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Addis Ababa University

School of Graduate Studies

College of Natural Science

Center for Food Science and Nutrition

**Determination of anemia, iron, folate and vitamin B₁₂ Status and
their association with the performance of Ethiopian professional
Athletes**

By

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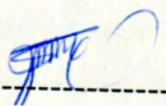
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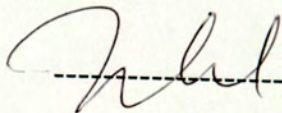
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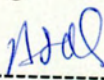
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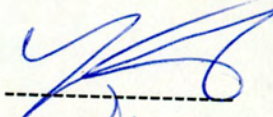
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Declaration

I the undersigned, declare that “this thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged”.

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Dr. Kaleab Baye

Dedication

This work is dedicated to my family who I want to extend my deepest love whenever I get good opportunity like this.

To my father and my mother, who not merely brought me physically into this world but they gave me strength and have thought me to be one of a person who can live for meaning and hope.

To my sister and brother, who they have supported and encouraged me in every progress of my life starting from the initial point to present,

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List of Abbreviations

AGP α_1	Acid Glyco-protein
ATP	Adenosine Tri Phosphate
CRP	C-reactive protein
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
DDS	Dietary Diversity Score
DMT-1	Divalent Metal Transporter-1
DNA	Deoxy Ribonucleic Acid
EAF	Ethiopian Athletics Federation
EDHS	Ethiopian Demographic and Health Survey
WHO	World Health Organization
EPHI	Ethiopian Public Health Institute
EPO	Erythropoietin
FAO	Food and Agriculture Organization
FFQ	Food Frequency Questioner
FSNRD	Food Science and Nutrition Research Directorate
FEP	Free erythrocyte protoporphyrin
HP	High performer athletes

Hs-CRP-----	Highly Sensitive C-Reactive Protein
IAAF-----	International Association of Athletics Federation
ID-----	Iron Deficiency
IDA-----	Iron Deficiency Anemia
LP-----	Low performer athletes
MCH-----	Mean Corpuscular Hemoglobin
MCV-----	Mean Corpuscular Volume
MCHC-----	Mean Corpuscular Hemoglobin Concentration
MTF-1-----	Metal Transcription Factor-1
NIH-----	National Institute of Health
PCV-----	Packed Cell Volume
RBC-----	Red Blood Cell
Hb-----	Hemoglobin
RDW-----	Red Blood Cell Distribution Width
RNA-----	Ribonucleic Acid
SOP-----	Standard Operation Procedures
SD-----	Standard deviation
THF-----	Tetra Hydro Folates
TIBC-----	Total Iron Binding Capacity
UIBC-----	Unsaturated Iron Binding Capacity
UL-----	Upper Limit

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Abstract

Better macro and micro nutrient status and their adequate intake of athletes has great role in balancing losses associated with strenuous exercise, then for better performance. The aim of the study was to determine anemia, iron, folate and vitamin B₁₂ status of Ethiopian professional athletes and to find if any association between body biochemical status and their performance. A cross sectional study was conducted using a convenient sample of 101 Ethiopian professional athletes from the period of February to April 2014. Biochemical samples, performance data, 24 hour dietary diversity and weekly food frequency were collected. The low, medium, and high dietary diversity terciles were 36.1, 60.9 and 3.3% respectively. The mean $\pm$ SD of dietary diversity was 5.44 ± 1.8 . Prevalence of iron overload (Serum ferritin >200 ng/ml) was 11%, whereas that of anemia and iron deficiency was 3% and 15%, respectively. The prevalence of moderate folate deficiency was 20.8%. The mean serum vitamin B₁₂ concentration was 561 ± 231 pg/ml and there was no deficiency for this nutrient. Running-distance was significantly associated with the iron and folate status of male athletes. In contrast, such association was absent for female athletes. Performance of the athletes was associated with their Red blood cell count (RBC) at ($p < 0.05$). The high performer athletes exhibited better micronutrient status and hematological variables than their counter parts. However, the RBC of the athletes was the only parameter whose association was statistically significant ($p = 0.03$). The observed gender difference in the association of running-distance category with iron and folate in this study needs further investigation. Given the 11% iron overload in the present study indicate there is a possibility to expose for hemochromatosis. Prescription of supplements such as iron-folate, multivitamins and minerals were based on broad spectrum of deficiency in the athletes. But these prescriptions should be based on clinical laboratory testing to turn back iron overload and other trace element toxicity. In general it is recommendable to conduct iron overload assessment in Ethiopia healthy population.

Chapter One

1. Introduction

1.1 Background and justification

Almost One-third of world populations suffer from micronutrient deficiencies. The most common micronutrient deficiencies include vitamin A, iron, iodine, vitamin B₁₂, zinc and folic acid. The cause of these micro nutrient deficiencies is primarily due to prolonged inadequate dietary intake of foods rich in these micronutrients (Uchendu, 2011). Micronutrient deficiency has got attention recently as their deficiency known to have negative effect on productivity, work capacity, school and work performance, birth weight and finally they lead to morbidity and mortality (World Health Organization (WHO, 2011)).

Iron deficiency anemia (IDA) is the most prevalent micronutrient deficiency in the world from all micro nutrient deficiencies and around 2 billion people are affected. It occurs in developing countries at greater rates. The rest of micronutrients prevalence such as folate and vitamin B₁₂ there is no sufficient data yet (WHO, 2011). Anemia is the decrease in number and size of red blood cells (RBCs) or decreased amount of hemoglobin (Hb) in RBCs. Iron deficiency (ID) occur when serum ferritin level below the normal range, that is below 15µg/L (WHO, 2011).

Anemia and ID affects oxygen and carbon dioxide exchange between blood and tissue cells thus limits a person's endurance and performance; because of this iron is an important nutrient in sports that require endurance for success (Ahmadi *et al.*, 2010; Driskell and Wolinsky, 2006).

Many micronutrients play key roles in energy metabolism and during strenuous physical activity, the rate of energy turnover in skeletal muscle can be increased up to 20-100 times the resting rate. Marginal deficiency states may have little effect on the sedentary individual, but may have profound consequences for the serious athlete. Regular intense exercise training may also increase micronutrient requirements, either by increasing frequency of use or by increasing

losses from the body which resulting in the need for an increased dietary intake (Maughan, 1999).

This cross sectional study was designed to assesses the dietary diversity, dietary habits of the athletes and to determine the anemia, Iron, Folate, Vitamin B₁₂ and complete blood count (CBC) level, which include (RBC count, Hb, MCV, MCH, MCHC & RDW) status of the Ethiopian athletes (distance runners), by using dietary and biochemical method of nutritional assessment.

The biomarkers which are strongly associated and used as good indicator of body Iron, Folate and Vitamin B₁₂ statuses of the athletes such as transferin, ferritin, serum-folate and serum-vitamin B₁₂ were analyzed. In addition to these the acid glycoprotein (α -1AGP) parameter, which is an acute phase proteins or inflammatory biomarkers was analyzed.

1.2 Statement of the problem

Micronutrient deficiencies affect nearly half of humanity; it remains a major public-health problem worldwide not only in developing but also in developed countries. WHO considers that more than 2 billion people worldwide suffer from vitamin and mineral deficiencies, primarily iodine, iron, vitamin A and zinc, which have important health consequences. In resource-poor areas, these micronutrient deficiencies are frequently exacerbated by infectious diseases (WHO, 2011).

Malaria, HIV/AIDS, hookworm infestation, schistosomiasis, and other infections such as tuberculosis are particularly important factors contributing to the high prevalence of anemia and micronutrient malnutrition in some areas of the world (WHO, 2007). ID only contributes 75% of anemia in the world, followed by folate and vitamin B₁₂ deficiencies. And identifying the magnitude of anemia and its determinants in high-risk groups would be essential for evidence based intervention modalities, particularly in developing countries (Haidar, 2010).

In Ethiopia the prevalence of anemia in women of age 15-49 were 17%. The prevalence in women who were neither pregnant nor breastfeeding were 15% and also a higher proportion of women in rural areas were anemic 18% than those in urban areas which was 11%. Nationally,

11% of men aged 15-49 were anemic. Younger men, age 15-19 were 18 % and older men, age 40-49 were 14% but age 50-59 had 19% prevalence of anemia. So it shows an increasing prevalence as age increased. Men residing in Addis Ababa have the lowest anemia prevalence 3% of any region, while men in Affar, Somali and Dire Dawa all 15% had the highest (EDHS, 2011).

A cross sectional study done in Ethiopia in 2010 shows that, in a total of 970 women, aged 15-49 years, the overall prevalence of anemia, ID, IDA, deficiency of folic acid and parasitic infestations were 30.4%, 50.1%, 18.1%, 31.3%, and 13.7% respectively (Haidar, 2010). There were many researches done such as a study in Korea by kim *et al.*, (2007), a study in Israel by Dubnov and Constantini, (2004) and a study in Sweden by Sandström in 2014, which they were documented relatively high prevalence of anemia, ID and IDA in athletes; but still not sufficient data available on folate and vitamin B₁₂ status related to the athletes.

There are very few researches done related to demographic characteristics (Scott *et al.*, 2003), food and macronutrient intake (Beis *et al.*, 2011), Y chromosome haplo groups (Moran, *et al.*, 2004) and mitochondrial DNA lineages of elite endurance Ethiopian runners (Scott *et al.*, 2005); but unfortunately there is no any research has been done before related to hematological indices, anemia and some special trace element status such as iron, folate, vitamin B₁₂, vitamin D & and also their intake by Ethiopian professional athletes.

1.3 Significance of the study

As there is no any previous research done on the micro nutrient status of the Ethiopian male and female athletes, especially the iron, folate and vitamin B₁₂ statuses, which relatively have high demand and expect to have effects on the health and performance of athletes is not studied, so this study would expected to fill the existing research gap and generate valuable base line information about these micro nutrient status, dietary diversity and the dietary habits of the athletes.

This research can be used for generating good information for nationally and internationally competitive citizens in sport, who can work for their excellence in their area of competition by

making aware of them about the healthy, adequate, nutritious and well balanced foods in relation to their macro and micro nutrient requirement against their day to day exercise stress.

The research can be useful in having good monitoring guideline for Folate, Vitamin B₁₂, iron and non iron-deficiency anemia for the Ethiopian athletes. This nutritional assessment will be very important to identify if any athletic group which is at risk of nutritional deficiencies or overload and promote early intervention, provide optimal nutritional care and counseling to those susceptible groups by providing better dietary options and follow up mechanisms and also to recommend nutritional supplements as necessary.

The research can provide useful information, used as a reference document, in identifying the gaps and it can serve as estimator of nutritional status and habits of other athletic sport groups including foot ball, cycling, swimming and it can initiate further study on populations whose life style is exercise related. Furthermore the study will have great input in using as diagnosing tools for their physicians, nutritionists and or dieticians in order to have good medical, nutritional and dietary follow up.

1.4 Objectives of the study

1.4.1 General Objective

- To determine anemia, Iron, Folate and Vitamin B₁₂ statuses of Ethiopian athletes and finding if there is any association with their performance.

1.4.2 Specific objectives

- To assesses the dietary diversity and food frequency consumption of the runners.
- To determine RBC count, Hb, Ferritin, Transferin, serum-folate and Vitamin B₁₂ level and then to compare with their respective normal reference values.
- To determine the level of AGP then deduce the level and percent of infection and inflammation persist in the athletic population.
- To categorize the performance of the athletes as their best time and then associate with their hematological & micro nutrient status.

Chapter Two

2. Literature Review

2.1 Prevalence of anemia and iron deficiency in athletes

There are many researches evidence which shows the prevalence of anemia and ID in professional athletes. ID is more prevalent in female's athletes than in males. The prevalence of ID in male and female athletes was reported to be 10 and 20 % respectively (Ahmadi *et al.*, 2010).

A study done in Poland of 126 female endurance athletes and 52 controlled subjects with the age of 16-20 years; showed that, 26% of the athletes were marginal iron deficient without anemia (malczewska-Lenczowska *et al.*, 2000). A survey done in Israel on 114 Olympic level athletes of different individual sports showed that 23% of females and 8% of males had iron depletion and 3.5% had IDA. And these percentages were much lower than those found in top-level basketball players of Israel populations in which there was prevalence of (35%, 15% and 7%) respectively (Dubnov and Constantini, 2004).

A research done in 64 professional female athletes (runners, badminton and shooting athletes) of age 13-19 years; the prevalence of Anemia, ID and IDA were 23.4%, 23.4% and 14.0% respectively. There was a significant difference found among runners and badminton versus shooting athletes, which the prevalence of ID was (33.3%, 25.0% and 19.2%) respectively (Kim *et al.*, 2007).

2.2 Mechanism/routes of iron loss in athletes

Athletes or distance runners may lose iron via several routes such as bleeding during injury, sweating, hematuria, and erythrocyte destruction due to exercise stress and others which often doubles the amount of lost (Peeling *et al.*, 2009). Prolonged exercise has also been shown to

increase the use and metabolism of iron. Furthermore decreased erythropoietin production and menstrual loss in female athletes may also cause anemia in athletes. The cellular level reduced oxygen transport capacity and lower aerobic capacity are obvious consequence of iron depletion (Beard and Tobin, 2000).

2.3 Importance of iron and hemoglobin for distance runners

Endurance activity and resistance to fatigue depend on many factors; one of among these is the capacity of blood for carrying oxygen which is basically determined by Hb concentration. As iron is an essential mineral involved in the transport of oxygen via Hb to the tissues, the storage of oxygen in muscle with myoglobin and numerous processes involved in the oxidative regeneration of adenosine tri phosphate (ATP), all of which affect an individual's capacity to perform physical work.

ID has deleterious effects on the physical performance, increases heart rate and elongates the recovery time after exercise in the athletes especially for athletes who train in endurance events such as distance running, swimming and cycling (Tsalis *et al.*, 2004). Iron is one of the most critical minerals with implications for sports performance and IDA impairs muscular performance. Iron is a component of hemoglobin, myoglobin, cytochromes and various enzymes in the muscle cells, all of which are involved in the transport and metabolism of oxygen for aerobic energy production during endurance exercise.

The benefits of iron supplementation may depend on the iron status of the athlete. Serum ferritin levels in the range of 20-30 ng/ml have been considered as an indicator of presence of ID or iron depletion (Williams, 2005). Given the importance of replacing this iron loss to the overall health and performance of the athlete, strategies to either reduce the severity of the iron loss or to maximize the intake and absorption of dietary iron become particularly important, when considering means by which to maintain iron balance.

Iron absorption is markedly affected by the intestinal mucosa, the chemical nature and amount of iron in the ingested food and a variety of dietary factors that increase or decrease the availability of iron (Anschuetz *et al.*, 2010).

2.4 Folate and Vitamin B₁₂ role in erythropoiesis and anemia

2.4.1 Role of folate

Folate is a central component of human erythropoiesis besides iron and vitamin B₁₂. One of the first clinical manifestations of folate deficiency is the production of megaloblastic marrow cells, macrocytic erythrocytes and ultimately macrocytic anemia (Scholl and Johnson, 2000). Folate is required for a number of enzymes that are critical for deoxyribonucleic acid (DNA) synthesis and amino acid metabolism. In addition, folate's role in assisting with cell division makes it a critical nutrient for growth, the synthesis of new cells, such as RBCs and for the repair of damaged cells and tissues.

Thus, folate requirements might be higher with exercise, since the damaged muscle tissue needs to be repaired (Woolf and Manore, 2006). The balance, between the loss of mature RBCs and new production is maintained by an oxygen-sensing system, which responds via erythropoietin (EPO) production. Cytokines, hormones, interactions with cells in the bone marrow and elements, such as iron, folate and vitamin B₁₂ are involved in control mechanisms of the process (Kyriazi, 2011). The two figures below are, among the most stable five types of folate structure.

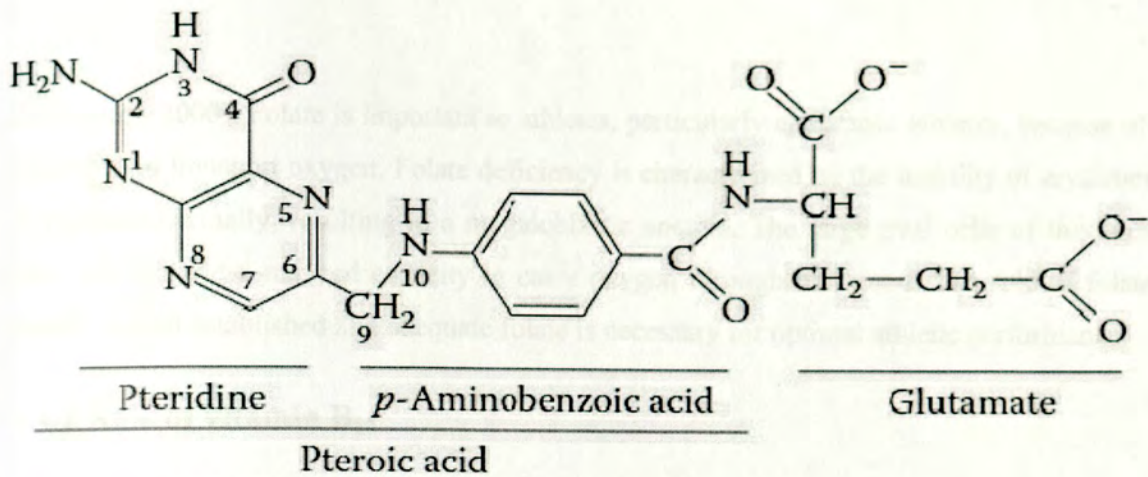


Figure 1: Chemical structure of Dietary Folic acid (Shane, 2010)

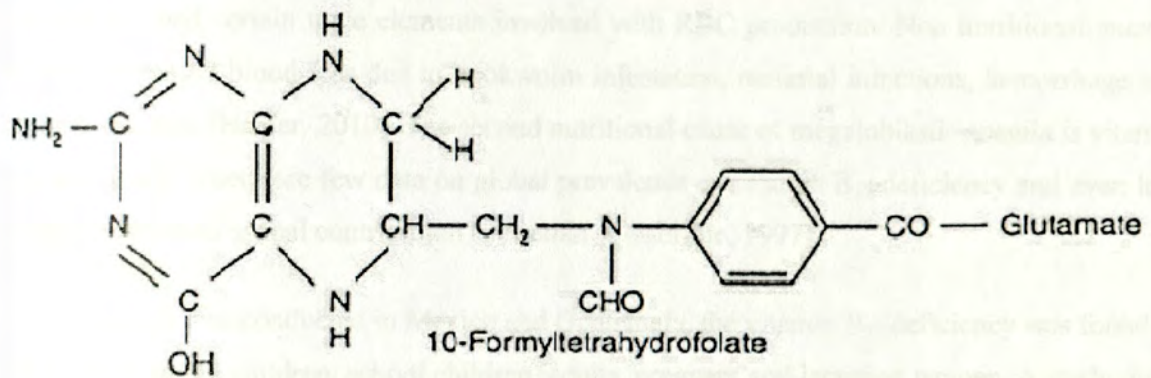


Figure 2: Chemical structure of dietary folate (FAO, 2001)

However, the global impact of folate on anemia is not clear. And also there is no sufficient data on folate status and prevalence related to professional athletes. But current research suggests that exercise may increase the requirements for riboflavin and vitamin B₆ and active individuals with poor or marginal nutritional status for B-vitamins may have decreased ability to perform exercise at high intensities (Woolf and Manore, 2006).

A study done in Ethiopian women, aged 15-19 years old; the overall prevalence of folate deficiency (<6ng/ml) was 31.3% (Haidar, 2010). Folate deficiency was found in 21% of pregnant women in Malawi and was frequently associated with other micronutrient deficiencies (Broek

and Letsky, 2000). Folate is important to athletes, particularly endurance athletes, because of the high need to transport oxygen. Folate deficiency is characterized by the inability of erythrocytes to replicate normally, resulting in a megaloblastic anemia. The large oval cells of this anemia have less Hb and a reduced capacity to carry oxygen through the blood. The role of folate in anemia is well established and adequate folate is necessary for optimal athletic performance.

2.4.2 Role of vitamin B₁₂

Nutritional anemia includes anemia due to deficiency in iron plus deficiencies in folate and vitamin B₁₂ and certain trace elements involved with RBC production. Non nutritional anemia such as increased blood loss due to hookworm infestation, malarial infections, hemorrhage and chronic diseases (Haidar, 2010). The second nutritional cause of megaloblastic anemia is vitamin B₁₂ deficiency. There are few data on global prevalence of vitamin B₁₂ deficiency and even less is known about its global contribution to anemia (Casterline, 1997).

A study which was conducted in Mexico and Guatemala, the vitamin B₁₂ deficiency was found in 19-47 % of young children, school children, adults, pregnant and lactating women. A study done in anemic preschoolers in Mexico showed that children with higher initial vitamin B₁₂ concentrations were more likely to respond to iron supplements with improved Hb concentrations than children with low vitamin B₁₂ status (Allen, 2004).

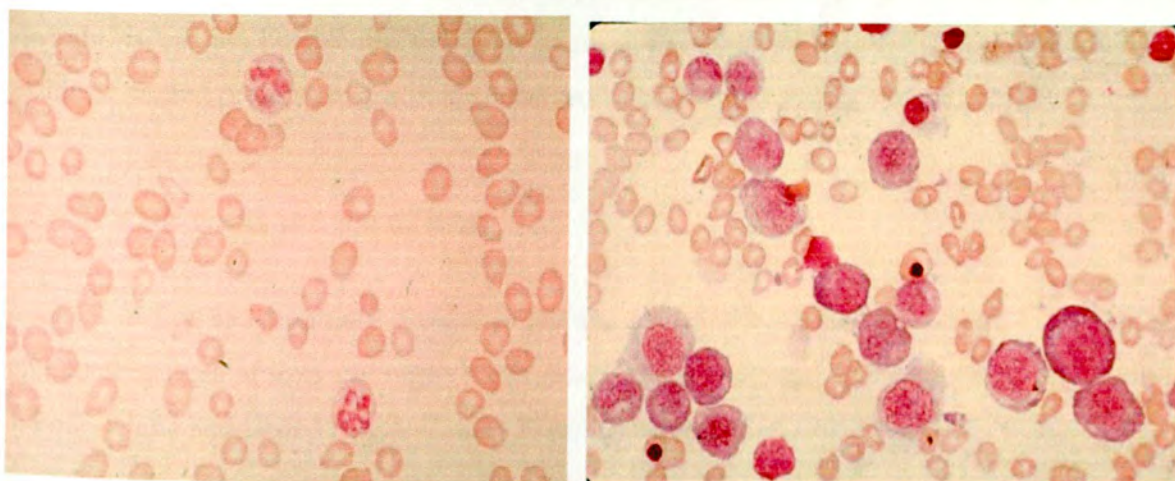


Figure 3: Blood film with macrocytic red cells (left), bone marrow producing red cells larger than normal with nuclei that are immature due to folate/B₁₂ deficiency, megaloblastic anemia (right) (provan, 2003).

Physiologically, Vitamin B₁₂ functions as a methyl donor and works with folic acid in the synthesis of DNA and RBCs (Ly *et al.*, 2012). Besides, it is vital in maintaining the health of the insulation sheath (myelin sheath) that surrounds nerve cells. Clinically, the major symptom of vitamin B₁₂ deficiency is pernicious anemia, a condition characterized by large, immature RBCs. Again, a deficiency may also manifest itself in neurological dysfunction that is almost indistinguishable from senile dementia and Alzheimer's disease (Dreskell and Wolinsky, 2006).

Vitamin B₁₂ is classified as a water soluble vitamin and is distinctive among all vitamins due to its large size, complexity and that it contains the metal ion cobalt. It is necessary for appropriate nervous system function and for the metabolism of carbohydrate, protein and fat. Deficiencies in B₁₂ can lead to inefficient erythropoiesis and megaloblastic anemia. Furthermore, neurological disorders such as neuropathy, myelopathy, memory impairment, dementia, depression and brain atrophy may occur in those with low B₁₂ status (Rusher and Pawlak, 2013).

Vitamin B₁₂ is essential for three enzymatic processes: the conversion of homocysteine to methionine; the conversion of methylmalonic acid to succinyl coenzyme A and the conversion of 5-methyltetrahydrofolate to tetrahydrofolate, a process necessary for DNA synthesis and RBC production. It cannot be manufactured by humans and must be regularly obtained from the ingestion of animal proteins or fortified cereal products (Langan and Zawistoski, 2011).

2.5 Food with good source of iron

The source of iron is commonly divided in to two, animal origin and plant origin. From animal origin, good sources of iron are: organ meats like (liver, kidney and heart), chicken, beef, Lamb, fish, Pork, whole and boiled Egg & Turkey breast (Saunders *et al.*, 2012).

From plant origins, the very common good source of dietary iron are Pumpkin seeds, Sundried tomatoes, raw spinach, apricots, broccoli, Sesame seeds, Cashew nuts, mixed-grain bread roll, tofu, fortified and malted chocolates, Lentils/soybeans/kidney beans, amaranth, tofu, teff, barely, Chickpeas, cassava, sorghum and millet (Saunders *et al.*, 2012; Tidemann-Andersen *et al.*, 2011).

2.6 Bioavailability of dietary iron

Bioavailability of dietary iron can be affected by various factors. The iron from non animal source foods generally has low bioavailability but can be significantly enhanced by adding foods rich in vitamin C. Other effective ways of increasing iron bioavailability include germination, soaking, fermentation and malting of grains, a process which activate phytases that breakdown phytic acid, as this is the most important anti-nutrient that inhibits iron absorption (Jain, 2013; Hunt, 2003).

Dietary iron bioavailability is low in populations consuming monotonous plant based diets with little meat. In meat, 30–70% of iron is heme iron, of which 15–35% is absorbed. However, in plant based diets in developing countries most dietary iron is non-heme iron and its absorption is often less than 10%. The absorption of non-heme iron is increased by meat and ascorbic acid, but inhibited by phytates, polyphenols and calcium (Zimmermann and Hurrell, 2007).

The bioavailability of heme iron is 2–3 times greater than that of non-heme iron. Average heme iron absorption is 23%. Heme iron absorption is affected by the amount of stored iron and is greatest when iron stores are depleted and it is enhanced by factors found in meat, fish and poultry that are present in the same meal with the non-heme iron. Absorption of non-heme iron can be greatest when iron stores are depleted and decreases as the body iron stores increase (Driskell and Wolinsky, 2006).

2.6.1 Dietary iron absorption enhancers

2.6.1.1 Vitamin C

Vitamin C is the only dietary constituent other than animal tissue that has been shown repeatedly to augment the absorption of non-heme iron in humans. The stimulating influence of ascorbic acid has been shown when ascorbic acid is given with inorganic iron alone and is even more pronounced when it is taken with food. The facilitating effect of vitamin C is directly related with dose (Cook and Reddy, 2001).

The highly enhancing effect of ascorbic acid on iron absorption is because of maintaining iron in the appropriate valence state for enzyme function. The potential also may exist for vitamin C induced release of ferric iron from ferritin, with subsequent reduction of iron to the ferrous form. The chelate (a ferrous ascorbate chelate in vitamin C served as the reducing agent), remains soluble in the small intestine and thus can improve intestinal absorption of non-heme iron (Hurrell and Egli, 2010).

Ascorbic acid will overcome almost all inhibitors of iron absorption, which include phytate, polyphenols, calcium and milk product proteins and able to increase the absorption of both native and fortified iron. In fruit and vegetables the enhancing effect of ascorbic acid is often cancelled out by the inhibiting effect of polyphenols.

Ascorbic acid is the only main absorption enhancer in vegetarian diets and iron absorption from vegan meals can be best optimized by the inclusion of ascorbic acid containing vegetables. Other organic acids such as citric acid, lactic acid and others have also been shown to enhance absorption, but for them to be effective, their molar ratio to iron needs to be very high (Hurrell and Egli, 2010; Teucher *et al.*, 2004).

2.6.1.2 Vitamin A

There are several lines of evidence to show that vitamin-A modulates iron metabolism (Semba and Bloem, 2002). Vitamin-A is needed for erythropoiesis and also Hb is not incorporated into

RBCs in a normal way in persons who suffer from vitamin A deficiency. Supplementation with vitamin A alone significantly increases Hb, hematocrit or plasma ferritin in children and pregnant women. In some populations, simultaneous supplementation with iron and vitamin A increases Hb concentrations to a greater extent in anemic pregnant women than does iron supplementation alone (Walczyk *et al.*, 2003).

Vitamin A deficiency may cause anemia by impairing iron metabolism, but the mechanism of this effect is not quite clear. In vitamin A deficient populations, improving vitamin A status reduces the prevalence of anemia in most but not all studies. EPO (produced mainly by renal peritubular cells) acts on the late stages of erythropoiesis, primarily on colony forming unit erythroid cells and stimulates maturation through the normoblast into reticulocytes and mature erythrocytes. The enhancer region of the EPO gene contains a response element that is regulated by retinoic acid (Zimmermann *et al.*, 2006).

One of the several suggested mechanisms of cause of anemia by vitamin A is; vitamin-A deficiency decreases transferrin synthesis and thus reduces iron transport to the bone marrow, reduces bone marrow iron uptake ; impairs the differentiation of blood cells due to lack of retinoic acid and impairs mobilization of iron from ferritin stores. Another Alternative high prevalence of infections, which frequently reported during vitamin A deficiency, is indirectly responsible for decreasing Hb concentrations because the body sequesters iron during infections (Walczyk *et al.*, 2003).

Furthermore, in non-heme iron meals, iron absorption is significantly increased because of presence of vitamin A. The mechanism of increasing iron absorption for non-heme iron containing foods likely to be; as iron solubility significantly decreased while go from gastric pH to intestinal pH which leads to very low iron absorption; but the solubility was increased tremendously as a result of presence of vitamin A or beta-carotene (Garcia-Casal *et al.*, 1998).

2.6.1.3 Animal tissues

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. Unlike ascorbic acid, there is only a modest rise in percentage absorption with increasing quantities of tissue protein. It has been suggested that peptides released during proteolytic digestion by pepsin in the stomach may increase the solubility of inorganic iron (Cook and Monsen, 1976).

It was proposed that the meat effect is a specific property of its amino acid composition and that; in particular cysteine containing residues are important. 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 un-oxidized (Hurrell and Egli, 2010).

2.6.2 Dietary iron absorption inhibitors

2.6.2.1 Phytates

In plant based diets, absorption and bioavailability of dietary iron can be considerably decreased by the phytic acid (Hunt, 2003). Chemically phytates are myo-inositol hexakisphosphate salts and they are found in all kinds of grains, seeds, nuts, vegetables, fruits and roots like potatoes. Other foods contain chemical entities (ligands) that strongly and irreversibly bind ferrous ions and thus inhibit absorption. Phytate and phosphates in bran products, bread made from high extraction flour, breakfast cereals, oats, rice especially unpolished rice, pasta products, cocoa, nuts, soya beans and peas contain high amounts.

The negative effect of phytate on iron absorption has been shown to be dose dependent and starts at very low amount of meal (Minihane and Rimbach, 2002). Cereals and legumes often have high contents of inhibitors of mineral absorption, including phytates and polyphenols, which

inhibit zinc and iron absorption by forming insoluble complexes in the intestine. Consequently, the availability of these micronutrients from diets based on cereals and legumes is often poor.

And such dietary components are thought to be in part responsible for the low absorption efficiency and high incidence of IDA evident in most developing countries, where largely vegetable and cereal diets are consumed (Luo and Xie, 2012). Several studies indicate a reduction of non-heme iron absorption at marginal dietary iron supply due to phytic acid, in humans and other mono-gastric animals. In addition phytic acid is also known to inhibit the availability of other divalent minerals such as Zn and Mg (Minihane and Rimbach, 2002).

Completely removing phytates from cereal based foods has been shown to increase the percentage of iron absorption as much as 12-folds, yet it is not practical to remove completely from those foods instead WHO recommends to use sodium iron with ethylene diamine tetra acetic acid (EDTA) in fortifications like flour (Zemmerman, 2013).

2.6.2.2 Polyphenols

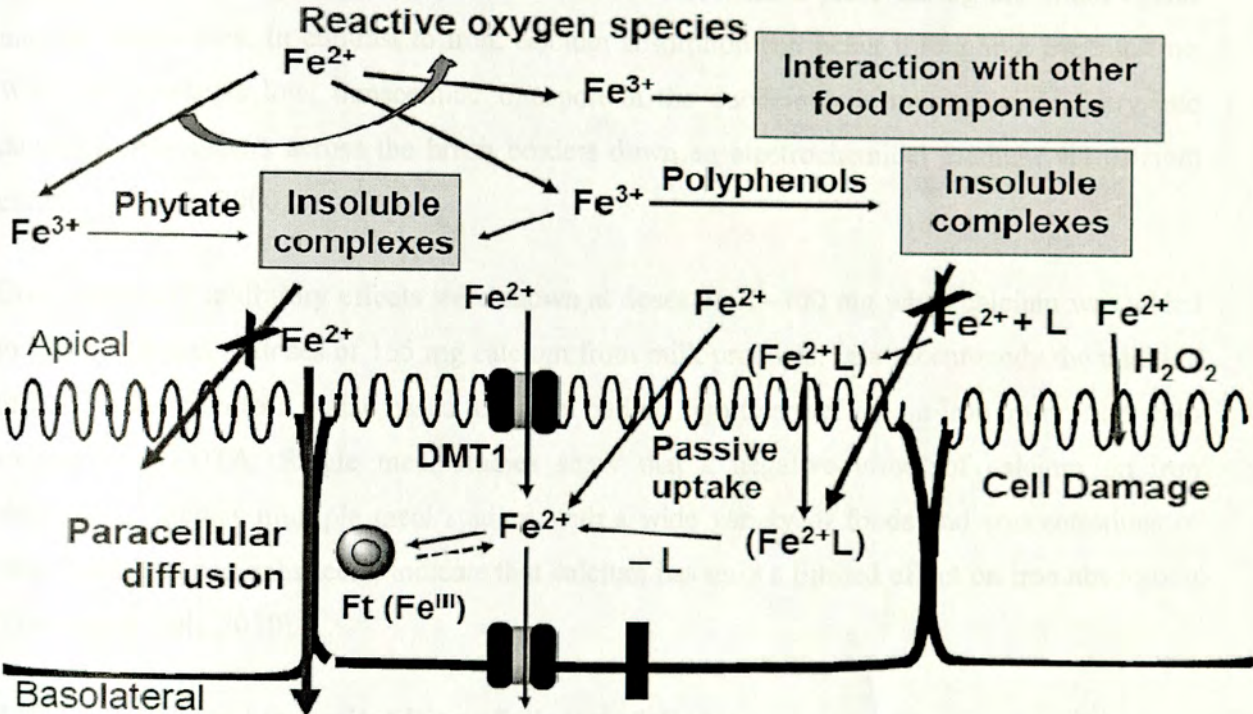
Polyphenols occur in various amounts in plant foods and beverages such as vegetables, fruit, cereals and legumes, tea, coffee and wine (Mascitelli and Goldstein, 2011). ID is particularly widespread in low-income populations where consumption of meat is low and consumption of cereal grains and legumes which contain inhibitors of iron absorption make up the bulk of a diet with little diversity. Thus, a major cause of ID is low bioavailability from plant-based diets containing iron bioavailability inhibitors such as polyphenols (Tako *et al.*, 2014).

Both phytic acid and polyphenolic compounds can be potent iron absorption inhibitors, forming un absorbable complexes in the gut lumen. The inhibiting effect of polyphenols on iron absorption has largely been demonstrated, but the capability of complex formation with iron in the intestine and there by the reduction of iron uptake into the body depends on their structure (Petry *et al.*, 2010). The phenolic compounds are released from food or beverages during digestion and can combine with iron in the intestinal lumen making it unavailable for absorption.

The polyphenols are such powerful inhibitors of non-heme iron that substantial changes in the amount of iron absorbed are more likely to occur if the timing of consumption is altered, rather

than the quantity. Among elderly participants in the Framingham Heart study, each cup of coffee (236 ml) consumed in a week was associated with 1% lower serum ferritin, was a good measurement of body iron stores.

It was noticed that a single serving of instant coffee contains 120mg of polyphenols. At comparable amounts, the polyphenols from black tea were shown to be more inhibiting than the polyphenols from herb teas and wine (Mascitelli and Goldstein, 2011). The figure below shows metabolism and toxicity of ferrous ions in cell in the presence of phytate and polyphenols.



Where, Ft-ferritin, L-ligand, DMT1-divalent metal transporter 1.

Figure 4: Mechanism of ferrous sulfate metabolism with inhibitors and its toxicity

2.6.2.3 Calcium

Absorption of iron can be inhibited by the presence of calcium in the same meal; the larger the dose of calcium, especially in supplements, the greater the reduction of both heme and non-heme iron absorption (Hunt, 2003).

In fact, calcium is the only dietary factor found to inhibit the absorption of both heme and non-heme iron. Maximal inhibition of non-heme iron absorption (50%) has been shown to occur at a 300mg dose of calcium. This inhibitory effect may present a public health problem because recommendations by the Institute of Medicine and National Institutes of Health for the prevention of osteoporosis have led to widespread use of calcium supplements and fortificants. These practices may exacerbate the effects of marginal iron intakes (Roughea *et al.*, 2005).

More recently, it was suggested that the inhibition effect takes place during the initial uptake into the enterocytes. In contrast to iron, calcium absorption can occur throughout the intestine. When the intake is low, transcellular transport in the duodenum is up regulated. Entry into duodenal cells occurs across the brush borders down an electrochemical gradient via calcium channels (Lynch, 2000).

Dose dependent inhibitory effects were shown at doses of 75–300 mg when calcium was added to bread rolls and at doses of 165 mg calcium from milk products. In a recent study the addition of 200 mg calcium to a maize based test meal had no significant effect on iron absorption from sodium iron EDTA. Single meal studies show that a negative effect of calcium on iron absorption, whereas multiple meal studies, with a wide variety of foods and concentrations of other inhibitors and enhancers, indicate that calcium has only a limited effect on iron absorption (Hurrell and Egli, 2010).

2.7 Sources and bioavailability of dietary foliate

Naturally folates are a group of water-soluble compounds with similar biological activity to the synthetic vitamin. The B-vitamin folate is essential for cell replication and occurs naturally in cereal based foods. Germinated cereals are reported to be very richer folate source, because of synthesis of folate in the growing seedling (Hefni and Witthöft, 2012).

The two most abundant natural folate classes are the 5-methyltetrahydrofolates (5-methyl THF), which is widely distributed in all foods and the 5- or 10-formyltetrahydrofolates (5- or 10-formyl THF), which tends to be found in foods of animal origin. The main dietary sources of folate are

cereal and cereal products such as fortified breads and breakfast cereals, leafy green vegetables like spinach and broccoli and dairy products (Finglas and Wright, 2002).

The bioavailability of folates from various foods is considered to be dependent on a number of potential factors, including intestinal hydrolysis of polyglutamyl folates, the extent of conjugation of food folate sources, folate absorption in the small and large intestine, the food matrix, the instability of certain labile folates during digestion (or before ingestion) and the presence of certain dietary constituents that may enhance or inhibit folate stability during digestion (McNulty and Pentieva, 2010).

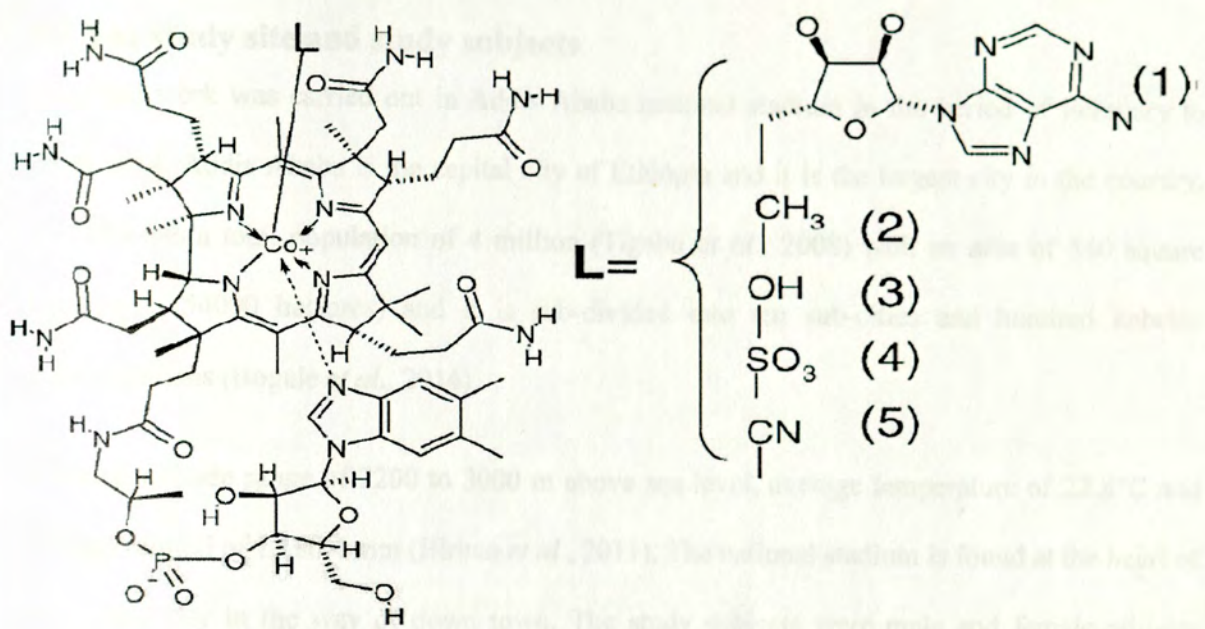
The recommendations for folates can be easily met by consumption of folic acid fortified foods, owing to the higher stability and bioavailability of synthetic folic acid compared with native food folates. The bioavailability of food folate is commonly estimated at 50% of folic acid bioavailability when establishing food recommendations, but this should be considered a rough estimate, as data on the bioavailability of food folate vary between 30% and 98% (Ohrvik and Withoft, 2011).

2.8 Sources and bioavailability of dietary vitamin B₁₂

Vitamin B₁₂ is synthesized only in certain bacteria and this synthesized vitamin is concentrated mainly in the bodies of higher predatory organisms in the natural food chain system (Watanabe *et al.*, 2014). It is found almost exclusively in animal based products including red meats, poultry, fish, shell fish, milk, cheese, other milk products and eggs but plants are not considered to be the major dietary sources of vitamin B₁₂. Some plant foods, such as edible algae or blue-green algae (cyanobacteria), however, contain large amounts of vitamin B₁₂ (Watanabe *et al.*, 2014; Zeuschner *et al.*, 2012).

Vitamin B₁₂ compounds in algae appear to be inactive in mammals. Foods contain various vitamin B₁₂ compounds with different upper ligands; methylcobalamin and 59-deoxyadenosylcobalamin function respectively, as coenzymes of methionine synthase, which is involved in methionine biosynthesis and of methylmalonyl CoA mutase, which is involved in amino acid and odd chain fatty acid metabolism in mammalian cells (Watanabe, 2007).

When the two B₁₂ dependent enzymes lack their B₁₂ cofactors, two metabolites, homocysteine and methylmalonic acid, increase. The usual dietary source of vitamin B₁₂ for omnivores is flesh of other animals. Feces contain large amounts of corrinoids, including some true vitamin B₁₂. Ruminants are unique in these microorganisms, which rumen synthesizes vitamin B₁₂ as long as they have a cobalt source (Stabler and Allen, 2004).



1; 59-deoxyadenosylcobalamin; 2, methylcobalamin; 3, hydroxocobalamin; 4, sulfitecobalamin; 5, cyanocobalamin or vitamin B₁₂

Figure 5: Structural formula of true vitamin B₁₂ and partial structures of the vitamin (Watanabe *et al.*, 2014).

Chapter Three

3. Materials and Methods

3.1 The study site and study subjects

The field work was carried out in Addis Ababa national stadium in the period of February to April 2014. Addis Ababa is the capital city of Ethiopia and it is the largest city in the country. The city has a total population of 4 million (Tigabu *et al.*, 2008) with an area of 540 square kilometers (54000 hectares) and it is sub-divided into ten sub-cities and hundred kebeles administrations (Bogale *et al.*, 2014).

It has an altitude range of 2200 to 3000 m above sea level, average temperature of 22.8°C and average rainfall of 1,180.4 mm (Birhan *et al.*, 2011). The national stadium is found at the heart of the capital city in the way of down town. The study subjects were male and female athletes (distance runners) of age 17-35 years, who registered and doing continuous training under the Ethiopian Athletics Federation and their respective coaches.



Figure 6: The study sites and study subjects.

3.2 The study design

The study design was cross-sectional method with socio-demographic, food frequency, dietary diversity and biochemical method of micronutrient status assessment of 101 Ethiopian national athletes (distance runners) registered in Ethiopia athletics federation.

3.3 Ethical clearance

The research proposal was submitted and presented at Ethiopian public health institute (EPHI) and ethical clearance was obtained from the institute scientific and ethical review office (SERO). Then before starting the field work and data collection, first the Ethiopia athletics federation (EAF) was informed by written paper from AAU and EPHI about the study objective study subjects the sampling and sampling procedures.

The federation coaches' and sport physiologists were also asked their informed consent by describing the aim of the research. Finally both male and female athletes (distance runners) were informed about the study objectives, its importance and also benefits of participation. Their volunteers to participate in the study, their rights to withdraw or continue to be part of the study were given to the study participants.

Detail written consents of the study was attached in appendix form (See appendix A). Then their agreement to participate in the study was approved by signature on the prepared consent form. According to their consent, they were asked structured and open ended questionnaires for socio-demographic, dietary diversity and food frequency questioner (FFQ) assessment by nutritionists and also they gave haematological samples for professional phlebotomist, which the detailed procedure was explained below in the sample collection section.

3.4 Sampling and sample size

According to EAF 2006 E.C national athletics team nomination, there were a total of two hundred ninety two (292) national athletes participating in different category of distance running. By using convenient sampling method, a total of 101 national athletes were selected to

participate in this study. The study participants were stratified based on their running distance by relying on information provided by the coaches of the athletes and data from the Ethiopian athletics federation. From a total of 101 athletes, 16 athletes were from marathon, 18 were from long distance, 14 were from 3000m steeple chase, 22 were from middle distance running and 31 were from short distance running.

3.5 Data collection, analysis and entry flow diagram.

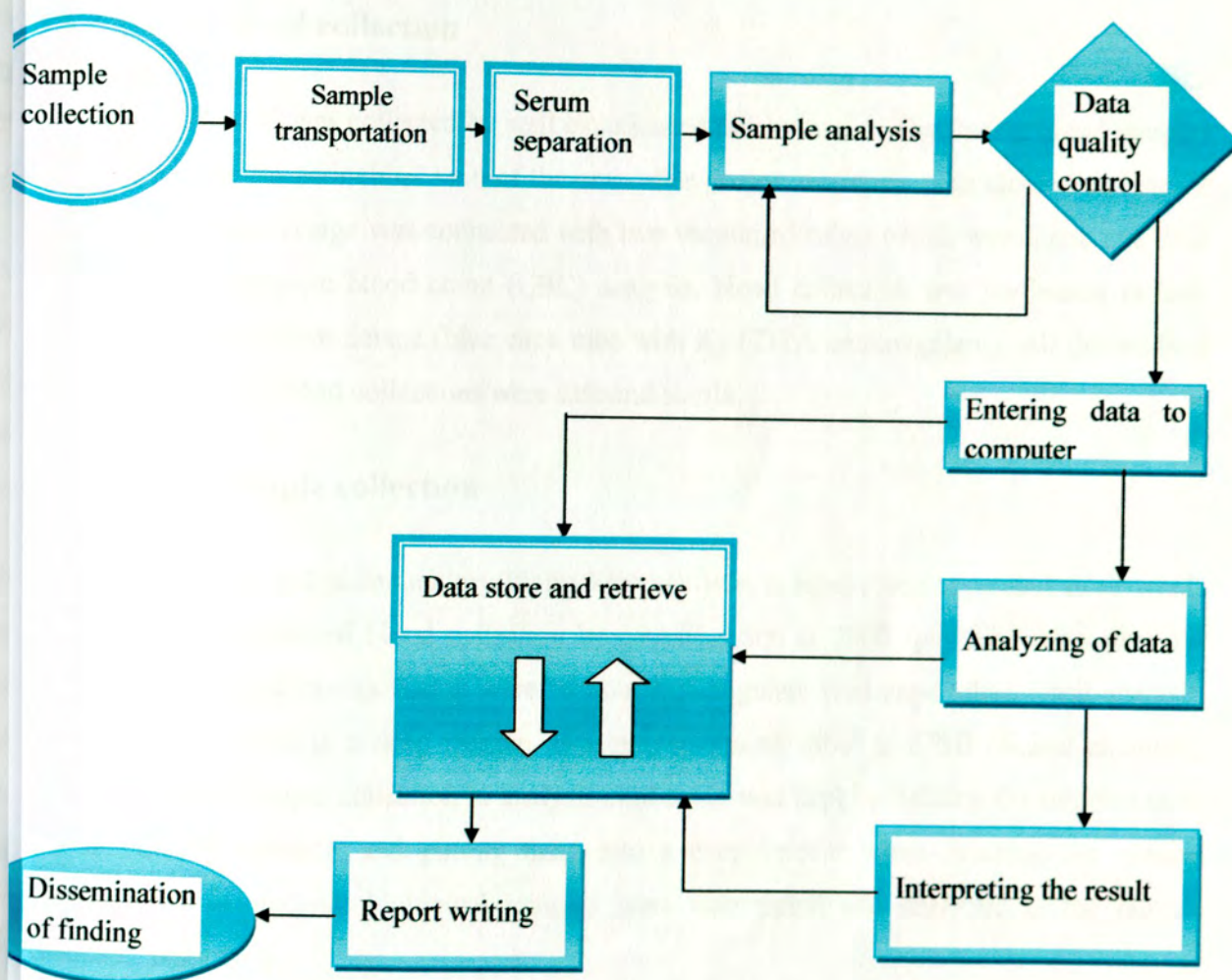


Figure 7: Data collection analysis and entry diagram

3.5.1 Dietary diversity data collection

Assessment of the dietary diversity was done using food frequency questionnaire (FFQ) method of assessment according to liu *et al.*, (2013) and individual dietary diversity (DD) method of assessment was based on the method described by Kennedy *et al.*, (2010) and these were used by adopting to the local context and scored according to method used by Ajani, (2010) for an assessment of dietary diversity. And the detail of dietary assesment interview questionnaires is attached in the appendix (see Appendix C).

3.5.2 Whole blood collection

The blood specimen was collected by well experienced phlebotomist. The fasting blood samples were taken from the anticubital vein of the arm, after proper antisepsis with alcohol and sterile cotton swabs. The syringe was connected with two vacuumed tubes which were done one after the other. For complete blood count (CBC) analysis, blood collection was performed in anti-coagulated venipuncture device (blue cape tube with K₂-EDTA anticoagulant). All the medical equipment used for blood collections were safe and sterile.

3.5.3 Serum sample collection

For micro nutrient and inflammatory biomarkers analysis, a serum was separated from whole blood after 30 minutes of blood collection by centrifugation at 3000 rpm. The tubes used for serum collection and transportation were without anticoagulant (red cape tube). Until analysis, the serum was stored in a deep freezer, at a temperature of -80c° at EPHI clinical chemistry laboratory. From sample collection to analysis cold chain was kept by holding the samples in an ice box during transport and putting them into a deep freezer when reaching the clinical chemistry laboratory. All biological samples were transported and analyzed in the clinical chemistry laboratory.

3.6 Laboratory analysis

3.6.1 Analysis of hematological indices (CBC)

The collected blood samples were analyzed immediately for complete blood counting, which included MCV, MCH, MCHC, RBC count, Hb level, red cell distribution width (RDW) and lymphocytes. The analysis was performed using a hematological analyzer (SYSMEX XT-1800i, ©sysmex corporation, Japan). The instrument uses fluorescent flow cytometry and cell counting methods of analysis.

Hb, RBC and other hematological indices were analyzed before eight hour of blood collection, as the standard hematological sample analysis and the EPHI laboratory analysis protocol.

3.6.2 Anemia status assessment method

Hemoglobin level was used to define anemia by adjusting for altitude according WHO Hb adjustment formula (Sullivan *et al.*, 2008).

$$\text{Hb adjustment} = -0.032 \times (\text{altitude} \times 0.0032808) + 0.022 \times (\text{altitude} \times 0.0032808)^2$$

In this study altitude adjustment was used for an altitude range of 2250m to 2750m, this altitude range taken from the commonly used training place which were Addis Ababa stadium and it is approximately 2400m above sea level and high altitude area such as Entoto and others are >2600m above sea level. As the Adjustment to individual Hb value (g/dL) for this altitude range (2250 to 2750m) is -1.3; so this value was used to calculate Hb adjusted anemia.

WHO Hb cut off points to define anemia in non-smoking, non-pregnant and non-African population living at altitude of <1000m; for men of age 15 years and above the Hb normal range is ≥130g/L and Hb of 110-129g/L as mild anemia, Hb level of 80-109 as moderate anemia and Hb<80g/L taken as severe anemia. For non pregnant female of age higher or equal to 15 years old, the Hb level of ≥120g/L is normal range, Hb of 110-119 is mild anemia, Hb of 80-109 is moderate anemia and Hb of less than 80g/L taken as severe anemia (WHO, 2011).

Before going to statistical analysis on hematological variables, the data has checked for its normal distribution and the kolmogorov-smirnov and Shapiro-wilk tests show that $p > 0.05$ which indicate the data was normally distributed. And the mean $\pm$ Sd of the Hb and RBC data used for inferential statistic analysis.

3.6.3 Iron, folate, vitamin B₁₂ and AGP analysis

Transferrin and AGP were measured by immunological analysis instrument (Cobas Intgera 400 plus Roche Diagnostics, GmbH, Germany). A fully automated analyzer was used for the determination of immunological variables by using immuno-turbidity-meteric method of analysis.

Serum-ferritin, folate, and vitamin B₁₂ were measured using Elecys 2010, Roche diagnostics, GmbH, Germany, an immunoassay analyzer using the Electrochemiluminescence immunoassay “ECLIA” method of analysis was used for immunological tests. All sealed standard reagents and controls were integrated in the analyzer during analysis. These products have high resolution and internationally accepted methods for ferritin, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), transferin, folate, vitamin B₁₂, including many other hormonal and immunological analysis.

3.6.4 Iron, folate, vitamin B₁₂ and AGP assessment method

Body Iron status is best assessed by measuring biomarkers like ferritin, soluble transferin, TIBC and serum iron level (WHO, 2011; WHO, 2001). In this study, serum feritin and transferin biomarkers were used to assess the iron status of Ethiopian athletes. As the ferritin data was skewed and has not met the assumption of normality, the log transformed mean of serum ferritin was used for statistical analysis and back transformed mean ferritin was used for inferential statistics.

Folate status can be assessed by measuring RBC folate, serum folate, and serum methyl malonic acid (MMA) (WHO, 2012 and WHO, 2005). In this study serum-folate was analyzed as an

indicator for the folate status of athletes. And the same way as ferritin, log transformed mean of serum folate and back transformed mean was used for inferential statistics. Vitamin B₁₂ status can be assessed by biochemical measurements, such as presence of macrocytic anemia, increased mean cell volume (MCV) and decreased Hb levels.

The most common biochemical indicators of B₁₂ status are, measuring serum cobalamin and methyl malonic acid (MMA) concentrations, which are usually associated with tissue content of the vitamin. The vitamin B₁₂ data was log transformed in order to meet the assumption of normality as in the case of ferritin and folate. Then the back transformed data was used for inferential statistics. Serum vitamin B₁₂ is considered as severely deficient when it is <150 pg/ml, moderately deficient when b/n 150-200 pg/ml range, and normal when it is >201 pg/ml (Whitney and Rolfes, 2007). The lower limit normal range for vitamin B₁₂ is 200–250 pg/ml but not has internationally agreed upper normal range (Driskell and wolnskey, 2006).

The presence of any disease infection or inflammation can be determined by commonly used proxy indicators like C-reactive protein (CRP) and acid glycoprotein (α 1-AGP). These parameters measure the extent of acute phase proteins present in the human body which spikes as a result of infection, chronic disease or inflammation. In the presence of disease or infection the acute phase proteins are increased and this has a direct relationship with CRP and AGP serum levels. In this study AGP was used to assess the inflammation status of the athletes.

3.7 Quality control and quality assurance in the lab analysis

To ensure the validity of the laboratory data, double checking was performed during laboratory sample analysis, data entry and analysis. During the laboratory analysis, two independently checked quality control runs were performed with study samples according to standard operation procedure (SOP) of the laboratory. Very well trained and experienced laboratory professionals participated in the biological sample collection and laboratory analysis. The fact that the field work was not far from the central laboratory (EPHI) has facilitated sample handing and transport in cold chain.

3.8 Statistical analyses

All data collected with questionnaires for food frequency, dietary diversity and the data obtained from biochemical analysis; the descriptive and anova analysis were using SPSS version 20. The results of each analyzed biomarkers were summarized in terms of the number of measurements (n), mean ($\bar{x}$), standard deviation (Sd), median ($\tilde{x}$), inter quartile range (Q1, Q3), correlation (r), coefficient of variation (CV), regression (r^2) and the analyzed statistical results were compared using their counterpart reference ranges, to decide whether they are in normal range, mild, moderate or severely deficient and also to find significant differences.

Given that the serum-iron, folate and vitamin B₁₂ data was skewed and did not meet assumptions of normality (p-value < 0.05 for Shapiro-wilk test), the data was log transformed then statistical analysis was done and the back transformed mean was used for inferential statistics. However AGP, RBC, Hb, and transferin datas were fit for normality assumption for Shapiro-wilk test, so non-transformed data were used for inferential statistics.

Chapter Four

4. Results and Discussion

4.1 Socio-demographic characteristics

The mean age ($\pm$ SD) was 21.3 ± 3.2 year, and 72 athletes (71.3%) of the athletes were in the range of 19-25 years, nineteen athletes (18.8%) were in the range of 17-18 years, and the remaining ten athletes (9.9%) were in the range of 26-35 years of age. Sixty eight athletes (67.3%) were orthodox by religion, ten athletes (9.9%) were Muslim, the 21.8% were protestant the rest 1% was follower of Hawariat religion. The largest ethnic group was Oromo 42 (41.6%) followed by Amhara 26 (25.7 %) and Tigrie 13(12.9%) but the rest were from other different ethnic groups.

Concerning the educational status of athletes, 23 (22.8%) had attended elementary school, sixty eight (67.3%) attended high school and ten athletes (9.9%) had at least some college/university level education. From 101 athletes, only two athletes (2%) were married and all the rest were single. Out of the total study subjects, forty seven athletes (46.5%) earn monthly income of 1501-1999 ETB, twenty four (23.8%) earn 1000-1500, 17 athletes (16.9%) earn 2000-2500, nine athletes (8.9%) less than 1000 and the rest four athletes (4%) earn 2501-3500 ETB by their clubs and Ethiopia Athletics Federation, see in (Table 2) below.

Table 1: Socio demographic characteristics of study participants (n=101) in Ethiopia athletics federation, 2014.

Socio demographic Variable	Frequency	Percent
Athletes age range		
17-18	19	18.8
19-25	72	71.3
26-35	10	9.9
Religion		
Orthodox	68	67.3
Muslim	10	9.9
Protestant	22	21.8
Others*	1	1
Ethnicity		
Oromo	42	41.6
Amahara	26	25.7
Tigre	13	12.9
Others**	20	19.8
Educational status		
Illiterate	0	0
Primary (1-8)	23	22.8
Secondary (9-12)	68	67.3
College/university	10	9.9
Marital status of athletes		
Single	99	98
Married	2	2
Divorced	0	0
Widowed	0	0
Sex of athletes		
Male	57	56.4
Female	44	43.6
Average monthly income (ETB)		
≤ 1000	9	9
1001-1500	24	23.8
1501-1999	47	46.5
2000-2500	17	16.9
2501-3500	4	4

*Hawariat; **Welaita, Sidama, Gurague, Harari, Hadya, Hamer & Benishangul

Regarding the intensity of their exercise; most of the athletes, seventy eight (78.2%) had 6 days training, eight had 5 days training, five had 4 days and three had 7 days training per week for an average of 2 hours of exercise per days. As the training intensity of the athletes, shown in (Table 2 is high, it indicates the need for the athletes to consume energy and nutrient-rich foods that would compensate their expenditure. However, whether their monthly income would allow them to get much diversified diets may be equivocal which requires further analysis.

Table 2: Training intensity, running-distance category and other related factors

Variables		Frequency	Percent (%)
Sport category and sex			
Marathon	Male	10	9.9
	Female	6	6
Long runners	Male	8	8
	Female	10	9.9
3000m runner	Male	9	9
	Female	5	5
Middle distance	Male	12	11.8
	Female	10	9.9
Short runners	Male	18	17.8
	Female	13	12.9
Weekly training days			
7 days per week		6	6
6 days per week		79	78
5 days per week		8	8
3-4 days per week		7	7
Training hour per day			
1:30-1:45 Hr		44	43.56
2:00-2:30 Hr		57	56.44
Sleeping hour per day			
>12Hrs		4	4
11-12 Hrs		29	28.7
9-10 Hrs		50	49.5
7-8 Hrs		16	15.8
6 or less Hrs		2	2
Training place			
Stadium, sululta, gefersa, holeta		28	27.7
Stadium, sebeta, Ararat, CMC		26	25.7

Stadium, entoto, mekele, A.kality	32	31.7
Mixture of all	15	14.8
Running experience in each sport category		
≥ 6 years	13	12.9
5 years	23	22.7
4 years	27	26.5
3 years	28	28.7
2 years	10	9.9

Majority of the athletes' (78%) that participated in the present research had 3-5 years experience in their running distance category; they have been doing intense and frequent exercise and sleep longer hours (9-10 hour) relative to other sedentary population. This may indicate the level of their fatigue due to the intense exercise.

4.2 Food frequency and dietary diversity of the athletes

The International Association of Athletic Federations (IAAF) consensus statement on nutrition for athletics published in 2007 states: "Well chosen foods will help athletes train hard, reduce risk of illness and injury and achieve performance goals, regardless of the diversity of events, environments, nationality and level of competitors." When we come to the diversity of foods which were commonly consumed by the Ethiopian athletic population was measured in a weekly food frequency (FF) and 24-hour dietary diversity, the result show that there is no special difference compared to what is usually consumed by the general Ethiopian population.

However, the athletes tended to have a higher consumption of animal source foods like egg and milk. Fruit consumption, like banana was frequent and with large serving portion. Usually the consumed foods were plant based products than animal source. Cereal consumption was high with frequent use of barely in the form of powder mixed with water and sugar, traditionally called "Beso". Barley was also consumed in the form of porridge and chiko (butter added hard paste of barely).

In contrast, consumption of meat, fish, poultry and beverages like coffee, tea, coca-cola, beer and other hard sprits were low. The present dietary intake findings are in agreement with the study

done by Beis *et al* (2011) on elite Ethiopian athletes. The previous study showed that consumption of meat was low and reported relatively high consumption of egg, milk, bread, “injera”, pasta, rice, lentils and porridge.

Table 3: Weekly food frequency % consumption of the athletes.

types of food consumed	1-2 times/ Week (%)	3-4 times/ Week (%)	5-6 times/ Week (%)	1 & >1 times/day (%)	Not cons/ Week (%)
Meat & meat products	28.7	30.7	3.0	10.9	26.7
Dairy products (milk, cheese)	32.7	16.9	11.7	9.0	29.7
Organ meats	4	2.0	0	1.0	93
Egg	49.5	24.8	6.0	7.0	12.9
Fish	9.9	2.0	0	0	88.1
Fruits & juices	34.6	31.7	10.9	18.9	4.0
Vegetables (spinach, lettuce, cabbage)	43.5	36.7	7.0	9.0	4.0
Legumes, nuts & seeds	42.5	31.7	9.9	11.9	4.0
Cereals (Teff, wheat, barley, maize)	3	14.9	14.9	67.3	0
Tubers & roots (carrot, potato etc)	30.7	34.7	16.8	15.9	2.0
Beverages (coffee, cola)	31.7	26.8	6.9	21.8	12.9

We can see from the above table, there was very low consumption of animal source foods like fish (11%), organ meats (7%) and no poultry food sources, with modest consumption of meat (73.3%) of the athletes in a weekly basis. In addition (74.3%) of them were consume eggs in 2 or 3 times per a week. The very usual daily consumption was includes; fermented *teff* in the form of

injera, barley; in the form of “beso”, “kolo,” porridge and “chiko” wheat; in the form of bread, cooked lentils with injera and “shiro wet”. But 1 to 2 times consumption of spaghetti was common per week in the form of pasta, macaroni and rice.

In addition compared to sedentary segments of the population, there was relatively high serving size of dairy products (milk), usually ½ to 1 lit per serving. Fruit and juices like mango, avocado, orange, pineapples, was consumed by (66.3%) of the population with 2 to 3 times per week. There was appreciable (80.2%) of the athletes consume vegetables like cabbage, lettuce, spinach and broccoli usually 2 to 3 times per week. legumes, nuts, seeds and roots were consumed with 2 to 3 times in a weekly basis by 74.2% of the population.

4.3 Dietary diversity score of athletes

Table 4: Food groups consumed by athletes (%) in the last 24-hour

Food groups	Short (%)	Middle (%)	Steeple chase (%)	Long (%)	Marathon (%)	Total
Cereals	90.3	86.4	100	100	100	95.3
Milk and milk products	8.7	40.9	42.9	11.1	37.5	34.2
Egg	32.3	31.8	42.9	27.8	50.0	36.9
Legumes, nuts and seeds	32.3	59.1	50.0	22.2	62.5	45.2
Other vegetables	68.9	67.8	61.2	63.1	65.7	65.3
Vitamin A rich fruits	48.4	81.8	50.0	61.1	62.5	60.8
Fats and oils	69.8	73.2	75.1	72.9	81.1	74.4
Organ meat	3.2	0	0	0	6.3	1.9
Flesh meat	38.7	40.9	42.9	11.1	37.5	34.2
Dark green leafy vegetables	38.7	27.3	57.1	33.3	37.5	38.8
Fish	3.2	0.0	0.0	5.6	0.0	1.8
Other fruits	52.4	84	53	64	65.5	63.8
White roots and tubers	58.1	45.5	71.4	61.1	62.5	59.7
Vitamin A rich vegetables	58	62	55.3	61	59	59.1
Beverages	58.1	22.7	50.0	33.3	31.3	39.1

As the above table shows that, the most consumed food groups in 24 Hour period were; cereal/grain products, vegetables, fruits and fats & oils. But the least consumed food types were fish and organ meats.

Table 5: Dietary diversity tercile scores among the five distance categories.

Dietary diversity score	Short (%)	Middle (%)	3000m (%)	Long (%)	Marathon (%)	Total (%)
2	0	4.55	0	0	0	1.31
3	6.45	13.64	0	11.11	6.25	7.49
4	32.26	22.73	14.3	44.44	25	27.75
5	22.6	9.1	21.43	27.8	25	21.2
6	12.9	22.73	42.9	5.55	6.25	18.1
7	16.13	9.1	7.14	5.55	6.25	9.9
8	0	9.1	0	5.55	18.75	6.7
9	6.45	4.55	7.14	0	12.5	6.13
10	3.23	0	7.14	0	6.25	3.3
Dietary Diversity Terciles						
Low (1 to 4)	38.71	40.92	14.3	55.55	31.25	36.1
Medium (5 to 9)	58.1	54.6	78.6	44.45	68.75	60.9
High (10 to 14)	3.23	0	7.14	0	6.25	3.3

The table above shows that except for long distance running (lies in low diversity), the rest running category were in medium dietary diversity per 24 hour. And when we see the total distance runners, 60.9% of the population lies in medium dietary diversity followed by 36.1% in the range of low diversity and 3.3% in high diversity range. The Dietary Diversity Scores (DDS) of individual subjects ranged from 2 to 10. The proportion of the subjects with the low (1-4), medium/average (5-9) and high (10-14) scores are shown in Table 4. The overall mean DDS among subjects was 5.44 with a standard deviation of 1.8.

4.4 Use of dietary supplement by athletes

Even though it is very common to use dietary supplements in most professional athletes especially in females, almost all of the research participants (91%) have not taken dietary

supplements like iron, folate, vitamin C, vitamin B-complex, vitamin A, fish oil/omega-3 oil, magnesium, copper, zinc, calcium, selenium, iodine including amino acids on a regular basis. Rather, male athletes take nutritional supplements, while there is training burden because of preparation for national or international competitions, but female athletes take the supplements according to their menstrual cycles as directed by their physician/sport physiologist.

4.5 Health and inflammation status of the athletes

Based on the interview conducted with the athletes (for questionnaire see appendix B), most, if not all, had no medical conditions and thus were healthy. As shown by the AGP figures, most athletes (98%) had no infection/inflammation. When come to $\alpha 1$ -AGP analysis, the normal reference range of the biomarker was 0.5-1.2g/L (Roche laboratory diagnostic manual, 2010).

In this study, only 2% athletes had higher than the upper reference value (>1.2 g/L) and 11% had an $\alpha 1$ -AGP less than the lower reference value (<0.5 g/L) and among the 11% of athletes with lower AGP, the 9% had an $\alpha 1$ -AGP value of ≥ 0.31 g/L, but the rest 2% had $\alpha 1$ -AGP of ≤ 0.02 g/L. Higher level of $\alpha 1$ -AGP is an indication of presence of inflammation or infection (Henry *et al.*, 2003).

4.6 RBC and Hemoglobin level of athletes

The mean $\pm$ SD of crude and altitude adjusted Hb of male and female athletes were (16.7 ± 0.82 g/dL Vs 15.4 ± 0.82 g/dL) and (15 ± 0.88 g/dL Vs 13.7 ± 0.88 g/dL), respectively. There was a significant difference ($p < 0.01$) between male and female Hb level at 99% confidence interval (CI) for which the difference among running-distance was not statistically significant.

The mean $\pm$ Sd of RBC count for male and female athletes were (5.45 ± 0.34 and 4.85 ± 0.35 RBC count $\times 10^{12}/L$), respectively. There was statistically significant correlation observed between Hb and RBC count in 99% CI and ($p < 0.001$). As in the case of Hb, there was significant difference ($p < 0.01$) between male and female RBC level at 99.9% CI.

Table 6: Mean variation of Hb and RBC level by sex

Sex	RBC	Hb
Male (n=57)	5.45 ± 0.34^a	16.7 ± 0.82^a
Female (n=44)	4.9 ± 0.35^b	15 ± 0.9^b

Words of different letters are statistically significant, $p < 0.05$

Even though it was not statistically significant among each distance categories, in general there was a physical mean difference between short and endurance runners, which indicate that long distance running can negatively affect the hematological variables like Hb and RBC, and Possibly other hematological indices. The table below shows the Mean $\pm$ SD of Hb and RBC level in each distance categories.

Table 7: Mean $\pm$ Sd of Hb and RBC by running distance categories

Distance category	RBC (male, n=57)	RBC (female, n=44)	Hb (male, n=57)	Hb (female, n=44)
Short distance (100-400m)	5.5 ± 0.4^a	4.9 ± 0.35^e	16.8 ± 0.8^b	14.8 ± 0.7^d
Middle distance (800-1500m)	5.6 ± 0.3^a	5.0 ± 0.3^e	17.2 ± 0.9^b	15.3 ± 1.1^d
Steeple cheese (3km)	5.3 ± 0.3^a	4.6 ± 0.1^e	16.3 ± 0.0^b	14.9 ± 0.8^d
Long distance (5& 10km)	5.4 ± 0.3^a	4.9 ± 0.4^e	16.5 ± 1.1^b	15.0 ± 1.0^d
Marathon (42.2km)	5.5 ± 0.3^a	4.7 ± 0.4^e	16.5 ± 1.0^b	14.9 ± 0.8^d

Superscript words of the same letters in the column are not statistically significant, $p > 0.05$

There was no statistically significant difference observed by running distance categories which was partitioned by sex with their red blood cells and hemoglobin level. Table above shows the Mean $\pm$ SD of Hb and RBC level of athletes in each distance categories. The result in this study showed that being run for different distance category (short, middle, 3km with steeple chase, long and marathon) has not significantly affected hematological variables, such as RBC and hemoglobin level of the athletes at 95% confidence interval.

4.7 Anemia status of athletes based on WHO Hb cut offs

According to mentioned cut off points for Hb with altitude adjustment in chapter three; the percentage of athletes which were anemic (Hb<12g/dL) was 3% (three athletes) and among them the two athletes, 1 male and 1 female runner were from long distance (5 and 10 km) running but the rest one male was from marathon running. Despite of relatively few runners participated, the presence of anemia in marathon and long distance runners contrast to short and middle runners, indicate that very long distance running can affect hemoglobin level negatively.

4.8 Micronutrient status

4.8.1 Iron status

The non-log-transformed mean and standard deviation of ferritin level for male (n=57) and female (n=44) athletes were 134.1 ± 86.8 Vs 63.9 ± 40.4 $\mu\text{g/L}$ respectively. According to the WHO guideline for iron assessment and ID by ferritin concentrations developed in 2011, Serum ferritin level of <15 $\mu\text{g/L}$ indicate the presence of ID in both female and male of age older than five years. WHO cut off points for iron overload stated as serum ferritin >200 $\mu\text{g/L}$ for male and >150 $\mu\text{g/L}$ for female in the absence of inflammation (WHO, 2011).

Based on the iron cut off points, the percentage of iron deficient, ferritin (<12 $\mu\text{g/L}$) were 2%. The iron deficient subjects were one male and one female athlete. Percentage of first stage iron depletion (ferritin <50 $\mu\text{g/L}$, but >30 $\mu\text{g/L}$) was 22% and second stage depleted iron store,

(ferritin<30 $\mu\text{g/L}$, but >12 $\mu\text{g/L}$) was 13% and among the 13% second stage iron deficiency, 11% were females athletes.

The mean ferritin value above and percentage of second stage iron deficiency indicates, female athletes had lower iron stores than male and this is an indication of more vulnerability than their counterparts. In this study iron overload (ferritin>200 $\mu\text{g/l}$) was 11%, which all of them were male and among all iron overload athletes 8% were from short distance runners. The graph below shows the general iron status of Ethiopian athletes by their ferritin level, according to WHO ferritin guideline, 2011.

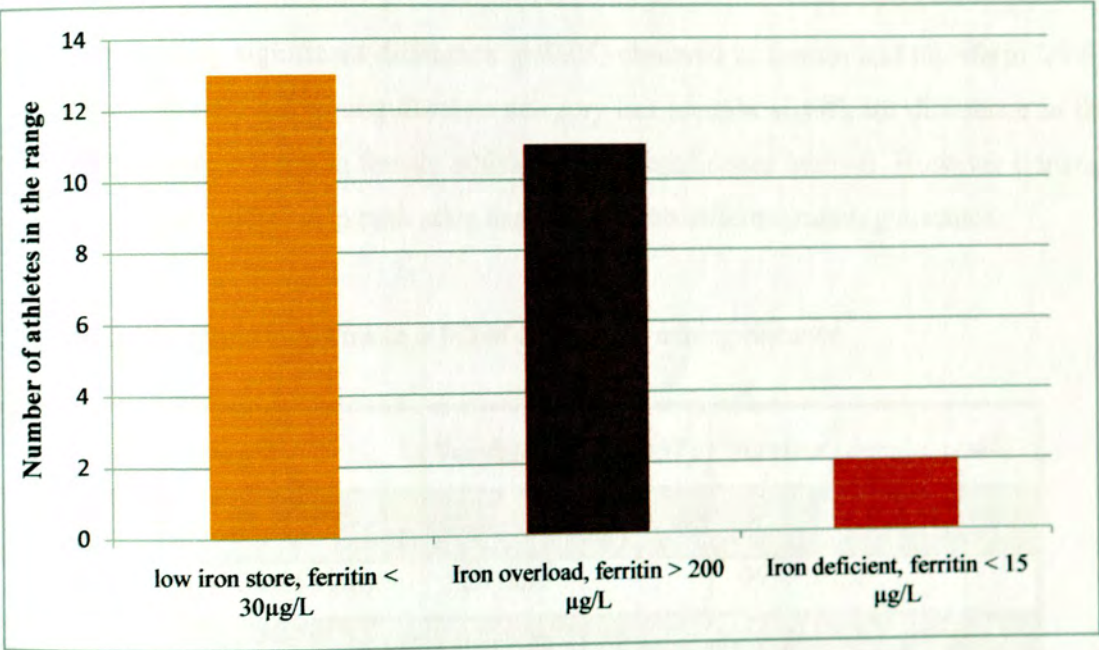


Figure 8: Iron status of the whole athletes

When the serum-ferritin data was partitioned by sex, among 44 female athletes 27.3% had low iron store (<30 $\mu\text{g/L}$). And this finding is fairly consistent with a study done in 42 Iranian female athletes participating in team ball sport in 2010, which showed low ferritin level in 33.3% and iron overload in 12.5% of the study participants (Ahmedi *et al.*, 2010). However, in the current

study there was no iron overload female athlete. The table below shows variation of ferritin and transferin by sex.

Table 8: The mean and standard deviation of ferritin and transferin by sex

sex	Mean $\pm$ SD of ferritin	Mean $\pm$ SD of transferin
Male (n=57)	107 $\pm$ 2.1 ^a	2.6 $\pm$ 0.4 ^a
Female (n=44)	51 $\pm$ 2.0 ^b	2.7 $\pm$ 0.44 ^b

Superscript words of different letters are statistically significant, $p < 0.05$

There was statistically significant difference ($p < 0.05$) observed in ferritin and transferin level of male and female athletes. Running distance category has brought significant difference in their ferritin level for male but not in female athletes at 95% confidence interval. However transferin was not significantly different in both male and female with different running distance.

Table 9: Gender partitioned mean $\pm$ SD of ferritin by running distance

Distance category	Ferritin (male, n=57)	Ferritin (female, n=44)
Short distance (100-400m)	171 $\pm$ 1.5 ^a	52.0 $\pm$ 2.1 ^a
Middle distance (800-1500m)	72 $\pm$ 2.2 ^b	39.0 $\pm$ 2.2 ^a
Steeple chase (3km)	111 $\pm$ 1.8 ^{ab}	64.0 $\pm$ 1.6 ^a
Long distance (5 & 10km)	124 $\pm$ 1.8 ^{ab}	67.6 $\pm$ 2.3 ^a
Marathon (42.2km)	65 $\pm$ 2.2 ^b	41.4 $\pm$ 1.1 ^a

Superscript words of different letters are statistically significant, $p < 0.05$

The ferritin level of male marathon runners was significantly lower than short male distance runners which prove the iron demand of marathon running is higher than short distance running. The absence of significant difference in ferritin level of male long distance and steeple chase

(running 3km with hurdles and water jump) runners explained by nearly the same running distance and presence of additional barrier in steeple chase can equalize the iron demand despite of their exercise intensity and dietary patterns.

However middle distance runners showed significantly lower mean ferritin level; compared to steeple chase and long distance was anomaly, which may be due to presence of high exercise volume. Exercise volume and intensity of the athletes were very subjective and vary from one coach to other despite of their food and micronutrient intake, this could affect and thought to bring difference in iron status. Even though the same trend in mean ferritin variation was observed in female athletes as male across distance categories, but none of them were statistically significant.

A study done in Korea by Kim *et al.*, (2007); of runners (n=30), badminton players (n=8) and shooting athletes (n=26) the prevalence of anemia, iron deficiency and iron deficiency anemia were 23.4%, 23.4% and 14.0% respectively. The analyzed free erythrocyte protoporphyrin (FEP) was $48.7 \pm 21.1 \mu\text{g/dL}$. A moderately negative correlation of Hb and FEP was noted and found statistically significant ($r=0.571$, $P < 0.001$).

There was a significant difference in the prevalence of iron deficiency among the three athletes; runners and badminton players tending to be affected more frequently with iron deficiency than static athletes such as the shooters. The iron deficiency in runners and badminton vs. shooting athletes was 33.3% & 25.0% Vs 19.2% respectively. And the results confirmed free erythrocyte protoporphyrin (FEP) to be the most significant factor for the diagnosis of iron deficiency in athletes. Proper nutritional counseling and monitoring need to be tailored to non-static sports like runners & badminton players against static athletes such as shooting.

An earlier study conducted on 970 Ethiopian women of reproductive age reported a 50.1% prevalence of depleted iron store ($<50 \mu\text{g/l}$) by Haider, (2010). Relatively low prevalence in the current study as compared to the study reported in women, probably because of the variation of study subjects.

Study subject difference and high helminthes & malaria infestation in the former study and also the socio economic status of the study participant expect to bring the variation in prevalence. Prevalence of the anemia and iron deficiency was lower than that of study by Jamal hider in 2010). This may partly be due to their relatively better socio-economic status than the study participant and also may be their relative attention to their diet (balancing energy input and output) account for it. However, this deficiency and overload prevalence was higher as compared from many reports of European and USA athletes and can bring deleterious effect on their health and performance.

For example a study done on iron status of experienced male and female distance runners in Canada showed that there were no anemic and iron deficient athletes, but presence of first stage ID in most of males and second stage ID in females were reported (Anschuetz *et al.*, 2010).

A study conducted to investigate the relation ship between hematological indices, iron status of athletes and their performance in Germany showed that anemia and iron deficiency was rare and the Hb and Hct of the athletes were not different with the untrained controls. Reduced Hb, Hct and RBC levels were observed in endurance athletes compared with power (boxing, weight lifting and soon) and mixture (power and endurance) type of event. These findings suggest that exercise-induced plasma volume expansion and to a lesser extent hemolysis was the reason for the low RBC.

This is further confirmed by the low haptoglobin which was only observed in runners and not in cyclist, further suggesting that not exercise *per se*, rather the “traumatic” movement of running might trigger the destruction of RBCs. Physical training by itself has no significant effect on selected hematological variables in athletes compared with untrained controls rather the specific type and duration of exercise was the major importance in the adaptations of the blood cell system and the iron metabolism (Schumacher *et al.*, 2002). In the current study, the prevalence of iron deficiency (ferritin<12 µg/L and second stage depleted iron store, ferritin <30 µg/L) was 15%.

Another study done to investigate the iron status of Spanish junior soccer and basketball players showed that the mean iron intake was 19.24 ± 4.58 mg/day and serum iron was within the reference range. Mean analytical data (RBC, Hb, Hct, MCV, ferritin and transferrin saturation) were all between reference ranges. However, basketball players had higher iron intake than soccer players (22.1 ± 6.4 and 19.2 ± 3.9 mg/day, respectively; $p < 0.05$). Nevertheless, basketball players had lower levels of mean RBCs, Hb, Hct and MCV than soccer players (Jara *et al.*, 2006).

But in contrast a study was done in Israel by Dubnov and Constantini, (2004) reported that, among male and female top-level basketball players ($N=103$) from 8 national basketball teams were screened for their anemia and iron store status. Iron depletion, defined by a ferritin level below $20 \mu\text{g/L}$ and it was found among 22% of study participants (15% in males Vs 35% in females, $p = 0.019$). Anemia was found among 25% of athletes (18% in males Vs 38% in females, $p = 0.028$). Iron deficiency anemia, defined by the presence of anemia ($\text{Hb} < 12 \text{g/dl}$) and ferritin below $12 \mu\text{g/L}$ in addition transferrin saturation below 16%, was found among 7% of players (3% in males Vs 14% in females, $p = 0.043$).

The detail interview and AGP parameters showed that presence of infection or parasitic infestation in athletes were lower than reports for the general population. Most of the female athletes were single and thus, factors that could lead to iron deficiency such as birth spacing, pregnancy and lactation are over-ruled. All the above mentioned factors would contribute to decrease the high prevalence situation in the present study.

The mean $\pm$ SD of serum transferrin was 2.6 ± 0.4 g/L. when it was partitioned by sex, the male mean transferrin level was 2.55 ± 0.38 g/L and for female it was 2.7 ± 0.44 g/L. According to Roche diagnostic laboratory; normal reference range for Serum transferrin is 2-3.6 g/L. Using this range, three athletes had serum transferrin of < 2.0 g/L (lower boundary) and two had serum transferrin of > 3.6 g/L (upper boundary).

A research done by department of family medicine and the University of South Carolina shows that, ingestion of large quantities of dietary iron and red meat in persons with high transferrin

saturation is associated with an increase in mortality (Mainous *et al.*, 2004). Given the increased probable risk of mortality with increase in transferring saturation, medical follow up may be required for study participants that have had high transferrin.

4.8.2 Folate status of athletes

The mean $\pm$ Sd of serum-folate for all athletes was 8.5 ± 3.1 ng/ml. With a mean values of 8.4 ± 3.2 ng/ml for male and 8.5 ± 3.0 ng/ml for female athletes. According to the WHO folate cut off points reported in 2012, a serum folate concentration of >20 ng/ml (>45.3 nmol/L) is considered elevated, 6-20 ng/ml (13.5-45.3nmol/L) is considered normal, 3-5.9 ng/ml (6.8-13.4nmol/L) is defined as moderate deficiency and concentration of <3 ng/ml (<6.8 nmol/L) are indicative of severe folate deficiency. Accordingly, there were 21 athletes (20.8%) that had low folate status (moderate deficiency), but 80 athletes (79.2%) had serum-folate within normal range. There were no gender differences in the prevalence of moderate folate deficiency. The figure below shows the folate status of the Ethiopian athletes.

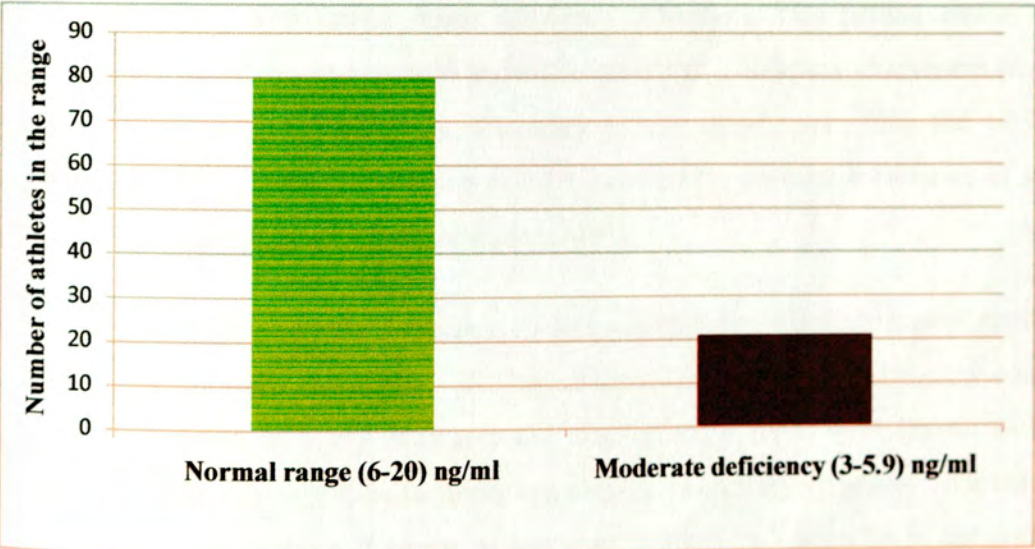


Figure 9: Folate status of the athletes.

4.8.2.2 Comparison of serum folate status with references for various population

The age range of reference population was 18 to 65 years. Pregnant women were excluded and the population was selected according to normal homocysteine values.

Table 10: Comparison of folate status of European and USA reference population with Ethiopian athletes.

Region	N	Median and (Q1, Q3) folate in ng/ml	2.5 th -97.5 th percentile
Europe	290	9.57	4.6-18.7
Australia	345	10.6	5.3-19.3
Ethiopian athletes (total)	101	7.74 (6.3 - 10.2)	4.2-16.8
Male athletes	57	7.7 (6.2 - 10.1)	3.5-17.5
Female athletes	44	7.8 (6.3 - 10.4)	4.4-17.1

In the current study, the prevalence of depleted folic acid (folate <6ng/ml) was 20.8%; however, there was no athlete with severe folate deficiency (<3ng/ml). This finding was in line with reports of folate status for recreational endurance athletes in Germany (Herrmann *et al.*, 2005) which showed a prevalence of folate deficiency (<5.38 ng/mL) was 15% and 14.9% in the present study. Among Ethiopian women of reproductive age, the total prevalence of severe and moderate folate deficiency was 31.3% (Haider, 2010).

This prevalence difference may be because of differences in the characteristics of study subjects and differences in food consumption patterns. There were common food variety consumed in weekly or daily basis by the participants and most of these foods were known to have high source of dietary folate such as milk, meat, egg, lentils, green leafy vegetables, banana and other fruits and juices. In addition Presence of low infection and inflammation in the present study could minimize the high deficiency prevalence. There was not statistically significant gender difference observed in their folate level ($P < 0.05$), but differences were expected for serum ferritin and hemoglobin as clearly illustrated by the different cut-off points for male and female.

Table 11: Analysis of micro nutrient and hematological variables by sex

Variation of Micro nutrient and Hematological variables by sex (Mean $\pm$ Sd)					
Sex	RBC	Hb	Ferritin	Folate	Vitamin B ₁₂
Male	5.45 $\pm$ 0.34 ^a	16.7 $\pm$ 0.82 ^a	107 $\pm$ 2.1 ^a	7.9 $\pm$ 1.45 ^a	542 $\pm$ 1.5 ^a
Female	4.9 $\pm$ 0.35 ^b	15 $\pm$ 0.9 ^b	51 $\pm$ 2.0 ^b	8.0 $\pm$ 1.4 ^a	496 $\pm$ 1.4 ^a

Different superscript letters in the same column indicate significant differences, $p < 0.05$

There was statistically significant difference b/n male and female for their red blood cell, hemoglobin and Ferritin which all these are expected naturally, from biological and physiological difference of sex (male and female), But this difference was not observed for folate and vitamin B₁₂.

Actually there is no sex dependent cut of points for vitamin B₁₂ and Folate by WHO, NIH, CDC or other known health organizations. However, a study conducted in Jordan showed no sex-specific differences in vitamin B₁₂ status among 20-40 years old Jordanians. Similarly, no association was observed with BMI and vitamin B₁₂ intake (Qutob *et al.*, 2011).

Table 12: Serum folate status of the athletes by running-distance category.

Distance category	Folate (n=101)	Folate (male, n=57)	Folate (female, n=44)
Short distance (100-400m)	7.3 $\pm$ 1.4 ^{ab}	6.8 $\pm$ 1.4 ^{ab}	7.9 $\pm$ 1.4 ^a
Middle distance (800-1500m)	7.7 $\pm$ 1.4 ^{ab}	7.4 $\pm$ 1.3 ^{ab}	7.9 $\pm$ 1.4 ^a
Steeple cheese (3km)	7.0 $\pm$ 1.4 ^a	6.4 $\pm$ 1.5 ^a	8.3 $\pm$ 1.2 ^a
Long distance (5 & 10km)	9.7 $\pm$ 1.45 ^b	12.2 $\pm$ 1.4 ^c	8.1 $\pm$ 1.3 ^a
Marathon (42.2km)	8.8 $\pm$ 1.44 ^{ab}	9.4 $\pm$ 1.3 ^{bc}	8.0 $\pm$ 1.7 ^a

Superscript words of the same letters in one column are not statistically significant, $p > 0.05$

The folate level of male runners was significantly varied by running distance category. But similar to iron, the association between female folate status and running distance category was

not statistically significant and these gender differences by running distance requires further investigation.

4.8.3 Vitamin B₁₂ status

In the present study, serum vitamin B₁₂ of the athletes was analyzed. The non-log-transformed mean and Sd of the athletes were 561 ± 231 pg/ml. And the mean male and female vitamin B₁₂ level was 586 ± 258 and 528 ± 189 pg/ml respectively. Thirty five percent of the athletes had vitamin B₁₂ of (≥ 601 pg/ml) but 9% of them had (>800 pg/ml). In the present study, the analyzed vitamin B₁₂ data had a minimum value of 210 pg/ml. Based on the borderline/cut off ranges mentioned in chapter three, all of the athletes were in the acceptable range (vitamin B₁₂ >201 pg/ml) which indicated the absence of severe or moderate deficiency for this nutrient.

4.8.3.1 The vitamin B₁₂ status in comparison to non-athlete population

There were some literatures which documented the normal range of serum B₁₂ as 200-900pg/ml and >900 pg/ml is indicating the vitamin overload, but the other do not specify the upper limit; this was some controversies observed for vitamin B₁₂ upper normal range (UL) and also there is no internationally agreed upper level to pose any health risk. But some literatures associate the higher cobalamin level with cancer risks.

For instance a Population-Based Cohort Study in Denmark reported by Arendt *et al.*, (2013), elevated Plasma Vitamin B₁₂ level used as a marker for cancer. Cancer risk increased with higher Cobalamin levels and was highest during the first year of follow-up (Cobalamin, 601–800 pmol/L: standardized incidence ratio (SIR) =3.44, 95% CI =3.14-3.76; Cobalamin >800 pmol/L: SIR = 6.27, 95% CI =5.70-6.88; both $P < 0.001$). The risks were particularly elevated for smoking-and alcohol-related cancers for persons with high cobalamin levels. But such type of finding needs further investigation on their validity and association strength.

The present study was fairly inconsistent with some very limited studies have done related to athlete's vitamin B₁₂ status and the various studies have been done on healthy sedentary population in different parts of the world.

A study done on vitamin B₁₂ level of recreational endurance Athletes in Germany shows that the median with (Q1, Q3) level was 343 (285–431) respectively and prevalence of vitamin B₁₂ deficiency (<211 pg/ml) was 10% (Herrmann *et al.*, 2005). Whereas a study among healthy subjects (n= 165) aged 20-40 years in Pakistan 27.3% had vitamin B₁₂ deficiency (<200 pg/ml), 41.8% had serum B₁₂ of 201-300 pg/ml and 30.9% had normal B₁₂ levels (>300 pg/ml). Among those whose serum vitamin B₁₂ was <300 pg/ml, 47.4% had confirmed deficiency using MMA (true deficiency indicator).

Among those with serum B₁₂ between 201-300 pg/ml, 44.9% had confirmed B₁₂ deficiency. Vitamin B₁₂ status was found to be negatively correlated with MCV and no significant associations were found between vitamin B₁₂ status with gender, body mass index (BMI) and total vitamin B₁₂ intake (Qutob *et al.*, 2011). A study done to assess the serum folate and vitamin B₁₂ status of healthy Iranian adults in a population based cross-sectional study of 984 individuals (507 men and 477 women) aged 20–80 years, the mean serum folate was 4.61 ± 2.40 ng/mL and the mean serum vitamin B₁₂ level was 265.6 ± 170.9 pg/mL.

Overall, 1.0 % were folate deficient and 25.8% had low vitamin B₁₂ levels according to the manufacturer's reference ranges (folate <1.5 ng/mL and vitamin B₁₂ <160 pg/mL). The mean serum folate and vitamin B₁₂ levels were significantly lower in male than female and the prevalence of vitamin B₁₂ deficiency was considerably higher than folate deficiency (Shams *et al.*, 2009). This finding was in contrast to the present one which indicated no significant gender difference in folate and vitamin B₁₂ level and absence of vitamin B₁₂ deficiency.

4.8.3.2 Comparing the Vitamin B₁₂ with reference population

The reference populations were in age range of 18 and 65 years. Pregnant women were excluded and the population was selected according to normal homocysteine values. Both the reference population and the current subjects were analyzed with the same instrument (Roche: Elecsys, 2010 instrument).

Table 13: Median and quartile range of the vitamin B₁₂ in comparison with European and USA reference population.

Region	N	Median and (Q1, Q3) in pg/ml	2.5 th -97.5 th percentile
Europe	291	357	191-663
USA	178	463	211-946
Ethiopian athletes (total)	101	516 (431-671)	234-1206
Male athletes	57	520 (434-678)	232-1534
Female athletes	44	508 (398-644)	215-1115

There was no statistically significant difference observed ($p > 0.05$) in the mean B₁₂ level of male and female athletes and among each running distance categories at 95% confidence interval. But there was physically observable mean difference between male and female athletes which it was 586 ± 258 and 528 ± 189 pg/ml respectively and also mean vitamin B₁₂ decrement with increases in running distance (short to marathon).

Table 14: Statistically analyzed Vitamin B₁₂ by distance categories

Distance category	Mean $\pm$ SD of Vitamin B ₁₂ (in pg/ml)
Short distance (100-400m)	565 ± 1.5^a
Middle distance (800-1500m)	560 ± 1.3^a
Steeple cheese (3km)	466 ± 1.5^a
Long distance (5 & 10km)	490 ± 1.5^a
Marathon (42.2km)	478 ± 1.4^a

Superscript words of the same letters are not statistically significant, $p > 0.05$

Deficiency of folate has been associated with improper function of nerve, brain, neural tube defects, neuropathy and anemia. However, cobalamin deficiency related to the synthesis of

serotonin and catecholamine neurotransmitters as well as S-adenosylmethionine which may then lead to various diseases which includes memory disturbance, delirium, mood disorders and psychosis. Therefore these vitamins may have a great impact on individuals working abilities and performance. Some studies have also associated them with several types of cancers.

The study participants have showed unexpectedly high serum B₁₂ level when compared not only to researches done in different parts of the world, but also when compared with the reference population. Given that no specified upper limit, whether such high values are normal or can pose health problem remain unknown (Wahlin *et al.*, 2001).

The high serum vitamin B₁₂ status of the athletes may be partly explained by their higher consumption of some vitamin B₁₂ rich foods like milk and eggs which were frequently consumed with large amounts (usually half or one liter at a time) of milk and an average of 4-eggs in one serving dish were consumed either in daily or every other day.

However a study reported from Denmark; the underlying pathogenesis leading to high Cobalamin levels is poorly elucidated, with a few exceptions. It is not thought to involve increased Cobalamin intake because intestinal absorption capacity is saturable and high physiological consumption does not increase plasma Cobalamin levels substantially.

Only Cobalamin therapy in the form of injections or extremely high oral doses can produce high circulating levels, but during the cohort study, these subjects were excluded, so we conclude that the mechanisms resulting in high Cobalamin levels may be related to malignant pathogenesis (Arendt *et al.*, 2013). But it needs further investigation on the association of high cobalamin and different pathogenesis and etiological factors.

4.9 Correlation between athletes' micronutrient status and hematological variables with their performance

To investigate whether hematological variables and micronutrient status are associated with athletic performance, data on performance (2013 and 2014) were obtained from the Ethiopian athletics federation (EAF). The athletes' best time archived was obtained from the coaches of the

athletes for each distance categories. Distance covered per time or speed of the athletes (an average of 2013 and 2014) was taken as performance measurement.

The data was then categorized into high and low performers. The mean and standard deviation (Sd) of their hematological variables was calculated and for each of hematological variables, the statistical analysis was done in order to find if there was any significant association ($p < 0.05$) between high and low performers.

Table 15: statistical association of Athletes' hematological values of and their performance.

Hematological Variables (Mean $\pm$ Sd) of high performer Vs low performer athletes					
Male & female	Hb (g/L)	RBC count x (10^{12})	Ferritin ($\mu\text{g/L}$)	Vitamin B ₁₂ (pg/ml)	Folate (ng/ml)
High performer	16.2 $\pm$ 1.2 ^a	5.3 $\pm$ 0.14 ^a	84.5 $\pm$ 63.3 ^a	539 $\pm$ 153 ^a	9.3 $\pm$ 3.8 ^a
Low performer	15.5 $\pm$ 1.0 ^a	5.0 $\pm$ 0.14 ^b	88.6 $\pm$ 60.8 ^a	487.6 $\pm$ 189.5 ^a	7.4 $\pm$ 3.3 ^a

Supper script words of different letters in one column are statistically significant ($p < 0.05$)

The statistical analysis in the above table shows that even though there was a positive association between the micronutrient and hemoglobin level of high performer against low performer, but except for RBC values, they were not statistically significant. Irrespective of gender differences, higher performers had higher RBC values than their low performing counterparts ($p < 0.05$).

Chapter Five

5. Conclusions and Recommendations

5.1 Conclusions

Athletes need relatively high fluid, macro and micro nutrient intakes to balance losses associated with strenuous exercise and stay healthy. In addition, micronutrient intake should be regulated to compensate for high altitude running, fatigue and traumatic movement of distance running which lead to losses of minerals and vitamins through sweating, urination, hemolysis or intense exercise-induced malabsorption in the stomach and intestine.

As the weekly food frequency assessment and the dietary diversity showed that the most frequent and largely consumed foods on a weekly and daily basis were: cereals, legumes/nuts/seeds, fruits & juices, vegetables, eggs, milk, meat and fat and oils. These food varieties are known to be good sources of micro nutrient provided that they are consumed in the right amount and frequency.

The present study assessed the most important micronutrients status such as iron, folate vitamin B₁₂ and hematological variable status which are known to have tremendous effect on athlete's health, performance and endurance and also AGP inflammation marker.

Although the micronutrients and other hematological variables were positively associated with performance however only the RBC of high performer athletes was significantly ($p < 0.05$) higher compared to the low performer ones. In contrast to other trace elements assessed, there was no vitamin B₁₂ deficiency. Rather the Ethiopian athletes exhibited higher vitamin B₁₂ status compared to previous research findings.

5.2 Recommendations

Ethiopian athletes, especially long-distance runners, have extraordinary performance and are known to win various international competitions and have broken many international records in Olympics, diamond and golden leagues. Monitoring the diet and fluid intake of the athletes for their day to day requirement in terms of energy and fluid demand is very crucial not only to sustain their success in marathon, long distance like 10km and 5km, but also to bring competency in middle and short distance running. Similarly, there is need for continuous monitoring of micronutrient intake to intervene whenever needed.

Given the 11% of the athletes have serum ferritin $>200\mu\text{g/L}$ which indicates iron overload. even though it quite common to give dietary supplements such as iron-folate, multivitamins and other trace minerals for athletes with a broad spectrum of suspecting micro nutrient deficiency, however it should be based on clinical testing for possible consequences of overload and toxicity. Generally it is very recommendable to conduct iron overload assessment in Ethiopia healthy populations.

Future studies should investigate the reason behind the observed high RBC in high performing athletes. Such study findings may need to be repeated in other athletes like Kenyans' top performer which most of them from kalejin-tribe and very supper athletes from Arsi-Bekoji or Assela in Ethiopia.

Strength and limitation of the study

This study has assessed the dietary diversity & food frequency, important hematological variables (CBC) including RBC, Hb level and micronutrients status such as iron, folate, vitamin B₁₂ status of the athletes and then their association with the performance of the professional Ethiopian athletes. As there was no previous study related with these athletes, so the study could fill the existing research gaps and can have its own great contribution for dieticians/nutritionist & physicians as a guideline in diagnosing the situation. The major constraints of this study were absence of enough budgets & high cost of reagents, scarcity of national base line data & reference value for any of the parameters, absence of related research works, nevertheless of all these challenges the study came to the ground. In the contrary the study have limitations and possible weakness; such as parameters like RBC-folate, C-reactive protein, transferrin saturation or total iron binding capacity (TIBC) are not included because of absence of reagents and their costs. In addition rather of using cross sectional study design (point of time data); time series study design may be more convenient for analyzing cause and effect relationship.

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Appendices

Appendix A

Consent form

Title: Determining Hematological indices, Iron, Folate and Vitamin B₁₂ Statuses and their relation with the performance of Ethiopian national athletes.

Purpose: The purpose of this study is to determine the dietary habits and the micronutrient statuses of Ethiopian athletes and to analyze if any iron and none iron deficiency anemia, its severity level, and based on the study result, to recommend/propose further strategies or mechanisms which best suit for the exciting problem.

Procedure: If you agree to take part in this study, the study will take about 30 minutes of your time. First we will ask you about your personal information, types of training you are performing & its load of exercise, kilometer cover in competition you are candidated with, your dietary intake, then we will ask you for hematological sample to test the level of Iron, Folate and Vitamin B-12 status in your body. The materials we will use to collect the blood specimens are clean, sterile and safe. The samples will be analyzed in EHNRI using a code so that no one will know your results. All information collected during this study will be kept private and will only be known by the investigator.

Benefits: This study will help you and others that are currently engaged athletics sports, particularly in running sports, in terms of promoting for better nutritional status, health and performance by providing necessary and reliable information to the Ethiopian athletic federation, physicians, dieticians' nutritionists, coaches and trainer which can enable to set, rules and regulations to improve the health, strength and performance of the athletes.

Risks: There is no risk to you by answering the questions or giving a blood specimen. Blood drawing may cause brief soreness from the needle stick, bruising, dizziness, and rarely, infection where the needle enters the vein. And well trained and professional phlebotomists will draw the blood.

Right to refuse or withdraw: You can choose whether or not you want to be in this study. You may refuse to be in any or all parts of the study. You may decide to stop participating at any time. Nothing bad will happen if you do not take part.

PERSONS TO CONTACT: Please if you have any questions you can ask at any time of need. You may direct your questions regarding this study to: Kifle Habte, Tel. 0913202001. We will give you the copy of this consent.

The information on this form has been explained to me. I have been given a chance to ask questions. I feel that all of my questions have been answered. Being in the study is my choice. I may change my mind and leave the study at any time.

Participant name: -----

Signature: -----

Date: -----

Person obtaining consent

Name: -----

Signature: -----

Date: -----

የኢትዮጵያ ጤናና ሥነ-ምግብ ምርምር ኢንስቲትዩት

የስምምነት ቅጽ እና የምርምር ስራ ማብራሪያ

የዚህ ጥናት ዓላማ የተለያዩ ሯጭ አትሌቶች የሚከተሉትን የአመጋገብ ስርአትና የምግብ መጠን አወሳሰድ ማጥናት፤ በሰውነታቸው ውስጥ ያለው የአይረን፣ፎስፎረስና ቫይታሚን ቢ 12 መጠን ማወቅና መለየት ሲሆን በተጨማሪም በጥናቱ የሚሳተፉትን ፈቃደኛ የሆኑ አትሌቶችን ቃለመጠይቅ በማድረግ የችግሩን መጠንና ምንጮቻቸውን በመለየት የችግሩን ደረጃ በጥናት መልክ ማቅረብ እንዲሁም ለአትሌቶቹ ከጤንነት አንጻርም ሆነ ከሀገር ውስጥና ከሀገር ውጭ በሚወዳደሩት ውድድሮች ላይ ዘለቂታ ያለው የተሻለ ተፎካካሪነት ለማምጣት የሚረዱ ምግቦችና ጤናማ የአመጋገብ ስርአቶችን እንዲከተሉ ማድረግ ነው።

በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ፡-

- በመጀመሪያ የግል ህይወቱን የተመለከተ ቃለመጠይቅ ይደረገልዎታል።
- ከዚያም የሚወስዱትን ምግብ አይነት፣ መጠንና የአመጋገብ ዘይቤዎትን የሚመለከት ቃለመጠይቅ ይደረግልዎታል።
- በመቀጠልም የንጥረ ምግብ መጠን ለማወቅ የሚያገለግል የደም ናሙና ይሰጣሉ። የሚሰጡት ናሙና ከተጠቀሰው ምርመራ ውጪ ለሌላ የላቦራቶሪ ምርመራ አይወልም።

ከጥናቱ የሚገኘው መረጃ እርሶ ያለዎትን የንጥረ ምግብ መጠን ለማወቅና የአመጋገብ ባህልዎን ለማሻሻል፣ እንደርሶ ላሉ ስፖርተኞች ከሚያደርጉት እንቅስቃሴ አንጻር ጤናማና በቂ የሆነ የአመጋገብ ስርአት ምን ማለት እንደሆነና ምን ምን መያዝ እንዳለበት ለማስተማርና ይህንኑ የአመጋገብ ዘይቤ ተከትለው በሀገራቸውም ሆነ ከሀገር ውጭ በሚያደርጉት ውድድሮች ላይ ብቁ፣ ጠንካራ፣ ጤናማና ተፎካካሪ ሆነው እንዲገኙ ለማድረግ ነው። እርሶ የሚሰጡት መረጃዎች በሙሉ ሚስጥርነታቸው በጥብቅ የተጠበቀ ነው።

የጥናቱን ሂደት በተመለከተ ጥያቄ ካልዎት በማንኛውም ጊዜ መጠየቅ ይችላሉ።

ከዚህ በላይ የተገለፀውን አንብቤ ወይም ተነቦልኝ የጥናቱ ተሳታፊ ለመሆን ተስማምቻለሁ።

የተሳታፊው ፊርማ.....

የተመራማሪው ፊርማ.....

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Appendix B



Ethiopian Health and Nutrition Research Institute

Determining Hematological indices, Iron, Folate and Vitamin B₁₂ Statuses and their relation with the performance of Ethiopian national athletes.

Demographic, Exercise and Health Related Questionnaires

Good morning/afternoon

Thank you for your interest in talking with me today. I am _____ who is a member of a team conducting a study entitled “Determining Iron deficiency anemia, Folate and Vitamin B₁₂ status of Experienced Athletes Registered in Ethiopia Athletics Federation, 2014”. The study is being carried out in Ethiopian elite athletes by Ethiopia Public Health Institute and Addis Ababa University, in order to determine the status of most important micronutrients and dietary intakes of high level endurance exercise, power and mixed athletes.

Your participation in the study is on voluntary bases; however, your positive response and honest answer will help us in understanding the situation better and will eventually contribute in designing appropriate interventions. All the information gathered will be kept confidential and your name will never be linked with any of the information that you provided. The interview will take about 30 minutes. Your participation is greatly appreciated.

Do you have any questions about the study?

Are you willing to participate in the study?

Yes ☐

No ☐

(Remember: if the study participant is not willing to take part in the study, stop the interview)

Sample Person ID:

Date of Interview: - 20

Time started : am / pm Time ended : am / pm

Part I Socio-demographic questionnaire

1. ____ Your sex 1. Male 2. Female
2. ____ When you were born (month & year)?-----
3. ____ In which region, town and village you were born?-----
4. ____ your ethnicity?-----
5. ____ What is your average monthly income in birr? 1. <1000 2. 1000-1500 3. 1501-1999
4. 2000-2500 5. 2501-2999 6. 3000-4000 7. specify if any other
6. ____ Number of dependant (father, mother, children and others)?-----
7. ____ Marital status? 1. Married 2. Single 3. Divorced 4. Widowed
8. ____ Do you have children? If yes how many? -----
9. ____ What's your religion? 1. Orthodox 2. Muslim 3. Protestant 4. others
10. ____ Literacy level? 1. Illiterate 2. 1-4Grade 3. 5-8 Grade 4. High school 5.
college/university 6. Others specify-----

Part II Exercise related questionnaire

1. ____ At what age, you actually started running?-----
2. ____ What is your running distance in national/international/ competition?-----
3. ____ How long is your experience in this running distance?-----
4. ____ When you perform exercise, usually what distance you try to run/cover?-----
5. ____ At what altitude do you usually make practice (in feet/km)?-----
If you don't know the altitude tell the exact name of your usual training place-----
6. ____ Usually for how many days a week do you practice? 1.) 3 days 2.) 4days
3.) 5 days 4.) 6 days 7. Specify if any other-----
7. ____ For how long do you usually practice/day? 1.) 30min 2.) 1Hr 3.) 2Hr 4.) 3Hr 5).
4Hr 6.) 5Hr 7. Specify if any other-----

Part III Health and Exercise related questionnaires

1. ____ If any ill feeling or problem you have during practice/competition? 1.yes 2.No

If yes, please explain it more-----.

2. ____ Do you have unconsciousness or fainting after or during training/competition? 1.yes

2.No

If yes, how usually it occurs?-----

3. ____ Do you have Fatigue, headache, shortness of breath and dizziness after or during exercise/competition/? 1. Yes 2. No

4. ____ If yes in Q.3 when and how frequently it occurs? 1. During/after every exercise 2. usually 3. sometimes 4. rarely

5. ____ And also, in your believe, what is the cause of these kind of symptoms?-----.

6. ____ Do you have recently experienced tissue damage, injury and bleeding? 1. Yes 2.No

If yes please explain its occurrence and duration-----.

7. ____ If you have diarrhea or vomiting in the last two weeks? 1. Yes 2. No

8. ____ Do you have Gastritis, Intestinal or other infection? 1. Yes 2.No

9. ____ If yes, in Q.9 which infection and when it started?-----.

11. ____ And, have you been examined clinically? 1. Yes 2.No

12. ____ Have you experienced parasitic infection (hookworm, tapeworm, and ascariasis) in the last month? 1. Yes 2.No.

If yes, have you been examined clinically? 1. Yes 2.No

13. ____ Do you have had malaria since the last two month? 1. Yes 2. No

14. ____ Are you using insecticide treated net in your home or when you go other place, where malarial cases are common? 1. Yes 2.No

15. ____ Do you have any organ disease or infection? If yes, which infection?-----.

16. ____ Did you have any fever in the last 2 weeks? 1. Yes 2.No

17. ____ Did you have any other problem in the last month? 1. Yes 2.No

If yes, please specify-----

19. ____ Have you taken antibiotics, since the last month? 1. Yes 2.No

19a. 1) what types of antibiotics?----- 2) For what type of disease?-----

20. ____ Have you taken pain killers since the last 3 days? 1. Yes 2. No

21. ____ Do you take alcohol? 1. Yes 2. No

22. ____ Do you smoke? 1. Yes 2.No

23. ____ In average, how many hours do you sleeping/day?-----.

Part IV. Questions below are for female athletes only

1. ____ Does your menstruation come in its normal schedule/menstruate regularly/? 1. Yes
2.No

2. ____ If yes in Q.1, for how many days usually bleeding persists? -----

3. ____ Do you have any amenorrhea (no menstruation) case? 1. Yes 2. No

4. ____ If yes in Q.3, which type of amenorrhea you have? 1. Primary amenorrhea

2. Secondary amenorrhea

5. ____ If you have secondary amenorrhea, over the last year, tell me the exact month of amenorrhea started & its duration.-----

6. ____ Do you have more than one child? 1. Yes 2.No If yes how many?-----

6a. ____ By how far apart your child births were? 1.) 1child/year 2.) 1child/2year 3.) 1child/3year

4.) ≥ 4 year/child

7. ____ Are you currently pregnant 1. Yes 2.No

8. ____ Are you currently lactating? 1. Yes 2.No

Note: **primary amenorrhea** (no menstruation while age ≥ 16 years old). **Secondary amenorrhea** (no menstruation for 3 months or more after normal menstruation).

Appendix c

Part V. Dietary diversity and food frequency questionnaires.

A. Dietary Diversity Questionnaire

Please describe the foods (meals and snacks) that you ate or drank yesterday during the day and night, whether at home or outside the home. Start with the first food or drink of the morning. *Write down all foods and drinks mentioned. When composite dishes are mentioned, ask for the list of ingredients. When the respondent has finished, probe for meals and snacks not mentioned.*

	Never	per mon	per mon	per wk.	per wk.	per wk.	per day	day	per day	size			
	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup or 240ml	☐	☐	☐
milk										1cup or 240ml			
yogurt										1cup or 240ml			
cheese										1 cup or 240ml			
soy cheese										2oz. or 56g			
										100g			

4.Fruits	Average use over the last week									Amount			
	Never	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
orange	☐	☐	☐	☐	☐	☐	☐	☐	☐	1medium or 150g	☐	☐	☐
lemon										1medium or 100g			
banana										1medium or 150g			
mango										1medium or 150g			
Guava/zeituna/										1medium or 150g			

pumpkin											1 cup or 245g			
pepaya											1 medium or 275g			
avocado											1 medium or 150g			
any fruit juices											1 glass or 250ml			

Vegetables	Mode of processing /raw or boiled/	Average use over the last week									Amount			
		Never	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
beach/qosta/		☐	☐	☐	☐	☐	☐	☐	☐	☐	1 cup or 225g	☐	☐	☐
celery/selata/											1 cup or 225g			
tomato											1 cup or 225g			
cabbage											1 cup or 225g			
broccoli											1 cup or 225g			
green beans/selata/											1 tbsp or 15g			
spinach											1 medium or 112g			
potato											1 medium or 112g			
sweet potato											1 medium or 112g			

Meat and organ meats	Average use over the last week									Amount			
	Never	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
Raw meat/	☐	☐	☐	☐	☐	☐	☐	☐	☐	200gm	☐	☐	☐
meats/liver, heart/										170gm			
other processed										1 serving or 280g			

Fish	Average use over the last week									Amount			
	Never	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
Fried fish/	☐	☐	☐	☐	☐	☐	☐	☐	☐	4oz. or 112g	☐	☐	☐
Other fish										170g			
Other form of processed fish										1 cup or 200g			

Poultry	Average use over the last week									Amount			
	Never	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
Chicken	☐	☐	☐	☐	☐	☐	☐	☐	☐	100g	☐	☐	☐
										1 whole egg or 84g scrambled			

Food items	Never	per mon	per mon	per wk.	per wk.	per wk.	per day	per day	per day	size			
bean / Akure ater/	☐	☐	☐	☐	☐	☐	☐	☐	☐	½ cup or 112g	☐	☐	☐
										½ cup or 112g			
Beans/ (boleqae/										½ cup or 112g			
baqela/										½ cup or 112g			
										½ cup or 112g			
pumpkin seeds										½ cup or 1 tea cup			
chilis/misir/										½ cup or 1 tea cup			
blackpeas/shimbira kure/										½ cup			
										½ cup			
										½ cup			
										½ cup			
										½ cup			
										½ cup			
										2tbsp. or 30g			
inga seeds										1/2 cups or 112g			
										1/2 cups or 112g			
araroni										1/2 cups or 112g			
zaghetti													

10. Tubers and roots	Average use over the last week								Amount			
	1	2-3	1-2	3-4	5-6	1-2	3-4 per	5 or >5	Medium serving size	S	M	L

	Never	per mon	per mon	per wk.	per wk.	per wk.	per day	day	per day				
pot / qey sir/	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 medium or 100g	☐	☐	☐
										3 large carrots or 85g			
										2tbsp or 30g			
										10g			
										20g			

11. sweets	Average use over the last week									Amount			
	Nev er	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
chocolates	☐	☐	☐	☐	☐	☐	☐	☐	☐	50g	☐	☐	☐
										1tbsp or 30g			
										1tsp or 5g			
marmalade										1tbsp or 15g			
12. Fats and oils	Average use over the last week									Amount			
	Nev er	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
	☐	☐	☐	☐	☐	☐	☐	☐	☐	1tbsp or 15g	☐	☐	☐
nut butter										2 tbsp or			

											30g				
											1tbsp or				
											15g				

3. Carbonated beverages	Average use over the last week									Amount			
	Never	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M	L
Cola	☐	☐	☐	☐	☐	☐	☐	☐	☐	1 bottle of coca or 300ml	☐	☐	☐
Bull/Energy drink/										1/2 bottle of red bull or 120ml			

4. Other beverages			Average use over the last week								Amount			
			Ne ver	1 per mon	2-3 per mon	1-2 per wk.	3-4 per wk.	5-6 per wk.	1-2 per day	3-4 per day	5 or >5 per day	Medium serving size	S	M
Beverage	Time often used the beverage?													
Tea	With meal	After meal	☐	☐	☐	☐	☐	☐	☐	☐	1 tea cup or 60ml	☐	☐	☐
Coffee											coffee cup or 60ml			
Beer											1 bottle beer or 360ml			
Wine											10 tbsp			

												or 150ml			
Card												3tbsp or			
erits												45ml			

Note. There are quick unit conversions and abbreviations below, use them as necessary.

1 cup=8 oz.=16 tablespoon/tbsp/=48 teaspoon/tsp, 1tbsp=15g=15ml and 1tsp=5g=5ml

1 cup=224g=240ml and $\frac{1}{2}$ cup=112g=120ml=1 full tea cup (medium tea cup)

1 ounce /oz./ =28gm=30ml Month (mon.) and Week (wk.)

You have answered all the questions I have. Thank you *very much* for your time and honest response. Please take a moment to fill in any questions you may have skipped. Have a good time!

Part VI. Dietary supplements information

A. Vitamins and minerals supplements

Type of Dietary supplement	Response	Brand name	Year & month of supplement started.	Dose per day or per week
1. Iron-folate	1. Yes 2. No			
2. B-complex/B-vitamins/	1. Yes 2. No			
3. Vitamin C	1. Yes 2. No			
4. Calcium	1. Yes 2. No			
5. Copper	1. Yes 2. No			
6. Zinc	1. Yes 2. No			
7. Magnesium	1. Yes 2. No			
8. Vitamin D	1. Yes 2. No			
9. Vitamin E	1. Yes 2. No			
10. Vitamin A	1. Yes 2. No			
11. Selenium	1. Yes 2. No			
12. Caffeine	1. Yes 2. No			
13. Omega 3-fatty acid/fish oil/	1. Yes 2. No			

4. Iodine	1. Yes 2. No				
5. B-caroten	1. Yes 2. No				
6. Coenzyme Q10	1. Yes 2. No				
7. Ephedrine	1. Yes 2. No				
8. Any other dietary supplement	1. Yes 2. No				

B. protein rich supplements /Protein pump/

No.	Type of supplement	Response	Brand name	Year & month of supplement started.	Dose per day or per week
1.	Ieucine	1. Yes 2. No			
2.	Isoleucine	1. Yes 2. No			
3.	Glutamine	1. Yes 2. No			
4.	Argenine	1. Yes 2. No			
5.	Glucosamine	1. Yes 2. No			
6.	Creatine	1. Yes 2. No			
7.	B-alanine	1. Yes 2. No			
8.	Casein	1. Yes 2. No			
9.	Whey protein	1. Yes 2. No			
10.	Soy protein	1. Yes 2. No			
11.	Any other supplement	1. Yes 2. No			

Appendix D

Statistical analyzed data to find if there was an association b/n performance versus hematological variables of male and female athletes.

Anova: Single Factor for male (hemoglobin)

SUMMARY

Groups	Count	Sum	Average	Variance
column 1 (Hb of H.P)	8	137.3	17.1625	0.468393
column 2 (Hb of L.P)	8	129	16.125	1.016429

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4.30563	1	4.305625	5.799519	0.0303866	4.600109908
Within Groups	10.3938	14	0.742411			
Total	14.6994	15				

Where: H.P-high performer athletes

L.P- low performer athletes

Anova: Single Factor for female (Hemoglobin)

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (Hb of HP)	8	121.9	15.2375	0.465536
Column 2 (Hb of LP)	8	119.5	14.9375	0.476964

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.36	1	0.36	0.763926	0.3968484	4.600109908
Within Groups	6.5975	14	0.47125			
Total	6.9575	15				

**Anova: Single Factor for male
(Red blood cell)**

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (RBC of HP)	8	44.56	5.57	0.13317
Column 2 (RBC of LP)	8	41.46	5.1825	0.13988

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.6005	1	0.60065	4.39938	0.054588081	4.6001
Within Groups	1.9115	14	0.13655			
Total	2.515	15				

**Anova: Single Factor
(Female)**

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (RBC of HP)	8	40.82	5.1025	0.03788
Column 2 (RBC of LP)	8	38.57	4.82125	0.08473

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.31640	1	0.316406	5.16138	0.0393931	4.6001
Within Groups	0.85823	14	0.061303			
Total	1.17464	15				

Anova: Single Factor
(Male)

SUMMARY

Groups	Count	Sum	Average	Variance
column 1 (ferritin of HP)	8	912.41	114.0513	4620.994
column 2 (ferritin of LP)	8	881.3	110.1625	4539.886

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	60.48951	1	60.48951	0.013206	0.910142	4.60011
Within Groups	64126.17	14	4580.44			
Total	64186.66	15				

Anova: Single Factor
(Female)

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (ferritin of HP)	8	439.98	54.9975	1976.399
Column 2 (ferritin of LP)	8	536.1	67.0125	2322.917

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	577.4409	1	577.4409	0.26862	0.612353	4.60011
Within Groups	30095.21	14	2149.658			
Total	30672.65	15				

Anova: Single Factor (Male)

Anova: Single Factor (Male)

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (Vitamin B ₁₂ of HP)	8	4396.6	549.575	39881.76
Column 1 (Vitamin B ₁₂ of LP)	8	3759.4	469.925	40037.48

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	25376.49	1	25376.49	0.635053	0.438814	4.60011
Within Groups	559434.7	14	39959.62			
Total	584811.2	15				

Anova: Single Factor (Female)

Anova: Single Factor (Female)

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (Vitamin B ₁₂ of H.P)	8	4234.2	529.275	9768.436
Column 2 (Vitamin B ₁₂ of L.P)	8	4041.4	505.175	36239.52

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2323.24	1	2323.24	0.100993	0.755332	4.60011
Within Groups	322055.7	14	23003.98			
Total	324378.9	15				

Anova: Single Factor **(male)**

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (folate of H.P)	8	81.14	10.1425	23.09036
Column 2 (folate of L.P)	8	52.5	6.5625	13.40691

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	51.2656	1	51.2656	2.809284	0.1159	4.600109908
Within Groups	255.4809	14	18.24864			
Total	306.7465	15				

Anova: Single Factor **(Female)**

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1 (folate of H.P)	8	67.08	8.385	6.18951
Column 2 (folate of L.P)	8	66.21	8.27625	7.91405

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.04730625	1	0.04730	0.00670	0.93588	4.600109908
Within Groups	98.7249875	14	7.05178			
Total	98.7722937	15				

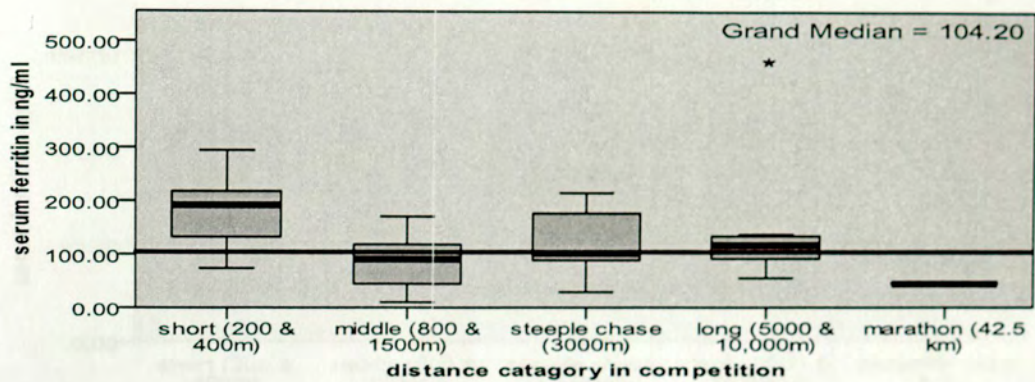
Statistical significance

Male ferritin by distance category

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.	Decision
1	The medians of serum ferritin in ng/ml are the same across categories of distance category in competition.	Independent-Samples Median Test	.001	Reject the null hypothesis.
2	The distribution of serum ferritin in ng/ml is the same across categories of distance category in competition.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.
Asymptotic significances are displayed. The significance level is .05.				

Male ferritin median test

Independent-Samples Median Test



Total N	57
Median	104.200
Test Statistic	19.933
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.001

1. More than 20% of the cells have expected values less than five.

1. More than 20% of the cells have expected values less than five.

Female ferritin by distance category

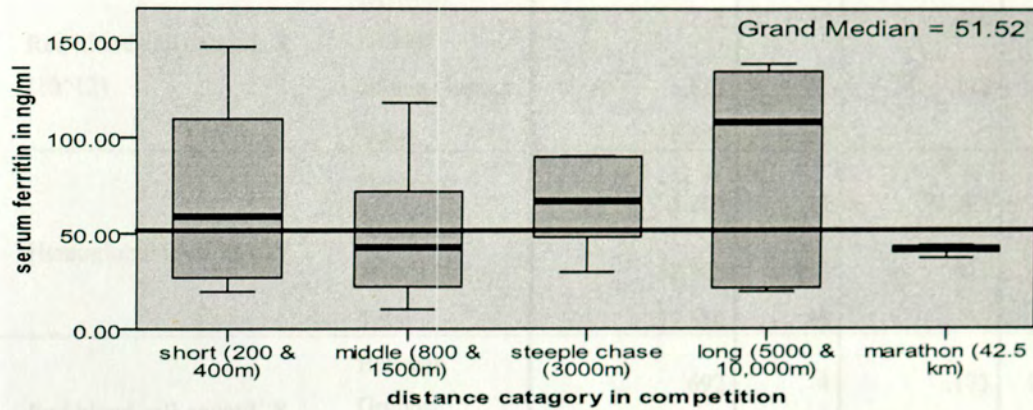
Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The medians of serum ferritin in ng/ml are the same across categories of distance category in competition.	Independent-Samples Median Test	.043	Reject the null hypothesis.
2	The distribution of serum ferritin in ng/ml is the same across categories of distance category in competition.	Independent-Samples Kruskal-Wallis Test	.427	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Female ferritin median test

Independent-Samples Median Test



Total N	44
Median	51.525
Test Statistic	9.877
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.043

1. More than 20% of the cells have expected values less than five.

Over all ANOVA

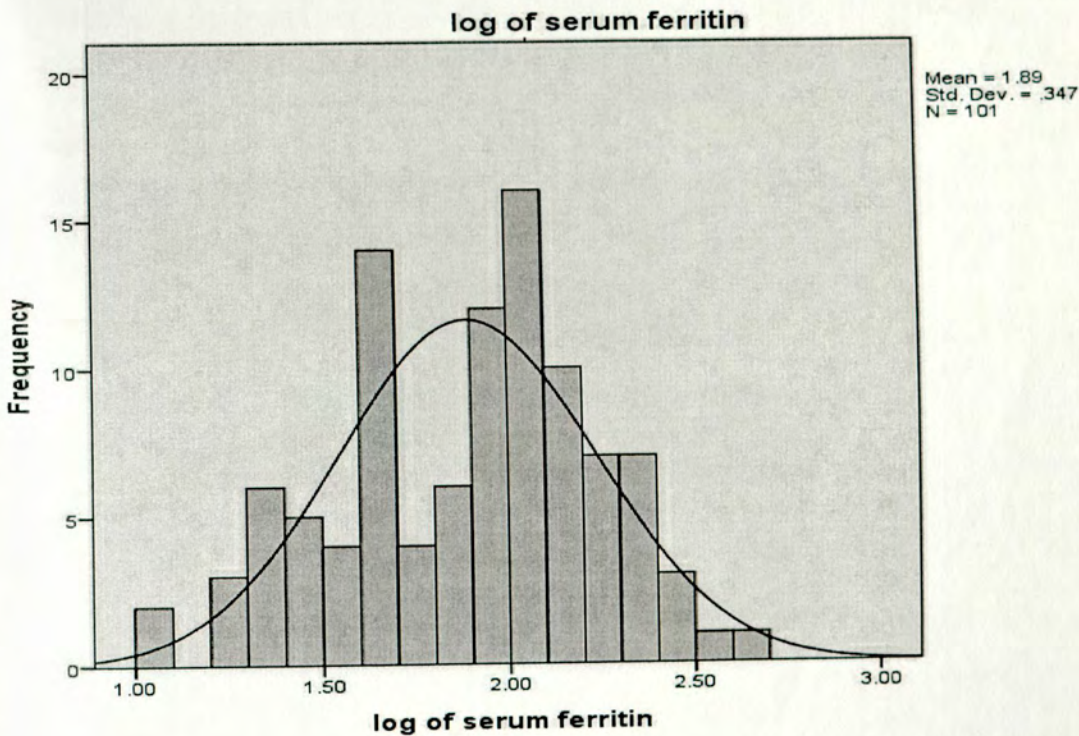
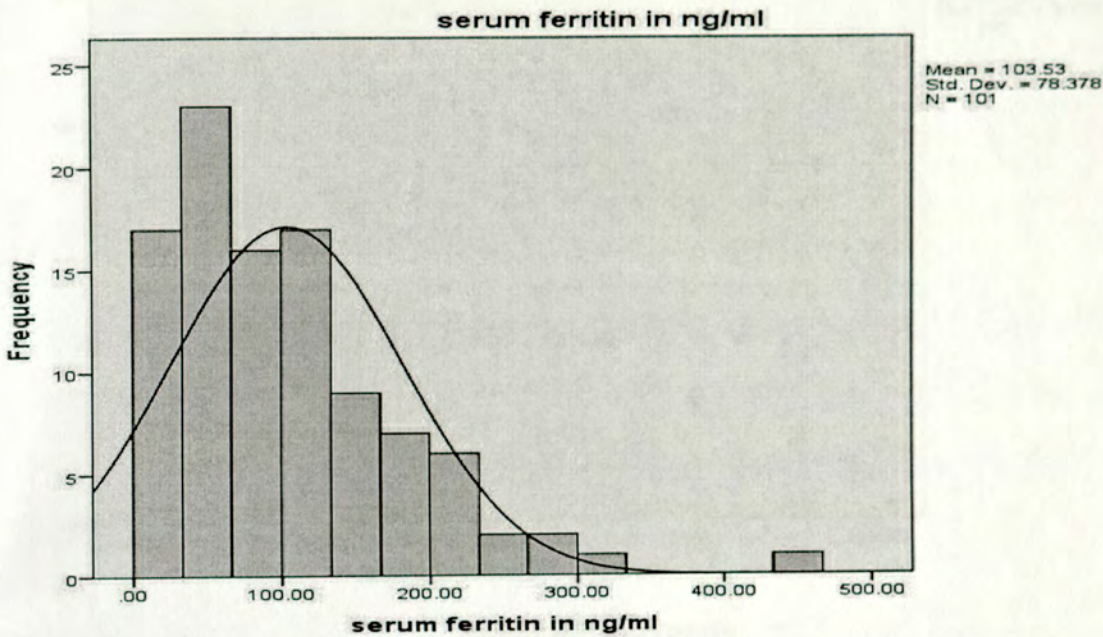
Male or Female			Sum of Squares	df	Mean Square	F	Sig.
Male	Red blood cell count/L X (10 ¹²)	Between Groups	.721	4	.180	1.613	.185
		Within Groups	5.811	52	.112		
		Total	6.532	56			
	Hemoglobin level in g/dl	Between Groups	5.204	4	1.301	2.094	.095
		Within Groups	32.305	52	.621		
		Total	37.510	56			
Female	Red blood cell count/L X (10 ¹²)	Between Groups	.692	4	.173	1.432	.242
		Within Groups	4.712	39	.121		
		Total	5.403	43			
	Hemoglobin level in g/dl	Between Groups	1.598	4	.399	.489	.743
		Within Groups	31.830	39	.816		
		Total	33.427	43			

Overall ANOVA for male and female with Hb & RBC count

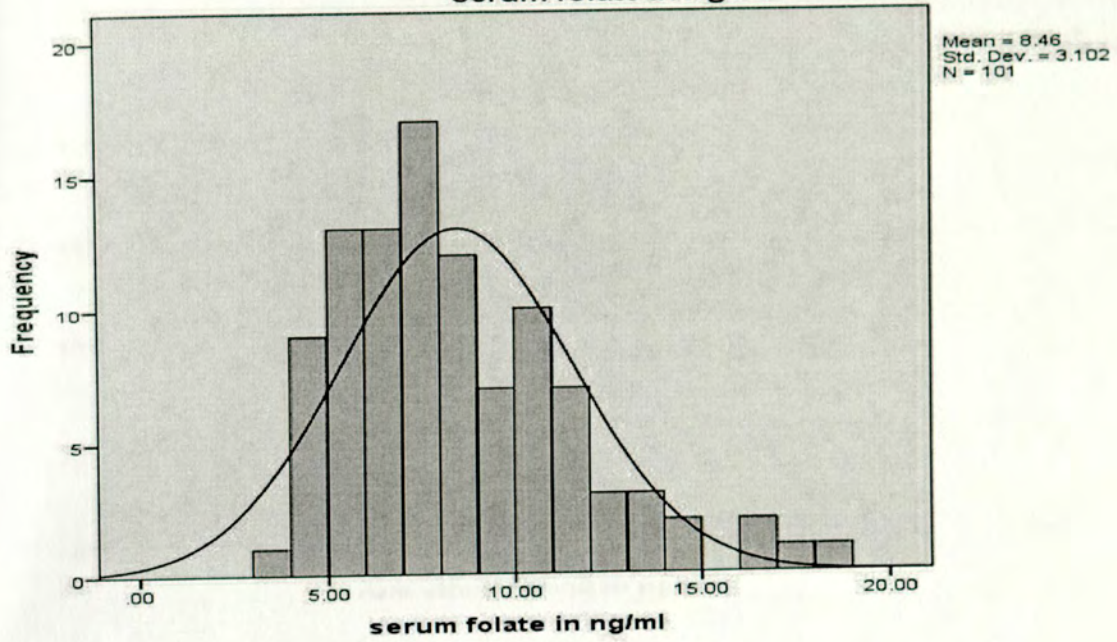
		Sum of Squares	df	Mean Square	F	Sig.
Red blood cell count/L X (10 ¹²)	Between Groups	8.79	1	8.79	72.9	.000
	Within Groups	11.9	99	.12		
	Total	20.73	100			
Hemoglobin level in g/dl	Between Groups	73.9	1	73.9	103.2	.000
	Within Groups	70.9	99	.72		
	Total	144.9	100			

P < 0.00, indicating there was statistically sig. difference observed of Hb & RBC count in male and female.

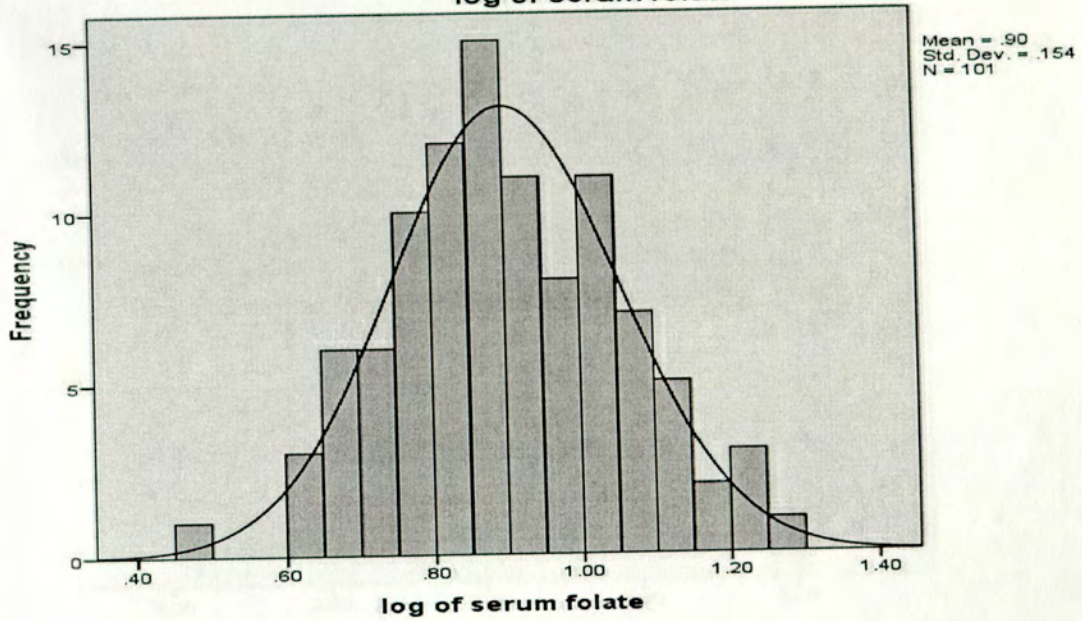
Normality curve for ferritin, folate and B₁₂ (before & after log transformation)



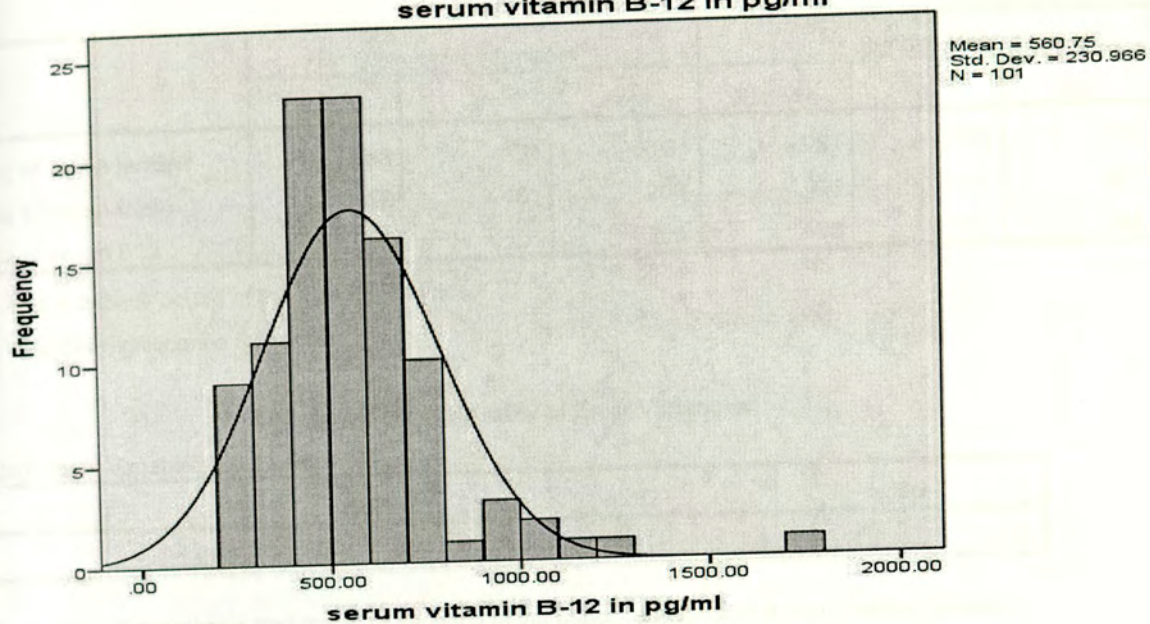
serum folate in ng/ml



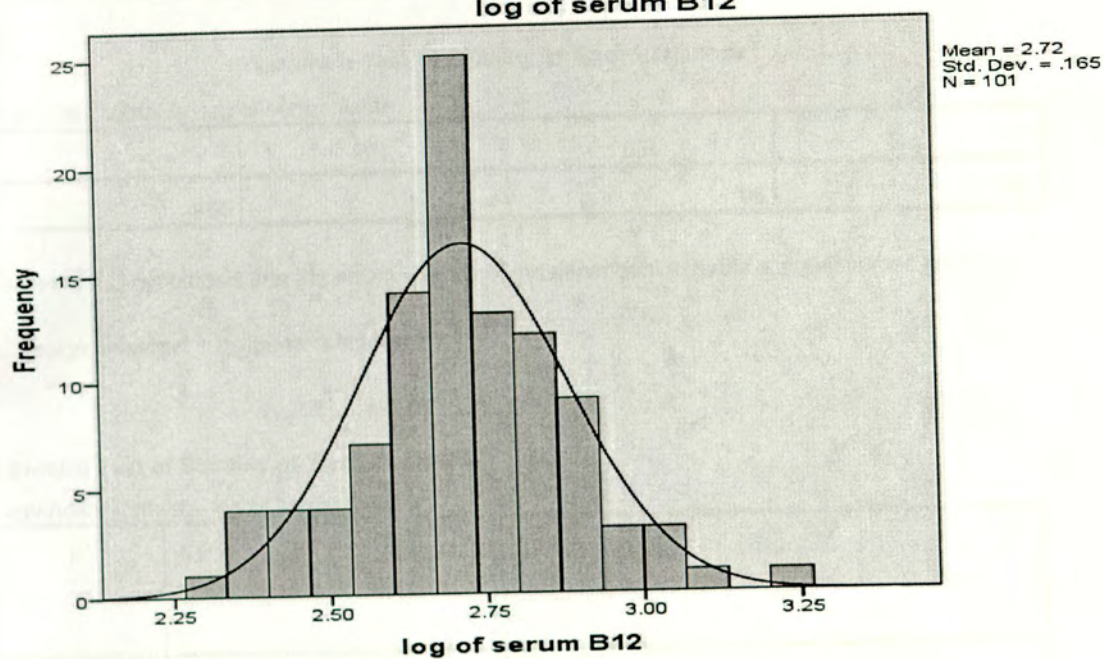
log of serum folate



serum vitamin B-12 in pg/ml



log of serum B12



Tests of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
log of serum ferritin	.093	101	.031	.981	101	.157
log of serum folate	.055	101	.200*	.994	101	.957
log of serum B12	.070	101	.200*	.986	101	.345

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Levene's Test of Equality of Error Variances^a

Dependent Variable: log of serum B12

F	df1	df2	Sig.
1.684	4	96	.160

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + distance category

Levene's Test of Equality of Error Variances^a

Dependent Variable: log of serum folate

F	df1	df2	Sig.
.465	4	96	.761

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + distance category

Levene's Test of Equality of Error Variances^a

Dependent Variable: log of serum ferritin

F	df1	Sig.
1.468	4	.218

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + distance category

Statistics

		identification number	log of serum folate	log of serum B12	log of serum ferritin
N	Valid	101	101	101	101
	Missing	0	0	0	0
Skewness		.042	.075	.074	-.312
Std. Error of Skewness		.240	.240	.240	.240
Kurtosis		-1.361	-.174	.646	-.420
Std. Error of Kurtosis		.476	.476	.476	.476

Statistics

		serum ferritin in ng/ml	serum vitamin B-12 in pg/ml	serum folate in ng/ml
N	Valid	57	57	57
	Missing	0	0	0
Skewness		1.249	2.053	1.087
Std. Error of Skewness		.316	.316	.316
Kurtosis		2.400	6.623	1.229
Std. Error of Kurtosis		.623	.623	.623