NUTRITIONAL ASSESSEMENT OF MOTHERS AND THEIR PRE-SCHOOL CHILDREN AND COGNITIVE FUNCTIONS OF CHILDREN IN NORTHERN JORDAN

By

MUNA ALGHARIBEH (GHARAIBEH)

Bachelor of Science in Pharmacy
University of Jordan
Amman, Jordan
1996

Master of Business Administration
University of Jordan
Amman, Jordan
2002

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2009

NUTRITIONAL ASSESSEMENT OF MOTHERS AND THEIR

PRE-SCHOOL CHILDREN AND COGNITIVE

FUNCTIONS OF CHILDREN IN

NORTHERN JORDAN

Dissertation Approved:

Dr. Barbara Stoecker

Dissertation Adviser

Dr. Tay Kennedy

Dr. Solo Kuvibidila

Dr. Janice Miller

Dr. A. Gordon Emslie

Dean of the Graduate College

AKNOWLEDGMENT

I would like to express my sincere gratitude and appreciation to all those who contributed to this work and supported me to bring this dissertation into existence. My deepest gratitude is to my advisor, Dr. Barbara Stoecker. With your enthusiasm in the field of nutrition, your high research standards, your persistence and patience in obtaining scientific knowledge, your professionalism, and above all your wisdom, you have helped me to develop greatly, both personally and professionally. Every suggestion you gave to me has proved to be valuable. Thank you, Dr. Stoecker, for your guidance, encouragement, and the independence you gave to me throughout my entire doctoral program.

I also extend my sincerest appreciation to the professors in my committee. Dr. Tay Kenndey, Dr. Solo Kuvibidila, and Dr. Janice Miller for their encouragement along the way and for the significant knowledge they shared with me. Thanks are also extended to Dr. Shiretta Ownbey whom I enjoyed working with for the last three years.

Dr. Tay Kennedy, your passion for nutrition is both inspirational and contagious. Because of you, I loved maternal and child nutrition. You always have given me a fresh perspective on research. Your spirit and laughter will forever stay with me.

So many people in Jordan deserve my heartfelt gratitude. The research team, who assisted in collecting data for my project, Mrs. Nawal El Halabi, Mrs. Hanan

Tubaishat, Ms. Hanan Bataineh, and Ms. Osama Ali, without you I would not have been able to complete this work. Thank you all for your assistance, generous contribution, and the endless hours you spent in this study. I will be always grateful to Mr. Ahmad Gharaibeh at Irbid Laboratories who handled the blood samples, and to Mr. Hasan Harb and Ms. Maysoon El Ndooj at Al Farouq Society. Lastly, my sincerest appreciation is extended to Mr. Hussein Badwan and his wife Majeda (the principals of Al Shomoo' Schools in Irbid). Your generosity and kindness are beyond words.

I would like to thank the faculty, staff, and my colleagues in the Department of Nutritional Sciences and in College of Human Environmental Sciences whose friendship was always encouraging. Thanks are extended to Judi Horn, Tina Castleberry, and Anne Mahoney who made the first floor a warm environment for me. I owe special thanks to my colleague Afework Mulugeta who lent me a helpful hand in the laboratory analysis. Thanks are also extended to my friends in Stillwater who made this journey enjoyable all these years.

My words can only fall short in expressing my sincerest gratitude and appreciation for my family: my dad, Ahmad; my mom, Ruyat; my brothers, Qusai, Luai, and Oday; my daughter, Lina; and my grandma Fatima who passed away while I was in the United States. I dedicate this dissertation to you. Without your love and patience I wouldn't have completed this journey, indeed, probably I wouldn't have started it. You were a constant source of love, concern, and strength all of these years. Thank you all for bringing joy into my life and for making the hard times bearable and the good times sweeter. Thank you the most for being such a wonderful family.

TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

Table Page

CHAPTER II

Table Page

Table Page

CHAPTER III

CHAPTER IV

CHAPTER V

Table Page

CHAPTER VI

Table Page

LIST OF FIGURES

Figure Page

CHAPTER II

CHAPTER IV

CHAPTER V

Figure Page

CHAPTER I

INTRODUCTION

Good nutrition is a prerequisite for human development. Many developing countries still have unacceptably high rates of undernutrition and are unlikely to reach their Millennium Development Goals by 2015. The wide-spread undernutrition and key vitamin and mineral deficiencies in developing countries will continue to be a serious threat to public health and to economic and social development in these countries without appropriate attention and health policies. The high rates of "hidden hunger" or micronutrient deficiency among women in Jordan are caused by many factors including early age at marriage, short birth intervals, poor dietary diversification, and lack of national policies on food fortification for nutrients such as zinc and vitamin D.

With abundant sunlight in the Middle East, one would have assumed that the populations of these countries would be secured from being vitamin D deficient, but research on the vitamin D status of Middle Eastern women has shown the opposite. On an average, 5–10 min of direct sunlight exposure of the arms and legs (half the minimal erythema dose) will produce about 3000 IU of vitamin D_3 (Holick 2007); however, the amount of body surface area that is actually exposed to sunlight depends on the clothing style, use of sunscreen and lifestyle. A growing body of research suggests

that vitamin D deficiency is widespread among women in the Middle Eastern countries (Ghannam et al 1999, Mishal 2001, Moussavi et al 2005, Olmez et al 2006, Siddiqui and Kamfar 2007), and limited research has been carried out to determine vitamin D status among children in the Middle East. No study has assessed the vitamin D status of both women and their preschool children in the region.

Anemia and iron deficiency are the most common nutritional disorders affecting children and women in both industrialized and developing countries. In the Middle East, about 47% of pre-school children (0.8 million) have hemoglobin (Hb) below 11 g/dL and almost 40 million women of child bearing age (non-pregnant) have Hb below 12 g/dL (WHO 2008). The prevalence of anemia in the Eastern Mediterranean region is considered to be a severe public health problem among pre-school children and moderate among women of child-bearing age (WHO/UNICEF/UNU 2001). In Jordan, it is estimated that more than 200,000 children under the age of five and almost 30% of non-pregnant women of reproductive age are anemic (WHO 2008). In 2003, the Ministry of Health in Jordan estimated the prevalence of anemia, iron deficiency, and iron deficiency anemia to be 32%, 40%, and 22%, respectively among non-pregnant women of reproductive age, and 20%, 26%, and 10%, respectively among pre-school children (Ministry of Health 2003).

The World Health Organization (WHO) identified the factors contributing most to burden of disease in developing countries with high mortality; zinc deficiency was ranked as the 5th factor followed by iron deficiency as the 6th (WHO 2002). Globally about ½ of the human population is at risk of low zinc intake (Brown and Wuehler

2000).The high prevalence of anemia in the Middle East North Africa (MENA) region despite the fortification of flour with iron supports the fact that there is a need to assess zinc status. Anemia is a marker for both iron and zinc deficiency (Shrimpton et al 2005). It is likely that iron deficiency exists with other micronutrient deficiencies like zinc (Dewey and Brown 2003). By examining the availability of zinc in the local diet in Afghanistan, Algeria, Djibouti, Egypt, Iraq, Morocco, Pakistan, Somalia, Sudan, and Yemen, the prevalence of zinc deficiency in the Middle East and North Africa region was estimated to be 46%, a percentage that makes the region rank third after South Asia (79%) and Sub-Saharan Africa (50%) (Caulfield and Black 2004). Currently, there are no estimates about the national prevalence and distribution of zinc deficiency in Jordan, and there is a serious lack of awareness of the importance of zinc in human nutrition not only in Jordan but also in the MENA region. Direct measurement of plasma zinc in a representative sample is required, so that knowledge about the prevalence and distribution of zinc deficiency can be established before setting steps to eliminate zinc deficiency.

Animal and human studies have suggested an association between iron and zinc deficiencies and delayed cognitive and developmental outcomes. There is a considerable body of research suggesting poor cognitive, motor, or social functioning in iron deficient infants and young children up to 24 months of age (Aukett et al 1986, Black et al 2004a, Friel et al 2003, Heywood et al 1989, Idjradinata and Pollitt 1993, Lozoff et al 1987, Oski et al 1983, Walter et al 1983, Walter et al 1989, Williams et al 1999). Longitudinal studies have also shown that infants with chronic iron deficiency fail

to catch up with the children with good iron status in developmental tests over time, despite correction of iron status (Algarin et al 2003, Corapci et al 2006, Dommergues et al 1989, Hurtado et al 1999, Lozoff et al 1991, Lozoff et al 2000, Lozoff et al 2006b, Palti et al 1985, Shafir et al 2006, Walter 2003). However, research on developmental outcomes and motor and cognitive functioning among iron deficient pre-school children is limited. The few available studies showed impaired motor, cognitive, and developmental outcomes among iron-deficient pre-school children (Lozoff et al 2007, Metallinos-Katsaras et al 2004, Pollitt et al 1983, Pollitt et al 1986, Soewondo et al 1989, Stoltzfus et al 2001).

Many zinc intervention studies that assessed the role of zinc in improving diminished cognitive ability and motor functioning have suggested relations between motor development in infants, toddlers, and school aged children and zinc supplementation (Ashworth et al 1998, Bentley et al 1997, Black et al 2004a, Castillo-Duran et al 2001, Friel et al 1993, Gardner et al 2005, Kirksey et al 1991, Kirksey et al 1994, Merialdi et al 1999, Sazawal et al 1996), although more research is needed to confirm such a relationship (Black 2003). On the other hand, other studies found no impact of zinc supplementation on infant and child mental development and behavior (Black et al 2004b, Cavan et al 1993, Gibson et al 1989, Hamadani et al 2001, Hamadani et al 2002, Lind et al 2004, Tamura et al 2003, Taneja et al 2005).

Malnutrition in Jordan should be addressed immediately if sustained economic growth will convert the country into a “developed” state, as there is a definite positive correlation between health and income per capita. Healthier children have better school

performance and higher cognitive capacity. Healthier populations have higher productivity, lower mortality rate, and higher national saving rates. It is a two-way causality where health improvements lead to economic growth, and economic growth facilitates health improvement.

The ***overall aim*** of this study was to examine the extent and causes of malnutrition and of selected micronutrient deficiencies (vitamin D, iron, and zinc) in women of childbearing age and their preschool children in northern Jordan.

The ***specific objectives*** of the study were:

1. To assess vitamin D status in women of childbearing age and their preschool children in Jordan during the summer.
2. To determine the prevalence of iron and zinc deficiency in mothers and their preschool children living in northern Jordan, and to evaluate the diagnostic efficiency (i.e., sensitivity and specificity) of serum TfR and different TfR/ferritin ratios in identifying iron deficiency in women of child-bearing age and preschool children in Jordan.
3. To examine possible associations between children's performance on cognitive tests, their socioeconomic variables and their iron and zinc status indicators.

The dissertation consists of six chapters that follow this introduction. Chapter 2 provides an overview of the current literature on vitamin D, iron, and zinc. It also presents research on cognitive and developmental outcomes related to zinc and iron

deficiency. Research methodology is addressed in chapter 3. The vitamin D study is presented in chapter 4 as it was published in the *European Journal of Clinical Nutrition*. Chapters 5 and 6 are organized in manuscript format. Chapter 5 assesses the prevalence of iron and zinc deficiency among study participants, and chapter 6 examines the association between children's performance on cognitive tests and their iron and zinc status indicators. Finally, chapter 7 provides a general discussion of the findings, conclusions, and recommendations for future research.

CHAPTER II

REVIEW OF LITERATURE

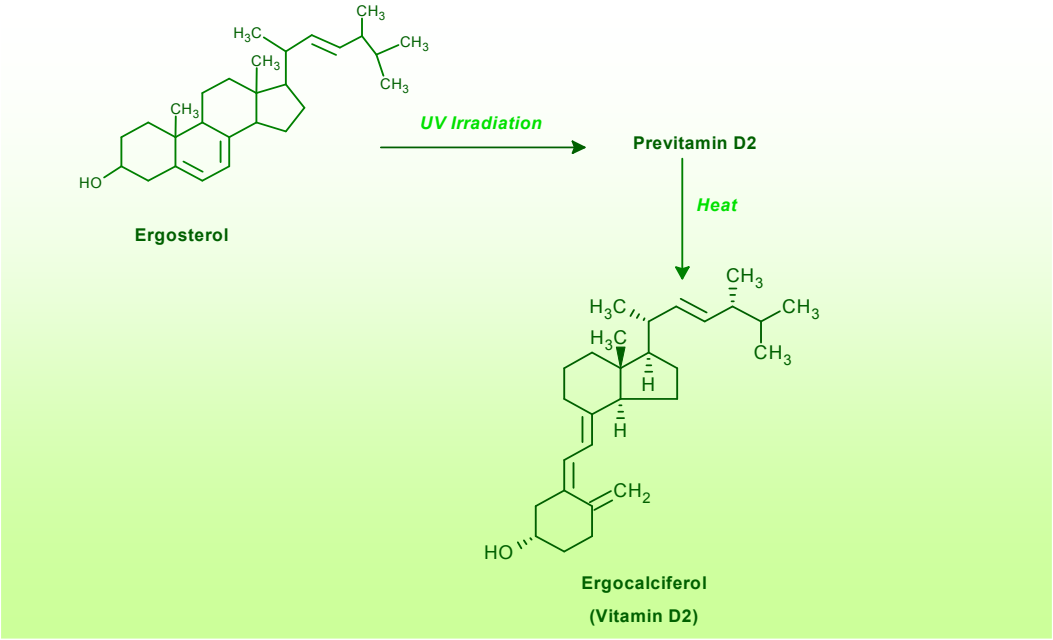
VITAMIN D CHEMISTRY, METABOLISM, AND CIRCULATION

The importance of vitamin D to human health began to be clearly understood during the 1930s. The chemical structure of vitamin D was identified in Germany by Adolf Windaus (Norman and Henry 2006, Windaus et al 1932), a Nobel prize winner in Chemistry in 1928 for his work on sterols and their connection to vitamins.

Vitamin D is derived from sterols. It is a generic term that represents four rings with differing side chain structure. Vitamin D contains three intact rings with a break in the fourth ring, and that is why it is considered to be a seco-steroid (Bouillon et al 1995). The two most important forms of vitamin D are: vitamin D_2 (ergocalciferol), and vitamin D_3 (cholecalciferol). Ergocalciferol is the synthetic form of vitamin D that is produced by the UV radiation of ergosterol in plants which leads to the formation of pre-vitamin D_2 (Figure 2.1). Then in a heat dependent process (Holick 2007), it is converted to vitamin D_2 (ergocalciferol). Vitamin D_3 is formed in the plasma membranes of human and animal skin (Figure 2.2).

When UVB radiation (290-315nm) hits the skin, it converts 7-dehydrocholesterol to pre-vitamin D_3, a thermodynamically unstable cis isomer which is rapidly converted to vitamin D_3 (Bouillon et al 1995, Holick 2007). Approximately 50% of pre-vitamin D_3 is converted to vitamin D_3 within 2 hours (Holick 2005). Excessive exposure to sun light is unlikely to cause vitamin D_3 toxicity because once vitamin D_3 is made, it enters the circulation, and the excess amounts of 7-dehydrochoelsterol and pre-vitamin D_3 are rapidly converted to inactive photoproducts like tachysterol and lumisterol (Holick 2005).

Figure 2.1
Vitamin D_2 synthesis in plants

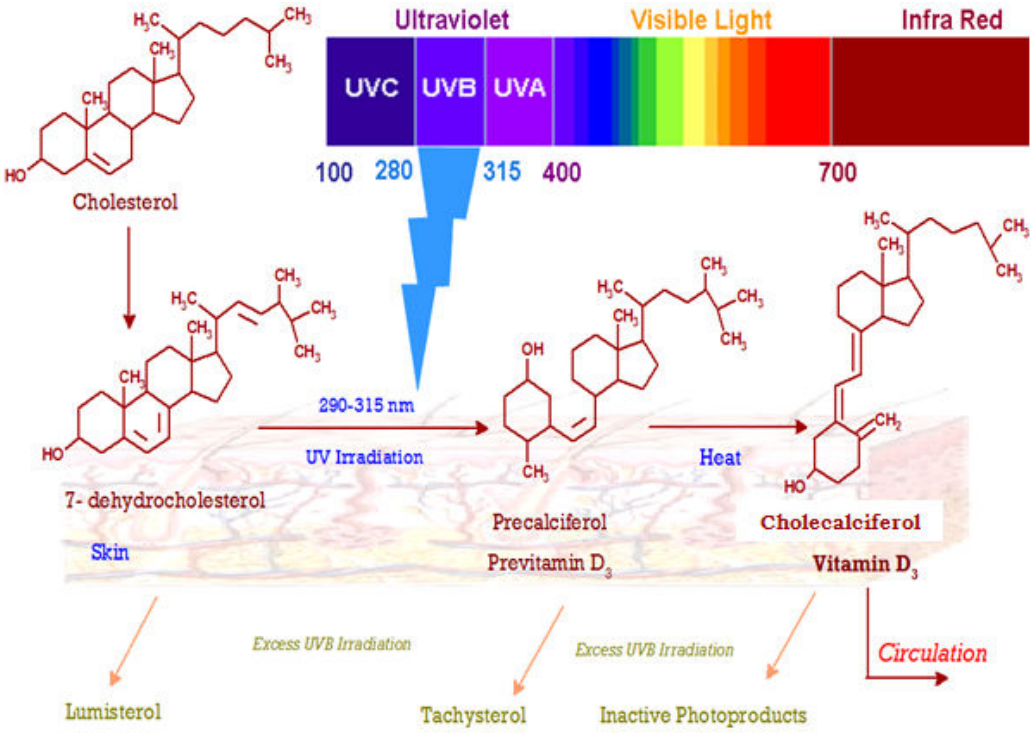


Vitamin D from both sources, the skin and the diet, is metabolized in the liver by 25-hydroxyvitamin D-24-hydroxylase (Figure 2.3) to [25(OH) D], a term that is used to reflect both 25(OH) D_2 and 25(OH) D_3 (Gascon-Barre 2005). Serum 25(OH) D is the form

that is used to assess vitamin D status in individuals (Holick 2007, Holick 2009). The 25(OH) D is the immediate precursor of the active metabolite: 1,25 dihydroxyvitamin D [1,25$(OH)_2$D], and the kidney is the primary site for production of 1,25$(OH)_2$D with the action of the enzyme 25-hydroxyvitamin D-1alpha-hydroxylase (Henry 2005). Serum 1,25 $(OH)_2$D does not reflect vitamin D status, it remains normal or even increased with vitamin D deficiency as a result of secondary hyperparathyroidism (Holick 2009)

Figure 2.2

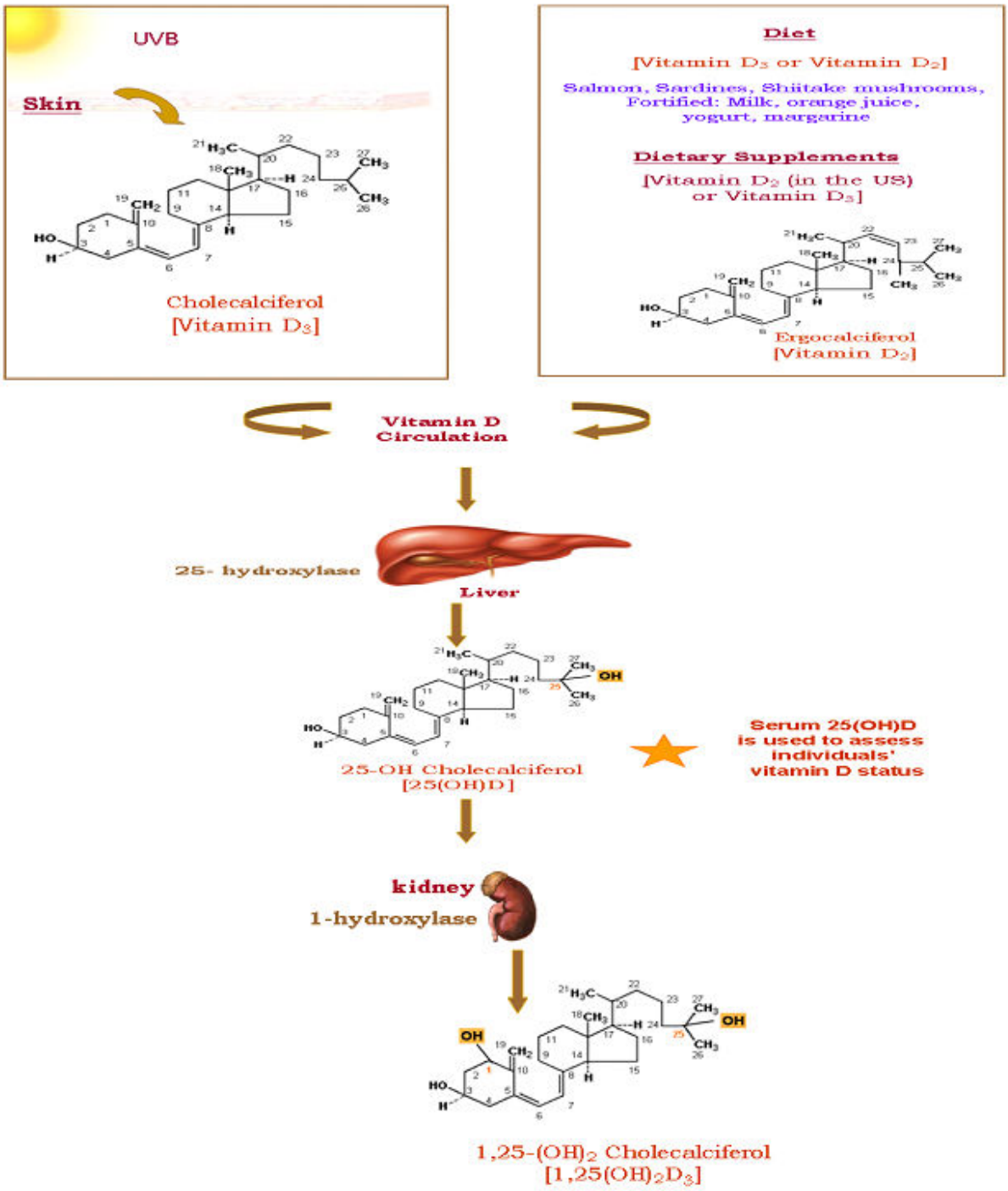
Vitamin D_3 synthesis in skin



.

Figure 2.3

Vitamin D metabolism in human

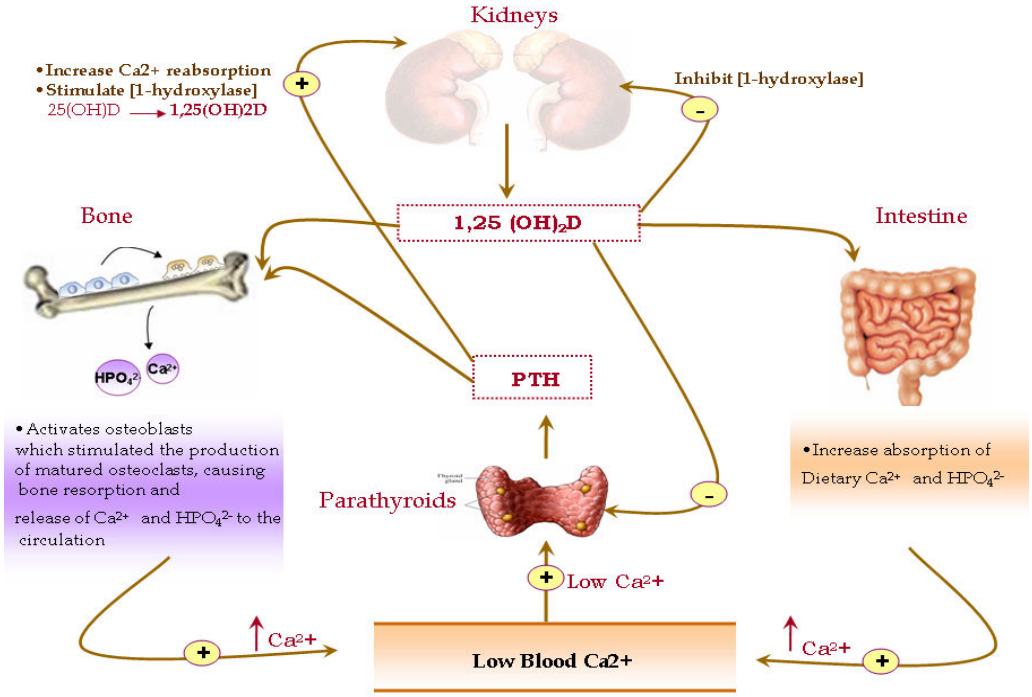


ROLE OF 1, 25(OH)$_2$ D IN CALCIUM HOMEOSTASIS

The production of [1, 25(OH)$_2$D] in the kidney is tightly regulated by plasma parathyroid hormone (PTH) and calcium and phosphorous concentrations (Figure 2.4). The initial stimulus to 1, 25(OH)$_2$D production in the kidneys is the decreased blood calcium concentration and the parathyroid hormone. When calcium concentration in blood decreases, the parathyroid glands are stimulated to secrete PTH. The PTH acts on two major sites: the kidneys and the bone. In kidneys PTH increases renal calcium reabsorption, and stimulates 25-hydroxyvitamin D-1alpha-hydroxylase, the enzyme that converts 25(OH) D to its active form, 1, 25(OH)$_2$D. In the bone, both PTH and 1, 25(OH)$_2$D act to increase calcium and phosphorous mobilization from bone to increase serum levels of calcium. This occurs through activation of osteoblasts (PTH interacts with its receptors in osteoblasts), which stimulates the conversion of pre-osteoclasts into mature osteoclasts, causing bone resorption and demineralization of the skeleton. A negative feedback mechanism is exerted by 1,25(OH)$_2$D on the kidney to decrease its own production. The third site where 1,25(OH)$_2$D acts is the intestine. The decrease in blood calcium stimulates 1,25(OH)$_2$D to interact with cell membrane vitamin D receptors (VDRs) in the intestine to stimulate the absorption of calcium and phosphorous, and increase the serum concentrations of both (Holick 2007, Norman and Henry 2006).

Figure 2.4

Regulation of calcium homeostasis by 1,25(OH)$_2$D



Kidneys, bone, and intestine are considered the classical targets for 1,25(OH)$_2$D. Recent research had shown that vitamin D receptors (VDRs) are present in different tissues in human body, and that 1,25(OH)$_2$D interacts with VDRs to affect functions other than calcium homeostasis. These tissues include: gastrointestinal cells like: esophagus, stomach, small intestine, large intestine, and colon; reproductive cells like: testis, ovary, placenta, and uterus; immune cells like: thymus, bone marrow, B-cells, and T-cells (Adams and Hewison 2008, Nagpal et al 2005, Wesley Pike and Shevde 2005) . Vitamin D receptors are also present in cardiac and smooth muscles, breast, brain, cancer cells, hair follicles, pancreatic β-cells, and pituitary gland (Norman and Silva

2001). Recent research on 1,25$(OH)_2$D has shown that it has immuno-modulatory effects that might be involved in multiple sclerosis, type II diabetes, upper respiratory tract infections, psoriasis, rheumatoid arthritis, and inflammatory bowel disease (Chen et al 2007, Ginde et al 2009, Holick 2006a, Mathieu and Adorini 2002, Nagpal et al 2005, Pittas et al 2006). It is also involved in cardiovascular functions through its effects on rennin-angiotensin regulation (Holick 2006a, Wang et al 2008). A recent meta-analysis showed vitamin D supplementation to be associated with decrease in total mortality rates from any cause (Autier and Gandini 2007).

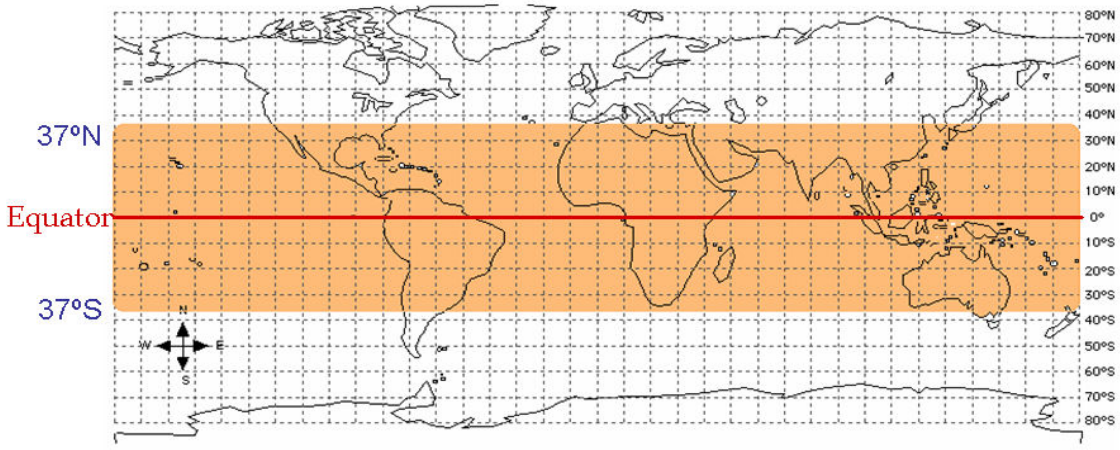
HOW MUCH VITAMIN D DO WE GET THROUGH SUN EXPOSURE?

The major source for vitamin D is skin synthesis in response to sunlight exposure, specifically to UVB (290-315 nm). The Australian and New Zealand Bone and Mineral Society recommends an exposure of hands, face, and arms to 1/3 of a minimal erythema dose (the amount of sunlight exposure that causes slight redness of the skin) for adequate endogenous synthesis of vitamin D_3 (Working Group of the Australian and New Zealand Bone and Mineral Society et al 2005). Exposure of the whole body for 10-15 min to sunlight during summer generates 20,000 IU of vitamin D_3 (Hollis 2005). On average 5-10 minutes of direct sunlight exposure of the arms and legs (half the minimal erythema dose) will produce about 3000 IU of vitamin D_3 (Holick 2007). This amount varies by several factors including: latitude, time of the year, clothing practice, use of sunscreen, skin pigmentation, obesity, and age.

At latitudes between 37°N and 37°S, sunlight should be sufficient to produce normal levels of 25(OH) D_3 (Figure 2.5), and it would be insufficient at latitudes above 37°N and below 37°S (Holick 2006a).

Figure 2.5

Map highlighting latitudes between 37°N and 37°S



However, amount of body surface area that is actually exposed to sunlight (this depends on clothing style, use of sunscreen, and lifestyle, i.e. indoor versus outdoor activities) and time of the year influence vitamin D status among individuals living under abundant sunlight. In two global studies that evaluated vitamin D status among postmenopausal women with osteoporosis from 18 countries at latitudes ranging 64°N-20°S (Lips et al 2001), and 25 countries on 5 continents (Lips et al 2006), it was found that vitamin D deficiency was higher among participants from countries "closer" to the equator, like countries in the Middle East, Central and Southern Europe, and Latin America. A major factor in the Middle Eastern countries would be the cultural dress style and the sedentary lifestyle of women. This is supported by several studies that

have been done in the Middle East (Basaran et al 2007, Dawodu et al 1998, Gannage-Yared et al 2000, Mishal 2001, Moussavi et al 2005, Saadi et al 2006).

Several studies have found that serum concentrations of 25(OH) D are lower in winter compared to the summer (Chapuy et al 1996, Goswami et al 2000, Lips et al 2001, Pal et al 2003, Pasco et al 2004), and among the elderly (Adami et al 2008, Chapuy et al 1996, Kudlacek et al 2003, Vieth et al 2003) due to less efficient cutaneous synthesis of vitamin D_3 and lack of sunlight exposure (Holick et al 1989, Lips 2001).

Skin pigmentation is another factor that affects vitamin D synthesis in the skin; higher prevalence of vitamin D deficiency was found in black Americans compared to white Americans (Looker 2005, Nesby-O'Dell et al 2002, Yanoff et al 2006). High melanin pigment concentration in the skin blocks UVB penetration and decreases the cutaneous production of vitamin D (Matsuoka et al 1991). People with darker skin needs longer duration of sunlight exposure to achieve the same level of vitamin D_3 synthesis in the skin (Hollis 2005, Matsuoka et al 1991, Nesby-O'Dell et al 2002).

Obesity is another factor that contributes to low serum 25(OH)D among individuals (Holick 2003, Liel et al 1988, Need et al 1993, Parikh et al 2004). Bioavailability of vitamin D decreases with obesity (Wortsman et al 2000), and percentage body fat is inversely proportional to serum 25(OH)D in healthy women (Arunabh et al 2003). While serum parathyroid hormone was found to be positively associated with body mass index (BMI) (Kamycheva et al 2004), total body fat was found to have a strong inverse relationship with serum levels of 25(OH)D, and a weaker

association was found between body mass index (BMI) and serum 25(OH)D (Snijder et al 2005).

DIETARY SOURCES OF VITAMIN D

Populations of developed countries are largely dependent on fortified food and dietary supplements to meet their dietary vitamin D requirements. Dietary vitamin D is mainly obtained from natural sources like salmon, sardines, tuna, egg yolk, and cod liver oil, and from fortified foods which include fortified milk, orange juice, cereals, butter, margarine, and yogurt. Natural sources of vitamin D, fortified foods and their vitamin D content, and vitamin D supplements are presented in Tables 2.1, 2.2, and 2.3, respectively.

Table 2.1

Natural sources of vitamin D and their vitamin D content

Food source		***Size***	***Vitamin D content***	***Reference***
Salmon	Fresh wild	3.5 oz	600-1000 IU	(Holick 2007)
	Farmed	3.5 oz	100-250 IU	
	Canned	3.5 oz	300-600 IU	
Sardines	-Canned in tomato sauce, drained solids, with bone	1 can (3.75 oz)	1776 IU	(U.S. Department of Agriculture and Agricultural Research Service 2007)
	-Canned in oil, drained solids, with bone	1 can (3.75 oz)	250 IU	
Tuna	Tuna light, canned in oil	3.5 oz	234 IU	
Cod Liver Oil		1 tsp	450 IU	
Egg yolk	Raw, fresh	large	18 IU	
liver	Lamb liver, raw	100 gm	16 IU	

In developing countries sun light exposure is considered the major source for vitamin D synthesis for many reasons: foods rich in vitamin D (fatty fish, liver) are not commonly consumed, dairy products are rarely fortified with vitamin D, frequent consumption of raw milk and dairy products derived from raw milk, and the relatively high prices of vitamin D supplements making them unaffordable for the majority. In addition, there is little awareness of the importance of vitamin D to health and there is a lack of nutrition education related to vitamin D.

Table 2.2

Fortified foods and their vitamin D content

Food type		*Estimates of fortified products in the US**	*Size*	*Vitamin D content*	*Reference*
Fortified Milk	1%, 2%, and non fat milk	All	1 cup	98 IU	(U.S. Department of Agriculture and Agricultural Research Service 2007)
	dry, non fat, instant	All	1 cup	299 IU	
	Soy milk	All	1 cup	100 IU	
Fortified cereals		Few	100 gm	74-300 IU	
Fortified Orange juice		Few	1 cup	100 IU	
Fortified Yogurt		Few	1 cup	100 IU	(Holick 2007)
Fortified butter		Few	3.5 oz	50 IU	
Fortified margarine		Few	3.5 oz	430 IU	
Fortified cheese		Few	3 oz	100 IU	

*(Calvo et al 2004)

Table 2.3

Vitamin D supplements

Oral vitamin D supplement type	*Vitamin D content per capsule*	*Reference*
Vitamin D_3	6, 10, 14, 40, and 80 IU	(Norman and Henry 2006)
Vitamin D_3	400, 800, 1000, 2000 IU	(Holick 2007)
Vitamin D_2	50,000 IU	

ADEQUATE INTAKES OF VITAMIN D

"Adequate intake (AI) refers to a recommended average daily nutrient intake level based on observed or experimentally derived approximations or estimates of the nutrient intake by a group (or groups) of apparently healthy people" (Institute of Medicine 2005). Adequate intake is used when there are insufficient available studies to define estimated average requirements (EAR), and therefore set the recommended daily allowance (RDA). The lack of accurate estimates for vitamin D dietary intake due to the absence of accurate measurements of food composition of vitamin D, and the difficulty in assessing the exact amount of vitamin D synthesized in the skin (due to variability in age, latitude, skin pigmentation, use of sunscreen, season, etc.) lead to the absence of consensus on the vitamin D recommendations for the general population.

The recommendations for adequate intakes of vitamin D published in 1997 by the Institute of Medicine (IOM) in the US as presented in Table 2.4.

Table 2.4

US vitamin D adequate intakes

Age	*AI*	*Upper limit*
Infants 0-12 months	200 IU or 5 µg/day	1000 IU or 25 µg/day
Ages (1-50) years	200 IU or 5 µg/day	2000 IU or 50 µg/day
Adults aged (50-70) years	400 IU or 10 µg/day	2000 IU or 50 µg/day
Adults aged > 71 years	600 IU or 15 µg/day	2000 IU or 50 µg/day
Pregnancy & Lactation	200 IU or 5 µg/day	2000 IU or 50 µg/day

Source: (Institute of Medicine 2005)

The more recently published "Dietary Guidelines for American 2005" stressed those values and recommends higher dietary vitamin D for specific population groups: elderly, individuals with dark skin, and those with limited cutaneous production of vitamin D due to insufficient exposure to UVB radiation (e.g., housebound individuals). The recommended vitamin D daily intake for this "high risk group" is 1000 IU (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2005). The report suggests the daily intake of: 3 cups of vitamin D-fortified milk (300 IU), 1 cup of vitamin D-fortified orange juice (100 IU), and 600 IU of vitamin D supplement to reach the vitamin D intake recommendation of 1000 IU.

Similar vitamin D adequate intakes are suggested in Australia and New Zealand. The minimum daily dietary intake of vitamin D that is required to prevent vitamin D deficiency is 200 IU for ages < 50 years, and 600 IU for age >70 years (Working Group of the Australian and New Zealand Bone and Mineral Society et al 2005). A vitamin D supplement of 400 IU is recommended when sun exposure is limited. The World Health

Organization and Food and Agricultural Organization of the United Nations recommend 200 IU of vitamin D as a minimum requirement for children and adults, 400 IU for older adults (51-65 years old) and 600 IU for ages >65 years (WHO/FAO 2002). Table 2.5 summarizes recommended vitamin D intakes by different organizations along with the term used by each country.

Unfortunately until now, there are no recommended dietary guidelines concerning vitamin D intake in the Middle East and North Africa region (MENA), despite the high prevalence of vitamin D deficiency. Recently, the Middle East and North Africa consensus on osteoporosis recommended a daily intake of 400 IU of vitamin D for women 50-70 years old, and 600 IU for women older than 70 years old (Maalouf et al 2007). There are no dietary guidelines for vitamin D intake for the remainder of the population, there are insufficient data to assess the dietary intake of vitamin D, and most importantly, there are no studies that assess the amount of vitamin D that is actually produced through sun exposure. One might assume that the abundant sunlight in the region would secure the population from being vitamin D deficient, but this may not be the case in the MENA region.

Table 2.5

Vitamin D intakes recommended by different organizations

WHO

Source: (WHO/FAO 2002)

Term used	*Age Groups (years) for both males and females*			*Pregnancy & Lactation*
	0-50	*51-65*	*>65*	
Recommended Nutrient Intake (RNI) of vitamin D/ day	200 IU (5µg)	400 IU (10µg)	600 IU (15µg)	200 IU (5µg)

United States & Canada, Australia and New Zealand

Sources: (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2005) and (National Health and Medical Research Council in Australia and New Zealand 2005)

Term used	*Age Groups (years) for both males and females*			*Pregnancy & Lactation*
	0-50	*51-70*	*>70*	
Adequate Intake (AI) of vitamin D/ day	200 IU (5µg)	400 IU (10µg)	600 IU (15µg)	200 IU (5µg)

Ireland

Source: (Food Safety Authority of Ireland 1999)

Term used	*Age Groups (years) for both males and females*					*Pregnancy & Lactation*
	1-3	*4-10*	*11-17*	*18-64*	*65+*	
Recommended Dietary Allowance (RDA) of vitamin D/ day	400 IU (10µg)	0- 400 IU (0-10µg)	0-600 IU (0-15µg)	0- 400 IU (0-10µg)	400 IU (10µg)	400 IU (10µg)

Netherlands

Source: (Health Council of the Netherlands 2008)

Term used	*Age Groups (years) for both males and females*				*Pregnancy & Lactation*
	0-3	*4-50*	*51-70*	*>70*	
Adequate Intake (AI) of vitamin D/ day					
With no exposure to sunlight	400 IU (10µg)	200 IU (5µg)	400 IU (10µg)	600 IU (15µg)	400 IU (10µg)
Light-colored skin, and remain outdoors for at least 15 minutes a day with at least hands and face uncovered	200 IU (5µg)	100 IU (2.5µg)	200 IU (5µg)	500 IU (12.5µg)	300 IU (7.5µg)

Table 2.5 *(continued)*

Vitamin D intakes recommended by different organizations

Commission of the European Communities
Source:(Commission of the European Communities 1993)

Term used	*Age Groups for both males and females*					
A population Reference Intake (PRI) of vitamin D/ day	*6-11 months*	*1-10 years*	*11-17 years*	*18-64 years*	*65+ years*	*Pregnancy & Lactation*
	>400 IU (>10µg) *Max: 900 IU (25 µg)*	0-400 IU (0-10µg)	0- 600 IU (0-15µg)	0-400 IU (0-10µg)	400 IU (10µg)	400 IU (10µg)

European Society for Pediatric Endocrinology (ESPE) Bone Club Recommendations
Source: (Hochberg et al 2002)

Term used	*Age Groups (years) for both males and females*					
Adequate Intake (AI) of vitamin D/ day	*Premature infants*	*Term-1st year*	*Childhood*	*Adolescence*	*Chronic disease*	*3rd trimester Pregnancy & Lactation*
	200-400 IU	200-800 IU	0-400 IU	0-1000 IU	400 IU	400-1000 IU
	The higher doses are recommended when sun exposure is limited and for dark skin					

VITAMIN D TOXICITY

Although excessive exposure to sunlight has the risk of causing skin cancer, there is no risk of vitamin D intoxication, as the sunlight destroys excess vitamin D and convert it into inactive photoproducts (Webb et al 1989). The tolerable upper intake level (UL) that is set by Food and Nutrition Board is 2,000 IU (50µg) per day (Institute of Medicine 2005) . A recent study that reviewed a set of well-designed human clinical trials, and applied a risk assessment based on the absence of toxicity, suggested a vitamin D UL dose of 10,000 IU (250µg) per day (Hathcock et al 2007).

MEASURING VITAMIN D STATUS

The major circulating metabolite of vitamin D is 25(OH) D; it is the most abundant and has the longest half-life of all vitamin D metabolites (Holick 2006b, Lips 2004). It reflects the total synthesis of 25(OH) D from the two sources, sunlight exposure and dietary intake (Holick 2006b).

As noted earlier, vitamin D along with parathyroid hormone plays a major role in the homeostatic regulation of serum calcium and phosphorous concentrations. "When calcium levels fall even slightly, levels are returned to normal by a PTH/vitamin D-controlled increase in calcium absorption, increase in renal tubular reabsorption, and bone resorption" (Connie 2006). Calcium absorption is reduced at lower concentrations of 25(OH)D, even within a normal reference range of 25(OH)D (Heaney et al 2003). Calcium levels were significantly reduced at serum 25(OH) D concentrations of 50 nmol/L relative to a mean serum concentration of 25(OH)D of 86 nmol/L. When serum calcium is reduced in response to vitamin D deficiency; hyperparathyroidism is triggered and PTH secretion is increased.

An inverse relationship between PTH level and serum 25(OH)D has been reported in many studies (Guillemant et al 1999, Holick et al 2005, Lamberg-Allardt et al 2001, Outila et al 2001, Vieth et al 2003), and it is suggested that the optimal range for 25(OH)D is the range where the PTH concentrations will be reduced to the minimum and theoretically attain the plateau value based on an exponential function. Different research groups have found similar but slightly different 25(OH)D concentration when PTH starts to plateau. The PTH plateau was reached at serum 25(OH) D concentration of

70 nmol/L (Vieth et al 2003), 75nmol/L (Holick et al 2005), 80 nmol/L (Lamberg-Allardt et al 2001), and 83 nmol/L (Guillemant et al 1999).

DEFINING VITAMIN D DEFICIENCY

There is an absence of consensus on the cut-off point that defines vitamin D deficiency. Even the term deficiency is not used consistently by researchers; other terms like vitamin D inadequacy, vitamin D insufficiency, and mild, moderate, or severe deficiency are also used. The absence of consensus is due to three main reasons; first, there is a great variation of serum 25(OH) D among different countries. Populations of many developed countries tend to have higher levels of serum 25(OH) D (due to fortification, use of supplements, and nutrition education), and therefore higher serum 25(OH) concentrations are used as cut-off points for deficiency. In countries where there is a high prevalence of low serum 25(OH) D, lower cut-off points are used.

Secondly, there are differences in the assay methods used to measure serum 25(OH) D between different laboratories. A cross calibration of three serum 25(OH) D assays: competitive protein binding (CPB), high-performance liquid chromatography (HPLC), and radioimmunoassay (RIA) administered by five international laboratories showed that although the assays were able to consistently identify low and high values of serum 25(OH) D, reported serum 25(OH) D was higher when measured by CPB than by HPLC, and intermediate vales were reported using RIA (Lips et al 1999). The highest correlation was found between HPLC and RIA ($r=.84$, $p<.01$).

A third reason for the absence of consensus on the cut-offs for vitamin D deficiency, is the differences in the impact of low serum 25(OH)D on the biomarkers that are used to define an adequate level of serum 25(OH)D. These biomarkers include: serum PTH, calcium absorption, bone mineral density (BMD), and fractures. For example, as noted in the previous section the threshold of serum 25(OH) D that will suppress high serum concentrations of PTH has varied from 70-83 nmol/L in different studies.

An evidence report on vitamin D status and bone health reviewed 167 studies and concluded that although there is a fair amount of evidence for some association between serum 25(OH) D and bone health outcomes (rickets, PTH, falls, fractures) in infants and children, women of reproductive age, postmenopausal women, and elderly, "it was difficult to define specific thresholds of circulating 25(OH)D for optimal bone health"(Cranney et al 2007).

Table 2.6 shows that the cut-off points for an optimal concentration of 25(OH) D in U.S. and Canada [25(OH)D > 75-80 nmol/L] are higher than the cut-off points used in most studies in European countries [25 (OH) D >50] and what is proposed by the Bone and Mineral Society, Endocrine Society, and Osteoporosis in Australia and New Zealand. In the Middle East; most of the studies set a lower cut-off point to define deficiency, such as 25(OH) D <30-37.5 nmol/L.

Table 2.6

Cut-off points used to define low serum 25(OH) D in different countries

USA & Canada				
USA	2007	vitamin D sufficiency	≥ 75 nmol/L	
		vitamin D insufficiency	52-74 nmol/L	(Holick 2007)
		vitamin D deficiency	< 50 nmol/L	
	2005	Optimal vitamin D concentration	>80 nmol/L	
		Osteoporosis	20-80 nmol/L	(Heaney 2004, Heaney 2005)
		Osteomalacia and rickets	<20 nmol/L	
Canada	2006	Optimal vitamin D concentration	>75 nmol/L	(Vieth 2006)
Europe				
Netherlands	2004	Vitamin D replete	>50 nmol/L	
		Mild vitamin D deficiency	25-50 nmol/L	(Lips 2004)
		Moderate vitamin D deficiency	12.5-25 nmol/L	
		Severe vitamin D deficiency	<12.5 nmol/L	
	2006	Vitamin D deficiency	<25 nmol/L	(van der Meer et al 2006)
Germany	2006	Vitamin D adequacy	70-100 to 250 nmol/L	
		Hypovitaminosis D	>50-70 to 100 nmol/L	(Zittermann et al 2006)
		Vitamin D insufficiency	>25-50 nmol/L	
		Vitamin D deficiency	0-25 nmol/L	
Irland	2008	Vitamin D in adequacy	<50 nmol/L	(Hill et al 2008)
		Vitamin D deficiency	<25 nmol/L	
Finland	2006	Vitamin D insufficiency	<40 nmol/L	(Laaksi et al 2006)
		Vitamin D deficiency	<25 nmol/L	
Australia & Newzealand				
	2005	Mild vitamin D deficiency	25-50 nmol/L	(Working Group of the Australian and New Zealand Bone and Mineral Society et al 2005)
		Moderate vitamin D deficiency	12.5-25 nmol/L	
		Severe vitamin d deficiency	<12.5 nmol/L	
Middle East				
Lebanon	2000	Hypovitaminosis D	<30nmol/L	(Gannage-Yared et al 2000)
		Severe hypovitaminosis D	< 12.5 nmol/L	
Turkey	2005	Vitamin D deficiency	<37.5 nmol/L	(Hatun et al 2005)
	2006	Vitamin insufficiency	<37.5 nmol/L	(Olmez et al 2006)
	2007	Optimal levels	≥ 75 nmol/L	
		Suboptimal	< 75 nmol/L	(Basaran et al 2007)
		Vitamin D insufficiency	< 50 nmol/L	
		Vitamin D deficiency	< 30 nmol/L	
Jordan	2001	Vitamin D insufficiency	>12.5-30 nmol/L	(Mishal 2001)
		Severe vitamin D deficiency	<12.5 nmol/L	
UAE	2006	Vitamin D insufficiency	>80 nmol/L	
		Moderately severe vitamin D deficiency	<20 nmol/L	(Saadi et al 2006)
Iran	2004	Mild vitamin D deficiency	25- ≤ 35 nmol/L	
		Moderate vitamin D deficiency	12.5- ≤ 25 nmol/L	(Hashemipour et al 2004)
		Severe vitamin D deficiency	≤12.5 nmol/L	

PREVALENCE OF VITAMIN D DEFICIENCY AMONG HEALTHY YOUNG WOMEN – AN INTERNATIONAL OVERVIEW

In the United States, hypovitaminosis D among nonpregnant African women and White women of reproductive age (15-49 years) was examined in data obtained from the Third National Health and Nutrition Examination Survey, 1988-1994 (NHANES III) (Nesby-O'Dell et al 2002). Mean serum 25(OH) D measured by radioimmunoassay was 44.2 ± 1.1 nmol/L among African American women, and 82.5 ± 1.5 nmol/L among white women. The prevalence of hypovitaminosis D (defined as serum 25(OH) D ≤ 37.5 nmol/L) was 42.4% among African Americans (n= 1546), and 4.2% among whites (n= 1426). When serum 25(OH)D cut-off point was defined as 25.0 nmol/L, the prevalence of vitamin D deficiency was 12.5% among African American women.

Scragg and colleagues (2007) examined the association between serum 25(OH)D and blood pressure among 12, 644 people aged ≥ 20 years in NHANES III (Scragg et al 2007). Mean serum 25(OH) D (measured by radioimmunoassay) was 78 nmol/L in males (n= 6097), and it varied in females (n= 6547) according to different age groups (Table 2.7). The concentration of 25(OH)D was lower with higher BMI values, and it was lowest in the non-Hispanic black

Another study assessed the association of 25(OH)D and the risk of cancer in 16818 participants (age > 17 years) in NHANES III (Freedman et al 2007). Mean serum 25(OH)D and the prevalence of low serum 25(OH)D are presented in Table 2.8.

Table 2.7

Serum 25(OH) D by age, ethnicity, and BMI in NHANES III

Females' age group (year)	***n***	***Mean serum 25(OH)D (nmol/L)***
20-29	3127	81
30-39	2901	78
40-49	2128	73
50-59	1295	72
60-69	1434	70
≥ 70	1759	67
Ethnicity (males & females)		
Non-Hispanic black	3479	49
Mexican American	3866	68
Non-Hispanic white	5299	79
BMI (males & females)		
≤ 22.1	2499	80
22.2-24.6	2511	79
24.7-27.1	2528	75
≥ 30.7	2523	67

Source: adapted from (Scragg et al 2007)

Table 2.8

Serum 25(OH) D by age, sex, and race in NHANES III

		Range of serum 25(OH)D (nmol/L)			
Characteristics		*<50*	*50- <62.5*	*62.5- <80*	*≥80*
n		5744	3143	3713	4218
Age (years)		45.2	45.9	44.1	40.8
Serum 25(OH)D (nmol/L)		38.3	56.6	71.3	104.4
Sex (%)	*Men*	34.7	46.5	49.7	55.3
	Women	65.3	53.5	50.3	44.7
Race (%)	*Non- Hispanic white*	51.6	69.7	81.2	90.4
	Non- Hispanic black	29.0	12.4	5.9	2.4
	Mexican American	7.2	7.4	5.4	2.9
	Other	12.2	10.5	7.6	4.3

Source: adapted from (Freedman et al 2007)

Vitamin D status of 200 black and 200 white pregnant women residing in northern United States and their neonates was assessed in Pittsburg, Pennsylvania (Bodnar et al 2007). Serum 25(OH)D was measured using ELISA and validated by HPLC. Two serum samples were taken from the mothers one at < 21 week of gestation and one before delivery, as well as a cord serum sample. Black women had lower serum 25(OH)D than white women, and both had high prevalence of vitamin D insufficiency before 21st week of gestation and before delivery. Black neonates had lower serum 25(OH) D compared to white neonates, and both had high levels of vitamin D insufficiency (Table 2.9).

Table 2.9

25(OH) D in pregnant women and their neonates in Pennsylvania

		serum 25(OH)D (nmol/L)		
	Mean	***< 37.5***	***37.8-80***	***> 80***
4-21 wk gestation				
White women	73.1	2.0%	60.3%	37.3%
Black women	40.2	44.9%	51.0%	4.1%
37-42 wk gestation				
White women	80.4	5.0%	41.2%	53.8%
Black women	49.4	29.2	54.1%	16.7%
Cord Blood	67.4	9.7%	56.4%	33.9%
	39.0	45.6%	46.8%	7.6%

Source: (Bodnar et al 2007)

In Canada; vitamin D insufficiency was assessed in 796 young women (age: 18-35 years) from Oct. 1995-Mar. 1997 (Vieth et al 2001). Serum 25(OH) D (measured by radioimmunoassay) was highest in August and lowest in February (Table 2.10). The prevalence of low serum 25(OH) D (less than 40 nmol/L) was higher in non-white (Asian, and Indo-Asian), and non-black subjects than in white women (although sample size for the black women was small).

Table 2.10

Serum 25(OH) D in young women in Canada

Women's group	*n*	*Mean 25(OH)D (nmol/L)*	*Prevalence of serum 25(OH)D < 40 nmol/L during winter*
White	380	58± 24	21.3%
Non-white	47	51± 22	31.9%
Black	8	68 ± 40	25
Women's group	*n*	*Mean 25(OH)D (nmol/L)*	*Prevalence of serum 25(OH)D < 40 nmol/L during summer*
White	322	76± 28	7.1%
Non-white	35	68± 33	17.1%
Black	4	68 ± 15	0

Source: (Vieth et al 2001)

Vitamin D status of a white British population was assessed from the 1958 British birth cohort (Hypponen and Power 2007). Vitamin D, measured by enzyme-linked immunosorbent assay, was assessed in 7437 whites (at 45 years of age) in four seasons between Sep. 2002 and Apr. 2004. Seasons were classified as: winter (December - February); spring (March - May); summer (June -August); and fall (September - November), and four geographical regions were: south, middle, north, and Scotland. Mean 25(OH) D was higher in summer and fall, and the prevalence of vitamin D deficiency was higher during winter and spring. Men had higher 25(OH) D concentrations than women during the summer and the fall but not during the winter and the spring (Table 2.11).

Table 2.11

25(OH) D in a British population

Characteristics		*n*	*mean*	*25(OH)D (nmol/L)* <25	<40	<75
All						
	Winter & Spring	2850	41.1	15.5%	46.6%	87.1%
	Summer & Fall	4587	60.3	3.2%	15.4%	60.9%
Winter & Spring						
	Men	*1413*	41.1	13.9%	47.0%	88.7%
	Women	*1437*	41.2	17.1%	46.3%	85.4%
Summer & Fall						
	Men	*2312*	61.9	2.2%	13.4%	58.1%
	Women	*2275*	58.6	4.3%	17.4%	63.7%

Source: (Hypponen and Power 2007)

Mean concentration of 25(OH) D was also lowest in Scotland compared to other regions in winter and spring (35.4 nmol/L in Scotland compared to 41.2, 40.6, and 42.6 nmol/L in south, middle, and north, respectively), and in summer and fall (45.6 nmol/L in Scotland compared to 62.4, 60.4, and 60.9 nmol/L in south, middle, and north, respectively).

In France, vitamin D status was assessed in 1569 adults selected from 20 French cities grouped into 9 geographical regions (Chapuy et al 1997) between November 1994 and April 1995. The mean serum 25(OH) D measured by radioimmunoassay was 62 ± 30 nmol/L in men (n= 765, aged 45-65 years) and 60 ± 30 nmol/L in women (n= 804, aged 35-60 years), with the lowest values in the North and the greatest in the South. The prevalence of low serum 25(OH)D (values ≤ 30 nmol/L) was 14% among all participants.

In Italy, a longitudinal study was performed to assess vitamin D status of healthy subjects in southern Italy (Carnevale et al 2001). Serum 25(OH) D was measured twice (in February and in August) by radioimmunoassay for a total of 90 participants (32 men, and 58 women). Mean age was 39.4 years for men and 36.9 for women. The prevalence of hypovitaminosis D (defined as serum 25(OH) D < 30 nmol/L) was 17.8% in winter and 2.2% in summer for all participants. Hypovitaminosis D among women was 27.6% in winter and 3.4% in summer. When 50 nmol/L was used as a cut-off for deficiency; the prevalence of vitamin D deficiency among women was 81% during winter, and 6.9% during summer.

In Germany, seasonal variation in vitamin D status was assessed in 38 women (mean age= 24.5 years) in winter (collected in February), and 38 females of the same age (mean age= 24.7 years) in summer (collected between July - October) in Bonn at latitude: 51°N (Zittermann et al 1998). Mean serum 25(OH) D measured by radioimmunoassay was 30.3 ± 19.1 nmol/L in winter and 69.8 ± 27.0 nmol/L in summer. In Finland, vitamin D deficiency was assessed in healthy young adults in winter in the southern area at 60°N latitude (Lamberg-Allardt et al 2001). Serum 25(OH) D was measured by radioimmunoassay in 202 women and 126 men (age: 31-43 years). Mean serum 25(OH) D was 47 ± 34 and 45 ± 35 in women and men, respectively. The prevalence of low serum 25(OH) D with a cut point value of 25 nmol/L was 26.2% (n=53) in women and 28.6% (n=36) in men. Another study in Finland (Helsinki, 60°N) assessed vitamin D in 178 female adolescents (aged 14-16 years) during winter (Outila et al 2001). Mean serum 25(OH)D measured by radioimmunoassay was 39.0 ± 14 nmol/L, and

vitamin D deficiency (defined as serum 25(OH)D ≤ 25.0 nmol/L) was prevalent among 13.5% of participants (n= 24).

In Austria, vitamin D was assessed in 654 women (age range: 21-74 years) and 435 men (age range: 26-76 years) (Kudlacek et al 2003). Mean serum 25(OH) D measured by enzyme-based protein binding assay was 52.3 ± 33.3 nmol/L with no significant difference between the two sexes. The prevalence of vitamin D deficiency (defined as serum 25(OH)D < 30.0 nmol/L) was 26% (n=271) among participants.

In India, vitamin D deficiency was assessed in healthy urban (n=943, mean age=46 ± 0.43 years) and suburban (n=205, mean age= 43 ± 1.01) subjects in southern India (13.4°N) (Harinarayan et al 2007). Serum 25(OH) D measured by competitive radioimmunoassay was assessed in 141 men, and 572 women. Mean serum 25(OH) D was higher in men compared to women, and it was higher in rural subjects in both men (59.3 nmol/L in rural subjects vs. 46.4 nmol/L in urban subjects), and women (47.5 nmol/L in rural subjects vs. 38.75 nmol/L in urban subjects). Vitamin D deficiency (defined as serum 25(OH) D < 50.0 nmol/L) was prevalent among 44% of men, and 70% of women in rural areas, and among 62% of men and 75% of women in urban areas.

Plasma 25(OH) D was assessed in young and old women and men using radioimmunoassay in north-east of China in Shenyang (Yan et al 2000). Participants were 48 young women and 48 young men (age: 25-35 years), and 48 old women and 50 old men (age; 65-75 years). Vitamin D deficiency (defined as plasma 25(OH) D < 25 nmol/L) was prevalent among 48%, 29%, 15%, and 13% of old men, young men, old women, and young women, respectively. A recent study in China has examined vitamin

D status of 441 young nonpregnant women (age: 20-35 years) in two Chinese cities (Beijing and Hong Kong) in the spring between February and June 2006 (Woo et al 2007). Mean serum 25(OH) D measured by radioimmunoassay was 32 nmol/L, and the prevalence of vitamin D insufficiency (defined as 25(OH)D concentration ≤ 50 nmol/L was 93% among all participants.

Vitamin D insufficiency among 77 healthy young women (aged 19-66 years) was assessed in Japan (Nakamura et al 2001a) during the winter of 1999-2000. Mean serum 25(OH)D measured by HPLC was 42.0 nmol/L for all subjects. The mean serum 25(OH)D for women aged <30 years (n=38) was 34.0 ± 11.0 nmol/L, and it was significantly lower than that for women aged 30 years or above (n=39) with a mean 25(OH) D of 50.0 ± 14.4 nmol/L. In another study that assessed serum 25(OH) D in 77 Japanese female college students (age: 19-24 years) (Nakamura et al 2001b). The mean serum 25(OH)D measured by HPLC was 34.2 ± 12.1 nmol/L, and 40.3% (n=31) of females had serum 25(OH)D less than 30.0 nmol/L.

Vitamin D status of nonpregnant women was assessed in 504 women (18-40 years old) living in two Asian cities: Jakarta (6°S) in Indonesia and Kuala Lumpur (2°N) in Malaysia (Green et al 2008). Mean plasma 25(OH) D measured by radioimmunoassay was 48nmol/L for all participants. Less than 1% of women had plasma 25(OH) D < 17.5 nmol/L, and over 60% of women had 25(OH) D less than 50 nmol/L (Table 2.12).

Table 2.12

25(OH) D of non-pregnant women in Indonesia and Malaysia

Group	*n*	*Mean age*	*Mean 25(OH)D (nmol/L)*	*Prevalence of serum 25(OH)D < 50 nmol/L*
All	504		48	61%
Jakarta	126	30	46	63%
Kuala Lumpur:				
Malay	133	26	43	74%
Chinese	123	23	58	38%
Indian	122	27	45	68%
All Kuala Lumpur	378	25	49	60%

Source: (Green et al 2008)

In Australia, a comparison of vitamin D status in women < 60 years of age from three regions across Australia was performed using data from three cross-sectional studies (van der Mei et al 2007). The authors reviewed three population studies [McGrath et al. 2001; Pasco et al. 2001; van der Mei et al. 2007]. Serum 25(OH) D was measured by radioimmunoassay in the three studies. Participants included in the analysis were 167 women from Southeast Queensland (27°S), 561 women from Geelong region (38°S), and 432 women from Tasmania (43°S). Samples were collected over all seasons for the three studies; summer (December - February), autumn (March - May), winter (January -August), and spring (September – November). In the three studies; vitamin D insufficiency was common in winter and spring. The prevalence of vitamin D deficiency in spring is shown in Table 2.13:

Table 2.13

Prevalence of vitamin D deficiency in Australia

Region	*n*	*Mean 25(OH)D (nmol/L)*	*Prevalence of serum 25(OH)D during Spring*	
			< 25 nmol/L	*< 50 nmol/L*
Southeast Queensland	167	67.0	7.1%	40.5%
Geelong	561	75.5	7.9%	37.4%
Tasmania	432	51.1	13.0%	67.3%

Source: (van der Mei et al 2007)

PREVALENCE OF VITAMIN D DEFICIENCY IN THE MIDDLE EAST AND NORTH AFRICA

The unexpected vitamin D deficiency in the Middle East came into view during the 1980s when a few studies in Saudi Arabia reported low concentrations of serum 25(OH)D among Saudi women (Fonseca et al 1984), Saudi mothers and their newborn infants (Serenius et al 1984, Taha et al 1984), men (Sedrani 1984) , and the elderly (Al-Arabi et al 1984). Plasma 25(OH)D was assessed in 31 adult Saudi women; the range was 5-45 nmol/L, with median of 15 nmol/L (Fonseca et al 1984), and significantly lower plasma 25(OH)D was found in women whose average sunlight exposure was less than 30 minutes per day.

Vitamin D status was assessed in 119 pregnant women at term and their newborns in Saudi Arabia (Serenius et al 1984); serum 25(OH)D was undetectable (< 7.5 nmol/L) in 11 of 119 maternal samples, and in 50 of 119 umbilical samples. It was extremely low (< 10 nmol/l) in a further 19 maternal and 31 umbilical samples. Significantly higher serum 25(OH) D was found in higher socioeconomic groups, and in mothers who had been given vitamin D supplementation during pregnancy.

In another study, plasma 25(OH) D was assessed in 100 Saudi mothers and their neonates within 24 hours after delivery (Taha et al 1984). Low plasma 25(OH) D (< 25 nmol/L) was found in 59 mothers and 70 neonates.

In 1999; serum 25(OH) D was assessed using radioimmunoassay in 321 healthy Saudi females (age: 10 - >50 years) with the majority between 20-50 years of age. Fifty-two percent of participants had severe hypovitaminosis D (25(OH) D ≤ 20 nmol/L) and parathyroid hormone (PTH) was correlated significantly (r=-0.28) with 25(OH) D. (Ghannam et al 1999).

Serum 25(OH)D was assessed in the western region of Saudi Arabia (Jeddah) using a competitive binding radioimmunoassay in 739 infants and pre-school children (aged 4-72 months). Serum 25(OH) D correlation with dietary intake of vitamin D and duration and frequency of sunlight exposure was determined (Bahijri 2001). Serum 25(OH)D ranged between 6.5-192 nmol/L. Infants and children were grouped by age into five groups: group 1, n= 110 (4 - < 6 months old); group 2, n= 170 (6 - < 12 months old); group 3, n= 166 (12 - < 24 months old); group 4, n= 166 (24 - < 36 months old); and group 5, n= 221 (36 - 72 months old). Prevalence of vitamin D deficiency, defined as serum 25(OH)D < 25 nmol/L, was: 16.4%, 15.3%, 16.3%, 2.4%, and 8.6% in the groups 1, 2, 3, 4, and 5, respectively.

The prevalence of vitamin D deficiency among women in United Arab Emirates (UAE) has been assessed in a few studies. The effect of duration of sunshine exposure weighted against the magnitude of clothing on serum 25(OH)D was assessed in 3 groups of women of child-bearing age. Women groups were: UAE nationals (n=33), non-gulf

Arabs (n=25), and Europeans (n=17) who lived in UAE (Dawodu et al 1998). Serum 25(OH) D was measured by HLPC, and PTH was measured by immunoradiometric assay. Lowest serum 25(OH) D was found among UAE nationals and non-gulf Arabs (21.5 nmol/L, and 31.5 nmol/L, respectively). Both values were significantly lower than serum 25(OH) D found in European women (160.8 nmol/L). PTH values were lower with lower serum 25(OH) D but without a significant difference between the three groups. UV exposure score was significantly lower in UAE nationals and non-gulf Arabs compared to European women. Nationality, clothing, and UV exposure score were major predictors of vitamin D status.

Serum 25(OH) D was measured in exclusively breast feeding infants and their mothers during summer in UAE (Dawodu et al 2003). Their 25(OH) D was assessed by HLPC in 90 mothers and 78 infants. Infants ranged in age from 4 to 16 weeks, and they were Arab (n=23) or South Asian (n=67). Fifty-five mothers (61%), and 64 infants (82%) had serum 25(OH) D < 25nmol/L. The median serum 25(OH)D was 21.5 nmol/L in women and 11.5 nmol/L in infants.

Serum 25(OH) D was assessed in 259 Emirati women (age range: 20-85 years) (Saadi et al 2006). All women had vitamin D deficiency (serum 25(OH)D < 80 nmol/L), and 35.1% of women had serum 25(OH)D < 20 nmol/L. Vitamin D was assessed in two groups [pre-menopausal (n= 175), and postmenopausal (n=84)] between Jan 1, 2003 and June 30, 2005. Mean serum 25(OH) D was significantly lower in pre-menopausal women (24.3 ± 10.4 nmol/L) compared to postmenopausal women (27.3 ± 11.2 nmol/L). There was no significant difference between serum 25(OH)D in women who covered all

their body except for face and hands (24.8 ± 11.1 nmol/L), and those who covered their whole body (25.7 ± 10.6 nmol/L). However mean serum 25(OH) D was significantly higher in women wearing western dress style (63.3 ± 26.2 nmol/L). Mean serum 25(OH)D was highest in April (29.2 ± 13.0 nmol/L) and lowest in August (18.2 ± 5.9 nmol/L).

A study in Kuwait has compared plasma 25(OH) D in 50 veiled and 22 unveiled women between 14-45 years of age (El-Sonbaty and Abdul-Ghaffar 1996). Mean plasma 25(OH) D was significantly lower in veiled women (14.4 nmol/L) compared to non veiled women (30.2 nmol/L). Another study in Kuwait assessed serum 25(OH) D by radioimmuniassay in 214 full-term pregnant mothers and their neonates (Molla et al 2005). Mean serum 25(OH) D was 36.5 nmol/L in mothers and 20.5 nmol/L in neonates. Eighty-six mothers (40%) and 142 neonates (66%) had serum 25(OH) D < 25nmol/L.

Prevalence of vitamin D deficiency was assessed in 318 (153 boys and 165 girls) high school students (aged 14-18 years) in Iran (Moussavi et al 2005). Serum 25(OH) D was < 50 nmol/L in 46.2 % of participants (18.3% in males, and 72.1% in females), and it was < 82 nmol/L in 72.2% of participants (49% in males, and 95.2% in females). Serum 25(OH)D was measured by HLPC in 73 early postmenopausal Iranian women in Tehran (Rassouli et al 2001). Mean age was 55.7 ± 3.6 years, and samples were collected during February -June 2000. Mean serum 25(OH) D was 42.8 ± 28.3 nmol/L. There was no significant difference between serum 25(OH) D levels measured in winter (33.3 ±12.5 nmol/L, n = 17) and spring (45 ± 30 nmol/L, n = 56). Overall, prevalence of vitamin D deficiency (defined as serum 25(OH) D < 30 nmol/L) was 36% (n= 26).

In Turkey, vitamin D deficiency was found in pre-menopausal women (Alagol et al 2000), early infancy (Hatun et al 2005), elderly (Atli et al 2005), healthy female adolescents (Olmez et al 2006), and among women with osteoporosis (Basaran et al 2007).

Alagol and colleagues (2000) assessed the vitamin D status among 48 pre-menopausal women (14-44 years of age) during summer. Women were grouped into three groups; group I: women wearing western dress style; group II: women wearing full covering clothing showing hands and face; and group III: women wearing full covering clothing including face and hands. (Alagol et al 2000). Mean serum 25(OH)D was : 56 ± 41.3 nmol/L, 31.9 ± 24.4 nmol/L, and 9 ± 5.7 nmol/L for the groups I, II, and III, respectively. Another study evaluated serum 25(OH) D in a total of 42 mothers who were diagnosed with vitamin D deficiency and their infants (27 boys and 15 girls) in two medical centers in Turkey (Hatun et al 2005). Serum 25(OH) D was measured only in 29 infants and 15 mothers by radioimmunoassay, and serum 25(OH) D <37.5 nmol/L was prevalent in all of the infants and the mothers, with mean serum 25(OH)D of 17.5 nmol/L in infants and 19.5 nmol/L in mothers.

Serum 25(OH)D was assessed by radioimmunoassay in 64 healthy female adolescents (14-18 years of age) from low and high socioeconomic settlements at the end of summer (September -October) and at the end of winter (February - March) in Turkey (Olmez et al 2006). Serum 25(OH) was higher at the end of summer compared to winter, and higher for the girls from the high socioeconomic settlement compared to the girls from the low socioeconomic settlement. Mean serum 25(OH) D was 65.3

nmol/L at the end of summer compared to 59.5 nmol/L at the end of winter for girls from the higher socioeconomic settlement, and it was 51.6 nmol/L at the end of summer compared to 34.3 nmol/L at the end of winter for girls from the lower socioeconomic settlement. The prevalence of vitamin D insufficiency (25(OH)D < 37.5 nmol/L) among girls from the higher socioeconomic settlement was 15.6% at the end of both seasons, and it was 59.4% at the end of winter, and 25% at the end of summer for girls from the lower socioeconomic settlement.

Serum 25(OH) D was assessed by HLPC in 259 women with osteoporosis in Turkey (Basaran et al 2007). Mean age was 61 ± 8.9 years. Mean 25(OH)D was 56.75 ± 31.5 nmol/L. Thirty-five women (13.5%) had serum 25(OH)D < 30 nmol/L, ninety-seven women (37.5%) had serum 25(OH)D between 30-50 nmol/L, seventy-nine women (30.5%) had serum 25(OH)D between 50-75 nmol/L, and forty-eight women (18.5%) had serum 25(OH)D more than 75 nmol/L. Quality of Life (QOL) was assessed using QOL Questionnaire of the European Foundation of Osteoporosis (QUALEFFO), and it was found that concentration of 25(OH)D is significantly associated with QOL.

The prevalence of vitamin D deficiency was assessed in an elderly Turkish population (Atli et al 2005). Serum 25(OH)D was measured by radioimmunoassay for 138 female and 87 male participants living in old age homes (OAH), and 171 female and 24 male participants living in own homes (OH). Elderly individuals were in good health. Concentrations of 25(OH) D < 37.5 nmol/L were observed among 33.4% of total participants. Low 25(OH) D concentrations were higher among women and among elderly living in an old age home (54.1% vs. 18.4% for females and males, respectively)

compared to those who lived in their own homes (27.9% and 4.2% for females and males, respectively). Mean serum 25(OH) D was 24.8 nmol/L for females and 37.6 nmol/L for males living in an old age home, and it was 41.3 nmol/L for females and 63.2 nmol/L for males living in their own home.

Prevalence of vitamin D inadequacy in Lebanon and Turkey was reported in an international study that investigated serum 25(OH)D among 2589 postmenopausal women with osteoporosis from 18 countries in 5 continents (Lips et al 2006). Serum 25(OH) D was measured by competitive binding immunoassay in 150 women from Turkey (mean age = 61 years), and in 251 women from Lebanon (mean age = 67.5 years). Mean serum 25(OH) D was the lowest among women in Turkey (54.5 nmol/L) and Lebanon (48.8 nmol/L) compared to the whole population study. Prevalence of vitamin D deficiency was measured at different cut-off points: 37.5 nmol/L, 50 nmol/L, 62.5 nmol/L, and 75 nmol/L. In Turkey the prevalence of vitamin D deficiency was 31.3%, 57.3%, 68%, and 76.7%, respectively with these cut offs. In Lebanon, the prevalence of vitamin D deficiency was 34.3%, 58.2 %, 76.5%, and 84.9% using these cut offs.

In Lebanon, hypovitaminosis D was examined in 99 men and 217 women (aged 30-50 years) between January and April 1999 (Gannage-Yared et al 2000). One hundred and fifty-six of participants were from five different rural centers, and 160 were from urban centers. Fifty-one of the women selected were veiled. Serum 25(OH) D was measured by radioimmunoassay. Mean serum 25(OH) D was 24.3 nmol/L for all participants (35.8 nmol/L in men, and 19.0 nmol/L in women). Serum 25(OH) D was < 30

nmol/L in 72.8% of all participants, and low 25(OH) D was more prevalent in women (83.9%) compared to men (48.5%). Severe hypovitaminosis D (25(OH)D < 12.5 nmol/L) was observed in 30.7% of all participants and was highly prevalent in women (41.5%). Style of clothing had a significant impact on 25(OH)D among women. Veiled women had a significantly lower 25(OH) D (12.8 nmol/L) compared to non veiled women (24.5 nmol/L). Women living in rural areas had significantly lower 25(OH)D compared to women living in urban areas (16.6, and 21.4 nmol/L, respectively), whereas men living in rural areas had significantly higher serum 25(OH)D compared to men living in urban areas (40.2, and 31.5 nmol/L, respectively).

Vitamin D was also assessed in healthy school children (aged 10-16 years) in Lebanon (El-Hajj Fuleihan et al 2001). Children participating were from different socioeconomic status, and the study was performed twice; at the end of spring 1999 (n=169), and at the end of summer 1999 (n=177). Overall, mean serum 25(OH) D (measured by competitive protein-binding assay) was lower in the spring (42.5 nmol/L) compared to the summer (55 nmol/L). Girls had lower serum 25(OH) D in both seasons (37.5 and 47.5 nmol/L in spring and summer, respectively) compared to boys (47.5 and 60.0 nmol/L in spring and summer, respectively). Among all participants, serum 25(OH) D < 50 nmol/L was prevalent among 65% in spring and 40% in summer. Socioeconomic status had a significant effect on 25(OH)D; in both seasons students in middle socioeconomic status had lower 25(OH)D (29.5 and 49.8 nmol/L in spring and summer, respectively) compared to students in higher socioeconomic status (53.5 and 57.5 nmol/L in spring and summer, respectively).

Only one study assessed the prevalence of vitamin D deficiency in Jordan (Mishal 2001). Serum 25(OH) D measured by radioimmunoassay was assessed in 22 men, and 124 women (18- 45 years of age) in Amman. The study also studied the effect of dress styles on 25(OH) D among young women, and it was conducted during two seasons: summer (July - September), and winter (January - March). Women were subdivided into 3 groups based on their dress style; women wearing full-covering clothing including hands and face (n= 11 in summer, n= 12 in winter), women wearing full covering clothing showing face and hands (n= 31 in summer, n= 49 in winter), and women wearing western-type dress style (n= 12 in summer, n=8 in winter). The male group included 11 participants in summer, and 11 in winter. In both seasons, there was a significant difference between all groups; serum 25(OH) D was highest in men (43.8 and 34.7 nmol/L in summer and winter, respectively), followed by women with western dress-style (36.7 and 30.9 nmol/L in summer and winter, respectively), followed by women wearing full covering showing face and hands (28.3 and 24.4 nmol/L in summer and winter, respectively), followed by women wearing full covering including face and hands (24.3 and 22.7 nmol/L in summer and winter, respectively). Overall, serum 25(OH) D < 30 nmol/L was prevalent among 72.5 % and 50% of all participants in winter and summer, respectively.

A study in Egypt assessed vitamin D status in 60 female patients (20-50 years) with chronic low back pain, matched with a control group of healthy females (n=20) of the same age (Lotfi et al 2007). Serum 25(OH) D assessed by radioimmunoassay was significantly lower in patients than controls (90.5 and 99.5 nmol/L, respectively). Forty-

nine patients (81.7%) and 12 controls (60%) had serum 25(OH)D between 50-90 nmol/L. Serum 25(OH)D positively correlated with fewer pregnancies, longer duration of sunlight exposure, and body area exposed to the sun, and negatively correlated with wearing black clothing, and clothing made of synthetic fibers.

In Tunisia, the first study to assess vitamin D status among a healthy section of the Tunisian population (males and females) appeared in 2005 (Meddeb et al 2005). Serum 25(OH) D was measured by radioimmunoassay between January and March 2002 in a total of 389 participants (67% females) aged 20-60 years chosen from an urban area near Tunis. More than 70% of women who participated in the study didn't wear the veil. The prevalence of hypovitaminosis D (25(OH) D <37.5 nmol/L) among the whole sample was 47.6% (n= 185). Among those with hypovitaminosis D, one case was found to have serum 25(OH) D < 12.5 nmol/L, and 66 cases (36%) had serum 25(OH)D between 12.5-25.0 nmol/L. Mean serum 25(OH) D in veiled women was 35.1 nmol/L compared to 42.5 nmol/L in non veiled women. The prevalence of hypovitaminosis D (25(OH) D <37.5 nmol/L) was significantly higher in veiled women compared to nonveiled women (70.5% and 48.9%, respectively). Having 25(OH) D< 37.5 nmol/L was higher in postmenopausal women compared to pre-menopausal women (74.5% and 50%, respectively), and it was higher with multiparity (parity ≥ 3) compared to nonparous groups (68.9% and 43.9%, respectively).

It worth mentioning here that the type of veil women wear in Tunisia and in Maghreb Countries (Libya, Algeria, Tunisia, and Morocco) is different from the one wore by women in the gulf region and Middle Eastern countries. In Tunisia, it is thin, white,

and doesn't cover the whole body (i.e. shows the face, hands, and legs), and the majority doesn't wear it. In Morocco, the veil is not limited to the Islamic code of dress style, but also is part of the Moroccan traditional clothing, where even the non-veiled women wear the traditional long and hooded garment with long sleeves. In the gulf region (UAE, SA, Kuwait, Iran), the veil is the thick, black, and long abayeh that covers the whole body, and most women cover their faces and hands.

In Morocco a case-control study evaluated the impact of clothing style on bone mineral density (BMD) among postmenopausal women (Allali et al 2006). The study recruited 178 postmenopausal women living in urban centers of Morocco, and 178 controls matched by age (mean age= 63.2 years) and body mass index (mean BMI= 32.1 kg/m^2). Veiled women constituted 83.7% of osteoporotic patients and 69.1% of nonosteoporotic patients. Wearing the concealing clothing was associated with a high risk of osteoporosis: OR= 2.29 (95% CI: 1.38-3.82). authors of the study highly recommended the need to assess vitamin D status among the population and particularly in women who wear concealing clothing.

Serum 25(OH) D measured by chemiluminescence was assessed in 415 women (aged 24-77 years) during summer in Rabat in Morocco (Allali et al 2008). Women's mean age was 50 ± 9.3 years, all were residing in urban areas, eighty-five percent had never practiced sports, and 74% were menopausal. Fifty-six percent of women were wearing the veil for a mean duration of 10 years. Mean serum 25(OH) D for all women was 46 nmol/L. The prevalence of vitamin D insufficiency (25(OH)D ≤ 75 nmol/L) was 91%. One hundred seventy-eight women (43%) had serum 25(OH)D < 37.5 nmol/L, and

17 women (4%) had values < 12.5 nmol/L. Prevalence of vitamin D insufficiency increased in higher ages with the highest prevalence in women >55 years of age (52%). Also, prevalence of vitamin D insufficiency was significantly higher in veiled women (47%) compared to non-veiled women (33%), and it was associated with time spent outdoors. Women who spent less than 30 minutes per day in outdoor activities had a higher prevalence of vitamin D insufficiency (25(OH)D ≤ 75 nmol/L) compared to women who spent more than 30 min/day (47% and 33%, respectively).

It might be relevant here to mention two studies that had assessed vitamin D status of Bangladeshi women, given the fact that Bangladesh is a Muslim country. The first study assessed the influence of socio-economic status on vitamin D status among Bangladeshi women *(Islam et al 2002).* Serum 25(OH) D was measured by radioimmunoassay in a low socioeconomic group (n= 99), and in a high socioeconomic group (n= 90). Women's age ranged between 16-40 years. Median value of serum 25(OH) D was 36.7 nmol/L and 43.5 nmol/L in the low socio-economic group and high socio economic group, respectively. Serum 25(OH)D <25 nmol/L was prevalent among 17% of women in low socio-economic group compared to 12% in the high socio-economic group. The prevalence of serum 25(OH) D < 37.5 nmol/L was 50%, and 38% among women in the low socio-economic group and high socio-economic group, respectively.

The second study evaluated serum 25(OH) D among three groups of women aged 18-60 years (Islam et al 2006). The first group included non-veiled young women (n= 36, mean age= 22.3 years), the second group included veiled women (n= 30, mean

age = 47.7 years), and the third group included non-veiled diabetic women (n= 55, mean age= 50.2 years). Mean 25(OH) D was not significantly different between the three groups. The prevalence of vitamin D deficiency (25(OH) D< 25 nmol/L) was 39%, 30%, and 38% among non-veiled young women, veiled women, and diabetic women, respectively. The prevalence of vitamin D insufficiency (25(OH) D< 40 nmol/L) was 78%, 38%, and 76% among non-veiled young women, veiled women, and diabetic women, respectively.

SOCIOECONOMIC AND HEALTH STATUS OF WOMEN OF CHILD-BEARING AGE AND CHILDREN UNDER-FIVE IN JORDAN

COUNTRY PROFILE

The population size of Jordan was 5.8 million in 2008, with a population growth rate of 2.4% (Population Reference Bureau 2009), and is expected to reach 6.1 million by 2010 (Department of Statistics 2008). Jordan is divided into 12 governorates, and the population density varies from 206 persons/km² in the middle region, 48 persons/km² in the northern region, and 11 persons/km² in southern region (Department of Statistics 2008). The percent of population living in urban areas reached 83% in 2004, and around 38% of the population lives in the capital city Amman (Population Reference Bureau 2009).

Life expectancy at birth was estimated to be 72 years (Males: 71, Females: 73) (United Nations Population Fund 2009). Children under the age of 15 constitute 37% of

the population, and the proportion of people over the age of 65 is 3% (Population Reference Bureau 2009).

The gross domestic product (GDP) per capita reached $2,850 in 2007 (World Bank 2009). The United Nations Development Programme (UNDP) gave Jordan a rank of 86th out of 177 countries with data on the Human Development Index (HDI). The HDI for Jordan is 0.773 (United Nations Development Programme 2008). The HDI provides a composite measure of three dimensions of human development: living a long and healthy life (measured by life expectancy), being educated (measured by adult literacy and enrolment at the primary, secondary and tertiary level), and having a decent standard of living (measured by purchasing power parity, income). With regard to education, illiteracy rate has declined during the past ten years to reach 7.9%, and females are more likely to be uneducated (Table 2.14).

Table 2.14

Illiteracy rate in Jordan

	2003	*2004*	*2005*	*2006*	*2007*
Adult male illiteracy rate (% 15+ years of age)	5.1	5.6	4.8	5.1	4.3
Adult female illiteracy rate (% 15+ years of age)	14.8	15.1	13.1	13.7	11.6
Total (Males and Females)	*9.9*	*10.3*	*9*	*9.3*	*7.9*

Source: (Ministry of Health 2007)

Although female literacy rate is high, only 12% of married women are employed, while labor force participation rate for male is 66.4% (United Nations Population Fund 2009)

HEALTH STATUS

Fertility rate dropped dramatically between 1983 and 2002 (6.6, and 3.7, respectively) and remained almost constant over 2002-2007 (Ministry of Health 2007). Family wealth seems to influence fertility, women living in poor households have almost twice as many children (4.8 children/woman) compared to women who live in wealthier households (2.5 children/woman) (Department of Statistics 2008).

Fifty-percent of women in Jordan are married by age 22.2, but only 18% are married by age 18. Childbearing begins at a relatively late age in Jordan compared to neighboring countries, half of women have their first birth by age 23.9 and only 8% of women had their first birth by age 18 (Department of Statistics 2008). Maternal mortality ratio is 41 per 100,000 live births (World Health Organization 2008) which is low compared to other countries in the Middle East.

Infant mortality rate has sharply declined during the last 20 years from 82 deaths per 1,000 live births in 1976 to 19 deaths in 2007. Under-five mortality rate is 25 deaths per 1,000 live births (UNICEF 2009)**.**

Mortality rates are slightly higher in rural than urban areas, and they also differ by governorate. For example, under-five mortality ranges from 10 in central governorates to 39 in southern governorates. Also, as women's education increases, childhood mortality decreases. Under-five mortality rate is twice as high among children whose mothers are uneducated compared to children whose mothers have at least a high school degree (Department of Statistics 2008).

Many factors influence infant mortality rate; mother's age at birth, birth order, and length of previous birth interval. Mother's age at delivery and infant mortality exhibit a U-shaped curve. Mortality rates are higher among infants born to mothers less than 20 years of age, and those aged 40 and over (Department of Statistics 2008). Birth weight is an important indicator for the infant's susceptibility to the risk of illness. The State of the World's Children 2009 report estimated that 12% of babies born in the year 2006-2007 in Jordan were born with low birth weight (<2.5 kilogram at birth) (UNICEF 2009).

THE PREVALENCE OF STUNTING AND WASTING AMONG CHILDREN UNDER-FIVE IN JORDAN AND THE REGION

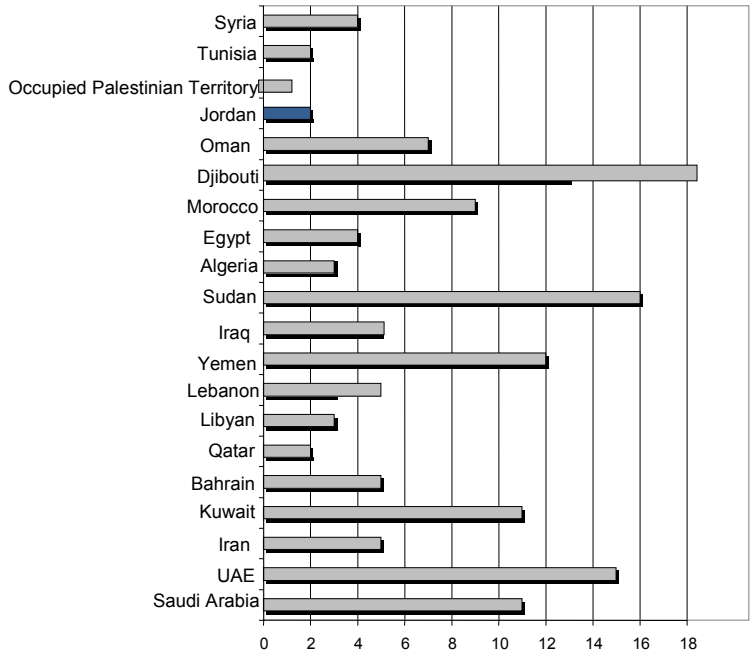
Two major indicators of child malnutrition are stunting and wasting. Stunting is defined as insufficient height relative to age (WHO 1995b). It is an indicator of chronic nutritional deprivation, as it results from extended periods of inadequate food intake, poor dietary quality, increased morbidity, or a combination of these factors (Gibson 2005) . Wasting results from a failure to gain sufficient weight relative to height or from weight loss (WHO 1995b).

The State of the World's Children 2009 reported (based on the most recent data available during the years 2000-2007) a prevalence of 5%, 2%, and 9% for underweight, wasting and stunting, respectively among children under five in Jordan (UNICEF 2009). Jordan has been successful in reducing the prevalence of stunting from 16% during the period 1980-1996 (UNICEF 1996) to 9% during the period 1996-2005 (UNICEF 2006).

Figures 2.6 and 2.7 show that protein-energy malnutrition is still a public problem in the Middle East North Africa (MENA) region. The prevalence of stunting and wasting in some countries (like United Arab Emirates, Saudi Arabia, and Kuwait) is shocking given the high GNI per capita for these countries. Poverty and food shortage are not strong causes for stunting and wasting among children in these countries. On the other hand, De Onis and Blossner (2000) analyzed 92 countries in a standardized way to allow comparisons between prevalence of undernutrition and obesity across countries and over time; many countries with a high level of obesity still reported significant rates of childhood stunting. These countries include: Egypt, Morocco, Bahrain, and Algeria from the MENA region (De Onis and Blossner 2000).

Figure 2.6

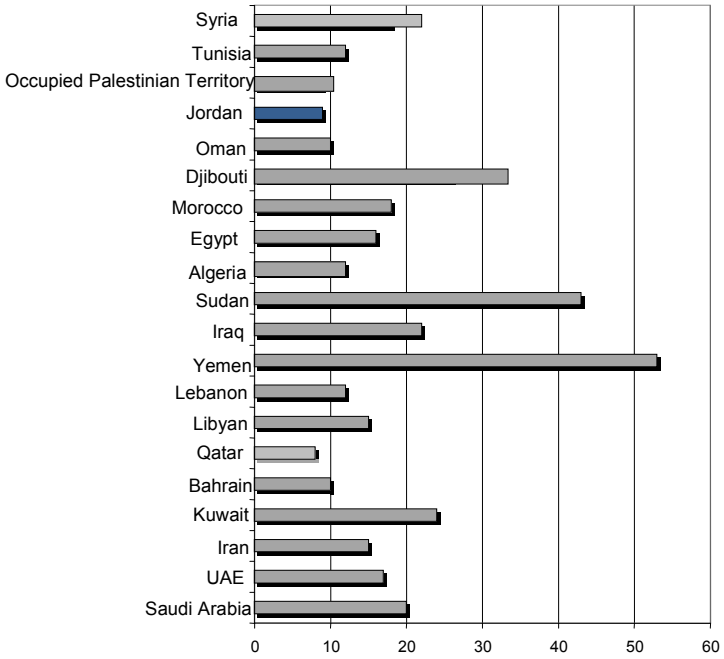
Percentage of children under-five suffering from wasting in the MENA region (2000-2007)



Data refer to the most recent year available during 2000-2007, source: (UNICEF 2009)

Figure 2.7

Percentage of children under-five suffering from stunting in the MENA region (2000-2007)



Data refer to the most recent year available during 2000-2007, source: (UNICEF 2009)

On the local level, the last estimates of the national prevalence of stunting, wasting and underweight were determined in 1997 by the Jordan Population and Family Survey. The nutritional status of children from birth to 59 months was determined using three indices: weight-for-height (wasting) reflecting acute growth disturbances, height-for-age (stunting) reflecting long term faltering and weight-for-age (underweight). The national prevalence rates of stunting, wasting, and underweight were 7.8%, 1.9%, and 5.1%, respectively, (Department of Statistics 1997). There was little difference in stunting between boys and girls in the whole sample (8% and 7% for males and females, respectively). Compared to males, a slightly higher proportion of females had wasting

(2.0% and 1.2% for females and males, respectively), and underweight (5.5% and 4.6% for females and males, respectively).

The nutritional status of children was better in urban than in rural areas: 13.7% of rural children were stunted compared to only 6.7% of urban children. The same trend was true in the case of underweight (8.5% and 4.3% for rural and urban children, respectively). However, there was no difference regarding wasting (around 2%) in each sector (Khatib et al 1998). In 2006, growth pattern was determined in 1695 healthy children (842 boys and 853 girls) between 3-6 years of age (Ibrahim et al 2008). Two percent of boys and 0.5% of girls were below -3 SD of weight for height z-score, and 7.4% of boys and 5.2% of girls fell in the group of (-3 SD < -2 SD).

NUTRITIONAL STATUS OF WOMEN OF CHILD-BEARING AGE IN JORDAN

The 2007 Jordan Population and Health Survey (JPFHS) collected data on the height and weight of 7,759 women aged 15-49 years (half of the households sampled). Almost 50% of women were overweight or obese (BMI≥ 25 kg/m^2), and only 4% were underweight (Department of Statistics 2008) (Table 2.15).

Jordan (as many developed and developing countries) is undergoing a "nutrition transition". According to Popkin (2006) this global phenomena is characterized by large shifts from traditional diet that is high in fiber, grains, fruits, and vegetables to more modern diet that is characterized by high fat, low fiber, and greater percentages of energy derived from sugars and animal protein and fats (Popkin 2006). This phenomenon has been attributed to the increased urbanization worldwide from 46% in 1965 to 83% in 2004,

(Population Reference Bureau 2009), and the health care reforms that decreased infant and child mortality rate and increased life expectancy.

Table 2.15

BMI distribution among Jordanian women

		Percentage of women in each BMI category (Total n= 7,759)			
	Mean BMI (kg/m^2)	***<18.5***	***18.5-24.9***	***25-29.9***	***≥30.0***
Age *(years)*					
15-19	23	7.3	71.2	15.7	5.7
20-29	24.3	3.7	60.9	23.3	12.1
30-39	26.4	3.4	41.4	33.1	22.2
40-49	27.3	3.7	38.2	28.4	29.7
Residence					
Urban	25.7	3.9	49.3	27.2	19.7
Rural	26.3	4.0	45.3	28.2	22.4
Total	25.8	3.9	48.6	27.4	20.1

Source: (Department of Statistics 2008)

Nutrition transition is accompanied by increase in obesity rates, and chronic diseases including diabetes, hypertension, and stroke. The total prevalence of obesity (BMI ≥ 30kg/m^2) was found to be 34.8% among 710 Jordanian adults in 2004 (Table 2.16), and obesity was significantly associated with diabetes, high blood pressure, high cholesterol, and asthma (Zindah et al 2008).

Table 2.16

Prevalence of obesity and related non-communicable diseases in a study in Jordan

	Sex		Age groups (years)				
	Male %	*Female %*	*18-34 %*	*35-49 %*	*50-64 %*	*≥65 %*	*Total %*
Obesity	21.1	41.5	19.1	42.7	45.3	43.4	34.8
High blood pressure	36.3	27.3	9.4	28.3	55.2	61.4	30.2
High cholesterol	25.6	21.9	8.0	25.5	38.0	39.2	23.1
Diabetes	17.7	16.5	6.1	12.7	32.3	39.0	16.9

Source: (Zindah et al 2008)

Another recent study assessed the prevalence of metabolic syndrome among 394 males and 727 females in Northern Jordan (Khader et al 2007). Participants' age was ≥ 25 years, and criteria for the metabolic syndrome were met if an individual met 3 or more of the following criteria: abdominal obesity, high fasting glucose, high blood pressure, hypertriglyceridemia, and low LDL cholesterol. Mean ± SD of BMI for women was 31.6 ± 5.8, and the prevalence of metabolic syndrome was 40.9% among women compared to 28.7% among men.

The 2007 Jordan Population and Family Health Survey (JPFHS) shows that most mothers of young children consume foods made from grains (92%), foods made with oil, fat or butter (88%), meat, fish, shellfish, poultry or eggs (84 %) and cheese or yogurt (82%) (Department of Statistics 2008). Two-thirds of mothers consume fruits or vegetables rich in vitamin A and three-fourths consume other types of fruits and vegetables. Consumption of iron-rich foods was found to be higher among mothers with a higher education and among women living in wealthier households. In addition, 50% of mothers reported taking iron supplementation for more than three months during

their last pregnancy, and 19% of mothers reported not taking any iron supplementation during their last pregnancy. Mothers with higher levels of education and those living in the wealthiest households were most likely to take at least 90 days worth of iron supplements.

Overall, Jordan has achieved good progress compared to the other countries in the region in reducing maternal and infant mortality rate, stunting and wasting among children less than five years of age, and in implementing some programs to eliminate micronutrient deficiency like fortification and supplementation of some micronutrients. Jordan is moving ahead toward the achievement of the Millennium Development Goals (Table 2.17).

Table 2.17

Examples of health indicators related to Millennium Development Goals (MDGs) in selected countries in the Middle East

	Jordan	*Lebanon*	*Egypt*
MDG 1: Eradicate poverty and hunger			
Prevalence of child malnutrition (U5 underweight - %)	4	4	5
Prevalence of child malnutrition (U5 stunting - %)	9	11	18
Prevalence of child malnutrition (U5 wasting - %)	2	5	4
MDG 2: Reduce child mortality			
Under 5 mortality rate (per 1,000)	24	29	36
Infant mortality rate (per 1,000 live births)	21	26	30
MDG 3: Improve maternal health			
Maternal mortality ratio (per 100,000 live births)	41	100	84
Births attended by skilled health staff (%)	99	96	74

Source: The State of the World's Children 2009: Maternal and Newborn Health. (UNICEF 2009)

PREVALENCE OF ANEMIA AND IRON DEFICIENCY IN JORDAN AND THE EASTERN MEDITERRANEAN REGION

Anemia and iron deficiency are the most common nutritional disorders affecting children and women in both industrialized and developing countries (Table 2.18). Globally, forty-seven percent of preschool-age children and 30.2% of non-pregnant women are anemic (WHO 2008). About 47% of pre-school children (0.8 million) have hemoglobin (Hb) levels below 11 g/dL and almost 40 million women of child bearing age (non-pregnant) have Hb levels below 12 g/dL in the Eastern Mediterranean region (WHO 2008).

Table 2.18

Global prevalence of anemia in WHO regions

Region	*Prevalence of anemia (%)*		
	Pre-school children *(0.00-4.99 yrs) Hb<11 g/dl*	Pregnant women *(no age range defined) Hb<11 g/dl*	Non-pregnant women *(15.00- 49.99 yrs) Hb<12 g/dl*
Africa	67.6	57.1	47.5
Americas	29.3	24.1	17.8
South-East Asia	65.5	48.2	45.7
Europe	21.7	25.1	19.0
Eastern Mediterranean	**46.7**	**44.2**	**32.4**
Western Pacific	23.1	30.7	21.5
Global	**47.4**	**41.8**	**20.2**

Source: adapted from " Worldwide prevalence of anemia 1993–2005: WHO global database on anemia", (WHO 2008)

The WHO classification of public health significance of anemia in populations based on prevalence estimated from hemoglobin or hematocrit is presented in Table

2.19. According to the WHO classification, the prevalence of anemia in the Eastern Mediterranean region is considered a severe public health problem among pre-school children and moderate among women of child-bearing age.

Table 2.19

WHO classification of public health significance of anemia

Prevalence of anemia (%)	*Category of public health significance*
≤ 4.9	Normal
5.0 - 19.9	Mild
20.0 - 39.9	Moderate
≥ 40.0	Severe

Source: (WHO/UNICEF/UNU 2001)

Anemia is a major nutritional problem among women and children in the countries of the Middle East and North Africa region regardless of the variation in the socioeconomic status among these countries. The prevalence of anemia among pre-school children ranges from 22% in Tunisia to almost 70% in Yemen and 85% in Sudan (Table 2.20). In Jordan, it is estimated that more than 28.3% children under the age of five are anemic (WHO 2008).

Table 2.20

Prevalence of anemia among *pre-school children* in the Eastern Mediterranean region

	Date of survey	*Age range (yrs)*	*Sample size*	*Prevalence of anemia % (Hb<11 g/dl)*	*Public health problem*
Low Income					
Yemen				68.3	Severe
Sudan	1994-1995	0.5-6.99	1970	84.6	Severe
Lower Middle Income					
Egypt	2000	0.5-4.99	4708	29.9	Moderate
Iraq				55.9	Severe
Morocco	2000	0.5-4.99	1486	31.5	Moderate
Syria				41.0	Severe
Jordan	***2002***	***0.5-4.99***	***2573***	***28.3***	***Moderate***
Iran				35.0	Moderate
Tunisia	1996-1997	0.0-5.99	965	21.7	Moderate
Lebanon	1997-1998	1.0-4.99	234	28.3	Moderate
Upper-middle Income					
Oman	1995	0.0-5.99	5015	50.5	Severe
Libya				33.9	Moderate
Saudi Arabia				33.1	Moderate
Bahrain				24.7	Moderate
High Income					
Qatar	1995	1.0-2.0	1449	26.2	Moderate
Kuwait	1998-2002	0.5-4.99	3693	32.4	Moderate
United Arab Emirates				27.7	Moderate

Source: "WHO Global Database on Anemia: World Wide Prevalence of Anemia 1993-2005" (WHO 2008)

The prevalence of anemia among non-pregnant women of reproductive age has been classified as a severe public health problem in some countries in the region even in countries with higher income like United Arab Emirates and Bahrain with prevalence of 44% and 51%, respectively (Table 2.21).

Table 2.21

Prevalence of anemia among non-pregnant women of reproductive age in the Eastern Mediterranean region

		Date of survey	*Age range (yrs)*	*Sample size*	*Prevalence of Anemia % (Hb<11 g/dl)*	*Public health problem*
Low Income	Yemen				51.0	Severe
	Sudan				43.5	Severe
Lower Middle Income	Egypt	2000	15-49.99	9210	27.6	Moderate
	Iraq				45.3	Severe
	Morocco	2000	15-49.99	1784	32.6	Moderate
	Syria				33.4	Moderate
	Jordan	***2002***	***15-49.99***	***2925***	***28.6***	***Moderate***
	Iran	1994-1995	15-49.99	1351	33.0	Moderate
	Tunisia	1996-1997	17-59.99	1951	26.3	Moderate
	Lebanon	1997-1998	15-49.99	539	25.2	Moderate
Upper-Middle Income	Oman	2000	15-49.99	2766	34.0	Moderate
	Libya				29.9	Moderate
	Saudi Arabia				32.2	Moderate
	Bahrain	2002	14-49.99	384	51.3	Severe
High Income	Qatar				36.2	Moderate
	Kuwait	1998-2002		2993	28.7	Moderate
	UAE				43.9	Severe

Source: "WHO Global Database on Anemia: World Wide Prevalence of Anemia 1993-2005" (WHO 2008)

Based on the WHO global database almost 30% of non-pregnant women of reproductive age in Jordan are anemic but some other studies show that the percentage of women with iron deficiency is even higher. The Ministry of Health in Jordan has assessed the prevalence of anemia, iron deficiency, and iron deficiency anemia among women of reproductive age (Table 2.22), and it was 32%, 40%, and 22%, respectively (Ministry of Health 2003).

Table 2.22

Prevalence of anemia, iron deficiency, and iron deficiency anemia among different age groups in Jordan, 2003

	Anemia	*Iron deficiency*	*Iron deficiency anemia*
Non-pregnant women of reproductive age	32%	40%	22%
Pre-school children	20%	26%	10%
School children (6-18 years)	9.3%	-	-

Source: (Ministry of Health 2003)

In 2003, anemia was assessed in 200 pregnant women in northern Jordan (Albsoul-Younes et al 2004). Eighty-four women (41%) women were anemic. Increased risk of anemia was associated with lower educational level, multiparity, and rural residence. Another study assessed the prevalence of anemia in 260 women aged 18-49 years and found that 40% were anemic (Mawajdeh et al 2003).

A study on the incidence of iron deficiency anemia in infants was conducted in 1999. The study examined the relationship between anemia during pregnancy and iron deficiency in 232 infants. The iron status of infants born to 107 anemic (Hb<11g/dl) and 125 non-anemic mothers was reviewed at 3, 6, 9 and 12 months. Indicators to define iron-deficiency were (Hb<11g/dl) and either plasma ferritin (<12 mg/dl) or zinc protoporphyrin (ZPP) (>35mg/dl) whole blood. The results indicate that anemia was significantly higher in infants born to anemic mothers (81%) compared with controls (65%). At 12 months of age, 72% were anemic, while 57% were identified as iron deficient (Kilbride et al 1999).

The prevalence of iron deficiency anemia as well as different types of Thalassaemia was investigated in 1020 school children (aged 6-15 years) living in the north-eastern Badia region in Jordan (Babiker et al 1999). Iron deficiency anemia was diagnosed in 54 children.

The Ministry of Health in Jordan conducted a national baseline survey on iron deficiency anemia along with WHO, UNICEF, and Centers for Disease Control and Prevention in 2002. The study found a different prevalence of iron deficiency among children based on age groups as well as between children living in urban and rural areas as described in Table 2.23 (Ministry of Health et al 2002).

Table 2.23

Prevalence of anemia among children by age groups in Jordan, 2002

Children's Age Group (yrs)	*n*	*Mean Hb (g/dl)*	*Prevalence of Anemia (%)*
1.00-1.99	245	11.2	34.4
2.00-2.99	258	11.7	23.3
3.00-3.99	281	11.9	13.2
4.00-4.99	276	12.0	10.6
1.00-4.99 (rural)	366	11.6	22.4
1.00-4.99 (urban)	695	11.7	19.4

Source: (Ministry of Health et al 2002)

The high prevalence of anemia among children under- five years of age is due to poor dietary intakes of iron, low iron absorption, and mothers' anemia during pregnancy. The high prevalence of anemia among women is caused by many factors including early age at marriage, short birth intervals, poor dietary diversification, lack of nutrition education, and perhaps by high consumption of tea containing tannins that reduce iron availability (Aoyama 1999).

PREVALENCE OF ZINC DEFICIENCY IN JORDAN AND THE EASTERN MEDITERRANEAN REGION

The World Health Organization (WHO) identified the factors contributing most to burden of disease in developing countries with high mortality; zinc deficiency was ranked as the 5th factor followed by iron deficiency as the 6th (WHO 2002). Globally about ½ of the human population is at risk of low zinc intake (Brown and Wuehler 2000). First cases of zinc deficiency in humans were reported in the Middle Eastern region in the sixties, specifically in Iran and Egypt (Prasad et al 1961, Prasad et al 1963), and zinc deficiency was highly correlated with severe anemia, growth retardation, and delay in sexual maturation.

In zinc deficiency, the first response is a reduction in growth without an apparent reduction in tissue concentration (Cousins 2006). Signs of zinc deficiency include: growth retardation, depression of immune function, decreased appetite, skeletal abnormalities, and impaired reproductive ability (Cousins 2006). According to a meta-analysis of 33 randomized-controlled zinc intervention trials in pre-pubertal children, zinc interventions have demonstrated that zinc supplementation produced positive responses including stunting reduction in children, weight gain, and linear growth (Brown et al 2002). Zinc supplementation is also associated with improved reproductive functions including fetal growth, increased birth weight, lack of congenital malformations, and term delivery (Black 2003).

According to the World Bank, zinc deficiency accounts for about 800,000 deaths annually from diarrhea, pneumonia, and malaria in children under five years of age (Caulfield et al 2006). While Sub-Saharan Africa accounts for the malaria burden, the

Middle East and North Africa region along with South Asia and Sub-Saharan Africa contribute to the other zinc deficiency outcomes: pneumonia and diarrhea.

The high prevalence of anemia in the MENA region despite the fortification of flour with iron supports the fact that there is a need to assess zinc status. Anemia is a marker for both iron and zinc deficiency (Shrimpton et al 2005), and it is likely that iron deficiency exists with other micronutrient deficiencies including zinc and vitamin B_{12} (Dewey and Brown 2003). Table 2.24 shows that the prevalence of zinc deficiency in the Middle East and North Africa region is estimated to be 46%. This figure was estimated by examining the availability of zinc in the local diet in the following countries: Afghanistan, Algeria, Djibouti, Egypt, Iraq, Morocco, Pakistan, Somalia, Sudan, and Yemen (Caulfield and Black 2004).

Table 2.24

Estimated zinc deficiency outcomes among children ages birth through four by region

Region	*Prevalence (%)*	*Deaths (Thousands)*	*DALYs loss* (Thousands)*
East Asia and the Pacific	7	15	1,004
Eastern Europe and Central Asia	10	4	149
Latin America and the Caribbean	33	15	587
Middle East and North Africa	**46**	**94**	**3,290**
South Asia	79	252	8,510
Sub-Saharan Africa	50	400	14,094
High Income Countries	5	0	2

*DALYs: Disability Adjusted Life Years = the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability.
Source: Adapted from (Caulfield et al 2006)

Currently, there are no estimates about the national prevalence and distribution of zinc deficiency in Jordan, and there is a serious lack of awareness of the importance

of zinc in human nutrition not only in Jordan but also in the MENA region. Direct measurement of plasma zinc in a representative sample is required, so that knowledge about the prevalence and distribution of zinc deficiency can be established before setting steps to eliminate zinc deficiency. Gibson (2006) recommended that: *"at risk countries either national or targeted Zn interventions such as supplementation, fortification, dietary diversification or modification, or biofortification should be implemented, where appropriate, by incorporating them into pre-existing micronutrient intervention programs" (Gibson 2006).*

Diets in the MENA region are characterized by low zinc bioavailability contributed to by diets high in phytate that inhibits zinc absorption. The incidence of zinc deficiency is similar to that of nutritional iron deficiency because both are caused by similar dietary patterns (Sandstead 2000). Zinc deficiency may be the underlying cause in the high prevalence of child growth retardation, low birth weight and anemia in the MENA region (Aoyama 1999).

IRON IN HUMANS

OVERVIEW

The approximate amount of iron in the human body is about 2.5-4.0 gm which is about 30-40 mg/kg body weight (Beard 2006). The largest constituent of body iron is in the form of functional iron which takes three forms: hemoglobin (about 70%), the oxygen carrying pigment of red blood cells, myoglobin (about 4%), the oxygen-binding storage protein in muscles; and various iron-containing enzymes like cytochromes which act as electron carriers within the cells. About 25% of body iron (0-15 mg/kg) is in the form of storage iron that is found primarily in the liver (60%), muscles, and cells of the reticuloendothelial system (Beard 2006). Most of the iron stored in the liver is in the form of ferritin (95%) and hemosiderin (5%) (Bothwell et al 1979). Finally, iron mobilized from tissues carried by the transport protein (transferrin) comprises less than 1% of body iron.

Iron balance in the human body is maintained based on the interrelationship between dietary iron intake, iron absorption and bioavailability, and body demand (WHO 1996). Disruption in any of these factors like low iron intake due to poverty or poor food choices, poor absorption and low iron bioavailability, or increase in body demand due to pregnancy, growth or blood loss in menstruation or diseases means that the body's physiological needs are not met by enough iron intake, and iron stores diminish leading to iron deficiency.

Dietary sources provide two forms of iron: heme and non-heme iron. Heme iron is derived from hemoglobin and myoglobin and found in animal sources of food like

liver, beef, turkey, and other kinds of meat. Non-heme iron refers to all non-animal sources of iron (plant sources, fortified food, and contaminant iron coming from dust, dirt, and soil). The two forms differ in their bioavailability. Heme iron is absorbed more effectively than non-heme iron and is considered the most bio-available form of iron (Bothwell et al 1979). During digestion, the pancreatic and gastrointestinal enzymes split the heme molecule from the globin molecule but the iron remains tightly bound within the porphyrin ring structure of the heme molecule and is taken up intact after binding to its receptors (Conrad et al 1967). Then the iron is released to the cytoplasm by the action of heme oxygenase (Raffin et al 1974). The average absorption of heme iron is about 25% (Hallberg et al 1979) whereas the absorption of non heme iron is often much less than that. The absorption of heme iron is regulated based on an individual's iron status. During iron deficiency, heme iron absorption in the proximal intestine increases up to 40%, but decreases to about 10% during iron repletion (Hallberg et al 1997), an important regulatory mechanism in preventing iron overload in case of increased dietary intakes of iron.

IRON REQUIREMENTS AND BIOAVAILABILITY

Iron losses vary with age, sex, and disease status (hemorrhage, infection with hookworms and other parasites, or peptic ulcer). A typical male loses about 0.8-0.9 mg iron per day (Beard 2006) while females of reproductive age lose an average estimated additional 0.48 mg/day due to menstruation (WHO/FAO 2005b). However, the menstrual iron loss can be as high as 2.0 mg/day (Beard 2006). Infants, children, and

pregnant women have higher requirements for iron due to growth and development. Iron stores are exhausted during rapid growth, and if physiological requirements are not met by adequate iron intake, a nutritional iron deficiency will develop.

Iron intake alone is not enough to ensure attaining adequate iron status for individuals. The bioavailability of dietary iron (how much is really absorbed) is very important. Unfortunately, in addition to the low intake by children and women of foods rich in iron in many developing countries, most of the iron intake is in the form of non heme iron that comes from non animal sources. Due to poverty, people rely more on plant-based diets like grains and legumes which contain high amounts of energy but low amount of bioavailable iron. The bioavailability of non-heme iron is affected by a number of inhibitors and facilitators of absorption. Absorption of non-heme iron is enhanced by ascorbic acid and animal proteins like meat, fish, and poultry (Cook and Monsen 1977, Davidsson et al 1998, Disler et al 1975a, Hallberg and Hulthen 2000) and greatly inhibited by phytate (grains, seeds, and beans), polyphenols (tannins in tea and coffee), organic acids (chlorogenic acid in coffee), and soy proteins (Cook et al 1981, Derman et al 1977, Disler et al 1975b, Gillooly et al 1983, Hallberg and Rossander 1982a, Hallberg and Rossander 1982b, Hallberg et al 1987, Hurrell et al 1992, Morck et al 1983). The highest iron bioavailability is associated with diets that are high in ascorbic acid and meat content, and low in phytate-rich cereals. Consuming no coffee or tea within 2 hours of the main meals also enhances iron bioavailability (Morck et al 1983, WHO/FAO 2005b).

Calcium has been found to interfere significantly with the absorption of both heme and non-heme iron although the precise mechanism is unknown (Gleerup et al 1993, Gleerup et al 1995, Hallberg et al 1991, Hallberg et al 1992).

Table 2.25 shows the bioavailability of iron from different type of diets that vary in their meat, phytate, and tannin content. The bioavailability is then expressed as amount of iron absorbed by a 55-kg woman with no iron stores, and then it is translated into the percentage absorbed for an iron intake of 15 mg/d.

Table 2.25

The potential bioavailability of iron in different diets types and estimated percentage absorbed for an iron intake of 15 mg/day

Type of Diet	*Bioavailability (µg/kg/d}*	*Absorption in 55-kg woman with no iron stores (mg/d)*	*Bioavailability (%) for an iron intake of 15 mg/d*
Very high meat in two main meals daily and high ascorbic acid (theoretical)	75.0	4.13	27.5
High meat/fish in two main meals daily	66.7	3.67	24.5
Moderate meat/fish in two main meals daily	53.2	2.93	19.5
Moderate meat/fish in two main meals daily; low phytate and calcium	42.3	2.32	15.5
Low meat intake; high phytate, often one main meal	25.0	1.38	9.2
Meat/fish negligible; high phytate; high tannin; and low ascorbic acid	15.0	0.83	5.5

Source: Adapted from (WHO/FAO 2005b)

In the Middle East, tea along with coffee is widely consumed with meals or immediately following meals. Tea is widely consumed by mothers, young girls, and even children. The high content of polyphenols in these beverages inhibits the absorption of non-heme iron in diet and greatly decreases its bioavailability. Iron absorption is decreased by 70-80% with consumption of 200 ml tea, and decreased by 60% with consumption of 150 ml coffee (Hallberg and Hulthen 2000).

Iron intake and iron bioavailability were studied in a longitudinal study in 126 iron-replete, non anemic 6-10 year old children in Morocco (Zimmermann et al 2005). The children consumed their regular cereal and legume-based diet for 15 months. The children's diets had sufficient energy, protein and most importantly had high iron content (5.4 mg/1000 kcal). Based on the children's iron intake, absorption of only 8% of their dietary iron would be sufficient to meet their requirements, but the absorption was estimated to be 2% only (0.22 mg/d). At the end of the study, 75% of children were iron deficient and about 33% had mild iron deficiency anemia.

Iron status and tea consumption were reviewed in 16 studies that evaluated iron deficiency in relation to tea consumption (Temme and Van Hoydonck 2002). Six studies included infants and children, and another six included pre-menopausal women. Subjects in these 12 studies had a high prevalence of iron deficiency. The other 4 studies included men and elderly with low prevalence of iron deficiency. The review found that tea consumption was inversely associated with serum ferritin and/or hemoglobin in populations with marginal iron status but the association was not found in people with adequate iron stores.

Recently, the WHO published recommended nutrient intakes (RNIs) for iron based on the bioavailability of the usual diet consumed by the population (WHO/FAO 2005b). For example, if the usually consumed diets of the population have an estimated bioavailability of 5.5% (form Table 2.25: diets that have a negligible content of meat/fish, high phytate, high tannin, and low ascorbic acid have a bioavailability of 5.5%), then the RNI for iron for children at 4-6 years of age for example should increase up to 12.6 mg/day compared to 4.2 mg/day for diets with 15% bioavailability (Table 2.26).

Table 2.26

Recommended nutrient intakes (RNIs) for iron for different dietary iron bioavailability

Group	*Mean body weight (kg)*	*Recommended nutrient intake (mg/day) for a dietary iron bioavailability of:*		
		15%	*10%*	*5%*
Children (1-3 years)	13	3.9	5.8	11.6
Children (4-6 years)	19	4.2	6.3	12.6
Females (11-14) pre-menarch	46	9.3	14.0	28.0
Females (11-14)	46	21.8	32.7	65.4
Females (15-17)	56	20.7	31.0	62.0
Women (18+ years)	62	19.6	29.4	58.8
Postmenopausal women	62	7.5	11.3	22.6
Lactating women	62	10.0	15.0	30.0
Men (18+ years)	75	9.1	13.7	27.4

Source: (WHO/FAO 2005b)

Women of child bearing age along with teenage girls (i.e., menstruating women) have an increased iron requirement due to menstrual iron losses. On average, a menstruating woman looses approximately 0.56 mg/day over 28 days of the menstrual cycle (WHO/FAO 2005b). The volume of menstrual blood loss varies from woman to woman but it remains fairly constant for the same woman throughout her fertile life

(Hallberg et al 1966). Hallberg and Rossander-Hulten studied the dietary iron requirements in menstruating women taking into account three factors: the average basal iron loss (0.8 mg/day), the menstrual loss, and three different iron bioavailabilities based on three diet types (14% for Swedish diet, 16% for French diet, and 16.6% for US diet). A bioavailability of 15% was used to represent the bioavailability of the general Western-type diet. The study concluded that the dietary iron requirements for the 95th percentiles were 18.9 mg for menstruating women (18+ years old) and 21.4 mg for menstruating teenagers (Hallberg and Rossander-Hulten 1991).

STAGES OF IRON DEFICIENCY

Iron deficiency is "*a state in which there is insufficient iron to maintain the normal physiological function of tissues such as blood, brain, and muscles*" (WHO/CDC 2004). It results from long-term negative iron balance (WHO/UNICEF/UNU 2001). The progression of iron deficiency occurs in three successive stages:

- Iron deficiency (ID): characterized by exhaustion of iron stores (diminished ferritin and hemosiderin) but functional iron is not compromised. This stage is marked by a decrease in serum ferritin concentration.
- Iron-deficient Erythropoieses (IDE): at this stage, the iron supply for eryhthropoiesis is reduced. This stage is marked by increases in serum transferrin receptors (TfR) and erythrocyte protoporphyrin but still anemia is not detected.

- Iron deficiency anemia (IDA): this is the final stage of iron deficiency which is characterized by low iron stores and sub-optimal hemoglobin concentration along with decreases in the following red cell indices: mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC).

ASSESSMENT OF IRON DEFICIENCY

The gold standard for the evaluation of iron status is the absence of stainable iron in the reticuloendothelial cells in the bone marrow (Bothwell et al 1979). However, this method is not practical for evaluation of iron status in field settings because it is expensive and invasive. The commonly used measures of iron status are mentioned in Table 2.27. Cook (2005) classifies the laboratory measures of iron deficiency into two major categories: screening and definitive measures (Cook 2005). The screening measures involve: hemoglobin; hematocrit; red cell indices; transferrin saturation; and erythrocyte zinc protoprphyrin. The definitive measures involves: ferritin, serum iron; transferrin receptor; and bone-marrow iron. The screening tests measure the iron supply to the circulating red blood cells (degree of hemoglobinization), while the definitive tests measure the depletion of iron stores and tissue iron deficiency.

Each of these tests has its limitation in term of sensitivity and specificity (as discussed in Table 2.27. Therefore the use of a multivariable approach is preferred in population-based assessment when measurement of multiple indices is feasible (WHO/UNICEF/UNU 2001). A discussion on defining iron deficiency and iron deficiency anemia using multiple indices is presented later.

Table 2.27

Description of iron deficiency and iron deficiency anemia indicators

<table>
<tr><th>Explanation and comments</th><th>Suggested cut-off values</th></tr>
<tr><td colspan="2">Hemoglobin (g/dl) and Hematocrit (PCV)%</td></tr>
<tr><td>Hemoglobin (Hb):
-Most widely used to assess iron deficiency anemia which is the final stage of iron deficiency, therefore it has a low sensitivity. Typically, 20-30% of body iron is lost before anemia develops (Cook 2005). Also, it has a low specificity. Measurement of Hb is indicative of anemia but not specifically iron deficiency anemia. Low Hb concentrations can result from deficiencies of vitamin A, B_6, B_{12}, and riboflavin, and folic acid (Fishman et al 2000, van den Broek and Letsky 2000).

Hematocrit [Packed cell Volume (PCV)]:
-Low sensitivity: it falls only in case of severe iron deficiency anemia
-Low specificity: affected by same factors that influence Hb</td><td>Hb and PCV cut-off values for iron deficiency anemia

<table><tr><th></th><th></th><th><u>Hb</u></th><th><u>PCV</u></th></tr><tr><td>Children</td><td>6-59 mo</td><td><11.0</td><td><0.33</td></tr><tr><td></td><td>5-11 y</td><td><11.5</td><td><0.34</td></tr><tr><td></td><td>12-14 y</td><td><12.0</td><td><0.36</td></tr><tr><td>Women> 15 y</td><td>Non-pregnant</td><td><12.0</td><td><0.36</td></tr><tr><td></td><td>Pregnant</td><td><11.0</td><td><0.33</td></tr><tr><td>Men >15 y</td><td></td><td><13.0</td><td><0.39</td></tr></table>Source: (WHO/UNICEF/UNU 2001)</td></tr>
</table>

Table 2.27-*continued*

Description of iron deficiency and iron deficiency anemia indicators

<table>
<tr><th>Explanation and comments</th><th>Suggested cut-off values</th></tr>
<tr><td colspan="2">Red Cell Indices: MCV, MCH, and MCHC</td></tr>
<tr><td>-Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) are relatively late indicators of IDA.
-In IDA, the reduction in MCV occurs in parallel with anemia, followed by a fall in MCH, then MCHC which is the least useful index.
-Microcytic RBCs (small MCV) is a common sign of iron deficiency anemia. Macrocytic RBCs (high MCV) indicate megaloblastic anemia resulting from folate or vitamin B_{12} deficiency.</td><td>Normal values for red cell indices for children and females
<table><tr><th>Age (y)</th><th>MCV</th><th>MCH</th><th>MCHC</th></tr><tr><td>Children</td><td></td><td></td><td></td></tr><tr><td>1-1.9</td><td>79 (67)</td><td>27.4 (22)</td><td>34.4 (32)</td></tr><tr><td>2-4.9</td><td>81 (73)</td><td>28.1 (25)</td><td>34.5 (32)</td></tr><tr><td>5-7.9</td><td>82 (74)</td><td>28.6 (25)</td><td>34.5 (32)</td></tr><tr><td>Females</td><td></td><td></td><td></td></tr><tr><td>12-14.9</td><td>86 (77)</td><td>29.4 (26)</td><td>34.1 (32)</td></tr><tr><td>15-17.9</td><td>88 (78)</td><td>30.0 (26)</td><td>33.9 (32)</td></tr><tr><td>>18.0</td><td>90 (81)</td><td>30.6 (26)</td><td>33.9 (32)</td></tr></table>Source: (WHO/UNICEF/UNU 2001)
- Values are mean (-2SD). Units are: MCV (fl), MCH (pg), and MCHC (g/l).</td></tr>
<tr><td colspan="2">Erythrocyte Zinc Protoporphyrin (ZnPP) or Erythrocyte Protoporphyrin</td></tr>
<tr><td>-When iron is insufficient in the bone marrow for heme biosynthesis, zinc is substituted for iron to form ZnPP instead of protoporphyrin IX. Therefore, high concentration of ZnPP in blood indicates a suboptimal supply of iron at the time of erythrocyte maturation (Gibson 2005).
- Low specificity: can be elevated in infection or inflammation, lead poisoning, or hemolytic anemia.
- In iron overload, normal iron levels, or mild iron deficiency without anemia, ZnPP remains normal.</td><td>Moderate and severe iron deficiency without anemia
<table><tr><th>Age < 5 y</th><th>Age >5y</th></tr><tr><td>>70 µg/dl RBC</td><td>>80 µg/dl RBC</td></tr><tr><td>>2.6 µg/g Hb</td><td>>3.0 µg/g Hb</td></tr><tr><td>>61 mmol/mol heme</td><td>>70 mmol/mol heme</td></tr></table>Source: (WHO/UNICEF/UNU 2001)</td></tr>
</table>

Table 2.27-*continued*

Description of iron deficiency and iron deficiency anemia indicators

Explanation and comments	*Suggested cut-off values*
Serum Iron, Transferrin (TIBC), and Transferrin Saturation	
-Both serum iron and transferrin saturation are subjected to wide diurnal variation. -The three variables are useful in determining nutritional iron deficiency where serum iron falls and TIBC increases causing a decrease in transferrin saturation [transferrin saturation= (Serum Iron/TIBC)100%] -Another limitation is that infections and chronic inflammatory conditions tend to lower transferrin saturation (due to decrease in both serum iron and TIBC).	Transferrin saturation <16% *(WHO/UNICEF/UNU 2001)*
Serum Transferrin receptor (TfR)	
- TfR regulate the uptake of iron (TfR-transferrin iron complex) into cells especially erythroid precursors which are necessary for Hb synthesis (Skikne 2008). - Cells express TfRs on their surface in proportion to their iron requirements (Cook et al 1993). Therefore, TfR increase in response to iron deficiency or increased erythropoiesis. -TfR is a sensitive and reliable measure of functional iron deficiency (Skikne et al 1990). - A major advantage of measuring TfR is that (unlike ferritin) it is not affected by infection or inflammation (Skikne et al 1990) therefore, TfR can be used to distinguish anemia of chronic disease from iron deficiency anemia. When TfR is performed in conjunction with serum ferritin, the TfR will *"be useful in establishing the true prevalence of iron deficiency anemia in population studies"(Cook et al 1993).* - Cut-off values vary according to the assay used. Currently there is no universally agreed reference value for TfR.	

Table 2.27-*continued*

Description of iron deficiency and iron deficiency anemia indicators

<table>
<tr><th>Explanation and comments</th><th>Suggested cut-off values</th></tr>
<tr><td colspan="2">Serum Ferritin</td></tr>
<tr><td>- Ferritin is a very sensitive indicator of iron status. Serum or plasma ferritin concentration is the most useful measure for storage iron (Cook et al 1974).
- In healthy adults, each 1µg/L of serum ferritin corresponds to 8-10 mg body iron, and it is equivalent to 120µg/L of storage iron per kg body weight in small adults or children (Finch et al 1986).
- “serum ferritin is the only measure of iron status that can reflect a deficient, excess, or normal iron status”(Gibson 2005).
-Ferritin is an acute-phase reactant protein. Therefore, it increases (independent of iron status) in response to acute/chronic infection or inflammation which makes it an unreliable indicator of iron status in population studies where there is high incidence of infection/inflammation.</td><td>Serum ferritin Cut-off Points (µg/L)
<table><tr><th></th><th>Children <5 y</th><th>Children >5 y and Adults</th></tr><tr><td>Depleted Iron Stores</td><td><12</td><td><15</td></tr><tr><td>Depleted Iron Stores + Infection</td><td><30</td><td>-</td></tr><tr><td>Risk of Iron Overload</td><td>-</td><td>>200 (adult male)
>150 (adult female)</td></tr></table>Source: (WHO/UNICEF/UNU 2001)</td></tr>
</table>

THE USE OF MULTIPLE INDICES TO ASSESS IRON STATUS

Many challenges exist in assessing iron status of populations. First of all, although the prevalence of anemia has been used as an indicator to assess the prevalence of iron deficiency, iron deficiency could occur with or without anemia. In addition to dietary iron deficiency, anemia could be caused by: infectious diseases (malaria, hookworm infections), micronutrients deficiencies (folate, vitamin B_{12}, and vitamin A), and inherited conditions like thalassaemia (WHO/CDC 2004). Therefore, a hemoglobin concentration below the recommended cut-offs may or may not reflect iron status. On the other hand, the absence of anemia doesn't necessarily mean an adequate iron concentration. Iron deficiency might exist even when anemia is not present in the cases where iron deficiency is not severe enough or didn't last long enough to cause anemia. The early stages of iron deficiency are characterized by low serum/plasma ferritin and elevated transferrin receptor concentrations.

The second challenge is assessing iron status in the presence of infections. The pro-inflammatory cytokines: cytokine tumor necrosis factor alpha (TNFα) and interleukin 1-α (IL-1α) stimulate ferritin synthesis through transcription induction of the H chain of ferritin in the presence or absence of exogenous iron (Torti and Torti 2002). This means that in the presence of infection, it is difficult to interpret iron status based only on ferritin levels because ferritin might be high despite low iron stores.

In the most recent report (2004) of the joint WHO/CDC technical consultation on the assessment of iron status at the population level, it was recommended that measurement of hemoglobin concentration is necessary to assess iron status of

populations even though anemia is not necessarily caused by iron deficiency, and that "measurement of serum ferritin and transferrin receptors provide the best approach to measuring iron status of populations" (WHO/CDC 2004). In areas where infection and inflammation are common, then serum ferritin alone is not a useful indicator of iron status, and therefore measurement of serum ferritin should be combined with the measurement of transferrin receptors to distinguish between iron deficiency and inflammation. The WHO/CDC consultation suggests the following interpretation of serum ferritin and TfR in assessing iron status of populations (Table 2.28):

Table 2.28
Interpretation of low serum ferritin and high transferrin receptor concentrations in population surveys

% serum ferritin values < threshold *(<12 µg/L in children, <15 µg/L in Adults)*	% TfR values > threshold *(Apply thresholds recommended by assay manufacturer)*	Interpretation
< 20% [a]	< 10%	Iron deficiency is not prevalent
< 20% [a]	≥ 10%	Iron deficiency is prevalent; inflammation is prevalent
≥ 20% [b]	≥ 10%	Iron deficiency is prevalent
≥ 20% [b]	< 10%	Iron depletion is prevalent

[a] <30% for pregnant women, [b] ≥30% for pregnant women, *Source: (WHO/CDC 2004)*

To control for the high serum ferritin concentration resulting from infection or inflammation, the WHO/CDC report suggests the measurement of other acute phase proteins to help interpret ferritin data. These include: c-reactive protein (CRP), α-1-antichymotrypsin (ACT), α-1- acid glycoprotein (AGP), serum amyloid A, fibrinogen, ceruloplasmin, complement factor-3 (C3), and haptoglobin. High values for an acute

phase protein indicates the presence of infection. With infection ferritin concentration is expected to be high despite low iron stores, and therefore individuals with such high values should be excluded from the analysis (if feasible) to define the population's iron status.

In a recent review on the interpretation of iron status indicators during infection (Northrop-Clewes 2008), the acute phase proteins are categorized into three groups:

1. Acute phase proteins which increase by ½ fold like ceruloplasmin and C3.
2. Acute phase proteins which increase by 2-5-fold, these include: haptoglobin, fibrinogen, AGP, and ACT.
3. Acute phase proteins which increase as much as 1000-10,000-fold like CRP and serum amyloid A.

Both CRP and amyloid A respond quickly to infection. CRP rises to concentrations > 5mg/L in the first 6 hours (Shine et al 1981) and peaks at 48-72 hours, but also returns to normal concentration (0.8 mg/L) quickly. While ACT rises quickly like CRP, it does stay at a higher concentration longer than CRP. AGP shows a slow increase in response to infection/inflammation but it remains at a high concentration for longer period than both CRP and ACT (WHO/CDC 2004). "*Ferritin was found to be the only biomarker of iron status that is consistently related to either CRP or AGP" (Beard et al 2006b).* Ferritin as an acute phase protein responds early to infection. An earlier study found that the increase in CRP concentrations at the initial phase of infection parallels those of ferritin, but in later stages of inflammation when CRP decreased, serum ferritin was maintained at a high level and gradually normalized over weeks (Baynes et al 1986). The WHO/CDC

consultation report stated that: "*the most commonly measured acute phase protein is CRP, but there is evidence that AGP may better reflect the change in concentration of ferritin in serum and may be the useful acute phase protein to measure*"(WHO/CDC 2004) .

Cogswell and colleagues (2009) compared two approaches used to identify iron deficiency (Cogswell et al 2009). The first one is a multivariable approach that is used by the National Health and Nutrition Examination Surveys (NHANES) which defines iron deficiency as: "*having abnormal results for two or more of the following tests: serum ferritin concentration, erythrocyte protoporphyrin, or transferrin saturation*" (US Department of Health and Human Services 2000). The second approach for the quantitative estimation of total body iron was proposed by Cook and colleagues (2003) using two indicators: transferrin receptor (TfR) and serum ferritin (SF) (Cook et al 2003). The logarithm of the ratio TfR/SF proposed in this model was found to be directly proportional (close to a linear relationship) to the amount of body iron expressed as mg/kg body weight as in the following formula:

Body Iron (mg/kg) = - [log (TfR/Ferritin ratio) - 2.8229]/0.1207

Positive values for body iron (> 0 mg/kg) indicates iron surplus in stores, and negative values (body iron <0 mg/kg) indicates tissue iron deficiency.

The Centers for Disease Control and Prevention (CDC) has phased in this model on NHANES beginning 2003-2004 among preschool children and women of child bearing age (Cusick et al 2008). The battery of indicators measured in the NHANES to assess iron status had traditionally included ferritin, transferrin saturation, erythrocyte

protoporphyrin, and complete blood count. After phasing in the new model, transferrin receptor along with the traditional indicators were measured through the 2005-2006 cycle, after which transferrin saturation and erythrocyte protoporphyrin were phased out (Cusick et al 2008). The WHO presented the model proposed by Cook and colleagues (2003) for the quantitative estimation of total body iron in its joint WHO/CDC technical consultation report on the assessment of iron status at the population level and pointed out that despite the significant advance of this method on previous ones, there is a difficulty of validating the relationship between TfR/ferritin and body iron stores in children and young women by phlebotomy (WHO/CDC 2004).

In the Cogswell study, iron status and inflammation were measured in 486 children aged 1-2 years, 848 children aged 3-5 years, and 3742 non-pregnant females aged 12-49 years from NHANES 2003-2006. The study found that there was fair to good agreement between both approaches in identifying iron deficiency in children (aged 3-5 years), and good agreement in females of child-bearing age. The important finding was that the second approach (the one based in TfR/ferritin ratio) found lower prevalence of iron deficiency, was less affected by inflammation, and better predicted anemia among females of child-bearing age.

In addition to the use of TfR/ferritin ratio, the TfR-F index (TfR/ log ferritin) has been also studied and shown to be valuable in assessing iron status and in differentiating between anemia of chronic disease and iron deficiency anemia (Punnonen et al 1997, Weiss and Goodnough 2005). The diagnostic sensitivity and specificity of ferritin and TfR were maximized when the TfR-F index was used to identify

iron deficient patients and the index provided an area under the ROC curve (AUC^{ROC}) value of 1.00 (Punnonen et al 1997). Other studies found that TfR/log ferritin could sensitively detect iron deficiency, however, the use of log (TfR/ferritin) ratio had the highest sensitivity in determining the stages of iron deficiency (Lin et al 2008, Malope et al 2001). With the lack of consensus on the use of TfR/ferritin ratio in defining iron status, there is a need for larger studies to validate the ratios of TfR/ferritin in detecting iron deficiency and establish definitive age-specific reference ranges that can be applied to population studies.

ZINC

OVERVIEW

The body content of zinc is estimated to range from 1.5-2.5 g, and this level is maintained with a daily absorption of approximately 5 mg of zinc in the small intestine (Cousins 2006). Approximately, thirty percent of total body zinc is in the skeletal muscles, and only 0.1% is in the form of plasma zinc (WHO/FAO 2005a). Almost 70% and 20-40% of plasma zinc is bound to albumin and α_2-macroglobulin, respectively, and less than 1% of plasma zinc is bound to cysteine and histidine. Blood zinc is found mostly in erythrocytes where more than 85% of zinc in red cells is found in carbonic anhydrase, and about 5% is present as Cu/Zn-superoxide dismutase (Cousins 2006). Zinc is involved in almost all metabolic pathways and plays a major role in DNA transcription, RNA translation, cellular division, and cellular activation. Further, it is required (as electron acceptor) for the catalytic function of more than 100 specific enzymes. These include

enzymes that belong to all enzyme classes (transferases, oxidoreductases, lysases, hydrolases, ligases, and isomerases) in addition to RNA polymerases (I, II, and III), alcohol dehydrogenase, carbonic anhydrase, and alkaline phosphatase (IZiNCG 2004). Plasma/serum zinc is maintained under tight homeostatic mechanisms that keeps normal levels of serum zinc concentrations within the range of 12-15 mmol/L (78-98 µg/dL) in healthy individuals (Gibson et al 2008).

Zinc is a key trace element in the immune system. Zinc deficiency affects the development of both innate immunity (natural killer cells and neutrophils), and acquired immunity (T-lymphocyte functions, Th1-cytokine production, B-lymphocytes and antibody production). In addition, zinc deficiency has a crucial effect on macrophages where phagocytosis, intracellular killing, and cytokine production are compromised (Shankar and Prasad 1998). Benefits of oral zinc supplementation in the prevention and/or treatment of diarrhea in children were recently reviewed. Zinc supplements improve immune functions, increase resistance to infections, and reduce the severity, risk, and duration of diarrhea in malnourished children living in developing countries, and low and middle income countries (Fischer Walker and Black 2004, Scrimgeour and Lukaski 2008). Zinc intervention studies that assessed the role of zinc in improving diminished cognitive ability and motor functioning suggest a relationship between zinc supplementation and motor development in infants, toddlers, and school aged children although more research is needed to confirm such relations (Black 2003).

ASSESSMENT OF ZINC DEFICIENCY

Recently, the International Zinc Nutrition Consultative Group (IZiNCG) published its second technical document on zinc intervention strategies and summarized the new guidelines on the assessment of population zinc status (Brown et al 2009). The guidelines on the assessment of zinc status were reviewed and published following an interagency meeting on zinc status indicators by WHO, UNICEF, the International Atomic Energy Agency (IAEA), and IZiNCG (de Benoist et al 2007). These guidelines considered three main types of zinc assessment: biochemical, dietary, and functional methods. Ideally, these three indicators are combined together to set up the best estimate of zinc deficiency. A discussion on these three methods is presented below.

Serum or plasma zinc concentration below the age/sex/time of the day/fasting status-specific cutoffs is considered the best available biochemical measure of zinc deficiency as recommended by the Joint WHO/UNICEF/IAEA/IZiNCG Interagency meeting on zinc status indicators (de Benoist et al 2007). Serum zinc is considered a good indicator of the population's zinc status because it reflects the dietary intakes of zinc, both individual and population mean serum zinc responds consistently to zinc supplementation, and finally reference data are available for most age/sex groups. However, it should be noted that *"serum zinc concentration may not be a reliable indicator of an individual's zinc status. Nevertheless, the distribution of serum zinc concentrations among a representative sample of a population can be used to assess the risk of zinc deficiency in that population."* (IZiNCG 2007a).

Serum zinc concentrations suggested to define zinc deficiency are lower than normal (2.5th percentile) since zinc status varies by age, sex, time of the day, and fasting status. These values are presented in Table 2.29.

Table 2.29
Suggested lower serum zinc cutoffs for deficiency by age, sex, time of the day, and fasting status

Time of the day and fasting status		*Suggested lower serum zinc cutoffs for deficiency (µg/dL)*[*]		
		< 10 years *Males & females*	***> 10 years*** *Non pregnant females*	*Males*
Morning	Fasting[†]	Not available	74	70
	Non-fasting	65	70	66
Afternoon, non-fasting		57	61	59

[*] Divide by 6.54 to convert to µmol/L.
[†] fasting defined as no food or beverage consumption for at least 8 hours
Source: (IZiNCG 2007a)

If the prevalence of zinc deficiency according to the suggested lower cutoffs in the table is greater than 20%, public health intervention should be addressed to improve zinc status (Hess et al 2009). While serum or plasma zinc concentrations fall during severe infections or stress, "common, acute infections encountered in community settings do not have a major effect on the mean plasma zinc concentrations of children in low-income countries" (Brown 1998).

The second approach for zinc status assessment is the dietary assessment which is done through the use of 24-hr recall or weighed dietary records to identify populations with sub-optimal zinc intake [zinc intake below the estimated average requirements (EAR)]. Risk of zinc deficiency increases when the prevalence of inadequate intakes is greater than 25% (de Benoist et al 2007). Zinc bioavailability is

highly variable with the type of food consumed. Protein found in meat, poultry, and fish enhances zinc absorption, and high levels of calcium, and high phytate: zinc ratios in the whole diet inhibit zinc absorption. The consumption of phytate-rich foods from plants greatly reduces the zinc bioavailability, therefore phytate: zinc molar ratio should be taken into account when determining dietary zinc bioavailability (IZiNCG 2007b).

The recommended dietary intakes (RNIs) proposed by the WHO/FAO were established in 1996 based on the WHO estimates of average daily requirements for zinc with the addition of 50% or two standard deviations to ensure that the RNIs meet zinc requirements from diets differing in zinc bioavailability (WHO/FAO 2005a). Zinc adjusted RNIs based on zinc bioavailability are presented in Table 2.30.

Table 2.30

The recommended nutrient intake (RNIs) for zinc for different dietary zinc bioavailability

Group	*Age (years)*	*Mean body weight (kg)*	*Recommended nutrient intake (mg/day) for a dietary zinc bioavailability of:*		
			50%	*30%*	*15%*
Children	1-3	12	2.4	4.1	8.3
	4-6	17	2.9	4.8	9.6
	7-9	25	3.3	5.6	11.2
Adolescents					
Females	10-18	47	4.3	7.2	14.4
Males	10-18	49	5.1	8.6	17.1
Adults					
Females	19-65	55	3.0	4.9	9.8
	65+	55	3.0	4.9	9.8
Males	19-65	65	4.2	7.0	14.0
	65+	65	4.2	7.0	14.0
Pregnant women					
1st trimester		-	5.8	9.5	19.0
2nd trimester		-	5.3	8.8	17.5
3rd trimester		-	4.3	7.2	14.4

Source: (WHO/FAO 2005a)

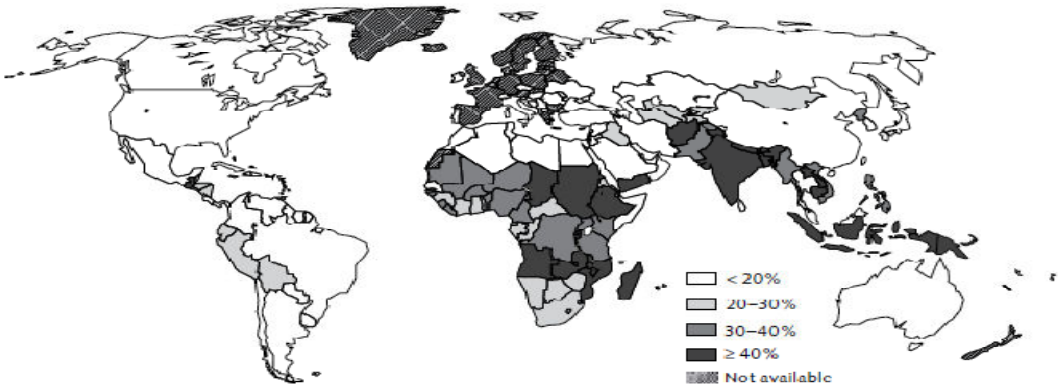
Finally, functional consequences of low zinc status could be used to suggest the need for zinc interventions or supplementation, although these consequences might not be related only to zinc deficiency. In other words, high incidence of infection or stunting among a population could be attributable to many other factors beside zinc deficiency. However, the meta-analysis by Brown and colleagues (2002) concluded that the severity of stunting predicted the response to zinc intervention, and growth responses were greater in children with low initial weight-for-age z scores and in those with low initial height-for-age z scores (Brown et al 2002). The IZiNCG suggests in its second report that the risk of zinc deficiency is of public health concern when the prevalence of low height-for-age (The percentage of children under 5 years of age with height-for-age z-score less than −2.0 SD with respect to the reference population) is greater than 20%, and in that case nutrition intervention strategies should address zinc status (Hess et al 2009). Fischer Walker and Black supported this recommendation in their earlier review (Fischer Walker and Black 2007). The following functional indicators for assessing the risk of zinc deficiency were reviewed in 46 randomized controlled trials (RCTs) of zinc supplementation: infectious diseases, growth, and development outcomes. The review found that zinc supplementation decreases both the incidence and the prevalence of infectious diseases (diarrhea and pneumonia), and that zinc supplementation had a remarkable effect on linear growth especially among pre-school children who started the RCTs with low height-for-age irrespective of the children's age. However, it was difficult to estimate the prevalence of zinc deficiency based on the incidence and prevalence of diarrhea and pneumonia. These results agree with Brown and colleagues

(2002) meta-analysis study mentioned earlier. Thus the prevalence of stunting among a population could be used as a functional indicator of population zinc status.

In its first technical report, the International zinc consultative Group (IZiNCG) recommended the use of a composite index for the estimation of national risk of zinc deficiency based on two indicators: the prevalence of stunting among pre-school children and the percent of individuals at risk of inadequate dietary zinc intake based on national food balance sheets (IZiNCG 2004). However, in its second report, the IZiNCG stated that "the estimate of the extent of zinc deficiency should rely on the prevalence of stunting among children less than 5 years because not much data is available from nationally representative surveys on inadequate dietary zinc intake, and assessment of dietary zinc intake and serum zinc can be used to confirm the risk of zinc deficiency" (IZiNCG 2009). A prevalence of stunting >20% of a population is a public health concern for zinc (WHO 1995a). Countries in South and Southeast Asia, sub-Saharan Africa, and Central America have the highest prevalence rates of stunting (>30%) as shown in Figure 2.8.

Figure 2.8

Prevalence of nutritional stunting in children under five years of age



Source: (IZiNCG 2004)

The two indicators (stunting rates among children under 5 years of age, and the risk of inadequate zinc intake) were used to estimate the global burden of zinc deficiency based on data from 52 countries in 12 regions and sub-regions in the world (Fischer Walker et al 2009). Almost 453,207 of deaths were attributed to zinc deficiency (which is 4.4% of childhood deaths). Around 14.4% of diarrhea deaths, 10.4% of malaria deaths, and 6.7% of pneumonia deaths among children (6 months-5 years of age) were attributed to zinc deficiency.

To sum up, in a review of evidence Gibson and colleagues (2008) have recommended three indicators of population zinc status: percentage of population with serum zinc below the cut-off points, percentage of stunted children (under 5 years of age), and the prevalence of inadequate zinc intakes i.e., below the estimated average requirements (Gibson et al 2008). The use of serum zinc to assess population zinc status has been confirmed in several ways including: 1) in zinc interventions serum zinc successfully predicted functional responses to zinc supplementation 2) the strong relation between serum zinc and dietary intakes of zinc 3) the strong relationship between serum zinc at baseline and change in serum zinc after zinc intervention. When studying the usefulness of serum zinc in predicting functional responses to zinc supplementation, linear growth or height-for-age was considered the best functional outcome compared to infectious diseases (diarrhea, malaria, and pneumonia), and developmental outcomes. The use of other zinc indicators has been also discussed as potential zinc biomarkers such as: zinc concentration in hair, cells (erythrocytes, platelets, leucocytes, and neutrophils), metalloenzymes, and zinc-binding proteins (like

metallothionein), but still more research is needed to verify the accuracy and validity of such biomarkers.

COGNITION, IRON, AND ZINC

IRON AND COGNITION- POSSIBLE MECHANISMS

Iron uptake in the brain occurs primarily through transferrin receptors found on brain capillary endothelial cells (Connor and Benkovic 1992, Fishman et al 1987). Transferrin is mainly found in oligodendrocytes in the brain and myelinating Schwann cells in the peripheral nervous system (Connor and Benkovic 1992). The brain responds to iron depletion and repletion through a homeostatic mechanism that is tightly regulated by the blood brain barrier. When an individual's iron status is low the rate of iron intake in the brain increases, and when the iron status is high, iron uptake in the brain decreases (Taylor et al 1991), a very important regulatory mechanism given the high iron requirements by the brain along with the high susceptibility of the brain to oxidative damage.

Iron deposition in the brain differs by brain region and by stages of development (Pinero et al 2000, Youdim and Yehuda 2000), with the highest concentrations found in the globus pallidus, caudate nucleus, puamen, and substantia nigra (Beard 2003). These regions are high in iron content during adulthood but not during early stages of life (Beard 2001). This has been found in both rats (Connor and Menzies 1996) and humans where it was only by age of 12- 15 years that the substantia nigra became rich in iron (Aoki et al 1989). This means that brain sensitivity to iron deficiency is affected by the

different regional needs in the brain and different stages of neurodevelopment (Beard 2001, Erikson et al 1997, Pinero et al 2000).

Iron is important for the proper myelination of the neurons (Kwik-Uribe et al 2000, Youdim and Yehuda 2000, Yu et al 1986). A decrease in iron bioavailability due to low dietary intakes or availability or due to changes in iron storage capacity results in hypomyelination, a decrease in myelin proteins including proteolipids, and chronic changes in myelin composition (Ortiz et al 2004). Kwik-Uribe and coworkers (2000) studied the behavioral and biochemical effects of chronic iron deprivation during early development in mice, and also studied effect of postnatal consumption of Fe adequate diets on iron-deficient offspring. The mice fed the marginal iron diet showed significant differences in brain iron concentrations, dopamine metabolism, and myelin fatty acid composition compared to control mice. The postnatal consumption of iron adequate diets didn't fully reverse all biochemical disturbances. The study found that chronic inadequate iron intakes during early development resulted in irreversible changes in brain biochemistry (Kwik-Uribe et al 2000).

The distribution of iron in brain is associated with the metabolism of gamma-aminobutyric acid (GABA) which modulates the activity of dopaminergic neurons. Some studies reported alteration in GABA metabolism as a result of iron deficiency (Li 1998, Shukla et al 1989). Hill (1988) pointed out that there is a great overlap between the iron distribution in the brain and the distribution of GABA, i.e., there is a similarity in the brain regions that are high in iron and brain regions that receive input from GABA nerve fibers (Hill 1988). These regions include: globus pallidus, substantia nigra, ventral

pallidus and the cerebellar nuclei. This is very important, because this overlap in the localization of iron and GABA occurs in the brain regions that regulate the cognitive (learning and memory), behavioral, and emotional functions (Youdim and Yehuda 2000).

Iron is a co-factor for the synthesis of neurotransmitters including tyrosine hydroxylase (norepinephrine, and dopamine), tryptophan hydroxylase (serotonin), and also is a co-factor for xanthine oxidase, and ribonucleotide reductase: the rate limiting step in DNA synthesis (Youdim and Green 1978, Youdim et al 1989). There is an overlap in the distribution of dopamine and iron in the brain which occurs mainly in the striatum which is the major input area of the basal ganglia system. The striatum is believed to have the highest concentration of both dopamine and iron (Beard and Connor 2003). The striatum which is rich in dopamine receptors and dopaminergic fibers is best known for its involvement in higher order cognitive and emotional processes, reward-related processes and motor functioning (Lozoff and Georgieff 2006). Studies have consistently shown that the dopamine system is sensitive to changes in iron status (Burhans et al 2005, Erikson et al 2000, Morse et al 1999, Unger et al 2008). Lower dopamine transporter density has been found in iron-deficient brain regions (Erikson et al 1997, Pinero et al 2000), and dietary iron deficiency was found to be significantly correlated with decrease in dopamine (D2) receptor density in the brain regions that are iron deficient (Erikson et al 2001). Other studies reported reductions in dopamine D1 and D2 receptors and monoamine transporters in iron deficient rats (Beard et al 2006a, Felt et al 2006). The changes in monoamine transporters and the neurochemical/behavioral abnormalities were persistent despite early repletion of iron status (Felt et al 2006,

Lozoff et al 2006a). In fact, Beard and Connor (2003) noted that delayed development as a consequence of iron deficiency during infancy persists despite iron repletion, whereas these consequences appear to be reversible if iron deficiency occurs during preschool and older childhood (Beard and Connor 2003).

IRON AND COGNITION-LOOKING FOR EVIDENCE

Grantham-McGregor and Ani (2001) looked for evidence of a causal relationship between iron deficiency and children's cognition and behavior through a review of more than 26 intervention studies and 10 longitudinal studies (Grantham-McGregor and Ani 2001). They found an association between iron deficiency anemia and poor cognitive and motor development. The longitudinal studies consistently showed that children who were anemic during infancy continued to have poor cognition and poor school performance at an older age (middle childhood). However, the review indicated that poor socioeconomic status prevents causal inferences from being made. Iannotti and colleagues (2006) reviewed 26 randomized controlled trials of oral iron supplementation in young children (0-59 months of age) living in developing countries, and found that iron deficient or anemic children had reduced cognitive and motor development, particularly if it was for longer duration (Iannotti et al 2006).

Sachdev and colleagues (2005) evaluated 17 randomized controlled trials with interventions that included oral or parenteral iron supplementation, fortified formula milk or cereals and examined psychomotor development, cognition, mental development, or school performance as an outcome measure in children (Sachdev et al

2005). An association was found between iron deficiency anemia and poor motor development in children. However, the review showed conflicting data regarding the role of iron supplementation in improving motor and cognitive functions in children. The meta-analysis showed a modest effect of iron supplementation on mental development among children above 7 years of age, but no convincing evidence was found for the effect of iron intervention on mental or motor development in children below 27 months of age.

Lozoff et al (2006) reviewed follow-up studies from around the world regarding long-term effect of iron deficiency on cognition, neurophysiological functions, motor functioning, and social-emotional functions in infants, pre-school children, and adolescents (Lozoff et al 2006b). All studies showed nearly consistent results: children who had iron deficiency during infancy continued to perform less well than children who had adequate iron status during infancy. Lozoff (2007) stated that: "*there is compelling evidence that 6-24 month-old infants with iron deficiency anemia are at risk for poorer cognitive, motor, social-emotional, and neurophysiological development in the short term. Iron-deficiency anemia in this age period is also consistently associated with poorer long term outcomes"*.(Lozoff 2007)

Adequate iron status during "critical periods" of development is necessary for "normal" development (Beard 2003). The question remains about what is the age that is considered the "critical period" of development for iron effects on the brain. Additionally, the time of onset of iron deficiency, the severity and duration of iron deficiency, and the overlap with other nutritional deficiencies are important

determinants of long-term consequences on cognitive, behavioral and developmental outcomes.

IRON DEFICIENCY- COGNITIVE AND DEVELOPMENTAL OUTCOMES IN PRESCHOOL CHILDREN

Research suggests poor cognitive, motor, or social functioning in iron-deficient infants and young children up to 24 months of age (Aukett et al 1986, Black et al 2004a, Friel et al 2003, Heywood et al 1989, Idjradinata and Pollitt 1993, Lozoff et al 1987, Oski and Honig 1978, Oski et al 1983, Walter et al 1983, Walter et al 1989, Williams et al 1999). Longitudinal studies also showed that infants with chronic iron deficiency fail to catch up to the children with good iron status in development tests despite correction of iron status (Algarin et al 2003, Corapci et al 2006, Dommergues et al 1989, Hurtado et al 1999, Lozoff et al 1991, Lozoff et al 2000, Lozoff et al 2006b, Palti et al 1985, Shafir et al 2006, Walter 2003). However, research on the developmental outcomes and motor and cognitive functioning among iron deficient pre-school children is limited. The few available studies showed impaired motor, cognitive, and developmental outcomes among iron deficient pre-school children (Lozoff et al 2007, Metallinos-Katsaras et al 2004, Pollitt et al 1983, Pollitt et al 1986, Soewondo et al 1989, Stoltzfus et al 2001).

Pollitt examined various psychological tests in mildly iron-deficient children aged 3-6 years at baseline and after 3 months of oral iron therapy (Pollitt et al 1983). Psychological tests included: the Stanford-Binet Intelligence Scale, measures of attention (discrimination learning), conceptual learning (oddity learning), and short term recall. The study found that iron deficient children had lower scores on tests of

discrimination learning and memory than the control group, and that iron supplementation improved attention in iron deficient children. Pollitt (1986) conducted another study in Guatemala that assessed the performance of 25 preschool children with iron deficiency anemia, and 25 iron-replete children aged 3-6 years on a series of cognitive tests (attention, memory, and learning tasks) before and after 3 months of oral iron therapy (Pollitt et al 1986). The study found that iron deficient children with or without anemia performed poorly on problem-solving situations and those with iron deficiency anemia showed more deficits in attention and higher order cognitive functions than non anemic children. The study also concluded that iron-deficient children are not likely to improve their performance on measures of cognitive functions following iron repletion therapy.

A double-blind clinical trial assessed the effect of iron supplementation on cognitive functions among 176 Indonesian children aged 3-6 years (Soewondo et al 1989). Half the children received iron supplementation for 8 weeks while the other half received placebo. Significant changes were found from pre to post intervention in the iron-deficient anemic children in cognitive processes related to verbal intelligence, visual attention, and concept learning. The study concluded that iron deficiency anemia but not iron deficiency was related to low scores on various cognitive tests.

The effect of iron supplementation on development of language and motor skills was assessed in 614 pre-school children aged 6-59 months in Zanzibar (Stoltzfus et al 2001). Although iron supplementation improved motor and language development, the effect on motor development was limited to children who were severely anemic

(Hb<9.0g/dL at baseline). The effect of two months supplementation of 15 mg iron on attention and conceptual thinking was examined in 49 children at 3-4 years of age in Greece (Metallinos-Katsaras et al 2004). Twenty-one children were anemic at baseline (Hb <11.2 g/dL, TS<16%, and ferritin <12 mcg/L. Twenty-eight children had good iron status (baseline Hb>12.0 g/dl, and either TS >20% or serum ferritin >12 mcg/L. Iron supplementation resulted in improvement in selective attention but not oddity learning tasks among anemic children. The effect of iron supplementation among children with good iron status was not significant.

Affect, social looking toward adults, and behavior as a function of iron status in pre-school children (aged 47-68 months) with or without iron deficiency anemia were recently evaluated in a cross sectional study in India (Lozoff et al 2007). Seventy-four children had iron deficiency anemia and 164 were non-anemic. Children with iron deficiency anemia showed a greater wariness and hesitancy as evidenced by less social looking toward their mothers, taking longer time to touch a stimulus toy, and being slower to smile than non anemic children.

Two follow-up studies assessed various development tests and intellectual functioning among Costa Rican pre-school children who had either chronic, severe iron deficiency or good iron status at infancy. All infants with iron deficiency were treated in infancy and none had iron deficiency after treatment. The first study tested 163 children at 5 years of age on motor and mental functioning (Lozoff et al 1991). All children had excellent hematologic status and growth at five years of age. The tests showed that children with moderate iron deficiency anemia as infants (n=30) had poorer

performance on perceptual speed, performance IQ, visual matching, visual motor integration, and gross and fine motor performance even after controlling for maternal IQ and home socioeconomic status. The second study compared child affect and behavior, and quality of mother-child interaction of 40 children at five years of age who formerly had chronic and severe iron deficiency (as infants) to 102 children with good iron status at infancy (Corapci et al 2006). Children with chronic iron deficiency at infancy showed lower levels of physical activity, positive affect, and verbalization at 5 years of age. Mother-child interaction (eye contact, shared positive affect, turn taking) was lower in children with chronic iron deficiency compared to children with good iron status at infancy.

In summary, almost all reviews of the current literature on the effects of iron deficiency on children's cognition and behavior (Beard and Connor 2003, Grantham-McGregor and Ani 2001, McCann and Ames 2007, Thomas et al 2009), and reviews on the role of iron supplementation in improving development and cognition outcomes (Iannotti et al 2006, Sachdev et al 2005), have supported an association between iron deficiency anemia and cognitive and behavioral performance. Yet, a direct and unequivocal causal connection has not been clearly established.

ZINC AND COGNITION- POSSIBLE MECHANISMS

The exact biological mechanisms by which zinc deficiency alters brain functions and relates to cognition and behavior are not yet clear, but research has shown that zinc is essential for neurogenesis, neuronal migration, and synaptogenesis (Bhatnagar and Taneja 2001). Zinc acts as a neuromodulator in synaptic transmission (Colvin et al 2003,

Frederickson and Bush 2001, Kay and Toth 2008). Zinc is highly concentrated in the synaptic vesicles of particular neurons called “zinc-containing” neurons which are subsets of glutamatergic or “gluzinergic” neurons (Frederickson et al 2000, Hesse 1979, Howell et al 1984, Smart et al 2004), and these neurons are found almost exclusively in the cerebral cortex and limbic structures of the forebrain (Bitanihirwe and Cunningham 2009, Brown and Dyck 2004, Frederickson et al 2000). Because of the abundance and exclusive presence of “gluzinergic” neurons in the mammalian cerebral cortex, it has been suggested that zinc might have a critical role in cortical communication (Frederickson et al 2000), cognitive and emotional functioning (Bitanihirwe and Cunningham 2009), as these zinc containing neurons contribute to the synaptic plasticity underlying learning and memory (Brown and Dyck 2004).

In addition to that, there is increasing evidence that zinc deficiency alters the expression of specific subunits of the neurotrophins and N-methyl-D-aspartate (NMDA) receptors for glutamate, and thus controlling glutamate as a neurotransmitter in the central nervous system. The NMDA receptors are involved in neuronal differentiation, neuronal migration, and synaptic plasticity in hippocampus (a brain region that is involved in learning and memory), especially during early periods of development where synaptic plasticity is crucial (Chowanadisai et al 2005, Levenson 2006, Smart et al 2004). Zinc deficiency can alter the developmental regulation of NMDA receptors and cause changes that persist long after the correction of zinc status, and this indicates the importance of having adequate dietary zinc intakes during fetal and neonatal development (Levenson 2006)

Zinc deficiency has been studied in animals. Rats and monkey models have been used to study the effect of zinc restriction or deprivation during different stages of the life cycle. Rats have been used in studies that involve the prenatal and infancy periods since they have a short period (2 weeks) between weaning and puberty, whereas rhesus monkeys have been used to study zinc deficiency during infancy, childhood and adolescence since they have relatively a longer period between weaning and puberty that reaches 3 years (Bhatnagar and Taneja 2001). Research on animals has shown that zinc deficiency is associated with reduced motor activity, decrease in performance on tasks of visual attention and short-term memory, impaired spatial learning and knowledge retention, and increased emotional response to stress (Bhatnagar and Taneja 2001, Black 2003, Golub et al 1995, Golub et al 2000, Tahmasebi Boroujeni et al 2009). Zinc deprivation in animals has been associated with impairments in learning and memory that are poorly reversible later in life (Sandstead 2003).

ZINC AND COGNITION-LOOKING FOR EVIDENCE

Relatively few studies have assessed the impact of zinc deficiency on cognition and behavior in humans. There are some observational studies but most of the research has involved zinc supplementation trials in pregnant women, infants, and school-age children with mixed results.

Merialdi and colleagues (1999) assessed the impact of improvement of maternal zinc status during pregnancy on fetal neurobehavioral development in Peru (Merialdi et al 1999). Pregnant women were randomly assigned to receive a daily supplement of 60

mg iron and 250 mcg folate, with or without 15 mg zinc (for 10-24 week of gestation, and 1 week post partum). Fifty-five fetuses were electronically monitored at 32 and 36 weeks of gestation where fetal heart and movement patterns were observed. Increased fetal heart range, minimal fetal heart variability, increased number of accelerations, and more vigorous fetal movements were observed in the fetuses of mothers who received zinc supplementation.

Zinc nutriture of 50 women living in a periurban Egyptian village was assessed over the last 6 months of pregnancy and the first 6 months of lactation (Kirksey et al 1991, Kirksey et al 1994). Estimated zinc intake was approximately 2mg/day from diets high in phytate and fiber. Performance on Bayley's Motor Development Scale at 6 months of age was negatively related to maternal intakes of zinc, phytate, and fiber.

A randomized controlled study has assessed developmental outcomes in 52 infants with very low birth weight (Friel et al 1993). Infants were randomly allocated to either 11 mg zinc/L of formula or 6.7 mg zinc /L for 6 months. Higher loco-motor development score on Griffiths mental development sub scales in infants using the formula with higher zinc content was reported. A randomized, double blind, placebo-controlled study has assessed the impact of daily supplementation of zinc (10 mg) for 7 months on activity patterns of 85 Guatemalan infants recruited at 6-9 months of age (Bentley et al 1997). Zinc supplemented infants were significantly more frequently observed sitting up or playing than lying, and less likely to be observed crying or whining.

To investigate the impact of zinc supplementation on observed activity levels among 93 children (aged 12-23 months), a randomized controlled trial was conducted in a low socioeconomic urban population in New Delhi (Sazawal et al 1996). Zinc (10 mg/day) was supplemented for at least one month along with selected vitamins. Children in the zinc group spent 72% more time performing higher movement activities. A greater effect was observed in boys.

In northeast Brazil, a double blind part-randomized efficacy trial was conducted to assess the impact of zinc supplementation on mental and psychomotor development of 205 term infants weighing 1500-2499 gm born to low income families (Ashworth et al 1998). Low birth weight infants were randomly allocated to receive 1 mg zinc (n=68) or placebo (n=66), and another group received 5 mg zinc (n=71). The supplementation started at birth and lasted for 8 weeks. Bayley Scales of Infant Development were used to assess mental and psychomotor development at 6 and 12 months of age and no difference was found in the scores of the three groups. However, at 12 months of age, child behavior was rated on 5 scales (responsiveness to tester, emotional tone, activity level, cooperation with the test procedure, and amount of vocalization). Children who received five mg of zinc supplement had significantly higher rating on behavior, particularly responsiveness.

The effect of zinc supplementation on neurodevelopment during infancy was assessed in Chile (Castillo-Duran et al 2001). Term infants (n=150) were randomly assigned to receive 5 mg zinc or placebo daily for 12 months. Solid food and iron were added at 5 months of age. Assessment of psychomotor development, mental

development, behavior rating, emotional regulation, and motor quality showed no difference between the two groups at 6 months of age. However, at 12 months of age, scores on motor quality were found to be significantly different with higher mean motor quality for the zinc supplemented group.

An interesting study assessed the impact of weekly supplements of iron (20 mg+ 1mg riboflavin), zinc (20 mg+ 1mg riboflavin), iron + zinc (20mg iron+20mg zinc+ 1 mg riboflavin), or micronutrient mix (MM) [twice the recommended dietary allowance of 16 vitamins and minerals in addition to 20 mg iron, 20 mg zinc, and 1 mg riboflavin] to 221 infants at age of 6 months to age of 12 months in Bangladesh (Black et al 2004a). Bayley Scales of Infant Development II and the Home Observation Measurement of Environment (HOME) scale were administered to children at 6 months of age (pre-intervention) and 12 months of age (post intervention). Maternal education, HOME score, months breastfed, anemia, growth by 6 months, and change in growth from 6 to 12 months were controlled. The study showed that although psychomotor development index scores (PDI) had decreased from 6 months to 12 months for all groups, it decreased less for the "iron+ zinc" and MM groups compared to the control group (1 mg riboflavin). That means that supplementation with iron and zinc together or with MM containing iron and zinc had protected the infants from the decline in the motor development. Moreover, the effect size for the (iron + zinc) group was similar to that for the MM group, which indicates that there was no additional benefit of the added vitamins and minerals on the children's motor development (Black et al 2004a). The

study concluded that there are beneficial effects of weekly combined iron and zinc supplementation on motor development and orientation-engagement.

The effect of zinc supplementation and psychosocial stimulation on the psychomotor development of undernourished children was studied in a randomized controlled trial among 114 Jamaican children aged 9-30 months (Gardner et al 2005). Children were randomly assigned into four groups: stimulation alone (weekly home visits for 30 minutes demonstrating maternal-child interactions), zinc supplementation alone (10 mg), both stimulation and zinc supplementation, and control (neither stimulation nor zinc supplementation). Griffith's mental development scales were used to assess development, and these involved: locomotors (large muscle activities such as walking or jumping), hand and eye coordination, hearing and speech, and performance on shape recognition, block construction, and block patterns. Zinc supplementation improved development only in children who received stimulation. Hand and eye coordination was improved in all children who received zinc supplementation, but the improvement was greater in those who received both stimulation and zinc supplementation.

Despite the mentioned studies that have shown the beneficial effects of zinc supplementation on child development, there are some conflicting results regarding the impact of zinc supplementation on infant and child mental development and behavior.

Gibson and colleagues (1989) found no zinc treatment effect on attention span in a double-blind, pair-matched study that involved schoolboys at 5-7 years of age in Ontario, Canada (Gibson et al 1989). Study participants were randomly assigned to

receive either 10 mg zinc or placebo daily for one year. Four subsets were used to measure attention span, these include: sentence imitation, word sequence, oral directions, and design reproduction. No difference was found in average attention span scores between treatment groups at baseline, 6 months, and 12 months of the study.

A double-blind zinc intervention study of 162 Guatemalan children (mean age 81.5 months) didn't find any significant effect for zinc supplement on cognition (Cavan et al 1993). Children were assigned to either zinc supplement group (10 mg zinc + micronutrients mix per day) or placebo group (micronutrients mix without zinc) for 12 months. No treatment effect was found for the total cognitive scores or for the three subsets administered (letter sequence, oral directions, and design reproduction).

A randomized, double-blind, controlled trial assessed the impact of 5 mg zinc supplementation for a period of 5 months on the mental development index (MDI) subscale of the Bayley Scales of Infant Development (Hamadani et al 2001). Three hundred one infants aged one month were randomly assigned to receive either zinc supplement or placebo, and developmental levels were assessed at 7 and 13 months of age in 212 infants. Unexpectedly, the zinc-supplemented group had slightly but significantly lower scores on the mental development index of the Bayley Scales than the placebo group, even after controlling for nutritional status and social background.

A follow-up study found no treatment effect of zinc supplementation during pregnancy on mental development and behavior of infants at 13 months of age (Hamadani et al 2002). Pregnant Bangladeshi women (n=559) were randomly allocated to receive either 30 mg zinc or placebo at 4 months of gestation each day until delivery.

Bayley Scales of Infant Development II and behavior rating were administered to infants at 13 months of age. Infants in the placebo group had higher scores on mental development index and psychomotor development index compared to zinc-supplemented group even after controlling for differences between the two groups.

Another study also found no effect of zinc supplementation on the mental or psychomotor development index in children aged 12-18 months (Taneja et al 2005). In a double-blind, randomized, placebo-controlled trial, infants received 10 mg zinc or placebo daily for 4 months. Bayley Scales of Infant Development II were administered at enrollment and at the end of the study. There was no difference in psychomotor index scores and adjusted mean mental scores in the treatment and the control groups.

A relatively long follow-up study found no effect of zinc supplementation of women in later stages of pregnancy on the neurological development of their children at five years of age (Tamura et al 2003). In a double-blind trial pregnant women received either 25 mg zinc or placebo daily during the second half of pregnancy. Three hundred fifty five children (mean age= 5.3 years) took six tests that included: the differential ability scales, visual sequential memory, auditory sequential memory, knox cube, gross motor scale, and grooved pegboard tests. Again, no difference in scores was found between children whose mothers received zinc supplementation and those children whose mothers received the placebo.

Lind and colleagues (2004) compared the effect of combined iron and zinc supplementation with effects of iron and zinc as single micronutrients on psychomotor development in 680 Indonesian infants at 6-12 months of age (Lind et al 2004). Infants

were randomly assigned into four groups that received daily supplementation of: 10 mg iron, 10 mg zinc, 10 mg iron and 10 mg zinc, or placebo. Results of the study showed that single supplementation with iron only improved psychomotor development, but neither single supplementation with zinc nor the combined supplementation of iron and zinc had a significant effect on psychomotor development.

Another study that found no effect of zinc supplementation on development and behavior of infants involved a nine months supplementation trial of 5 mg zinc on small for gestational age (SGA) infants in New Delhi (Black et al 2004b). Two hundred infants were randomized to receive daily supplements of micronutrients mix with or without zinc from one to nine months of age. Bayley Scales of Infant Development II were used to measure infants' development and behavior at 6 and 10 months. Zinc supplementation had no effect on infants' development or behavior at either 6 or 10 months.

In summary, research suggests a beneficial effect of zinc supplementation on infants and child mental development and behavior. However, some studies that involved zinc supplementation trials in pregnant women, infants, and school-age children have shown conflicting results.

CHAPTER III

METHODOLOGY

STUDY DESIGN AND RECRUITMENTS OF PARTICIPANTS

A convenience sample of 93 women and their children aged 4–5 years from urban/suburban areas in Northern Jordan was recruited for the study. Participants were approached by direct contact with the help of local community centers. Potential participants included mothers of ages between 18-45 years who had children of age 4-5 years. Mothers were called by the local community centers and a brief explanation of the study was given on the phone, after which mothers who were interested in participation were scheduled to come with their children to the community center where they were given a detailed explanation of every aspect of the study. After completion of the consent form for the mothers and the assent form for children, mothers were screened for pregnancy and mothers and children were screened for illness and date of birth. Any child or mother with high temperature, acute illness (illness in the same day or in the previous three days), or serious health problem was excluded from the study. Pregnant women were also excluded from participation.

Mothers reported date of birth for themselves, and date of birth for children was determined based on governmental birth certificates. In the community center, anthropometric measurements were collected for mothers and children, a questionnaire was administered individually to the mothers, and cognitive tests were administered to children. After that, participants were transferred to a local clinical laboratory where non-fasting venous blood samples were drawn from each participant. All samples were taken between June and July 2007. Hematological tests that require fresh blood were done immediately, and results were reported to participants. Serum and plasma samples were transferred into trace element-free tubes which were then frozen at -20 °C, shipped in dry ice to the laboratories of the Nutritional Sciences Department at Oklahoma State University in the USA and stored at -20°C until analyzed.

ETHICAL REVIEW

All procedures of the study were reviewed and approved by the Oklahoma State University Institutional Review Board (IRB Application No. HE0731). An informed written consent was obtained from the mothers, and verbal assent was obtained from the children using the content in the assent form.

RESEARCH ASSISTANTS

Four university graduates research assistants were trained on anthropometric measurements, questionnaire administration to mothers, and cognitive test administration to children. Two of the research assistants held masters degrees, one in

education and one in sociology. Two research assistants had bachelors degrees, one in pharmacy and one in chemistry. The research assistants received training to ensure high quality and standardized performance in the field work.

ANTHROPOMETRIC MEASUREMENTS

The anthropometric measurements collected from participants were weight and height for mothers and children, in addition to mid upper arm circumference (MUAC) for children. Height and MUAC were reported to the nearest millimeter, and weight was reported to the nearest 100 gram. To ensure standardization in anthropometric measurements, height and MUAC measurements were taken by the same individual for all participants, and weight was taken by the same individual for all participants. All measurements were taken in duplicate and averages of the duplicates were reported. When there was a difference between two measurements, a third measurement was taken for verification. Body mass index (BMI) was calculated for mothers as body weight (kg)/height (m^2). For chapter four, anthropometric data for children were expressed as z-scores based on the CDC/WHO 1978 reference using Epi Info, v. 3.4.3 (Centers for Disease Control, USA). For chapters five and six, anthropometric data for children were expressed as *z*-scores based on the WHO (World Health Organization) 2007 reference using AnthroPlus, version 1.0.2 (WHO 2009).

THE QUESTIONNAIRE

The questionnaire was validated by a group of academic experts in research, statistics and dietetics who reviewed the questions to ensure face and content validity. Second, a focus group discussion with local women was carried out and some items of the questionnaire were modified to eliminate ambiguity. The questionnaire was filled for each mother by the researcher or a research assistant based on one to one interview that lasted from 40-60 minutes for each participant. The questionnaire was bilingual, i.e., the questions and potential answers (multiple choices) were written in both English and Arabic. Although the research assistants were fluent in English, to avoid any spontaneous and unsystematic translation that might result in bias, they were asked and trained to administer the questions as they were written in Arabic.

The questionnaire consisted of four sections, and each section consisted of two parts; one part concerned the mother and the other part concerned her child, and mother's responses for her self and her child were reported. The questionnaire consisted of the following sections:

Section 1: General health status description for the mother and the child and family health histories.

Section 2: Socioeconomic and demographic characteristics. The questions concerning the mothers in this section involved: age, marital status, parental education and occupation, average household income (defined as total monthly family income after rent deduction), number of children, and number of pregnancies. Questioning

concerning the child involved: child's gender, date of birth, child order in his/her siblings, and type of family the child lives in (nuclear or joint family).

Section 3: Life style. Questions concerning the mothers in this section involved: hours spent daily in housework, hours spent on field/gardening work, sports performance (if yes, where and for how long), daily exposure to sun light, and dress style. Questions concerning the child in this section involved: time spent on watching TV, time spent in kindergarten (KG) if the child goes to KG, time spent in active play, and daily exposure to sunlight.

Section 4: Nutrition and dietary intakes. Questions concerning mothers and children in the section involved: type and amount of bread consumed, type of flour used to make baked goods, consumption of various food categories like: milk; milk derivatives; tea; fruits; vegetables; chicken; meat; liver; sardines; and fish, smoking, and the use of supplements. For dietary questions that involved amount consumed, participants were shown sample measures to ensure consistency and accuracy in reporting. These involved: 150 ml cup to represent cup of tea, 250 ml cup to represent cup of milk, shaken milk, or yogurt, dish containing 250 gm labna (similar to cream cheese), a piece of white cheese that weighed 250 gm, and a piece of bread that weighed 40 gm.

BLOOD DRAWING

Non fasting venous blood samples were obtained from mothers and children in a clinical laboratory in the center of Irbid city in Northern Jordan. Blood was drawn using trace-element free evacuated blood collection tubes (Sarstedt, Newton, NC). One

aliquot was immediately used before centrifuging for the determination of complete blood count (CBC). Blood samples were refrigerated and allowed to clot for 30 minutes. Samples then were centrifuged at 3000 rpm for 10 minutes. Separated serum and plasma were aliquoted into trace-element free vials. Around 2 ml serum and 2 ml plasma were obtained from each mother and child. All steps followed the protocol recommended by the International Zinc Nutrition Consultative Group for the assessment of population zinc status (IZiNCG 2007a).

ASSESSMENT OF VITAMIN D

Serum 25(OH)D and intact serum parathyroid hormone (PTH) were measured using enzyme-linked immunoabsorbent assay (ELISA) kits (IDS, Fountain Hills, AZ, USA). The assay defined expected values for 25(OH) D were: 47.7-144 nmol/L. Normal range for PTH was 0.8-3.9 pmol/L as defined by the assay. For 25 (OH) D and PTH, standards and serum samples were analyzed in duplicate, and values for the manufacturer's controls fell within the certified ranges. Coefficient of variation (CV) for the low and high controls for 25(OH) D were 15.4% and 9.0%, respectively. For PTH the CV for the low and high controls were 18.7% and 8.4%, respectively.

ASSESSMENT OF ANEMIA

Hemoglobin concentration less than 12 mg/dl was used to define anemia in mothers. For children, Hb <11 mg/dl was used to define anemia in children less than 60

months of age, and Hb < 11.5 mg/dl was used to define anemia in children aged 60 months or older(WHO/UNICEF/UNU 2001).

ASSESSMENT OF IRON INDICATORS

Plasma ferritin, transferrin receptor (TfR), and α-1-acid glycoprotein (AGP) were determined in 93 mothers and their children. Plasma ferritin was measured using immunoradiometric assay (Ramco Laboratories, Inc. Stafford, Texas); transferrin receptor was measured using enzyme immunoassay (Ramco Laboratories, Inc. Stafford, TX); α-1-acid glycoprotein was assayed using radial immunodiffusion (Kent laboratories, Redmond, Washington). For ferritin, AGP, and TfR, standards and serum samples were analyzed in duplicate, and values for the manufacturer's controls fell within the certified ranges. A coefficient of variation (CV) for the low, medium, and high controls for ferritin was 1.5%, 2.0%, and 1.9%, respectively. For TfR, the CV for the medium and high controls was 15.1% and 15.9%, respectively. For AGP the CV for the low, medium, and high controls was 4.1%, 9.8%, and 6.1% respectively.

For plasma ferritin cut-off points of 15 μg/L and 12 μg/L were used to define iron deficiency in absence of inflammation in mothers and children (WHO/UNICEF/UNU 2001), respectively. An AGP concentration ≥1.2 was used to indicate the presence of inflammation (Paracha et al 2000, Wieringa et al 2002).

Quantitative estimation of body iron (mg/kg) was based on the model proposed by Cook and colleagues (Cook et al 2003) using the following formula:

$$\text{Body Iron (mg/kg)} = -[\log_{10}(\text{TfR/Ferritin ratio}) - 2.8229]/0.1207$$

Positive values for body iron (> 0 mg/kg) indicate iron surplus in stores, and negative values (body iron <0 mg/kg) indicate tissue iron deficiency.

Plasma ferritin, TfR, and the ratio of TfR to plasma ferritin TfR/ferritin (TfR in mg/L and ferritin in µg/L) were used to assess iron deficiency in mothers and children. Sensitivity, specificity, and area under the curves were calculated using the receiver operator characteristics (ROC) curves to assess the performance and define the cut-off points of TfR, TfR/Ft and TfR/Log_{10} ferritin in the diagnosis of iron deficiency and the differentiation between anemia of chronic disease (ACD) and iron deficiency anemia (IDA) (Lin et al 2008, Malope et al 2001, Punnonen et al 1997).

Sensitivity (SN) was defined as proportion of people with disease who test positive:

$$SN = TP/(TP+FN)$$

And specificity (SP) was defined as proportion of people without a disease who test negative

$$SP = TN/(TN+FP)$$

Where: TP is true positive, FN is false negative, TN is true negative, and FP is false positive.

The ROC curve was obtained by plotting sensitivity on the x-axis against 1-specificty on the y-axis for every possible cut-off value for the diagnostic test. Ideally, a perfect diagnostic test will yield 100% sensitivity and 100% specificity with an area under the curve (AUC^{ROC}) of one. An area of 0.5 is a poor indicator and represents a test result that was obtained by random chance. Usually AUC^{ROC} will fall between 0.5 and 1.0 and the closer the AUC^{ROC} is to one the better the discriminant ability of the diagnostic test. Based on AUC^{ROC}, tests can be non informative (AUC^{ROC} =0.5), less accurate (AUC^{ROC} between 0.5 and 0.7), moderately accurate (AUC^{ROC} between 0.7 and 0.9), highly

accurate (AUC^{ROC} between 0.9 and 1.0), and perfectly accurate (AUC^{ROC} =1.0) (Swets 1988).

The optimal sensitivity and specificity were defined as the minimal value of: $(1-SN)^2 + (1-SP)^2$. The likelihood ratio for a positive test (LR+) was defined as: SN/(1-SP) and was defined as the probability of individual with disease to test positive divided by the probability of an individual without a disease to test positive. The likelihood ratio for a negative test (LR-) was defined as: (1-SN)/SP and was defined as the probability of individual with disease to test negative divided by the probability of an individual without disease to test negative.

ASSESSMENT OF ZINC STATUS

Plasma zinc was determined using inductively coupled plasma mass spectrometry (ICPMS) (Elan 9000, Perkin Elmer, Norwalk, CT). All plasma samples were diluted (1:20) with 0.1% nitric acid (GFS Chemicals, Powell, OH). Gallium (10 µg/L) was used as the internal standard (Perkin Elmer Pure Atomic Absorption Standard, Norwalk, CT). A sample check (concentration of 50 µg/L) was measured repetitively (once after 10 participants' samples), and the mean, standard deviation, and CV were 52.0 µg/L, 0.76, and 1.5%, respectively. Plasma zinc concentration below the age/sex/time of the day/fasting status-specific cutoffs is considered the best available biochemical measure of zinc deficiency as recommended by the Joint WHO/UNICEF/IAEA/IZiNCG Interagency meeting on zinc status indicators (de Benoist et al 2007). A cut-off point of 70 µg/dL for mothers and 65 µg/dL for children was used to define deficiency (IZiNCG 2007a).

COGNITIVE TESTS

Cognitive function was assessed in children using components of the Peabody Picture Vocabulary Test-IV (PPVT-IV) - Form A and of the Kaufman Assessment Battery for Children (K-ABC-II). The PPVT-IV test measures the receptive vocabulary of children and adults (Dunn and Dunn 2007). Each PPVT item consists of two parts: the stimulus word and a display of four pictures, one of which describes the stimulus word. The PPVT set consists of 150 picture pages along with 150 stimulus words. Two school teachers and two mothers in Jordan helped in the translation of the stimulus words to the Arabic language, and identifying the pictures that might be unfamiliar to children in Jordan due to cultural differences. Then, the test was evaluated by having it administered to children around 4-5 years of age (other than study participants), and based on children responses, the test was revised again, some words were changed, and the test was re-administered to another group of children. After that the researcher along with the group of school teachers and mothers agreed on the Arabic translation for the stimulus words used in the test and these words were written down in Arabic on the response forms. Very few questions were manipulated to suit the children's culture, for example: children were asked to point at the drawer instead of the fence (item 30), the mouse instead of the squirrel (item 35), the clown instead of the knight (item 64), and the piano instead of the harp (item 92). To ensure consistency, the test was administered to the children by the same person. Children were asked to point to the picture that best described the stimulus word, and their responses were written on the response form.

Raw scores were calculated by subtracting the total number of errors from the number of the ceiling item. Then raw scores were transformed into the corresponding standard scores for each age group.

The Kaufman Assessment Battery for Children (K-ABC-II) is a measure of the processing and cognitive abilities of children and adolescents aged three through eighteen (Kaufman and Kaufman 2004). The children were tested on two of the five scales of KABC-II: sequential and simultaneous processing. The core subtests on the sequential K-ABC-II scale included: number recall (memory span) and word order (memory span, and working memory). The core subtests on the simultaneous K-ABC-II scale included: conceptual thinking (visualization and induction), face recognition (visual memory), triangles (spatial relations, and visualization), and pattern reasoning (induction, and visualization). In addition, the hand movement (memory span and visual memory) which is a supplementary test was also administered to children. In addition, the non verbal index of the K-ABC-II (NVI) was measured and was used to assess processing and cognitive abilities of children using both core and supplementary subtests that can be communicated with gestures and responded to without speaking.

The PPVT and K-ABC-II subtests were administered to all children n the same order: PPVT, followed by conceptual thinking, followed by face recognition, followed by number recall, followed by triangles, followed by word order, followed by pattern reasoning, and finally hand movements. Children took the tests under similar settings, and each test was administered by the same examiner for all children. The following table shows a description of the K-ABC subtests:

Table 3.1

Description of the K-ABC-II subtests

subtest	Description
Conceptual thinking	The child views a set of 4 or 5 pictures and identifies the one picture that doesn't belong with the others.
Face recognition	The child attends closely to photographs of one or two faces that are exposed briefly and then selects the correct face or faces, shown in different pose, from a group photograph.
Number recall	The child repeats a series of numbers in the same sequence as the examiner said them, with series ranging from 2 to 9 numbers. The numbers are single digits. (All numbers used in the number recall subtest are of one syllable. However, numbers used in the test were the Arabic numbers which are pronounced in two or more syllables. To ensure consistency, all numbers used in the subtest were numbers with 2 syllables only. These include the numbers 1, 2, 5, 6, 7, 9, and 10 in Arabic.
Triangles	The child assembles several identical foam triangles (blue on one side, yellow on the other) to match a picture of an abstract design; for easier items, the child assembles a set of colorful plastic shapes to match a model constructed by the examiner.
Word order	The child touched a series of silhouettes of common objects in the same order as the examiner said the names of the objects.
Pattern reasoning	The child is shown a series of stimuli that form a logical linear pattern, but one stimulus is missing; the child completes the pattern by selecting the correct stimulus from an array of 4-6 options at the bottom of the page. Most stimuli are abstract, geometric shapes, but some easy items use meaningful pictures.
Hand movement	The child copies the examiner's precise sequence of taps on the table with the fist, palm, or side of the hand.

Source: adapted from (Kaufman and Kaufman 2004)

CONVERSION OF K-ABC RAW SCORES INTO STANDARD SCORES

Raw scores for the seven subtests (conceptual thinking, face recognition, number recall, triangles, word order, pattern reasoning, and hand movement) were reported, and then transformed into the corresponding scaled scores for each age group. Then the sum of the scaled scores for the three K-ABC scales was calculated as the following:

For children < 5years of age, (n=39, age (min-max) = 53-59 months):

- Nonverbal index K-ABC-II = conceptual thinking + face recognition + triangles + hand movement
- Simultaneous K-ABC-II = conceptual thinking + face recognition + triangles
- Sequential K-ABC-II = number recall + word order

For children ≥ 5 years of age, (n=50, age (min-max) = 60-71 months):

- Nonverbal index K-ABC-II= conceptual thinking + face recognition + triangles + hand movement + pattern reasoning
- Simultaneous K-ABC-II for children ≥ 5 years of age = conceptual thinking + triangles + pattern reasoning
- Sequential K-ABC-II = number recall + word order

For children ≥ 6 years of age, (n=4, age (min-max) = 72-73 months):

- Nonverbal index K-ABC-II = conceptual thinking + triangles + hand movement + pattern reasoning + story completion (scaled scores for those four children were not added for this scale since children were not tested on story completion).

- Simultaneous K-ABC-II = conceptual thinking + triangles + pattern reasoning + Rover (scaled scores for those four children were not added up for this scale since children were not tested on Rover).
- Sequential K-ABC-II = number recall + word order

STATISTICAL ANALYSIS

For vitamin D and PTH analysis, variables were checked for normality, and natural logarithm (ln) transformations were performed for skewed variables which included: mothers' 25(OH)D and PTH, and children's PTH. Means ± standard deviations or percentages were used to express results unless otherwise stated. Analysis of variance was used to compare subgroups of the participants, and a p-value of ≤ 0.05 was significant. The relationships between serum 25(OH) D and serum PTH in both mothers and children were evaluated with linear and non-linear regression models using GraphPad Prism, v. 5, (GraphPad Software, La Jolla, CA). Other statistical analyses were conducted using the Statistical Package for the Social Sciences, v.15.0 (SPSS Inc., Chicago, IL, USA).

For iron indicators and zinc analysis, variables that had skewed distribution (ferritin, TfR, TfR/Ferritin, AGP and Hb) were transformed to logarithms and geometric means ± 1SD were reported for those variables. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS/PASW), version 18.0 (SPSS Inc, Chicago, IL, USA). Analysis of variance was used to compare subgroups of the participants. Stepwise multiple regression analysis was performed to determine the

variance contributed by different socioeconomic variables identified in correlation analysis for the prediction of cognitive scores.

CHAPTER IV

ASSESSMENT OF SERUM 25(OH) D IN WOMEN OF CHILDBEARING AGE AND THEIR PRESCHOOL CHILDREN IN NORTHERN JORDAN DURING SUMMER

ABSTRACT

Objective**:** To assess vitamin D status in women and their children at 4-5 years of age in northern Jordan during summer.

Design**:** A cross-sectional study; 93 mother-child dyads volunteered through local community centers between June-July 2007. Anthropometric measurements were performed and information on socioeconomic status, health issues, lifestyle factors, and nutritional intake were obtained from mothers by questionnaire.

Main measures: Serum 25(OH) D and serum PTH

Results: Mean age (SD) was 34.0 (6.0) years for mothers and 60.7 (5.4) months for children. Maternal body mass index (BMI) was 29.6 (5.6) with 77% of women having BMI ≥ 25 kg/m^2. Mean concentration of 25(OH)D was 25.6 (9.6) nmol/L in mothers; Only two women (2.2%) had 25(OH)D concentrations less than 12.5 nmol/L, but 48.9% of women were below 25.0 nmol/L, and 97.8% had serum 25(OH)D less than 50 nmol/L. No woman had values above 75 nmol/L. Children had higher ($p<.0001$) 25(OH) D than

their mothers with a mean of 55.8 nmol/L. The children also had lower (p<.0001) mean serum PTH than their mothers (1.47 vs. 3.12 pmol/L, respectively). Only three children had serum 25(OH) D less than 25 nmol/L, but 39% (n=34) had 25(OH)D less than 50.0 nmol/L. Older women and those with five or more pregnancies had significantly reduced mean 25(OH)D. Children living in families with lower incomes had significantly higher mean serum 25(OH)D as did children consuming fortified milk compared to non-fortified milk.

Conclusion: Despite the abundant sunlight during summer, vitamin D status is a concern for mothers and children in Jordan.

Key words: vitamin D deficiency; 25(OH)D; parathyroid hormone; women of child-bearing age; Jordan

INTRODUCTION

There is a growing body of research suggesting that vitamin D deficiency is widespread among women in the Middle Eastern countries, and while considerable research has been done to determine the prevalence of vitamin D deficiency among *adolescent females* (Moussavi et al 2005, Olmez et al 2006, Siddiqui and Kamfar 2007), *women of child bearing age* (Dawodu et al 1998, Fonseca et al 1984, Gannage-Yared et al 2000, Ghannam et al 1999, Mishal 2001), *veiled/non veiled women* (Alagol et al 2000, Allali et al 2008, El-Sonbaty and Abdul-Ghaffar 1996, Islam et al 2006, Meddeb et al 2005), *postmenopausal women* (Rassouli et al 2001), and *women with osteoporosis*

(Basaran et al 2007, Lips et al 2006). Limited research has been done to determine vitamin D status among children in Middle Eastern countries.

A few studies reported inadequate vitamin D status among: *infants and their mothers* in United Arab Emirates (Dawodu et al 2001, Dawodu et al 2003), Turkey (Hatun et al 2005), Kuwait (Molla et al 2005) , and Saudi Arabia (Taha et al 1984); *infants and pre-school children* (aged 4-72 months) in Saudi Arabia (Bahijri 2001); and *school children* (aged 10-16 years) in Lebanon (El-Hajj Fuleihan et al 2001).

With the abundant sunlight in the region, one would assume that populations of these countries would be secured from being vitamin D deficient, but the research on vitamin D status of Middle Eastern women has shown the opposite. On average 5-10 minutes of direct sunlight exposure of the arms and legs (half the minimal erythema dose) will produce about 3000 IU of vitamin D_3 (Holick 2007); however, amount of body surface area that is actually exposed to sunlight depends on clothing style, use of sunscreen, and lifestyle.

To our knowledge no study has assessed the vitamin D status of both women and their pre-school children in the region. This study assessed serum 25(OH)D and parathyroid hormone in women and their children at 4-5 years of age in Jordan, and investigated the variables that might influence vitamin D status in the study population.

METHODS

A convenience sample of 93 women and their children at 4-5 years of age from urban/suburban areas in northern Jordan (31°N) was recruited for the study.

Participants were approached by direct contact with the help of local community centers. Exclusion criteria were acute illness or pregnancy in mothers. The study was approved by Oklahoma State University Institutional Review Board, and informed written consent was obtained from the mothers. Four university graduate research assistants in Jordan were trained and assisted in taking anthropometric measurements and in administration of the questionnaire.

Weight (kg) and height (cm) were measured for each mother and child, and middle upper arm circumference (cm) was measured for children. A questionnaire covering health status, demographic characteristics, income (defined as total monthly family income after rent deduction), life style, and nutrition of mother and child was administered individually to mothers. The questionnaire was validated by a group of academic experts in research, statistics, and dietetics who reviewed the questions to ensure face and content validity. Secondly a focus group discussion with local women was carried out and some items of the questionnaire were modified to eliminate ambiguity.

After collection of anthropometric measurements and questionnaire data in the field, participants were transferred to a local clinical laboratory where venous blood samples were drawn from each participant. All samples were taken between June and July, 2007. Serum samples were frozen at -20°C, shipped in dry ice to our laboratories at Oklahoma State University in the USA, and stored at -20°C until analysis. Serum 25(OH)D and intact serum PTH were measured using enzyme-linked immunoabsorbent assay (ELISA) kits (IDS, Fountain Hills, AZ, USA).

Body mass index (BMI) was calculated for mothers as body weight (kg)/height (m^2). Anthropometric data for children were expressed as z-scores based on the CDC/WHO 1978 reference using Epi Info, v. 3.4.3 (Centers for Disease Control, USA). The relationships between serum 25(OH) D and serum PTH in both mothers and children were evaluated with linear and non-linear regression models using GraphPad Prism, v. 5, (GraphPad Software, La Jolla, CA). Other statistical analyses were conducted using the Statistical Package for the Social Sciences, v.15.0 (SPSS Inc., Chicago, IL, USA). Variables were checked for normality, and natural logarithm (ln) transformations were performed for skewed variables which included: mothers' 25(OH) D and PTH, and children's PTH. Means ± standard deviations or percentages were used to express results unless otherwise stated. Analysis of variance was used to compare subgroups of the participants, and a p-value of ≤ 0.05 was significant.

RESULTS

Study group characteristics

The characteristics of the participants are summarized in Table 4.1. The mean age of the women was 33.9 y and their children (51 boys and 42 girls) had a mean age of 60.7 mo. The children's middle upper arm circumference (MUAC) ranged from 14.0-19.5 cm, four children only (4.3%) were below -2 standard deviations for weight-for-age (-2z), and two children (2.2%) were below -2 for the height-for-age z-score.

The women had high BMIs with a mean of 29.6, and 77% of women were overweight with a BMI ≥ 25 kg/m^2. Seventy-four women (79.6%) were currently

married, and 17 women (18.3%) were widows; widows had a significantly higher BMI ($p < .05$) than other women (32.5 vs. 28.9 kg/m^2, respectively). The mean number of children per woman was 4.1. Eighteen percent of women had completed 8 years or less of school, but most had completed high school. Ninety-one percent didn't have a job, and the monthly income for each family (gross income-housing) was equivalent to 325 USD.

The women reported 5.3 sun exposure hours per week, and 70% of women said that they never tended to avoid sun exposure. However, most women (86%) were wearing clothing that showed only their hands and faces, 10 women (10.8%) were wearing full- covering clothing, and only 3 women were wearing western dress style.

Food consumption patterns of mothers and children

Seventy percent of women and 87% of children reported milk consumption, but consumption was only 4.1 cups per week for women and 5.6 cups per week for children. Additionally, 23% of mothers and 11% of children reported consumption of milk derivatives like yogurt and cheese but not milk (Table 4.2). The majority of mothers (73.8%) and children (82.7%) who drank milk consumed the commercially processed milk, while 26.2% of women and 17.3% of children consumed raw (unpasteurized) milk. Most of the commercially processed milk is in the powder form and is fortified with vitamin D.

Fish consumption was relatively rare; more than 75% of both the mothers and the children reported that they never or rarely consumed fish. Approximately half of

women and children had never or rarely consumed liver. None of the children had used multivitamin supplements. Only 7 women reported taking supplements, and their 25(OH)D of 30.4 nmol/L was not significantly different from the 25.2 nmol/L for those women not using multivitamin supplements.

Serum 25(OH) D and PTH concentrations

Serum 25(OH) D, the major circulating metabolite of vitamin D, was used to assess vitamin D status (Holick 2006b, Lips 2004). Serum 25(OH) D reflects the total vitamin D from the two sources, synthesis from sun light exposure and dietary intake (Holick 2006b).

Mean concentration of 25(OH) D was 25.6 nmol/L in women (Table 4.3). Forty-five women (48.9%) were below 25.0 nmol/L, and 90 women (97.8%) has serum 25(OH)D less than 50 nmol/L. Mothers' mean PTH was 3.12 pmol/L with 27.2 % above 3.9 pmol/L, the upper range of normal for the analysis. Children had a higher mean 25(OH)D than their mothers with a mean concentration of 55.8 nmol/L ($p < .0001$) (Figure 4.1). The children also had a lower mean serum PTH (1.47 pmol/L) than their mothers ($p < .0001$). Only three children (3.4%) had serum 25(OH) D less than 25 nmol/L, and more than 61% (n=53) had serum 25(OH) D higher than 50.0 nmol/L.

In mothers, the negative relationship between serum 25(OH) D and serum PTH did not reach significance in the linear regression model ($p= .086$). In the nonlinear regression model, serum PTH didn't reach a plateau as all serum 25(OH) D observations were below 80 nmol/L.

In children the relationship between serum 25(OH) D and PTH was studied using a linear regression analysis; for 1 unit increase in serum 25(OH) D concentrations on the ln-scale, serum PTH concentrations on the ln-scale decreased by 0.226 units ($p<0.000$). In addition to that, a non-linear regression model was fitted between serum 25(OH)D and serum PTH; based on the non-linear weighted least squares regression analysis, the plateau for serum PTH was reached at 1.36 pmol/L in children [PTH (pmol/L) = 1.363 + 0.987 exp (-0.0435 * 25(OH) D nmol/L), and the asymptote for the curve was reached in the 25(OH) D range of 69.9- 80 nmol/L.

Effect of age, number of pregnancies, BMI, dress style, and income on mean 25(OH) D

Women less than 29 years of age had mean 25(OH) D of 30.5 nmol/L compared to a mean in all women older than 29 years of 24.3 nmol/L ($p<0.05$). Women who had five or more pregnancies had lower ($p <0.05$) mean serum 25(OH) D (22.3 nmol/L) compared to women reporting less than 5 pregnancies (27.9 nmol/L). Women who had five or more pregnancies had a 63.2% prevalence of serum 25(OH)D less than 25.0 nmol/L compared to 38.9% prevalence among women who had less than 5 pregnancies. There was no significant difference in mean 25(OH) D concentration between women in normal, over weight, and obese BMI categories. Likewise dress style had no significant effect on mean serum 25(OH) D concentrations in women although the number of women in western dress was very small (Table 4.4).

Serum 25(OH) D concentration in children was negatively correlated with income ($r= - 0.230$, $p < .05$) but this was not so in mothers. Children living in families with

income less than $212 per month had a significantly higher mean serum 25(OH) D than children living in families with income more than $212 per month (61.6 vs. 52.6 nmol/L, respectively), while mothers with monthly income less than $212 had a significantly higher mean serum PTH than mothers living with monthly income less than $212 (3.6 vs. 2.9 pmol/L, respectively).

Milk type consumed relation to serum 25(OH) D and serum PTH

In children there was a significant difference in serum 25(OH) D concentrations and serum PTH according to milk type consumed by the children, whereas this difference was not significant among mothers. Children who consumed the unfortified fresh milk had lower ($p < .05$) mean serum 25(OH)D (43.0 nmol/L) and higher mean serum PTH (2.0 pmol/L) compared to those children who consumed the fortified milk who had a mean serum 25(OH)D concentration of 56.0 nmol/L and mean serum PTH of 1.4 pmol/L (Table 4.5).

DISCUSSION

The results showed high prevalence of serum 25(OH) D less than 25.0 and 50.0 nmol/L among women of child-bearing age in Jordan during summer. Mean serum 25(OH)D concentrations in mothers were much lower than those of their 4-5 years old children, although 39.1% of children still had 25(OH)D concentrations less than 50.0 nmol/L. In Jordan one would assume that the abundant sun light during summer would be sufficient to produce adequate levels of 25(OH) D among the population. Although

70% of women said they did not avoid sun exposure, the daily sun exposure hours reported by mothers were less than 1 hr/day. In addition to that, 74% of women (n=69) reported that they avoid sun exposure during the time from 11:00 am to 3:00 pm. Only 8 women had jobs, whereas more than 50% of children were attending pre-schools where most of them walk home at noon. The differences in time and frequencies of sun exposure hours between mothers and their children might be a factor contributing to the higher mean concentrations of 25(OH) D in children compared to their mothers.

Dietary vitamin D sources were limited; more than 75% of the mothers and 80% of children reported that they never or rarely consumed fresh fish or sardines, and only 7.5% of mothers used multivitamin supplements. While 70% of women and 87% of children did consume milk, a considerable percentage of mothers and their children consumed the raw unfortified milk. The data illustrate the significant differences in mean serum 25(OH) D and PTH concentrations between children consuming the raw unfortified milk and those consuming the fortified milk.

The higher 25(OH) D in young women and in women in women with less than five pregnancies may be explained by younger women having a more outgoing lifestyle and those with fewer pregnancies being less housebound. A study in Lebanon found that a high degree of parity (≥ 4) negatively impacted vitamin D status (Gannage-Yared et al 2000).

Several studies in the Middle East and North Africa found a significant impact of dress style on serum 25(OH) D status among women (Alagol et al 2000, Allali et al 2008, Dawodu et al 1998, El-Sonbaty and Abdul-Ghaffar 1996, Gannage-Yared et al 2000,

Meddeb et al 2005), but in some studies dress was not significant (Islam et al 2006, Mishal 2001). In our study 86% of the women wore clothing that covered the whole body except for hands and face, and only 3% wore western clothing, so a clothing style comparison could not be made statistically.

Mothers had high BMI value, which perhaps could be explained by their sedentary lifestyle, and high number of pregnancies (42% of women had 5 or more pregnancies). Although literature suggests that obesity is related to low serum 25(OH) D among individuals (Holick 2003, Liel et al 1988, Need et al 1993, Parikh et al 2004), and percentage body fat is inversely proportional to serum 25(OH) D in healthy women (Arunabh et al 2003), due to decrease in bioavailability of vitamin D with obesity (Wortsman et al 2000), in this study BMI has no significant impact on mean serum 25(OH) D among women.

In this study children living in families with lower income had higher mean serum 25(OH) D. Perhaps this may be explained partially by these children spending more of their day outside the house because of the poor housing environment, which supports the important role of sun exposure for vitamin D status.

The fact that children had higher mean serum 25(OH) D concentrations than their mothers should not mask the fact that these children still had low concentrations of serum 25(OH) D, especially since the study was conducted in the middle of summer. There is not yet a consensus on what would be normal 25(OH) D values for children. However, a very recent study by (Looker et al 2008) reported on US NHANES data. The sample included 895 children aged 1-5 years, and mean ± SEM was 76.43 ± 1.58 nmol/L.

In our study, the relationship between serum 25(OH) D and serum PTH in children showed that PTH started to increase at 25(OH)D concentrations lower than 69.9 nmol/L; based on this value almost 78.5% of children may have had inadequate vitamin D status. Children in our study had a mean serum 25(OH)D (55.8 nmol/L) during summer that was lower than reported for pre-school children of similar age (4.8 years) in Greece during the fall (Nicolaidou et al 2006) where the immigrant children from Eastern Europe had a mean 25(OH)D concentration of 69.7 nmol/L, and the Greek children had a mean 25(OH)D of 83.8 nmol/L.

To our knowledge this was the first study to assess 25(OH)D in mothers of child bearing age and their children at 4-5 years of age in the region. The study was conducted during summer, and values of 25(OH) D are expected to be even lower during winter based on the literature (Chapuy et al 1996, Goswami et al 2000, Pal et al 2003, Pasco et al 2004). Natural foods rich in vitamin D are not commonly consumed by the larger population, and there are no governmental regulations that mandate vitamin D fortification for the processed milk and milk products produced in Jordan. Unfortunately until now, there are no recommended dietary guidelines concerning vitamin D intake in the Middle East and North Africa region (MENA), despite the high prevalence of vitamin D deficiency. Recently, the MENA consensus on osteoporosis recommended the daily intake of 400 IU of vitamin D for women 50-70 years old, and 600 IU for women older than 70 years (Maalouf et al 2007), but there remain no guidelines for younger women and for children. Despite the abundant sunlight during summer, vitamin D status is a concern for young mothers and children in Jordan.

Nutrition education programs should be implemented to inform women about the consumption of vitamin D fortified milk products as well as the importance of sufficient exposure to sunlight to attain optimal vitamin D status for themselves and their children. There is a need to assess vitamin D status at the national level so that strategies for vitamin D fortification and supplementation are developed and implemented.

REFERENCES

Alagol F, Shihadeh Y, Boztepe H, Tanakol R, Yarman S, Azizlerli H *et al* (2000). Sunlight exposure and vitamin D deficiency in Turkish women. *J Endocrinol Invest* **23:** 173-177.

Allali F, El Aichaoui S, Khazani H, Benyahia B, Saoud B, El Kabbaj S *et al* (2008). High Prevalence of Hypovitaminosis D in Morocco: Relationship to Lifestyle, Physical Performance, Bone Markers, and Bone Mineral Density. *Semin Arthritis Rheum*.

Arunabh S, Pollack S, Yeh J, Aloia JF (2003). Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab* **88:** 157-161.

Bahijri SM (2001). Serum 25-hydroxy cholecalciferol in infants and preschool children in the Western region of Saudi Arabia. Etiological factors. *Saudi Med J* **22:** 973-979.

Basaran S, Guzel R, Coskun-Benlidayi I, Guler-Uysal F (2007). Vitamin D status: effects on quality of life in osteoporosis among Turkish women. *Qual Life Res* **16:** 1491-1499.

Chapuy MC, Schott AM, Garnero P, Hans D, Delmas PD, Meunier PJ (1996). Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. *J Clin Endocrinol Metab* **81:** 1129-1133.

Dawodu A, Absood G, Patel M, Agarwal M, Ezimokhai M, Abdulrazzaq Y *et al* (1998). Biosocial factors affecting vitamin D status of women of childbearing age in the United Arab Emirates. *J Biosoc Sci* **30:** 431-437.

Dawodu A, Dawson KP, Amirlak I, Kochiyil J, Agarwal M, Badrinath P (2001). Diet, clothing, sunshine exposure and micronutrient status of Arab infants and young children. *Ann Trop Paediatr* **21:** 39-44.

Dawodu A, Agarwal M, Hossain M, Kochiyil J, Zayed R (2003). Hypovitaminosis D and vitamin D deficiency in exclusively breast-feeding infants and their mothers in summer: a justification for vitamin D supplementation of breast-feeding infants. *J Pediatr* **142:** 169-173.

El-Hajj Fuleihan G, Nabulsi M, Choucair M, Salamoun M, Hajj Shahine C, Kizirian A *et al* (2001). Hypovitaminosis D in healthy schoolchildren. *Pediatrics* **107:** E53.

El-Sonbaty MR, Abdul-Ghaffar NU (1996). Vitamin D deficiency in veiled Kuwaiti women. *Eur J Clin Nutr* **50:** 315-318.

Fonseca V, Tongia R, el-Hazmi M, Abu-Aisha H (1984). Exposure to sunlight and vitamin D deficiency in Saudi Arabian women. *Postgrad Med J* **60:** 589-591.

Gannage-Yared MH, Chemali R, Yaacoub N, Halaby G (2000). Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. *J Bone Miner Res* **15:** 1856-1862.

Ghannam NN, Hammami MM, Bakheet SM, Khan BA (1999). Bone mineral density of the spine and femur in healthy Saudi females: relation to vitamin D status, pregnancy, and lactation. *Calcif Tissue Int* **65:** 23-28.

Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N (2000). Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. *Am J Clin Nutr* **72:** 472-475.

Hatun S, Ozkan B, Orbak Z, Doneray H, Cizmecioglu F, Toprak D *et al* (2005). Vitamin D deficiency in early infancy. *J Nutr* **135:** 279-282.

Holick MF (2003). Vitamin D: A millenium perspective. *J Cell Biochem* **88:** 296-307.

Holick MF (2006). High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* **81:** 353-373.

Holick MF (2007). Vitamin D deficiency- Review Article. *N Engl J Med* **357:** 266-281.

Islam MZ, Akhtaruzzaman M, Lamberg-Allardt C (2006). Hypovitaminosis D is common in both veiled and nonveiled Bangladeshi women. *Asia Pac J Clin Nutr* **15:** 81-87.

Liel Y, Ulmer E, Shary J, Hollis BW, Bell NH (1988). Low circulating vitamin D in obesity. *Calcif Tissue Int* **43:** 199-201.

Lips P (2004). Which circulating level of 25-hydroxyvitamin D is appropriate? *J Steroid Biochem Mol Biol* **89-90:** 611-614.

Lips P, Hosking D, Lippuner K, Norquist JM, Wehren L, Maalouf G *et al* (2006). The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *J Intern Med* **260:** 245-254.

Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA (2008). Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. *Am J Clin Nutr* **88:** 1519-1527.

Maalouf G, Gannage-Yared MH, Ezzedine J, Larijani B, Badawi S, Rached A *et al* (2007). Middle East and North Africa consensus on osteoporosis. *J Musculoskelet Neuronal Interact* **7:** 131-143.

Meddeb N, Sahli H, Chahed M, Abdelmoula J, Feki M, Salah H *et al* (2005). Vitamin D deficiency in Tunisia. *Osteoporos Int* **16:** 4.

Mishal AA (2001). Effects of different dress styles on vitamin D levels in healthy young Jordanian women. *Osteoporos Int* **12:** 931-935.

Molla AM, Al Badawi M, Hammoud MS, Molla AM, Shukkur M, Thalib L *et al* (2005). Vitamin D status of mothers and their neonates in Kuwait. *Pediatr Int* **47:** 649-652.

Moussavi M, Heidarpour R, Aminorroaya A, Pournaghshband Z, Amini M (2005). Prevalence of vitamin D deficiency in Isfahani high school students in 2004. *Horm Res* **64:** 144-148.

Need AG, Morris HA, Horowitz M, Nordin C (1993). Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. *Am J Clin Nutr* **58:** 882-885.

Nicolaidou P, Kakourou T, Papadopoulou A, Kavadias G, Dimitriou E, Georgouli H *et al* (2006). Low vitamin D status in preschool children in Greece. *Nutr Res* **26:** 620-625.

Olmez D, Bober E, Buyukgebiz A, Cimrin D (2006). The frequency of vitamin D insufficiency in healthy female adolescents. *Acta Paediatr* **95:** 1266-1269.

Pal BR, Marshall T, James C, Shaw NJ (2003). Distribution analysis of vitamin D highlights differences in population subgroups: preliminary observations from a pilot study in UK adults. *J Endocrinol* **179:** 119-129.

Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J *et al* (2004). The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* **89:** 1196-1199.

Pasco JA, Henry MJ, Kotowicz MA, Sanders KM, Seeman E, Pasco JR *et al* (2004). Seasonal periodicity of serum vitamin D and parathyroid hormone, bone resorption, and fractures: the Geelong osteoporosis study. *J Bone Miner Res* **19:** 752-758.

Rassouli A, Milanian I, Moslemi-Zadeh M (2001). Determination of serum 25-hydroxyvitamin D(3) levels in early postmenopausal Iranian women: relationship with bone mineral density. *Bone* **29:** 428-430.

Siddiqui AM, Kamfar HZ (2007). Prevalence of vitamin D deficiency rickets in adolescent school girls in Western region, Saudi Arabia. *Saudi Med J* **28:** 441-444.

Taha SA, Dost SM, Sedrani SH (1984). 25-Hydroxyvitamin D and total calcium: extraordinarily low plasma concentrations in Saudi mothers and their neonates. *Pediatr Res* **18:** 739-741.

Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF (2000). Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* **72:** 690-693.

Figure 4.1

Relation between serum 25(OH) D and serum PTH in mothers and children in Jordan

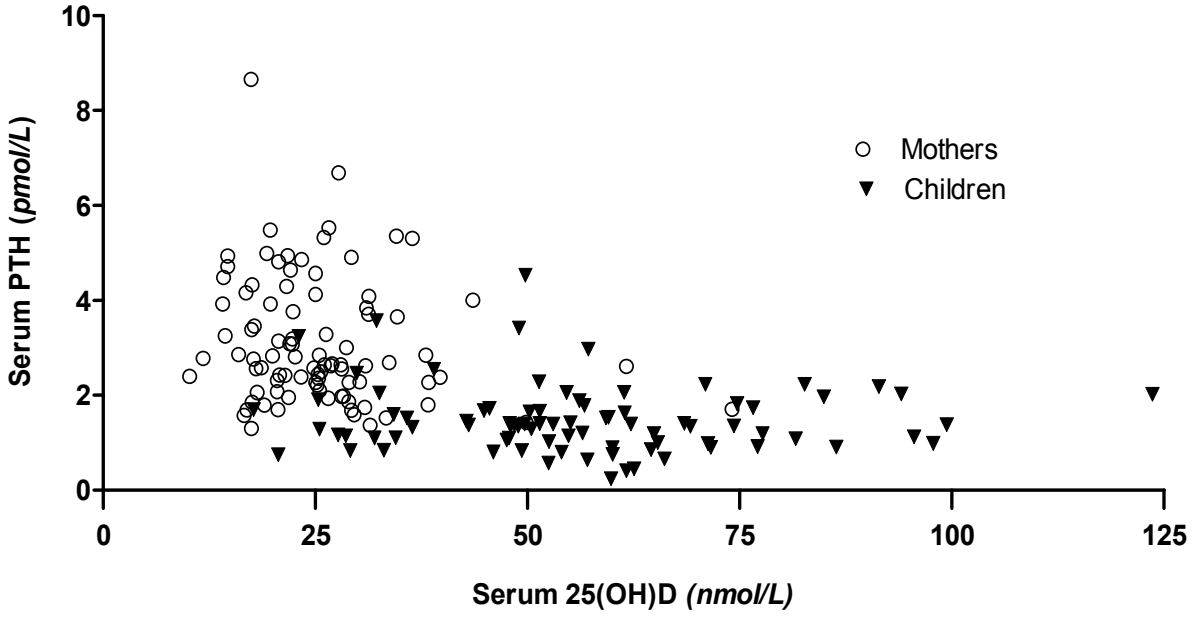


Table 4.1

Characteristics of the study participants

	N	mean (SD)	Median	Percentile	
				25th	***75th***
Mothers					
Age (years)	93	33.9 (5.9)	35.0	29.0	38.0
BMI (kg/m^2)	93	29.6 (5.6)	29.0	25.2	33.5
Number of pregnancies	93	4.8 (2.5)	4.0	3.0	6.0
Number of children	93	4.1 (1.9)	4.0	3.0	5.0
Income (USD/month)*	92	325 (272)	254	174	353
Years of schooling	92	10.7 (2.3)	12.0	9.0	12.0
Time spent in garden or field work (hr/wk)	15	2.6 (2.4)	2.0	1.0	3.0
Sun exposure (hr/wk)	93	5.3 (4.6)	3.5	1.8	7.0
Children					
Age (months)	93	60.7 (5.4)	61.0	55.0	65.0
Weight- for-age (z)	93	-0.0 (1.1)	0.1	-0.6	0.6
Height–for-age (z)	93	0.5 (1.2)	0.6	-0.3	1.4
Child MUAC** (cm)	93	16.7 (1.2)	16.7	16.0	17.5

*Income is defined as total monthly family income after rent deduction in US $.

**MAUC: Middle upper arm circumference.

Table 4.2

Food consumption patterns by mothers and children

		Mothers (%)	Children (%)
Milk and milk products*			
• No		7.5	2.2
• Yes:	▸ Fluid milk, powdered milk, and milk products	70.0	87.0
	▸ Milk products only but not fluid or powdered milk	22.5	10.8
Milk type consumed**			
• Raw milk		26.2	17.3
• Commercial milk:	▸ Powdered (vitamin D fortified)	63.1	72.8
	▸ Liquid (not all vitamin D fortified)	10.8	9.9
Liver consumption			
• Never or rarely		43.4	51.6
• Once a month or more		56.6	48.4
Multivitamin use			
• No		92.5	100
• Yes		7.5	0

* Milk products included: cheese, yogurt, labneh (sour cream).

**Milk type consumed by the 65 mothers and 81 children who consume milk

Table 4.3

Serum 25(OH)D distribution in mothers and children and means of serum PTH in these categories

Serum 25(OH)D Category (nmol/L)	%N	PTH *(pmol/L)* Mean (SD)
Mothers		
Mean = 25.6 (9.6)nmol/L		
<12.5	2.2	2.59 (0.27)
12.5-24.9	46.7	3.35 (1.40)
25-49.9	48.9	2.97 (1.28)
50-75	2.2	2.16 (0.64)
>75	0	-
Total (n=92)	***100***	***3.12 (1.32)***
Children		
Mean = 55.8 (19.8)nmol/L		
<12.5	0	-
12.5-24.9	3.4	1.89 (1.25)
25-49.9	35.6	1.64 (0.87)
50-75	46.0	1.31 (0.58)
>75	14.9	1.52 (0.52)
Total (n=87)	***100***	**1.48** ***(0.72)***

Table 4.4

Differences in mean serum 25(OH) D in mothers by age, BMI, dress style, and number of pregnancies

Variable		N	25(OH)D *(nmol/L)* Mean	Prevalence of 25 (OH)D <25 nmol/L
Age *(years)*	< 29	19	30.5[a]	47.4 % *(n=9)*
	≥ 29	74	24.3[b]	48.6% *(n=36)*
BMI *(kg/m^2)*	<18.5	1	28.1	0% *(n=0)*
	18.5-24.99	20	24.6	55.0% *(n=11)*
	25.0-29.99	33	26.8	30.3% *(n=10)*
	≥ 30.0	48	24.9	50.0 % *(n=24)*
Dress Style	Full covering	10	28.4	50.0% *(n=5)*
	Showing only face & hands	79	24.9	50.6% *(n=40)*
	Western style	3	31.9	0% *(n=0)*
Number of Pregnancies	<5	54	27.9[a]	38.9% *(n=21)*
	≥ 5	38	22.3[b]	63.2% *(n=24)*

Note: Variables with different superscripts were significantly different, p< 0.05

Table 4.5

Milk type consumed and mean serum 25(OH) D and PTH in mothers and children

	Milk type consumed	25(OH) D (nmol/L)		PTH (pmol/L)	
		n	Mean (SD)	n	Mean (SD)
Mothers	unfortified	17	26.6 (14.5)	16	3.1 (1.4)
	fortified	41	26.0 (7.4)	41	3.0 (1.1)
Children	unfortified	14	43.0 (12.8) [a]	13	2.0 (1.1) [a]
	fortified	53	56.0 (19.8) [b]	58	1.4 (0.6) [b]

Note: Variables with different superscripts were significantly different, p< 0.05

CHAPTER V

ASSESSMENT OF IRON AND ZINC STATUS OF WOMEN OF CHILDBEARING AGE AND THEIR CHILDREN IN JORDAN

ABSTRACT

Prevalence of iron and zinc deficiency among women and their preschool children was assessed in Jordan in July 2007. Hemoglobin and plasma ferritin, zinc, transferrin receptors and α-1-acid glycoprotein (AGP) were determined in 93 mothers and their children. Measurements of hemoglobin, ferritin, and zinc showed 29% of mothers and 10% of children to be deficient by at least two parameters, and 24% of mothers and 3% of children were deficient by three parameters. Thirty-eight women (41%) and 24 children (26%) were anemic. Mothers with ferritin <15 μg/L were 54%, and 16% of children had ferritin <12 μg/L; half of children were below 22 μg/L. Mothers body iron (mean ± SD) was estimated to be significantly lower (0.5 ± 4.9) mg/kg than their children (3.5 ± 3.8) mg/kg; 45% of mothers had an iron deficit of 4.0 mg/kg body weight while 18% of children had an iron deficit of 0.71 mg/kg body weight. More than 50% of mothers and 30% of children had AGP≥1.2 g/L. Plasma zinc was 67.2 ± 11.4 μg/dL in mothers and 64.6 ± 9.4 μg/dL in children. More than half of women (58%) and children (56%) were below IZiNCG cutoffs for zinc deficiency. Zinc was correlated for mothers and children ($r=0.33$, $p<.005$). The performance of three parameters: TfR, TfR/ferritin,

and TfR/Log_{10} ferritin in detecting iron deficiency among mothers and children was evaluated using receiver operator characteristics (ROC) curves. TfR/ferritin and TfR/Log_{10}ferritin appeared to have better diagnostic efficiency in detecting iron deficiency than the use of TfR alone. In summary**,** co-existing micronutrient deficiencies were widespread among mothers and children in northern Jordan.

INTRODUCTION

Iron deficiency

Anemia and iron deficiency are among the most common nutritional disorders affecting children and women in both industrialized and developing countries. In the Middle East, about 47% of pre-school children (0.8 million) have hemoglobin (Hb) levels below 11 g/dl and almost 40 million women of child bearing age (non-pregnant) have Hb levels below 12 g/dL (WHO 2008). According to the WHO classification of public health significance of anemia in populations based on prevalence estimated from hemoglobin or hematocrit (WHO/UNICEF/UNU 2001), the prevalence of anemia in the Eastern Mediterranean region is considered a severe public health problem among pre-school children (>40%) and moderate among women of child-bearing age (20%-40%).

In Jordan, it is estimated that more than 200,000 children under the age of five years and almost 30% of non-pregnant women of reproductive age are anemic (WHO 2008). In 2003, the Ministry of Health estimated the prevalence of anemia, iron deficiency, and iron deficiency anemia to be 32%, 40%, and 22%, respectively among non-pregnant women of reproductive age, and 20%, 26%, and 10%, respectively among

pre-school children (Ministry of Health 2003). Some studies found a prevalence of anemia that reaches 41% among pregnant women (Albsoul-Younes et al 2004), and 40% among women of reproductive age (Mawajdeh et al 2003). Ferritin is the most useful measure for storage iron in absence of inflammation (Cook et al 1974). However, in populations with high incidence of inflammation it becomes an unreliable indicator of iron status and serum transferrin receptor (TfR) has shown promise in identifying iron status without being influenced by acute or chronic inflammation (Skikne et al 1990). In addition to the use of TfR, both TfR/ferritin and TfR/Log ferritin had been demonstrated to be valuable in assessing iron status (Lin et al 2008, Malope et al 2001, Punnonen et al 1997, Vazquez Lopez at al 2006).

Zinc deficiency

The World Health Organization (WHO) identified the factors contributing most to burden of disease in developing countries; zinc deficiency was ranked as the 5th factor followed by iron deficiency as the 6th (WHO 2002). Worldwide, 16% of lower respiratory tract infections, 10% of diarrheal disease, and 18% of malaria, are attributed to zinc deficiency (WHO 2002). Globally about half of the human population is at risk of low zinc intake (Brown and Wuehler 2000). The first cases of zinc deficiency in humans were reported in the Middle East region in the 1960s, specifically in Iran and Egypt (Prasad et al 1961, Prasad et al 1963), and zinc deficiency was highly correlated with severe anemia, growth retardation, and delay in sexual maturation. The prevalence of zinc deficiency in the Middle East North Africa (MENA) region is estimated to reach 46%, a

percentage that makes the region ranked third after South Asia (79%) and Sub-Saharan Africa (50%) (Caulfield et al 2006). The prevalence of zinc deficiency in the MENA region was estimated by examining the availability of zinc in the local diet in the following countries: Afghanistan, Algeria, Djibouti, Egypt, Iraq, Morocco, Pakistan, Somalia, Sudan, and Yemen (Caulfield and Black 2004). Diets in the MENA region are characterized by low zinc bioavailability contributed to by diets high in phytate that inhibits zinc absorption. Currently, there are no estimates of the national prevalence and distribution of zinc deficiency in Jordan, and there is a serious lack of awareness of the importance of zinc in human nutrition not only in Jordan but also in the MENA region.

This study aims to assess the prevalence of iron deficiency and zinc deficiency in mothers and their preschool children living in northern Jordan. A second aim of this study is to evaluate the diagnostic efficiency and assess the sensitivity and specificity of serum TfR and different TfR/ferritin ratios in identifying iron deficiency in women of child-bearing age and preschool children in Jordan.

METHODS

A convenience sample of 93 women and their children aged 4–5 years from urban/suburban areas in Northern Jordan was recruited for the study. Participants were approached by direct contact with the help of local community centers. Exclusion criteria were acute illness or pregnancy in mothers. The study was approved by the Oklahoma State University Institutional Review Board, and an informed written consent was obtained from the mothers. Four university graduate research assistants in Jordan

were trained and assisted in taking anthropometric measurements and in administration of the questionnaire and the cognitive tests.

Weight (kg) and height (cm) were measured for each mother and child, and middle upper arm circumference (MUAC) (cm) was measured for children. Height and MUAC were reported to the nearest millimeter, and weight was reported to the nearest 100 gram. A questionnaire covering health status, demographic characteristics, income (defined as total monthly family income after rent deduction), lifestyle and dietary intakes of mother and child was administered individually to mothers. The questionnaire was validated by a group of academic experts in research, statistics and dietetics who reviewed the questions to ensure face and content validity. Second, a focus group discussion with local women was carried out and some items of the questionnaire were modified to eliminate ambiguity.

After collection of anthropometric measurements and questionnaire data in the field, participants were transferred to a local clinical laboratory where non-fasting venous blood samples were drawn from each participant. All samples were taken between June and July 2007. Serum and plasma samples were transferred into trace element-free tubes which were then frozen at -20 °C, shipped in dry ice to our laboratories at Oklahoma State University in the USA and stored at -20 °C until analysis. In addition to hemoglobin (Hb), the following were measured: plasma ferritin (Ft) using immunoradiometric assay (Ramco Laboratories, Inc. Stafford, TX); transferrin receptor (TfR) using enzyme linked immunoassay (Ramco Laboratories, Inc. Stafford, TX); α-1-acid

glycoprotein (AGP) using radial immunodiffusion (Kent Laboratories, Redmond, WA); and plasma zinc (Zn) using inductively coupled plasma mass spectrometry (ICPMS).

For serum ferritin (Ft), AGP, and TfR, standards and serum samples were analyzed in duplicate, and values for the manufacturer's controls fell within the certified ranges. Coefficients of variation (CV) for the low, medium, and high controls for AGP were 4.1%, 9.8%, and 6.1%, respectively. For serum ferritin, the CV for the low, medium, and high controls were 1.5%, 2.0%, and 1.9%, respectively. For TfR, the CV for the medium and high controls was 15.1% and 15.9%, respectively. For plasma zinc, a sample check (concentration of 50 µg/L) was measured repetitively (once after 10 participants' samples), and the mean, standard deviation, and CV were 52.0 µg/L, 0.76, and 1.5%, respectively.

Plasma zinc concentration below the age/sex/time of the day/fasting status-specific cutoffs is considered the best available biochemical measure of zinc deficiency as recommended by the Joint WHO/UNICEF/IAEA/IZiNCG Interagency meeting on zinc status indicators (de Benoist et al 2007). A cut-off point of 70 µg/dL for mothers and 65 µg/dL for children was used to define deficiency (IZiNCG 2007a).

Hemoglobin concentration less than 12 g/dl was used to define anemia in mothers. In children, Hb <11 g/dl was used to define anemia in children less than 60 months of age (n= 39), and Hb < 11.5 g/dl was used to define anemia in children aged 60 months or older (n=54) (WHO/UNICEF/UNU 2001).

Quantitative estimation of body iron (mg/kg) was based on the model proposed by Cook and colleagues (2003) using the following formula:

Body Iron (mg/kg) = - [log (TfR/Ferritin ratio) - 2.8229]/0.1207

Positive values for body iron (> 0 mg/kg) indicate iron surplus in stores, and negative values (body iron <0 mg/kg) indicate tissue iron deficiency (Cook et al 2003).

Serum ferritin, TfR, and the ratio of TfR to serum ferritin TfR: Ft (TfR in mg/L and Ft in μg/L) were used to assess iron deficiency in mothers and children. For serum ferritin, cut-offs of 15 μg/L for mothers and 12 μg/L for children were used to define iron deficiency in absence of inflammation (WHO/UNICEF/UNU 2001). An AGP concentration ≥1.2 g/L was used to indicate the presence of inflammation (Paracha et al 2000, Wieringa et al 2002).

Sensitivity, specificity, and area under the curves were calculated using the receiver operator characteristics (ROC) curves to assess the performance and define the cut-off points of TfR, TfR/Ft and TfR/Log Ft in the diagnosis of iron deficiency and the differentiation between anemia of chronic disease (ACD) and iron deficiency anemia (IDA) (Lin et al 2008, Malope et al 2001, Punnonen et al 1997). Sensitivity (SN) was defined as proportion of people with disease who test positive: SN= TP/ (TP+FN). Specificity (SP) was defined as proportion of people without the disease who test negative SP = TN/ (TN+FP), where: TP is true positive, FN is false negative, TN is true negative, and FP is false positive.

The ROC curve was obtained by plotting sensitivity on the x-axis against 1 minus specificity on the y-axis for every possible cut-off value for the diagnostic test. Ideally, a perfect diagnostic test will yield 100% sensitivity and 100% specificity with an area under the curve (AUC^{ROC}) of one. An area of 0.5 is a poor indicator and represents a test

result that was obtained by random chance. Usually AUC^{ROC} will fall between 0.5 and 1.0 and the closer the AUC^{ROC} is to 1 the better the discriminant ability of the diagnostic test. Based on AUC^{ROC}, tests can be non informative (AUC^{ROC} =0.5), less accurate (AUC^{ROC} between 0.5 and 0.7), moderately accurate (AUC^{ROC} between 0.7 and 0.9), highly accurate (AUC^{ROC} between 0.9 and 1.0), and perfectly accurate (AUC^{ROC} =1.0) (Swets 1988).

The optimal sensitivity and specificity were defined as the minimal value of $(1\text{-}SN)^2 + (1\text{-}SP)^2$. The likelihood ratio for a positive test (LR+) was defined as: SN/(1-SP). The likelihood ratio for a negative test (LR-) was defined as: (1-SN)/SP and was defined as the probability of an individual with disease to test negative divided by the probability of an individual without a disease to test negative.

Body mass index (BMI) was calculated for mothers as body weight (kg)/height (m^2). Anthropometric data for children were expressed as *z*-scores based on the WHO (World Health Organization) 2007 reference using AnthroPlus, version 1.0.2 (WHO 2009). Variables that have skewed distribution were transformed to logarithms and geometric means ± 1SD were reported for those variables. Variables with skewed distribution included: ferritin, TfR, and TfR/ferritin in mothers and children, and AGP and Hb in children. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS/PASW), version 18.0 (SPSS Inc, Chicago, IL, USA). Analysis of variance was used to compare subgroups of the participants.

RESULTS

The characteristics of the participants are summarized in Table 5.1. The mean age of the women was 33.9 years and their children (51 boys and 42 girls) had a mean age of 60.7 months. The children's mid-upper arm circumference ranged from 14.0 to 19.5 cm; only four children (4.3%) had less than -2 SD for weight-for-age (-2 *z*), whereas two children (2.2%) had <-2 for the height-for-age *z*-scores.

Women had high BMIs with a mean of 29.6 kg/m^2, and 77% of women were classified as overweight or obese with a BMI ≥25 kg/m^2. In total, 74 women (79.6%) were married and 17 women (18.3%) were widows; widows had a significantly higher BMI (P<0.05) than did other women (32.5 vs 28.9 kg/m^2, respectively). The mean number of children per woman was 4.1. A total of 18% of women had completed 8 years or less of school, but most had completed high school. In total, 91% of the participants were not working outside the home, and the monthly income for each family (gross income-housing) was equivalent to 325 US$. Mothers of 48 children reported that their children went to kindergarten.

Hemoglobin

The biochemical indices are presented in Table 5.2. Thirty-eight women (41%) were anemic with a mean ± SD Hb concentration of 12.2 ± 1.0 g/dL. In children, the prevalence of anemia was higher in the older children (age ≥ 60 months) than the younger ones (age <60 months). Twenty children aged >59 months (37%) had Hb <11.5 g/dL, whereas around 10% (n=4) of children aged ≤ 59 months had Hb <11 gm/dl. In

mothers, hemoglobin concentration had a significant negative correlation with TfR (r=-0.32, p<.005), and a significant positive correlation with plasma ferritin and zinc (r=0.51, p<.000 and r=0.30, p<.005 respectively), and with estimated body iron (r=0.52, p<.000). In children Hb correlated negatively with TfR (r=-.21, p<.05).

Plasma ferritin, TfR, and body iron

Plasma ferritin concentrations ranged from 0.6 µg/L to 122.0 µg/L in mothers, and from 1.6 µg/L to 160.2 µg/L in children. More than half of the women (54%, n=50) were below the defined cut-off points for ferritin deficiency (15 µg/L), and approximately 75% (n=70) were below 32 µg/L (Table 5.2). Sixteen percent of children had ferritin below 12 µg/L, and half were below 22 µg/L. In mothers, as expected, plasma ferritin negatively and significantly correlated with TfR (r=-0.38, p<.000). But interestingly, in children, plasma ferritin and TfR were not significantly correlated (Table 5.3). Mothers had significantly lower mean serum ferritin and higher TfR (13.1 ± 3.1 µg/L, and 7.6 ± 1.5 mg/L, respectively) than their children (21.9 ± 2.0 µg/L, and 5.9 ± 1.4 mg/L, respectively). Forty-one women (44%) and 13 children (14%) had TfR above the normal range of TfR (2.9-8.3 mg/L) as defined by the assay.

Mothers had significantly lower mean body iron compared to their children (p <.000) as noted in the cumulative frequency plot (Figure 5.1). The mean ± SD body iron for mothers was 0.5 ± 4.9 mg/kg, and 45% of mothers (n=42) had an iron deficit of 4.0 mg/kg body weight. Children had a mean body iron of 3.5 ± 3.8 mg/kg and 18% of children (n=17) had an iron deficit of 0.71 mg/kg body weight.

Zinc

Mean ± SD of plasma zinc was 67.2 ± 11.4 in mothers and 64.6 ± 9.4 in children with no significant difference between mothers and children. Fifty-four women and 53 children were below the defined cut-off points for zinc deficiency (Table 5.2). Seventy-five percent of both mothers and children had plasma zinc below 73.1 μg/dL and 70.1 μg/dL, respectively. Mothers' zinc correlated positively with children's zinc (r=0.33, p<.005), and only in mothers, zinc had a positive correlation with Hb (r=0.30, p<.005).

AGP, ferritin, and BMI

Inflammation was prevalent among study participants. More than half of the mothers (n=50), and 30% of children (n=28) had AGP≥1.2 g/L. Mothers had significantly higher mean AGP (p<.000) than their children, with a mean AGP of 1.2 ± 0.4 g/L, and 1.0 ± 1.5 g/L in mothers and children, respectively. In children, AGP was positively correlated with ferritin (r= 0.35, p<.005). In mothers, BMI was significantly correlated with AGP (r=.22, p<.05), ferritin (r=0.21, p<.05), number of pregnancies (r=0.33, p<.000), and age (r= 0.42, p<.000). In all mothers with inflammation (AGP≥1.2 g/L), only eight mothers had WBC count >10,000mm^3, and only two of the eight mothers were anemic. However, in children, only six children of those with high AGP had WBC count >10,000mm^3, and none were anemic.

When mothers were grouped according to BMI into 3 groups (BMI <25.0 kg/m^2, n= 21, BMI=25.0-29.99 kg/m^2, n= 34, and BMI ≥30.0 kg/m^2, n=38), there was no

significant difference between the three BMI groups on all biochemical indices (ferritin, TfR, AGP, Iron, Hb, and zinc). However, when overweight women (BMI=25.0-29.99 kg/m^2, n=34) and obese women (BMI≥30 kg/m^2, n= 38) were combined in one group (BMI≥25 kg/m^2, n=72), and tested against women with normal BMI, AGP was significantly higher in the combined group (overweight and obese) compared to women with normal BMI, Table 5.4. Although there was no significant difference between the two groups on all other indices, mean ferritin was higher in the overweight and obese mothers (13.8 ± 3.2 µg/L) compared to the mothers with normal weight (10.9 ± 2.8 µg/L). At the same time mean TfR was almost the same in the two groups (7.3 ± 1.7 mg/L) and (7.7 ± 1.5 mg/L) in mothers with BMI <25 kg/m^2, and the combined overweight and obese mothers, respectively.

Prevalence of anemia, iron deficiency, and iron deficiency anemia among mothers

As presented above, there was a widespread prevalence of anemia, inflammation, and low iron stores among mothers. Figure 5.2 provides the method that was followed to identify mothers with iron deficiency anemia (IDA), anemia of chronic disease (ACD), iron deficiency (ID), and women with normal indices. At the same time the performance of the three parameters (TfR, TfR/ferritin, and TfR/Log_{10}ferritin) in detecting iron deficiency among these groups was evaluated using receiver operator characteristics (ROC) curves.

Mothers were grouped first into 2 categories: anemic mothers (Hb<12 mg/dL, n=38) and non-anemic mothers (Hb≥12 mg/dL, n= 55). Non-anemic mothers were

further grouped into two groups based on the presence or absence of inflammation. Those non-anemic mothers without inflammation (AGP<1.2 g/L), ferritin ≥ 15 µg/L, and with no abnormal findings in the complete blood count (WBC and RBC count, PCV, MCV, MCH, and MCHC) were considered the control group (n=18). The control group had the following measures (mean ± SD): ferritin: 32.3 ± 1.8 µg/L; TfR, 5.9 ± 1.5 mg/L; TfR/Ferritin, 0.2 ± 1.6; TfR/Log_{10}ferritin, 4.0 ± 1.3; AGP, 0.9 ± 0.2 g/L; and Hb, 13.1 ± 0.6 mg/dL.

The iron deficient group of the non-anemic mothers was determined based on having ferritin < 15 µg/L in presence or absence of inflammation. Thirteen mothers with AGP ≥ 1.2 g/L and nine mothers with AGP <1.2 g/L also had ferritin < 15 µg/L. These two groups were combined together as non-anemic iron deficient mothers (n=22).

Before determining the prevalence of iron deficiency among the remaining 15 non-anemic mothers who had high AGP and adequate ferritin, the performance of three parameters (TfR, TfR/ferritin, and TfR/Log_{10}ferritin) in detecting iron deficiency was evaluated using receiver operator characteristics (ROC) curves among the control group and the iron deficient group. As shown in Figure 5.3, the area under the curve (AUC^{ROC}) was highest for TfR/Ferritin (AUC^{ROC} =0.985, p=.000), followed by TfR/Log_{10}ferritin (AUC^{ROC} =0.856, p=.000), and the least was for TfR (AUC^{ROC} =0.727, p=.016). The sensitivity of TfR >6.7 mg/L, TfR/Ferritin >0.37, and TfR/Log_{10}ferritin >5.5 in the diagnosis of iron deficiency was 72.7%, 95.5% and 81.8%, respectively. The specificity was 76.5%, 94.1%, and 82.4% for TfR, TfR/Ferritin, and TfR/Log_{10}ferritin, respectively.

The TfR/Ferritin showed the highest efficiency in determining iron deficiency with AUC^{ROC} =0.985 and 95% CI= 0.977-1.00.

The optimal TfR/Ferritin cut-off point was determined at 0.37, at this point the ROC curve reached the closest distance (0.006) to the upper left-hand corner (0, 1) of the graph. The likelihood ratio for a positive test (LR+) for this point was 16.2, a high value (LR+ >10 significantly increases the probability of a disease) which means that a mother with iron deficiency is about 16 times more likely to be "ruled in" as having iron deficiency using the TfR/ferritin cut-off point of 0.37 than a mother who is not iron deficient. The same cut-off point had also a very low LR- value <0.1 which "rules out" that the mother is iron deficient. In other words, LR- represents the probability of an iron deficient mother to test negative (falsely identified as iron-sufficient) divided by the probability of an iron-sufficient mother to have a negative test (correctly identified as iron-sufficient). At TfR/ferritin cut-off point of 0.37, a mother with iron deficiency has a probability of .05 or about 1/20 of those without iron deficiency to be detected as not having iron deficiency. In other words, an iron-sufficient mother is 20 times more likely to be detected as iron–sufficient compared to an iron-deficient mother using a TfR/ferritin cut-off point of 0.37 to define deficiency.

The optimal cut-off point of TfR/Ferritin= 0.37 was used to identify iron deficiency in the remaining non-anemic mothers with inflammation (n=15). Only one mother was identified as being iron deficient (TfR/ferritin<.37) while the rest were iron sufficient despite inflammation. An independent sample t-test was used to see if there was any significant difference between the control group (n=18) and the group of

mothers who were identified as iron sufficient in presence of inflammation (n=14), and there was no significant difference in all biochemical indices (ferritin, TfR, TfR/ferritin, iron, and Hb) except of course for AGP which was higher in the group of mothers who were iron sufficient with inflammation. Non-anemic mothers who were identified as iron sufficient were grouped together (n=14+17=31) and were named group 1 (G1): iron-sufficient and not anemic. Non-anemic mothers who were identified as iron deficient were grouped together (n= 22+1=23), and were named G2 (iron deficient and not anemic).

Among anemic mothers, the performance of three parameters (TfR, TfR/ferritin, and TfR/Log_{10}ferritin) in differentiating between iron deficiency anemia (IDA) and anemia of chronic disease (ACD) was evaluated using the receiver operator characteristics (ROC) curves (excluding those women with both ferritin≥ 15 µg/L and AGP≥ 1.2 g/L). As shown in Figure 5.4, the three indicators were significant in identifying iron deficiency among anemic mothers with TfR/ferritin having the highest AUC^{ROC} (.985, p=.000), followed by TfR/Log_{10}ferritin (AUC^{ROC}=.935, p=.000), and then TfR with AUC^{ROC}=.812, p=.005). The sensitivity of TfR >7.3 µg/ml, TfR/Ferritin >0.42, and TfR/Log_{10}ferritin >5.8 in the differentiation between IDA and ACD was 82.8%, 96.6% and 89.7%, respectively. The specificity was 77.8%, 100%, and 88.9% for TfR, TfR/Ferritin, and TfR/Log_{10}ferritin, respectively. Both TfR/ferritin and TfR/Log_{10}ferritin showed high efficiency in differentiating between IDA and ACD, but TfR/ferritin had the highest AUC^{ROC} and the highest specificity and sensitivity were reached at a cut-off point of 0.42. At this cut-point, the TfR/ferritin had a likelihood ratio for a negative test (LR-) of 0.03,

which means that a mother who is not iron deficient is about 33 times more likely to be detected as iron-sufficient than an iron deficient mother using a TfR/ferritin cut-off point of 0.42.

Based on a TfR/ferritin cut-off point of 0.42, anemic mothers with a TfR/ferritin ≥ 0.42 were classified as mothers with iron deficiency anemia (IDA), n=28, and were named group 3 (G3), and anemic mothers with TfR/ferritin < 0.42 were classified as mothers with anemia of chronic disease (ACD), n=10, and were named group 4 (G4). Significant differences in means for all biochemical indices of the four mothers groups are described in Table 5.5.

Prevalence of anemia, iron deficiency, and iron deficiency anemia among children

A similar way of analysis (that was used with mothers) was followed to identify iron status of children. As described (Figure 5.5), sixty-nine children were non-anemic. Those non-anemic children with ferritin ≥ 12 µg/L, AGP <1.2 g/L, and with no abnormal findings in the TfR and the complete blood count were considered the control group (n=36). The control group had the following measures (mean ± SD): ferritin: 22.3 ± 1.5 µg/L; TfR: 4.8 ± 1.4 mg/L; TfR/Ferritin: 0.2 ± 1.8; TfR/Log_{10}ferritin: 3.8 ± 1.4; AGP: 0.8 ± 0.2 g/L; Hb: 12.1 ± 0.6 mg/dL; and zinc: 65.5 ± 9.2 µg/dL. Of the non-anemic children only eight children had low ferritin (<12 µg/L).

The performance of three parameters (TfR, TfR/ferritin, and TfR/Log_{10}ferritin) in detecting iron deficiency was evaluated using receiver operator characteristics (ROC) curves among the control group and the iron deficient group. As shown in Figure 5.6,

the area under the curve (AUC^{ROC}) was highest for TfR/Ferritin (AUC^{ROC} =0.986, 95%CI=0.947-1.0, p=.000). The TfR/ Log_{10}ferritin was also significant (p<.005) with AUC^{ROC} (95%CI)= 0.907(0.750-1.0), while transferrin receptor was not significant with AUC^{ROC} (95%CI)= 0.667(0.453-0.880). The sensitivity of TfR/Ferritin >0.45, and TfR/Log_{10}ferritin >5.9 in the diagnosis of iron deficiency was 100% and 75.0%, respectively. The specificity was 91.7% and 94.4% for TfR/Ferritin, and TfR/Log_{10}ferritin, respectively.

The optimal TfR/Ferritin cut-off point of 0.45 had the closest distance (0.007) to the upper left-hand corner (0, 1) of the ROC graph. The likelihood ratio for a positive test (LR+) for this point was 12.0, a high value (LR+ >10) which means that an iron-deficient child is about 12 times more likely to be "ruled in" as having iron deficiency using the TfR/ferritin cut-off point of 0.45 than a child who is not iron deficient. Since sensitivity determined at this cut-off point equals one, LR- also will be zero (LR-= 1-sesntivity/specificity). A sensitivity of one or LR- of zero means that there is no probability of a child with iron deficiency to be identified as iron sufficient using TfR/ferritin>0.45 as a cut-off point to define deficiency. Based on this, non-anemic children were grouped into group 1: iron sufficient and not anemic (n=55), and group 2: iron deficient but not anemic (n=13).

Among anemic children, the performance of three parameters (TfR, TfR/ferritin, and TfR/Log_{10}ferritin) in differentiating between iron deficiency anemia (IDA) and anemia of chronic disease (ACD) was evaluated using the receiver operator characteristics (ROC) curves (excluding those children with both ferritin≥ 12 µg/L and

AGP≥ 1.2 g/L). As shown in Figure 5.7, only TfR/ferritin and TfR/ Log_{10}ferritin indicators were significant in identifying iron deficiency among anemic children with TfR/ferritin having the highest area under the curve (AUC^{ROC},95%CI)= (.986, .942-1.0, p=.001), followed by TfR/Log_{10}ferritin (AUC^{ROC}, 95%CI)= (.833,.782-.912, p=.025), while transferrin receptor was not significant with AUC^{ROC} (95%CI) = 0.556(0.227-0.884). The TfR/ferritin showed the highest efficiency in differentiating between IDA and ACD among anemic children, with the highest AUC^{ROC} and highest sensitivity and specificity (1.00 and 91.7, respectively) at a cut-off point of 0.43. At this cut-point, the TfR/ferritin had a high likelihood ratio for a positive test (LR+) =12.0, which means that a child with iron deficiency is about 12 times more likely to be "ruled in" as having iron deficiency using the TfR/ferritin cut-off point of 0.43 than a child who is not iron deficient. The same cut-off point had LR- = 0. Based on the TfR/ferritin cut-off point of 0.43, anemic children were grouped into two groups: group 3 (G3): children with iron deficiency anemia (IDA), n=9; and group 4 (G4): children with anemia of chronic disease (ACD), n=15. Significant differences in means for all biochemical indices of the four groups of children are described in Table 5.6.

Iron Indices and Food consumption patterns of mothers and children

Meat and liver consumption

Approximately half of the women and children had never or rarely consumed liver. Sixty percent of women (n=56) and 54% of children (n=50) reported eating meat at least twice a week. No significant differences were found on all biochemical indices for

mothers based on amount of liver or meat consumed. In children, no significant differences were found on all biochemical indices based on meat consumption, while liver consumption correlated positively with iron (mg/kg), $r=.24$, $p<.05$. A significant difference on iron status was found based on how often the child consumes liver. Those children who consumed liver at least twice a month had higher estimated means ± SD for body iron (4.9 ± 5.4 mg/kg) compared to those children who consumed liver once a month or less (iron= 2.9 ± 2.7 mg/kg) as shown in Table 5.7.

Bread consumption

Eighty-three women reported consumption of bread that was made from fortified flour (fortified with iron and folic acid), only six women reported consumption of bread that was made from whole grain (unfortified), and four women consumed both bread types. Despite having no significant difference among these three groups for ferritin, TfR, TfR/ferritin, zinc, and iron (comparison is difficult due to the small sample size of mothers consuming whole grain and both flour types), mothers consuming the fortified flour had higher mean ferritin, iron, and zinc concentrations and lower TfR and TfR/ferritin than mothers consuming bread made from unfortified flour and mothers consuming bread made from both flour types (Table 5.8). For children, almost all children (94%) were consuming bread that was made from fortified flour, one child was consuming bread made from whole grain (unfortified), and four children were consuming bread made from both flour types.

Mineral supplements

None of the children had taken mineral supplements. Of the 93 women studied, only 16 women reported taking supplements and most of these supplement were either iron alone or multivitamin/mineral supplements that contained iron. Although there was a trend for higher mean plasma ferritin and lower TfR in women taking supplements compared to those not taking supplements, the differences were small and not statistically significant (Table 5.9).

Tea consumption

Eighty-three percent of women (n=77) reported consumption of tea with a mean ± SD of 21.8 ± 16.3 cups per week. Consumption among mothers ranged between 0.5-70.0 cups per week, while consumption for children ranged between 2-35 cups per week for 67 children with mean ± SD of 11.1 ± 8.2 cups per week. Whether tea was consumed with/without meals was also reported for mothers and children. Thirty-one mothers reported consumption of tea with meals, 17 women reported tea consumption between meals, and 28 women reported consumption of tea both with meals and between meals.

Interestingly, among mothers iron indices didn't differ by the amount of tea consumed but it did differ by whether the tea was consumed with or without meals. As shown in Table 5.10, means ± SD of ferritin and iron were significantly lower, and means ± SD of TfR/ferritin and TfR/Log_{10}Ferritin were significantly higher in mothers consuming tea strictly with meals (n=31) compared to the group of mothers who consume tea

between and with meals (n=28). In addition, TfR was significantly higher in the groups of mothers who consume tea strictly with meals (8.8 ± 1.5 mg/L) compared to those who don't consume tea (6.4 ± 1.6 mg/L).

Women's Marital Status

There was a trend for lower means of plasma ferritin, Hb, and zinc in the combined subgroup of widowed (n=17) and separated women (n=2) than in the subgroup of currently married women (n=74). However the differences were not statistically significant (Table 5.11). Perhaps the trend could be explained by the lower discretionary income of widowed/separated women compared to the currently married women. In fact, widowed/separated women had significantly ($p<.005$) lower mean monthly discretionary income ($160) compared to currently married women who have mean monthly discretionary income of $373.

DISCUSSION

The data suggest that there are widespread co-existing micronutrient deficiencies among mothers and to a lesser extent among children based on low values for hemoglobin, ferritin, and zinc. Among mothers, fifty-four percent had ferritin< 15 µg/L, 58% had zinc< 70 µg/dL, 41% had Hb< 12 g/dL, 34% had both low ferritin and low zinc, at least 29% had two or more co-existing micronutrient deficiencies, and about 24% were deficient on the three parameters: Hb, ferritin, and zinc. These results

indicate that micronutrient deficiencies are still a major public health problem for women living in suburban areas of Jordan.

Among children, zinc deficiency was very prevalent with 52 children (56%) below 65 µg/dL. Sixteen percent of children (n=15) had ferritin < 12 µg/L, twenty-six percent (n=24) were anemic, ten percent of children had both low ferritin and low zinc, 14% were both anemic and had low zinc, and only three children were deficient on all three parameters (Hb, ferritin, and zinc). The high prevalence of zinc deficiency among children is striking given that the percentage of stunting among those children is very low. Only two children (2.2%) had <-2 for the height-for-age *z*-scores. This means that the absence of stunting should not be perceived as sufficient evidence for adequate zinc levels among children. The IZiNCG suggests in its second report that the risk of zinc deficiency is of a public health concern when the prevalence of low height-for-age (The percentage of children under 5 years of age with height-for-age z-score less than −2.0 SD with respect to the reference population) is greater than 20%, and in this case nutrition intervention strategies should address zinc status (Hess et al 2009). In our case, stunting was almost absent among study participants but still zinc deficiency is of a high concern. In fact, this emphasized the need to use the three indicators: prevalence of stunting among children under five, biochemical assessment (serum/plasma zinc< cut off points), and the prevalence of inadequate zinc intakes (Gibson et al 2008, Hess et al 2009, IZiNCG 2007a) to ideally reflect zinc status of children.

Another important finding in this study is the high prevalence of inflammation among study participants and especially among women. Nearly half of the mothers

(n=46), and 28% of children (n=26) had AGP>1.2 g/L, despite the fact that when mothers and children were recruited for the study any potential participant with any sign of infection and/or inflammation was excluded, and none of the participants had any sign of infection at time of blood collection. Any participant with high temperature, having a disease at time of blood collection or three days earlier was immediately excluded from participation. Ten women and 15 children were classified as having anemia of chronic disease (ACD). Anemia of chronic disease is the second most prevalent type of anemia after iron deficiency anemia, it is caused by chronic activation of the immune system (Agarwal and Prchal 2009), and it has the characteristic of an adaptive immune response (Zarychanski and Houston 2008). AGP was not correlated with ferritin or iron in mothers, while in children AGP positively correlated with ferritin ($r=0.35$, $p<.000$). Perhaps one potential explanation is that among mothers there was a high prevalence of inflammation, high prevalence of overweight and obesity, and a high prevalence of iron deficiency, so even though ferritin, as an acute phase protein, would be expected to increase in presence of inflammation, it also might decrease due to the high prevalence of obesity (which is also an inflammatory state that will decrease iron absorption as discussed below). In children, with the absence of obesity, high levels of AGP correlated positively with ferritin without the presence of any opposing factor that would decrease ferritin status. One limitation of this study is that C-reactive protein was not measured, and it is possible that a combination of the two measures (AGP and CRP) would have given a better estimation on the prevalence of inflammation in the study population.

The high prevalence of inflammation in mothers could be explained by the fact that those mothers had a high prevalence of overweight (37%, n=34) and obesity (40%, n=37). Literature suggests a link between excess body fat and an increase in pro-inflammatory cytokines, particularly TNF-α (Greenberg and Obin 2006, Hotamisligil et al 1995). The adipose tissue secreted cytokines can drive a state of chronic low-grade inflammation that plays an important role in obesity related disorders (Bekri et al 2006, Nathan 2008). In fact, the data in this study showed that overweight and obese women had significantly higher AGP (mean ± SD= 1.3 ± 0.4 g/L) compared to mothers with normal weight (mean ± SD= 1.1 ± 0.4 g/L).

Many studies have shown that there is a great overlap between overweight and/or obesity and iron deficiency among adults (Yanoff et al 2007), children (Nead et al 2004, Zimmermann et al 2008) and women (Bentley and Griffiths 2003, Eckhardt et al 2008, Tussing-Humphreys et al 2009). In addition to the fact that overweight individuals might have higher iron requirements, hepcidin has been proposed as a potential mediator between increased adiposity and iron deficiency (Miraglia Del Giudice et al 2009, Schulze et al 2008). Hepcidin has been shown to be expressed in the adipose tissue (in addition to the liver). Adipose tissue in obese patients showed increased expression of C-reactive protein and interleukin 6 which in turn were shown to promote hepcidin expression (Bekri et al 2006). In addition to that, it has been found that leptin (production of which increases in overweight and obese people) up-regulates hepatic tissue expression of hepcidin (Chung et al 2007). The hepcidin secreted from both

adipose tissue and liver acts as a negative regulator of intestinal iron absorption and reduces dietary iron absorption (Laftah et al 2004).

The data in our study have shown a significantly higher inflammatory state in obese and overweight mothers compared to mothers with normal weight, but no significant difference was found in ferritin, TfR, TfR/ferritin, and iron between the two groups. Mean ferritin levels tended to be higher in the obese and overweight mothers (13.8 ± 3.2 µg/L) compared to the mothers with normal weight (10.6 ± 2.8 µg/L) although the difference was not significant. Transferrin receptor was very close in both groups. The percentage of women with deficit body iron estimates was also very close, i.e. 45% in mothers with normal weight, and 44% in mothers who are overweight or obese, respectively. Further analysis is needed to understand and explore the relation between the high levels of AGP, the high incidence of obesity, and the low iron status of mothers in this study.

The ratio of TfR/ferritin has been used to quantitatively estimate total body iron (Cook et al 2003). This approach has been used by several investigators (Beard et al 2006b, Cook et al 2005, Iannotti et al 2005, Zimmermann et al 2005), and the Centers for Disease Control and Prevention (CDC) has phased in this approach in NHANES beginning in the years 2003-2004 (Cusick et al 2008). In this study, the mean ± SD body iron for mothers was 0.5 ± 4.9 mg/kg, and 45% of mothers (n=42) had an average iron deficit of 4.0 mg/kg body weight. Children had a mean body iron of 3.5 ± 3.8 mg/kg and 18% of children (n=17) had an average iron deficit of 0.71 mg/kg body weight.

In settings where there is no co-existing inflammation/infection, serum ferritin remains a useful indicator of iron status. However, in case of high prevalence of infection/inflammation, the use of serum ferritin alone is not sufficient to detect iron deficiency. In these settings, TfR is most valuable in assessing iron status since it has the distinct advantage over ferritin of not being affected by the acute phase response (Cook et al 1993). The ratio of TfR/ferritin had been demonstrated to be valuable in assessing iron deficiency. This ratio takes advantage of the increase in TfR and the decrease in ferritin in the case of iron deficiency (Punnonen et al 1997). Some studies found that TfR/Log_{10} ferritin had the highest efficiency in determining the extent of iron deficiency. For example, Punnonen et al (1997) found that while TfR/ferritin improved the diagnostic sensitivity and specificity in detecting iron deficiency compared to TfR or ferritin alone, the TfR/Log ferritin index provided an outstanding indicator of iron depletion (Punnonen et al 1997). Malope et al (2001) found that while TfR/Log ferritin could sensitively reflect iron deficiency, the use of Log (TfR/ferritn) ratio had the highest sensitivity in determining stages of iron deficiency (Malope et al 2001). Lin et al (2008) similarly showed that although both Log (TfR/ferritin) and TfR/Log ferritin could sensitively reflect stages of iron deficiency, the use of Log (TfR/ferritin) provided the highest sensitivity in determining iron deficiency (Lin et al 2008). Another study recommended both TfR/ferritin and LogTfR/ferritin for the discrimination of iron deficiency in the absence of anemia (Vazquez Lopez et al 2006). On the other hand, there are some studies that didn't find any improvement in accuracy in detecting iron

deficiency by the use of TfR/Log ferritin over ferritin (Lewis et al 2007), TfR alone (Markovic et al 2005), or both ferritin and TfR (Lee et al 2002).

This study has shown that both TfR/ferritin and TfR/Log_{10}ferritin could sensitively detect iron deficiency and could serve as reliable indices for determining the extent of iron deficiency in women of child-bearing age and preschool children. TfR/ferritin had higher diagnostic efficiency with the highest area under the curve (which reached 99%) and the highest sensitivity and specificity at the determined cut-off point in both mothers and children. This study didn't aim to specify cut-off points for TfR/ferritin or TfR/Log ferritin to identify iron deficiency, instead it aimed to examine whether the use of TfR/ferritin or TfR/Log ferritin can improve the diagnosis of iron deficiency, especially in presence of high rates of inflammation. In fact, the different studies that examined the use of TfR/ferritin or TfR/log ferritin had determined the highest efficiency of either or both ratios at different cut off points. This is may be due to two reasons: first, until now there are no universally agreed reference values for TfR, and cut-off values vary according to the assay used. Secondly, the ROC analysis is complex with no single analytic approach that can be applied for all studies. The area under the curve of a diagnostic test produced from a group of participants doesn't have a fixed value, and if the same diagnostic test was repeated on a different group of participants with similar characteristics, the AUC produced might be different (Hanley and McNeil 1983). Therefore, the performance of the diagnostic test should be judged within the context of the study. A second important aspect for a valid use of ROC curves in biomedical analysis is to have an accurate reference standard. In the case of defining iron deficiency

the use of the gold standard (the absence of stainable iron in the reticuloendothelial cells in the bone marrow) to define the reference groups is not always feasible, and only few studies have used stained bone marrow aspirates to determine iron deficient and iron sufficient groups. A third factor related to ROC curves is determining the optimal cut-off point of a diagnostic test. A perfect diagnostic test would have 100% sensitivity and 100% specificity with an area under the curve of one. Unfortunately, this is not usually the case. The researcher determines the optimal cut-off point based on a trade-off between sensitivity and specificity over a continuous range of points.

Given the impracticality of using the gold standard for the evaluation of iron status in the field setting, the study relied on normal values for ferritin, Hb, TfR, and blood indices in absence of inflammation to establish the control group. In fact, the Centers for Disease Control and Prevention (CDC) has traditionally relied upon the use of ferritin, transferrin saturation, erythrocyte protoporphyrin, and complete blood count in assessing iron status in NHANES. However, when the CDC phased in the use of the TfR/ferritin ratio based (Cook et al 2003) model beginning in 2003-2004, it also phased out the use of transferrin saturation and erythrocyte protoporphyrin after the 2005-2006 cycle (Cusick et al 2008).

There is a need for larger studies to further evaluate the TfR/ferritin and reach consensus on definitive age- specific cut-off values or reference ranges that can be applied to population studies.

REFERENCES

Agarwal, N. and J. T. Prchal (2009). "Anemia of chronic disease (anemia of inflammation)." Acta Haematol **122**(2-3): 103-8.

Albsoul-Younes AM, Al-Ramahi RJ, Al-Safi SA (2004). Frequency of anemia in pregnancy in Northern Jordan. *Saudi Medical Journal* **25:** 1525-1527.

Beard JL, Murray-Kolb LE, Rosales FJ, Solomons NW, Angelilli ML (2006). Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: the role of biomarkers for the acute phase response. *Am J Clin Nutr* **84:** 1498-1505.

Bekri S, Gual P, Anty R, Luciani N, Dahman M, Ramesh B *et al* (2006). Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* **131:** 788-796.

Bentley ME, Griffiths PL (2003). The burden of anemia among women in India. *Eur J Clin Nutr* **57:** 52-60.

Bothwell TH, Charlton RW, Cook JD, Finch CA (1979). *Iron Metabolism in Man*. Blackwell Scientific Publications: Oxford.

Brown KH, Wuehler SE. Zinc and Human Health: the Results of Recent Trials and Implications for Program Interventions and Research. Ottawa, Ontario, Canada, Micronutrient Initiative, 2000.

Caulfield L, Black RE (2004). *Zinc Deficiency. Comparative Quantification of Health Risks: The Global and Regional Burden of Disease due to 25 Selected Major Risk Factors.* World Health Organization/Harvard University Press: Cambridge, MA.

Caulfield LE, Richard SA, Rivera JA, Musgrove P, Black RE (2006). Stunting, Wasting, and Micronutrient Deficiency Disorders In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB *et al* (eds). *Disease Control Priorities in Developing Countries*, 2 edn. Oxford University Press New York. pp 552-553.

Chung B, Matak P, McKie AT, Sharp P (2007). Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J Nutr* **137:** 2366-2370.

Cook JD, Lipschitz DA, Miles LE, Finch CA (1974). Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* **27:** 681-687.

Cook JD, Skikne BS, Baynes RD (1993). Serum transferrin receptor. *Annu Rev Med* **44:** 63-74.

Cook JD, Flowers CH, Skikne BS (2003). The quantitative assessment of body iron. *Blood* **101:** 3359-3364.

Cook JD, Boy E, Flowers C, Daroca Mdel C (2005). The influence of high-altitude living on body iron. *Blood* **106:** 1441-1446.

Cusick SE, Looker AC, Cogswell ME, Pfeiffer CM, Grummer-Strawn L (2008). Iron-status indicators. *Pediatrics* **121:** 651-652; author reply 652.

de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C (2007). Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. *Food Nutr Bull* **28:** S480-484.

Eckhardt CL, Torheim LE, Monterrubio E, Barquera S, Ruel MT (2008). The overlap of overweight and anaemia among women in three countries undergoing the nutrition transition. *Eur J Clin Nutr* **62:** 238-246.

Gibson RS, Hess SY, Hotz C, Brown KH (2008). Indicators of zinc status at the population level: a review of the evidence. *Br J Nutr* **99 Suppl 3:** S14-23.

Greenberg AS, Obin MS (2006). Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr* **83:** 461S-465S.

Hanley JA, McNeil BJ (1983). A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* **148:** 839-843.

Hess SY, Lonnerdal B, Hotz C, Rivera JA, Brown KH (2009). Recent advances in knowledge of zinc nutrition and human health. *Food Nutr Bull* **30:** S5-11.

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* **95:** 2409-2415.

Iannotti LL, O'Brien KO, Chang SC, Mancini J, Schulman-Nathanson M, Liu S *et al* (2005). Iron deficiency anemia and depleted body iron reserves are prevalent among pregnant African-American adolescents. *J Nutr* **135:** 2572-2577.

IZiNCG. Techincal Brief No.2: Assessing population zinc status with serum zinc concentration 2007.

Laftah AH, Ramesh B, Simpson RJ, Solanky N, Bahram S, Schumann K *et al* (2004). Effect of hepcidin on intestinal iron absorption in mice. *Blood* **103:** 3940-3944.

Lee EJ, Oh EJ, Park YJ, Lee HK, Kim BK (2002). Soluble transferrin receptor (sTfR), ferritin, and sTfR/log ferritin index in anemic patients with nonhematologic malignancy and chronic inflammation. *Clin Chem* **48:** 1118-1121.

Lewis DK, Whitty CJ, Epino H, Letsky EA, Mukiibi JM, van den Broek NR (2007). Interpreting tests for iron deficiency among adults in a high HIV prevalence African setting: routine tests may lead to misdiagnosis. *Trans R Soc Trop Med Hyg* **101:** 613-617.

Lin XM, Zhang J, Zou ZY, Long Z, Tian W (2008). Evaluation of serum transferrin receptor for iron deficiency in women of child-bearing age. *Br J Nutr* **100:** 1104-1108.

Malope BI, MacPhail AP, Alberts M, Hiss DC (2001). The ratio of serum transferrin receptor and serum ferritin in the diagnosis of iron status. *Br J Haematol* **115:** 84-89.

Markovic M, Majkic-Singh N, Subota V (2005). Usefulness of soluble transferrin receptor and ferritin in iron deficiency and chronic disease. *Scand J Clin Lab Invest* **65:** 571-576.

Mawajdeh SM, Al-Qutob R, Schmidt A (2003). Measuring reproductive morbidity: a community-based approach, Jordan. *Health Care For Women International* **24:** 635-649.

Ministry of Health. Food and Nutrition Policy Report. “Toward a Comprehensive Food and Nutrition Policy in Jordan”. A Preliminary Report on a Collaborative Project with the Ministries of Health and Agriculture. . Amman, Jordan, 2003.

Miraglia Del Giudice E, Santoro N, Amato A, Brienza C, Calabro P, Wiegerinck ET *et al* (2009). Hepcidin in Obese Children as a Potential Mediator of the Association between Obesity and Iron Deficiency. *J Clin Endocrinol Metab*.

Nathan C (2008). Epidemic inflammation: pondering obesity. *Mol Med* **14:** 485-492.

Nead KG, Halterman JS, Kaczorowski JM, Auinger P, Weitzman M (2004). Overweight children and adolescents: a risk group for iron deficiency. *Pediatrics* **114:** 104-108.

Paracha PI, Jamil A, Northrop-Clewes CA, Thurnham DI (2000). Interpretation of vitamin A status in apparently healthy Pakistani children by using markers of subclinical infection. *Am J Clin Nutr* **72:** 1164-1169.

Prasad AS, Halsted JA, Nadimi M (1961). Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *Am J Med* **31:** 532-546.

Prasad AS, Miale A, Jr., Farid Z, Sandstead HH, Schulert AR (1963). Clinical and experimental. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. . *J Lab Clin Med* **116:** 737-749.

Punnonen K, Irjala K, Rajamaki A (1997). Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood* **89:** 1052-1057.

Sandstead HH (2000). Causes of iron and zinc deficiencies and their effects on brain. *J Nutr* **130:** 347S-349S.

Schulze KJ, Christian P, Ruczinski I, Ray AL, Nath A, Wu LS *et al* (2008). Hepcidin and iron status among pregnant women in Bangladesh. *Asia Pac J Clin Nutr* **17:** 451-456.

Skikne BS, Flowers CH, Cook JD (1990). Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* **75:** 1870-1876.

Swets JA (1988). Measuring the accuracy of diagnostic systems. *Science* **240:** 1285-1293.

Tussing-Humphreys LM, Liang H, Nemeth E, Freels S, Braunschweig CA (2009). Excess adiposity, inflammation, and iron-deficiency in female adolescents. *J Am Diet Assoc* **109:** 297-302.

Vazquez Lopez MA, Carracedo A, Lendinez F, Munoz FJ, Lopez J, Munoz A (2006). The usefulness of serum transferrin receptor for discriminating iron deficiency without anemia in children. *Haematologica* **91:** 264-265.

WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO, 2009 (http://www.who.int/growthref/tools/en/).

WHO. The World Health Report 2002: Reducing risks, promoting healthy life. Geneva, 2002.

WHO. Worldwide prevalence of anaemia 1993-2005: WHO Global Database on Anaemia/ Edited by Bruno de Benoist, Erin McLean, Ines Egli and Mary Cogswell., 2008.

WHO/UNICEF/UNU. Iron Deficiency Anaemia: Assessment, Prevention and Control. A guide for programme managers. Geneva, World Health Organization; UNICEF; United Nations University, 2001.

Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal (2002). Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. *J Nutr* **132:** 3061-3066.

Yanoff LB, Menzie CM, Denkinger B, Sebring NG, McHugh T, Remaley AT *et al* (2007). Inflammation and iron deficiency in the hypoferremia of obesity. *Int J Obes (Lond)* **31:** 1412-1419.

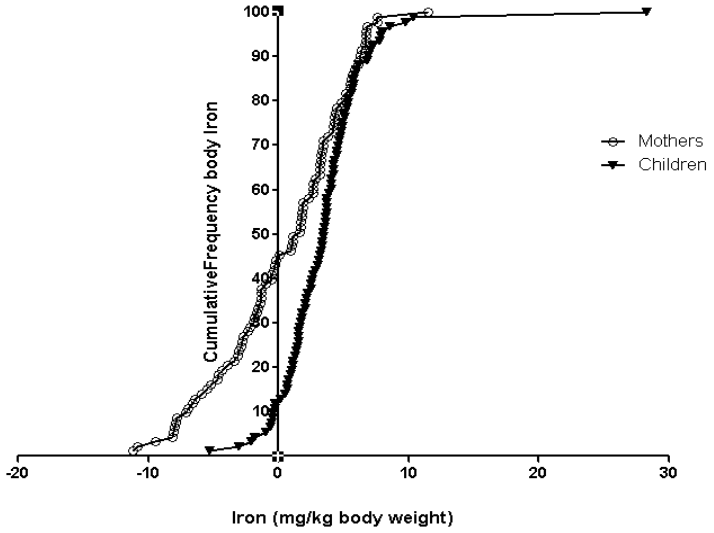
Zarychanski, R. and D. S. Houston (2008). "Anemia of chronic disease: a harmful disorder or an adaptive, beneficial response?" CMAJ **179**(4): 333-7.

Zimmermann MB, Chaouki N, Hurrell RF (2005). Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. *The American Journal Of Clinical Nutrition* **81:** 115-121.

Zimmermann MB, Zeder C, Muthayya S, Winichagoon P, Chaouki N, Aeberli I *et al* (2008). Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. *Int J Obes (Lond)* **32:** 1098-1104.

Figure 5.1

The cumulative frequency of body iron in mothers and children



Body iron (mg/kg) for mothers and children calculated using cook and colleagues (2003) approach based on the following equation: Body Iron (mg/kg) = [log (TfR/ferritin ratio)-2.8229]/0.1207.

Figure 5.2

Schematic diagram of mothers' groups based on Hb, AGP, and iron status

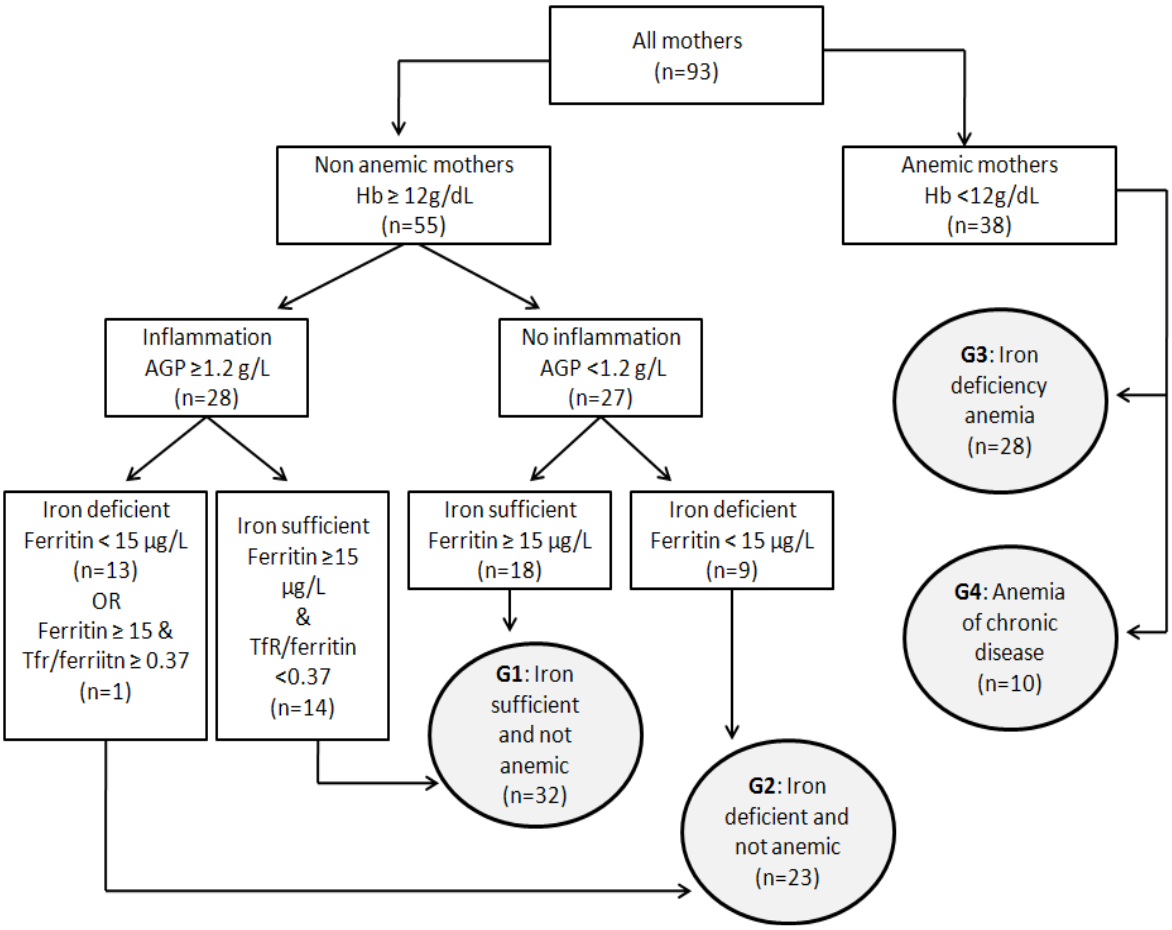
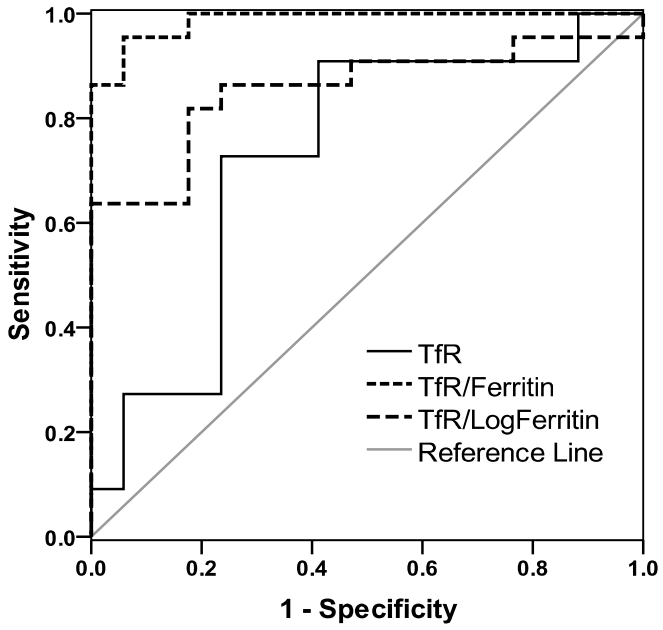


Figure 5.3

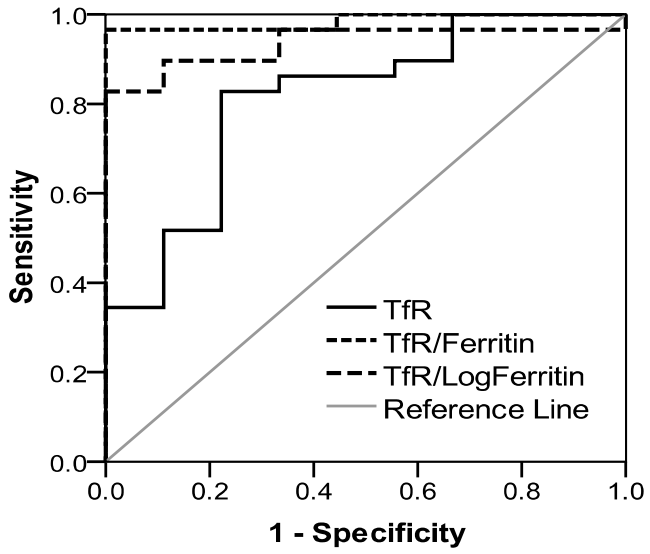
ROC curves for TfR, TfR/ferritin, and TfR/Log_{10} ferritin identifying iron deficiency in non-anemic mothers



	AUC^{ROC} (SE)	Sig	Sensitivity	Specificity	LR+	LR-
TfR	.727 (.09)	.016	72.7	76.5	3.1	.36
TfR/ferritin	.985 (.01)	.000	95.5	94.1	16.2	.05
TfR/Log_{10}ferritin	.856 (.06)	.000	81.8	82.4	4.6	.22

Figure 5.4

ROC curves for TfR, TfR/ferritin, and TfR/log_{10} ferritin identifying iron deficiency in anemic mothers



	AUC (SE)	Sig	sensitivity	specificity	LR+	LR-
TfR	.812 (.08)	.005	82.8	77.8	3.7	.22
TfR/Ferritin	.985 (.02)	.000	96.6	100	-	.03
TfR/Log_{10}ferritin	.935 (.04)	.000	89.7	88.9	8.1	.12

Figure 5.5

Schematic diagram of children' groups based on Hb, AGP, and iron status

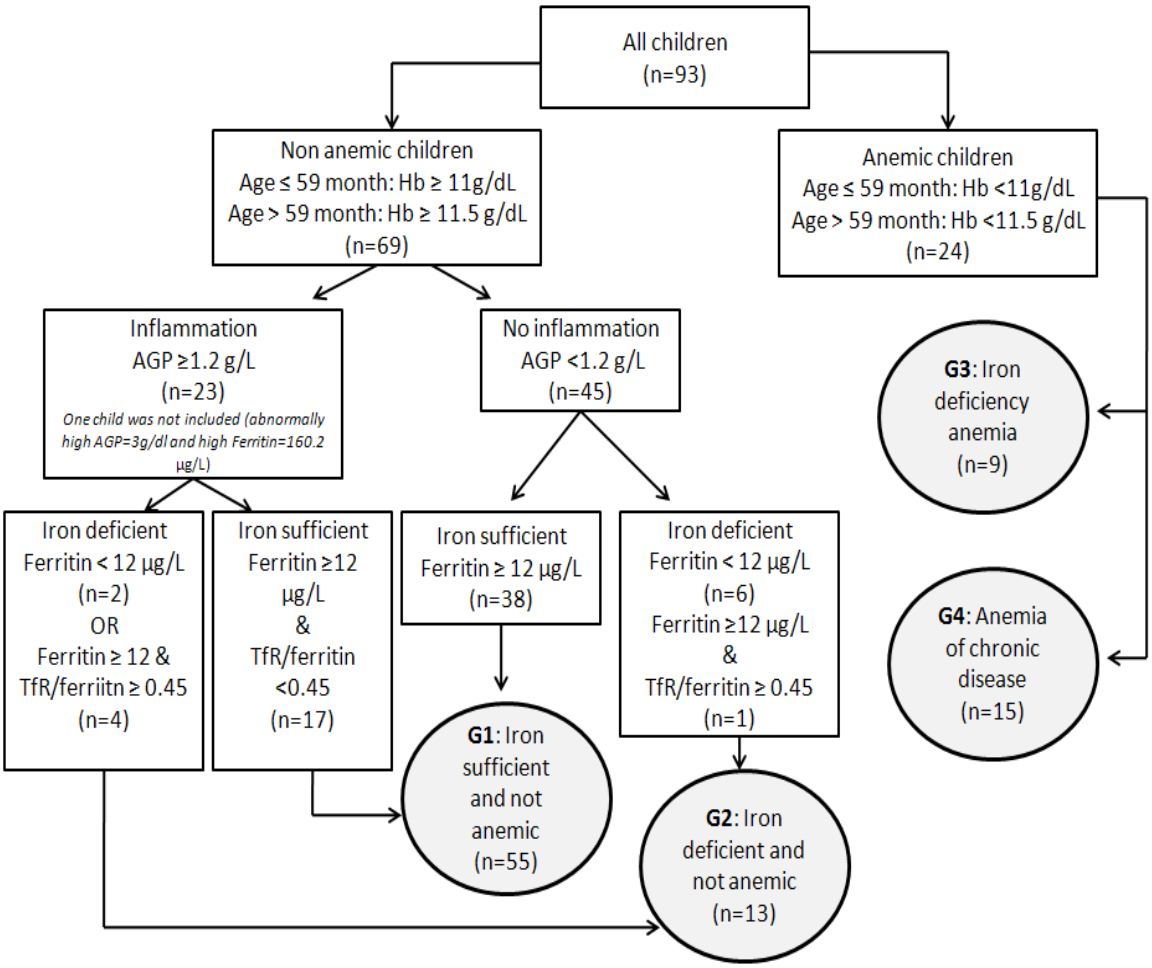
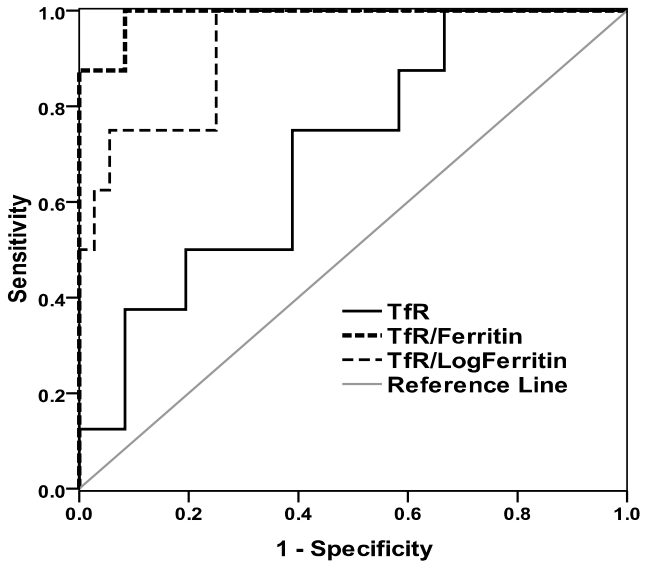


Figure 5.6

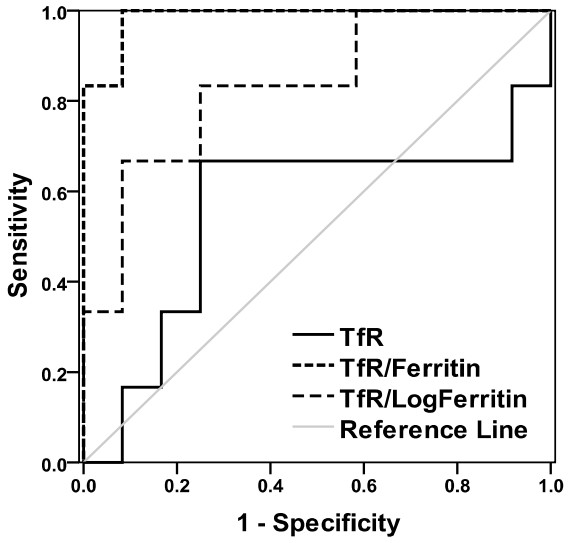
ROC curves for TfR, TfR/Ferritin, and TfR/Log_{10}ferritin identifying iron deficiency in non-anemic children



	AUC (SE)	Sig	sensitivity	specificity	LR+	LR-
TfR	.667 (.11)	.196	75.0	61.1	1.9	.41
TfR/Ferritin	.986 (.02)	.000	100	91.7	12.0	-
TfR/Log_{10}ferritin	.907 (.06)	.002	75.0	94.4	13.4	.26

Figure 5.7

ROC curves for TfR, TfR/Ferritin, and TfR/log_{10}ferritin identifying iron deficiency in anemic children



	AUC (SE)	Sig	sensitivity	specificity	LR+	LR-
TfR	.556 (.17)	.708	66.7	75.0	2.7	.44
TfR/Ferritin	.986 (.02)	.001	100	91.7	12.0	-
TfR/Log_{10}ferritin	.833 (.10)	.025	83.3	75.0	3.3	.22

Table 5.1

Characteristics of the study participants

	N	Mean ± SD	Median	Percentile	
				25th	***75th***
Mothers					
Age (years)	93	33.9 ±5.9	35.0	29.0	38.0
BMI (kg/m^2)	93	29.6 ±5.6	29.0	25.2	33.5
Number of pregnancies	93	4.8 ±2.5	4.0	3.0	6.0
Number of children	93	4.1 ±1.9	4.0	3.0	5.0
Income (USD/month)*	92	325 ±272	254	174	353
Years of schooling	92	10.7 ±2.3	12.0	9.0	12.0
Children					
Age (months)	93	60.7 ±5.4	61.0	55.0	65.0
Weight- for-age (z)	93	-0.0 ±1.1	0.1	-0.6	0.6
Height–for-age (z)	93	0.5 ±1.20	0.6	-0.3	1.4
Child MUAC** (cm)	93	16.7 ±1.2	16.7	16.0	17.5

*Income is defined as total monthly family income after rent deduction in US $.

**MAUC: Mid-upper arm circumference.

Table 5.2
Means, SD, and percentiles of various biochemical indices in mothers and children

		Ferritin (µg/L)	**TfR** (mg/L)	**Zinc** (mg/L)	**AGP** (g/L)	**TfR/ Ferritin**	**Hb** (g/dL)
Mothers	Mean	13.1	7.6	67.2	1.2	0.6	12.2
	S.D.	3.1	1.5	11.4	0.4	3.9	0.99
	P25	*5.6*	*5.6*	*57.8*	*1.0*	*0.2*	*11.6*
	P50	*14.0*	*7.8*	*67.8*	*1.2*	*0.4*	*12.2*
	P75	*31.8*	*10.3*	*73.1*	*1.5*	*1.5*	*13.0*
	Prevalence (%)	<15 µg/L = 53.8	≥8.5mg/L =44.4	<70mg/L =58.1	≥1.2 g/L =53.8		<12 g/dL = 40.9
Children	Mean	21.9	5.92	64.6	1.0	0.3	11.7
	S.D.	2.0	1.4	9.4	1.5	2.1	1.1
	P25	*14.6*	*4.8*	*58.1*	*0.8*	*0.2*	*11.3*
	P50	*21.9*	*6.2*	*63.2*	*1.0*	*0.3*	*11.6*
	P75	*31.3*	*7.3*	*70.1*	*1.3*	*0.4*	*12.2*
	Prevalence (%)	<12 µg/L =16.1	≥8.5mg/L =14.0	<65mg/L =55.9	≥1.2 g/L =30.0		<11 g/dL= 10.3 (age≤ 59m) <11.5 g/dL= 37.0 (age> 59m)
Mean comparison (mothersVchildren)		p <.005	p <.000	N.S.	p <.000	p <.000	p <0.005

Table 5.3

Correlations between biochemical indices in mothers and children

		Children *(n=93)*					**Mothers** *(n=93)*			
		Ft	**TfR**	**AGP**	**Zn**	**Hb**	**Ft**	**TfR**	**AGP**	**Zn**
Children	**Ft**									
	TfR	.10								
	AGP	**.35**[†]	.17							
	Zn	.08	-.02	-.17						
	Hb	.12	**-.21**[‡]	-.02	.14					
Mothers	**Ft**	.14	.06	-.12	.03	-.01				
	TfR	.04	**.38**[†]	**.26**[†]	-.02	.01	**-.38***			
	AGP	-.04	.02	.-.01	.04	.12	.004	.15		
	Zn	.15	-.07	-.12	**.33**[†]	.07	.14	-.04	.13	
	Hb	.06	-.16	-.03	-.02	-.06	**.51***	**-.32**[†]	.01	**.30**[†]

*Sig. at P<.000, [†]Sig. at p<.005, and, [‡] Sig. at p<.05

Table 5.4

Various biochemical indices in mothers according to BMI

	BMI <25.0 kg/m^2 *(n=21)* Mean ± SD	BMI≥25.0 kg/m^2 *(n=72)* Mean ± SD
BMI (kg/m^2)	23.0 ± 1.1[b]	31.6 ± 1.2 [a]
Ferritin (µg/L)	10.9 ± 2.8	13.8 ± 3.2
TfR (mg/L)	7.3 ± 1.7	7.7 ± 1.5
TfR/Ferritin	0.7 ± 4.1	0.6 ± 3.9
Zinc (mg/L)	68.6 ± 11.4	67.0 ± 11.4
AGP (g/L)	1.1 ± 0.4[c]	1.3 ± 0.4[d]
Hb (g/dL)	12.2 ± 0.9	12.2 ± 1.0

[a,b] Sig. at p<.000 ; [c,d] Sig. at p<.05

Table 5.5
Differences in biochemical indices among mothers' iron status groups

	G1: iron sufficient, not anemic *(n=32)*	**G2:** iron deficient, not anemic (ID) *(n=23)*	**G3:** iron deficiency anemia (IDA) *(n=28)*	**G4:** anemia of chronic disease (ACD) *(n=10)*	
	Mean ± SD	Mean ± SD	Mean ±SD	Mean ±SD	
Ferritin (µg/L)	34.3 ±1.7	7.8 ± 2.2	4.5 ±2.2	37.1 ±1.8	G1 v G2, p<.000 G1 v G3, p<.000 G2 v G4, p<.000 G3 v G4, p<.000
TfR (mg/L)	6.2 ± 1.5	8.1 ± 1.5	9.8 ± 1.4	6.0 ±1.5	G1 v G3, p<.000 G3 v G4, p<.01
TfR/Ft	0.2 ± 1.6	1.0 ± 2.6	2.2 ± 2.5	0.2 ± 2.2	G1 v G2, p<.000 G1 v G3, p<.000 G2 v G4, p<.000 G3 v G4, p<.000
TfR/Log$_{10}$Ferritin	4.3 ± 1.4	8.3 ± 12.8	16.4 ± 2.2	3.9 ±1.6	G1 v G3, p<.01
AGP (g/L)	1.2 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	1.3 ± 0.4	
Zinc (mg/L)	70.0 ± 11.5	67.3 ± 10.7	63.5 ± 8.5	68.5 ±17.8	
Hb (g/dL)	13.1 ±0.6	12.6 ±0.5	11.2 ±0.6	11.5 ± 0.3	G1 v G3, p<.000 G1 v G4, p<.000 G2 v G3, p<.000 G2 v G4, p<.000
BMI (kg/m^2)	29.0 ± 1.2	28.6 ± 1.2	28.9 ± 1.2	31.0 ± 1.2	
Iron (mg/kg)	Iron Surplus 4.7 ± 1.7	Iron Deficit 1.6 ± 3.4	Iron Deficit 4.2 ± 3.3	Iron Surplus 5.1 ± 2.8	G1 v G2, p<.000 G1 v G3, p<.000 G2 v G3, p<.05 G2 v G4, p<.000 G3 v G4, p<.000

Table 5.6
Differences in biochemical indices among children's iron status groups

	G1: Iron sufficient, not anemic *(n=55)* Mean ± SD	**G2:** Iron deficient, not anemic (ID) *(n=13)* Mean ± SD	**G3:** Iron deficiency anemia (IDA) *(n=9)* Mean ± SD	**G4:** anemia of chronic disease (ACD) *(n=15)* Mean ± SD	
Ferritin (μg/L)	27.2 ± 1.7	11.3 ± 2.3	10.0 ± 1.2	24.7 ± 1.4	G1 v G2, p<.000 G1 v G3, p<.000 G2 v G4, p<.05 G3 v G4, p<.05
TfR (mg/L)	5.3 ± 1.4	7.5 ± 1.5	7.2 ± 1.4	6.4 ± 1.4	G1 v G2, p<.05
TfR/Ft	0.2 ± 1.8	0.7 ± 1.8	0.7 ± 1.4	0.3 ± 1.5	G1 v G2, p<.000 G1 v G3, p<.000 G2 v G4, p<.000 G3 v G4, p<.005
TfR/Log$_{10}$Ferritin	3.9 ± 1.3	8.6 ±4.7	7.5 ±2.2	4.8 ±1.4	G1 v G2, p<.000 G1 v G3, p<.000 G2 v G4, p<.000 G3 v G4, p<.05
AGP (g/L)	1.0 ± 1.6	1.0 ± 1.4	1.1 ± 1.3	1.0 ± 1.4	
Zinc (mg/L)	65.2 ± 9.0	63.3 ± 8.6	64.4 ± 11	63.9 ± 12	
Hb (g/dL)	12.1 ± 1.1	12.0 ± 1.0	10.7 ± 1.1	11.0 ± 1.0	G1 v G3, p<.000 G1 v G4, p<.000 G2 v G3, p<.000 G2 v G4, p<.000
Iron (mg/kg)	Iron Surplus 4.9 ± 3.8	Iron Surplus .0008 ± 2.1	Iron Deficit 0.29 ± 1.3	Iron Surplus 3.4 ±1.3	G1 v G2, p<.000 G1 v G3, p<.005 G2 v G4, p<.000

Table 5.7

Differences in means ± SD of iron indices according to liver consumption among children

	Eat liver at least twice a month (n=27)	Eat liver once a month or less (n=66)
	Mean ±SD	Mean ±SD
Ferritin (µg/L)	25.5 ± 2.1	20.5 ± 2.1
TfR (mg/L)	5.6 ± 1.5	6.1 ± 1.4
TfR/Ft	0.2 ± 2.2	0.3 ± 2.1
TfR/Log_{10}Ferritin	4.4 ± 1.7	5.4 ± 3.1
AGP (g/L)	1.0 ± 1.6	1.0 ± 1.5
Zinc (mg/L)	65.2 ± 10.3	64.3 ± 9.1
Hb (g/dL)	11.6 ± 1.1	11.8 ± 1.1
Iron (mg/kg)	4.9 ± 5.4 [a]	2.9 ± 2.7 [b]

[a,b] sig at p<.05

Table 5.8

Means ± SD of iron indices in mothers consuming bread made from different flour types

	Unfortified whole grain (n=6)	Fortified white flour (n=83)	Both flour types (n=4)
	Mean ± SD	Mean ± SD	Mean ± SD
Ferritin (μg/L)	6.4 ±2.9	14.0 ±3.1	9.0 ±3.1
TfR (mg/L)	8.7 ±1.4	7.5 ±1.5	9.2 ±1.9
TfR/Ft	1.4 ±3.2	0.5 ±3.9	1.0 ±5.2
AGP (g/L)	1.2 ±0.4	1.3 ± 0.4	1.1 ±0.5
Zinc (mg/L)	64.6 ±13.8	67.6 ±11.5	63.7 ±8.4
Hb (g/dL)	11.0 ±1.0[b]	12.3 ±0.9[a]	12.3 ±1.0
Iron (mg/kg)	-2.6 ±4.2	0.8 ±4.8	-1.5 ±6.0

[a,b] sig at p<.005

Table 5.9

Means ± SD of iron indices in mothers using and not using supplements

	Mothers taking supplements (n=16)	Mothers not taking supplements (n=77)
	Mean ± SD	Mean ± SD
Ferritin (μg/L)	15.4 ± 2.8	12.6 ± 3.2
TfR (mg/L)	7.2 ± 1.5	7.7 ± 1.5
TfR/Ft	0.4 ± 3.4	0.6 ± 4.0
TfR/Log_{10}Ferritin	8.9 ± 8.6	10.8 ± 20.3
AGP (g/L)	1.2 ± 0.5	1.3 ± 0.4
Zinc (mg/L)	65.6 ± 13.3	67.5 ± 11.1
Hb (g/dL)	11.8 ± 1.0	12.3 ± 1.0
Iron (mg/kg)	1.3 ± 4.4	0.3 ± 5.0

Table 5.10

Differences in means ± SD of iron indices according to tea consumption among mothers

	Strictly with meals (n=31)	Between meals (n=17)	Both: between and with meals (n=28)	Don't drink tea (n=17)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Ferritin (μg/L)	9.5 ± 2.9[b]	13.8 ± 3.8	17.6 ± 3.1[a]	13.4 ± 2.8
TfR (mg/L)	8.8 ± 1.5 [a]	7.6 ± 1.5	7.0 ± 1.5	6.4 ± 1.6 [b]
TfR/Ft	0.9 ± 3.8[a]	0.6 ± 4.6	0.4 ± 3.5 [b]	0.5 ± 3.6
TfR/Log_{10}Ferritin	17.5 ±27.7 [a]	7.4 ± 16.9	6.2 ± 8.9 [b]	7.8 ± 6.0
AGP (g/L)	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.4	1.1 ± 0.4
Zinc (mg/L)	66.4 ± 9.6	69.7 ± 13.3	66.1 ± 11.1	68.3 ± 13.2
Hb (g/dL)	11.9 ± 0.9	12.4 ± 1.1	12.4 ± 0.9	12.3 ± 1.1
Iron (mg/kg)	-1.2 ± 4.8 [b]	0.7 ± 5.5	1.8 ± 4.5 [a]	1.2 ± 4.6
Tea consumed (cups)	14.6 **±** 9.4	16.6 **±** 18.7	32.5 **±** 15.4	-

[a,b] sig at p<.05

Table 5.11

Differences in means ± SD of iron indices among currently married women and widowed/separated women

	Currently married women (n=74)	Widowed and separated women (n=19)
	Mean ± SD	Mean ± SD
Ferritin (μg/L)	14.5 ± 3.1	8.8 ± 3.0
TfR (mg/L)	7.3 ± 1.5 [b]	9.1 ± 1.5 [a]
TfR/Ft	0.5 ± 3.9[b]	1.0 ± 3.4[a]
TfR/Log_{10}Ferritin	10.3 ± 20	11.4 ± 13.9
AGP (g/L)	1.2 ± 0.4	1.3 ± 0.4
Zinc (mg/L)	67.7 ± 11.9	65.4 ± 9.4
Hb (g/dL)	12.3 ± 1.0	11.9 ± 0.8
Iron (mg/kg)	1.0 ± 4.9[a]	-1.6 ± 4.4 [b]

[a,b] sig at p<.05

CHAPTER VI

ASSESSMENT OF IRON AND ZINC INDICATORS AND COGNITIVE FUNCTIONS OF PRESCHOOL CHILDREN IN JORDAN

ABSTRACT

The association between iron and zinc deficiency and children's performance on cognitive tests was assessed in Jordan in July 2007. Hb and plasma ferritin, transferrin receptor (TfR), zinc, α-1- acid glycoprotein (AGP) were determined in 93 preschool children. In addition, tests to assess the non verbal cognitive functions (PPVT-IV and K-ABC-II) were administered to the children, and their relation to the children's iron status, nutritional status and socioeconomic status of the children and their mothers were analyzed. Mean age ± SD for children was 60.7 ± 5.4 months. Mean concentrations of plasma ferritin, TfR, AGP, and Hb were 21.9 ± 2.0 µg/L; 5.9 ± 1.4 mg/L; 1.0 ± 1.5 g/L; and 11.7 ± 1.1 g/dL, respectively. Number (%) of children who had ferritin concentrations <12 µg/L and TfR concentrations ≥ 8.5 µ/mL were 15 (16.1%) and 13 (14%), respectively, and 28 children had AGP> 1.2 g/L. Mean plasma zinc was 64.6 ± 9.4 µg/dL, 53 children (56%) had plasma zinc < 65.0 µg/dL, and 75% had plasma zinc <70.1 µg/dl. The analysis of results of cognitive tests performed by children showed no significant difference between girls and boys on all dimensions of the tests. Neither

ferritin nor zinc predicted child performance on the tests, except for the number recall subtest. Children who had plasma zinc ≥57.0 μg/dL had significantly higher scores on the number recall subtest (9.9 ± 2.3) compared to children who had plasma zinc< 57.0 μg/dL (8.5 ± 2.4). Children's scores on all cognitive tests were not different among groups of children with different iron status, but socioeconomic variables predicted many of the cognitive tests. Stepwise multiple regression showed that father's education predicted children performance on the PPVT-IV, mother's education and family income predicted children's performance on the nonverbal index of the K-ABC-II, mother's education and father's education predicted children performance on the simultaneous scale of K-ABC-II, only mother's education predicted children's performance on triangles, hours children spent watching TV predicted their performance on word order, and finally kindergarten attendance predicted children's performance on hand movement.

A possible explanation for the lack of an association between iron deficiency and cognitive measures is that children didn't have severe iron deficiency. More research is needed to assess short-term and long-term developmental outcomes in Jordanian preschool children with low iron and zinc status.

INTRODUCTION

Research suggests poor cognitive, motor, or social functioning in iron deficient infants and young children up to 24 months of age (Aukett et al 1986, Black et al 2004a, Friel et al 2003, Heywood et al 1989, Idjradinata and Pollitt 1993, Lozoff et al 1987, Oski et al 1983, Walter et al 1983, Walter et al 1989, Williams et al 1999). Longitudinal

studies also showed that infants with chronic iron deficiency failed to catch up to the children with good iron status in developmental tests overtime despite correction of iron status (Algarin et al 2003, Corapci et al 2006, Dommergues et al 1989, Hurtado et al 1999, Lozoff et al 1991, Lozoff et al 2000, Lozoff et al 2006b, Palti et al 1985, Shafir et al 2006, Walter 2003). However, research on the developmental outcomes and motor and cognitive functioning among iron-deficient preschool children is limited. The few available studies showed impaired motor, cognitive, and developmental outcomes among iron deficient pre-school children (Lozoff et al 2007, Metallinos-Katsaras et al 2004, Pollitt et al 1983, Pollitt et al 1986, Soewondo et al 1989, Stoltzfus et al 2001).

Animal studies have shown that zinc deficiency is associated with reduced motor activity, decrease in performance on tasks of visual attention and short-term memory, impaired spatial learning and knowledge retention, and increased emotional response to stress (Bhatnagar and Taneja 2001, Black 2003, Golub et al 1995, Golub et al 2000, Tahmasebi Boroujeni et al 2009). Zinc deprivation in animals has been associated with impairments in learning and memory that are poorly reversible later in life(Sandstead 2003).

Few research studies have been conducted to assess the impact of zinc deficiency on cognition and behavior in humans. There are some observational studies but most of the research has involved zinc supplementation trials in infants and school-age children with mixed results. Many zinc intervention studies that assessed the role of zinc in improving diminished cognitive ability and motor functioning have suggested a relationship between zinc supplementation and motor development in infants, toddlers,

and school aged children (Ashworth et al 1998, Bentley et al 1997, Black et al 2004a, Castillo-Duran et al 2001, Friel et al 1993, Gardner et al 2005, Kirksey et al 1991, Kirksey et al 1994, Merialdi et al 1999, Sazawal et al 1996), although more research is needed to confirm such relations (Black 2003). On the other hand, other studies found no impact of zinc supplementation on infant and child mental development and behavior (Black et al 2004b, Cavan et al 1993, Gibson et al 1989, Hamadani et al 2001, Hamadani et al 2002, Lind et al 2004, Tamura et al 2003, Taneja et al 2005).

No previous study has investigated the relation between nutritional status and cognition of preschool children in Jordan. This study aimed to assess the association between children's performance on tests of non-verbal cognitive functions and their iron and zinc indicators.

METHODS

A convenience sample of 93 women and their children aged 4–5 years from urban/suburban areas in Northern Jordan was recruited for the study. Participants were approached by direct contact with the help of local community centers. An exclusion criterion was acute illness. The study was approved by the Oklahoma State University Institutional Review Board, and an informed written consent was obtained from the mothers. Four university graduate research assistants in Jordan were trained and assisted in taking anthropometric measurements, administration of the questionnaire, and in the administration of cognitive tests.

Weight (kg), height (cm), and mid-upper arm circumference (cm) were measured for children. A questionnaire covering health status, demographic characteristics, income (defined as total monthly family income after rent deduction), lifestyle and dietary intakes of children was administered individually to mothers. The questionnaire was validated by a group of academic experts in research, statistics and dietetics who reviewed the questions to ensure face and content validity. Second, a focus group discussion with local women was carried out and some items of the questionnaire were modified to eliminate ambiguity.

After collection of anthropometric measurements and questionnaire data in the field, participants were transferred to a local clinical laboratory where non-fasting venous blood samples were drawn from each participant. All samples were taken between June and July 2007. Hemoglobin concentration was measured immediately, and serum and plasma samples were transferred into trace element-free tubes which were then frozen at -20 °C, shipped in dry ice to our laboratories at Oklahoma State University in the USA and stored at -20 °C until analysis. In addition to Hemoglobin (Hb), the following were measured in 93 mothers and their children: plasma ferritin (Ft) was measured using immunoradiometric assay (Ramco Laboratories, Inc. Stafford, Texas); transferrin receptor (TfR) was measured using enzyme immunoassay (Ramco Laboratories, Inc. Stafford, TX); α-1-acid glycoprotein (AGP) was assayed using radial immunodifusion (Kent Laboratories, Redmond, WA); and plasma zinc (Zn) was measured using inductively coupled plasma mass spectrometry (ICPMS).

For plasma ferritin, AGP, and TfR, standards and serum samples were analyzed in duplicate, and values for the manufacturer's controls fell within the certified ranges. Coefficients of variation (CV) for the low, medium, and high controls for AGP were 4.1%, 9.8%, and 6.1%, respectively. For plasma ferritin, the CV for the low, medium, and high controls were 1.5%, 2.0%, and 1.9%, respectively. For TfR, the CV for the medium and high controls was 15.1% and 15.9%, respectively. For plasma zinc, a sample check (concentration of 50 µg/L) was measured repetitively (once after 10 participants' samples), and the mean, standard deviation, and CV were 52.0 µg/L, 0.76, and 1.5%, respectively.

Hemoglobin concentration less than <11 mg/dl was used to define anemia in children less than 60 months of age (n= 39), and Hb < 11.5 mg/dl was used to define anemia in children aged 60 months or older (n=54) (WHO/UNICEF/UNU 2001). A cut-off point of 65 µg/dL was used to define zinc deficiency in children (IZiNCG 2007a). Quantitative estimation of body iron (mg/kg) was based on the model proposed by Cook and colleagues (2003) using the following formula: Body Iron (mg/kg) = -[log(TfR/Ferritin ratio) - 2.8229]/0.1207 (Cook et al 2003).

Plasma ferritin, TfR, and the ratio of TfR to plasma ferritin TfR/Ft (TfR in mg/L and Ft in µg/L) were used to assess iron deficiency in children. A ferritin cut-off point of 12 µg/L was used to define iron deficiency in absence of inflammation (WHO/UNICEF/UNU 2001). Normal range for TfR was between 2.9-8.3 µg/ml as defined by the assay. An AGP concentration ≥1.2 was used to indicate the presence of inflammation (Paracha et al 2000, Wieringa et al 2002). Based on values of plasma

ferritin, TfR, TfR/ferritin, AGP, and Hb, children were classified into four groups (described in chapter 5): iron sufficient and not anemic (n=55), iron deficient but not anemic (n=13), iron deficiency anemia (n=9), and children with anemia of chronic disease (n=15).

Cognitive function was assessed in children using components of the Peabody Picture Vocabulary Test-IV (PPVT-IV) and of the Kaufman Assessment Battery for Children (KABC-II). The children were tested on two of the five scales of KABC-II: sequential and simultaneous processing. The core subtests on the K-ABC-II sequential scale included: number recall (memory span) and word order (memory span and working memory). The core subtests on the K-ABC-II simultaneous scale included: conceptual thinking (visualization and induction), face recognition (visual memory), triangles (spatial relations and visualization), and pattern reasoning (induction and visualization). In addition, the hand movement which is a supplementary test was also administered to children (it measures memory span and visual memory). The non verbal index K-ABC-II (NVI) is used to assess processing and cognitive abilities of children using both core and supplementary subtests that can be communicated with gestures and responded to without speaking.

Raw scores for PPVT-IV were reported and then transformed into the corresponding standard scores for each age group. For K-ABC-II, raw scores for the seven subtests (conceptual thinking, face recognition, number recall, triangles, word order, pattern reasoning, and hand movement) were reported, and then transformed into the corresponding scaled scores for each age group. Then the sum of the scaled

scores for the three K-ABC-II scales was calculated. Sequential K-ABC-II = (number recall + word order) for all children. For children < 5years of age: NVI = (conceptual thinking + face recognition + triangles + hand movement), and simultaneous K-ABC-II = (conceptual thinking + face recognition + triangles). For children ≥ 5 years of age: NVI = (conceptual thinking + face recognition + triangles + hand movement + pattern reasoning), and simultaneous K-ABC-II = (conceptual thinking + triangles + pattern reasoning). Scaled scores for four children were not added for NVI and simultaneous K-ABC-II because those children were not tested on story completion (part of the NVI for this age group), and rover (part of the simultaneous K-ABC-II for this age group). Finally, the sum of the scaled scores for the three K-ABC-II scales (NVI, simultaneous, and sequential) was transformed to the corresponding standard score for each age group.

Anthropometric data for children were expressed as *z*-scores based on the WHO (World Health Organization) 2007 reference using AnthroPlus, version 1.0.2 (WHO 2009).Variables that had skewed distribution (ferritin, TfR, and TfR/Ferritin, AGP and Hb) were transformed to logarithms, and geometric means ± 1SD were reported for those variables. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS/PASW), version 18.0 (SPSS Inc, Chicago, IL, USA). Analysis of variance was used to compare subgroups of the participants. Stepwise multiple regression analysis was performed to determine the variance contributed by different socioeconomic variables identified in correlation analysis for the prediction of cognitive scores.

RESULTS

Characteristics of study participants

Fifty-one boys and 42 girls participated in the study with a mean ± SD age of 60.7 ± 5.4 months (Tables 6.1 & 6.2). The children's mid-upper arm circumference ranged from 14.0 to 19.5 cm; only four children (4.3%) had less than -2 SD for weight-for-age (-2 *z*), whereas two children (2.2%) had <-2 for the height-for-age *z*-scores. The mean age of mothers was 33.9 ± 5.9 years, and each mother had 4.1 ± 1.9 children with 50% of mothers having 5 children or more. A total of 18% of mothers had completed 8 years or less of school, 63% of mothers had completed high school, and 72% of fathers had completed high school. In total, 91% of mothers and only 5% of fathers did not have a job, and the monthly income for each family (gross income-housing) was equivalent to 325 US$.

Based on mothers' report, seventy-nine children had breast fed for 13.9 ± 6.3 months, fifty children (54%) were attending kindergarten for 5.1 ± 0.9 hr/day, all children watched TV for 11.0 ± 8.6 hr/wk, and 45% of children (n=42) spent 1 hr/day or more on TV. Mothers also reported that their children spent 24.3 ±14.4 hr/wk in active play, and 60% of children spent at least three hours in active play daily.

Biochemical indices

As described in Table 6.1, twenty-four children (25.8%) were anemic based on their hemoglobin concentrations. Plasma ferritin ranged between 1.6-160.2 µg/L with a mean ± SD of 21.9 ± 2.0 µg/L. Fifteen children (16%) had ferritin below 12 µg/L, and half

were below 22 µg/L. Mean ± SD forTfR was 5.9 ± 1.4 mg/L and 13 children (14%) had TfR above the normal range for the assay (2.9-8.3 mg/L). Children's estimated mean body iron 3.5 ± 3.8 mg/kg and 18% of children (n=17) had an iron deficit of 0.71 mg/kg body weight. Twenty-eight children (30%) had AGP≥1.2 g/L. There was no significant difference between boys and girls for zinc or for all iron indices (ferritin, TfR, iron, and Hb). Plasma zinc ranged between 46.3-87.8 µg/dL with a mean of 64.6 ± 9.4 µg/dL. Fifty-three children (56%) were below 65.0 µg/dL, and 75% had plasma zinc below 70.1 mg/L.

Correlations between cognitive tests, zinc and iron indices, and socioeconomic variables

Correlations between cognitive tests are presented in Table 6.3. No significant correlation was found between ferritin, TfR/ferritin, and zinc and children scores on all cognitive tests (Table 6.4). Transferrin receptor correlated negatively only with scores on conceptual thinking ($r=-.23$, $p<.05$). Quantitatively estimated iron correlated negatively with scores on hand movement ($r=-.23$, $p<.05$), and AGP correlated positively with scores on PPVT-IV.

Socioeconomic variables showed correlations with some of the cognitive tests. The hours that children spend in active play correlated negatively with non verbal index K-ABC-II scale ($r=-.23$, $p<.05$), sequential K-ABC-II scale ($r=-.22$, $p<.05$), triangles subtest ($r=-.25$, $p<.05$), and hand movement subtest ($r=-.24$, $p<.05$). Kindergarten attendance correlated positively with non verbal index K-ABC-II scale ($r=.32$, $p<.01$), simultaneous K-ABC-II ($r=.27$, $p<.05$), face recognition subtest ($r=.22$, $p<.05$), and hand movement subtest ($r=.29$, $p<.01$).

Hours the child spent watching TV correlated positively only with the word order subtest (r=.23, p<.05). Family income (after rent deduction) correlated positively with PPVT-IV (r=.26, p<.005), nonverbal index of the K-ABC-II (r=.32, p<.005), the simultaneous K-ABC-II scale (r=.32, p<.005), the triangles subtest (r=.32, p<.005), and the hand movement subtest (r=.23, p<.05). Highest level of education for mothers and fathers correlated positively with the non verbal K-ABC-II scale (r=.28, p<.01 and r=.29, p<.05, respectively), the simultaneous K-ABC-II scale (r=.34, p<.005 and r=.32, p<.05, respectively), the triangles subtest (r=.33, p<.005 and r=.27, p<.05, respectively), and the hand movement subtest (r=.28, p<.05 and r=.25, p<.05, respectively), while father's highest level of education (but not mothers' highest level of education) correlated positively with PPVT-IV (r=.45, p<.005).

Table 6.5 shows the correlations between socioeconomic variables. Hours of active play was negatively correlated with KG attendance (r=-.21, p<.05) and with father's education (r=-.27, p<.05). Kindergarten attendance correlated positively with income (r=.41, p<.01), mother's education (r=.25, p<.05), and father's education (r=.33, p<.01). Finally, both fathers' education and mothers' education were positively correlated with each other(r=.35, p<.01), and with their income (r=.43, p<.000 for mothers, and r=.51, p<.000 for fathers).

Performance of children on cognitive tests and iron and zinc indices

Means ± SD of PPVT-IV and K-ABC-II scales and subtests are reported in Table 6.6. Children's scores on PPVT-IV ranged between 47-118. Scores for 21 children were

below 2SD, scores for 37 children were below average, twenty-six children were within the normal range, and only one child scored above average (Table 6.7). Analysis of variance showed no significant difference in the means of all iron and zinc indices for the children in the three groups: lower extreme, below average, and normal range. Also no significant difference was found in the means of iron and zinc indices for children whose scores fell within or above the normal range (n=27), and those whose scores were below normal range (n=58).

In the nonverbal index of the K-ABC-II, seven children scored <-2SD, scores for 34 children were below average, scores for 40 children were within the normal range, and only one child scored above average (Table 6.8). There was no significant difference in the means of all iron and zinc indices between the three groups. Although zinc was slightly higher in the group of children who scored in the normal range (65.6 ± 9.9 µg/dl) than both groups who scored below average (62.2 ± 8.7µg/dl) and in the lower extreme (62.2 ± 8.7µg/dl), the difference was not significant.

On the simultaneous K-ABC-II scale, more children scored in the normal range (n=43), scores for 35 children were below average, and 3 children scored in the lower extreme (<-2SD) (Table 6.9). Only TfR showed a significant difference between those children who scored in the lower extreme (9.3 ± 1.7 mg/L) than children who scored below average (5.8 ± 1.6 mg/L), and those who scored within the normal range (5.7 ± 1.4 mg/L). Ferritin and estimated body iron were also lower in the three children who scored in the lower extreme but the difference was not significant.

On the Sequential K-ABC-II, more children were within the normal range (n=54), thirty children were below average, two children scored in the lower extreme, and no score was above average (Table 6.10). Zinc was lower in the two children who scored in the lower extreme, but the difference was not significant. Ferritin was lower and TfR was higher in the same group but also the difference was not significant.

For the eight K-ABC-II subtests (conceptual thinking, face recognition, triangles, word order, pattern reasoning, number recall, and hand movement), means for all iron and zinc indices were compared for children below the 20th percentile and those above the 80th percentile using the students't-test (Table 6.11). No significant difference was found in all iron and zinc indices between the two groups.

Children's performance on cognitive tests was evaluated in the children who are grouped based on iron status into four groups: iron sufficient and not anemic, iron deficient but not anemic, children with iron deficiency anemia, and children with anemia of chronic disease Children's performance on all cognitive tests showed no significant difference between children with different iron status (Table 6.12).

Children's performance on cognitive tests was also evaluated in the children who were grouped based on zinc status into two groups: those with plasma zinc < 65.0 µg/dL (n= 37), and those with zinc ≥ 65.0 µg/dL (n= 49). No significant difference was found in children's performance between the two groups on all cognitive tests. However, when a lower cutoff for zinc was used (57.0 µg/dL), the cutoff for zinc deficiency recommended by the IZiNCG for non-fasting afternoon zinc samples, there was a trend of lower scores on most of the cognitive tests for children who had plasma zinc < 57.0 µg/dL compared

to children who had plasma zinc ≥57.0 µg/dL, but the difference was not statistically significant except for number recall subtest. Children who had plasma zinc ≥57.0 µg/dL had significantly higher score on number recall subtest (9.9 ± 2.3) compared to children who had plasma zinc < 57.0 µg/dL (8.5 ± 2.4) as shown in Table 6.13.

Performance of children on cognitive tests and socioeconomic variables

There was no significant difference between girls and boys on all cognitive tests scores: PPVT-IV, and K-ABC-II scales and subtests (Table 6.14). Also no significant difference was found on all cognitive tests between children who breast fed and those who didn't breast fed (Table 6.15).

Children's performance on some cognitive tests was different for children who attended kindergarten (KG) and those who did not (Table 6.16). Children attending KG had significantly higher scores than children not attending KG on the following tests: non verbal index K-ABC–II scale (89.3 ± 12.1 v 81.5 ± 11.4), simultaneous K-ABC-II (90.3 ± 11.0 v 84.2 ± 11.3), word order subtest (6.1 ± 1.7 v 5.9 ± 1.1), and hand movement subtest (9.0 ± 2.4 v 7.6 ± 2.1).

Hours spent in active play also influenced children's performance on cognitive tests (Table 6.17). Children who spent more than 5 hours a day in active play had significantly lower scores on PPVT-IV than children who played less than 5 hours a day (75.8 ± 10.0 and 79.5 ± 14.6, respectively), sequential K-ABC-II subscale (82.1 ± 8.2 and 89.0 ± 9.2, respectively), number recall subtest (8.3 ± 2.2 and 10.1 ± 2.2, respectively), and hand movement subtest (7.4 ± 2.0 and 8.7 ± 2.4, respectively).

Highest level of education fathers and mothers affected children's performance on cognitive tests (Table 6.18). Children's scores on nonverbal index K-ABC-II scale and simultaneous K-ABC-II scale differed significantly between children whose mothers had completed high school or above (88.6 ± 11.7 and 90.3 ± 10.7, respectively), and those children whose mothers had less than high school education (80.6 ± 11.9 and 82.6 ± 11.3, respectively). Children's performance on these same two scales also differed by fathers' highest level of education. Children whose fathers completed high school or above had significantly higher scores on non verbal index K-ABC-II scale and simultaneous K-ABC-II scale (89.0 ± 12.4 and 91.1 ± 11.1, respectively) than children whose fathers had an education of less than high school (80.5 ± 10.4 and 82.6 ± 10.0, respectively).

Children's performance on PPVT-IV, the conceptual thinking subtest, and the face recognition subtest differed by fathers' (but not mothers') highest level of education. On the other hand, children's performance on triangles subtest, word order subtest, and hand movement subtest differed by mothers' (but not fathers') highest level of education. Neither fathers' nor mothers' highest level of education affected children's performance on the sequential K-ABC-II scale and number recall subtest (Table 6.18).

Cognitive tests scores also varied by family's income. Children living in families with higher income (income ≥$350) had significantly higher scores than children living in families with income < $350 on PPVT-IV, non verbal index K-ABC-II scale, simultaneous K-ABC-II scale, triangles subtest, and hand movement subtest (Table 6.19).

Children's performance on conceptual thinking only differed by hours spent watching TV. Children who watched TV more than one hour daily (n=41) had significantly higher scores (9.1± 4.3) than children who watched TV for less than an hour a day (n=45) with mean ±SD of 7.3 ± 3.6, $p<.05$.

Since socioeconomic variables were highly correlated, and these variables correlated with many of the scores on cognitive tests, a stepwise multiple regression analysis was conducted to determine the socioeconomic variables that best predict children's performance on cognitive tests. PPVT-IV and K-ABC-II scales and subtests were simultaneously regressed on the set of six predictors: family income after rent deduction, mother's highest level of education, father's highest level of education, kindergarten attendance, hours spent in active play, and hours spent watching TV. The variances of cognitive tests scores accounted for by the predictors, the standardized and the unstandardized regression coefficients, along with p-value are presented in Table 6.20. Only father's education significantly predicted children's scores on PPVT-IV (R^2=.19, β=.44, and $p<.000$). Twenty percent of the variability in the non verbal index K-ABC-II scale was accounted for by both mother's education (β=.30, $p<.05$), and family income (β =.26, $p<.05$). Mother's education and father's education jointly explained 20% of the variability in children's scores on the non verbal index K-ABC-II scale, while mothers' education only accounted for 12% of the variability of children scores on triangles subtest. Hours child spend on TV accounted for 7% of the variability in children scores on word order subtest, and kindergarten attendance accounted for 13% of variability in hand movement subtest.

DISCUSSION

Iron and zinc indicators didn't show any significant correlation with any of the administered cognitive tests. For PPVT-IV, children's performance fell mostly within normal range, below average, and lower extreme categories, and there was no significant difference between these three groups for all iron and zinc indicators. For K-ABC-II scales (non verbal index K-ABC-II, simultaneous K-ABC-II, and sequential K-ABC-II) most of the children were either within the normal range or below average, and few children were in the lower extreme. Again, no significant difference was found for all iron and zinc indicators between these groups. Only the three children in the lower extreme in simultaneous K-ABC-II had significantly higher TfR than each of the children in the below average category and the normal range category, but the low number of children who performed in the lower extreme (n=3) doesn't allow for a sufficient power to detect a statistically significant difference. For all K-ABC-II subtests (conceptual thinking, face recognition, number recall, triangles, word order, pattern reasoning, and hand movement), iron and zinc indicators didn't differ between children below the 20^{th} percentile and those above the 80^{th} percentile.

When children were classified according to their iron and Hb status into four groups (iron sufficient and not anemic, iron deficient but not anemic, iron deficiency anemia, and anemia of chronic disease), and their performance on cognitive tests was evaluated, no significant difference was found between children with different iron status on all cognitive tests.

On the other hand, children's performance on cognitive tests was associated with socioeconomic variables, namely: father's education, mother's education, income, hours the child spent on TV, and kindergarten attendance.

This study found no association between iron status in children at 4-5 years of age and their performance on cognitive tests. Almost all reviews of the current literature on the effect of iron deficiency on children's cognition and behavior (Beard 2003, Grantham-McGregor and Ani 2001, Lozoff et al 2006a, McCann and Ames 2007, Thomas et al 2009), and reviews on the role of iron supplementation in improving development and cognition outcomes (Iannotti et al 2006, Sachdev et al 2005), have supported an association between iron deficiency anemia and poor cognitive and behavioral performance. Yet, a direct and unequivocal causal connection could not be clearly established.

In this study, only 16% of children had ferritin < 12 μg/L and 26% were anemic. The low prevalence of iron deficiency among children may be a possible explanation for the lack of an association between iron deficiency and cognitive measures. Only nine children had iron deficiency anemia (the latest stage of iron deficiency). If the study had more children in this category, this would allow for a better comparison that might have shown the impact of iron deficiency on children's performance on cognitive tests. This agrees with Metallinos-Katsaras and colleagues (2004) who found that although iron supplementation improved motor and language development, the effect on motor development was limited to children who were severely anemic (Hb<9.0g/dl at baseline). In our study none of the children had Hb below 9.0 g/dl. In addition, the study

of Metallinos-Katsaras and colleagues (2004) found that although iron supplementation resulted in improvement in selective attention among anemic children, there were no significant differences in cognitive outcomes at baseline between those children who were anemic and those with good iron status (Metallinos-Katsaras et al 2004).

Although zinc deficiency was more prevalent among children than iron deficiency, neither zinc deficiency nor iron deficiency was associated with children's performance on cognitive tests, except for the number recall subtest, where children with higher zinc status showed significantly better performance on the number recall. Fifty-two children (56%) had zinc below 65 µg/dL zinc, and despite the high prevalence of zinc deficiency only two children (2.2%) had <-2 for the height-for-age *z*-scores. A possible explanation is that zinc deficiency may have occurred recently in children's lives, which means they didn't develop the signs of chronic zinc deficiency that might contribute to a larger percentage of stunting among children. Beside that, although more than half of the children were below the defined cut off point for zinc deficiency; their zinc concentration was not extremely low. Zinc concentration ranged between (46.3 – 64.9 µg/dL) for the zinc deficient children. Most importantly, there is absence of reliable measures that could sensitively measure plasma zinc. It could be that the actual prevalence of zinc deficiency is less than the reported figure, especially because when a lower cutoff for zinc deficiency was used, children's performance on one of the subtests (number recall) became significantly different. These factors could explain the lack of any association between zinc status in children and their performance on cognitive tests. With that being said, the study findings are consistent with previous studies that

found no association between zinc status and infant and child mental development and behavior (Black et al 2004b, Cavan et al 1993, Gibson et al 1989, Hamadani et al 2001, Hamadani et al 2002, Lind et al 2004, Tamura et al 2003, Taneja et al 2005).

A major limitation in this study is that the cognitive tests utilized in this study were developed for the US population, not for the population in Jordan. To overcome this limitation, we worked with a team of local school teachers and mothers to adapt the PPVT-IV and K-ABC-II subtests so that they would be culturally appropriate. All stimulus words and measures were translated into Arabic for training and administration. All cognitive measures were pretested on a group of preschoolers in Jordan; the *cultural differences* in the interpretation of some of the measures were evaluated and adjusted, and then cognitive measures were tested again on another group of preschoolers before administering them to the study participants.

The study of cognitive psychology and micronutrients is an emerging research area. The exact biological mechanisms by which zinc deficiency alters brain functions and relates to cognition and behavior are not yet clear. It could be that there might be specific cognitive defects associated with zinc deficiency that were not captured by the administered cognitive tests. Perhaps an important finding of this study that relates to populations with lower socioeconomic status in Jordan is that parent's education and especially mothers' education was an important component (more than income) of children performance on cognitive tests. Children whose parents had higher education had better performance on most of the tests than children whose parents were less educated.

This is the first study to assess the effect of iron and zinc deficiency on cognition in Jordan. There is an extreme lack of data not only on preschool children, but also on younger children, infants, and adolescents. Certainly, larger studies are needed to assess short-term and long-term developmental outcomes in iron deficient infants, young children, preschool children, school children, and adolescents in Jordan. The long term effects of iron and zinc deficiency may have not been captured by this study, but certainly rigorous randomized controlled trials of iron and zinc supplements with large sample size can provide detailed information and help to make firm conclusions that can be used to develop ways for enhancing child development in Jordan.

REFERENCES

Algarin C, Peirano P, Garrido M, Pizarro F, Lozoff B (2003). Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. *Pediatr Res* **53:** 217-223.

Ashworth A, Morris SS, Lira PI, Grantham-McGregor SM (1998). Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. *Eur J Clin Nutr* **52:** 223-227.

Aukett MA, Parks YA, Scott PH, Wharton BA (1986). Treatment with iron increases weight gain and psychomotor development. *Arch Dis Child* **61:** 849-857.

Beard J (2003). Iron deficiency alters brain development and functioning. *J Nutr* **133:** 1468S-1472S.

Bentley ME, Caulfield LE, Ram M, Santizo MC, Hurtado E, Rivera JA *et al* (1997). Zinc supplementation affects the activity patterns of rural Guatemalan infants. *J Nutr* **127:** 1333-1338.

Bhatnagar S, Taneja S (2001). Zinc and cognitive development. *Br J Nutr* **85 Suppl 2:** S139-145.

Black MM (2003). The evidence linking zinc deficiency with children's cognitive and motor functioning. *Journal of Nutrition* **133 (Suppl 1):** 1473-1476.

Black MM, Baqui AH, Zaman K, Ake Persson L, El Arifeen S, Le K *et al* (2004a). Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. *Am J Clin Nutr* **80:** 903-910.

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

Castillo-Duran C, Perales CG, Hertrampf ED, Marin VB, Rivera FA, Icaza G (2001). Effect of zinc supplementation on development and growth of Chilean infants. *J Pediatr* **138:** 229-235.

Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW (1993). Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. *Am J Clin Nutr* **57:** 344-352.

Cook JD, Flowers CH, Skikne BS (2003). The quantitative assessment of body iron. *Blood* **101:** 3359-3364.

Corapci F, Radan AE, Lozoff B (2006). Iron deficiency in infancy and mother-child interaction at 5 years. *J Dev Behav Pediatr* **27:** 371-378.

Dommergues JP, Archambeaud MP, Ducot B, Gerval Y, Hiard C, Rossignol C *et al* (1989). [Iron deficiency and psychomotor development tests. Longitudinal study between 10 months and 4 years of age]. *Arch Fr Pediatr* **46:** 487-490.

Friel JK, Andrews WL, Matthew JD, Long DR, Cornel AM, Cox M *et al* (1993). Zinc supplementation in very-low-birth-weight infants. *J Pediatr Gastroenterol Nutr* **17:** 97-104.

Friel JK, Aziz K, Andrews WL, Harding SV, Courage ML, Adams RJ (2003). A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. *J Pediatr* **143:** 582-586.

Gardner JM, Powell CA, Baker-Henningham H, Walker SP, Cole TJ, Grantham-McGregor SM (2005). Zinc supplementation and psychosocial stimulation: effects on the development of undernourished Jamaican children. *Am J Clin Nutr* **82:** 399-405.

Gibson RS, Vanderkooy PD, MacDonald AC, Goldman A, Ryan BA, Berry M (1989). A growth-limiting, mild zinc-deficiency syndrome in some southern Ontario boys with low height percentiles. *Am J Clin Nutr* **49:** 1266-1273.

Golub MS, Keen CL, Gershwin ME, Hendrickx AG (1995). Developmental zinc deficiency and behavior. *J Nutr* **125:** 2263S-2271S.

Golub MS, Keen CL, Gershwin ME (2000). Moderate zinc-iron deprivation influences behavior but not growth in adolescent rhesus monkeys. *J Nutr* **130:** 354S-357S.

Grantham-McGregor S, Ani C (2001). A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr* **131:** 649S-666S; discussion 666S-668S.

Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM (2001). Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. *Am J Clin Nutr* **74:** 381-386.

Hamadani JD, Fuchs GJ, Osendarp SJ, Huda SN, Grantham-McGregor SM (2002). Zinc supplementation during pregnancy and effects on mental development and behaviour of infants: a follow-up study. *Lancet* **360:** 290-294.

Heywood A, Oppenheimer S, Heywood P, Jolley D (1989). Behavioral effects of iron supplementation in infants in Madang, Papua New Guinea. *Am J Clin Nutr* **50:** 630-637; discussion 638-640.

Hurtado EK, Claussen AH, Scott KG (1999). Early childhood anemia and mild or moderate mental retardation. *Am J Clin Nutr* **69:** 115-119.

Iannotti LL, Tielsch JM, Black MM, Black RE (2006). Iron supplementation in early childhood: health benefits and risks. *Am J Clin Nutr* **84:** 1261-1276.

Idjradinata P, Pollitt E (1993). Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *Lancet* **341:** 1-4.

IZiNCG. Techincal Brief No.2: Assessing population zinc status with serum zinc concentration 2007.

Kirksey A, Rahmanifar A, Wachs TD, McCabe GP, Bassily NS, Bishry Z *et al* (1991). Determinants of pregnancy outcome and newborn behavior of a semirural Egyptian population. *Am J Clin Nutr* **54:** 657-667.

Kirksey A, Wachs TD, Yunis F, Srinath U, Rahmanifar A, McCabe GP *et al* (1994). Relation of maternal zinc nutriture to pregnancy outcome and infant development in an Egyptian village. *Am J Clin Nutr* **60:** 782-792.

Lind T, Lonnerdal B, Stenlund H, Gamayanti IL, Ismail D, Seswandhana R *et al* (2004). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *Am J Clin Nutr* **80:** 729-736.

Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E *et al* (1987). Iron deficiency anemia and iron therapy effects on infant developmental test performance. *Pediatrics* **79:** 981-995.

Lozoff B, Jimenez E, Wolf AW (1991). Long-term developmental outcome of infants with iron deficiency. *N Engl J Med* **325:** 687-694.

Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW (2000). Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics* **105:** E51.

Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T (2006a). Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev* **64:** S34-43; discussion S72-91.

Lozoff B, Jimenez E, Smith JB (2006b). Double burden of iron deficiency in infancy and low socioeconomic status: a longitudinal analysis of cognitive test scores to age 19 years. *Arch Pediatr Adolesc Med* **160:** 1108-1113.

Lozoff B, Corapci F, Burden MJ, Kaciroti N, Angulo-Barroso R, Sazawal S *et al* (2007). Preschool-aged children with iron deficiency anemia show altered affect and behavior. *J Nutr* **137:** 683-689.

McCann JC, Ames BN (2007). An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr* **85:** 931-945.

Merialdi M, Caulfield LE, Zavaleta N, Figueroa A, DiPietro JA (1999). Adding zinc to prenatal iron and folate tablets improves fetal neurobehavioral development. *Am J Obstet Gynecol* **180:** 483-490.

Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, Lonnerdal B, Stamoulakatou A, Pollitt E (2004). Effect of iron supplementation on cognition in Greek preschoolers. *Eur J Clin Nutr* **58:** 1532-1542.

Oski FA, Honig AS, Helu B, Howanitz P (1983). Effect of iron therapy on behavior performance in nonanemic, iron-deficient infants. *Pediatrics* **71:** 877-880.

Palti H, Meijer A, Adler B (1985). Learning achievement and behavior at school of anemic and non-anemic infants. *Early Human Development* **10:** 217-223.

Paracha PI, Jamil A, Northrop-Clewes CA, Thurnham DI (2000). Interpretation of vitamin A status in apparently healthy Pakistani children by using markers of subclinical infection. *Am J Clin Nutr* **72:** 1164-1169.

Pollitt E, Leibel RL, Greenfield DB (1983). Iron deficiency and cognitive test performance in preschool children. *Nutr Behav* **1:** 137-146.

Pollitt E, Saco-Pollitt C, Leibel RL, Viteri FE (1986). Iron deficiency and behavioral development in infants and preschool children. *Am J Clin Nutr* **43:** 555-565.

Sachdev H, Gera T, Nestel P (2005). Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public Health Nutr* **8:** 117-132.

Sandstead HH (2003). Zinc is essential for brain development and function. *J Trace Elem Exp Med* **16:** 165-173.

Sazawal S, Bentley M, Black RE, Dhingra P, George S, Bhan MK (1996). Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. *Pediatrics* **98:** 1132-1137.

Shafir T, Angulo-Barroso R, Calatroni A, Jimenez E, Lozoff B (2006). Effects of iron deficiency in infancy on patterns of motor development over time. *Hum Mov Sci* **25:** 821-838.

Soewondo S, Husaini M, Pollitt E (1989). Effects of iron deficiency on attention and learning processes in preschool children: Bandung, Indonesia. *Am J Clin Nutr* **50:** 667-673; discussion 673-664.

Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM *et al* (2001). Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. *BMJ* **323:** 1389-1393.

Tahmasebi Boroujeni S, Naghdi N, Shahbazi M, Farrokhi A, Bagherzadeh F, Kazemnejad A *et al* (2009). The effect of severe zinc deficiency and zinc supplement on spatial learning and memory. *Biol Trace Elem Res* **130:** 48-61.

Tamura T, Goldenberg RL, Ramey SL, Nelson KG, Chapman VR (2003). Effect of zinc supplementation of pregnant women on the mental and psychomotor development of their children at 5 y of age. *Am J Clin Nutr* **77:** 1512-1516.

Taneja S, Bhandari N, Bahl R, Bhan MK (2005). Impact of zinc supplementation on mental and psychomotor scores of children aged 12 to 18 months: a randomized, double-blind trial. *J Pediatr* **146:** 506-511.

Thomas DG, Grant SL, Aubuchon-Endsley NL (2009). The role of iron in neurocognitive development. *Dev Neuropsychol* **34:** 196-222.

Walter T, Kovalskys J, Stekel A (1983). Effect of mild iron deficiency on infant mental development scores. *J Pediatr* **102:** 519-522.

Walter T, De Andraca I, Chadud P, Perales CG (1989). Iron deficiency anemia: adverse effects on infant psychomotor development. *Pediatrics* **84:** 7-17.

Walter T (2003). Effect of iron-deficiency anemia on cognitive skills and neuromaturation in infancy and childhood. *Food Nutr Bull* **24:** S104-110.

WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO, 2009 (http://www.who.int/growthref/tools/en/).

WHO/UNICEF/UNU. Iron Deficiency Anaemia: Assessment, Prevention and Control. A guide for programme managers. Geneva, World Health Organization; UNICEF; United Nations University, 2001.

Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal (2002). Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. *J Nutr* **132:** 3061-3066.

Williams J, Wolff A, Daly A, MacDonald A, Aukett A, Booth IW (1999). Iron supplemented formula milk related to reduction in psychomotor decline in infants from inner city areas: randomised study. *BMJ* **318:** 693-697.

Table 6.1

Characteristics of the study participants

	N	Mean ±SD	Percentile		
			25th	***50th***	***75th***
Children					
Age (months)	93	60.7 ± 5.4	55.0	61.0	65.0
Weight- for-age (z)	93	0.0 ±1.1	-0.6	0.1	0.6
Height–for-age (z)	93	0.5 ±1.2	-0.3	0.6	1.4
Child MUAC* (cm)	93	16.7 ± 1.2	16.0	16.7	17.5
Ferritin (µg/L)	93	21.9 ± 2.0	14.6	21.9	31.3
Transferrin receptor (mg/L)	93	5.9 ± 1.4	4.8	6.2	7.3
Iron (mg/kg)**	93	3.5 ± 3.8	1.5	3.5	5.0
Zinc (µg/dL)	93	64.6 ± 9.4	58.1	63.2	70.1
AGP (g/L)	93	1.0 ± 1.5	0.8	1.0	1.3
Hemoglobin (g/dL)	93	11.7 ± 1.1	11.3	11.6	12.2
Months child breast fed	79	13.9 ± 6.3	10.0	14.0	18.0
Child's order between siblings	93	3.1 ±1.8	2.0	3.0	4.0
Hours spent in KG(hr/d)	50	5.1 ± 0.9	4.4	5.0	6.0
Hours spent on TV (hr/wk)	93	11.0 ± 8.6	5.5	7.0	14.0
Hours spent in active play (hr/wk)	93	24.3 ±14.4	14.0	21.0	33.8
Mothers					
Age (years)	93	33.9 ± 5.9	29.0	35.0	38.0
Number of pregnancies	93	4.8 ± 2.5	3.0	4.0	6.0
Number of children	93	4.1 ± 1.9	3.0	4.0	5.0
Income (USD/month) ***	92	325 ± 272	174	254	353
Years of schooling	92	10.7 ± 2.3	9.0	12.0	12.0
Number of household members	93	6.3 ±2.2	5	6	7

*MAUC: Mid-upper arm circumference.

** Iron measured quantitatively using Cook and Colleagues method

***Income is defined as total monthly family income after rent deduction in US $.

Table 6.2
Characteristics of the study population- qualitative variables

Variable	N (%)
Children's gender *(Total= 93)*	
Male	51 (54.8%)
Female	42 (45.2%)
Child go to KG *(Total= 93)*	
No	43 (46.2%)
Yes	50 (53.8%)
Child breast fed *(Total= 93)*	
No	14 (15.1%)
Yes	79 (84.9%)
Mothers' marital status *(Total= 93)*	
Currently married	74 (79.6%)
Separated	2 (2.2%)
Widowed	17 (18.3%)
Mothers' education *(Total= 92)*	
Secondary education or less	33 (35.9%)
High school completed	33 (35.5%)
College/University completed	26 (28.0 %)
Mothers' work status *(Total= 93)*	
Working	8 (8.7%)
Not working	85 (91.4%)
Fathers' education *(Total= 76)*	
Secondary education or less	21 (27.6%)
High school completed	34 (44.7%)
College/University completed	21 (27.6%)
Fathers' work status *(Total=76)*	
Working	72 (94.7%)
Not working	4 (5.2%)

Table 6.3
Correlations between cognitive tests

		Cognitive tests									
		1	2	3	4	5	6	7	8	9	10
Cognitive Tests	1										
	2	.53†									
	3	.47†	.88†								
	4	.45†	.46†	.33††							
	5	.37††	.54†	.67†	.34†						
	6	.32††	.64†	.48†	.27††	.21					
	7	.35†	.38††	.27*	.92†	.24*	.28*				
	8	.44†	.71†	.77†	.23*	.32††	.19	.17			
	9	.45	.43†	.32††	.78††	.40†	.16	.48†	.25*		
	10	.07	.25	.33*	.16	.18	.00	.14	-.01	.14	
	11	.32††	.58†	.28*	.32††	.02	.11	.24*	.40†	.33††	-.02

†Sig. at p<.000, ††Sig. at p<.005, *Sig. at p<.05

Numbers represent the following cognitive tests:

1. PPVT-IV
2. Non verbal index K-ABC-II
3. Simultaneous K-ABC-II
4. Sequential K-ABC-II
5. Conceptual thinking
6. Face recognition
7. Number recall
8. Triangles
9. Word order
10. Pattern reasoning
11. Hand movement

Table 6.4

Correlations between cognitive tests, zinc and iron indices, and socioeconomic variables

Cognitive Tests	Iron & Zinc indices							Socio-economic variables					
	Ferritin	TfR	TfR/Ft	Iron	Zn	Hb	AGP	Hours in active play	KG attendance	Hours watch TV	Income	Mothers' education	Fathers' education
1	.02	.06	.04	.03	-.04	-.01	**.29***	-.14	.20	.15	**.26**†	.21	**.45**†
2	-.10	-.03	.12	-.17	.11	-.10	.14	**-.23***	**.32**††	.10	**.32**†	**.28**††	**.29***
3	-.07	-.13	.02	-.08	.19	-.08	.12	-.18	**.27***	.06	**.32**†	**.34**†	**.32***
4	-.10	.19	.19	-.11	.10	-.16	.05	**-.22***	.11	.03	.06	.08	.23
5	-.18	**-.23***	.18	-.09	.06	-.12	.18	-.17	.18	.13	.01	.14	.15
6	.08	.18	-.04	-.07	.16	.04	.17	-.12	**.22***	.05	.14	-.01	.10
7	-.05	.21	.05	-.03	.16	**-.23***	.01	**-.25***	.12	-.10	.02	-.01	.23
8	-.12	-.15	-.10	-.03	.01	-.15	.05	-.08	**.27***	.02	**.32**†	**.33**†	**.27***
9	-.16	.03	.20	.20	-.03	.01	.07	-.12	.06	**.23***	.09	.20	.16
10	-.17	.03	.18	-.18	-.07	.01	-.06	-.07	-.27	-.10	-.23	-.28	-.15
11	-.14	-.01	.17	**-.23***	-.05	-.05	.01	**-.24***	**.29**††	.07	**.23***	**.28***	**.25***

†Sig. at p<.005, †† Sig. at p<.01, *Sig. at p<.05

Numbers on the left column represent the following cognitive tests:

1. PPVT-IV 2. Non verbal index K-ABC-II 3. Simultaneous K-ABC-II 4. Sequential K-ABC-II 5. Conceptual thinking
6. Face recognition 7. Number recall 8. Triangles 9. Word order 10. Pattern reasoning 11. Hand movement

Table 6.5
Correlations between socioeconomic variables

	Hours in active play	KG attendance	Hours watch TV	Income	Mothers' education
Hours in active play					
KG attendance	**-.21***				
Hours watch TV	.13	-.03			
Income	-.17	**.41††**	.07		
Mothers' education	-.16	**.25***	.17	**.43¥**	
Fathers' education	**-.27***	**.33††**	.07	**.51¥**	**.35††**

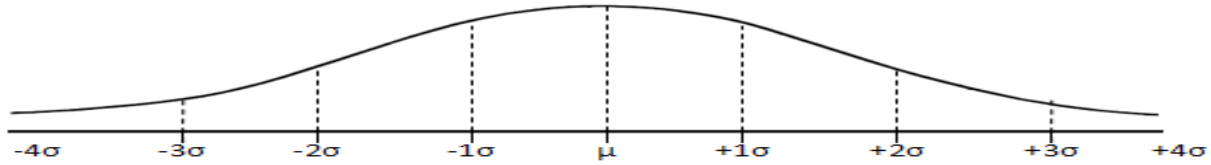
¥ Sig. at p<.000, † Sig. at p<.005, †† Sig. at p<.01, * Sig. at p<.05

Table 6.6
Descriptive statistics of cognitive tests

Test	N	Mean ± SD	Percentile			Min - Max
			25th	*50th*	*75th*	
PPVT-IV	85	78.6 ± 13.7	69.5	78.0	87.0	47 – 118
K-ABC-II Scales:						
Non verbal index K-ABC-II	82	85.7± 12.3	75.5	84.5	94.3	62 - 118
Simultaneous K-ABC-II	82	87.5 ± 11.5	80.0	85.0	97.0	68 – 116
Sequential K-ABC-II	86	78.2 ± 9.5	79.3	88.0	94.0	68 – 106
K-ABC-II Subtests:						
Conceptual thinking	86	7.9 ± 2.6	6.0	8.0	9.0	1 – 15
Face recognition	82	8.1 ± 2.9	6.0	8.0	10.0	2 – 15
Number recall	86	9.6 ± 2.3	8.0	10.0	11.0	5 – 14
Triangles	86	7.8 ± 2.7	6.0	7.0	9.0	2 – 16
Word order	86	6.0 ± 1.5	5.0	6.0	7.0	3 - 10
Pattern reasoning	50	8.9 ± 1.6	8.0	9.0	10.0	5 – 12
Hand movement	85	8.4 ± 2.3	7.0	8.0	10.0	4 - 14

Table 6.7

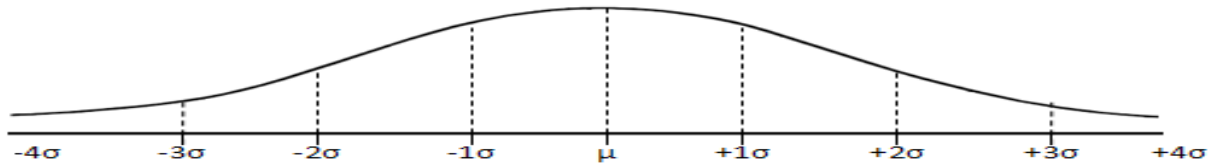
Iron and zinc indices of children by their performance on Peabody Picture Vocabulary Test (PPVT-IV)



	Lower extreme	**Below average**	**Normal Range**	**Above Average**	**Upper extreme**	Total
n (%)	21 (24.7)	37 (43.5)	26 (30.6)	1 (1.2)	0 (0)	N= 85
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Hb (g/dL)	11.8 ± 1.1	11.7 ± 1.1	11.6 ± 1.1	11.4	-	11.7 ± 1.1
Ferritin (µg/L)	22.2 ± 1.9	21.6 ± 2.3	23.5 ± 1.8	27.1	-	22.4 ± 2.0
TfR (µg/mL)	5.6 ± 1.4	6.2 ± 1.5	6.1 ± 1.4	6.2	-	6.0 ± 1.5
TfR/Ferritin	0.3 ± 1.9	0.3 ± 2.0	0.3 ± 1.9	0.2	-	0.3 ± 2.2
Iron (mg/kg)	3.5 ± 2.4	3.0 ± 3.4	4.3 ± 5.4	3.9	-	3.6 ± 3.9
Zinc (mg/L)	66.6 ± 8.3	62.0 ± 9.8	65.7 ± .9	61.1	-	64.3 ± 9.3
AGP (g/L)	0.8 ± 1.6	1.1 ± 1.6	1.2 ± 1.4	2.2	-	1.1 ± 1.6

Table 6.8

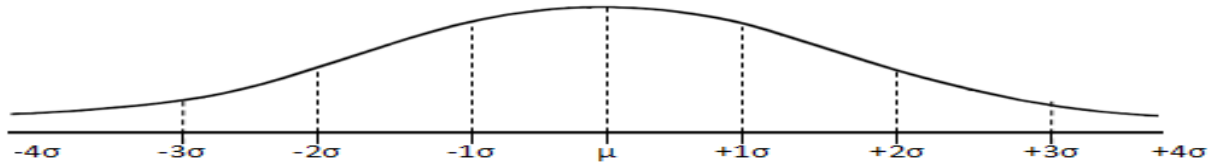
Iron and zinc indices of children by their performance on non verbal index K-ABC-II



	Lower extreme	**Below average**	**Normal Range**	**Above Average**	**Upper extreme**	Total
n (%)	7 (8.5)	34 (41.5)	40 (48.9)	1 (1.2)	0 (0)	N= 82
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Hb (g/dL)	11.9 ± 1.1	11.7 ± 1.1	11.7 ± 1.1	10.5	-	11.7 ± 1.1
Ferritin (µg/L)	21.7 ± 1.9	23.8 ± 2.0	21.0 ± 2.0	32.6	-	22.1 ± 2.0
TfR (µg/mL)	4.3 ± 1.6	6.1 ± 1.6	5.9 ± 1.3	6.1	-	5.9 ± 1.5
TfR/Ferritin	0.2 ± 2.3	0.3 ± 2.3	0.3 ± 2.0	0.2	-	0.3 ± 2.2
Iron (mg/kg)	4.3 ± 2.9	4.2 ±5.2	3.0 ± 2.6	4.5	-	3.6 ± 3.9
Zinc (mg/L)	62.1 ± 5.2	62.2 ± 8.7	65.6 ± 9.9	66.4	-	63.9 ± 9.1
AGP (g/L)	0.9 ± 1.4	1.0 ± 1.6	1.1 ± 1.5	0.9	-	1.0 ± 1.5

Table 6.9

Iron and zinc indices of children by their performance on simultaneous K-ABC-II

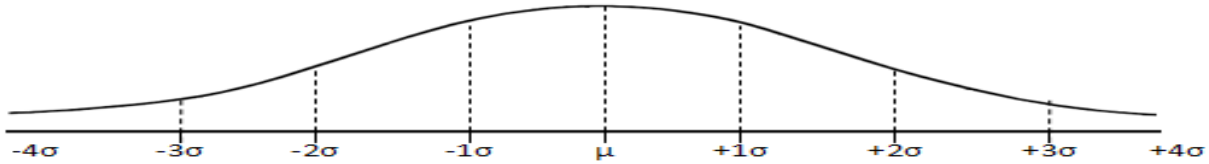


	Lower extreme	Below average	Normal Range	Above Average	Upper extreme	Total
n (%)	3 (3.7)	35 (42.7)	43 (52.4)	1 (1.2)	0 (0)	N= 82
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Hb (g/dL)	12.0 ± 1.1	11.8 ± 1.1	11.7 ± 1.1	10.5	-	11.7 ± 1.1
Ferritin (µg/L)	17.5 ± 1.9	24.3 ± 2.1	20.5 ± 2.0	32.6	-	22.1 ± 2.0
TfR (µg/mL)	9.3 [a] ± 1.7	5.8 [b] ± 1.6	5.7 [b] ± 1.4	6.1	-	5.9 ± 1.5
TfR/Ferritin	0.5 ± 1.3	0.2 ± 2.4	0.3 ± 2.0	0.2	-	0.3 ± 2.2
Iron (mg/kg)	0.8 ± 1.0	4.4 ±5.2	3.1 ± 2.5	4.5	-	3.6 ± 3.9
Zinc (mg/L)	63.1 ± 3.5	62.8 ± 7.6	64.8 ± 10.5	66.4	-	63.9 ± 9.1
AGP (g/L)	0.8 ± 1.4	1.0 ± 1.6	1.1 ± 1.5	0.9	-	1.0 ± 1.5

[a,b] Sig. at p<.05

Table 6.10

Iron and zinc indices of children by their performance on sequential K-ABC-II



	Lower extreme	Below average	Normal Range	Above Average	Upper extreme	Total
n (%)	2 (3.7)	30 (42.7)	54 (52.4)	0 (0)	0 (0)	N= 86
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Hb (g/dL)	12.2 ± 1.1	11.9 ± 1.1	11.6 ± 1.1	-	-	11.7 ± 1.1
Ferritin (µg/L)	29.6 ± 1.1	24.8± 1.9	21.3 ± 2.1	-	-	22.6 ± 2.0
TfR (µg/mL)	3.1 ± 1.2	5.8 ± 1.5	6.1 ± 1.4	-	-	5.9 ± 1.5
TfR/Ferritin	0.1 ± 1.3	0.2 ± 2.1	0.3 ± 2.2	-	-	0.3 ± 2.2
Iron (mg/kg)	6.6 ± 1.0	3.7 ± 2.7	3.5 ± 4.4	-	-	3.6 ± 3.9
Zinc (mg/L)	52.4 ± 1.9	64.1 ± 8.5	64.4 ± 9.1	-	-	64.0 ± 9.0
AGP (g/L)	1.0 ± 1.2	1.1 ± 1.5	1.0 ± 1.6	-	-	1.1 ± 1.5

Table 6.11

Iron and zinc indices of children classified by subtest performance

(below 20th percentile versus above 80th percentile)

Subtest		Iron and zinc indices (Mean ±SD) 20th percentile	80th percentile
Conceptual thinking			
(n: lower=17, upper=17)	Hb (g/dL)	11.8 ± 1.1	11.5 ± 1.1
	Ferritin (µg/L)	17.2 ± 2.6	23.2 ± 2.0
	TfR (µg/mL)	5.4 ± 1.6	6.7 ± 1.4
	TfR/Ferritin	0.3 ± 2.3	0.3± 2.5
	Iron (mg/kg)	3.0 ± 3.0	2.7 ± 3.3
	Zinc (µg/dL)	65.1 ± 9.5	65.5 ± 8.9
	AGP (g/L)	0.9 ± 1.7	1.2 ± 1.4
Face recognition			
(n: lower=17, upper=16)	Hb (g/dL)	11.9 ± 1.1	11.8 ± 1.1
	Ferritin (µg/L)	20.1 ± 1.9	23.8 ± 1.8
	TfR (µg/mL)	4.7 ± 1.5	6.4 ± 1.4
	TfR/Ferritin	0.2 ± 2.4	0.3 ± 1.9
	Iron (mg/kg)	3.9 ± 3.1	3.3 ± 2.3
	Zinc (µg/dL)	62.4 ± 2.4	68.1 ± 2.3
	AGP (g/L)	7.8 ± 8.0	7.8 ± 12.4
Number recall			
(n: lower=17, upper=18)	Hb (g/dL)	11.8 ± 1.1	11.6 ± 1.1
	Ferritin (µg/L)	28.9 ± 1.8	24.6 ± 2.1
	TfR (µg/mL)	5.1 ± 1.5	6.1 ± 1.4
	TfR/Ferritin	0.2 ± 1.9	0.2 ± 2.1
	Iron (mg/kg)	4.7 ± 2.3	5.0 ± 6.6
	Zinc (µg/dL)	62.0 ± 10.2	65.5 ± 10.8
	AGP (g/L)	1.2 ± 1.5	1.1 ± 1.5
Triangles			
(n: lower=18, upper=17)	Hb (g/dL)	11.9 ± 1.1	11.6 ± 1.1
	Ferritin (µg/L)	26.8 ± 2.2	22.7 ± 1.6
	TfR (µg/mL)	6.1 ± 1.5	5.3 ± 1.3
	TfR/Ferritin	0.2 ± 2.2	0.2 ± 1.7
	Iron (mg/kg)	3.8 ± 2.9	3.7 ± 1.9
	Zinc (µg/dL)	66.1 ± 8.7	65.6 ± 7.4
	AGP (g/L)	1.1 ± 1.6	1.1 ± 1.4

Table 6.11-*continued*

Iron and zinc indices of children classified by subtest performance

(below 20th percentile versus above 80th percentile)

Subtest		Iron and zinc indices (Mean ± SD) 20th percentile	80th percentile
Word order			
(n: lower=18, upper=17)	Hb (g/dL)	11.8 ± 1.1	11.8 ± 1.1
	Ferritin (µg/L)	26.7 ± 1.9	21.0 ± 2.0
	TfR (µg/mL)	5.7 ± 1.6	6.9 ± 1.3
	TfR/Ferritin	0.2 ± 2.2	0.3 ± 2.1
	Iron (mg/kg)	4.1 ± 2.9	2.6 ± 2.6
	Zinc (µg/dL)	65.3 ± 9.9	62.9 ± 7.4
	AGP (g/L)	1.1 ± 1.7	1.1 ± 1.4
Pattern reasoning			
(n: lower=10, upper=10)	Hb (g/dL)	11.9 ± 1.1	11.9 ± 1.1
	Ferritin (µg/L)	24.5 ± 2.2	20.1 ± 1.5
	TfR (µg/mL)	6.5 ± 1.6	7.1 ± 1.3
	TfR/Ferritin	0.3 ± 2.5	0.3 ± 1.5
	Iron (mg/kg)	3.3 ± 3.4	1.5 ± 2.8
	Zinc (µg/dL)	67.5 ± 7.1	63.0 ± 5.4
	AGP (g/L)	1.4 ± 1.3	1.1 ± 1.5
Hand movement			
(n: lower=18, upper=17)	Hb (g/dL)	11.8 ± 1.1	11.7 ± 1.1
	Ferritin (µg/L)	26.0 ± 2.0	16.1 ± 2.3
	TfR (µg/mL)	5.5 ± 1.5	5.6 ± 1.3
	TfR/Ferritin	0.2 ± 2.2	0.3 ± 2.4
	Iron (mg/kg)	5.5 ± 6.3	2.3 ± 3.2
	Zinc (µg/dL)	66.3 ± 11.2	62.9 ± 7.8
	AGP (g/L)	1.1 ± 1.5	1.1 ± 1.4

Table 6.12

Mean scores of cognitive tests for children iron status groups

	G1: Iron sufficient, not anemic Mean ±SD	**G2:** Iron deficient, not anemic (ID) Mean ±SD	**G3:** Iron deficiency anemia (IDA) Mean ±SD	**G4:** Anemia of chronic disease (ACD) Mean ±SD
	(n=55)	*(n=13)*	*(n=9)*	*(n=15)*
Ferritin (µg/L)	27.2 ± 1.7	11.3 ± 2.3	10.0 ± 1.2	24.7 ± 1.4
TfR (µg/mL)	5.3 ± 1.4	7.5 ± 1.5	7.2 ± 1.4	6.4 ± 1.4
TfR/Ft	0.2 ± 1.8	0.7 ± 1.8	0.7 ± 1.4	0.3 ± 1.5
AGP (g/L)	1.0 ± 1.6	1.0 ± 1.4	1.1 ± 1.3	1.0 ± 1.4
Hb (g/dL)	12.1 ± 1.1	12.0 ± 1.0	10.7 ± 1.1	11.0 ± 1.0
Zinc (mg/L)	65.2 ± 9.0	63.3 ± 8.6	64.4 ± 11	63.9 ± 12
PPVT-IV	77.0 ± 14.2 *(n=49)*	80.9 ± 10.1 *(n=13)*	78.3 ± 13.1 *(n=8)*	82.8 ± 15.6 *(n=14)*
Non verbal index K-ABC-II	84.4 ± 12.9 *(n=48)*	86.1 ± 13.9 *(n=12)*	87.6 ± 8.7 *(n=7)*	89.5 ± 10.9 *(n=14)*
Simultaneous K-ABC-II	87.1 ± 11.7 *(n=48)*	86.6 ± 13.5 *(n=12)*	85.9 ± 9.5 *(n=7)*	90.9 ± 10.5 *(n=14)*
Sequential K-ABC-II	85.8 ± 9.3 *(n=51)*	90.6 ± 8.2 *(n=12)*	90.4 ± 7.6 *(n=8)*	88.4 ± 11.5 *(n=14)*
Conceptual thinking	7.6 ± 2.4 *(n=51)*	9.2 ± 3.0 *(n=12)*	7.6 ± 3.6 *(n=8)*	7.8 ± 2.3 *(n=14)*
Face recognition	7.7 ± 3.1 *(n=48)*	7.7 ± 2.4 *(n=12)*	9.3 ± 3.4 *(n=7)*	9.3 ± 2.0 *(n=14)*
Number recall	9.2 ± 2.2 *(n=51)*	10.1 ± 2.5 *(n=12)*	10.8 ± 1.7 *(n=8)*	10.1 ± 2.8 *(n=14)*
Triangles	7.9 ± 2.8 *(n=51)*	7.1 ± 2.5 *(n=12)*	6.8 ± 1.4 *(n=8)*	8.8 ± 2.8 *(n=14)*
Word order	5.9 ± 1.5 *(n=51)*	6.7 ± 1.3 *(n=12)*	6.0 ± 1.2 *(n=8)*	5.9 ± 1.7 *(n=14)*
Pattern reasoning	8.9 ± 1.7 *(n=27)*	9.2 ± 1.9 *(n=5)*	8.4 ± 1.6 *(n=7)*	9.1 ± 1.1 *(n=11)*
Hand movement	8.3 ± 2.4 *(n=50)*	8.4 ± 2.3 *(n=12)*	9.0 ± 2.0 *(n=8)*	8.3 ± 2.5 *(n=14)*

Table 6.13

Mean scores of cognitive tests by zinc status

	Plasma zinc	
	≥ 57.0 µg/dL	**< 57 µg/dL**
	Mean ± SD	Mean ± SD
PPVT-IV	78.5 ± 14.6 *(n=67)*	79.2 ± 9.6 *(n=18)*
Non verbal index K-ABC-II	86.0 ± 12.8 *(n=64)*	84.4 ± 10.8 *(n=18)*
Simultaneous K-ABC-II	88.0 ± 12.0 *(n=64)*	85.7 ± 9.4 *(n=18)*
Sequential K-ABC-II	88.2 ± 9.1 *(n=68)*	83.4 ± 10.3 *(n=18)*
Conceptual thinking	7.9 ± 2.5 *(n=68)*	7.6 ± 2.3 *(n=18)*
Face recognition	8.0 ± 2.9 *(n=64)*	8.3 ± 2.8 *(n=18)*
Number recall	9.9 [a] ± 2.3 *(n=68)*	8.5 [b] ± 2.4 *(n=18)*
Triangles	7.8 ± 2.7 *(n=68)*	7.8 ± 2.4 *(n=18)*
Word order	6.1 ± 1.5 *(n=68)*	5.8 ± 1.4 *(n=18)*
Pattern reasoning	9.0 ± 1.7 *(n=37)*	8.0 ± 0.9 *(n=13)*
Hand movement	8.5 ± 2.4 *(n=67)*	8.1 ± 2.3 *(n=18)*

[a,b] Sig.at p<.05

Table 6.14

Mean scores of cognitive tests by sex

	Gender	
	Males Mean ±SD	**Females** Mean ±SD
PPVT-IV *(M=47, F=38)*	79.5 ± 12.9	77.6 ± 14.7
Non verbal index K-ABC-II *(M=46, F=36)*	84.4 ± 11.6	87.3 ± 13.2
Simultaneous K-ABC-II *(M=46, F=36)*	86.7 ± 10.9	88.6 ± 12.2
Sequential K-ABC-II *(M=48, F=38)*	86.7 ±9.8	87.8± 9.1
Conceptual thinking *(M=48, F=38)*	7.7 ± 2.9	8.1± 2.2
Face recognition *(M=46, F=36)*	7.4 ± 2.8	9.0 ± 2.8
Number recall *(M=48, F=38)*	9.4 ± 2.4	9.8 ± 2.3
Triangles *(M=48, F=38)*	7.8 ± 2.7	7.8 ± 2.6
Word order *(M=48, F=38)*	6.0 ± 1.5	6.0 ± 1.4
Pattern reasoning *(M=32, F=18)*	9.0 ± 1.5	8.8 ± 1.7
Hand movement *(M=47, F=38)*	8.5 ± 2.1	8.2 ± 2.6

Table 6.15

Mean scores of cognitive tests by breast feeding history

	Did the child breast fed?	
	Yes	**No**
	Mean ±SD	Mean ±SD
PPVT-IV *(Yes=73, No=12)*	79.0 ± 14.1	76.0 ± 11.2
Non verbal index K-ABC-II *(Yes=70, No=12)*	86.0 ± 12.3	83.5 ± 12.8
Simultaneous K-ABC-II *(Yes=70, No=12)*	88.2 ± 11.6	83.4 ± 10.0
Sequential K-ABC-II *(Yes=74, No=12)*	87.3 ± 9.6	86.9 ± 9.3
Conceptual thinking *(Yes=74, No=12)*	8.0 ± 2.7	7.3 ± 1.8
Face recognition *(Yes=70, No=12)*	8.1 ± 2.9	8.2 ± 2.8
Number recall *(Yes=74, No=12)*	9.7 ± 2.4	9.2 ± 2.2
Triangles *(Yes=74, No=12)*	7.9 ± 2.7	7.1 ± 2.3
Word order *(Yes=74, No=12)*	6.0 ± 1.4	6.3 ± 1.7
Pattern reasoning *(Yes=43, No=7)*	9.0 ± 1.6	8.7 ± 0.8
Hand movement *(Yes=73, No=12)*	8.3 ± 2.2	8.5 ± 3.0

Table 6.16

Mean scores of cognitive tests by kindergarten attendance

	Kindergarten attendance	
	Yes	**No**
	Mean ±SD	Mean ±SD
PPVT-IV *(Yes=47, No=38)*	81.2 ± 15.2	75.6 ± 11.0
Non verbal index K-ABC-II *(Yes=44, No=38)*	89.3[a] ± 12.1	81.5[b] ± 11.4
Simultaneous K-ABC-II *(Yes=44, No=38)*	90.3[c] ± 11.0	84.2[d] ± 11.3
Sequential K-ABC-II *(Yes=44, No=38)*	88.2 ± 10.2	86.1 ± 8.5
Conceptual thinking *(Yes=48, No=38)*	8.3 ± 2.9	7.3 ± 2.1
Face recognition *(Yes=44, No=38)*	8.7 ± 2.7	7.4 ± 3.0
Number recall *(Yes=44, No=38)*	9.9 ± 2.4	9.3 ± 2.2
Triangles *(Yes=44, No=38)*	8.4 ± 2.8	7.0 ± 2.2
Word order *(Yes=44, No=38)*	6.1[c] ± 1.7	5.9[d] ± 1.1
Pattern reasoning *(Yes=44, No=38)*	8.7 ± 1.6	9.6 ± 1.3
Hand movement *(Yes=44, No=38)*	9.0[a] ± 2.4	7.6[b] ± 2.1

[a,b] Sig. at p<.005, [c,d] Sig. at p<.05

Table 6.17

Mean scores of cognitive tests by children's hours of active play

	hours child spend in active play	
	≥ 5 hr/day	**< 5 hr/day**
	Mean ± SD	Mean ± SD
PPVT-IV	75.9[d] ± 10.0 *(n=21)*	79.5[c] ± 14.6 *(n=60)*
Non verbal index K-ABC-II	81.3[b] ± 12.2 *(n=22)*	87.3[a] ± 12.1 *(n=60)*
Simultaneous K-ABC-II	85.4 ± 12.7 *(n=22)*	88.3 ± 11.0 *(n=64)*
Sequential K-ABC-II	82.1 ± 8.2 *(n=22)*	89.0 ± 9.3 *(n=64)*
Conceptual thinking	7.2 ± 2.1 *(n=22)*	8.1 ± 2.7 *(n=64)*
Face recognition	7.2 ± 2.7 *(n=22)*	8.4 ± 2.9 *(n=60)*
Number recall	8.3[b] ± 2.2 *(n=22)*	10.1[a] ± 2.2 *(n=64)*
Triangles	7.9 ± 2.7 *(n=22)*	7.8 ± 2.7 *(n=64)*
Word order	5.6 ± 1.1 *(n=22)*	6.2 ± 1.4 *(n=54)*
Pattern reasoning	9.1 ± 1.4 *(n=11)*	8.9 ± 1.6 *(n=39)*
Hand movement	7.4[d] ± 2.0 *(n=22)*	8.7[c] ± 2.4 *(n=63)*

[a,b] Sig. at p<.005, [c,d] Sig. at p<.05

Table 6.18

Mean scores of cognitive tests by parental level of education

	Mothers' highest level of education		Fathers' highest level of education	
	High school or above	**Less than high school**	**High school or above**	**Less than high school**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
PPVT-IV	80.6 ± 13.5 *(n=52)*	75.0 ± 13.4 *(n=30)*	82.2 [a] ± 12.6 *(n=74)*	72.6 [b] ± 10.9 *(n=20)*
Non verbal index K-ABC-II	88.6 [a] ± 11.7 *(n=52)*	80.6 [b] ± 11.9 *(n=30)*	89.0 [c] ± 12.4 *(n=47)*	80.5 [d] ± 10.4 *(n=20)*
Simultaneous K-ABC-II	90.3 [a] ± 10.7 *(n=56)*	82.6 [b] ± 11.3 *(n=30)*	91.1 [a] ± 11.1 *(n=51)*	82.6 [b] ± 10.0 *(n=20)*
Sequential K-ABC-II	88.0 ± 9.6 *(n=55)*	85.8 ± 9.2 *(n=30)*	88.2 ± 9.9 *(n=50)*	84.1 ± 9.6 *(n=20)*
Conceptual thinking	8.2 ± 9.6 *(n=56)*	7.3 ± 9.2 *(n=30)*	8.4 ± 2.7 *(n=51)*	7.0 ± 1.9 *(n=20)*
Face recognition	8.3 ± 2.7 *(n=52)*	7.8 ± 3.2 *(n=30)*	8.6 [g] ± 3.0 *(n=47)*	7.1 [f] ± 2.2 *(n=20)*
Number recall	9.6 ± 2.4 *(n=56)*	9.6 ± 2.3 *(n=30)*	9.8 ± 2.4 *(n=51)*	8.9 ± 2.5 *(n=20)*
Triangles	8.4 [c] ± 2.7 *(n=56)*	6.7 [d] ± 2.2 *(n=30)*	8.42 ± 3.0 *(n=51)*	7.1 ± 2.0 *(n=20)*
Word order	6.3 [g] ± 1.5 *(n=56)*	5.6 [f] ± 1.3 *(n=30)*	6.2 ± 1.5 *(n=51)*	5.7 ± 1.2 *(n=20)*
Pattern reasoning	8.7 ± 1.6 *(n=35)*	9.3 ± 1.3 *(n=15)*	8.6 ± 1.5 *(n=33)*	9.4 ± 1.5 *(n=9)*
Hand movement	8.8 [g] ± 2.4 *(n=56)*	7.5 [f] ± 1.9 *(n=29)*	8.7 ± 2.3 *(n=50)*	7.8 ± 2.5 *(n=20)*

[a,b] Sig. at p<.005, [c,d] Sig. at p<.01, [g,f] Sig. at p<.05

Table 6.19

Mean scores of cognitive tests by family income after rent deduction

	Family income after rent deduction	
	Income ≥ $ 350	**Income < $ 350**
	Mean ± SD	Mean ± SD
PPVT-IV	84.5 [g] ± 13.9 *(n=23)*	76.2 [f] ± 13.0 *(n=61)*
Non verbal index K-ABC-II	92.8 [c] ± 13.4 *(n=20)*	83.5 [d] ±11.2 *(n=61)*
Simultaneous K-ABC-II	94.8 [c] ± 12.2 *(n=20)*	85.2 [d] ± 10.4 *(n=61)*
Sequential K-ABC-II	87.9 ± 10.2 *(n=24)*	86.9 ± 9.3 *(n=61)*
Conceptual thinking	8.2 ± 3.1 *(n=24)*	7.7 ± 2.4 *(n=61)*
Face recognition	8.9 ± 3.1 *(n=20)*	7.8 ± 2.8 *(n=61)*
Number recall	9.7 ± 2.5 *(n=24)*	9.5 ± 2.3 *(n=61)*
Triangles	9.5 [a] ± 3.5 *(n=24)*	7.1 [b] ± 1.9 *(n=61)*
Word order	6.2 ± 1.7 *(n=24)*	6.0 ± 1.4 *(n=51)*
Pattern reasoning	8.5 ± 1.8 *(n=18)*	9.2 ± 1.3 *(n=61)*
Hand movement	9.4 [g] ± 2.0 *(n=23)*	8.0 [f] ± 2.3 *(n=51)*

[a,b] Sig. at p<.000, [c,d] Sig. at p<.005, [g,f] Sig. at p<.05

Table 6.20

Stepwise regression of children's performance on cognitive tests in relation to socioeconomic variables

Model Summary					Coefficients			
	R^2	ΔR^2	p-value	Variables	B	SE	β	p-value
Test# 1: PPVT-IV, (N=69)								
Father education	.19	.19	.000	Father education	4.0	1.0	.44	.000
				Income			.13	.316
				Mother education			.14	.229
				KG attendance			.08	.491
				Hours in active play			-.02	.873
				Hours on TV			.11	.305
Test# 2: Non verbal index K-ABC-II (N=66)								
Mother education	.14	.14	.002	Mother education	3.0	1.2	.30	.014
Mother education, income	.20	.06	.031	income	.02	.01	.26	.031
				Father education			.14	.272
				KG attendance			.13	.314
				Hours in active play			-.13	.279
				Hours on TV			.08	.515
Test# 3: Simultaneous K-ABC-II, (N=66)								
Mother education	.14	.14	.002	Mother education	2.9	1.1	.31	.011
Mother education, Father education	.20	.06	.039	Father education	2.1	1.0	.25	.039
				income			.15	.248
				KG attendance			.08	.504
				Hours in active play			-.08	.513
				Hours on TV			.02	.898

Table 6.20-*continued*

Stepwise regression of children performance on cognitive tests in relation to socioeconomic variables

Model Summary					Coefficients			
	R^2	ΔR^2	p-value	Variables	B	SE	β	p-value
Test # 8: Triangles, (N=70)								
Mother education	.12	.12	.003	Mother education	.79	.26	.35	.003
				income			.21	.084
				Father education			.18	.126
				KG attendance			.18	.143
				Hours in active play			-.002	.983
				Hours on TV			-.02	.877
Test# 9: Word order, (N=70)								
Hours on TV	.07	.07	.032	Hours on TV	.04	.02	.26	.032
				income			.08	.511
				Mother education			.20	.087
				Father education			.15	.209
				KG attendance			.16	.180
				Hours in active play			-.16	.168
Test # 11: Hand movement, (N=69)								
KG attendance	.13	.13	.003	KG attendance	1.7	.54	.36	.003
				income			.08	.571
				Mother education			.21	.080
				Father education			.17	.161
				Hours in active play			-.15	.210
				Hours on TV			.04	.717

CHAPTER VII

CONCLUSION

This study had three objectives corresponding to the three manuscripts in the dissertation. The 1st objective was to assess vitamin D status in women of childbearing age and their preschool children in Jordan during the summer. The study found a high prevalence of vitamin D deficiency among women of childbearing age (48.9% had 25(OH) D <25.0 nmol/L, and 97.8% had 25(OH) D <50.0 nmol/L), and to a lesser extent among their children (39% had 25(OH) D <50.0 nmol/L). Natural foods rich in vitamin D are not commonly consumed by the larger population in Jordan, and there are no governmental regulations that mandate vitamin D fortification for the processed milk and milk products produced in Jordan. Unfortunately, there still are no recommended dietary guidelines concerning vitamin D intake in the Middle East and North Africa region (MENA), despite the high prevalence of vitamin D deficiency.

Despite the abundant sunlight during summer, the vitamin D status is a concern for young mothers and children in Jordan. Nutrition education programs should be implemented to inform women about the consumption of vitamin D fortified milk products as well as the importance of sufficient exposure to sunlight to attain optimal vitamin D status for themselves and their children. There is a need to assess vitamin D

status at the national level so that strategies for vitamin D fortification and supplementation are developed and implemented.

The 2nd objective of the study was to determine the prevalence of iron and zinc deficiency in mothers and their preschool children, and to evaluate the diagnostic efficiency of serum TfR and different TfR/ferritin ratios in identifying iron deficiency in women of child-bearing age and preschool children in Jordan. The study found widespread co-existing micronutrient deficiencies among mothers and to a lesser extent among children based on low values for hemoglobin, ferritin, and zinc. Besides, there was a high prevalence of inflammation among study participants and especially among women. Flour fortification with iron has been implemented in Jordan few years ago and still anemia and iron deficiency are a concern for mothers in Jordan. Given that there are no estimates of the national prevalence and distribution of zinc deficiency in Jordan, and that no previous study has assessed plasma zinc in the region, the high prevalence of zinc deficiency among study participants (58% and 56% among women and children, respectively) is alarming. There is a need to assess zinc status on the national level to provide information for developing a national public health policy that ensures fortification of staple foods with zinc. Along with food fortification, there is a need to conduct studies to examine the availability of zinc in the local diet.

An important finding in this study was the high prevalence of overweight (37%) and obesity (40%) among women. This could be explained by sedentary life style and high number of pregnancies. This highlights the need for the implementation of

nutrition education and health awareness programs that stimulate behavior change and promote healthy lifestyles especially in women.

The 3rd aim of the study was to examine any association between socioeconomic variables, measures of iron and zinc status, and children's performance on tests of cognitive functions. Although zinc deficiency was more prevalent among children than iron deficiency, children's performance on only one subtest was affected by their zinc status, while for the rest of cognitive tests neither zinc deficiency nor iron deficiency was associated with children's performance on any of the administered cognitive tests. An important finding of this study that relates to populations with lower socioeconomic status in Jordan and developing countries is that parent's education and especially mothers' education was an important component of children performance on cognitive tests. Women's education may not only improve their lives but also the lives of their children.

The interaction between cognitive psychology and micronutrients is an emerging research area. This is the first study to assess the effect of iron and zinc deficiency on cognition in Jordan. Certainly, larger studies are needed to assess short-term and long-term developmental outcomes in iron deficient infants, young children, preschool children, school children, and adolescents in Jordan. The long term effect of iron and zinc deficiency may not have been captured by this study, but certainly rigorous randomized controlled trials of iron and zinc supplements with larger sample size can provide detailed information and help to make firm conclusions that can be used to develop means to enhance child development in Jordan.

REFERENCES

Adami S, Viapiana O, Gatti D, Idolazzi L, Rossini M (2008). Relationship between serum parathyroid hormone, vitamin D sufficiency, age, and calcium intake. *Bone* **42:** 267-270.

Adams JS, Hewison M (2008). Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab* **4:** 80-90.

Agarwal, N. and J. T. Prchal (2009). "Anemia of chronic disease (anemia of inflammation)." Acta Haematol **122**(2-3): 103-8.

Al-Arabi KM, Elidrissy AW, Sedrani SH (1984). Is avoidance of sunlight a cause of fractures of the femoral neck in elderly Saudis? *Trop Geogr Med* **36:** 273-279.

Alagol F, Shihadeh Y, Boztepe H, Tanakol R, Yarman S, Azizlerli H *et al* (2000). Sunlight exposure and vitamin D deficiency in Turkish women. *J Endocrinol Invest* **23:** 173-177.

Albsoul-Younes AM, Al-Ramahi RJ, Al-Safi SA (2004). Frequency of anemia in pregnancy in Northern Jordan. *Saudi Medical Journal* **25:** 1525-1527.

Algarin C, Peirano P, Garrido M, Pizarro F, Lozoff B (2003). Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. *Pediatr Res* **53:** 217-223.

Allali F, El Aichaoui S, Saoud B, Maaroufi H, Abouqal R, Hajjaj-Hassouni N (2006). The impact of clothing style on bone mineral density among post menopausal women in Morocco: a case-control study. *BMC Public Health* **6:** 135.

Allali F, El Aichaoui S, Khazani H, Benyahia B, Saoud B, El Kabbaj S *et al* (2008). High prevalence of hypovitaminosis D in Morocco: relationship to lifestyle, physical performance, bone markers, and bone mineral density. *Semin Arthritis Rheum*.

Aoki S, Okada Y, Nishimura K, Barkovich AJ, Kjos BO, Brasch RC *et al* (1989). Normal deposition of brain iron in childhood and adolescence: MR imaging at 1.5 T. *Radiology* **172:** 381-385.

Aoyama A (1999). *Towards a virtuous circle : a nutrition review of the Middle East and North Africa. Human development network. Health Nutrition and Population Series.* . World Bank: Washington, D. C.

Arunabh S, Pollack S, Yeh J, Aloia JF (2003). Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab* **88:** 157-161.

Ashworth A, Morris SS, Lira PI, Grantham-McGregor SM (1998). Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. *Eur J Clin Nutr* **52:** 223-227.

Atli T, Gullu S, Uysal AR, Erdogan G (2005). The prevalence of Vitamin D deficiency and effects of ultraviolet light on Vitamin D levels in elderly Turkish population. *Arch Gerontol Geriatr* **40:** 53-60.

Aukett MA, Parks YA, Scott PH, Wharton BA (1986). Treatment with iron increases weight gain and psychomotor development. *Arch Dis Child* **61:** 849-857.

Autier P, Gandini S (2007). Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* **167:** 1730-1737.

Babiker MM, Bashir N, Sarsour N (1999). Prevalence of thalassaemia in schoolchildren in north-eastern Badia, Jordan. *Eastern Mediterranean Health Journal = La Revue De Sante De La Mediterranee Orientale = Al-Majallah Al-Sihhiyah Li-Sharq Al-Mutawassit* **5:** 1165-1170.

Bahijri SM (2001). Serum 25-hydroxy cholecalciferol in infants and preschool children in the Western region of Saudi Arabia. Etiological factors. *Saudi Med J* **22:** 973-979.

Basaran S, Guzel R, Coskun-Benlidayi I, Guler-Uysal F (2007). Vitamin D status: effects on quality of life in osteoporosis among Turkish women. *Qual Life Res* **16:** 1491-1499.

Baynes R, Bezwoda W, Bothwell T, Khan Q, Mansoor N (1986). The non-immune inflammatory response: serial changes in plasma iron, iron-binding capacity, lactoferrin, ferritin and C-reactive protein. *Scand J Clin Lab Invest* **46:** 695-704.

Beard J (2003). Iron deficiency alters brain development and functioning. *J Nutr* **133:** 1468S-1472S.

Beard J (2006). Iron. In: Bowman BA, Russell MR (eds). *Present Knowledge in Nutrition*, 9 edn. International Life science institute: Washignton, DC. pp 430-444.

Beard JL (2001). Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr* **131:** 568S-579S; discussion 580S.

Beard JL, Connor JR (2003). Iron status and neural functioning. *Annu Rev Nutr* **23:** 41-58.

Beard JL, Felt B, Schallert T, Burhans M, Connor JR, Georgieff MK (2006a). Moderate iron deficiency in infancy: biology and behavior in young rats. *Behav Brain Res* **170:** 224-232.

Beard JL, Murray-Kolb LE, Rosales FJ, Solomons NW, Angelilli ML (2006b). Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: the role of biomarkers for the acute phase response. *Am J Clin Nutr* **84:** 1498-1505.

Bekri S, Gual P, Anty R, Luciani N, Dahman M, Ramesh B *et al* (2006). Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* **131:** 788-796.

Bentley ME, Caulfield LE, Ram M, Santizo MC, Hurtado E, Rivera JA *et al* (1997). Zinc supplementation affects the activity patterns of rural Guatemalan infants. *J Nutr* **127:** 1333-1338.

Bentley ME, Griffiths PL (2003). The burden of anemia among women in India. *Eur J Clin Nutr* **57:** 52-60.

Bhatnagar S, Taneja S (2001). Zinc and cognitive development. *Br J Nutr* **85 Suppl 2:** S139-145.

Bitanihirwe BK, Cunningham MG (2009). Zinc: the brain's dark horse. *Synapse* **63:** 1029-1049.

Black MM (2003). The evidence linking zinc deficiency with children's cognitive and motor functioning. *Journal of Nutrition* **133 (Suppl 1):** 1473-1476.

Black MM, Baqui AH, Zaman K, Ake Persson L, El Arifeen S, Le K *et al* (2004a). Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. *Am J Clin Nutr* **80:** 903-910.

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

Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM (2007). High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. *J Nutr* **137:** 447-452.

Bothwell TH, Charlton RW, Cook JD, Finch CA (1979). *Iron Metabolism in Man*. Blackwell Scientific Publications: Oxford.

Bouillon R, Okamura WH, Norman AW (1995). Structure-function relationships in the

vitamin D endocrine system. *Endocr Rev* **16:** 200-257.

Brown CE, Dyck RH (2004). Distribution of zincergic neurons in the mouse forebrain. *J Comp Neurol* **479:** 156-167.

Brown KH (1998). Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. *Am J Clin Nutr* **68:** 425S-429S.

Brown KH, Wuehler SE. Zinc and Human Health: the Results of Recent Trials and Implications for Program Interventions and Research. Ottawa, Ontario, Canada, Micronutrient Initiative, 2000.

Brown KH, Peerson JM, Rivera J, H. AL (2002). Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **75:** 1062-1071.

Brown KH, Hess SY, guest editors (2009). International Zinc Nutrition Consultative Group (IZiNCG) technical document # 2. Systematic Reviews of Zinc Intervention Strategies. *Food Nutr Bull* **30:** S3-185.

Burhans MS, Dailey C, Beard Z, Wiesinger J, Murray-Kolb L, Jones BC *et al* (2005). Iron deficiency: differential effects on monoamine transporters. *Nutr Neurosci* **8:** 31-38.

Calvo MS, Whiting SJ, Barton CN (2004). Vitamin D fortification in the United States and Canada: current status and data needs. *Am J Clin Nutr* **80** 1710S-1716S.

Carnevale V, Modoni S, Pileri M, Di Giorgio A, Chiodini I, Minisola S *et al* (2001). Longitudinal evaluation of vitamin D status in healthy subjects from southern Italy: seasonal and gender differences. *Osteoporos Int* **12:** 1026-1030.

Castillo-Duran C, Perales CG, Hertrampf ED, Marin VB, Rivera FA, Icaza G (2001). Effect of zinc supplementation on development and growth of Chilean infants. *J Pediatr* **138:** 229-235.

Caulfield L, Black RE (2004). *Zinc Deficiency. Comparative Quantification of Health Risks: The Global and Regional Burden of Disease due to 25 Selected Major Risk Factors.* World Health Organization/Harvard University Press: Cambridge, MA.

Caulfield LE, Richard SA, Rivera JA, Musgrove P, Black RE (2006). Stunting, Wasting, and Micronutrient Deficiency Disorders In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB *et al* (eds). *Disease Control Priorities in Developing Countries*, 2 edn. Oxford University Press New York. pp 552-553.

Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW (1993). Growth and

body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. *Am J Clin Nutr* **57:** 344-352.

Chapuy MC, Schott AM, Garnero P, Hans D, Delmas PD, Meunier PJ (1996). Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. *J Clin Endocrinol Metab* **81:** 1129-1133.

Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S *et al* (1997). Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* **7:** 439-443.

Chen S, Sims GP, Chen XX, Gu YY, Lipsky PE (2007). Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol* **179:** 1634-1647.

Chowanadisai W, Kelleher SL, Lonnerdal B (2005). Maternal zinc deficiency reduces NMDA receptor expression in neonatal rat brain, which persists into early adulthood. *J Neurochem* **94:** 510-519.

Chung B, Matak P, McKie AT, Sharp P (2007). Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J Nutr* **137:** 2366-2370.

Cogswell ME, Looker AC, Pfeiffer CM, Cook JD, Lacher DA, Beard JL *et al* (2009). Assessment of iron deficiency in US preschool children and nonpregnant females of childbearing age: National Health and Nutrition Examination Survey 2003-2006. *Am J Clin Nutr* **89:** 1334-1342.

Colvin RA, Fontaine CP, Laskowski M, Thomas D (2003). Zn2+ transporters and Zn2+ homeostasis in neurons. *Eur J Pharmacol* **479:** 171-185.

Commission of the European Communities. Nutrient and energy Intaks for the European community. Luxembourg, Office for the official Publications of the European Communities
1993.

Connie M. Weaver (2006). Calcium. In: Barbara A. Bowman, Robert M. Russell (eds). *Present Knowledge in Nutrition*, 9th edn. ILSI (International Life Science Institute): Washington, DC.

Connor JR, Benkovic SA (1992). Iron regulation in the brain: histochemical, biochemical, and molecular considerations. *Ann Neurol* **32 Suppl:** S51-61.

Connor JR, Menzies SL (1996). Relationship of iron to oligodendrocytes and myelination. *Glia* **17:** 83-93.

Conrad ME, Benjamin BI, Williams HL, Foy AL (1967). Human absorption of hemoglobin-iron. *Gastroenterology* **53:** 5-10.

Cook JD, Lipschitz DA, Miles LE, Finch CA (1974). Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* **27:** 681-687.

Cook JD, Monsen ER (1977). Vitamin C, the common cold, and iron absorption. *Am J Clin Nutr* **30:** 235-241.

Cook JD, Morck TA, Lynch SR (1981). The inhibitory effect of soy products on nonheme iron absorption in man. *Am J Clin Nutr* **34:** 2622-2629.

Cook JD, Skikne BS, Baynes RD (1993). Serum transferrin receptor. *Annu Rev Med* **44:** 63-74.

Cook JD, Flowers CH, Skikne BS (2003). The quantitative assessment of body iron. *Blood* **101:** 3359-3364.

Cook JD (2005). Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol* **18:** 319-332.

Cook JD, Boy E, Flowers C, Daroca Mdel C (2005). The influence of high-altitude living on body iron. *Blood* **106:** 1441-1446.

Corapci F, Radan AE, Lozoff B (2006). Iron deficiency in infancy and mother-child interaction at 5 years. *J Dev Behav Pediatr* **27:** 371-378.

Cousins RJ (2006). Zinc. In: Bowman BA, Russell MR (eds). *Present Knowledge in Nutrition*, 9 edn. International Life science institute: Washignton, DC. pp 445-457.

Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D *et al* (2007). Effectiveness and safety of vitamin D in relation to bone health
*Evid Rep Technol Assess (Full Rep)***:** 1-235.

Cusick SE, Looker AC, Cogswell ME, Pfeiffer CM, Grummer-Strawn L (2008). Iron-status indicators. *Pediatrics* **121:** 651-652; author reply 652.

Davidsson L, Walczyk T, Morris A, Hurrell RF (1998). Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. *Am J Clin Nutr* **67:** 873-877.

Dawodu A, Absood G, Patel M, Agarwal M, Ezimokhai M, Abdulrazzaq Y *et al* (1998). Biosocial factors affecting vitamin D status of women of childbearing age in the United Arab Emirates. *J Biosoc Sci* **30:** 431-437.

Dawodu A, Dawson KP, Amirlak I, Kochiyil J, Agarwal M, Badrinath P (2001). Diet, clothing, sunshine exposure and micronutrient status of Arab infants and young children. *Ann Trop Paediatr* **21:** 39-44.

Dawodu A, Agarwal M, Hossain M, Kochiyil J, Zayed R (2003). Hypovitaminosis D and vitamin D deficiency in exclusively breast-feeding infants and their mothers in summer: a justification for vitamin D supplementation of breast-feeding infants. *J Pediatr* **142:** 169-173.

de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C (2007). Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. *Food Nutr Bull* **28:** S480-484.

De Onis M, Blossner M (2000). Prevalence and trends of overweight among preschool children in developing countries. *Am J Clin Nutr* **72:** 1032-1039.

Department of Statistics. Jordan Population and Family Health Survey. Statistical Year Book, Amman, Jordan, 1997.

Department of Statistics. Jordan Population and Family Health Survey 2007, Department of Statistics and Macro International Inc. Calverton, Maryland, USA, 2008.

Derman D, Sayers M, Lynch SR, Charlton RW, Bothwell TH, Mayet F (1977). Iron absorption from a cereal-based meal containing cane sugar fortified with ascorbic acid. *Br J Nutr* **38:** 261-269.

Dewey KG, Brown KH (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *United Nations University Food and Nutrition Bulletin* **24:** 5-28.

Disler PB, Lynch SR, Charlton RW, Bothwell TH, Walker RB, Mayet F (1975a). Studies on the fortification of cane sugar with iron and ascorbic acid. *Br J Nutr* **34:** 141-152.

Disler PB, Lynch SR, Charlton RW, Torrance JD, Bothwell TH, Walker RB *et al* (1975b). The effect of tea on iron absorption. *Gut* **16:** 193-200.

Dommergues JP, Archambeaud MP, Ducot B, Gerval Y, Hiard C, Rossignol C *et al* (1989). [Iron deficiency and psychomotor development tests. Longitudinal study between 10 months and 4 years of age]. *Arch Fr Pediatr* **46:** 487-490.

Dunn LM, Dunn DM (2007). *Peabody Picture Vocabulary Test*, 4 edn. NCS Pearson, Inc.: Minneapolis, MN.

Eckhardt CL, Torheim LE, Monterrubio E, Barquera S, Ruel MT (2008). The overlap of overweight and anaemia among women in three countries undergoing the nutrition transition. *Eur J Clin Nutr* **62:** 238-246.

El-Hajj Fuleihan G, Nabulsi M, Choucair M, Salamoun M, Hajj Shahine C, Kizirian A *et al* (2001). Hypovitaminosis D in healthy schoolchildren. *Pediatrics* **107:** E53.

El-Sonbaty MR, Abdul-Ghaffar NU (1996). Vitamin D deficiency in veiled Kuwaiti women. *Eur J Clin Nutr* **50:** 315-318.

Erikson KM, Pinero DJ, Connor JR, Beard JL (1997). Regional brain iron, ferritin and transferrin concentrations during iron deficiency and iron repletion in developing rats. *J Nutr* **127:** 2030-2038.

Erikson KM, Jones BC, Beard JL (2000). Iron deficiency alters dopamine transporter functioning in rat striatum. *J Nutr* **130:** 2831-2837.

Erikson KM, Jones BC, Hess EJ, Zhang Q, Beard JL (2001). Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. *Pharmacol Biochem Behav* **69:** 409-418.

Felt BT, Beard JL, Schallert T, Shao J, Aldridge JW, Connor JR *et al* (2006). Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for perinatal iron deficiency anemia in rats. *Behav Brain Res* **171:** 261-270.

Finch CA, Bellotti V, Stray S, Lipschitz DA, Cook JD, Pippard MJ *et al* (1986). Plasma ferritin determination as a diagnostic tool. *West J Med* **145:** 657-663.

Fischer Walker C, Black RE (2004). Zinc and the risk for infectious disease. *Annu Rev Nutr* **24:** 255-275.

Fischer Walker CL, Black RE (2007). Functional indicators for assessing zinc deficiency. *Food Nutr Bull* **28:** S454-479.

Fischer Walker CL, Ezzati M, Black RE (2009). Global and regional child mortality and burden of disease attributable to zinc deficiency. *Eur J Clin Nutr* **63:** 591-597.

Fishman JB, Rubin JB, Handrahan JV, Connor JR, Fine RE (1987). Receptor-mediated transcytosis of transferrin across the blood-brain barrier. *J Neurosci Res* **18:** 299-304.

Fishman SM, Christian P, West KP (2000). The role of vitamins in the prevention and control of anaemia. *Public Health Nutr* **3:** 125-150.

Fonseca V, Tongia R, el-Hazmi M, Abu-Aisha H (1984). Exposure to sunlight and vitamin D deficiency in Saudi Arabian women. *Postgrad Med J* **60:** 589-591.

Food Safety Authority of Ireland. Recommended Dietary Allowances for Ireland 1999, 1999.

Frederickson CJ, Suh SW, Silva D, Thompson RB (2000). Importance of zinc in the central nervous system: the zinc-containing neuron. *J Nutr* **130:** 1471S-1483S.

Frederickson CJ, Bush AI (2001). Synaptically released zinc: physiological functions and pathological effects. *Biometals* **14:** 353-366.

Freedman DM, Looker AC, Chang SC, Graubard BI (2007). Prospective study of serum vitamin D and cancer mortality in the United States. *J Natl Cancer Inst* **99:** 1594-1602.

Friel JK, Andrews WL, Matthew JD, Long DR, Cornel AM, Cox M *et al* (1993). Zinc supplementation in very-low-birth-weight infants. *J Pediatr Gastroenterol Nutr* **17:** 97-104.

Friel JK, Aziz K, Andrews WL, Harding SV, Courage ML, Adams RJ (2003). A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. *J Pediatr* **143:** 582-586.

Gannage-Yared MH, Chemali R, Yaacoub N, Halaby G (2000). Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. *J Bone Miner Res* **15:** 1856-1862.

Gardner JM, Powell CA, Baker-Henningham H, Walker SP, Cole TJ, Grantham-McGregor SM (2005). Zinc supplementation and psychosocial stimulation: effects on the development of undernourished Jamaican children. *Am J Clin Nutr* **82:** 399-405.

Gascon-Barre M (2005). The Vitamin D 25-Hydroxylase. In: Feldman D, Wesley Pike J, Glorieux FH (eds). *Vitamin D*. Elsevier. pp 47-67.

Ghannam NN, Hammami MM, Bakheet SM, Khan BA (1999). Bone mineral density of the spine and femur in healthy Saudi females: relation to vitamin D status, pregnancy, and lactation. *Calcif Tissue Int* **65:** 23-28.

Gibson RS, Vanderkooy PD, MacDonald AC, Goldman A, Ryan BA, Berry M (1989). A growth-limiting, mild zinc-deficiency syndrome in some southern Ontario boys with low height percentiles. *Am J Clin Nutr* **49:** 1266-1273.

Gibson RS (2005). *Principles of Nutritional Assessment*, 2 edn. Oxford university Press: Oxford New York.

Gibson RS (2006). Zinc: the missing link in combating micronutrient malnutrition in developing countries. *Proceedings of the Nutrition Society* **65:** 51-60.

Gibson RS, Hess SY, Hotz C, Brown KH (2008). Indicators of zinc status at the population level: a review of the evidence. *Br J Nutr* **99 Suppl 3:** S14-23.

Gillooly M, Bothwell TH, Torrance JD, MacPhail AP, Derman DP, Bezwoda WR *et al* (1983). The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *Br J Nutr* **49:** 331-342.

Ginde AA, Mansbach JM, Camargo CA, Jr. (2009). Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med* **169:** 384-390.

Gleerup A, Rossander-Hulten L, Hallberg L (1993). Duration of the inhibitory effect of calcium on non-haem iron absorption in man. *Eur J Clin Nutr* **47:** 875-879.

Gleerup A, Rossander-Hulthen L, Gramatkovski E, Hallberg L (1995). Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *Am J Clin Nutr* **61:** 97-104.

Golub MS, Keen CL, Gershwin ME, Hendrickx AG (1995). Developmental zinc deficiency and behavior. *J Nutr* **125:** 2263S-2271S.

Golub MS, Keen CL, Gershwin ME (2000). Moderate zinc-iron deprivation influences behavior but not growth in adolescent rhesus monkeys. *J Nutr* **130:** 354S-357S.

Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N (2000). Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. *Am J Clin Nutr* **72:** 472-475.

Grantham-McGregor S, Ani C (2001). A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr* **131:** 649S-666S; discussion 666S-668S.

Green TJ, Skeaff CM, Rockell JE, Venn BJ, Lambert A, Todd J *et al* (2008). Vitamin D status and its association with parathyroid hormone concentrations in women of child-bearing age living in Jakarta and Kuala Lumpur. *Eur J Clin Nutr* **62:** 373-378.

Greenberg AS, Obin MS (2006). Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr* **83:** 461S-465S.

Guillemant J, Taupin P, Le HT, Taright N, Allemandou A, Peres G *et al* (1999). Vitamin D status during puberty in French healthy male adolescents. *Osteoporos Int* **10:** 222-225.

Hallberg L, Hogdahl AM, Nilsson L, Rybo G (1966). Menstrual blood loss--a population study. Variation at different ages and attempts to define normality. *Acta Obstet Gynecol Scand* **45:** 320-351.

Hallberg L, Bjorn-Rasmussen E, Howard L, Rossander L (1979). Dietary heme iron absorption. A discussion of possible mechanisms for the absorption-promoting effect of meat and for the regulation of iron absorption. *Scand J Gastroenterol* **14:** 769-779.

Hallberg L, Rossander L (1982a). Effect of soy protein on nonheme iron absorption in man. *Am J Clin Nutr* **36:** 514-520.

Hallberg L, Rossander L (1982b). Effect of different drinks on the absorption of non-heme iron from composite meals. *Hum Nutr Appl Nutr* **36:** 116-123.

Hallberg L, Rossander L, Skanberg AB (1987). Phytates and the inhibitory effect of bran on iron absorption in man. *Am J Clin Nutr* **45:** 988-996.

Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* **53:** 112-119.

Hallberg L, Rossander-Hulten L (1991). Iron requirements in menstruating women. *Am J Clin Nutr* **54:** 1047-1058.

Hallberg L, Rossander-Hulten L, Brune M, Gleerup A (1992). Calcium and iron absorption: mechanism of action and nutritional importance. *Eur J Clin Nutr* **46:** 317-327.

Hallberg L, Hulten L, Gramatkovski E (1997). Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr* **66:** 347-356.

Hallberg L, Hulthen L (2000). Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *Am J Clin Nutr* **71:** 1147-1160.

Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM (2001). Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. *Am J Clin Nutr* **74:** 381-386.

Hamadani JD, Fuchs GJ, Osendarp SJ, Huda SN, Grantham-McGregor SM (2002). Zinc supplementation during pregnancy and effects on mental development and behaviour of infants: a follow-up study. *Lancet* **360:** 290-294.

Hanley JA, McNeil BJ (1983). A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* **148:** 839-843.

Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PV, Sarma KV *et al* (2007). High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. *The American Journal Of Clinical Nutrition* **85:** 1062-1067.

Hashemipour S, Larijani B, Adibi H, Javadi E, Sedaghat M, Pajouhi M *et al* (2004). Vitamin D deficiency and causative factors in the population of Tehran. *BMC Public Health* **4:** 38.

Hathcock JN, Shao A, Vieth R, Heaney RP (2007). Risk assessment for vitamin D. *Am J Clin Nutr* **85:** 6-18.

Hatun S, Ozkan B, Orbak Z, Doneray H, Cizmecioglu F, Toprak D *et al* (2005). Vitamin D deficiency in early infancy. *J Nutr* **135:** 279-282.

Health Council of the Netherlands. Towards an adequate intake of vitamin D, The Hague: Health Council of the Netherlands, 2008.

Heaney RP, Dowell MS, Hale CA, Bendich A (2003). Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D
J Am Coll Nutr **22:** 142-146.

Heaney RP (2004). Functional indices of vitamin D status and ramifications of vitamin D deficiency. *Am J Clin Nutr* **80:** 4.

Heaney RP (2005). The Vitamin D requirement in health and disease. *J Steroid Biochem Mol Biol* **97:** 13-19.

Henry HL (2005). The 25-Hydroxyvitamin D 1α-Hydroxylase. In: Feldman D, Wesley Pike J, Glorieux FH (eds). *Vitamin D*. Elsevier. pp 69-83.

Hess SY, Lonnerdal B, Hotz C, Rivera JA, Brown KH (2009). Recent advances in knowledge of zinc nutrition and human health. *Food Nutr Bull* **30:** S5-11.

Hesse GW (1979). Chronic zinc deficiency alters neuronal function of hippocampal mossy fibers. *Science* **205:** 1005-1007.

Heywood A, Oppenheimer S, Heywood P, Jolley D (1989). Behavioral effects of iron supplementation in infants in Madang, Papua New Guinea. *Am J Clin Nutr* **50:** 630-637; discussion 638-640.

Hill JM (1988). The distribution of iron in the brain. In: MBH Youdim (ed). *Brain Iron: Neurochemistry and Behavioural Aspects* Taylor & Francis: London, UK. pp 1-24.

Hill TR, Cotter AA, Mitchell S, Boreham CA, Dubitzky W, Murray L *et al* (2008). Vitamin D status and its determinants in adolescents from the Northern Ireland Young Hearts 2000 cohort. *Br J Nutr***:** 1-7.

Hochberg Z, Bereket A, Davenport M, Delemarre-Van de Waal HA, De Schepper J, Levine MA *et al* (2002). Consensus development for the supplementation of vitamin D in childhood and adolescence. *Horm Res* **58:** 39-51.

Holick MF, Matsuoka LY, Wortsman J (1989). Age, vitamin D, and solar ultraviolet. *Lancet* **2:** 1104-1105.

Holick MF (2003). Vitamin D: A millenium perspective. *J Cell Biochem* **88:** 296-307.

Holick MF (2005). Photobiology of Vitamin D. In: Feldman D, Wesley Pike J, Glorieux FH (eds). *Vitamin D*. Elsevier. pp 37-45.

Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT *et al* (2005). Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab* **90:** 3215-3224.

Holick MF (2006a). High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* **81:** 353-373

Holick MF (2006b). High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* **81:** 353-373.

Holick MF (2007). Vitamin D deficiency- Review Article. *N Engl J Med* **357:** 266-281.

Holick MF (2009). Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* **19:** 73-78.

Hollis BW (2005). Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* **135:** 317-322.

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* **95:** 2409-2415.

Howell GA, Welch MG, Frederickson CJ (1984). Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature* **308:** 736-738.

Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD (1992). Soy protein,

phytate, and iron absorption in humans. *Am J Clin Nutr* **56:** 573-578.

Hurtado EK, Claussen AH, Scott KG (1999). Early childhood anemia and mild or moderate mental retardation. *Am J Clin Nutr* **69:** 115-119.

Hypponen E, Power C (2007). Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* **85:** 860-868.

Iannotti LL, O'Brien KO, Chang SC, Mancini J, Schulman-Nathanson M, Liu S *et al* (2005). Iron deficiency anemia and depleted body iron reserves are prevalent among pregnant African-American adolescents. *J Nutr* **135:** 2572-2577.

Iannotti LL, Tielsch JM, Black MM, Black RE (2006). Iron supplementation in early childhood: health benefits and risks. *Am J Clin Nutr* **84:** 1261-1276.

Ibrahim AI, Hawamdeh ZM, Al-Smadi JT, Ammari BA (2008). Prevalence of overweight and obesity in urban and semi-urban Jordanian children aged 3-6 years. *Child Care Health Dev* **34:** 464-469.

Idjradinata P, Pollitt E (1993). Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *Lancet* **341:** 1-4.

Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, DC National Academy Press, 2005.

Islam MZ, Lamberg-Allardt C, Karkkainen M, Outila T, Salamatullah Q, Shamim AA (2002). Vitamin D deficiency: a concern in premenopausal Bangladeshi women of two socio-economic groups in rural and urban region. *Eur J Clin Nutr* **56:** 51-56.

Islam MZ, Akhtaruzzaman M, Lamberg-Allardt C (2006). Hypovitaminosis D is common in both veiled and nonveiled Bangladeshi women. *Asia Pac J Clin Nutr* **15:** 81-87.

IZiNCG (2004). The International Zinc Nutrition Consultative Group:Technical Document #1: Assessment of the Risk of Zinc Deficiency in Populations and Options for Its Control. *United Nations University Food and Nutrition Bulletin* **25:** S94-S204.

IZiNCG. Techincal Brief No.2: Assessing population zinc status with serum zinc concentration 2007a.

IZiNCG (2007b). Determining the prevalence of zinc deficiency: Assessment of dietary zinc intake. *IZiNCG Technical Brief No 3: Davis, CA*.

IZiNCG (2009). International Zinc Consultative Group. Technical Document # 2.

Systematic Reviews of Zinc Intervention Studies. *Food Nutr Bull* **30:** S3-S185.

Kamycheva E, Sundsfjord J, Jorde R (2004). Serum parathyroid hormone level is associated with body mass index. The 5th Tromso study. *Eur J Endocrinol* **151:** 167-172.

Kaufman AS, Kaufman NL (2004). *Kaufman Assessment Battery for Children*, 2 edn. AGS Publishing: Circle Pines, MN.

Kay AR, Toth K (2008). Is zinc a neuromodulator? *Sci Signal* **1:** re3.

Khader Y, Bateiha A, El-Khateeb M, Al-Shaikh A, Ajlouni K (2007). High prevalence of the metabolic syndrome among Northern Jordanians. *J Diabetes Complications* **21:** 214-219.

Khatib I, Hijazi S, Zoubi A. Nutritional Status of Jordanian Children. A Standardized Analysis of Anthropometric Data of Jordan's 1990 and 1997 Population and Family Health Survey (JPHS): . Columbia, Maryland, USA, A Report of IRD/Macro-International Inc., 1998.

Kilbride J, Baker T, Parapia L, Khoury S (1999). Incidence of Iron Deficiency Anemia in Infants in a Propective Study in Jordan. *European Journal of Haematology* **63:** 6.

Kirksey A, Rahmanifar A, Wachs TD, McCabe GP, Bassily NS, Bishry Z *et al* (1991). Determinants of pregnancy outcome and newborn behavior of a semirural Egyptian population. *Am J Clin Nutr* **54:** 657-667.

Kirksey A, Wachs TD, Yunis F, Srinath U, Rahmanifar A, McCabe GP *et al* (1994). Relation of maternal zinc nutriture to pregnancy outcome and infant development in an Egyptian village. *Am J Clin Nutr* **60:** 782-792.

Kudlacek S, Schneider B, Peterlik M, Leb G, Klaushofer K, Weber K *et al* (2003). Assessment of vitamin D and calcium status in healthy adult Austrians. *Eur J Clin Invest* **33:** 323-331.

Kwik-Uribe CL, Gietzen D, German JB, Golub MS, Keen CL (2000). Chronic marginal iron intakes during early development in mice result in persistent changes in dopamine metabolism and myelin composition. *J Nutr* **130:** 2821-2830.

Laaksi IT, Ruohola JP, Ylikomi TJ, Auvinen A, Haataja RI, Pihlajamaki HK *et al* (2006). Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men
Eur J Clin Nutr **60:** 1035-1038.

Laftah AH, Ramesh B, Simpson RJ, Solanky N, Bahram S, Schumann K *et al* (2004). Effect of hepcidin on intestinal iron absorption in mice. *Blood* **103:** 3940-3944.

Lamberg-Allardt CJ, Outila TA, Karkkainen MU, Rita HJ, Valsta LM (2001). Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? *J Bone Miner Res* **16:** 2066-2073.

Lee EJ, Oh EJ, Park YJ, Lee HK, Kim BK (2002). Soluble transferrin receptor (sTfR), ferritin, and sTfR/log ferritin index in anemic patients with nonhematologic malignancy and chronic inflammation. *Clin Chem* **48:** 1118-1121.

Levenson CW (2006). Regulation of the NMDA receptor: implications for neuropsychological development. *Nutr Rev* **64:** 428-432.

Lewis DK, Whitty CJ, Epino H, Letsky EA, Mukiibi JM, van den Broek NR (2007). Interpreting tests for iron deficiency among adults in a high HIV prevalence African setting: routine tests may lead to misdiagnosis. *Trans R Soc Trop Med Hyg* **101:** 613-617.

Li D (1998). Effects of iron deficiency on iron distribution and gamma-aminobutyric acid (GABA) metabolism in young rat brain tissues. *Hokkaido Igaku Zasshi* **73:** 215-225.

Liel Y, Ulmer E, Shary J, Hollis BW, Bell NH (1988). Low circulating vitamin D in obesity. *Calcif Tissue Int* **43:** 199-201.

Lin XM, Zhang J, Zou ZY, Long Z, Tian W (2008). Evaluation of serum transferrin receptor for iron deficiency in women of child-bearing age. *Br J Nutr* **100:** 1104-1108.

Lind T, Lonnerdal B, Stenlund H, Gamayanti IL, Ismail D, Seswandhana R *et al* (2004). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *Am J Clin Nutr* **80:** 729-736.

Lips P, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF (1999). An international comparison of serum 25-hydroxyvitamin D measurements
Osteoporosis International: A Journal Established As Result Of Cooperation Between The European Foundation For Osteoporosis And The National Osteoporosis Foundation Of The USA
9: 394-397.

Lips P (2001). Vitamin D Deficiency and Secondary Hyperparathyroidism in the Elderly: Consequences for Bone Loss and Fractures and Therapeutic Implications *Endocr Rev* **22:** 477-501

Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D *et al* (2001). A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. *J Clin*

Endocrinol Metab **86:** 1212-1221.

Lips P (2004). Which circulating level of 25-hydroxyvitamin D is appropriate? *J Steroid Biochem Mol Biol* **89-90:** 611-614.

Lips P, Hosking D, Lippuner K, Norquist JM, Wehren L, Maalouf G *et al* (2006). The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *J Intern Med* **260:** 245-254.

Looker AC (2005). Body fat and vitamin D status in black versus white women. *The Journal Of Clinical Endocrinology And Metabolism*
90: 635-640.

Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA (2008). Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. *Am J Clin Nutr* **88:** 1519-1527.

Lotfi A, Abdel-Nasser AM, Hamdy A, Omran AA, El-Rehany MA (2007). Hypovitaminosis D in female patients with chronic low back pain
Clin Rheumatol **26:** 1895-1901.

Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E *et al* (1987). Iron deficiency anemia and iron therapy effects on infant developmental test performance. *Pediatrics* **79:** 981-995.

Lozoff B, Jimenez E, Wolf AW (1991). Long-term developmental outcome of infants with iron deficiency. *N Engl J Med* **325:** 687-694.

Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW (2000). Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics* **105:** E51.

Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T (2006a). Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev* **64:** S34-43; discussion S72-91.

Lozoff B, Georgieff MK (2006). Iron deficiency and brain development. *Semin Pediatr Neurol* **13:** 158-165.

Lozoff B, Jimenez E, Smith JB (2006b). Double burden of iron deficiency in infancy and low socioeconomic status: a longitudinal analysis of cognitive test scores to age 19 years. *Arch Pediatr Adolesc Med* **160:** 1108-1113.

Lozoff B (2007). Iron deficiency and child development. *Food and Nutrition Bulletin* **28:**

S560-S571.

Lozoff B, Corapci F, Burden MJ, Kaciroti N, Angulo-Barroso R, Sazawal S *et al* (2007). Preschool-aged children with iron deficiency anemia show altered affect and behavior. *J Nutr* **137:** 683-689.

Maalouf G, Gannage-Yared MH, Ezzedine J, Larijani B, Badawi S, Rached A *et al* (2007). Middle East and North Africa consensus on osteoporosis. *J Musculoskelet Neuronal Interact* **7:** 131-143.

Malope BI, MacPhail AP, Alberts M, Hiss DC (2001). The ratio of serum transferrin receptor and serum ferritin in the diagnosis of iron status. *Br J Haematol* **115:** 84-89.

Markovic M, Majkic-Singh N, Subota V (2005). Usefulness of soluble transferrin receptor and ferritin in iron deficiency and chronic disease. *Scand J Clin Lab Invest* **65:** 571-576.

Mathieu C, Adorini L (2002). The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. *Trends Mol Med* **8:** 174-179.

Matsuoka LY, Wortsman J, Haddad JG, Kolm P, Hollis BW (1991). Racial pigmentation and the cutaneous synthesis of vitamin D. *Arch Dermatol* **127:** 536-538.

Mawajdeh SM, Al-Qutob R, Schmidt A (2003). Measuring reproductive morbidity: a community-based approach, Jordan. *Health Care For Women International* **24:** 635-649.

McCann JC, Ames BN (2007). An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr* **85:** 931-945.

Meddeb N, Sahli H, Chahed M, Abdelmoula J, Feki M, Salah H *et al* (2005). Vitamin D deficiency in Tunisia. *Osteoporos Int* **16:** 4.

Merialdi M, Caulfield LE, Zavaleta N, Figueroa A, DiPietro JA (1999). Adding zinc to prenatal iron and folate tablets improves fetal neurobehavioral development. *Am J Obstet Gynecol* **180:** 483-490.

Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, Lonnerdal B, Stamoulakatou A, Pollitt E (2004). Effect of iron supplementation on cognition in Greek preschoolers. *Eur J Clin Nutr* **58:** 1532-1542.

Ministry of Health, WHO, UNICEF, Centers for Disease Control and Prevention. National baseline survey on iron deficiency anemia and vitamin A deficiency, 2002.

Ministry of Health. Food and Nutrition Policy Report. “Toward a Comprehensive Food

and Nutrition Policy in Jordan". A Preliminary Report on a Collaborative Project with the Ministries of Health and Agriculture. . Amman, Jordan, 2003.

Ministry of Health. Annual Statistical Report, 2007.

Miraglia Del Giudice E, Santoro N, Amato A, Brienza C, Calabro P, Wiegerinck ET *et al* (2009). Hepcidin in Obese Children as a Potential Mediator of the Association between Obesity and Iron Deficiency. *J Clin Endocrinol Metab*.

Mishal AA (2001). Effects of different dress styles on vitamin D levels in healthy young Jordanian women. *Osteoporos Int* **12:** 931-935.

Molla AM, Al Badawi M, Hammoud MS, Molla AM, Shukkur M, Thalib L *et al* (2005). Vitamin D status of mothers and their neonates in Kuwait. *Pediatr Int* **47:** 649-652.

Morck TA, Lynch SR, Cook JD (1983). Inhibition of food iron absorption by coffee. *Am J Clin Nutr* **37:** 416-420.

Morse AC, Beard JL, Jones BC (1999). A genetic developmental model of iron deficiency: biological aspects. *Proc Soc Exp Biol Med* **220:** 147-152.

Moussavi M, Heidarpour R, Aminorroaya A, Pournaghshband Z, Amini M (2005). Prevalence of vitamin D deficiency in Isfahani high school students in 2004. *Horm Res* **64:** 144-148.

Nagpal S, Na S, Rathnachalam R (2005). Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* **26:** 662-687.

Nakamura K, Nashimoto M, Matsuyama S, Yamamoto M (2001a). Low serum concentrations of 25-hydroxyvitamin D in young adult Japanese women: a cross sectional study. *Nutrition* **17:** 921-925.

Nakamura K, Nashimoto M, Tsuchiya Y, Obata A, Miyanishi K, Yamamoto M (2001b). Vitamin D insufficiency in Japanese female college students: a preliminary report. *Int J Vitam Nutr Res* **71:** 302-305.

Nathan C (2008). Epidemic inflammation: pondering obesity. *Mol Med* **14:** 485-492.

National Health and Medical Research Council in Australia and New Zealand. Nutrient Reference Values for Australia and New Zealand; in: Health Mo (ed), © Commonwealth of Australia 2006, 2005, pp 127-138.

Nead KG, Halterman JS, Kaczorowski JM, Auinger P, Weitzman M (2004). Overweight children and adolescents: a risk group for iron deficiency. *Pediatrics* **114:** 104-108.

Need AG, Morris HA, Horowitz M, Nordin C (1993). Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. *Am J Clin Nutr* **58:** 882-885.

Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC *et al* (2002). Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* **76:** 187-192.

Nicolaidou P, Kakourou T, Papadopoulou A, Kavadias G, Dimitriou E, Georgouli H *et al* (2006). Low vitamin D status in preschool children in Greece. *Nutr Res* **26:** 620-625.

Norman AW, Silva FR (2001). Structure functions studies: Identification of vitamin D analogs for the ligand-binding domains of important proteins in the vitamin D-endocrine system. *Rev Endocr Metab Disord* **2:** 229-238.

Norman AW, Henry HH (2006). Vitamin D. In: Bowman BA, Russell RM (eds). *Present Knowledge in Nutrition*, 9th edn. ILSI Press: Washington, DC. pp 198-113.

Northrop-Clewes CA (2008). Interpreting indicators of iron status during an acute phase response--lessons from malaria and human immunodeficiency virus. *Ann Clin Biochem* **45:** 18-32.

Olmez D, Bober E, Buyukgebiz A, Cimrin D (2006). The frequency of vitamin D insufficiency in healthy female adolescents. *Acta Paediatr* **95:** 1266-1269.

Ortiz E, Pasquini JM, Thompson K, Felt B, Butkus G, Beard J *et al* (2004). Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in three different animal models. *J Neurosci Res* **77:** 681-689.

Oski FA, Honig AS (1978). The effects of therapy on the developmental scores of iron-deficient infants. *J Pediatr* **92:** 21-25.

Oski FA, Honig AS, Helu B, Howanitz P (1983). Effect of iron therapy on behavior performance in nonanemic, iron-deficient infants. *Pediatrics* **71:** 877-880.

Outila TA, Karkkainen MU, Lamberg-Allardt CJ (2001). Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. *Am J Clin Nutr* **74:** 206-210.

Pal BR, Marshall T, James C, Shaw NJ (2003). Distribution analysis of vitamin D highlights differences in population subgroups: preliminary observations from a pilot study in UK adults. *J Endocrinol* **179:** 119-129.

Palti H, Meijer A, Adler B (1985). Learning achievement and behavior at school of anemic and non-anemic infants. *Early Human Development* **10:** 217-223.

Paracha PI, Jamil A, Northrop-Clewes CA, Thurnham DI (2000). Interpretation of vitamin A status in apparently healthy Pakistani children by using markers of subclinical infection. *Am J Clin Nutr* **72:** 1164-1169.

Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J *et al* (2004). The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* **89:** 1196-1199.

Pasco JA, Henry MJ, Kotowicz MA, Sanders KM, Seeman E, Pasco JR *et al* (2004). Seasonal periodicity of serum vitamin D and parathyroid hormone, bone resorption, and fractures: the Geelong osteoporosis study. *J Bone Miner Res* **19:** 752-758.

Pinero DJ, Li NQ, Connor JR, Beard JL (2000). Variations in dietary iron alter brain iron metabolism in developing rats. *J Nutr* **130:** 254-263.

Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE *et al* (2006). Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* **29:** 650-656.

Pollitt E, Leibel RL, Greenfield DB (1983). Iron deficiency and cognitive test performance in preschool children. *Nutr Behav* **1:** 137-146.

Pollitt E, Saco-Pollitt C, Leibel RL, Viteri FE (1986). Iron deficiency and behavioral development in infants and preschool children. *Am J Clin Nutr* **43:** 555-565.

Popkin BM (2006). Global Nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. *Am J Clin Nutr* **84:** 9.

Population Reference Bureau. 2008 World population., 2009.

Prasad AS, Halsted JA, Nadimi M (1961). Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *Am J Med* **31:** 532-546.

Prasad AS, Miale A, Jr., Farid Z, Sandstead HH, Schulert AR (1963). Clinical and experimental. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. . *J Lab Clin Med* **116:** 737-749.

Punnonen K, Irjala K, Rajamaki A (1997). Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood* **89:** 1052-1057.

Raffin SB, Woo CH, Roost KT, Price DC, Schmid R (1974). Intestinal absorption of

hemoglobin iron-heme cleavage by mucosal heme oxygenase. *J Clin Invest* **54:** 1344-1352.

Rassouli A, Milanian I, Moslemi-Zadeh M (2001). Determination of serum 25-hydroxyvitamin D(3) levels in early postmenopausal Iranian women: relationship with bone mineral density. *Bone* **29:** 428-430.

Saadi HF, Nagelkerke N, Benedict S, Qazaq HS, Zilahi E, Mohamadiyeh MK *et al* (2006). Predictors and relationships of serum 25 hydroxyvitamin D concentration with bone turnover markers, bone mineral density, and vitamin D receptor genotype in Emirati women. *Bone* **39:** 1136-1143.

Sachdev H, Gera T, Nestel P (2005). Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public Health Nutr* **8:** 117-132.

Sandstead HH (2000). Causes of iron and zinc deficiencies and their effects on brain. *J Nutr* **130:** 347S-349S.

Sandstead HH (2003). Zinc is essential for brain development and function. *J Trace Elem Exp Med* **16:** 165-173.

Sazawal S, Bentley M, Black RE, Dhingra P, George S, Bhan MK (1996). Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. *Pediatrics* **98:** 1132-1137.

Schulze KJ, Christian P, Ruczinski I, Ray AL, Nath A, Wu LS *et al* (2008). Hepcidin and iron status among pregnant women in Bangladesh. *Asia Pac J Clin Nutr* **17:** 451-456.

Scragg R, Sowers M, Bell C (2007). Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens* **20:** 713-719.

Scrimgeour AG, Lukaski HC (2008). Zinc and diarrheal disease: current status and future perspectives. *Curr Opin Clin Nutr Metab Care* **11:** 711-717.

Sedrani SH (1984). Vitamin D status of Saudi men. *Trop Geogr Med* **36:** 181-187.

Serenius F, Elidrissy AT, Dandona P (1984). Vitamin D nutrition in pregnant women at term and in newly born babies in Saudi Arabia. *J Clin Pathol* **37:** 444-447.

Shafir T, Angulo-Barroso R, Calatroni A, Jimenez E, Lozoff B (2006). Effects of iron deficiency in infancy on patterns of motor development over time. *Hum Mov Sci* **25:** 821-838.

Shankar AH, Prasad AS (1998). Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr* **68:** 447S-463S.

Shine B, de Beer FC, Pepys MB (1981). Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta* **117:** 13-23.

Shrimpton R, Gross R, Darnton-Hill I, Young M (2005). Zinc Deficiency: what are the most appropriate interventions? . *British Medical Journal* **330:** 347-349.

Shukla A, Agarwal KN, Shukla GS (1989). Latent iron deficiency alters gamma-aminobutyric acid and glutamate metabolism in rat brain. *Experientia* **45:** 343-345.

Siddiqui AM, Kamfar HZ (2007). Prevalence of vitamin D deficiency rickets in adolescent school girls in Western region, Saudi Arabia. *Saudi Med J* **28:** 441-444.

Skikne BS, Flowers CH, Cook JD (1990). Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* **75:** 1870-1876.

Skikne BS (2008). Serum transferrin receptor. *Am J Hematol* **83:** 872-875.

Smart TG, Hosie AM, Miller PS (2004). Zn2+ ions: modulators of excitatory and inhibitory synaptic activity. *Neuroscientist* **10:** 432-442.

Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM *et al* (2005). Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab* **90:** 4119-4123.

Soewondo S, Husaini M, Pollitt E (1989). Effects of iron deficiency on attention and learning processes in preschool children: Bandung, Indonesia. *Am J Clin Nutr* **50:** 667-673; discussion 673-664.

Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM *et al* (2001). Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. *BMJ* **323:** 1389-1393.

Swets JA (1988). Measuring the accuracy of diagnostic systems. *Science* **240:** 1285-1293.

Taha SA, Dost SM, Sedrani SH (1984). 25-Hydroxyvitamin D and total calcium: extraordinarily low plasma concentrations in Saudi mothers and their neonates. *Pediatr Res* **18:** 739-741.

Tahmasebi Boroujeni S, Naghdi N, Shahbazi M, Farrokhi A, Bagherzadeh F, Kazemnejad

A *et al* (2009). The effect of severe zinc deficiency and zinc supplement on spatial learning and memory. *Biol Trace Elem Res* **130:** 48-61.

Tamura T, Goldenberg RL, Ramey SL, Nelson KG, Chapman VR (2003). Effect of zinc supplementation of pregnant women on the mental and psychomotor development of their children at 5 y of age. *Am J Clin Nutr* **77:** 1512-1516.

Taneja S, Bhandari N, Bahl R, Bhan MK (2005). Impact of zinc supplementation on mental and psychomotor scores of children aged 12 to 18 months: a randomized, double-blind trial. *J Pediatr* **146:** 506-511.

Taylor EM, Crowe A, Morgan EH (1991). Transferrin and iron uptake by the brain: effects of altered iron status. *J Neurochem* **57:** 1584-1592.

Temme EH, Van Hoydonck PG (2002). Tea consumption and iron status. *Eur J Clin Nutr* **56:** 379-386.

Thomas DG, Grant SL, Aubuchon-Endsley NL (2009). The role of iron in neurocognitive development. *Dev Neuropsychol* **34:** 196-222.

Torti FM, Torti SV (2002). Regulation of ferritin genes and protein. *Blood* **99:** 3505-3516.

Tussing-Humphreys LM, Liang H, Nemeth E, Freels S, Braunschweig CA (2009). Excess adiposity, inflammation, and iron-deficiency in female adolescents. *J Am Diet Assoc* **109:** 297-302.

U.S. Department of Agriculture and Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 20. , Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata 2007.

U.S. Department of Health and Human Services and U.S. Department of Agriculture (2005). *Dietary Guidelines for Americans, 2005 6th Edition*. US Government Printing Office: Washington DC.

Unger EL, Wiesinger JA, Hao L (2008). Dopamine D2 Receptor Expression Is Altered by Changes in Cellular Iron Levels in PC12 Cells and Rat Brain Tissue. *The Journal of Nutrition* **138**.

UNICEF. The State of the World's Children. New York, Oxford University Press, 1996.

UNICEF. Progress for Children. A Report Card on Nutrition, 2006, p 32.

UNICEF. The State of the World's Children 2009: Maternal and Newborn Health, United Nations Children's Fund, 2009.

United Nations Development Programme. Jordan Human Development Report, United Nations Development Programme, 2008.

United Nations Population Fund. 2009.

US Department of Health and Human Services. Healthy People 2010: Understanding and Improving Health Washington, DC, US Department of Health and Human Services, 2000, vol 2.

van den Broek NR, Letsky EA (2000). Etiology of anemia in pregnancy in south Malawi. *Am J Clin Nutr* **72:** 247S-256S.

van der Meer IM, Karamali NS, Boeke AJ, Lips P, Middelkoop BJ, Verhoeven I *et al* (2006). High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands
Am J Clin Nutr **84:** 350-353; quiz 468-359

van der Mei IA, Ponsonby AL, Engelsen O, Pasco JA, McGrath JJ, Eyles DW *et al* (2007). The high prevalence of vitamin D insufficiency across Australian populations is only partly explained by season and latitude. *Environ Health Perspect* **115:** 1132-1139.

Vazquez Lopez MA, Carracedo A, Lendinez F, Munoz FJ, Lopez J, Munoz A (2006). The usefulness of serum transferrin receptor for discriminating iron deficiency without anemia in children. *Haematologica* **91:** 264-265.

Vieth R, Cole DE, Hawker GA, Trang HM, Rubin LA (2001). Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. *Eur J Clin Nutr* **55:** 1091-1097.

Vieth R, Ladak Y, Walfish PG (2003). Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. *J Clin Endocrinol Metab* **88:** 185-191.

Vieth R (2006). What is the optimal vitamin D status for health? *Prog Biophys Mol Biol* **92:** 26-32.

Walter T, Kovalskys J, Stekel A (1983). Effect of mild iron deficiency on infant mental development scores. *J Pediatr* **102:** 519-522.

Walter T, De Andraca I, Chadud P, Perales CG (1989). Iron deficiency anemia: adverse effects on infant psychomotor development. *Pediatrics* **84:** 7-17.

Walter T (2003). Effect of iron-deficiency anemia on cognitive skills and neuromaturation in infancy and childhood. *Food Nutr Bull* **24:** S104-110.

Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K *et al* (2008). Vitamin D deficiency and risk of cardiovascular disease. *Circulation* **117:** 503-511.

Webb AR, DeCosta BR, Holick MF (1989). Sunlight regulates the cutaneous production of vitamin D3 by causing its photodegradation. . *J Clin Endocrinol Metab* **68:** 882-887

Weiss G, Goodnough LT (2005). Anemia of chronic disease. *N Engl J Med* **352:** 1011-1023.

Wesley Pike J, Shevde NK (2005). The Vitamin d Receptor. In: Feldman D, Wesley Pike J, Glorieux FH (eds). *Vitamin D*. Elsevier. pp 167-191.

WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO, 2009 (http://www.who.int/growthref/tools/en/).

WHO. Physical Status: The use and interpretation of anthropometry. Report of a WHO expert committee. Technical Report Series No. 854. Geneva, WHO, 1995a.

WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert committee. WHO technical report series Geneva, World Health Organization, 1995b.

WHO. Guidelines for the Control of Iron Deficiency in Countries of the Eastern Mediterranean Middle East and North Africa, World Health Organization, 1996.

WHO. The World Health Report 2002: Reducing risks, promoting healthy life. Geneva, 2002.

WHO. Worldwide prevalence of anaemia 1993-2005: WHO Global Database on Anaemia/ Edited by Bruno de Benoist, Erin McLean, Ines Egli and Mary Cogswell., 2008.

WHO/CDC. Assessing the Iron Status of Populations; Report of a Joint WHO/CDC Technical Consultation on the Assessment of Iron Status at the Population Level. Geneva, World Health Organization and Centers for Disease Control and Prevention 2004.

WHO/FAO. Human vitamin and mineral requirements. Report of a Joint FAO/WHO Expert Consultation. Bangkok, Thailand. FAO Rome, 2002.

WHO/FAO (2005a). Zinc. *Vitamin and Mineral Requirements in Human Nutrition*, 2 edn. World Health Organization and Food and Agriculture Organization of the United Nations: Geneva, Switzerland.

WHO/FAO (2005b). Iron. *Vitamin and Mineral Requirements in Human Nutrition*, 2 edn. World Health Organization and Food and Agriculture Organization of the United Nations: Geneva, Switzerland.

WHO/UNICEF/UNU. Iron Deficiency Anaemia: Assessment, Prevention and Control. A guide for programme managers. Geneva, World Health Organization; UNICEF; United Nations University, 2001.

Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal (2002). Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. *J Nutr* **132:** 3061-3066.

Williams J, Wolff A, Daly A, MacDonald A, Aukett A, Booth IW (1999). Iron supplemented formula milk related to reduction in psychomotor decline in infants from inner city areas: randomised study. *BMJ* **318:** 693-697.

Windaus A, Linsert O, Luttringhaus A, Weidlich G (1932). Uber das krystallisierte Vitamin D2. *Justus Liebigs Ann Chem***:** 226-231.

Woo J, Lam CW, Leung J, Lau WY, Lau E, Ling X *et al* (2007). Very high rates of vitamin D insufficiency in women of child-bearing age living in Beijing and Hong Kong. *Br J Nutr***:** 1-5.

Working Group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia, Osteoporosis Australia (2005). Vitamin D and adult bone health in Australia and New Zealand: a position statement. *Med J Aust* **182:** 281-285.

World Bank. World Development Report 2009: Reshaping Economic Geography 2009.

World Health Organization. World Health Statistics 2008, 2008.

Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF (2000). Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* **72:** 690-693.

Yan L, Prentice A, Zhang H, Wang X, Stirling DM, Golden MM (2000). Vitamin D status and parathyroid hormone concentrations in Chinese women and men from north-east of the People's Republic of China. *Eur J Clin Nutr* **54:** 68-72.

Yanoff LB, Parikh SJ, Spitalnik A, Denkinger B, Sebring NG, Slaughter P *et al* (2006). The prevalence of hypovitaminosis D and secondary hyperparathyroidism in obese Black

Americans. *Clin Endocrinol (Oxf)* **64:** 523-529.

Yanoff LB, Menzie CM, Denkinger B, Sebring NG, McHugh T, Remaley AT *et al* (2007). Inflammation and iron deficiency in the hypoferremia of obesity. *Int J Obes (Lond)* **31:** 1412-1419.

Youdim MB, Green AR (1978). Iron deficiency and neurotransmitter synthesis and function. *Proc Nutr Soc* **37:** 173-179.

Youdim MB, Ben-Shachar D, Yehuda S (1989). Putative biological mechanisms of the effect of iron deficiency on brain biochemistry and behavior. *Am J Clin Nutr* **50:** 607-615; discussion 615-607.

Youdim MB, Yehuda S (2000). The neurochemical basis of cognitive deficits induced by brain iron deficiency: involvement of dopamine-opiate system. *Cell Mol Biol (Noisy-le-grand)* **46:** 491-500.

Yu GS, Steinkirchner TM, Rao GA, Larkin EC (1986). Effect of prenatal iron deficiency on myelination in rat pups. *Am J Pathol* **125:** 620-624.

Zarychanski, R. and D. S. Houston (2008). "Anemia of chronic disease: a harmful disorder or an adaptive, beneficial response?" CMAJ **179**(4): 333-7.

Zimmermann MB, Chaouki N, Hurrell RF (2005). Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. *The American Journal Of Clinical Nutrition* **81:** 115-121.

Zimmermann MB, Zeder C, Muthayya S, Winichagoon P, Chaouki N, Aeberli I *et al* (2008). Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. *Int J Obes (Lond)* **32:** 1098-1104.

Zindah M, Belbeisi A, Walke H, Mokdad AH (2008). Obesity and diabetes in Jordan: findings from the behavioral risk factor surveillance system, 2004. *Prev Chronic Dis* **5:** A17.

Zittermann A, Scheld K, Stehle P (1998). Seasonal variations in vitamin D status and calcium absorption do not influence bone turnover in young women. *Eur J Clin Nutr* **52:** 501-506.

Zittermann A, Schleithoff SS, Koerfer R (2006). Vitamin D insufficiency in congestive heart failure: why and what to do about it? *Heart Fail Rev* **11:** 25-33.

APPENDICES

Oklahoma State University Institutional Review Board

Date:	Monday, June 18, 2007
IRB Application No	HE0731
Proposal Title:	Assessment of Nutritional Status and Cognitive Functions in Children 4-5 Years Old and Nutritional Status of their Mothers in Northern Jordan
Reviewed and Processed as:	Expedited (Spec Pop)

Status Recommended by Reviewer(s): Approved Protocol Expires: 6/17/2008

Principal Investigator(s

Muna Algharibeh
301 HES
Stillwater, OK 74078

Barbara J 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,

Sue C. Jacobs, Chair
Institutional Review Board

VITA

MUNA AHMAD GHARAIBEH

Candidate for the Degree of

DOCTOR OF PHILOSOPHY

Dissertation: NUTRITIONAL ASSESSEMENT OF MOTHERS AND THEIR PRE-SCHOOL CHILDREN AND COGNITIVE FUNCTIONS OF CHILDREN IN NORTHERN JORDAN

Major Field: HUMAN ENVIRONMENTAL SCIENCES (OPTION: NUTRITIONAL SCIENCES)

Biographical:

Education:

- Doctor of Philosophy (Ph.D.) in Human Environmental Sciences, Major: Nutritional Sciences, Department of Nutritional Sciences, Oklahoma State University, USA. August 2005- December 2009
- Master of Business Administration (MBA), Faculty of Business Administration, University of Jordan, Jordan. August 1999- June 2002
- Bachelor of Science (BS) in Pharmacy, Faculty of Pharmacy, University of Jordan, Jordan. August 1991- June 1996

Experience:

Worked as a pharmacist in a local pharmacy in Jordan for five years (1996-2002) and as a medical representative (2002-2003). Taught consumer behavior and principles of marketing at University of Jordan (2003-2004), and promotional strategy at Spears' School of Business at Oklahoma State University (2004-2006). Worked as teaching assistant for principles of human nutrition and nutrition and physical activity in aging at Nutritional Sciences Department at Oklahoma State University (2007-2009).

Professional Memberships:

- American Society of Nutrition
- Jordan Pharmaceutical Association

Name: MUNA ALGHARIBEH (GHARAIBEH) Date of Degree: December, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: NUTRITIONAL ASSESSEMENT OF MOTHERS AND THEIR PRE-SCHOOL CHILDREN AND COGNITIVE FUNCTIONS OF CHILDREN IN NORTHERN JORDAN

Pages in Study: 281 Candidate for the Degree of Doctor of Philosophy

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

Scope and Method of Study:

Nutritional status of women of childbearing age and their preschool children was assessed in a cross-sectional study in summer 2007. A convenience sample of 93 women and their preschool children from urban/suburban areas in northern Jordan was recruited for the study with the help of local community centers. Anthropometric measurements were performed and information on socioeconomic status, health issues, lifestyle factors, and nutritional intakes were obtained from mothers by questionnaire. Serum 25(OH)D and intact serum PTH were measured using enzyme-linked immunoabsorbent assay (ELISA) kits. In addition to hemoglobin (Hb), the following were measured: plasma ferritin using immunoradiometric assay; transferrin receptor using enzyme linked imunoassay; α-1-acid glycoprotein using radial immunodiffusion, and plasma zinc using inductively coupled plasma mass spectrometry. Body iron (mg/kg) was quantitatively estimated. Cognitive function was assessed in children using components of the Peabody Picture Vocabulary Test-IV (PPVT-IV) and of the Kaufman Assessment Battery for Children (K-ABC-II).

Findings and Conclusions:

Mean age of women was 33.9 years and of children was 60.7 months. The mean ± SD for 25(OH) D was 25.6 ± 9.6 nmol/L in women and 55.8 ± 19.8 nmol/L in children. A total of 48.9% of women were below 25.0 nmol/L and 97.8% were below 50 nmol/L. Mothers with ferritin <15 µg/L were 54%, and 16% of children had ferritin <12 µg/L. Mothers had significantly lower mean serum ferritin and higher TfR (13.1 ± 3.1 µg/L, and 7.6 ± 1.5 mg/L, respectively) than their children (21.9 ± 2.0 µg/L, and 5.9 ± 1.4 mg/L, respectively). More than 50% of mothers and 30% of children had AGP≥1.2 g/L, and 77% of women were classified as overweight or obese with a BMI≥25kg/m^2. Plasma zinc was 67.2 ± 11.4 µg/dL in mothers and 64.6 ± 9.4 µg/dL in children. More than half of mothers (58%) and children (56%) were below IZiNCG cutoffs for zinc deficiency. Three parameters: TfR, TfR/ferritin, and TfR/Log_{10} ferritin were evaluated using receiver operator characteristic (ROC) curves. TfR/ferritin and TfR/Log_{10}ferritin had better diagnostic efficiency in detecting iron deficiency than the use of TfR alone. Cognitive function was assessed in children, and neither ferritin nor zinc predicted child performance on most of the tests. Co-existing micronutrient deficiencies (in terms of vitamin D, iron, and zinc) were widespread among mothers and children in northern Jordan.

ADVISER'S APPROVAL: DR. BARBARA STOECKER