inc., Stafford, TX).

Serum soluble transferrin receptors (sTfR): Serum soluble transferrin receptors (sTfR) on a subsample of the girls were determined using an in vitro enzyme immunoassay based upon the double antibody sandwich method (Ramco Laboratories, Inc., Stafford, TX).

Serum zinc: Serum zinc was analyzed by Inductively Coupled Plasma Mass Spectrometer (ICPMS). All serum samples were diluted 20 times (200 μl diluted to 4 ml) with 0.1% HNO_3 (GFS Chemicals, Powell, OH) in Millipore water.

Nutritional status: Determination of the nutritional status of the school girls was based on anthropometric (stunting, thinness, MUAC) variables, biochemical indicators and clinical examinations. Biochemical vitamin A status was based on serum retinol. All girls were examined by a health officer experienced in diagnosing clinical manifestations due to vitamin A deficiency (ocular signs such as conjunctival xerosis, Bitot's spots, corneal xerosis, etc).

Statistical analysis: Statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC, USA). Student's t tests, chi square tests, correlation analysis, nonparametric tests, ANOVA, multiple and logistic regressions were employed for statistical analyses. The height, weight, age, hematocrit values, height-for-age z scores, BMI, BMI-for-age z scores, serum ferritin, serum sTfR, serum retinol, serum α -carotene, serum β -carotene, serum zinc, CRP and urinary iodine concentrations were the continuous variables. Groups including ages; schools; elevation; stunted and nonstunted; goitrous and nongoitrous; thin and normal; nutrient deficient and sufficient; anemic and nonanemic were treated as categorical variables. Socioeconomic and sociodemographic variables including location (school), home garden, water source, education level, mother's and father's occupation, type of residence, family food source, impact of food shortage, workload/physical activity, type of salt, latrine facilities and shoe wearing practices were treated as independent variables of different levels. Student's t tests were used to compare the means of dependent variables of two groups of an independent variable. Chi square tests were employed to analyze categorical variables. Correlation analysis was employed to indicate the strength of a linear relationship between two continuous variables. A test for normality was done using univariate analysis. Parameters that were not normally distributed including urinary iodine concentration, β -carotene and CRP, were presented as medians and were compared by Wilcoxon-Mann-Whitney and Kruskal-Wallis tests. Analysis of Variance (ANOVA) was used to compare the means of continuous dependent variables of three or more groups of an independent variable. Multiple regression analysis was employed to relate two or more continuous independent variables to a continuous dependent variable. Logistic regression was used to relate continuous independent variables to dichotomous dependent variables such as nutrient deficient and sufficient; stunted and nonstunted; thin and normal; goitrous and

nongoitrous; and anemic and nonanemic groups. Statistical significance was set at $p < 0.05$.

Results

The median (25th, 75th) family size of the households was 6 (5, 7). The mothers of 88.6% of the girls had no formal education. Major occupation for 81% of the mothers and 70% of the fathers was farming. The staple diets for the girls and their households were plant-based foods. Daily meal frequency was three or more times for the majority of the girls (95%). Only 5% reported that they eat only twice a day. The common source of food to the households was their own agricultural produce suggesting that the study subjects are mostly from farming households. Most households (71%) owned domestic animals but the frequency of the consumption of foods of animal origin was very low (6%) indicating that the domestic animals were used for other purposes or activities. A large proportion of the households (62%) had no home garden to grow vegetables, important sources of carotenoids.

The prevalence of vitamin A deficiency was assessed according to the recommendations of WHO (WHO 1996). Bitot's spots was diagnosed if clinical signs of vitamin A deficiency were exhibited in one or both eyes. School girls with serum retinol levels $< 20 \mu\text{g/dL}$ ($0.7 \mu\text{mol/L}$) were characterized as vitamin A deficient and those having $20 - 30 \mu\text{g/dL}$ ($0.7 - 1.05 \mu\text{mol/L}$) were characterized as low vitamin A status (WHO 1996). Mean (sd) levels of serum retinol and α -carotene were $35.9 \pm 9.6 \mu\text{g/dL}$ and $10.5 \pm 3.8 \mu\text{g/dL}$, respectively, and the median (25th, 75th) of β -carotene was 46.4 ($24.5, 77.9$) $\mu\text{g/dL}$. The mean (sd) of hematocrit, serum ferritin, serum sTfR and serum zinc were 42.9 (3.3), 48.4 (25.1), 8.2 (2.6) and 72.3 (14.4), respectively.

Sociodemographic variables such as maternal education, maternal occupation, family size, housing quality, access to toilet facilities and feeding frequency were not significantly associated with serum retinol levels in our study communities. Prevalence of vitamin A deficiency tended to be higher at 10 years of age. Our results indicated that the mean serum vitamin A concentrations increase with age ($p = 0.0003$) in the range from 10 – 14 years and decreased afterwards. The 10 year old school girls had the highest prevalence of vitamin A deficiency (6.5%) and the lowest mean serum retinol levels

(29.8±7.0 µg/dL); whereas, the 14 year old girls had the highest mean serum retinol levels (38.2±7.7 µg/dL) and none of them were vitamin A deficient.

Our biochemical analyses and clinical assessment indicated that vitamin A deficiency was a public health concern in the study communities. Two and half percent of the school girls were vitamin A deficient (<20 µg/dL) and 25.6% had serum retinol levels indicative of marginal vitamin A status (20 – 30 µg/dL) and thus were vulnerable to deficiency. Nearly 3.7% had Bitot’s spots, 3.2% had night blindness and 27.4% of the school girls mentioned the word “HIMA”, a local term for night blindness suggesting that night blindness is a common occurrence in the communities (Table 5.1).

Table 5.1: Prevalence of vitamin A deficiency disorders from clinical and biochemical indicators.

Assessment	Indicator/cutoff	% Prevalence
Ocular examination	Night blindness	3.2
	Bitot’s spots	3.7
Serum retinol	% < 20 µg/dL	2.5
	% (20 – 30) µg/dL	25.6
	% ≥30 µg/dL	71.7

As expected, serum retinol levels were significantly correlated with hematocrit ($r = 0.16$, $p = 0.0003$), α - carotene ($r = 0.19$, $p = 0.0003$) and β -carotene ($r = 0.23$, $p < 0.0001$) levels. However, we found no significant correlation between serum zinc and serum retinol levels. Serum retinol concentrations were found to vary by school ($F = 6.5$, $p < 0.0001$). The lowest mean retinol level was noted in Hareqo (30.1±8.0 µg/dL) and Debrehaila (30.5±8.1 µg/dL) and the highest in Qel’ae (39.8±7.9 µg/dL) and Debub (39.6±10.1 µg/dL) (Table 5.2).

Table 5.2: Vitamin A levels (µg/dL) of girls by study schools.

School	n	Mean(sd)*
Adigudom	34	33.2±8.2 ^b ^c
Debrehaila	28	30.5±8.1 ^c
Debub	42	39.6±10.1 ^a
Finarua	19	31.4±6.9 ^c
Hagereselam	53	37.5±9.4 ^{ab}
Hareqo	36	30.1±8.0 ^c
Qel’ae	51	39.8±7.9 ^a
Samre	42	36.7±8.1 ^{ab}
Tikul	54	36.9±11.4 ^{ab}
p	<0.0001	

*: Schools with different letters differ significantly ($r^2 = 0.16$, $F = 6.4$, $p < 0.0001$).

We observed no statistically significant effect of home gardening on vitamin A status of the girls ($p = 0.1208$). However, girls from households with home gardens had slightly higher mean serum retinol levels ($36.8\mu\text{g/dL}$) than those from households who didn't possess home gardens ($35.2\mu\text{g/dL}$). Serum retinol was positively correlated with the β -carotene ($r = 0.23$, $p < 0.000$) and α -carotene ($r = 0.19$, $p = 0.0003$). According to our multiple regression analysis, the significant predictors of serum retinol were age, hematocrit and β -carotene ($R^2 = 0.14$, $F = 9.53$, $p < 0.0001$).

Discussion

The prevalence of clinically evident vitamin A deficiency among preschool children in Ethiopia has decreased significantly over the past two decades. The decrease can be attributed largely to the routine biannual vitamin A supplementation of 200,000 IU to under-five children. However, it was not possible to identify trends in vitamin A status in school age children. This is the first report on the biochemical indicators of school age children and so there were no studies with which to compare our results.

We found moderate vitamin A deficiency based on our clinical assessments ($1\% \leq \text{XN} \leq 5\%$) and a biochemical evidence for mild vitamin A deficiency ($\geq 2\% - < 10\%$ for serum retinol levels below $20\mu\text{g/dL}$) in the school girls from our study communities. The findings of the present study showed that 2.5% of the girls were vitamin A deficient ($< 20\mu\text{g/dL}$) and 25.6% of the school girls had marginal vitamin A deficiency ($20\text{-}30\mu\text{g/dL}$). Moreover, the prevalence of night blindness and Bitot's spot were 3.2% and 3.7%, respectively, indicating the public health significance of vitamin A deficiency in the school girls. Moreover, the fact that many school girls mentioned the local term for night blindness suggested that night blindness is a well recognized phenomenon in the communities.

Location had a significant influence on serum retinol concentrations of the school girls ($F = 6.5$, $p < 0.0001$). The reasons for such spatial differences were not apparent which of course might have been identified by the collection of dietary intake data.

No report is available on vitamin A status of adolescents from Ethiopia. However, the prevalence of xerophthalmia (night blindness and Bitot's spot in our study subjects (6.8%) was slightly higher compared to the 5.8% of xerophthalmia (defined as any clinical signs of vitamin A deficiency in one or both eyes) in 6 – 9 year old school age

children from Tigray, Northern Ethiopia (Kassaye, Receveur et al. 2001). Serum retinol concentrations of 10 $\mu\text{g}/\text{dL}$ or less are indicative of severe vitamin A deficiency or depleted liver stores of the vitamin (WHO 1996). Because vitamin A is stored in the liver, low serum concentrations of vitamin A reflect not only a low intake of the nutrient but also depleted liver stores. However, none of the school girls from our study subjects had serum retinol levels below 10 $\mu\text{g}/\text{dL}$ compared to 8.4% from the previous study by Kassaye and colleagues (Kassaye, Receveur et al. 2001). Compared to previous reports of 0.9% rates of night blindness (Haidar, Demisse et al. 1999) and 0.5% Bitot's spots (Haidar, Tsegaye et al. 2003) in preschool children from adjacent communities, the older adolescent girls from our study had higher rates of night blindness (3.2%) and Bitot's spots (3.7%). This could partly be explained by the relatively good coverage for biannual provision of 200,000 IU of vitamin A supplementation for all one to five year old children from Tigray (Haidar, Tsegaye et al. 2003; Semba, Pee et al. 2008) confirming the notion that the gains achieved during the preschool age years could only be sustained by investing in the school age years as well. The increase in the prevalence in night blindness and Bitot's spots with increasing age could also be due to vitamin A deficiency in the past.

Low household socioeconomic status is typically associated with xerophthalmia (Tielsch and Sommer 1984; Tielsch, West et al. 1986; Cohen, Rahman et al. 1987; Mele, West et al. 1991; West 1991; Hussain, Kvale et al. 1993; Nestel, Herrera et al. 1993; Rosen, Sloan et al. 1994). However, we found no significant association between serum retinol and socioeconomic variables such as maternal education, maternal occupation, family size, housing quality, access to toilet facilities and feeding.

School girls from households with home gardens had mean serum retinol of 36.8 $\mu\text{g}/\text{dL}$ compared to the 35.2 $\mu\text{g}/\text{dL}$ for girls from households who didn't report home gardening. The non-significant difference in mean serum retinol was not surprising as the bio-conversion of carotenoids to retinol is a complex process influenced by numerous factors including the species of the carotenoid and molecular linkage; amount of carotenoid consumed in a meal; the source food matrix; fat in the diet; enhancers or inhibitors of digestion, absorption and bioconversion; nutritional status; genetic makeup and health status of the host and nutrient interactions (Castenmiller and West 1998; West

and Castenmiller 1998; West, Eilander et al. 2002; Blomhoff and Blomhoff 2006). Despite the low bioavailability of carotenoids, girls with higher serum carotenoids had higher concentrations of serum retinol. β -carotene was the only significant predictor of serum retinol levels among the carotenoids. Regardless of the low bioavailability of the carotenoids, promotion of dark green leafy vegetables seems to be an effective measure to improve vitamin A status of adolescents who have little access to foods containing preformed vitamin A.

Different studies (Molla, Khurhsid et al. 1993; Strube, Beard et al. 2002) have shown anemia to be a predictor of vitamin A deficiency. This was confirmed in this study. Multiple regression analysis showed hematocrit to be a predictor of serum retinol concentrations ($R^2 = 0.15$, $p = 0.0124$) suggesting unimpaired hepatic mobilization of retinol due to the relatively good iron nutritional status of the adolescent school girls.

Among the limitations of this study was the cross-sectional nature of the study, failure to collect dietary intake and the fact that school girls were not representative of all adolescent girls in the study communities. Adolescent girls who didn't attend schools are from relatively poor families and thus will be more vulnerable to food shortage and micronutrient deficiencies than the girls who had the chance to attend schools.

In summary, the high prevalence of night blindness and Bitot's spots together with the proportion of girls (25.6%) with marginal vitamin A deficiency (low serum retinol levels), suggested the existence of a serious public health risk in the school girls from Southeast Tigray, who had little access to preformed vitamin A sources. Vitamin A deficiency has been associated with increased risk of morbidity and mortality in pregnant women (Christian, West et al. 1998; Christian, West et al. 2000). In our study communities, where early marriage is common practice, adolescent school girls should thus be considered as high priority groups in terms of their vitamin A nutriture. Our results demonstrated high levels of marginal vitamin A deficiency in an age group not usually considered to be at risk. Approaches including nutrition education to increase knowledge as well as attitudes and practices concerning the consumption of vitamin A rich foods and encouraging home and school gardening are recommended. Our results could serve as a template for further research on vitamin A deficiency and other health related problems in adolescents in Ethiopia. Moreover, the information generated from

this study is of considerable value in indicating the magnitude of the problem and especially providing an indication of the magnitude of marginal vitamin A deficiency in this age group. Because of the diverse cultural differences in food preparation, food consumption and food taboos in different parts of the country, our study is not generalizable to all regions from Ethiopia and thus multicenter studies to establish the vitamin A status of adolescents is recommended.

CHAPTER VI

Severe iodine deficiency in adolescent school girls from Northern Ethiopia in the face of the decades-old knowledge to prevent it

Abstract

The present school-based cross-sectional study was designed to assess the severity of iodine deficiency in school age girls from Tigray, Northern Ethiopia. A total of 413 school girls (10-15 years old) were randomly drawn from nine schools. A large proportion of the school girls (52%) didn't know the cause of goiter, and none of them mentioned the effect of iodine deficiency on cognition or reproductive losses. Only 16% of the households utilized adequately iodized salt. The total goiter rate was 45.3%. The median (25th, 75th percentile) urinary iodine level was 50.2 (28.1, 135.5) $\mu\text{g/L}$. Nearly 67% of the girls had biochemical evidence of iodine deficiency (UI levels $< 100 \mu\text{g/L}$). None of the girls had UI levels $>300 \mu\text{g/L}$. The goiter prevalence in the iodine deficient and sufficient girls was 52.1 and 20.2%, respectively. School girls with goiter had significantly lower median UI levels (36.0 $\mu\text{g/L}$) than the girls without thyroid enlargement, 89.7 $\mu\text{g/L}$ ($p<0.0001$). Girls from households who utilized adequately iodized salt had higher median UI levels compared to those girls from households who consumed noniodized salt ($p=0.0251$). Our ANOVA revealed a significant association between the UI levels and the water source of the households ($p<0.0001$). Our results substantiated that iodine deficiency is a widespread public health problem in the study communities. It is likely that the population from the study communities suffers from some degree of impaired cognition. We suggest implementation of educational programs about IDD prevention in schools as well as intervention measures to ensure accessibility of adequately iodized salt to the study communities.

Severe iodine deficiency in school age girls from Northern Ethiopia in the face of the decades-old knowledge to prevent it

Introduction

Iodine deficiency disorders (IDD) still remain public health problems globally. Amongst the earliest clinical feature of suboptimal iodine nutrition is goiter, and cretinism is an extreme form of neurological damage from fetal hypothyroidism (Escobar, Obregón et al. 2007; Glinöer 2007; Zimmermann 2007). Various studies have correlated suboptimal iodine intake with increased incidence of mental retardation and have estimated mean IQ score may be lowered by 0.9 SD, or 13.5 IQ points, in iodine deficient areas (Bleichrodt and Born 1994). Iodine deficiency is also associated with increased reproductive failure (Pharoah, Butfield et al. 1971; Potter, McMichael et al. 1979; Bernal and Nunez 1995; Glinöer 1997; Chan and Kilby 2000; Dillon and Milliez 2000; Glinöer and Delange 2000; Abuye and Berhane 2007).

It is estimated that 36.5% (285.4 million) of school age children are at risk of iodine deficiency. The largest numbers of school age children with low iodine intake are from Southeast Asia (96 million) and Africa (50 million) (de Benoist, McLean et al. 2008). Various epidemiological studies have shown the public health significance of IDD in Ethiopia (Woldegebriel, West et al. 1993; Abuye and Urga 2000; Cherinet and Kelbessa 2000; Takele, Belachew et al. 2003; Abuye and Berhane 2007; Bezabih, Assefa et al. 2007). About three quarters of the Ethiopian population was estimated to be at risk for IDD in 1995 (MOH 1995). In 1995, nearly 78% (42 million) of the total population were exposed to iodine deficiency, 62% (35 million) were iodine deficient and 26% (14 million) had goiter with about 50,000 prenatal deaths attributable to iodine deficiency (MOH 1995). The first nationally representative IDD survey from Ethiopia was conducted in 1981-82 (Woldegebriel, Demeke et al. 1993). This household and school-based survey from 38 provinces, 19,158 households and 35,635 school children revealed a total goiter rate (TGR) of 36.1% in females and 25.1% in males (Woldegebriel, Demeke et al. 1993). Another cross-sectional study carried out in the late 1990s on 2485 school children from 10 villages across four regions of the country reported that 53.3% had goiter. Based on urinary iodine excretion, 70% had moderate iodine deficiency and 30% of the children had mild iodine deficiency (Abuye and Urga 2000). Recently, Abuye

and colleagues revealed a weighted prevalence rate for total goiter of 39.9% in children 6 to 12 yrs of age (Abuye, Berhane et al. 2007) and a total goiter prevalence of 35.8% among 15-49 years old Ethiopian women (Abuye and Berhane 2007).

Despite a known effective intervention strategy to prevent it, iodine deficiency has been increasing and getting worse in Ethiopia (Abuye, Berhane et al. 2008). This was corroborated by the recent press release from the Ethiopian Ministry of Health announced at the inaugural ceremony of the establishment of a central iodization facility at Afdera (MOH 2009). According to this press release, less than 5% of Ethiopian households have access to adequately iodized salt (MOH 2009). An accompanying press release from the Micronutrient Initiative, Ethiopia, further indicated the worsening of the country's goiter rate from 26% in the 1980s to about 40% today (MI 2009). Women of child bearing age with poor iodine nutrition are at increased risk for maternal losses. Considering the traditional practice of early marriage in the study communities, the adolescent period could be a perfect window of opportunity to address future reproductive failures and other IDD associated consequences with many socioeconomic ramifications. Thus, the objective of this study was to investigate the risk factors and severity of iodine deficiency in school age girls and examine the relationship of iodine deficiency with other micronutrient deficiencies.

Methods

Presurvey preparation: Prior to the actual data collection, the principal investigator visited the three local Wereda Education and Health Offices to explain the purpose of the study and to randomly select the nine Complete Primary Schools (CPS) for the study. Immediately after the random selection of the schools, support letters were written to the school directors from the Wereda Education Offices (WEO). After the support letters were sent to the schools, the research team visited each school to further explain the purpose and importance of the study to the school principals and teachers and to randomly select the schoolgirls.

Design: The study design was cross-sectional. A structured questionnaire was used to collect sociodemographic information. Blood and urine were collected using standard techniques to assess the nutritional status of the school girls from biochemical indicators. The interviewers were trained to standardize the questionnaire administration. Close

follow-up was made by the principal investigator of the research project during the data collection. At the end of the data collection in every study unit, a meeting was held among the research team to discuss practical problems and issues of major concern. The study was conducted in Southeastern zone of Tigray, Northern Ethiopia. The zone is one of the five zones of the National State of Tigray.

Ethical consideration: The study has been approved by the Institutional Ethics Review Committee of the College of Health Sciences at Mekelle University, the Health Research Ethics Committee at Mekelle University, the National Health Research Ethics Review Committee at the Ethiopian Science and Technology Commission and the Institutional Review Board (IRB) of Oklahoma State University, USA.

Study subjects: The study subjects were 10 – 15 year old adolescent school girls, recruited from the nine CPS. The school roster was used as a frame for randomization of the school girls. The sample size determination was based on an estimated 50% prevalence (p) of micronutrient (vitamin A, iron, iodine and zinc) deficiencies, a 95% confidence interval for the true prevalence and a relative precision (d) of 5%. The formula adopted was $z^2p(1-p)/d^2$ where $z = 1.96$, $p = 0.5$ and $d = 0.05$. The calculated sample size was 385. Assuming a dropout rate of 15%, a total of 50 adolescent school girls were recruited from the nine CPS. Girls who volunteered to participate in the study were requested to come with their parents the next day or given a consent form to take to their parents for signature as evidence that the parents allowed the girls to participate in the study.

Anthropometrics: Weights of the school girls were measured to the nearest 0.1 kg on a battery powered digital scale (SECA, UNICEF, Copenhagen) and heights were measured to the nearest 0.1 cm using a wooden height-measuring board with a sliding head bar following standard anthropometric techniques (WHO 1995). For weight and height measurements, study subjects removed their shoes, emptied their pockets, removed their jackets and wore light clothing. Mid upper arm circumference (MUAC) was also measured using a non-stretching tape. Anthropometric results were calculated using the WHO Anthroplus software (WHO AnthroPlus v1.0.2, Geneva, Switzerland) and presented as z-scores for height-for-age, BMI and BMI-for-age z scores (BAZ). School girls below -2 HAZ and BAZ scores were classified as stunted and thin, respectively.

Urine collection and analysis: Each girl was given a screw-capped plastic cup in which she collected 5 – 10 mL of middle urine. The urine samples were then transported to the nearby clinic for refrigeration and later in the day were transported to the Microbiology Laboratory at Mekelle University. The urine samples were aliquoted into 500 μ L eppendorf tubes and stored at -20°C at the Microbiology Department of the College of Health Sciences at Mekelle University until transported to the laboratory of the Department of the Nutritional Sciences at Oklahoma State University, USA for iodine analysis.

The Urinary Iodine (UI) concentration was determined by digesting the urine with ammonium persulfate following the Sandel-Kolthoff reaction (WHO 2001). In this method, iodine is determined by employing its catalytic role in the reduction of ceric ammonium sulfate to cerrous ion coupled to the oxidation of arsenous acid (i.e. As^{+3} to As^{+5}). As the reduction proceeds, the intensity of color decreases. The concentration of iodine was determined using a spectrometer at 405 nm.

Specifically, the urinary iodine concentration was determined as follows: 250 μ L urine samples and standards or calibrators were added into pyrex glass test tubes (13x100 mm) and digested with 1 mL of 1M ammonium persulfate at 95°C for 60 minutes in a heating block. After cooling the tube contents to room temperature, 2.5 ml of arsenous acid was added to the tubes containing samples and standards and vortexed briefly. After 15 minutes of reaction time, 300 μ L of ceric ammonium sulfate was added, vortexed briefly and the rate of the yellow color disappearance was measured after exactly 30 minutes using a Beckmann spectrometer (DU 800, Fullerton, CA). The color intensity was due to the catalytic reduction of ceric ammonium sulfate in the presence of arsenous acid. WHO cutoff points for urinary iodine levels were used to define iodine status: deficiency $< 100 \mu\text{g/L}$ (severe deficiency $<20\mu\text{g/L}$; moderate deficiency 20-49 $\mu\text{g/L}$; mild deficiency 50-99 $\mu\text{g/L}$), optimal 100 – 199 $\mu\text{g/L}$, more than adequate 200-299 $\mu\text{g/L}$, excessive $\geq 300 \mu\text{g/L}$ (WHO 2007).

Nutritional status: Determination of the nutritional status of the school girls was based on anthropometric (stunting, thinness, MUAC) variables, biochemical indicators and clinical examinations. Biochemical iodine status was based on urinary iodine levels; iron status on ferritin levels; zinc status on serum zinc and vitamin A status on serum retinol.

All girls were examined by a health officer experienced in diagnosing clinical manifestations due to iodine deficiencies (thyroid size by palpation). Thyroid size was graded according to WHO criteria (WHO 2007) as follows: grade 0 (no palpable or visible goiter), Grade 1 (palpable but not visible goiter), grade 2 (visible goiter with neck in the normal position).

Statistical analyses: Statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC, USA). Student's t tests, chi square tests, correlation analysis, nonparametric tests, ANOVA, multiple and logistic regressions were employed for statistical analyses. The height, weight, age, hematocrit values, height-for-age z scores, BMI, BMI-for-age z scores, serum ferritin, serum sTfR, serum retinol, serum α -carotene, serum β -carotene, serum zinc, CRP and urinary iodine concentrations were the continuous variables. Groups including ages; schools; elevation; stunted and nonstunted; goitrous and nongoitrous; thin and normal; nutrient deficient and sufficient; anemic and nonanemic were treated as categorical variables. Socioeconomic and sociodemographic variables including location (school), home garden, water source, education level, mother's and father's occupation, type of residence, family food source, impact of food shortage, workload/physical activity, type of salt, latrine facilities and shoe wearing practices were treated as independent variables of different levels. Student's t tests were used to compare the means of dependent variables of two groups of an independent variable. Chi square tests were employed to analyze categorical variables. Correlation analysis was employed to indicate the strength of a linear relationship between two continuous variables. A test for normality was done using univariate analysis. Parameters that were not normally distributed including urinary iodine concentration, β -carotene and CRP, were presented as medians and were compared by Wilcoxon-Mann-Whitney and Kruskal-Wallis tests. Analysis of Variance (ANOVA) was used to compare the means of continuous dependent variables of three or more groups of an independent variable. Multiple regression analysis was employed to relate two or more continuous independent variables to a continuous dependent variable. Logistic regression was used to relate continuous independent variables to dichotomous dependent variables such as nutrient deficient and sufficient; stunted and nonstunted; thin and normal; goitrous and

nongoitrous; and anemic and nonanemic groups. Statistical significance was set at $p < 0.05$.

Results

Nearly 5.6% of the girls were from lowland areas (< 2000 masl) and 94.4% were from highland areas (\geq 2000 masl). The median family size of the households was six. The mothers of 88.6% of the girls had no formal education. Major occupation for 81% of the mothers and 70% of the fathers was farming. The staple diets for the girls and their households were plant-based foods. Three quarters of the girls reported that the food source for their households was their own agricultural production. Most households (71%) owned domestic animals but the frequency of the consumption of foods of animal origin was very low (6%) suggesting that the domestic animals were used for other purposes or activities. None of the households had access to sea foods, the good sources of dietary iodine. Nearly 84% of the households utilized noniodized salt and 60% of the households use ganfur or geilla (local terms for the noniodized, locally mined salt which is transported to local communities by camel caravans of local traders) for household consumption. Avoidance of bread for girls with goiter has been mentioned as the most common food taboo associated with goiter and tattooing as the preventive method against goiter in the study communities.

Anthropometric characteristics and biochemical indicators are given in Table 6.1.

Table 6.1: Anthropometric and biochemical indicators of iodine deficient and sufficient study subjects.

Variables	Iodine (< 100 $\mu\text{g/L}$)		Iodine (\geq 100 $\mu\text{g/L}$)		p
	n	Mean (sd)	n	Mean(sd)	
Age, yr	213	12.8(1.5)	104	12.4(1.4)	0.03
HAZ	211	-1.3(1.1)	104	-1.2(1.0)	0.38
BAZ	211	-1.5(1.0)	104	-1.5(0.9)	0.93
Hct, %	179	42.9(3.6)	93	42.7(3.0)	0.65
Ferritin, $\mu\text{g/L}$	182	48.9(24.0)	92	48.4(26.3)	0.88
Zinc, $\mu\text{g/dL}$	180	74.1(14.1)	90	68.5(14.4)	0.003
Retinol, $\mu\text{g/dL}$	181	35.8(9.2)	92	36.4(10.2)	0.64

Goiter (thyroid enlargement) was used as the clinical variable and urinary iodine excretion (UIE) as the biochemical variable to evaluate the iodine status of the adolescent school girls. Clinical examination of the school girls revealed that 45.3% had goiter indicating a severe iodine deficiency on the basis of the WHO criteria (WHO 2007).

School girls with goiter had significantly lower median urinary iodine levels (36.0 $\mu\text{g/L}$) than the girls without thyroid enlargement (89.7 $\mu\text{g/L}$) ($\chi^2 = 36.9$, $p < 0.0001$). Moreover, the median (25th, 75th percentile) urinary iodine level, indicator of recent iodine intake, was 50.2 (28.1, 135.5) $\mu\text{g/L}$ which was far less than the optimal iodine intake (100 – 200 $\mu\text{g/L}$). These data suggest a high percentage of adolescent girls living with the curse of iodine deficiency. On the basis of the WHO criteria (WHO 2007), 67.2% of the school girls were iodine deficient (UIE < 100 $\mu\text{g/L}$) and 32.8% were iodine sufficient ≥ 100 $\mu\text{g/L}$). Mild, moderate and severe iodine deficiency was detected in 17.7%, 33.1% and 16.4%, respectively, of the school girls. None of the girls had UI >300 $\mu\text{g/L}$ indicating the low risk of iodine toxicity in the population. The median UI concentrations of the girls from the high altitude areas were lower 49.8 (27.9, 143.9) $\mu\text{g/L}$ than those living in the low altitude areas, 65.5 (27.9, 94.5) $\mu\text{g/L}$ ($p > 0.05$). The median was used instead of the mean as indicator of the status of current iodine nutrition because its frequency distribution was skewed towards low values. Our results revealed striking differences in the rate of goiter in adjacent schools ranging from 0% in Qel'ae to 80% in Samre. Three schools, namely Hagereselam, Tikul and Qel'ae, are from adjacent districts with a striking difference in goiter rate ranging from a complete absence of goiter in Qel'ae to 76.4% in Hagereselam (Table 6.2). Median UI levels were high in Adigudom (202 $\mu\text{g/L}$) and lowest in Debub (16.7 $\mu\text{g/L}$) (Table 6.2). However, the highest percentages of school girls with a UI level less than 50 $\mu\text{g/L}$ were from Samre (100%) and Debub (95.6%). Similarly, the highest percentage of girls from Debub (57.8%) had severe iodine deficiency.

Table 6.2: Prevalence of IDD in adolescent school girls from Tigray, Northern Ethiopia.

School	Goiter		UIE, $\mu\text{g/L}$				
	N	%	n	< 20 $\mu\text{g/L}$, %	20 - <50 $\mu\text{g/L}$, %	50 -< 100 $\mu\text{g/L}$,%	Median, $\mu\text{g/L}$
Adigudom	44	29.6	43	2.3	0.0	2.3	202.2
Debrehaila	38	52.6	30	13.3	73.3	13.3	33.9
Debub	48	68.8	45	57.8	37.8	4.4	16.7
Finarua	23	13.0	21	4.8	28.6	47.6	65.5
Hagereselam	55	76.4	40	20.0	35.0	30.0	47.0
Harego	44	63.6	32	3.1	71.9	25.0	44.7
Qeel'ae	53	0.0	33	3.0	39.4	33.3	64.3
Samre	50	80.0	20	50.0	50.0	0.0	19.8
Tikul	56	12.5	53	0.0	0.0	15.1	149.4
Total	411	45.3	317	16.4	33.1	17.7	50.2

Our results demonstrated a statistically significant association between UI levels and age ($\chi^2 = 11.6495$, $p=0.0399$). The rate of goiter was highest at 11 years of age (60.0%) and the lowest frequency of goiter was observed in the 10 year old girls (37.5%) (Table 6.3). None of the 10 year old school girls had a visible goiter and the highest percentages of girls with visible goiter were from the 15 year age category (Table 6.3).

Table 6.3: Distribution of total goiter rate by age in adolescent school girls.

Age	N	Goiter grade, %							
		0		1		2		Total Goiter rate (TGR)	
		N	%	n	%	n	%	n	%
10	32	20	62.5	12	37.5	0	0	12	37.5
11	60	24	40.0	34	56.7	2	3.3	36	60.0
12	76	46	60.5	26	34.2	4	5.3	30	39.5
13	110	60	54.6	39	35.5	11	10.0	50	45.5
14	83	50	60.2	29	34.9	4	4.8	33	39.8
15	50	25	50.0	19	38.0	6	12.0	25	50.0
Total	411	225	54.7	159	38.7	27	6.6	186	45.3

School girls from households utilizing iodized salt had higher median UI levels (93.6 $\mu\text{g/L}$) than those who used nonrefined noniodized (45.4 $\mu\text{g/L}$) and refined noniodized salt (58.9 $\mu\text{g/L}$) ($\chi^2 = 9.34$, $p = 0.0251$). According to the results from the questionnaire, nearly 84% of the households used noniodized salt (Table 6.4). Nearly 60% of the households used ganfur or geilla (local terms for the noniodized and non-refined locally mined salt) for household consumption. Compared to the African average

of 66.6% for access to iodized salt (de Benoist, McLean et al. 2008), the percentage of households which utilized iodized salt (16.2%) was much lower in our study communities.

Table 6.4: Effect of type of salt used for household consumption on the median urinary iodine levels.

Salt type	Households, n(%)	Median UIE values, $\mu\text{g/L}$ (n)
Noniodized, nonrefined (Ganfur)	235(60.4)	45.4(175)
Noniodized, refined	91(23.4)	58.9(72)
Iodized salt	63(16.2)	93.6(53)

A total of 82 (25.9%) of the adolescent school girls were at risk of both iodine ($< 100 \mu\text{g/L}$) and vitamin A ($< 30 \mu\text{g/dL}$) deficiencies. Among the sociodemographic variables, the water source to the households showed a strong association with urinary iodine concentrations ($p < 0.0001$). School girls from households who used springs as their source of water had higher median UIE levels ($135.1 \mu\text{g/L}$) than those girls from households consuming water from wells ($75.2 \mu\text{g/L}$) or tap/hand pump water sources ($43.1 \mu\text{g/L}$) ($\chi^2 = 23.79$, $p < 0.0001$).

Discussion

Our findings demonstrated that iodine deficiency and its health consequences comprised a major public health problem in the adolescent school girls from the study communities. The high goiter rate (45.3%), an obvious sign of chronic iodine deficiency, suggested sustained inadequate iodine nutrition in the study communities. What is worrisome is that the situation is worse when compared to the gross goiter prevalence of 30.6% in 1980-1981 (Woldegebriel, Demeke et al. 1993) and the total goiter weighted prevalence rate of 39.9% in 2005 (Abuye, Berhane et al. 2007). The median urinary iodine level ($50.2 \mu\text{g/L}$) was much lower than the WHO cut off with a high proportion (67.2%) of girls having UI levels lower than $100 \mu\text{g/L}$. Median urinary iodine excretion obtained in the present study was much higher ($50.2 \mu\text{g/L}$) than the median UIE reported earlier (median = $24.5 \mu\text{g/L}$) in 2007 (Abuye, Berhane et al. 2007). However, the difference in the value for urinary iodine excretion could be attributed to differences in methods of analysis as well as to an increased intake of iodine.

The prevalence of goiter in the schoolgirls as assessed by palpation (45.3%) was lower than the prevalence of iodine deficiency as determined by urinary iodine levels (67.2%). Considering the low access of the girls and their households to iodized salt and iodine rich foods and the increased sensitivity and accuracy of urinary iodine levels, the results of the UI seem to better describe the magnitude of the problem in the study communities. Therefore, despite the large variation in daily iodine excretion, assessment of iodine concentration from casual urine samples remains a valuable method for evaluating iodine status.

Urinary iodine levels in the present study varied with age. There was a significant difference in UI levels across the age range ($\chi^2 = 11.65$, $p=0.0399$). It is possible that more of the older girls were experiencing their pubertal growth spurt and thus had higher iodine requirements for growth than their younger counterparts. Whether these age-related differences had any adverse functional health consequences for the girls is unknown. Considering the practice of early marriage in the study areas, their offspring will face the serious consequences of maternal iodine deficiency if the deficiencies are not corrected before their first pregnancy.

Our study showed that the prevalence of goiter varied among the girls from the different schools. Prevalence rates were high in the highlands. Previous reports from Ethiopia (Nekatebeb 1993; Woldegebriel, Demeke et al. 1993; Cherinet and Kelbessa 2000) have reported an increase in prevalence of iodine deficiency with elevation. Such differences could be explained by differences in environmental factors, including iodine in drinking water, leaching out of iodine and iodine intake rather than differences in the habits of the population and their economic resources for the purchase of iodized salt. This may suggest the involvement of other factors in the genesis of thyroid enlargement besides iodine deficiency, which warrants further investigation.

The iodine status of our study girls and utilization of iodized salt of the households was far worse compared to their counterparts from around Sub-Saharan Africa. According to de Benoist and colleagues (de Benoist, McLean et al. 2008) , the prevalence of biochemical iodine deficiency (UI < 100 $\mu\text{g/L}$) in 6-12 yrs old African school age children was 40.8% and the percentage of households with access to iodized salt was 66.6%. However, in the present study, the prevalence of biochemical iodine

deficiency in the school girls was 67.2% and households who utilized iodized salt were 16.2%.

Awareness of iodine nutrition among the adolescent population was generally low. Nearly, 52.4% of the school girls didn't know the cause of goiter. During the field survey, the school girls indicated that they believed the unrefined salt (ganfur or geilla) had a better flavor than the iodized salt. Such unawareness in the adolescent school girls might imply that the communities are possibly ignorant of other health and nutrition issues as well. Moreover, we observed during the survey time that the cost of iodized and noniodized salt was the same. Thus, lack of awareness of the importance seems to be the main reason for failure to choose iodized salt for household consumption. This finding is disappointing in view of the decades old health and nutrition education efforts in the region. Without sustained health and nutrition education, the acceptability of the unrefined salt in the communities will continue to be a major challenge to increasing iodized salt coverage. Sustained nutrition education to create awareness of the importance of adequate iodine intake and ensuring the utilization of an adequately iodized salt at an affordable price should be important components of the iodine program in the region. Even brief reports in the media about iodine deficiency and the benefits of using iodized salt might influence health related decisions in these communities (Lie, Chapman et al. 2008). The nutrition education should focus on the damage to reproduction and mental retardation and its contribution to at least five of the Millennium Development Goals (Zimmermann, Jooste et al. 2008) rather than equating iodine deficiency with goiter, commonly perceived as a cosmetic problem by the girls and the communities.

School girls who reported knowing someone with goiter from their community or family had significantly lower mean UI levels than those who reported otherwise. This might reflect the possibility that there has been a legacy of preexisting iodine deficiency in their family and/or community that has not yet been resolved by adequate intake of iodine.

Cross-sectional studies in Senegalese adults (Ingenbleek, Luypaert et al. 1980) and Ethiopian children (Woldegebriel, West et al. 1993) have shown that vitamin A deficiency is associated with increased risk for goiter. Vitamin A deficiency interferes with iodine metabolism by decreasing thyroidal uptake of iodine, impairing thyroglobulin

synthesis, increasing TSH stimulation and increasing size of thyroid (Raz and Goodman 1969; Morley, Damassa et al. 1978; Morley, Melmed et al. 1980; Oba and kimura 1980; Higuieret and Garcin 1984; Higuieret, Pailler et al. 1989; Zimmermann, Wegmuller et al. 2004). The relatively good vitamin A status in our study subjects could explain the failure to see a noticeable effect of vitamin A on goiter rate. Vitamin A – iodine interaction has only been reported when the prevalence of clinical and biochemical signs of vitamin A deficiency are high (Zimmermann, Wegmuller et al. 2004).

Studies in humans and animals have shown that iron deficiency impairs thyroid metabolism but we found no correlation between iron status and goiter prevalence supporting an earlier report from Ethiopia (Woldegebriel, West et al. 1993). The effect of iron could have been masked by the relatively adequate iron status of the school girls in our study.

Women with poor iodine nutrition are at increased risk for reproductive losses. Various epidemiological studies have shown a direct proportionality between the frequency of reproductive failure and severity of iodine deficiency (Pharoah, Butfield et al. 1971; Potter, McMichael et al. 1979; Bernal and Nunez 1995; Glinoe 1997; Chan and Kilby 2000; Dillon and Milliez 2000; Glinoe and Delange 2000; Abuye and Berhane 2007). Moreover, impaired synthesis of thyroid hormones is associated with greater incidence of brain damage, reproductive losses and congenital abnormalities (Pharoah, Butfield et al. 1971; Bernal and Nunez 1995; Chan and Kilby 2000; Glinoe and Delange 2000). Considering the high prevalence rates of IDD and parental preference for early marriage in the study communities, the adolescent period is the perfect window of opportunity to address future reproductive failures and other IDD associated consequences with many socioeconomic ramifications in our study communities. So, we suggest that severe consequences of iodine deficiency, including cretinism and reproductive losses such as neonatal deaths, stillbirths and miscarriages, could be frequent in the study communities.

Amongst the limitations of this study was the higher refusal rate of the school girls from Finarua. In this school, 54% of the girls failed to return signed parental consent forms. Secondly, we didn't collect salt from each household and hence we were not able to determine the iodine content of the salt from the households. Third, the school girls

were not representative of the entire economic strata. Adolescent girls who didn't go to school were likely to be more disadvantaged and were not included.

In conclusion, this study was conducted to investigate the prevalence and severity of iodine deficiency in adolescent school girls. Iodine deficiency was a widespread health problem in these communities as evidenced by the high rate of goiter and low concentration of urinary iodine. Thus, serious consequences of iodine deficiency are likely to occur in these communities. Though we don't have the information regarding cretin rates and maternal losses, goiter rates were as high as 80% in some schools. Thus, further investigation to determine the extent of these consequences in the study communities is warranted. Moreover, a system for checking thyroxine levels as a routine screening procedure with the aim of detecting congenital abnormalities of the thyroid is recommended. Noniodized salt was the preferred product in most of the households demonstrating the importance of ensuring the utilization and accessibility of iodized salt at an affordable price, a medium term alternative approach for salt iodization technologies for small scale salt traders and sustained health and nutrition education to create awareness of the importance of optimal intake to prevent the health consequences of suboptimal iodine nutrition. Failure to recognize the problem will eventually rob the intelligence of the future generations from the study communities.

CHAPTER VII
ZINC DEFICIENCY WAS A SIGNIFICANT PUBLIC HEALTH PROBLEM IN
ADOLESCENT SCHOOL GIRLS FROM NORTHERN ETHIOPIA

Abstract

We conducted a cross-sectional study to investigate the magnitude and severity of zinc deficiency in 413 (10 – 15 year old) randomly selected adolescent school girls from Tigray, Northern Ethiopia. Stunting and thinness rates were 23.1%, and 26.8%, respectively. Mean (sd) zinc concentration of the adolescent school girls was 72.3 (14.3) $\mu\text{g/dL}$ and the prevalence of zinc deficiency ($< 70 \mu\text{g/dL}$) was 49.2%. Stunted ($72.3 \pm 15.9 \mu\text{g/dL}$) and non-stunted ($72.3 \pm 13.9 \mu\text{g/dL}$) girls had comparable serum zinc concentrations. Zinc deficiency was severe in 12 and 13 year old adolescent girls as were stunting and thinness. The fact that girls who were 12 and 13 years old were more zinc deficient, stunted and thin than those in the other age categories may reflect the higher growth rate and zinc requirements related to the growth spurt. Location had an effect on the zinc status of the girls ($p = 0.0001$) as reflected by the significant differences in mean serum zinc concentrations among the girls from the various schools. No significant associations were observed between zinc status and demographic characteristics such as maternal occupation ($p = 0.24$), maternal education level ($p=0.60$) and family size ($p = 0.57$). However, the water source to households ($R^2 = 0.04$, $p = 0.0007$) and access to household toilet facilities ($R^2 = 0.02$, $p=0.0110$) were significantly, albeit weakly, associated with zinc status. In conclusion, zinc deficiency is common in adolescent school girls from our study communities. We suggest the inclusion of zinc in the activities of vitamin A, iodine and iron in the context of ongoing general health and nutrition programs using existing personnel and infrastructure. Moreover, more national surveys of zinc intakes and serum zinc concentrations are needed to better determine the extent of zinc deficiency in Ethiopian adolescent populations and to serve as baselines against which to judge the effectiveness of future zinc intervention programs.

ZINC DEFICIENCY WAS A SIGNIFICANT PUBLIC HEALTH PROBLEM IN ADOLESCENT SCHOOL GIRLS FROM NORTHERN ETHIOPIA

Introduction

Various epidemiological and zinc supplementation studies have provided evidence that zinc deficiency is a worldwide problem (Castillo-Duran, Heresi et al. 1987; Gibson 1993; Sazawal, Black et al. 1996; Sempertegui, Estrella et al. 1996; Sian, Mingyian et al. 1996; Rosado, Lopez et al. 1997; Ruel, Rivera et al. 1997; Ruz, Castillon-Duran et al. 1997; Gibson and Huddle 1998; Gibson, Manger et al. 2007; Abebe, Bogale et al. 2008; Gibson, Abebe et al. 2008; Stoecker, Abebe et al. 2009). Some have even argued that suboptimal zinc nutrition is one of the ten most important factors contributing to the burden of disease in the developing world (Shrimpton, Gross et al. 2005). According to a recent report on global and regional child mortality and burden of disease attributable to zinc deficiency, Africa suffers 58% of child deaths attributable to zinc deficiency (Walker, Ezzati et al. 2009). When the African data are further disaggregated, about 32% of the deaths were from Eastern Africa, 42% from Western, 21% from Middle, 4% from Northern and 1% from Southern Africa. Ethiopia is one of five countries who together contribute 47% of the child deaths due to zinc deficiency in Africa (Walker, Ezzati et al. 2009).

Though the extent of zinc deficiency and its consequences are yet to be established, the limited available reports indicate that zinc deficiency is of public health concern in Ethiopian children (Umeta, West et al. 2000) and mothers (Haidar, Umeta et al. 2005; Hambidge, Abebe et al. 2006; Abebe, Bogale et al. 2008; Gibson, Abebe et al. 2008; Kassu, Yabutani et al. 2008; Stoecker, Abebe et al. 2009). Nearly 48% of the Ethiopian under-five children are stunted (EDHS 2006). Stunting in children is considered as an indirect indicator of zinc nutritional status (Hotz and Brown 2004) as zinc has a particular role in physical growth (Gibson 1993; Brandao-Neto, Stefan et al. 1995; Nishi 1996; Gibson, Manger et al. 2007) and stunted children respond to zinc supplementation with rapid increases in growth (Umeta, West et al. 2000; Gibson, Hess et al. 2008). Pregnant and nonpregnant mothers are also affected by zinc deficiency. A study by Kassu and colleagues (Kassu, Yabutani et al. 2008) showed that 66.7% of pregnant women from Gonder, Northwest Ethiopia were zinc deficient. Haidar and

colleagues (Haidar, Umata et al. 2005) reported a prevalence rate of 11.3% for marginal zinc deficiency in lactating women from metropolitan Addis Ababa. Recently, high prevalence rates of zinc deficiency (74%) among women in their third trimester have also been reported from Southern Ethiopia (Abebe, Bogale et al. 2008; Gibson, Abebe et al. 2008; Stoecker, Abebe et al. 2009).

Zinc deficiency is not a nutritional problem restricted to children and mothers only. It is a widespread nutritional problem in adolescents as well. Udomkesmalee and colleagues (Udomkesmalee, Dhanamitta et al. 1990) reported a 70% prevalence of zinc deficiency in school children from Thailand. Similarly, a recent study from the same region, reported 57% of the school children to be zinc deficient (Thurlow, Winichagoon et al. 2006). Another recent study (Abdelrahim, Mahgoub et al. 2009) on zinc deficiency from Eastern Sudan, a neighboring country to our study communities, reported a 9% prevalence of zinc deficiency in adolescent school girls. However, to the best of our knowledge, there are no reports regarding the zinc status of adolescents from Ethiopia. Thus, this study was conducted to investigate the magnitude and severity of zinc deficiency in adolescent school girls from Tigray, Northern Ethiopia.

Methods

Presurvey preparation: Prior to the actual data collection, the principal investigator visited the three local Wereda Education and Health Offices to explain the purpose of the study and to randomly select the nine Complete Primary Schools (CPS) for the study. Immediately after the random selection of the schools, support letters were written to the school directors from the Wereda Education Offices (WEO). After the support letters were sent to the schools, the research team visited each school to further explain the purpose and importance of the study to the school principals and teachers and to randomly select the schoolgirls.

Design: The study design was cross-sectional. A structured questionnaire was used to collect sociodemographic information. Blood were collected using standard techniques to assess the nutritional status of the school girls from biochemical indicators. Stool samples were collected to determine the parasite load in the school age girls. The interviewers were trained to standardize the questionnaire administration. Close follow-up was made by the principal investigator of the research project during the data collection. At the end

of the data collection in every study unit, a meeting was held among the research team to discuss practical problems and issues of major concern. The study was conducted in the Southeastern zone of Tigray, Northern Ethiopia. The zone is one of the five zones of the National State of Tigray.

Ethical consideration: The study has been approved by the Institutional Ethics Review Committee of the College of Health Sciences at Mekelle University, the Health Research Ethics Committee at Mekelle University, the National Health Research Ethics Review Committee at the Ethiopian Science and Technology Commission and the Institutional Review Board (IRB) of Oklahoma State University, USA.

Study subjects: The study subjects were 10 – 15 year old adolescent school girls, recruited from the nine CPS. The school roster was used as a frame for randomization of the school girls. The sample size determination was based on an estimated 50% prevalence of micronutrient (vitamin A, iron, iodine and zinc) deficiencies, a 95% confidence interval for the true prevalence and a relative precision of 5%. The formula adopted was $z^2p(1-p)/d^2$ where $z = 1.96$, $p = 0.5$ and $d = 0.05$. The calculated sample size was 385. Assuming a dropout rate of 15%, a total of 50 adolescent school girls were recruited from the nine CPS. Girls who volunteered to participate in the study were requested to come with their parents the next day or were given a consent form to take to their parents for signature as evidence that the parents allowed the girls to participate in the study.

Anthropometrics: Weights of the school girls were measured to the nearest 0.1 kg on a battery powered digital scale (SECA, UNICEF, Copenhagen) and heights were measured to the nearest 0.1 cm using a wooden height-measuring board with a sliding head bar following standard anthropometric techniques (WHO 1995). For weight and height measurements, study subjects removed their shoes, emptied their pockets, removed their jackets and wore light clothing. Mid upper arm circumference (MUAC) was also measured using a non-stretching tape. Anthropometric results were calculated using the WHO Anthroplus software (WHO AnthroPlus v1.0.2, Geneva, Switzerland) and presented as z-scores for height-for-age, BMI and BMI-for-age z scores (BAZ). School girls below -2 HAZ and BAZ scores were classified as stunted and thin, respectively.

Blood collection and analysis: Nonfasting capillary and venous blood samples were

collected aseptically by a phlebotomist following standard procedures. Disposable lancets and syringes were used to collect blood specimens. Blood samples were collected and processed inside rooms with lights turned off and serum samples were kept in an ice box after separation and during transport. The capillary blood obtained from a finger prick was used for blood film and for hematocrit determination while the venous blood obtained from antecubital area of the arm was used for serum retinol, β -carotene, α -carotene, ferritin, zinc, sTfR and CRP. About 5 – 10 ml of venous blood was collected from each school girl and allowed to clot in plain glass vacutainer blood collection tubes. After 15 minutes, serum was separated by centrifugation at 2500 rpm for 10 minutes. Serum was aliquoted into plastic sample vials and stored at -20°C at the Microbiology Laboratory of Mekelle University, Ethiopia, until transported to the laboratory of the Department of Nutritional Sciences at Oklahoma State University, USA. Frozen samples were shipped in an insulated ice box while being air freighted to the US. On arrival samples were thawed but quite cool. The serum samples were analyzed for retinol, α -carotene, β -carotene, ferritin, zinc and soluble transferrin receptors (sTfR). Trace mineral free gloves and pipette tips were used during analysis of the serum samples.

Serum zinc: Serum zinc was analyzed by Inductively Coupled Plasma Mass Spectrometer, ICPMS (Elan 9000, Perkin Elmer, Norwalk, CT). All serum samples were diluted 20 fold (200 μ l diluted to 4 ml) with 0.1% HNO₃ (GFS Chemicals, Powell, OH) in deionized water (Milli-Q, Advantage A10, Millipore, France). Standard solutions of zinc were prepared by dilution of certified standard solutions (Perkin Elmer, Norwalk, CT). Dilute working standards were prepared immediately prior to their use by diluting an intermediate stock standard solution. All samples and standards were spiked with 10 μ g/L gallium as an internal standard. To avoid zinc contamination, only plasticwares were used for reagent and sample preparation. Quality control samples (Utak Laboratories, Inc., Valencia, CA) utilized in order to verify method performance was within recommended ranges.

C - reactive protein: CRP was analyzed to screen for infection using ELISA (Helica Biosystems, Inc, Fullerton, CA) following the procedure provided with the kit. All controls were within recommended ranges. Serum concentrations \geq 3 mg/L were taken to indicate the presence of inflammation or infection (Beard, Murray-Kolb et al. 2006).

Nutritional status: Determination of the nutritional status of the school girls was based on anthropometric (stunting, thinness, MUAC) variables and biochemical indicators.

Statistical analysis: Statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC, USA). Student's t tests, chi square tests, correlation analysis, nonparametric tests, ANOVA, multiple and logistic regressions were employed for statistical analyses. The height, weight, age, hematocrit values, height-for-age z scores, BMI, BMI-for-age z scores, serum ferritin, serum sTfR, serum retinol, serum α -carotene, serum β -carotene, serum zinc, CRP and urinary iodine concentrations were the continuous variables. Groups including ages; schools; elevation; stunted and nonstunted; goitrous and nongoitrous; thin and normal; nutrient deficient and sufficient; anemic and nonanemic were treated as categorical variables. Socioeconomic and sociodemographic variables including location (school), home garden, water source, education level, mother's and father's occupation, type of residence, family food source, impact of food shortage, workload/physical activity, type of salt, latrine facilities and shoe wearing practices were treated as independent variables of different levels. Student's t tests were used to compare the means of dependent variables of two groups of an independent variable. Chi square tests were employed to analyze categorical variables. Correlation analysis was employed to indicate the strength of a linear relationship between two continuous variables. A test for normality was done using univariate analysis. Parameters that were not normally distributed including urinary iodine concentration, β -carotene and CRP, were presented as medians and were compared by Wilcoxon-Mann-Whitney and Kruskal-Wallis tests. Analysis of Variance (ANOVA) was used to compare the means of continuous dependent variables of three or more groups of an independent variable. Multiple regression analysis was employed to relate two or more continuous independent variables to a continuous dependent variable. Logistic regression was used to relate continuous independent variables to dichotomous dependent variables such as nutrient deficient and sufficient; stunted and nonstunted; thin and normal; goitrous and nongoitrous; and anemic and nonanemic groups. Statistical significance was set at $p < 0.05$.

Results

The mothers of 88.6% of the girls had no formal education. Major occupation for 81% of the mothers and 70% of the fathers was farming. The median family size was six. The staple diets for the girls and their households were cereal-based. Meal frequency was three or more times a day for the majority of the girls (95%). But, 5% of the girls reported that they eat only twice a day as they stay away from home for schooling. Three quarters of the girls reported that the food source to their households was their own agricultural production suggesting that the study subjects are mostly from farming households. Most households (71%) owned domestic animals but the frequency of the consumption of foods of animal origin was very low (6%) suggesting that the domestic animals were used for other purposes or activities.

Using 70 µg/dL as a cutoff, the prevalence of zinc deficiency in the school girls was 49.2%. Mean zinc levels of the adolescent school girls was 72.3(14.3) µg/dL. Stunted (72.3 ±15.9 µg/dL) and non-stunted (72.3±13.9 µg/dL) girls had comparable serum zinc concentrations. The zinc sufficient girls had higher mean ferritin and retinol levels but lower CRP and hematocrit levels than the zinc deficient girls. Anthropometric and biochemical indicators of the zinc deficient and sufficient adolescent school girls are summarized in table 7.1.

Table 7.1: Anthropometric and biochemical indicators of study subjects.

Variables	Zinc deficient (<70 µg/dL)		Zinc sufficient (≥70 µg/dL)		p
	n	Mean (sd)	n	Mean (sd)	
Age, yr	174	12.9(1.3)	180	12.6(1.5)	0.13
Height, cm	172	146.3(9.2)	180	144.6(9.2)	0.08
Weight, kg	172	34.5(7.2)	180	32.9(6.7)	0.03
BMI in kg/m ²	172	16.0(2.1)	180	15.6(1.7)	0.05
MUAC, cm	170	18.9(2.2)	180	18.4(2.0)	0.05
HAZ	172	-1.2(1.1)	180	-1.2(1.0)	0.60
BAZ	172	-1.4(0.9)	180	-1.5(0.9)	0.23
Hct, %	155	43.2(2.8)	162	42.6(3.5)	0.13
Retinol, µg/dL	174	35.0(9.7)	179	36.6(9.4)	0.12
UIE, µg/L	132	94.4(77.2)	138	79.8(74.4)	0.11
Ferritin, µg/L	174	45.7(24.7)	179	50.6(25.4)	0.06

Stunting, thinness and zinc deficiency were serious nutritional problems particularly at 12 and 13 years of age (Table 7.2). Girls of 10 years of age had the highest (78.4µg/dL) mean serum zinc levels and the lowest prevalence of zinc deficiency (27.6%) compared to the 12 year old school girls with the lowest (68.2 µg/dL) mean serum zinc levels and highest prevalence of zinc deficiency (59.7%).

Table 7.2: Relationship between stunting and zinc status of adolescent school girls (n = 354).

Age, yrs	n	% Stunted	% Thin	% Zinc deficient
10	30	12.9	12.9	27.6
11	49	19.6	26.8	44.4
12	62	30.3	31.6	59.7
13	97	27.5	28.4	52.1
14	75	19.3	27.7	48.0
15	41	19.2	19.2	50.0
Total	354	23.1	26.4	49.2

About 7.1% (n=25) of the school girls had higher CRP levels than the cut off (3 mg/L). We observed no significant difference in the mean serum zinc levels between school girls with CRP values below and above the 3 mg/L cutoff (71.2 vs. 72.3µg/dL). No girls were excluded from the data analysis as the CRP values were not remarkably high (Max value 4.2 mg/L).

Table 7.3: Variation of zinc levels (µg/dL) and the prevalence (%) of zinc deficiency by study sites (n = 174).

School	n	Mean (sd)	% Zinc deficient
Adigudom	13	75.9(16.7) ^a	37.5
Debrehaila	18	69.6(18.6) ^{ab}	63.0
Debub	19	72.6(13.3) ^a	46.3
Finarua	7	74.7(13.9) ^a	38.9
Hagereselam	25	74.0(16.5) ^a	49.0
Hareqo	14	76.9(12.7) ^a	41.2
Qel'ae	21	73.8(10.9) ^a	40.8
Samre	17	73.4(12.4) ^a	42.5
Tikul	40	63.3(10.9) ^b	73.6
p		0.0001	

*: Schools sharing the same letters were not significantly different.

Location had an effect on the zinc status of the girls ($p = 0.0001$) as reflected by the significant differences in mean serum zinc concentrations among the girls from the various schools (Table 7.3). Zinc deficiency was severe in Tikul. Adolescent school girls from Tikul had a significantly lower mean serum zinc levels ($63.3\mu\text{g/dL}$) and higher prevalence of zinc deficiency (73.6%) than the girls from the other schools ($p = 0.0001$). Mean serum zinc levels were higher in adolescent girls from Hareqo ($76.9\mu\text{g/dL}$) and the prevalence of zinc deficiency was lower in Adigudom (37.5%) (Table 7.3).

Vitamin A deficient girls had two and half fold ($\text{OR} = 2.61$, $\chi^2 = 105.7$, $p = 0.0012$) greater risk of being zinc deficient compared to those with serum retinol levels indicative of adequate vitamin A status. Water source to households ($F = 7.41$, $p = 0.0007$) and access to household toilet facilities ($p=0.0110$) were significantly associated with zinc status. Girls from households consuming tap water had higher mean serum zinc levels ($74.2 \mu\text{g/dL}$) than those from households consuming water from wells ($68.3 \mu\text{g/dL}$) and springs ($67.4 \mu\text{g/dL}$). Girls who had access to toilet facilities had significantly higher serum zinc levels ($74.6 \mu\text{g/dL}$) than those who didn't ($70.7 \mu\text{g/dL}$). We found significant positive correlation between serum zinc and serum ferritin ($r = 0.11$, $p=0.0432$) and significant negative correlation with urinary iodine ($r = -0.14$, $p = 0.0195$). Iodine deficient school girls had significantly higher mean serum zinc levels ($74.1 \mu\text{g/dL}$) than iodine sufficient girls ($68.5 \mu\text{g/dL}$) ($p=0.0025$). However, the prevalence of goiter was higher in the zinc deficient girls (55.25%) than in the zinc sufficient girls (44.75%).

Discussion

Human zinc deficiency is of public health significance in many developing countries. About one third of the world's population is estimated to be affected by zinc deficiency (Dhingra, Hiremath et al. 2009). However, prevalence of zinc deficiency is still unknown in many populations particularly adolescents from the developing world. To investigate the magnitude and severity of zinc deficiency in adolescents, we conducted a cross-sectional study in adolescent school girls from Tigray, Northern Ethiopia. To the best of our knowledge, this is the first study presenting serum zinc concentrations in adolescent school girls from Ethiopia. Our results provide evidence for the existence of widespread zinc deficiency in adolescent school girls from the study

communities. About half (49.3%) of the school girls were zinc deficient on the basis of the IZiNCG cutoff, 70 µg/dL (Hotz and Brown 2004).

Zinc nutrition depends on dietary intake and its bioavailability in foods. We were not able to establish the cause for the low biochemical zinc status of the girls because data on dietary intake were not collected. But, the heavy dependence on cereal-based staple diets, which contain inhibitors of zinc absorption (Black 2003; Gibson 2006) and minimal intake of foods of animal origin, which are good sources of bioavailable zinc, could suggest the role of anti-nutritional factors such as phytate in exacerbating the deficiency of zinc in our study. The cereal-based foods do contribute substantial amounts of zinc but also contribute importantly to phytate intake. Thus, the high prevalence of zinc deficiency in our study subjects could be partially explained by the low bioavailability of zinc from the cereal-based diets. However, we suggest that inadequate intake of zinc rather than poor bioavailability might be the main risk factor for the low biochemical zinc status in the school age girls. The high zinc deficiency in many Southeast Asian countries has been reported to occur predominantly in areas where soils are deficient in plant available zinc (Cakmak 2009). Ethiopian soils are low in zinc content (Lambein, Haque et al. 1994). As a result, crops grown locally will have low zinc concentration and households who depend entirely on locally grown crops will be at risk of low intake. The low intakes of foods of animal origin are likely to be an additional factor compromising zinc nutrition in our study communities.

In the present study, high prevalence of stunting was noted. This is expected because stunting is common in zinc deficient areas. Based on our stepwise logistic regression analysis, vitamin A deficient girls had two and half fold (OR = 2.61, $\chi^2 = 10.57$, $p = 0.0012$) greater risk of being zinc deficient compared to those with serum retinol levels indicative of adequate vitamin A status. A similar relationship between low serum zinc and reduced serum vitamin A concentrations have been reported by other studies (Rahman, Vermund et al. 2001; Rahman, Wahed et al. 2002). Zinc is involved in cellular proliferation and growth and its deficiency affects growth through its influence on the metabolism of hormones such as androgens, thyroid hormones, insulin and especially IGF-I and growth hormone. However, in this study, we did not find any

significant association between the indicators for iodine (UIE and goiter) and zinc nutritional status of the schoolgirls.

Location had an effect on the zinc status of the girls ($p = 0.0003$) as reflected by the significant differences in mean serum zinc concentrations among the girls from different schools (Table 7.3). Reasons for the association between school and serum zinc concentrations are not apparent. It is unlikely to be due to variability in sample handling, processing and transportation because they were thoroughly controlled during sample collection in the field and processing and analysis in the laboratory. Red blood cells are high in zinc content and they were separated from serum within 30 minutes and the necessary care was observed to avoid hemolysis during blood drawing. Moreover, fasting blood samples were collected at similar times of the day to avoid any time variation as plasma zinc concentration is higher in the morning than in the afternoon and higher following an overnight fast than in nonfasted subjects (King, Hambidge et al. 1994).

Zinc deficiency was most severe in 12 and 13 year old adolescent girls. The ages of 12 and 13 years of age were also the years where we observed higher prevalence of stunting and thinness. That school girls who were 12 and 13 years old were more zinc deficient, stunted and thin than those in the other age categories was possibly a reflection of the higher zinc requirements and growth rate related to the growth spurt. This might provide an observational support for the period of growth spurt in adolescent school girls from the study communities. Studies have shown that zinc supplementation improved linear growth in under-five children (Umeta, West et al. 2000) and adolescents (Castillo-Duran, Garcia et al. 1994). Despite the biochemical evidence of a high risk for zinc deficiency, our results do not suggest that zinc has a crucial role in stunting in adolescents. Support for this suggestion stems from the failure to establish a correlation between stunting and zinc deficiency ($p=0.9998$). Failure to observe a significant association may be due to the age group studied and the confounding effects of the coexistence of the other micronutrient (iodine, vitamin A and iron) deficiencies.

Major strengths of this study were the use of ICPMS, the gold standard for serum zinc analysis and the random selection of the school girls. Generalization could be made to adolescent school girls from the study communities as an attempt was made to identify randomized girls from the study schools. The major limitation of this study was the cross-

sectional nature of its design as we can't establish causal relationships between the independent variables and zinc status of the school girls. Secondly, blood specimens were not collected in certified trace mineral free syringes. Blood samples were collected into ordinary vacutainer tubes commonly used in Ethiopian health institutions. This might have overestimated the serum zinc levels and underestimated the severity of zinc deficiency in the study subjects as the glass tubes and rubber stoppers of the vacutainer tubes might contain zinc that can contaminate the blood samples. Third, zinc status was assessed from serum zinc levels only and the dietary intake of zinc was not determined.

In conclusion, zinc deficiency is common in adolescent school girls from our study communities. The high prevalence of zinc deficiency might have far reaching consequences. Numerous studies have established that zinc deficiency aggravates the disease burden and places under-five children at an increased risk from diarrheal diseases and pneumonia (Black 2003; Walker, Ezzati et al. 2009). Moreover, zinc deficiency has been implicated for retardation in sexual maturity in adolescent boys (Prasad, Halsted et al. 1961). Considering the practice of early marriage in the study communities and the zinc deficiency associated complications of pregnancy such as growth retardation and congenital abnormalities (Black 2001), decline in circulating zinc (Tamura, Goldenberg et al. 2000), and low birth weight (Krebs 2000; Black 2001), planning an effective intervention to control zinc deficiency is essential before the first birth to the adolescent girls from the study communities. Almost nothing is being done to address a zinc deficiency of this magnitude in comparison to vitamin A, iodine and iron deficiency except for the short-term zinc supplementation targeting children with diarrhea. We suggest the inclusion of zinc in the educational activities for vitamin A, iodine and iron in the context of ongoing general health and nutrition programs using existing personnel and infrastructure. Moreover, more national surveys of zinc intakes and serum zinc concentrations are needed to better determine the extent of zinc deficiency in Ethiopian adolescent populations and to serve as baseline against which to judge the effectiveness of zinc intervention programs. These suggestions call for the need to undertake multicenter studies to substantiate the data obtained so that intervention measures can be initiated.

CHAPTER VIII

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

Summary

Adolescence, the period between the ages of 10 and 19 years, is the second most critical period of physical growth in the human life cycle. During adolescence, growth and development are rapid and thus nutritional requirements are maximal (Tanner 1972; Giuseppina 2000; Abalkhail and Shawky 2002; Soekarjo, Pee et al. 2004). Despite the growing body of evidence about the importance of adolescent health, adolescents from the developing world in general, and Ethiopia in particular, have typically been considered a low risk group for poor nutrition. Nutrition related efforts in the country put much emphasis on early childhood and on pregnancy and lactation. Thus, this research was initiated to investigate the magnitude and severity of the deficiency of micronutrients in 10 – 15 years old adolescent school girls from rural communities of Tigray, Northern Ethiopia. The study described in this dissertation was concerned with protein energy malnutrition and micronutrient deficiencies including iodine, iron, vitamin A and zinc in adolescent school girls.

Various reports have shown that the deficiencies of the three micronutrients namely iodine, iron and vitamin A, are of public health significance in Ethiopian preschool children, pregnant and nonpregnant women. Though the extent of zinc deficiency and its consequences are not fully established yet, the available reports indicate that zinc deficiency is a growing public health concern in Ethiopia. Because, the nutrition situation of adolescents has not been addressed by any of the studies conducted so far, this study was designed to fill the gap in knowledge regarding the nutritional situation of adolescent girls from Ethiopia.

The study described in this thesis investigated the coexistence of multiple micronutrient deficiencies in 10-15 year old adolescent school girls from Tigray, Northern Ethiopia. Protein energy malnutrition (PEM) was found to be a common

occurrence in the adolescent school girls. Stunting rate was 23.1%, and 26.8% of the school girls were thin on the basis of their BMI. Nearly 35.9% of the adolescent school girls had MUAC less than 17.8 cm. PEM was not the only nutritional problem in our study subjects. Deficiency in micronutrients was also prevalent. Our study demonstrated that adolescent school girls from the study communities were affected by iodine, iron, vitamin A and zinc deficiencies. Among the four micronutrients, the deficiencies of iodine and zinc were severe compared to iron and vitamin A deficiencies.

Contrary to our expectation, only 7% of the school girls had anemia on the basis of their hematocrit levels. In 8.9% of the school girls, iron stores were depleted (ferritin < 15 µg/L) and 15.3% of them had low iron stores (ferritin < 30 µg/L) suggesting that large proportions of the adolescent girls were at risk of developing iron deficiency. However, parasites commonly associated with anemia, including hookworm, schistosomiasis and malaria, were rarely detected in the school girls.

Our results further demonstrated a high level of marginal vitamin A deficiency in an age group which is not usually considered to be at risk. About 2.5% of the school girls had depleted vitamin A stores (<20 µg/dL) and 25.6% had serum retinol levels indicative of marginal vitamin A stores (20 – 30 µg/dL) suggesting their vulnerability to vitamin A deficiency. The prevalence of clinically evident vitamin A deficiency was significant in the school girls. High prevalence of functional consequences of vitamin A deficiency (3.7% of Bitot's spots and 3.2% of night blindness) was observed from clinical assessments confirming the legacy of vitamin A deficiency in the communities.

Zinc deficiency was present in 49.2% of the girls (Serum zinc levels < 70 µg/dL). Zinc deficiency was particularly common in 12 and 13 year old adolescent girls. A higher prevalence of stunting and thinness also was observed in the 12 and 13 year old adolescents. School girls who were 12 and 13 year old were more likely to be zinc deficient, stunted and thin than those in the other age categories. This is possibly a reflection of the higher zinc requirements and growth rate related to the growth spurt. A prevalence of this magnitude will undoubtedly have far reaching consequences to the study communities. Numerous studies have established the fact that zinc deficiency aggravates the disease burden and places under-five children at an increased risk from diarrheal diseases and pneumonia. Moreover, zinc deficiency has been implicated for

retardation in sexual maturity in adolescent boys and pregnancy complications in girls. Considering the traditional practice of early marriage in the study communities and the zinc deficiency associated complications of pregnancy, planning an effective intervention strategy to control zinc deficiency before the first pregnancy of the adolescent girls is suggested.

Iodine deficiency was a widespread health problem in these communities as evidenced by the high rate of goiter (45.3%), median urinary iodine excretion (50.2 µg/L) and high rate (67%) of biochemical iodine deficiency (UI levels < 100 µg/L). Goiter rates were as high as 80% in some study sites (schools). With such a magnitude of iodine deficiency disorders, serious consequences of iodine deficiency are likely to occur in these communities. Surprisingly, only 16% of the households utilized iodized salt. Our results substantiated that iodine deficiency is a widespread public health problem in the study communities. It is likely that a large proportion of the population suffers from some degree of loss of mental capacity, which warrants further investigation.

Table 8.1 Summary table for nutrient deficiencies.

Nutrient/Condition	Indicator	Range	n	%	Remark
PEM	Height-for-age scores z	< -2Z	95	23.1	Stunted
	BMI-for-age scores z	< -2Z	110	26.8	Thin
	MUAC	< 17.8 cm	147	35.9	
Iodine	Urinary iodine	< 20 µg/L	52	16.4	Severe deficiency
		20–50µg/L	105	33.1	Moderate deficiency
		50-100µg/L	56	17.7	Mild deficiency
		≥100 µg/L	104	32.8	Adequate
	Goiter	Grade I	159	38.7	Palpable
		Grade II	27	6.6	Visible
Iron	Hematocrit (adj)*	< 37, 38, 39%	25	7.1	Anemia
	Serum ferritin	< 15 µg/L	32	8.9	Depleted stores
		15 – 30 µg/L	55	15.3	Low stores
		≥ 30 µg/L	273	75.8	Adequate stores
	Pallor		26	6.3	Clinical sign
Vitamin A	Serum retinol	< 20 µg/dL	9	2.5	Mild deficiency
		20 – 30 µg/dL	92	25.6	Marginal deficiency
		≥30 µg/dL	258	71.9	Adequate
	Night blindness		13	3.2	Clinical signs
	Bitot's spots		15	3.7	Clinical signs
Zinc	Serum zinc	< 70 µg/dL	174	49.2	Zinc deficiency
		≥ 70 µg/dL	180	50.8	Adequate

*: Cutoff = 37% for Finarua, 38% for Samre and Adigudom and 39% for all the rest of the schools.

Conclusions

In conclusion, concomitant deficiencies of more than one micronutrient were common in the adolescent school girls. Stunting and micronutrient deficiencies including iodine, iron, vitamin A and zinc were of serious public health significance in the adolescent school girls from the study communities. Our results demonstrated a high level of micronutrient deficiencies in an age group which is not usually considered to be at risk for nutritional deficiencies. Recognizing the intergenerational effect of undernutrition and the fact that the prevalence of adolescent macro and micronutrient undernutrition was high in the study communities, we recommend consideration of adolescent girls as a group of high priority in terms of their nutrition. Furthermore, emphasizing the importance of health and nutrition education of school girls as a strategy to address macro and micronutrient deficiencies in the study communities is suggested.

Suggestions for further research

Ethiopia is a country with diverse cultural differences in food taboos, and in food preparation and consumption. The country is endowed with different agro-climatic zones creating differences in staple foods across the different regions of the country. Therefore, our study results might not be generalizable to all regions and thus multicenter studies are needed to establish the nutritional status of adolescents and to substantiate our results.

Higher prevalence rates of stunting, thinness and zinc deficiency were observed in the 12 and 13 year old adolescent school girls than in the other age categories. We hypothesize that this possibly might be a reflection of the higher zinc requirements during the adolescence growth spurt. The time for the adolescent growth spurt in Ethiopian adolescents is not well defined. Therefore, carefully designed longitudinal studies are recommended to substantiate our hypothesis.

Contaminant iron (from the soil) has been implicated to contribute to the relatively lower prevalence of iron deficiency anemia in Ethiopia. However, its contribution has been a controversial issue among experts in the field. We suggest a carefully designed study to investigate the contribution of contaminant iron to iron nutrition in Ethiopia.

Iodine deficiency was a widespread health problem in these communities as evidenced by the high rate of goiter and low levels of urinary iodine. Based on the

biochemical analyses, serious consequences of iodine deficiency are likely to occur in these communities. But, no attempt has been made to collect information regarding cretinism rates and maternal losses. Thus, further investigation to determine the extent of these consequences in the study communities is suggested.

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APPENDICES

APPENDIX A

Mekelle University, Ethiopia

And

Oklahoma State University, USA

***Multiple micronutrient deficiencies in adolescent school girls from
Southeast Tigray, Northern Ethiopia***

Questionnaire

Code number: _____

1. Geographic information
Country: Ethiopia
Region: Tigray
Zone: Southeastern Zone
Woreda _____
School: _____
2. Age: _____
3. Educational level: _____
4. What is the educational level of your mother?
 - a. Illiterate
 - b. Primary education
 - c. High school education
 - d. College/university education
5. What is the educational level of your father?
 - a. Illiterate
 - b. Primary education
 - c. High school education
 - d. College/university education
6. What is your mother's occupation?
 - a. House wife
 - b. Small business (shop)
 - c. Civil servant
 - d. Others: _____
7. What is your mother's occupation?
 - a. Farmer
 - b. Civil servant
 - c. Small business (shop)
 - d. Others: _____

8. What is your current marital status?
 - a. Never married
 - b. On promise
 - c. Married
 - d. Divorced
 - e. Widowed
9. What is the size of your family/household? _____
10. What type of house do you live in?
 - a. Thatched roof (hut)
 - b. Tin roof (corrugated iron)
 - c. Other: _____
11. What is your religion?
 - a. Orthodox
 - b. Muslim
 - c. Catholic
 - d. Protestant
 - e. Other: _____
12. How do you compare your workloads to your brother or any boy of your age in your area?
 - a. Under loaded
 - b. Equally loaded
 - c. Overloaded
13. In which of the following domestic activities are you involved?
 - a. Cooking
 - b. Sanitation
 - c. Water fetching
 - d. Wood collection
 - e. Agricultural activities
 - f. Other household activities: _____
14. Among adolescents, who do you think is most affected by food shortage in your family?
 - a. Girls
 - b. Boys
 - c. Equally affected
15. How does food shortage affect adolescent's food consumption?
 - a. Reduction in the number of meals/servings
 - b. Reduction in the quantity of food at each meal
 - c. Reduction in the quality of food
 - d. No change
16. Has anyone told you what is best to feed yourself?
 - a. No
 - b. Yes
17. Do you know food taboos against adolescents?
 - a. No
 - b. Yes

18. How many times daily do you eat?
- One
 - Two
 - Three
 - More than three
19. Since this time yesterday, how many times have you fed yourself? _____
20. How do you compare your numbers of servings with your brother/ any boy of your age in your area?
- Less
 - Same
 - More
21. If yes, could you tell us few of them
- _____
 - _____
 - _____
22. How does your family meet its food needs?
- Grow their own
 - Buy/purchase
 - Subsidies/food aid
 - Others: _____
23. Does your family grow vegetables/fruits in the backyard?
- No
 - Yes
24. What types of foods are usually consumed by adolescent girls in your area?
- _____
 - _____
 - _____
25. How many times did you eat kale in the past three days?
- Once
 - Twice
 - Three times
 - Four or more
26. How many times did you eat potato in the past three days?
- Once
 - Twice
 - Three times
 - Four or more
27. How many times did you eat carrot in the past three days?
- Once
 - Twice
 - Three times
 - Four or more
28. Does your family own domestic animals (goat, sheep, cow, poultry, ox)?
- No
 - Yes

29. Have you consumed a diet of animal origin in the past three days?
- No
 - Yes
30. What is the source of drinking water to your family?
- Tap
 - Wells
 - Spring
31. What is the source of drinking water in your school?
- None
 - Tap
 - Wells
 - Spring
32. What type of latrine facilities do you use in your house?
- Open air
 - Pit latrine
33. What type of latrine facilities do you use in your school?
- Open air
 - Pit latrine or flush toilet
34. How often do you wear shoe?
- Never
 - Rarely
 - Some of the time
 - Most of the time
 - Always
35. Is there a feeding program in your school?
- No
 - Yes
36. How do you rate your current state of health?
- Bad
 - Good
 - Very good
37. What kinds of health services for adolescent girls do exist in your area?
- Curative services
 - Immunization
 - De-worming
 - Family planning/reproductive health
 - Micronutrient supplementation (vitamin A, iron, iodine, zinc)
 - Health education
38. Is malaria common in your area?
- No
 - Yes
39. Do you have insecticide treated net (ITN) in your house?
- No
 - Yes

40. How often is your family's house sprayed per year with an insecticide?
- None
 - Once
 - Twice
41. Do you have any problem seeing in the day time?
- No
 - Yes
42. Do you hear complaints from any one about not seeing well at night or at dusk/dawn?
- No
 - Yes
43. Do you have trouble seeing at night or dawn/dusk?
- No
 - Yes
44. If you have problems seeing in the night time, what is the name of this condition in Tigrigna? _____
45. What do you think is the cause of the seeing difficulty in the night time?

46. What do you think is the remedy for the seeing difficulty in the night time?

47. Do you know anyone with goitre in your area?
- No
 - Yes
48. Does someone from your family has goiter?
- No
 - Yes
49. What do you think is the cause of goiter? _____
50. How do we prevent goitre? _____
51. What type of salt is consumed by the household?
- Rock salt
 - Uniodized white salt
 - Iodized salt
 - I don't know
52. Have you ever received micronutrient (vitamin A, iron, iodine) supplement from a health professional or a health facility?
- No
 - Yes
53. Are you aware of the fact that there is a synergy between health and nutrition?
- No
 - Yes
54. Did you start menstruating?
- No
 - Yes

Thank you very much for your time and cooperation!

Appendix B
Frequency tables

1. List of Weredas

Wereda	Frequency	Percent	Cum. Frequency	Cum. Percent
Doguae Tembien	164	39.71	164	39.71
Hintalo Wojerat	138	33.41	302	73.12
Seharti Samre	111	26.88	413	100.00

2. List of the schools

School	Frequency	Percent	Cum. Frequency	Cum. Percent
Adigudom	44	10.65	44	10.65
Debre Haila	38	9.20	82	19.85
Debub	50	12.11	132	31.96
Finarua	23	5.57	155	37.53
Hagereselam	55	13.32	210	50.85
Hareqo	44	10.65	254	61.50
Qelaetogoga	53	12.83	307	74.33
Samre	50	12.11	357	86.44
Tikul	56	13.56	413	100.00

3. How old are you?

Age, yrs	Frequency	Percent	Cum. Frequency	Cum. Percent
10	32	7.75	32	7.75
11	60	14.53	92	22.28
12	76	18.40	168	40.68
13	110	26.63	278	67.31
14	85	20.58	363	87.89
15	50	12.11	413	100.00

4. What is your educational level?

Grade	Frequency	Percent	Cum Frequency	Cum Percent
4	46	11.53	46	11.53
5	142	35.59	188	47.12
6	112	28.07	300	75.19
7	81	20.30	381	95.49
8	18	4.51	399	100.00

5. What is the educational level of your mother?

Mother's education	Frequency	Percent	Cum Frequency	Cum Percent
No formal education	344	88.89	344	88.89
Primary education	25	6.46	369	95.35
Secondary education	10	2.58	379	97.93
College education	8	2.07	387	100.00

6. What is the educational level of your father?

Father's education	Frequency	Percent	Cum Frequency	Cum Percent
No formal education	320	82.69	320	82.69
Primary education	30	7.75	350	90.44
Secondary Education	19	4.91	369	95.35
College education	18	4.65	387	100.00

7. What is your mother's occupation?

Mother's Occupation	Frequency	Percent	Cum frequency	Cum Percent
House wife	318	81.54	318	81.54
Small business	41	10.51	359	92.05
Civil servant	25	6.41	384	98.46
Other	6	1.54	390	100.00

8. What is your father's occupation?

Father's occupation	Frequency	Percent	Cum. Frequency	Cum. Percent
Farmer	274	70.44	274	70.44
Civil servant	60	15.42	334	85.86
Small business	40	10.28	374	96.14
Others	15	3.86	389	100.00

9. What is the size of your family/household?

Family size	Frequency	Percent	Cum frequency	Cum percent
2	1	0.28	1	0.28
3	19	5.26	20	5.54
4	54	14.96	74	20.50
5	79	21.88	153	42.38
6	90	24.93	243	67.31
7	65	18.01	308	85.32
8	36	9.97	344	95.29
9	9	2.49	353	97.78
10	5	1.39	358	99.17
11	1	0.28	359	99.45
12	2	0.55	361	100.00

10. What is your current marital status?

Marital status	Frequency	Percent	Cum Frequency	Cum Percent
Never married	385	98.72	385	98.72
Promise	5	1.28	390	100.00

11. What type of house do you live in?

Type	Frequency	Percent	Cum Frequency	Cum Percent
Thatched roof	106	27.32	106	27.32
Corrugated iron	210	54.12	316	81.44
Soil roof	72	18.56	388	100.00

12. What is your religion?

Religion	Frequency	Percent	Cum Frequency	Cum Percent
Orthodox	380	97.69	380	97.69
Muslim	9	2.31	389	100.00

13. How do you compare your workloads to your brother or any boy of your age in your area?

Workload	Frequency	Percent	Cum Frequency	Cum Percent
Under loaded	177	46.21	177	46.21
Equally loaded	65	16.97	242	63.19
Over loaded	141	36.81	383	100.00

14. Involvement in cooking

Cooking	Frequency	Percent	Cum Frequency	Cum Percent
No	88	22.80	88	22.80
Yes	298	77.20	386	100.00

15. Involvement in sanitation

Sanitation	Frequency	Percent	Cum frequency	Cum Percent
No	283	73.32	283	73.32
Yes	103	26.68	386	100.00

16. Involvement in water fetching

Water fetch	Frequency	Percent	Cum Frequency	Cum Percent
No	132	34.20	132	34.20
Yes	254	65.80	386	100.00

17. Involvement in wood collection

Wood collection	Frequency	Percent	Cum Frequency	Cum Percent
No	274	70.98	274	70.98
Yes	112	29.02	386	100.00

18. Involvement in agricultural activities

Agri activity	Frequency	Percent	Cum Frequency	Cum Percent
No	362	93.78	362	93.78
Yes	24	6.22	386	100.00

19. Involvement in other household activities

Other HH	Frequency	Percent	Cum Frequency	Cum Percent
No	315	81.61	315	81.61
Yes	71	18.39	386	100.00

20. Among adolescents, who do you think is most affected by food shortage in your family?

Member	Frequency	Percent	Cum Frequency	Cum Percent
Girls	151	40.48	151	40.48
Boys	130	34.85	281	75.34
Both	92	24.66	373	100.00

21. How does food shortage affect adolescent's food consumption?

Impact	Frequency	Percent	Cum Frequency	Cum Percent
Reduce # meals	39	10.26	39	10.26
Reduce quantity	148	38.95	187	49.21
Reduce quality	172	45.26	359	94.47
No change	21	5.53	380	100.00

22. Has anyone told you what is best to feed yourself?

Nutr. info	Frequency	Percent	Cum Frequency	Cum Percent
No	372	96.88	372	96.88
Yes	12	3.13	384	100.00

23. How many times do you eat daily?

Feed freq	Frequency	Percent	Cum Frequency	Cum Percent
1	1	0.26	1	0.26
2	19	4.88	20	5.14
3	298	76.61	318	81.75
3 or more	71	18.25	389	100.00

24. Since this time yesterday, how many times have you fed yourself?

Feed freq.	Frequency	Percent	Cum Frequency	Cum Percent
2	4	1.04	4	1.04
3	18	4.70	22	5.74
4	259	67.62	281	73.37
5	83	21.67	364	95.04
6	18	4.70	382	99.74
7	1	0.26	383	100.00

25. How do you compare your numbers of servings with your brother/ any boy of your age in your area?

Comparison	Frequency	Percent	Cum Frequency	Cum Percent
Less	35	9.21	35	9.21
More	235	61.84	270	71.05
Same	110	28.95	380	100.00

26. Do you know food taboos against adolescents?

Food taboo	Frequency	Percent	Cum Frequency	Cum Percent
No	348	92.80	348	92.80
Yes	27	7.20	375	100.00

27. How does your family meet its food needs?

Source	Frequency	Percent	Cum Frequency	Cum Percent
Own produce	295	75.64	295	75.64
Purchase	91	23.33	386	98.97
Subsidies/food aid	4	1.03	390	100.00

28. Does your family grow vegetables/fruits in the backyard?

Home garden	Frequency	Percent	Cum Frequency	Cum Percent
No	240	61.70	240	61.70
Yes	149	38.30	389	100.00

29. What types of foods are usually consumed by adolescent girls in your area?

Food	Frequency	Percent	Cum Frequency	Cum Percent
Cereal-based	115	99.14	115	99.14
Animal	1	0.86	116	100.00

30. How many times did you eat kale in the past three days?

Kale	Frequency	Percent	Cum Frequency	Cum Percent
Once	186	47.57	186	47.57
Twice	85	21.74	271	69.31
3x	69	17.65	340	86.96
4 or more	51	13.04	391	100.00

31. How many times did you eat potato in the past three days?

Potato	Frequency	Percent	Cum Frequency	Cum Percent
Once	228	58.31	228	58.31
Twice	85	21.74	313	80.05
3x	46	11.76	359	91.82
4 or more	32	8.18	391	100.00

32. How many times did you eat carrot in the past three days?

Carrot	Frequency	Percent	Cum Frequency	Cum Percent
Once	254	65.13	254	65.13
Twice	93	23.85	347	88.97
3x	17	4.36	364	93.33
4 or more	26	6.67	390	100.00

33. Does your family own domestic animals (goat, sheep, cow, poultry, ox)?

Ownership	Frequency	Percent	Cum Frequency	Cum Percent
No	103	29.43	103	29.43
Yes	247	70.57	350	100.00

34. Have you consumed a diet of animal origin in the past three days?

Animal food	Frequency	Percent	Cum Frequency	Cum Percent
No	108	93.10	108	93.10
Yes	8	6.90	116	100.00

35. What is the water source to the household?

Water source	Frequency	Percent	Cum Frequency	Cum Percent
Tap	291	74.62	291	74.62
Wells	38	9.74	329	84.36
Spring	61	15.64	390	100.00

36. What is the drinking water source at your school?

Water source	Frequency	Percent	Cum Frequency	Cum Percent
None	319	81.59	319	81.59
Carry from home	62	15.86	381	97.44
Tap	2	0.51	383	97.95
Borehole	7	1.79	390	99.74
Well	1	0.26	391	100.00

37. What type of latrine facilities do you use in your house?

Toilet	Frequency	Percent	Cum Frequency	Cum Percent
None	197	50.64	197	50.64
Pit latrine	192	49.36	389	100.00

38. What type of latrine facilities do you use in your school?

Toilet	Frequency	Percent	Cum Frequency	Cum Percent
None	42	10.80	42	10.80
Pit latrine	347	89.20	389	100.00

39. How often do you wear shoe?

Shoe wear	Frequency	Percent	Cum Frequency	Cum Percent
Never	1	0.26	1	0.26
Rarely	1	0.26	2	0.51
Sometime	5	1.29	7	1.80
Mostly	2	0.51	9	2.31
Always	380	97.69	389	100.00

40. Is there a school feeding program in your school?

Feeding program	Frequency	Percent	Cum Frequency	Cum Percent
No	379	98.70	379	98.70
Yes	5	1.30	384	100.00

41. How do you rate your current health status?

Health status	Frequency	Percent	Cum Frequency	Cum Percent
Bad	51	13.60	51	13.60
Good	137	36.53	188	50.13
Very good	187	49.87	375	100.00

42. What kinds of health services for adolescent girls do exist in your area?

Health service	Frequency	Percent	Cum Frequency	Cum Percent
Curative	313	80.67	313	80.67
Immunization	5	1.29	318	81.96
Curative and immzn	50	12.89	368	94.85
Cura, immzn, deworming	12	3.09	380	97.94
Curative, immzn, suppl, deworm, health educ, reproductive health	8	2.06	388	100.00

43. Is malaria common in your area?

Malaria	Frequency	Percent	Cum Frequency	Cum Percent
No	171	43.96	171	43.96
Yes	218	56.04	389	100.00

44. Do you have insecticide treated net (ITN) in your house?

Bed net	Frequency	Percent	Cum Frequency	Cum Percent
No	245	62.82	245	62.82
Yes	145	37.18	390	100.00

45. How often is your family's house sprayed per year with an insecticide?

Spray	Frequency	Percent	Cum Frequency	Cum Percent
None	257	66.41	257	66.41
Once	125	32.30	382	98.71
Twice	5	1.29	387	100.00

46. Do you have a problem seeing in the day time?

Seeing problem	Frequency	Percent	Cum Frequency	Cum Percent
No	324	83.08	324	83.08
Yes	66	16.92	390	100.00

47. Do you hear complaints from any one about not seeing well at night or at dusk/dawn?

Seeing problem	Frequency	Percent	Cum Frequency	Cum Percent
No	254	65.30	254	65.30
Yes	135	34.70	389	100.00

48. Do you have a trouble seeing at dusk/down?

Seeing problem	Frequency	Percent	Cum Frequency	Cum Percent
No	307	87.22	307	87.22
Yes	45	12.78	352	100.00

49. If you have problems seeing in the night time, what is the name of this condition in Tigrigna?

Name	Frequency	Percent	Cum Frequency	Cum Percent
I don't know	229	60.42	229	60.42
Eye disease	6	1.58	235	62.01
Trachoma	40	10.55	275	72.56
Hima	104	27.44	379	100.00

50. What do you think is the cause of the seeing difficulty in the night time?

Cause	Frequency	Percent	Cum Frequency	Cum Percent
I don't know	72	46.15	72	46.15
Vit A def	21	13.46	93	59.62
Vitamin def	10	6.41	103	66.03
Lack of sanitation	28	17.95	131	83.97
Food shortage	4	2.56	135	86.54
Iron def	2	1.28	137	87.82
Iodine def	2	1.28	139	89.10
Lack of vaccination	1	0.64	140	89.74
Aging	1	0.64	141	90.38
Reading outside	9	5.77	150	96.15
Smoke	2	1.28	152	97.44
Trachoma	2	1.28	154	98.72
Wind	2	1.28	156	100.00

51. What do you think is the remedy for the seeing difficulty in the night time?

Remedy	Frequency	Percent	Cum Frequency	Cum Percent
I don't know	15	17.65	15	17.65
Sanitation	29	34.12	44	51.76
Vegetables	8	9.41	52	61.18
Vit A rich foods	17	20.00	69	81.18
Reading under a shed	7	8.24	76	89.41
Immunization	1	1.18	77	90.59
Medical care	2	2.35	79	92.94
Balanced diet	1	1.18	80	94.12
Supplementation	1	1.18	81	95.29
Others	4	4.71	85	100.00

52. Do you know anyone with goiter from your area?

Goiter	Frequency	Percent	Cum Frequency	Cum Percent
No	163	42.12	163	42.12
Yes	224	57.88	387	100.00

53. Do someone in your family has goiter?

Goiter	Frequency	Percent	Cum Frequency	Cum Percent
No	293	75.52	293	75.52
Yes	95	24.48	388	100.00

54. What do you think is the cause of goiter?

Cause	Frequency	Percent	Cum Frequency	Cum Percent
I don't know	200	52.36	200	52.36
Iodine def	143	37.43	343	89.79
Vitamin def	10	2.62	353	92.41
Dirty water drinking	8	2.09	361	94.50
Cold water drinking	1	0.26	362	94.76
Water under shed of tree	1	0.26	363	95.03
Food shortage	6	1.57	369	96.60
Dung making	1	0.26	370	96.86
Genetic	4	1.05	374	97.91
Breath	1	0.26	375	98.17
Iron def	2	0.52	377	98.69
Lack of hair butter	1	0.26	378	98.95
Lack of sanitation	3	0.79	381	99.74
Spitting saliva on dirt	1	0.26	382	100.00

55. How do we prevent goiter?

Prevention	Frequency	Percent	Cum Frequency	Cum Percent
I don't know	52	26.94	52	26.94
Iodized salt	122	63.21	174	90.16
Tattooing	2	1.04	176	91.19
Warm water drinking	5	2.59	181	93.78
Balanced diet	4	2.07	185	95.85
Iodine rich foods	1	0.52	186	96.37
Vitamin rich foods	1	0.52	187	96.89
Protein rich foods	1	0.52	188	97.41
Adding salt to food	1	0.52	189	97.93
Salt consumption	1	0.52	190	98.45
Sanitation	1	0.52	191	98.96
Supplements	1	0.52	192	99.48
Medical treatment	1	0.52	193	100.00

56. What type of salt is consumed in your household?

Salt type	Frequency	Percent	Cum Frequency	Cum Percent
Noniodized salt, ganfur	235	60.41	235	60.41
Noniodized white salt	91	23.39	326	83.80
Iodized salt	63	16.20	389	100.00

57. Have you ever received micronutrient (vitamin A, iron, iodine) supplement from a health professional or a health facility?

Supplementation	Frequency	Percent	Cum Frequency	Cum Percent
No	362	95.26	362	95.26
Yes	18	4.74	380	100.00

58. Are you aware of the fact that there is a synergy between health and nutrition?

Synergy	Frequency	Percent	Cum Frequency	Cum Percent
No	150	38.96	150	38.96
Yes	235	61.04	385	100.00

59. Did you start menstruating?

Menstruation	Frequency	Percent	Cum. Frequency	Cum. Percent
No	398	96.84	398	96.84
Yes	13	3.16	411	100.00

Appendix C

Ethiopian Science and Technology Commission, Ethics Approval



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The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

Mekelle University
Mekelle

ቁጥር: R.DHE/53-86/2009
Ref. No. **- 3 FEB 2009**
ቀን:
Date

Re Multiple micronutrient deficiencies in adolescent girls from South Eastern Tigray,
Northern Ethiopia

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
03 Feb. 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 Tibret
Secretary of NERC

cc: Afework Mulugeta
Mekelle University
Mekelle

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You may Contact

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Appendix D

Oklahoma State University Institutional Review Board

Oklahoma State University Institutional Review Board

Date: Monday, December 15, 2008
IRB Application No: HE0848
Proposal Title: Multiple Micronutrient Deficiencies in Adolescent Girls from the SE Zone of Tigrai

Reviewed and Processed as: Full Board

Status Recommended by Reviewer(s): Approved Protocol Expires: 9/9/2009

Principal Investigator(s):

Afewerk Mulugeta
330 HES
Stillwater, OK 74074

Barbara Stoecker
421 HES
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,



Shella Kennison, Chair
Institutional Review Board

VITA

Afework Mulugeta Bezabih

Candidate for the Degree of

Doctor of Philosophy

Thesis: MULTIPLE MICRONUTRIENT DEFICIENCIES IN ADOLESCENT SCHOOL GIRLS FROM TIGRAY, NORTHERN ETHIOPIA

Major Field: Human Environmental Sciences (Option: **Nutritional Sciences**)

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2009.

Completed the requirements for the Graduate Academic Degree in Food Science and Nutrition at Gent University, Gent, Belgium in 2002.

Completed the requirements for the Master of Science in Chemistry at Addis Ababa University, Addis Ababa, Ethiopia in 1995.

Completed the requirements for the Bachelor of Science in Chemistry at Addis Ababa University, Addis Ababa, Ethiopia in 1991.

Experience:

Worked as a chemist at the Ethiopian Health and Nutrition Research Institute from 1992 to 1993. Worked as a Chemist and Head of the Tigray Health and Research Laboratory Center in Mekelle from 1993 to 1998. Served as a Lecturer at Mekelle University, Ethiopia from 10/01/1998 to 08/15/2005. Worked as a research assistant at OSU from August 2005 to December 2009.

Professional Memberships:

Food and Nutrition Society of Ethiopia (FONSE), Chemical Society of Ethiopia (CSE) and Ethiopian Student Association at Oklahoma State University (ESA-OSU).

Name: Afework Mulugeta Bezabih

Date of Degree: December, 2009

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: MULTIPLE MICRONUTRIENT DEFICIENCIES IN ADOLESCENT
SCHOOL GIRLS FROM TIGRAY, NORTHERN ETHIOPIA

Pages in Study: **153** Candidate for the Degree of Doctor of Philosophy

Major Field: **Nutritional Sciences**

Scope and Method of Study: This cross-sectional study investigated the extent and severity of protein energy malnutrition and deficiencies of vitamin A, iron, iodine and zinc nutrition in 10 – 15 year old school girls from Tigray, Northern Ethiopia using anthropometric, biochemical and clinical indicators. A structured questionnaire was used to collect sociodemographic information. Serum retinol was analyzed by High Pressure Liquid Chromatography (HPLC); Urinary iodine (UI) concentrations were determined by chemical and spectroscopic methods; ferritin was analyzed by immunoradiometric assay (IRMA); serum zinc was analyzed using an inductively coupled plasma mass spectrometer (ICPMS); soluble transferrin receptors (sTfR) and C- Reactive Protein (hsCRP) were determined using enzyme linked immunosorbent assays (ELISA).

Findings and Conclusions: Prevalence of stunting and thinness in school girls was 23% and 27%, respectively. The prevalence of anemia in the school girls was 7% after adjusting hematocrit levels for elevation. Nearly 9% had depleted and 15% had low iron stores, and 6% had clinical signs of anemia. Parasites commonly associated with anemia were rarely detected. Nearly 3% were vitamin A deficient and 26% had serum retinol levels indicative of marginal vitamin A status. Clinical assessments showed that Bitot's spots and night blindness were prevalent in 3.7% and 3.2% of the girls, respectively. Total goiter rate as assessed by palpation was 45.3%, and 67% of the girls had biochemical iodine deficiency (UI < 100 µg/L). Only 16% of the households utilized iodized salt. More than 49% of the school girls were zinc deficient on the basis of serum zinc levels. In conclusion, protein energy malnutrition and micronutrient deficiencies including iodine, iron, vitamin A and zinc were of public health significance with deficiencies of iodine and zinc being more severe than iron and vitamin A. Our results demonstrated a high level of concomitant micronutrient deficiencies in an age group which is not usually considered to be at risk for nutritional deficiencies.

ADVISER'S APPROVAL: Dr Barbara Stoecker
