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

DIETARY IRON INTAKE OF FEMALE UNIVERSITY STUDENTS AND THEIR IRON STORE AS FUTURE MOTHERS: THE CASE OF COLLEGE OF NATURAL SCIENCE

By

Saron Nigussie

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

July, 2015

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PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

Under the Supervision of

Kaleab Baye (PhD)

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July, 2015

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Center for Food Science and Nutrition

This is to certify that the thesis presented by Saron Nigussie, entitled ‘Dietary iron intake of female university students and their iron store as future mothers: The case of the College of Natural Sciences’ submitted in partial fulfillment of the requirements for the degree of Master of Science (Food Science and Nutrition) complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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Advisor ……... Signature …… Date……..

Co- Advisor …….. Signature …… Date……..

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DECLARATION

I the undersigned, declare that this thesis is my original work and has not been presented for the award of a degree at any university and all the sources of materials used for this thesis have been duly acknowledged.

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ABSTRACT

Dietary iron intake of female university students and their iron store as future mothers: The case of the College of Natural Sciences.

Saron Nigussie, Kaleab Baye and Dawd Gashu

Iron deficiency (ID) is common single nutrient deficiency of great public health significance affecting children, adolescents and women of reproductive age worldwide. Women of reproductive age are at higher risk of developing iron deficiency and iron deficiency anemia. Therefore, this study was designed to determine the prevalence of iron deficiency and iron deficiency anemia among regular female university students studying at Addis Ababa University College of Natural Science in relation to their dietary iron intake and iron deficiency risk factors. Data on socio-demographic, anthropometric and iron deficiency risk factors such as social habits, and diseases symptoms, menstrual blood flow, dietary and medical history were collected from female students in the College of Natural Science, Addis Ababa University. Similarly, a one week weighed food record was obtained to estimate energy and micronutrient intake of students. In addition, blood samples were collected from female students (*n=45*) for analysis of hemoglobin level, serum ferritin, serum soluble transferrin receptor (sTfr) and C-reactive protein. The participants were in the age of 18-23 years old with mean value of 20.35 ± 1.3. In addition, they had body mass index in the range of 18.37-26.03 with mean value of 21.85 ± 1.7, suggesting normal fat-lean mass ratio of the study participants. The majority (95.5%) of present study participant consumed coffee and or tea, but had low (24.4%) intake frequency of fruits indicating the habit of consuming iron absorption inhibitors but low consumption of enhancers. In addition, consumption of fruits was recommended to be followed just short time after meal to support iron absorption, however, it was observed that the present study students consume fruits relatively long time after meal. About 9% of students often experienced heavy menstrual blood

flow. On the other hand, 20.0% of students often experienced Amenorrhea. However, neither excessive blood flow predispose participants for iron deficiency nor the missing of menstrual cycle were significant ($p > 0.05$) contributors to preserve hemoglobin or iron status. The majority of the study participants (84.4%) practiced fasting. The common Ethiopian fasting excludes animal source foods. However, in the present study fasting didn't affect the iron status of the subjects. The mean mineral intakes were: Ca (219.32 ± 15.0 mg/day), Zn (3.70 ± 0.3mg/day) and Fe (18.01 ± 0.8mg/day). In addition, the mean energy intake was 1965.98 ± 86.7kcal/day. Based on the result of dietary weight record, about 91% of participants had inadequate energy and all of the subjects had inadequate Ca and Zn intake. In addition, carbohydrates contributed the greater daily energy (72.3%) beyond the daily recommended value. On the other hand, the contribution of fats (14.4%) for daily energy requirement was low. The mean hemoglobin, sTfr and ferritin values were: 12.88±1.1g/dl, 2.60±0.3µg/l and 86.40±6.3µg/l, respectively. Only 4.5% of the participants were anemic (low hemoglobin) and had depleted iron store (serum ferritin<12µg/l). sTfR shows significant association With CRP ($p=0.15$) and BMI shows a significant correlation with serum ferritin ($p=0.006$).

Key words: Iron deficiency anemia, iron deficiency, female university students and Cafeteria meal

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LIST OF ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
DCytb	Duodenal Cytochrome b
DMT1	Divalent Metal Transporter
DNA	Deoxyribonuceic Acid
DRVs	Daily Requirement Value
ELISA	Enzyme-Linked Immunosorbant Assay
HCP1	Heme-Carrier Protein 1
IDA	Iron Deficiency Anemia
IRE	Iron Regulatory Element
LfR	Lactoferrin Receptor
LIP	Labile Iron Pool
NADH	Nicotinamide Adenine Dinucleotide
NICE	National Institute for Clinical Excellence
RNA	Ribonuclic Acid
TfR	Transferrin Receptor
USPSTF	US Preventive Services Task Force
WHO	World Health Organization
WRA	Women of Reproductive Age

1. INTRODUCTION

Iron deficiency with or without anemia has important consequences on human health and child development. Anemic women and their fetus are at greater risk of dying during the prenatal period. Children's mental and physical development can be delayed or impaired by iron deficiency. In addition, the physical work capacity and productivity of the work force may be reduced (WHO, 2004).

Iron play several physiological role including a cofactor to enzymes involved in oxidation reduction reactions which are common reactions in cell metabolism (Whitney *et al*., 2009). The immune system is also dependent on iron for its efficient functioning (Lieu *et al*., 2001).

Requirement of iron differs in age and sex. Females of reproductive age require more iron than men at the same age due to menstrual cycle blood lose. Pregnant women require additional iron to support added blood volume, growth of the fetus and blood loss during child birth. Infants and young children also demand high amounts of iron to support their rapid growth (Whitney *et al*., 2009).

About 50 % of women worldwide enter pregnancy without adequate iron reserves or are already iron deficient (Scholl, 2005). The amount of iron required during pregnancy is substantial and can lead to iron deficiency anemia (IDA) if iron reserves are not adequate (Viteri and Berger, 2005). Therefore, maintaining preconception iron stores is crucial by correcting inadequate intake to prevent iron deficiency anemia (IDA) and its associated complications such as reduced infant growth and increased risk of adverse pregnancy outcome (Ronnenberg *et al.*, 2004).

The most common approaches in preventing iron deficiency are: dietary diversification or iron supplementation (Thompson, 2007). Food-based approaches aim to improve iron status through

increasing the bioavailability, consumption of a nutritionally adequate and micronutrient rich diet made up from a variety of available foods. Food based approaches are recognized as an essential part of an urgently needed more comprehensive strategy to combat iron and other micronutrient deficiencies. As food-based strategies aim to improve the quality of the overall diet by increasing the availability and consumption of a wider range of foods, they address multiple nutrient deficiencies simultaneously (Thompson, 2007).

Although diets in Ethiopia especially *teff* contains high iron content, studies shows that iron deficiency and iron deficiency anemia ranges from mild to moderate and perceived as a public health problem (Umeta *et al*., 2008). Although, previous studies show that iron deficiency exists in Ethiopia, correlations with the dietary intake of the population are lacking. On the other hand, there is no or little information on the nutrient adequacy of student's cafeteria meal. This study therefore is intended to assess female student's body iron status and their dietary iron intake and also to correlate dietary intake and iron status of female students.

2. OBJECTIVES

2.1. General Objective

To assess dietary iron intake and body iron stores of female students at Addis Ababa University College of Natural Science in relation to risk factors for iron deficiency and iron deficiency anemia.

2.2. Specific Objectives

- To determine nutrient intakes of regular female students of Addis Ababa University College of natural sciences
- To assess iron status of female students of Addis Ababa University College of natural sciences using serum markers
- To investigate the relationship between dietary iron intake, and iron deficiency risk factors with iron status

3. LITERATURE REVIEW

3.1. Roll of Iron in the Body

Iron is a transition metal which exists in two biologically relevant oxidation or valency states: the reduced ferrous form (Fe^{2+}) and the oxidized ferric form (Fe^{3+}). The ability of iron to readily accept or donate single electrons means it is an efficient catalyst for electron transfer and free-radical reactions (Beliles, 1994). Although these properties lend themselves to fundamental metabolic processes, the reactivity of iron also means that "free iron" i.e., when it is not bound to protein or other organic molecules is potentially toxic (Graf *et al*., 1984).

Iron has a crucial role in the body. It is used as a cofactor to enzymes involved in oxidation reduction reactions which are wide spread in metabolism that they occur in all cells which mean it is crucial in energy making (Whitney *et al*., 2009).

Iron is required for the production of red blood cells and it is also part of hemoglobin binding to oxygen and thus facilitating its transport from lungs via the arteries to all cells throughout the body. Once oxygen is delivered, iron (as a part of hemoglobin) binds the carbon dioxide which is then transported back to the lung from where it gets exhaled. Iron plays an important role also in synthesis of DNA and RNA proteins (Conrad *et al*., 1999).

The production of enzymes (which play a vital role in the production of new cells, amino acids, hormones and neurotransmitters) also depends on iron, this aspect becomes crucial during the recovery process from illnesses because It is an important component of peroxide-generating enzymes and nitrous oxide-generating enzymes that are critical for proper enzymatic functioning

of immune cells (Beard, 2001). It is also involved in regulation of cytokine production and action as well as in the development of cell mediated immunity (Spear and Sherman, 1992).

Iron is the key component of the many enzymes that involve essential oxidation-reduction reactions, synthesis of neurotransmitters, catabolism of neurotransmitters and synthetic processes such as the production of myelin development of neuronal dendrite tree. Therefore iron homeostasis is critical for normal brain function especially in learning and memory (Beard *et al.*, 1993).

3.2. Metabolism Overview

3.2.1. Distribution of Iron in the Human Body

Iron represents approximately 35 and 45 mg/kg of body weight in adult women and men, respectively the majority of total body iron, about 60-70%, is present in hemoglobin in circulating erythrocytes. Another 10% of essential body iron is present in the forms of myoglobins, cytochromes and iron containing enzymes, amounting to no more than 4-8 mg of iron. In healthy individual, the remaining 20-30% of surplus iron is stored as ferritin and hemosidrin in hepatocytes and reticuloendothelial macrophages (Conrad *et al*., 1999).

Hemoglobin: Iron is a key functional component of this oxygen transporting molecule. About 60% to 70% total body iron is found in heme group of hemoglobin. A heme group consists of iron (Fe^{2+}) ion held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four parole molecules cyclically linked together by methene bridges with the iron ion bound in the center (Arora and Kapoor, 2012).

Ferritin and Hemosidrin: Ferritin is the major protein involved in the storage of iron. The protein consists of an outer polypeptide shell also termed apoferritin composed of 24 symmetrically placed protein chains (subunits), the average outside diameter is approximately 12.0 nm in hydrated state. The inner core approximately 6.0 nm contains an electron-dense and chemically inert inorganic ferric "iron-core" made of ferric oxyhydroxyhydroxide phosphate $[(FeOOH)_8(FeO\text{-}OPO_3H_2)]$ (Theil, 1987).

The ferritins are extremely large proteins (450 kDa) which can store up to 4500 iron atoms as hydrous ferric oxide. The ratio of iron to polypeptide is not constant, since the protein has the ability to gain and release iron according to physiological needs. Channels from the surface permit the accumulation and release of iron. All iron-containing organisms including bacteria, plants, vertebrates and invertebrates have ferritin (Theil, 1987).

The bulk of the iron storage occurs in hepatocytes, reticuloendothelial cells and skeletal muscle. When iron is in excess, the storage capacity of newly synthesized apoferritin may be exceeded. This leads to iron deposition adjacent to ferritin spheres. This amorphous deposition of iron is called hemosiderin and the clinical condition is termed as hemosiderosis (Arora and Kapoor, 2012).

Myoglobin: Myoglobin is an iron- and oxygen-binding protein found in the muscle tissue of vertebrates in general and in almost all mammals. It is a single-chain globular protein of 153 or 154 amino acids containing a heme prosthetic group in the center around which the remaining apoprotein folds (Hendgen-Cotta, 2009). It has eight alpha helices and a hydrophobic core. It has a molecular weight of 17,699 daltons (with heme), and is the primary oxygen-carrying pigment of muscle tissues (Ordway and Garry, 2004).

Transferrin: Transferrin is a protein involved in the transport of iron. The transferrins are glycoproteins with molecular weight of approximately 80000 Da, consisting of a single polypeptide chain of 680 to 700 amino acids and no subunits (Anderson *et al*., 1989). Transferrin delivers iron to cells by binding to specific cell surface receptors (TfR) that mediate the internalization of the protein. The TfR is a transmembrane protein consisting of two subunits of 90000 Da each, joined by a disulfide bond (Jing and Trowbridge, 1987).

Labile Iron Pool: The uptake and storage of iron is carried out by different proteins, hence a pool of accessible iron ions, called labile iron pool (LIP) exists, that constitutes crossroads of the metabolic pathways of iron containing compounds (Kruszewski and Iwaneñko, 2003). The LIP is localized primarily but not exclusively, within the cytoplasm of the cells. It is bound to low-affinity ligands (Pruse *et al*., 2008) and is accessible to per meant chelators and contains the cells' metabolically and catalytically reactive iron. LIP is maintained by a balanced movement of iron from extra- and intracellular source (Epsztejn *et al*., 1997).

Flavoprotiens and Other Heme Proteins: Certain enzymes also contain heme as part of their prosthetic group, e.g. catalase, peroxidases, tryptophan pyrrolase, guanylate cyclase, Nitric oxide synthase and mitochondrial cytochromes. Iron readily forms clusters linked to the polypeptide chain by thiol groups of cysteine residues or to non-proteins by inorganic sulphide and cysteine thiols leading to generation of iron- sulphur clusters. Examples of iron-sulphur proteins are the ferredoxins, hydrogenases, nitrogenases, NADH dehydrogenases and aconitases. Structure of most of these proteins dictates their function (Arora and Kapoor, 2012).

3.2.2. Iron Homeostasis

Finely tuned mechanisms maintain iron homeostasis by balancing the body's need for iron with minimizing the risk of iron toxicity. Absorption of iron from the gastrointestinal tract is regulated by the systemic need for iron. The risk of tissue damage by free reactive iron is limited by a series of organic molecules which have specific roles in binding free iron, carrying it in the circulation and delivering it to functional sites or to depots in which iron that are not immediately needed is deposited in a safe form. This is the basis for the systemic recycling of iron released during tissue and enzyme turnover. A key element of this salvage system is the deposition of iron in ferritin. The principal pool of ferritin is in the liver which serves as a buffer pool for any iron excess to immediate requirements. Approximately 25% of body iron is found in the liver: two-thirds as ferritin and up to one-third as insoluble hemosiderin (Bothwell and Finch, 1962).

Since the body has no means of excreting excess iron, control of the body iron burden is by regulation of iron absorption. The only way in which iron is lost from the body is adventitiously in desquamated skin cells and sweat (0.2–0.3 mg/day), urine (< 0.1 mg/day), gastrointestinal secretions, and hair. In premenopausal women, iron is also lost in menses (Salonen *et al*., 1992)

Iron turnover is also driven by the formation and destruction of hemoglobin present in erythrocytes, which have a life span of approximately 120 days; senescent erythrocytes are then engulfed and destroyed by the macrophages of the reticulo endothelial system. Their hemoglobin is broken down in the lysosomes, where iron is released from heme by heme oxygenase and transferred to the protein, apotransferrin, to form transferrin which is the iron carrier molecule in the plasma. Transferrin-bound iron is transported to the erythroblasts in the bone marrow for incorporation into heme for new erythrocytes, or delivered to cells in tissues undergoing growth

and development or to tissue ferritin depots. The macrophages of the reticulo endothelial system recycle approximately 30 mg/day of iron from senescent erythrocytes. This demonstrates the efficiency of the cyclic conservation and reutilization of systemic iron (Bothwell and Finch, 1962).

3.2.3. Absorption

Since iron is required for a number of diverse cellular functions, a constant balance between iron uptake, transport, storage and utilization is required to maintain iron homeostasis (Lieu *et al*., 2001). As the body lacks a defined mechanism for the active excretion of iron, it is mainly regulated at the point of absorption (Hurrell and Egli, 2010; Finberg, 2011). The fraction of iron absorbed from the amount ingested is typically low, but may range from 5% to 35% depending on circumstances and type of iron (McDowell, 2003).

Iron absorption occurs by the enterocytes by divalent metal transporter 1 (DMT1), , transports inorganic iron, i.e., iron that is not part of the haem molecule, and is specific for ferrous iron. This takes place predominantly in the duodenum and upper jejunum (Muir and Hopfer, 1985). It is then transferred across the duodenal mucosa into the blood, where it is transported by transferrin to the cells or the bone marrow for erythropoiesis (producing red blood cells). Non-haem iron uptake requires an acid pH, which is provided by gastric hydrochloric acid, to make it more soluble and to produce the protons that are required for its co-transport by DMT1. A haem enzyme, duodenal cytochrome B reductase (DcytB), located on the luminal surface of the enterocytes, converts dietary ferric iron to the ferrous state which is more soluble (Hurrell, 1997; Frazer and Anderson, 2005; Nadadur *et al.*, 2008).

Dietary heme can also be transported across the apical membrane by a yet unknown mechanism and subsequently metabolized in the enterocytes by heme oxygenase 1 (HO-1) to liberate Fe^{+2}. This process is more efficient than the absorption of inorganic iron and is independent of duodenal pH. It is thus not influenced by inhibitors such as phytate and polyphenols (Wang and Pantopoulos, 2011).

A feedback mechanism exists that enhances iron absorption in people who are iron deficient. In contrast, people with iron overload dampen iron absorption via hepcidin. It is now generally accepted that iron absorption is controlled by ferroportin which allows or not iron from the mucosal cell into the plasma. Hepcidin controls ferroportin. Hepcidin is high with good iron status (and infection) and degrades the ferroportin so preventing iron absorption. Hepcidin is low in iron deficiency and ferroportin remains intact so iron can be absorbed (Nemeth *et al.*, 2004).

The physical state of iron entering the duodenum greatly influences its absorption. At physiological pH, ferrous iron (Fe^{2+}) is rapidly oxidized to the insoluble ferric (Fe^{3+}) form. Gastric acid lowers the pH in the proximal duodenum reducing Fe^{3+} in the intestinal lumen by ferric reductases and the subsequent transport of Fe^{2+} across the apical membrane of enterocytes. This enhances the solubility and uptake of ferric iron. When gastric acid production is impaired (for instance by acid pump inhibitors such as the drug, prilosec), iron absorption is reduced substantially (Mackenzie and Garrick, 2005).

Consequently red meats are excellent nutrient sources of iron. Directly internalized Fe^{+2} is processed by the enterocytes and eventually exported across the basolateral membrane into the bloodstream via the solute carrier and Fe^{+2} transporter ferroportin. The ferroportin-mediated efflux of Fe^{+2} is coupled by its re-oxidation to Fe^{+2}, catalyzed by the membrane-bound

ferroxidase hephaestin that physically interacts with ferroportin and possibly also by its plasma homologue ceruloplasmin (Yeh *et al.*, 2009).

Exported iron is scavenged by transferrin, which maintains Fe^{+3} in a redox-inert state and delivers it into tissues. The total iron content of transferrin (3mg) corresponds to less than 0.1% of body iron, but it is highly dynamic and undergoes more than ten times daily turnover to sustain erythropoiesis. The transferrin iron pool is replenished mostly by iron recycled from effete red blood cells and, to a lesser extent, by newly absorbed dietary iron. Senescent red blood cells are cleared by reticuloendothelial macrophages, which metabolize hemoglobin and heme, and release iron into the bloodstream. By analogy to intestinal enterocytes, macrophages export Fe^{+2} from their plasma membrane via ferroportin, in a process coupled by re-oxidation of Fe^{+2} to Fe^{+3} by ceruloplasmin and followed by the loading of Fe^{+3} to transferrin (Wang and Pantopoulos, 2011).

Theil *et al.*, (2012) discovered a new mechanism for absorption of iron from vegetables and legumes that could provide a solution to iron deficiency problems. They state that ferritin iron from food is readily bioavailable to humans and has the potential for treating iron deficiency.

Hoppler *et al*., (2008) showed that ferritin iron is readily released from ferritin molecule during cooking and at gastric digestion. They state that the iron is dissolved in the gastric juice and would be expected to be absorbed by DTM1. In their study, they investigated changes of ferritin iron and protein during cooking and in vitro gastric digestion. Water soluble, native ferritin iron, measured in different legumes represented 18% (soybeans) to 42% (peas) of total seed iron. Ferritin iron was no longer detectable after boiling the legumes for 50 min in excess water. When they applied the same cooking treatment to recombinant bean ferritin propagated in Escherichia

coli, some ferritin iron remained measurable. They found that during in vitro gastric digestion of recombinant bean ferritin and red kidney bean extract, ferritin iron was fully released from the protein and dissolved at pH 2. Their stability tests at varying pH at 37ºC showed that the release of ferritin iron starts at pH 5 and is complete at pH 2. Hoppler *et al*., (2008) concluded that ferritin iron should be absorbed as efficiently as all other non heme iron in food.

3.3. Dietary Iron, Iron Absorption Enhancers and Inhibitors

3.3.1. Dietary Iron

Dietary iron exists in two main forms: heme iron and non-heme iron. Heme iron is found almost entirely in food of animal origin. Non-heme iron is found in animal and plant tissues as Fe^{2+} bound to insoluble proteins, phytates, oxalates, phosphates and carbonates, and as ferritin. The richest sources of non-heme iron include cereals, vegetables and nuts (Whitney *et al*., 2009).

In some developing countries, contamination iron from mechanical or chemical reactions occurring during food production and preparation may be an important source of iron (Adish *et al.*, 1999; Kroger-Ohlsen *et al.*, 2002). The iron content of food is increased by being cooked in cast-iron cookware, particularly when acidic foods are cooked for extended periods of time (Brittin and Noassaman, 1986; Fairweather- Tait *et al.*, 1995).

Processes that are most likely to increase the amount of iron available for absorption by degrading inhibitors of iron uptake (e.g. phytates and phosphates) include milling, soaking, cooking, germination, fermentation and heat.

3.3.2. Bioavailability of Dietary Iron

The intestinal uptake and transfer of iron is dependent on the body's need for iron. The availability of dietary iron for uptake by the gut mucosa is affected by the chemical form of iron (heme iron or inorganic non-heme iron) and the character of the complexes of inorganic iron with other dietary constituents.

Dietary components that affect iron absorption have been identified and partly characterized from single meal studies using isotope labels. Single meal studies have shown that heme iron is more efficiently absorbed from the diet (20–30%) than non-heme iron (5–15%) (Martinez-Torres and Layrisse, 1971; FAO, 1988). Non-heme iron absorption in single meal studies is very variable and is influenced by the balance of dietary factors enhancing (e.g., meat, ascorbic acid) and inhibiting (e.g., phytic acid, soy protein, polyphenols, calcium) absorption. With the exception of calcium, which has an inhibitory effect on both heme and non-heme iron (Hallberg *et al.*, 1991).

Homeostatic control of non-heme iron absorption is more pronounced than that of heme iron. Lynch *et al.,* (1989) reported that absorption of non-heme iron from a standard meal containing 4.8 mg/L of iron was nearly 10-fold higher with decreasing serum ferritin concentrations (from 100 to 10 µg/L) compared to a 2–3-fold increase for absorption of heme iron. Hallberg *et al.*, (1997) reported that fractional absorption of heme and non-heme iron was similar (about 40%) at serum ferritin concentrations of 10 µg/L. At higher serum ferritin concentrations, absorption of both heme and non-heme iron was decreased but the reduction was greater for non-heme. At serum ferritin concentrations of 15, 20 and 30 µg/L, heme iron absorption was 40%, 80% and 140% higher than that of non-heme iron. Non-heme and heme iron form a common pool after

uptake into the enterocyte, so the difference in their absorption is probably due to differences in the processes involved in the uptake of the two forms of iron by the enterocytes.

Since the systemic need for iron is the major determinant of iron uptake and transfer, bioavailability is not an absolute characteristic of a food or diet. However, as systemic needs for iron increase, the type of diet and its influence on the bioavailability of iron becomes increasingly relevant. For example, it could be of greater importance for growing children or in populations with iron deficiency secondary to intestinal parasites and infection, more particularly when the iron content of the diet is low. The presence of iron deficiency in a population where there is no evidence of any diseases that affect iron metabolism suggests that dietary iron supply may be inadequate in terms of absolute amounts of absorbable iron relative to iron requirements (Hurrell, 1997).

In general, iron compounds that are water-soluble have the highest bioavailability, followed by those that are soluble in dilute acid (equivalent to gastric conditions). Compounds that are insoluble in water or dilute acid have a lower bioavailability because the iron precipitates and cannot be taken up by the enterocyte (Hurrell, 1997).

3.3.3. Iron Absorption Enhancers

Ascorbic Acid: When ascorbic acid is added to a vegetable meal, percentage absorption is increased in approximate proportion to the molar ratio of ascorbic acid to iron irrespective of whether the ascorbic acid is introduced as the purified compound or in the form of fruits with high ascorbic acid content (Lynch and Cook, 1980). In one study, as little as 20 mg ascorbic acid added to a maize porridge meal fortified with 2 mg or 4 mg iron increased absorption 1.7-fold and 1.8-fold, respectively (Disler *et al.,* 1975). Absorption from vegetable-based meals may be

increased as much as six fold by larger quantities of ascorbic acid (Lynch and Cook, 1980). The influence of ascorbic acid is most pronounced in inhibitory meals, and it is effective in meals that contain high levels of the two main inhibitors of non home iron absorption, phytates and polyphenols (Cook and Monsen, 1977; Siegenberg, 1991). A much smaller promoting effect occurs when it is added to a high bioavailability meat-containing meal. Ascorbic acid is effective in promoting iron absorption only if eaten together with the iron. Cook and Monsen, (1975) demonstrated that 500 mg ascorbic acid taken with a meal increased absorption about six fold, whereas the same quantity had little effect when taken 4 or 8 hours before the meal. Methods of food preparation, especially baking at high temperatures (Disler *et al.,* 1975) or prolonged rewarming may lead to oxidation of the vitamin and the loss of its beneficial properties (Hallberg, 1982).

Ascorbic acid acts by maintaining iron in a soluble bioavailable form as the luminal pH rises once the gastric contents enter the duodenum. Iron, particularly when it is in the ferric form, is soluble only at acid pH. Ferric iron has a coordinating valence of 6.In aqueous solutions metal ions are bound to each other through water bridges. If the pH rises, hydroxide ions become available and metallic polymers or precipitated metallic hydroxides are formed. Above a pH of 4 almost all the iron is precipitated from a solution of ferric chloride. However, if ascorbic acid is added to soluble ferric chloride in an acid solution, a complex of iron and ascorbic acid is formed that remains soluble over a wide pH range (Conrad and Schade, 1968).

Organic Acids: Although less well studied than ascorbic acid, several other organic acids appear to have comparable enhancing effects in single-meal studies. Gillooly *et al*., (1983) measured iron absorption from 17 vegetable meals. All the vegetables associated with good iron

bioavailability contained appreciable amounts of one or more of the organic acids citric, malic, or ascorbic acids. The addition of citric, malic, or tartaric acids to a rice-based meal improved iron absorption two- to four fold (Gillooly *et al*., 1983; Ballot *et al.,* 1987). The high absorption of iron from maize and sorghum beers in sub-Saharan Africa is due to the presence of lactic acid (Derman *et al.,* 1980).

Animal Tissue: Several animal tissues, including beef, chicken, fish, lamb, liver, and pork, improve iron status both by supplying highly available heme iron and by improving absorption from the non heme iron pool (Cook and Monsen, 1976; Lynch *et al.*, 1989). Unlike ascorbic acid, there is only a modest rise in percentage absorption with increasing quantities of tissue protein. The factor(s) in animal tissue responsible for these beneficial properties remain poorly characterized. It has been suggested that peptides released during proteolytic digestion by pepsin in the stomach may increase the solubility of inorganic iron. (Kane and Miller, 1984; Slatkavitz and Clydesdale, 1988; Martinez-Torres and Layrisse, (1970) proposed that the meat effect is a specific property of its amino acid composition and that, in particular, cysteine-containing residues are important (Martinez-Torres *et al.,* 1981).

It is unlikely that the cysteine itself is an important enhancer of iron absorption, but enzymatic digestion of the two major myofibrillary proteins of meat, actin and myosin, produces a large number of cysteine-containing peptides that are stable in the gastrointestinal tract with thiol groups that tend to remain unoxidized (Taylor *et al.,* 1986). These peptides could bind iron, maintaining its solubility and availability for absorption. However, vitro studies have provided contradictory evidence suggesting that iron in the presence of enzymatic digestion products of meat is complexed with carboxyl but not thiol groups (Fitzsimmons *et al.,* 1985; Shears *et al.,*

1987). The effects of animal tissue may be more complex; several mechanisms could be involved. Torrance *et al.,* (1982) proposed that meat may act primarily by reducing the inhibitory effect of polyphenols. Kapsokefalou and Miller, (1991) found an increase in stable ferrous iron during in vitro beef digestion, suggesting that meat has a reducing effect. Finally, Zhang *et al*., (1990) proposed that meat may act by stimulating gastric acid production.

3.3.4. Iron Absorption Inhibitors

Phytate: The reduced iron absorption from meals containing wheat bran led Widdowson and McCance37 to suspect that phytate is an important inhibitory factor. A large number of subsequent studies have demonstrated that phytate is indeed a major inhibitor in cereal foods such as wheat, oats, sorghum, unpolished rice, and beans (Gillooly *et al*., 1983; Hurrell *et al.,* 1992). Studies using both wheat (Hallberg *et al*., 1989) and soybeans (Hurrell *et al.,* 1992) show that even small quantities of phytate are strongly inhibitor. Moreover, in the study reported by Hurrell *et al.,* (1992) using soybean protein isolates, there was no increase in the inhibitory effect of phytate at phytic acid concentrations above 4 mg per gram of soy protein isolate.

The details of the mechanisms by which phytates inhibit iron absorption have not been characterized. Monoferric phytate, which constitutes only a small proportion of the phytate in bran, is not inhibitory (Simpson *et al.,* 1981), but the formation of diferric and tetraferric phytate complexes in the gastrointestinal tract may render iron unavailable for absorption (Morris *et al.,* 1982).

Polyphenols: Tea was found to be a powerful inhibitor of iron absorption by Disler *et al.,* (1975). Subsequent studies indicated that this is primarily the result of its tannin content (Disler *et al.,* 1975). Polyphenols are present in other popular beverages and are common constituents of

many vegetables including several cereals. They appear to be equal in importance to phytates as inhibitors of non heme iron absorption. The extent of inhibition varies inversely with the condensed polyphenol content (Gillooly *et al*., 1983). As is the case for phytate, the maximal effect occurs at relatively low polyphenol concentrations. This has been demonstrated both by measuring non heme iron absorption from meals containing foods with varying polyphenol contents and by evaluating absorption from wheat rolls or dephytinized bread to which increasing quantities of tannic acid were added (Brune *et al*., 1989; Siegenberg *et al*., 1991). Polyphenols are thought to act through the formation of complexes between the hydroxyl groups of the phenolic compounds and iron molecules, rendering the iron unavailable for absorption.

Protein Digestion Product: While animal tissues improve non heme iron absorption, other proteins of both animal and vegetable origin exert an inhibitory effect. When tested in a semi purified meal of hydrolyzed maize starch, corn oil, and a protein source, animal protein foods such as whole milk, casein, and whey proteins derived from milk, cheese, whole egg, and egg white as well as purified bovine serum albumin diminished absorption to between 50% and 10% of the value obtained using a meal comprising only hydrolyzed maize starch and corn oil. (Lynch *et al*., 1989; Hurrell *et al*., 1989). Bovine serum albumin was the least inhibitory and whey protein the most. Two vegetable proteins tested (wheat gluten and soy protein) also had a marked inhibitory effect.

Lynch *et al*., (1994) were able to demonstrate the presence of a protein-related moiety in soybean protein isolate that inhibits non heme iron absorption independently from the effect of phytate. The two major protein fractions in soybeans (7S conglycinin and 11S glycinin) were purified and dephytinized. The essentially phytate-free 7S conglycinin fraction had an inhibitory effect

approximately equal to that of phytate, whereas the 11S glycinin fraction was only inhibitory in the presence of phytate. The recent observation of McFarlane *et al*., (1990) and Baynes *et al*., (1990) also suggest an important role for protein. They found that improved iron bioavailability in fermented soy products can be correlated with diminishing size of the predominant polypeptides remaining in a given product after fermentation.

Calcium: The addition of calcium in the form of milk or an inorganic salt to a meal reduces percentage nonheme iron absorption in human beings. However, the effect of calcium is complex and the mechanisms by which it interferes with iron uptake are poorly understood. An early study was based on a simple meal of semi purified ingredients that contained little calcium and had low iron bioavailability (Monsen *et al*., 1976).

Other two studies (Hallberg *et al*., 1991; Cook *et al*., 1991) suggest that calcium interferes with iron absorption by interactions that occur in the lumen. They appear to be complex and involve other dietary components. The inhibitory effect depends on the meal type, its normal calcium content, and, in some instances, the inhibition of phytate degradation during preparation. These observations may have particular importance for women taking calcium supplements.

3.4. Iron Deficiency

Iron deficiency is the most common form of malnutrition in the world, affecting more than 2 billion people globally. It is the most common micronutrient deficiencies in the world affecting 20% or more of women and children especially in developing countries (Caballero *et al*., 2005).

Iron deficiency occurs when dietary iron intake is low or the bioavailability is low. Due to low intake body iron starts to fall which will follow by decreasing of serum ferittin. When iron.

When lower iron intake goes on there will be a decrease in iron transport which will lead to lower iron serum and higher in transferrin. And finally the lower iron in the body will limit hemoglobin production in bone marrow and lead to lower hemoglobin level which is known as iron deficiency anemia (Whitney *et al*., 2009).

Cognitive Motor and Behavioral Development: In experimental animals, iron has been shown to play a key role in brain function. Several areas of the brain contain iron, sometimes in large quantities. Iron-deficient animals show alterations both in neurotransmitters and behavior that do not usually respond to iron replenishment (WHO, 2001).

Physical Work capacity: A linear relationship has been reported between iron deficiency and work capacity for agricultural workers in Colombia, Guatemala, Indonesia, Kenya, and Sri Lanka. Work capacity returned rapidly to normal with iron supplementation. Compared with non-anemic women, anemic female workers in China were 15% less efficient in performing their work. They spent 6% less energy on their out-of-work activities, had 4% lower maximal work capacity, and had 12% lower overall productivity, as compared to levels achieved after anemia was corrected by iron treatment for 4 months (WHO, 2001).

Pregnancy: Iron Deficiency Anemia increases the rate for maternal morbidity and mortality. It is estimated that 50% of women do not have adequate iron stores for pregnancy. Because the iron required for pregnancy (3–4 mg/d) is substantial, risk of iron deficiency and IDA increases with gestation (Scholl, 2005; Yip & Ramakrishnan, 2002). Some of the increase in anemia and iron deficiency anemia with gestation is an artifact of the normal physiologic changes of pregnancy. Hemoglobin and hematocrit decline throughout the 1st and 2nd trimesters, reach their lowest point late in the second to early in the 3rd trimester and then rise again nearer to term. However, IDA

during the first and third trimester resulted in a two fold increase in the risk of inadequate weight gain for gestation (Scholl, 2005).

Immune function: A number of studies have assessed the impact of iron deficiency anemia (typically hemoglobin <100g/L plus one or more measure of iron deficiency including serum iron, total iron-binding capacity, transferrin saturation) on different aspects of immune function. Results from these studies have shown decreases in: the proportion of T lymphocytes in the blood (Swarup-Mitra and Sinha, 1984; Kemahli *et al.*, 1988); the lymphocyte proliferative response to antigens (Swarup-Mitra and Sinha, 1984; Ahluwalia, 2004); and secretion of IL-2 (Galan *et al.*, 1992; Thibault *et al.*, 1993). Neutrophil function (respiratory burst, bacterial killing) is also impaired (Yetgin *et al.*, 1979; Walter *et al.*, 1986).

B lymphocyte functions (antibody responses) seem little affected by iron deficiency (MacDougall *et al.*, 1975). There are a limited number of studies on the impact of iron deficiency on the functions of monocyte/macrophages, none on antigen presentation, and few studies of cytokines other than IL-2. 8.6 Several studies have reported that immune impairments in iron deficient subjects were restored to control values after iron therapy (MacDougall *et al.*, 1975; Swarup-Mitra and Sinha, 1984), suggesting a pathogenic role of iron deficiency.

One study carried out among women of reproductive age in Ethiopia shows that mild to moderate anemia (30.4%), iron deficiency (49.7%) and iron deficiency anemia (17%) are common while severe anemia is rare (0.9%) with distinct regional variations. Furthermore, the study revealed the predominant age groups affected to be 15 - 24 and >35years (Melaku *et al.*, 2008).

Other study on the prevalence rate of IDA among pregnant and lactating women in the rural communities shows that prevalence rate of 18.7% (Hidar and Urga, 1999).

3.4.1. Vulnerable Groups

Infants: Eighty percent of the iron present in a newborn term infant is accreted during the third trimester of pregnancy. Infants born prematurely miss this rapid accretion and are deficient in total body iron. A number of maternal conditions, such as anemia, maternal hypertension with intrauterine growth restriction, or diabetes during pregnancy, can also result in low fetal iron stores in both term and preterm (Lozoff *et al.,* 1991).

Introduction of cows' milk to infants aged 6 months has also been associated with small losses of blood from the intestinal tract (Ziegler *et al.,* 1990). Several studies have reported that consumption of cows' milk, especially in the first six months of life (Sullivan, 1993; Robson, 1993) but also in the second six months (Michaelsen *et al.,* 1995; Zlotkin, 1993), is associated with lower hemoglobin and ferritin concentrations.

Children Aged 6 Months to 3 Years: Iron requirements of infants are highest during this period of rapid growth. After 6 months of age, dietary requirements for iron are increased as iron stores diminish and the amount of iron provided from breast milk is no longer sufficient, even with up regulation of intestinal absorptive mechanisms, to meet the increasing demands for growth and blood volume expansion (SACN, 2010)

Children Aged 3 Years and Above: Iron is required for children aged over 3 years to meet the needs for an expanding red cell mass, for growth, and to replace basal losses like it does for children (Dallman, 1990).

Adolescents: Increased iron requirements for this age group are driven by the pubertal growth spurt and increased blood volume, hemoglobin and lean tissue synthesis. Compared with boys, these changes are less for girls due to their lower growth velocity and differences in body composition. Whereas lean tissue, as a percentage of body mass, increases during puberty in boys, there is actually a small decrease in girls (Molgaard *et al.*, 1998). The peripheral use of iron due to increased growth rate means that serum ferritin concentrations may fall to levels at or below the usual reference range. For adolescent girls, iron requirements also increase to cover additional losses due to menstruation. However, the growth spurt precedes menarche and as a consequence the decline in serum ferritin concentrations in pubertal girls also precedes menarche. Although menstrual blood losses are initially small, the menstrual blood loss of adolescent girls aged 15 years, is only marginally lower than in older women and it is generally assumed that adolescent menstrual losses are at the same level as those for adults (Hallberg and Rossander-Hulten, 1991).

Menstruation: Menstrual blood loss is an important determinant of iron depots in women of reproductive age. A number of studies have observed an association between serum ferritin concentration and length of the menstrual period (Galan *et al.*, 1985; Soustre *et al.*, 1986; Milman *et al.*, 1993). Harvey *et al.*, (2005) reported a strong inverse correlation between menstrual blood loss and serum ferritin concentration with higher menstrual iron losses resulting in lower serum ferritin concentrations ($p < 0.001$); on average, an increase in menstrual iron loss of 1 mg/day resulted in a decrease in serum ferritin of 7 μg/L. There is little intra-individual variation between menstrual cycles (Hallberg and Nilsson, 1964) but considerable inter-individual variation which follows a right-skewed distribution in a population (Hallberg *et al.*, 1966).

The long term implications of menstrual iron losses over 20–30 years in the development of iron deficiency anemia in women of reproductive age and the volume of menstrual blood loss which should be regarded as abnormal and needing clinical management to treat iron deficiency anemia are not well characterized (SACN, 2010)

Pregnancy and Lactation: During pregnancy, there is a significant increase in iron requirement due to the rapid growth of the placenta and the fetus and the expansion of the globular mass (Dallman, 1990). Physiological adaptations during pregnancy and lactation ensure an adequate supply of iron to the fetus and developing infant, even in the presence of iron deficiency. However, severe iron deficiency and anemia can affect reproductive efficiency. The effect of pregnancy on iron metabolism Adaptations during pregnancy ensure the supply of nutrients to the fetus and other products of conception and sustain the additional metabolic burden of pregnancy. Maternal plasma volume and red cell mass increase early in pregnancy in advance of the systemic metabolic changes and significant growth that occurs in the fetus during the latter half of gestation. Iron supply to the conceptus is favored by changes in the structural and compositional characteristics of transferrin which facilitate preferential delivery of iron to placental rather than systemic transferrin receptors (SACN, 2010).

It has been suggested that women with short interpregnancy intervals (<18 months) may not have sufficient time to replace nutrients used during the previous pregnancy and that this may compromise their nutritional status at conception and their ability to support fetal development (King, 2003).

3.4.2. How to Diagnose Iron Deficiency

Due to low intake of dietary iron, iron store in the body falls and serum ferittin also falls. Measure of serum ferittin is the most sensitive and valuable indicator of iron store at this early age. However serum ferritin is also an acute phase protein, which means that its concentration rises during inflammation, so the customary thresholds to indicate an iron deficiency of <12–15 μg/l may no longer apply. One way of controlling for a high serum ferritin concentration resulting from infection would be to use the concentration of another acute phase protein to exclude individuals whose measurements of both indicators are above a certain threshold (WHO and CDC, 2004).

When lower iron intake goes on there will be a decrease in iron transport which will lead to lower iron serum and higher in transferring which are known as iron transporting proteins. Measurement of both serum iron and transferrin indicate the severity of iron deficiency. The more transferrin and the less iron in the blood the more advanced the deficiency (WHO, 2001).

Major advantages of measuring serum transferrin receptors involve the facts that the assay is not significantly affected by infection or inflammatory processes, and it does not vary with age, gender, or pregnancy. However, serum transferrin receptor levels may be elevated when there is increased red cell production, turnover, or both, such as in the case of haemolytic anaemia (WHO, 2001).

There are several methods for measuring serum transferrin. The most commonly used method is based on the ELISA assay (enzyme-linked immunosorbant assay). The values obtained will vary according to the method used, however, since there is no uniform standard available for their measurement (WHO, 2001).

When iron lower and limit hemoglobin production hemoglobin precursor erythrocyte protoporphyrin begins to accumulate and hematocrit values decline. Hemoglobin and hemmatocrit tests are easy and inexpensive but these tests are late indicators of iron deficiency (Whitney *et al*., 2009).

3.4.3. Prevention of Iron Deficiency

The four principle strategies are recommended for correcting iron deficiency in populations, alone or in combination: education combined with dietary modification or diversification, or both, to improve iron intake and bioavailability; iron supplementation (provision of iron, usually in higher doses, without food), iron fortification of foods and the new approach of biofortification. However, there are some difficulties in the application of some of these strategies when considering iron (Yip 2002).

Food Diversification: Dietary modifications for reducing IDA involve increased intake of iron rich foods such as flesh foods, consumption of fruits and vegetables rich in ascorbic acid to enhance non-heme 65 iron absorption and reduce intake of tea and coffee, which inhibit non-heme iron absorption. Food based approaches are recognized as an essential part of an urgently needed more comprehensive strategy to combat iron and other micronutrient deficiencies. As food-based strategies aim to improve the quality of the overall diet by increasing the availability and consumption of a wider range of foods, they address multiple nutrient deficiencies simultaneously (Hurrell, 2002; FAO/WHO, 2004).

Supplementation: Oral iron supplementation, ferrous iron salts (ferrous sulfate and ferrous gluconate) are preferred because of their low cost and high bioavailability (Zimmerman and Hurrell, 2007). Although iron absorption is enhanced when iron supplements are given on an

empty stomach, nausea and epigastric pain might develop. If these side-effects arise, lower doses between meals should be attempted or iron should be provided with meals, though food reduces absorption of medicinal iron by about two-thirds (Cavalli-Sforza *et al*., 2005).

Iron supplementation during pregnancy is advisable in developing countries, where women often enter pregnancy with low iron stores (CDC, 2002). Although the benefits of iron supplementation have generally been considered to outweigh the putative risks, there is some evidence to suggest that supplementation at levels recommended for otherwise healthy children carries the risk of increased severity of infectious disease in the presence of malaria and/or under nutrition (Oppenheimer, 2001; Sazawal *et al.*, 2006).

Fortification: Fortification of foods with iron is more difficult than it is with other nutrients, such as zinc in flour, iodine in salt and vitamin A in cooking oil (Zimmerman and Hurrell, 2007). The most bioavailable iron compounds are soluble in water or diluted acid, but often react with other food components to cause off-flavors, color changes or fat oxidation (Hurrell, 2002). Thus, less soluble forms of iron, although less well absorbed, are often chosen for fortification to avoid unwanted sensory changes (Zimmerman and Hurrell, 2007). Fortification with low iron doses is more similar to the physiological environment than in supplementation and might be the safest intervention (WHO, 2007).

Iron compounds recommended for food fortification by the WHO, (2006) include ferrous sulfate, ferrous fumarate, ferric pyrophosphate, and electrolytic iron powder. Hallberg and Rossander-Hulthen, (1991) estimated that 25% of the total iron intake in Sweden and the United States comes from fortification iron. When they calculated the bioavailability factors for the complete diet, they assumed the fortification iron was mainly low-bioavailability elemental iron powders

and they estimated that it was only 15% as well absorbed as native food iron. Food-fortification practices vary nationally and the need to adjust the dietary iron bioavailability factor for fortification iron will depend on the proportion of fortification iron in the total iron intake and the iron compounds used.

Biofortification: Iron contents vary from 25 to 56 mg/kg in wheat and 7 to 23 mg/kg in rice grains. However, most of this iron is removed during the milling process. Iron absorption from cereals and legumes, many of which have high native iron content, is generally low because of their high contents of phytate and polyphenols (Hurrell *et al.,* 1999).

In a biofortification study Lucca *et al.,* (2002) increased the iron content in rice endosperm to improve its absorption in the human intestine by means of genetic engineering. They introduced a ferritin gene from Phaseolus vulgaris into rice grains, increasing their iron content up to twofold. To increase iron bioavailability, they introduced a thermo-tolerant phytase from Aspergillus fumigatus into the rice endosperm. They indicated that this rice, with higher iron content and rich in phytase has a great potential to substantially improve iron nutrition in those populations where iron deficiency is so widely spread (Lucca *et al*., 2002).

Another strategy is to reduce anti-nutrient contents in order to make the iron supplied from their food sources more available. Iron availability may also increase by some techniques such as soaking, germination and fermentation, which promote enzymatic hydrolysis of phytic acid in whole grain cereals and legumes by enhancing the activity of endogenous or exogenous phytase enzyme (Schlemmer, 2009). Even use of non-enzymatic methods such as thermal processing, soaking, and milling for reducing phytic acid content in plant-based staples has been successful to improve the bioavailability of zinc and iron (Cook, 2005; Liang, 2008).

Because Ethiopia has a wide range of agro-climatic conditions, a wide variety of cereals, root crops and *enset* (E*nset ventricosum*) are grown; some of these yields are not fully exploited by the general population. There seems to be dependency on a single crop, resulting in shortage of minerals and vitamins implying that the bioavailability of much of the iron in the average Ethiopian diet is restricted, presumably affecting the iron status of the community (Hidar and Urga, 1999)

Studies showed that some crops commonly consumed in Ethiopia notably *teff,* are high in iron (Kaluski, Ophir and Amede, 2002) and fermented *enset* may increase non-heme iron absorption (Bering *et al*., 2006). Other study shows that the reason in slightly more than half of the subjects with anemia is the low intake of meat which is a source of heme iron. Heme iron is not only better absorbed than non-heme obtained from plant source food, whose absorption may range from 1- 10%, but also has an enhancing effect on absorption (Haidar and Pobocik, 2009). In one study finding, about one-third of the women with anemia had vegetables less than once a day suggests low consumption of vitamin C and could be another substantiating factor for the existence of iron deficiency anemia (Gibson *et al.,* 2008). A study with children in Ethiopia (Adish *et al.,* 1999) which included a thorough assessment of dietary intake showed that dietary iron was adequate but bioavailability was restricted because the type of iron was non-heme and there was inadequate vitamin C, additionally, absorption was further reduced due to the presence of inhibitory factors such as tannins and phenols.

3.5. Iron Overload

Iron overload occurs when excess iron accumulates in the body. It can be caused by increased absorption of dietary iron or by parenteral iron loading (repeated blood transfusions) as there is no mechanism to excrete excess iron (SACN, 2010).

3.5.1. Increased Iron Absorption

Iron absorption is increased with the haemochromatoses, rare genetic disorders of iron metabolism which include atransferrinaemia and aceruloplasminaemia, and with sub-Saharan dietary iron overload (SACN, 2010).

Chronic liver disease like alcoholic cirrhosis and porphyria cutanea tarda may also be associated with iron loading. Ineffective erythropoiesis is associated with increased iron absorption in severe thalassemia disorders (SACN, 2010)

3.5.2. Parenteral Iron Loading

The rate of iron loading through regular transfusions is considerably greater than the maximum possible increased iron absorption. One unit of red blood cells delivers approximately 200 mg iron, which means that individuals with transfusion dependent anemias are at significant risk of iron overload. With congenital anemias, such as s-thalassemia major, this can lead to the accumulation of up to 100 g iron by 20 years of age, by which time most patients would have died from toxic effects of the excess iron (Modell, 1979). Complications associated with iron overload include cardiac arrhythmias and heart failure, diabetes, delayed onset of puberty, and cirrhosis (Pippard, 1994).

4. MATERIALS AND METHODS

4.1. Study Area

The study was conducted in the College of Natural Sciences, Addis Ababa University on female regular students. The College of Natural Sciences, Arat-kilo campus comprises eight departments, two schools, two institutes, and three multidisciplinary programs offering undergraduate and postgraduate degrees.

4.2. Subjects

Sample size calculation formula will not hold in such context given the homogeneity of the population (same university, same status, same cafeteria, etc.). This study therefore, aimed to represent at least 5% - 10% of the female students of the College of Natural Sciences. Subjects were recruited through wall post advertisement. Only 83 Subjects (n = 83) gave their consent to take part in the study. Information on their socio-demographic characteristics and their dietary consumption and dietary practices were collected from all subjects through semi structured questionnaire (Appendix A). Only (n=45) subjects gave their consent for blood collection. In addition, subjects with no chronic illness, not taking any medication that affects iron status and university students' cafeteria users were considered to take part in the study.

4.3. Food Sampling and Weighed Recording

Complete menu food served by students cafeteria was collected and transferred to the laboratory of food science and nutrition for further analysis. Weighed record data for one day was also collected. The served meals were weighed and the leftovers (if any) were collected, weighed and deducted from the amount of food served to the subjects (Gibson 2005). Moisture content was

done for the raw food samples before drying. The food samples were dried at 50 ^{0}C for three days before other analysis was done.

4.4. Laboratory Analysis

4.4.1. Moisture Determination

Moisture content of the samples was determined according to AOAC (2000). A crucible dish was cleaned and placed in an oven (DHG90SSA, China) to dry at 105^{o}C for an hour and was placed in desiccators to cool down at least for 30 min. The crucible dish weight (W1) was measured after cooling. 5g of sample was measured on the crucible dish (W2) and dried at 105^{o}C for 3 hrs. After cooling in desiccators to room temperature the weight was measured (W3).

The moisture content was determined using the following equation;

$$\text{Moisture content in percent } (\%) = \frac{(W2 - W3)}{(W2 - W1)} * 100$$

4.4.2. Crude Protein Determination

Protein content of the samples was determined using kejeldhal method according to AOAC (2000). About 0.5 g of the sample was weighed by analytical balance and added to the digestion flask. Then 6 ml of acid mixture (concentrated ortho phospsoric acid and concentrated sulphuric acid) and 3.5 ml of 30% hydrogen peroxide solution were added in to the digestion flask step by step. The tubes were shaked until the violet reaction disappeared. About 3g of the catalyst mixture made of 0.5 g of selenium and 100 g of potassium sulphate was added in to the digestion flask. The solution was then digested at 370^{0}C for 1 hr by Tecator digester. After digestion was completed, the content in the flask

was diluted by water and 40% sodium hydroxide was added to neutralize the acid and to make the solution slightly alkaline.

$$NH_4SO_4 + 2NaOH \Longrightarrow 2NH_3 + 2H_2O + Na_2SO_4$$

The ammonia was then distilled into receiving flask that consisted solution of excess 4% boric acid solution for reaction with ammonia. The borate ion was formed as the result of the reaction of the boric acid and the ammonia and this was titrated with standard acid (0.1N sulphuric acid solution).

$$NH_3 + H_3BO_3 \text{ (boric acid)} \Longrightarrow NH_4^+ + H_2BO_3^- \text{ (borate ion)}$$

The nitrogen content was calculated from the equation:

Calculation

The Nitrogen content was determined as

$$\text{Nitrogen (\%)} = \frac{\text{V HCL in L} * \text{N HCL (ca.0.1)}}{\text{Wo}} * 14.007 * 100$$

The % of nitrogen is converted to % of protein by using appropriate conversion factor.

$$\text{Protein (\%)} = 6.25 * \text{\%Nitrogen}$$

Where:

V -The volume of HCL in L consumed to the end point of titration

N - The normality of HCL (used often is 0.1N)

W_o - Sample weight on dry matter basis

14.007 - The molecular weight of nitrogen.

4.4.3. Crude Fat Determination

The crude fat was extracted according to AOAC (2000) official method 4.5.01. The extraction cylinder was cleaned and dried in an oven at 105^0 C for an hour cooled and weighed (W_1). Briefly, triplicates of 2.00 g of the homogenized samples were weighed (W) in pre dried porous ceramic extraction thimble plugged with cotton. The thimble was placed in the extraction chamber (S2C-C, China). 50 ml of diethyl ether was added by using measuring cylinder and move in to the heating plank. The extraction continued for four hours at 55^0C. At 30 minute interval the extractor was checked to know whether the solvent is evaporated or not. After the extraction, the cylinder was placed in drying oven at 70^0C for 30 minute to remove the solvent and be cooled in desiccator for 30 minute. The extraction cylinder and the fat were weighed immediately after taken from the desiccators (W_2).

The crude fat was determined using the equation;

$$\text{Crude fat, percent by weight} = \frac{(W2 - W1)}{W} * 100$$

4.4.4. Ash Content Determination

The ash content was determined by AOAC (2000) using official method 923.03. The porcelain crucible dish was cleaned and dried in a muffle furnace (CSS1200, England) for 30 mins at 550^oC. The dishes was cooled in desiccators (with granular silica gel) for about 30 minutes at

room temperature and weighed to the nearest milligram (M_1). 2.5g of sample was weighed and placed in the dish (M_2). The sample was charged on hot plate under a fume-hood with increasing the temperature slowly until the smoke ceases. The sample was converted into ash in muffle furnace at 550^{o}C for 5 hours. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram (M_3).

$$\text{Total Ash (\%)} = \frac{(M3-M1)}{(M2-M1)} * 100$$

Where:

(M_2-M_1) - Sample mass in g on dry base

(M_3-M_1) - Mass of ash in g.

M_3= Mass of crucible and sample after ashing

M_2 = Mass of crucible and sample before ashing

M_1= Mass of crucible

4.4.5. Carbohydrate Determination

The carbohydrate content was determined by difference method. Briefly, subtraction of the sums of the weights of crude protein, total fat, moisture, and ash in the total weight of the sampled food and were subtracted from 100%.

Carbohydrate content was determined by the equation;

$$\% C = 100 - (\% M + \% P + \% F + \% A + \%Fb)$$

Where:

C- Carbohydrate content,

M - Moisture content,

P - Protein content,

Fb – Fiber content,

F- Fat content, and

A- Ash content.

4.4.6. Energy Determination

Energy calculation was based on the actual intake of the subjects from the weighed record data.

The energy yields of the samples were calculated as:

Energy Value = Carbohydrate + Fat + Protein; and

Where; Fat = 9 kcal/gram; Protein and carbohydrate each =4 kcal/gram

4.4.7. Calcium, Iron and Zinc Determination

Ashed samples were placed in dessicator to be cooled. After cooling to room temperature, the ash was moistened with deionized water and dried on hot plate. The dried samples were then placed in the furnace for 30 min. at 550^0c and placed in dessicator afterwards to be cooled. The samples were wetted with some drops of deionized water and 5 drops of concentrated nitric acid

and placed on low heat hot plate to evaporate the water and the acid. The samples were wetted completely with 5ml of 6N HCl, and dried on a low temperature hot plate. 7ml of 3N HCl was added to dried ash and heated on the hot plate until the solution boiled. The ash solution cooled to room temperature in a dessicator and was filtered into a 500ml graduated flask using filter paper (Whatman45, 125mm). The crucibles were washed three times with deionized water and filtered into a flask. The solution was diluted to 100 ml with de-ionized water. A blank which contains 12ml 3N HCl and deionized water in 100ml volumetric flask was also prepared for FAAS. (Tizazu *et al*., 2010)

Standard solutions: four series of standard metal solution was prepared by appropriate dilution of the metal stock solution with deionized water containing 2.4ml of 3N HCl in 10 ml volumetric flask. Calibration curve (absorbance verses concentration) for each element using the prepared standard solution was prepared. For the determination of calcium, lanthanum chloride (10% w/v) was added to both standards and samples to suppress interference from phosphorus (Tizazu *et al*., 2010).The sample concentration was analyzed using FAAS by aspirating de-ionized water. Sample blank solution was run with the sample solution.

$$\text{Metal content}\left(\frac{mg}{100g}\right) = \frac{[(a-b)*V]}{10W}$$

Where;

W = Weight (g) of samples

V = 50ml = volume (v) of extract

a = Concentration (µg/ml) of sample solution.

4.4.8. Determination of Phytate

Phytate was determined following Latta and Eskin, (1980) as later modified by Vantraub and Lapteva, (1988). Briefly, 0.1 g of dried sample was extracted with 10ml of 0.2N HCl for 1 hr at an ambient temperature and centrifuge (3000 rpm /30min). The clear supernatant was used for the phytate estimation. 2ml of wade reagent was added to 3ml of the supernatant sample solution then homogenize and centrifuged the solution (3000 rpm/10 minute). The absorbance at 500nm was measured using UV–Vis spectrophotometer. The phytate concentration was calculated from the difference between the absorbance of the blank (3ml of 0.2N HCl and 2ml of wade of reagent) and that of assayed sample. The amount of phytic acid was calculated using phytic acid standard curve and result was expressed as phytic acid in µg/g fresh weight.

A series of standard solution was prepared containing 4-40 µg/g phytic acid in 0.2N HCL. 3 ml of each standard was pipetted into 15 ml centrifuge tubes and 3 ml 0.2N HCl (blank). 2 ml of the wade reagent was added to each tube, and the solution was mixed on a vortex mixer for 5 seconds. The mixture was centrifuged (3000 rpm/10 min.) and the supernatant read at 500 nm by using water to make zero the spectrophotometer. Calibration curve (absorbance Vs concentration) was plotted using Microsoft excel 2007 to find out the slope and intercept.

$$\text{Phytic acid in } \mu g/g = \frac{[(Ab-As)-\text{Intercept}]}{\text{Slope} \times W \times 3} * 10$$

Where As = Sample absorbance, Ab= Blank absorbance, W= Weight of sample

4.5. Determination of iron and zinc bioavailability

The contents of phytic acid, iron and zinc were converted into moles by dividing by their respective molar mass and atomic weight (660.04, 55.85 and 65.01 g/ mol, respectively). The molar ratios of phytic acid/Fe and phytic acid/Zn were then calculated (Baye *et al.*, 2014).

4.6. Blood Collection and Analysis

Blood was collected from July/ 8 /2014 to July /11 /2014. About 5ml of blood was drawn from the anticubetal vein of the upper arm. Blood samples were analyzed for hemoglobin using hemocue (Hb 301 System) on site. The rest of blood samples were left to coagulate for 30 min. at room temperature. The coagulated blood was centrifuged to separate the serum. Serum was stored at lower temperature in the department until it was transported to the Ethiopian public health institute (EPHI) and stored at -80^{o}C until for further analyses.

Serum Ferritin

The serum was analyzed for ferritin in EPHI, HIV National Reference Laboratory, Clinical Chemistry section. The thawed serum samples were transferred into labeled test vials. Serum ferritin analysis (Gupta *et al*. 2009) was performed on Cobas e-411 (Roche Diagnostics GmbH, Mannheim, Germany) based on the electrochemiluminescent (ECL) reaction of ruthenium (II) tris (bipyridyl) with tripropylamine, according to standard operating procedure and manufacturer recommendation. All components and reagents for routine analysis are integrated in or on the analyzer.

Soluble Transferrin Receptor

Serum transferrin was determined based on the immunological agglutination principle (Wiwanitkita *et al*., 2007) with clinical chemistry analyzer Cobas Integra 400 plus (Roche Diagnostics GMBH, Menheim, Germany). The concentration of transferrin were determined through photometric measurement at 340 nm magnitude of scattering light by the precipitate formed from the reaction between human serum transferrin with a specific antiserum.

C - Reactive Protein (CRP)

Serum CRP was quantified by particle enhanced turbidimetric assay with clinical chemistry analyzer Cobas Integra 400 Plus (Roche Diagnostics GMBH, Menheim, Germany) in which human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate was determined turbidimetrically at 552 nm (Riordan and McWilliam, 2010).

4.7.Ethical consideration

The study was conducted following the Helsinki declaration guideline on human research ethics. The study was approved by the institutional review board at Addis Ababa University. In addition, written consent (Appendix B) was obtained from the participant.

4.8. Statistical Analysis

Data were entered and analyzed using Statistical Package for Social Sciences program (SPSS) Version 16. Descriptive statistics like range, and arithmetic mean ± SD were used to express the level of variables. Pearson correlation was conducted to test correlation of between variables. Level significance was set at $p< 0.05$.

4. RESULTS AND DISCUSSION

5.1. Socio-Demographic Status

The academic year of the students ranged from first year to third year. The anthropometric status of the subjects is summarized in table 1. Only four of the subjects had BMI above the recommended cutoff value.

Table 1. Anthropometric status of female university students`(n = 45)

Parameter	Mean±SD	Range
Age	20.35 ± 1.4	18.00 – 23.00
Height (m)	1.58 ± 0.1	1.48 - 1.70
Weight(Kg)	54.91± 5.6	46.00 – 70.00
BMI *(Kg/m^2)	21.85 ± 1.7	18.37 - 26.00

* BMI- Body Mass Index

5.2. Frequency of Eating Outside Students' Cafeteria

The frequency of subjects eating outside student's cafeteria is summarized in table 2. Subjects who ate more than three servings per week were considered as frequently and below three servings were considered infrequent. As shown in the table, the majority of subjects (86.6%) ate outside the students cafeteria infrequently, which shows their diet and any effect on iron status is mainly dependent on students' cafeteria meal.

Table 2. Frequency of eating outside student's cafeteria of female university students

Frequency	(n = 45)	
	N	%
No	3	6.7
Infrequently	39	86.6
Frequently	3	6.7

5.3. Coffee/Tea Consumption of Subjects

Frequency of coffee/tea consumption is summarized in table 3. Only two (4.4%) of the subjects did not drink coffee or tea. The majority of the subjects (53.3%) drink coffee/tea less than one hour after a meal. There was no significant association (p=0.211) between coffee or tea consumption and ferritin status of the subjects and also there was no significant association between hemoglobin value and consumption of tea/coffee (p=.710).

Table 3. Timing of drinking coffee/tea of female university students

Frequency	(n = 45)	
	N	%
Less than one hour after a meal	24	53.3
After one hour a meal	8	17.9
After two hours a meal and above	11	24.4

5.4. Fruit Consumption of Subjects

Frequency of fruit consumption is summarized in table 4. Only one (2.2%) of the subjects consume fruit once a daily. Eleven (24.4%) of the subjects consume fruit less than one hour before/after a meal. There was no significant association found between fruit consumption and ferritin status of the subjects (p = 0.105).

Table 4. Timing of fruit consumption of female university students

Frequency	(n=45)	
	N	%
Less than one hour before/after a meal	11	24.4
Before /after one hour a meal	11	24.4
Two hours and above before/after a meal	15	33.4

5.5. Menstrual Bleeding

Menstrual status of the Participants is summarized in table 5. Four (8.9%) of the subjects were demonstrated to have heavy blood loss. Among these four two of them have depleted iron store and iron deficiency anemia. Duration of menstrual cycle between subjects varies from two days to seven days.

Table 5. Menstrual status of female university students

	(n = 45)	
	N	%
Heavy Blood Loss	4	8.9
Missing For One Month	2	4.4
Missing For Two Months and above	7	15.6
No abnormality	32	71.1

5.6. Fasting Status

One year fasting history was gathered and summarized in table 6. The majority of the participants (84.4%) did practice fasting and the rest 15.6% did not practice fasting partially in the past year.

As for the degree of fasting, eighteen (40.0%) of the subjects reported that they missed breakfast and abstained from all animal source foods. In addition to missing breakfast and abstaining from animal source foods, sixteen (35.5%)of the subjects abstained lunch. There was no significant correlation between fasting and serum ferritin of the subjects (P = 0.701).

Table 6. Frequency distribution of university girls practiced fasting.

	N=45	
	N	%
All ASF*	4	8.9
All ASF and break fast	18	40.0
All ASF, breakfast and lunch	16	35.5

* ASF- Animal source foods

5.7. Dietary pattern, Energy and Nutrient Intake

Table 7. Dietary pattern of Addis Ababa University College of Natural Sciences student's cafeteria

	Breakfast	Lunch	Dinner
Monday	Firfir,Tea,bread	Minchet,Bread,Enjera	Gulash,Bread,Enjera
Tuesday	Kinche,Tea,Bread	Minchet,Bread,Enjera	Gulash,Bread,Enjera
Wednesday	Marmalada,Bread,Tea	Misir,Atikilt,Enjera,Bread	Ater,Bread,Enjera
Thursday	Kinche+Tea+Bread	Minchet,Bread,Enjera	Gulash,Bread,Enjera
Friday	Marmalad,Bread,Tea	Misir,Atikilt,Enjera,Bread	Ater,Bread,Enjera
Saturday	Kinche,Tea,Bread	Misir, Bread, Enjera	Shiro,Bread,Enjera
Sunday	Boiled egg,Bread,Tea	Bread,Pasta,Sigo	Gulash,Bread, Enjera

The energy and proximate composition of foods served by the cafeteria is summarized in table 8.Bread contributes the highest energy per 100 gram (268.13±1.8kcal) and Gulash contributes the lowest energy per 100 gram (73.35±2.1kcal) as for iron, enjera had the highest iron content of all (13.39±0.9 mg/100gm) and marmalade had the lowest (0.29±0.01 mg/100gm).

Table 8. Proximate composition of foods served by Addis Ababa University College of Natural Sciences Students' cafeteria

Sample	Moisture %	Fat %	Protein %	Ash %	Carbohydrate %	Fiber %	Phytate (µg/g)	Iron mg/100gm	Calcium mg/100gm	Zinc mg/100gm	Mean Energykcal/100 gm
Injera	61.36±2.7	1.66±0.1	10.08±0.4	1.91±0.3	83.48±3.0	1.43±0.2	545.85±19.1	13.39±0.9	83.25±6.0	1.25±0.1	148.28±1.1
Dabo	33.06±4.8	2.12±0.2	14.16±0.5	2.51±1.3	78.83±1.9	1.19±0.1	649.92±41.3	1.03±0.2	33.33±7.3	0.54±0.3	263.37±1.8
Firfir	71.11±1.2	30.86±1.0	7.49±0.1	3.47±1.5	54.87±1.3	1.65±0.1	545.94± 7.2	12.07±0.6	83.20±6.7	0.90±0.2	149.52±1.4
Marmalade	34.50±0.3	-	0.50±0.4	0.50±0.3	62.86± 1.3	0.82±0.1	343.41±15.1	0.29±0.1	38.70±0.9	0.04±0.1	256.72±2.3
kinche	64.12±1.2	19.29±2.5	10.47±0.1	1.50±0.4	64.58±3.1	2.08 ±0.9	504.13±16.6	1.39±0.1	45.95±2.6	0.98±0.2	167.65±1.4
pasta	63.31±0.4	3.90±2.1	14.68±0.5	1.11±0.1	77.69±2.3	1.31 ±0.2	615.35±22.0	1.10±0.1	37.34±1.1	1.18±0.1	147.04±2.7
Boild Egg	71.77±0.8	33.39±2.3	58.53±4.8	3.10±0.3	4.54±3.7	0.22±0.1	-	3.32±0.1	97.73±1.5	1.77±0.1	155.67±3.5
Atikilt	78.28±1.3	22.42±0.3	8.17±0.1	3.62±0.5	61.19±0.4	2.30± 0.9	431.93±2.0	3.13±2.2	78.24±0.1	1.20±0.1	98.88±1.5
Gulash	87.59±1.2	41.97±1.1	22.00±1.0	4.70±0.2	29.55± 0.9	0.89± 1.4	357.54±18.2	3.25±0.1	57.84±4.2	2.55±0.1	69.79±2.3
Menchet	84.14±0.8	32.37±1.0	25.17±1.5	5.78±0.6	34.86±0.8	0.91± 0.3	402.02±28.1	5.04±0.6	71.55±2.8	2.04±0.2	81.80±3.5
Misir	82.31±0.8	28.87±0.8	16.35±0.06	2.66±1.5	49.53±0.5	1.43±0.9	631.92±25.5	4.73±0.1	62.43±2.4	0.98±0.1	88.69±1.1
Shiro	80.26±5.5	36.62±0.4	14.99±0.44	4.16±0.3	41,52±0.3	1.35±0.5	520.04±26.5	3.13±1.7	48.58±9.1	0.89±0.1	106.41±0.9
Sigo	83.83±0.5	40.74±0.1	17.69±1.15	10.07±0.2	29.59±1.1	0.95±0.5	647.59±31.6	2.08±2.3	70.30±1.2	1.47±0.1	87.30±4.3
Ater	79.46±0.2	27.28±0.3	20.22±2.34	6.47±0.3	43.38±2.1	1.32±0.8	681.24±16.7	5.22±0.1	65.31±0.3	1.10±0.1	99.57±1.9

- Not Analzed

The daily nutrients intake of the overall subjects studied are shown in Tables 9. Based on the result of dietary weight record, 91.3% of participants had inadequate energy and all of the subjects had inadequate Ca and Zn intake. In addition, carbohydrates contributed the greater daily energy (72.3%) beyond the daily recommended value. On the other hand, the contribution of fats (14.4%) for daily energy requirement was low.

Table 9. Daily macro and micronutrient intake against recommended values

Nutrients	Actual intake	Range	Recommended Intake
Energy (kcal)	*1912.58 ± 83.7	1887.78 - 2092.63	2090.0[a]
Fat (g)	31.57 ± 1.4	30.76 - 30.54	43.6 [b]
Protein(g)	65.25 ± 3.2	62.78 – 72.35	46.0 [c]
Carbohydrate (g)	341.85 ± 22.5	335.87 – 386.81	130.0[d]
Fiber (g)	13.35 ± 1.4	11.80 – 15.04	25.0[d]
Iron (mg)	18.01 ± 0.8	17.96 – 19.98	18.0 [e]
Calcium (mg)	219.32 ± 15.0	207.02 – 240.71	1000.0 [e]
Zinc (mg)	3.71 ± 0.3	3.40 - 3.96	4.9[e]

* mean±SD
a:Joint FAO/WHO/UNU Expert Consultation on human energy requirements.2001.
b:FAO Fats and fatty acids in human nutrition: Report of an expert consultation. 2010.
c:Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition.2002.
d:Whitney *et al*., 2009
e:Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirement.2004.

Table 10. Molar ratio of phytate to iron and zinc of foods served by AAU, college of natural sciences students' cafeteria.

Sample	Phytate:Iron	Phytate:Zinc
Atikilt	1.17	3.54
Misir	1.13	6.35
Sigo	2.63	4.33
Firfir	0.38	5.97
Gulash	0.97	1.45
Shiro	1.40	5.75
Minchet	0.67	1.92
Ater	1.10	6.10
Enjera	0.34	4.33
Pasta	4.73	5.13
Kinche	3.07	5.10
Dabo	5.34	11.85
Marmalade	10.02	24.55

Molar ratio of phytate to iron and zinc is summarized in table 10. Marmalade had the highest molar ratio of phytate to zinc and phytate to iron. Only marmalade had phyatate to zinc ratio above the cutoff. Sigo, Pasta, Kinche, Dabo and maramalade was demonstrated to have phytate to iron ration above the cutoff value.

5.8. Blood Analysis Results

The hematological results are summarized in table 11. The result shows that 43 of the subjects had normal status and 2 of the subjects had both ferritin and hemoglobin below the cutoff (<15 µg/l and <12 g/dl respectively). CRP analysis was done to exclude subjects with inflammation but all subjects had normal CRP status. All subjects had normal sTfr status. There was

significant correlation between CRP and sTfR (p = 0.015) and also significant correlation was observed between BMI and serum ferritin status (p = 0.006).

Table 11. Blood analysis result of female university students

Parameters	Mean±SD	Range	Cutoff values
Hemoglobin (g/dl)	12.88±1.1	7.30 – 15.10	12[c]
Ferritin (µg/l)	86.40±6.3	5.16 – 370.7	15[c]
[a]CRP (µg/l)	0.96±1.1	0.12 – 3.98	5[c]
[b]sTfR (µg/l)	2.60±0.3	1.90 – 3.10	5[c]

[a] C- reactive protein
[b] soluble transferrin receptor
[c] WHO/CDC (2004)

Discussion

Iron deficiency anemia is the most common nutritional deficiency worldwide. Woman at reproductive age are at risk of developing iron deficiency. Iron deficiency could cause complications in pregnancy and reduces the work productivity of non pregnant women (Viteri and Burger, 2005).

Body mass index in the range of 18.37-26.03 with mean value of 21.85 ± 1.7, suggesting normal fat-lean mass ratio of the study participants (Table 1). Though BMI has its own limitation to show fat-lean mass ratio it is an important indicator for the risk of obesity.(whitney *et.al.*, 2009)

In the present study there was no significant association (p = 0.211) between the consumption of tea /coffee and ferritin status of the subjects and also there was no significant association between hemoglobin value and consumption of tea/coffee (p=.710). Polyphenol compounds are widely present in the human diet, and they are especially high in tea and coffee (Kuehnau, 1979).

The phenolic compounds are released from the food or beverage during digestion, and can complex with Fe in the intestinal lumen making it unavailable for absorption (Disler *et al*. 1975).

Morck *et al*., (1983) shows that drinking coffee with meal or within one hour have significant effect on iron absorption. In the present study the majority of the subjects use coffee/tea one hour after a meal(Table 3) which could be one reason that coffee/tea consumption didn't affect their iron status.

There was no significant association found between fruit consumption and ferritin status of the subjects ($p = 0.105$). The richest natural sources of vitamin C are fruits and vegetables. Vitamin C has been repeatedly shown in radioisotopic studies to enhance nonheme iron absorption (Diaz *et al*., 2003; Cook and Monsen, 1977; Ballot *et al*., 1987). The present study results indicate that serum ferritin was not specifically associated with fruit intake. In agreement with the present study, other study did not show any association between serum ferritin and fruit intake (Cade *et al.*, 2005). In general, it was suggested that the facilitating effect of vitamin C on iron absorption from a complete diet was less pronounced than that from single meals (Cook and Reddy, 2001) and that vitamin has an enhancing effect only if it is ingested with meals (Fleming *et al.*, 1998; Cook and Monsen, 1977). In the present study majority of the subjects consumed fruit one hour after a meal (Table 4) which could explain the result.

Total body iron stores contain about 2 to 4 g, with substantial differences between the sexes. A 60-kg woman may lose an additional 10 mg of iron per day during menstruation. Iron loss of 42 mg per menstrual cycle has been reported in females with heavy blood flow (Roland and Kelleher, 1989). In the present study, both subjects with IDA reported of experiencing heavy menstrual blood loss which could explain their low iron status. Other study among females of

childbearing age considered to have heavy menstrual cycle and duration of ≥ 8 days as one of the important risk factors. Moreover, history of clots and flooding increased the risk of having IDA (Al-Quaiz, 2001).

There was no significant correlation between fasting and serum ferritin of the subjects (P = 0.701). The blood sample was collected during fasting season, but the results did not show depletion of iron store. There is no or little data on fasting diets and iron status especially in Ethiopia, so it was difficult to compare the results with other findings. The common fasting diet in Ethiopia excludes all animal source foods which makes the fasting diets similar to vegetarian diets. Studies showed that iron from plant source is less bioavailable than iron from animal source which could be one factor for iron deficiency. At least two months between each fasting season could be one possible reason for the result of the present study. Further study to confirm that fasting has no impact on iron status is needed.

Calcium, zinc and fat are below the recommended daily intake values. Lack of dairy food sources in the student cafeteria dietary pattern could explain the low amount of Ca in the diet where as low amount of Zn in the diet may be due to the results of limited intake of animal source food as *Siga wot*. Recommended intake of fat is 20% of total energy intake for women of reproductive age and adults with BMI <18.5, especially in developing countries in which dietary fat may be important to achieve adequate energy intake in malnourished populations (FAO, 2010).

Phytate begins to lose its inhibitory effect on iron absorption when phytate:iron molar ratio is less than1.0, although even ratios as low as 0.2 exert some negative effect (Hurrell *et al*., 2003). The result of the present study (Table 10) indicates that phytate:iron molar ratio of most foods

served in students cafeteria are around the critical limit (1.0), which implies the foods consumed in students cafeteria have moderate bioavailability of iron.

The importance of foodstuffs as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytate may reduce the bioavailabity of dietary zinc by forming insoluble mineral chelates at a physiological pH (Oberleas, 1983). The phytate-to-zinc molar ratio has been proposed as an indicator of zinc bioavailability (Sirkka, 1997). Phytate:zinc molar ratios >15, indicative of poor zinc bioavailability (Morris and Ellis, 1989). Most foods analyzed in this research work had Phytate:zinc molar ratio below 15 which could imply foods consumed in students cafeteria have moderate zinc bioavailability.

Majority of the study subjects (95.5%) have normal iron status (Table 11). Adequate daily iron intake and moderate bioavailability of the foods could be the major causes for the normal iron status. Other studies conducted nationwide in Ethiopia shows mild to moderate form of iron deficiency anemia among women of reproductive age (Umeta *et al*., 2008; Haidar and pobocik, 2009). sTfR and CRP shows positive correlation ($p = 0.015$). CRP and sTfR shows positive correlation due to malaria infection and megaloblastic anemia (WHO/CDC, 2004). Though CRP, sTfR and Serum ferritin are below the cutoff point the positive correlation could suggest the prevalence and further study is required to conform it.

A linear regression shows that a significant correlation between BMI and serum ferritin ($p = 0.006$). BMI was shown to be a risk factor for iron deficiency in several studies with higher ferritin and lower other iron deficiency markers such as transferrin saturation and serum iron. Although the biological mechanism behind iron deficiency and higher BMI is not clear, some

mechanisms have been hypothesized. As Bekri *et al.,* (2006) shows higher BMI could be associated with sequestration of iron through inflammatory mediated mechansm. The other mechanism is as suggested by Menzie *et al.,* (2008) and Yanoff *et al.,* (2007) an increased requirement of iron attributed to larger blood volume. Further study is required to support BMI as a risk factor of iron deficiency between female students.

5. CONCLUSION AND RECOMMENDATION

Iron is one of the most important elements in nutrition and is of fundamental importance to life. As a constituent of heme, it is present in hemoglobin, myoglobin and in a variety of heme and non-heme iron enzymes. These compounds affect many vital functions. When iron deficiency is widespread and severe, effects on the individual's resistance to infectious disease are significant.

Iron deficiency and its severity are considerably greater in women during their reproductive years than in men. This is explained by the fact that in women there are increased requirements related to menstruation, pregnancy and lactation. One way to address this problem is adequate iron intake by diversifying food intake.

In the present study the majority of the subjects (95.56%) had normal iron status. This result could be explained by adequate dietary iron intake provided by the students' cafeteria. Coffee/tea intake and fruit intake shows no correlation with their iron status. Fasting habit also did not affect the iron status of the subjects. Although the iron intake of the subjects was adequate, the energy, Ca and Zn intake was inadequate. Subjects with IDA had experienced heavy menstrual blood loss which could be one possible cause for their iron status. Most of the subjects' BMI fall below 24 suggesting that normal lean-fat mass ratio

Energy deficiency is a major problem in developing countries like Ethiopia. Universities can be one way to address this problem by providing adequate macro and micro nutrients through cafeteria meal by improving the cafeteria meal quality towards nutritional value.

Though this study tried to cover many aspects of university female students nutritional status, the small sample size was a limitation. Studies should be conducted in different campuses and with larger sample size could come up with stronger result.

Limitation of the Study

Although the students are in controlled environment and live in almost similar condition, the small sample size is a limitation of the study. Since there was no conventional method to measure the menstrual blood the study participants report their menstrual blood flow based on their experiences this could also be one limitation of the study.

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7. APPENDICES

Appendix A: Questionnaire

ADDIS ABABA UNIVERSITY, CENTER FOR FOOD SCIENCE AND NUTRITION

Dietary iron intake of female university students and their iron store as future mothers: the case of college of Natural Sciences

I. Socio-demographic questions

1. Name: _____ _____ _____
2. Year: freshman ☐ 2nd year ☐ 3rd year ☐ 4th year ☐ Older ☐
3. Age :_____
4. Place of birth (woreda, zone & region):_____ _____ _____
5. Do you have children? YES ☐ NO ☐

5.1. If YES to question above, how many and how old are they? _____

II. Food Consumption Outside Cafeteria

1. Do you eat outside of student's cafeteria? YES ☐ NO ☐
2. If YES to question above, how often do you eat outside the cafeteria?
   a. < 1 per week
   b. 1 – 2 times per week
   c. 3 – 4 times per week
   d. > 5 times per week
   e. 1 per day
   f. > 1 per day
   g. Others? Specify _____
3. What type of food do you usually consume when eating outside cafeteria?
   a. Meat & its products: _____
   b. Milk and dairy products: _____
   c. Poultry: _____
   d. Fish: _____
   e. Fruits: _____
   f. Vegetables: _____

g. Cereal and legume based foods: _____

h. Sweets (Chocolates, Honey, Sugar, Marmalade): _____

4. Which meal of the day do you miss usually (from students cafeteria)?

Type	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Breakfast							
Lunch							
Dinner							

5. Which type of food do you miss usually?

_____

6. Do you drink coffee/tea? YES ☐ NO ☐

7. If YES to the question above, how often do you drink coffee/tea?
   a. Occasionally
   b. 1 per week
   c. 2 – 3 times per week
   d. 1 - 2 per day
   e. ≥ 3 per day

8. At what time do you usually drink coffee/tea?
   a. < 1 hr before meal
   b. < 1 hr after meal
   c. 2 – 3 hrs after meal
   d. > 3 hrs after meal

9. Do you consume fruits? YES ☐ NO ☐

10. If YES to question above, how often do you consume fruits?
    a. < 1 in a month
    b. 1 – 2 times in a month
    c. Once in two weeks
    d. Once a week
    e. 2 times in a week

f. 3 times in a week

g. Once daily

h. 2 – 3 times a day

11. At what time do you usually consume fruits

a. < 1 hr before meal

b. < 1 hr after meal

c. 2 – 3 hrs before/after meal

d. 3 hrs before/after meal

e. ≥3 hrs before/after meal

12. What are the fruits you usually consume?

_____

_____

III. <u>Health Related Questions</u>

1. Have you experienced any sickness in the past six months? Yes ☐ No ☐
2. If YES to the question above, what was your sickness and when did it took place?

_____

3. Have you experienced the following health problems in the last two weeks?

Health condition	YES	NO	Remark
3.1. Diarrhea			
3.2. Respiratory diseases			
3.3. Vomiting			

4. Have you experienced parasite related- infections in the past six month? Yes ☐ No ☐
5. Do you smoke? YES ☐ NO ☐
6. If YES to question above, could you indicate how many pieces per day you smoke, and for how long you have been smoking?

_____

7. Are you currently taking any medication/Supplements? YES ☐ NO ☐
8. If YES to question above, could you indicate what medication/supplement you are taking?

9. Have you ever practiced any fasting in the past one year? YES ☐ NO ☐

10. If YES to question above, please choose which one you practiced.

a. Gena
b. Fasika
c. Nenewe
d. Wednesday and Friday
e. Sene
f. Filseta
f. Tsege
g. Other

11. During fasting, to what extent do you limit your intake?

a. Deprivation of ASF except fish
b. Deprivation of all ASF
c. Deprivation of ASF+ no breakfast
d. Deprivation of ASF + fasting until 9:00 PM (local time)

12. Does your menstruation comes in its normal schedule/menstruate regularly/?

Yes ☐ No ☐

13. If NO to question above, have you experienced amenorrhea (menstrual disorder) in the last six months?

Yes ☐ No ☐

13.1. If YES, please describe your situation.

_____

14. Have you ever got pregnant? YES ☐ NO ☐

15. If YES to question above, have you ever had an abortion? ☐ ☐

16. Are you pregnant currently? YES ☐ NO ☐

17. If YES to question above, how old (in months) is your pregnancy? _____

APPENDIX B: Consent Form

Consent Form

Title: Dietary iron intake of female university students and their iron store as future mothers: the case of College of Natural Sciences

Principal Investigator: Saron Nigussie

Introduction

Iron is a nutrient in our food that helps our body to make energy, recovery from illness and normal brain function. Because of blood loss due to menstrual cycle women at reproductive age are exposed to iron deficiency. If iron is low in the diet the body iron store will diminish and it would lead to anemia which will lead to complications especially during pregnancy for both the fetus and mother.

We came from the Center for Food Science and Nutrition, AAU. In the present MSc research project, we would like to evaluate the dietary intake of female students and correlate this to their serum iron status.

Procedures

If you agree to participate, we will be asking you some basic information, collect data on the type and amount of food you consume during the day, and collect blood (< 5mL) by health professionals.

Risks

The risk will be minimal and not more than what is routinely encountered during blood collection in health care centers.

Benefits

Free screening of their iron status and for those found to be iron deficient; they will be referred to the College of Natural Sciences Clinic for follow up. The results from this research may help improve the cafeteria service and devise strategies for continuous screening and treatment of iron deficiency.

Cost

There is no cost to you for participating.

Compensation

There will be no financial compensation for participating but you will be able to know your serum iron status.

Participant Rights

If I have said things that are not clear to you, you may ask without hesitation and I will answer. You may feel free and ask questions. Your participation in the study is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. If you don't agree to participate, you can say 'no' without worries.

Confidentiality

Test results and any information about you will be kept private. Only the research team will have access to your information. When I write a report, everyone's information will be put together so that information about you or any other individual cannot be seen. You will be identified with random numbers on serum vials. A list with the names and numbers will be kept in a private, locked file cabinet.

Persons to contact:

If you have any questions, you can ask at any time. If you have additional questions about the study, you may contact:

Saron Nigussie

Phone No. 0912006761

If you agree to participate in the study, please sign at the space indicated below.

Thank you for your cooperation.

The study has been explained to me and my questions have been answered to my satisfaction.

I agree to participate in this study.

_____	_____	_____
Signature	Name	Date
_____	_____	_____
Signature of study representative	Name	Date