EFFECT OF PRE VERSUS POST EXERCISE IRON SUPPLEMENTATION AND AEROBIC EXERCISE ON HEMATOLOGICAL, ANTHROPOMETRIC AND PHYSIOLOGICAL PROFILE OF MEKELLE UNIVERSITY FEMALE STUDENTS, ETHIOPIA

M.Sc. THESIS

HAILU GEBRESLASSIE WELETATYOS

NOVEMBER 2019

HARAMAYA UNIVERSITY, HARAMAYA

Effect of Pre versus Post Exercise Iron Supplementation and Aerobic Exercise on Hematological, Anthropometric and Physiological Profile of Mekelle University Female Students, Ethiopia

A Thesis Submitted to the Department of Sport Science,

Postgraduate Program Directorate

HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirement for the Degree of

MASTERS OF SCIENCE IN SPORT NUTRITION

Hailu Gebreslassie Weletatyos

November 2019

Haramaya University, Haramaya

HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

As thesis research advisors, we here by certifying that we have read and evaluated the thesis entitled **“Effect of Pre versus Post Exercise Iron Supplementation and Aerobic Exercise on Hematological, Anthropometric and Physiological Profile of Mekelle University Female Students, Ethiopia”** prepared under our guidance by Hailu Gebreslassie Weletatyos and recommended that it is submitted as fulfilling the thesis requirement.

Negussie Bussa (BPharm, PhD)	________________	______________
Major Advisor	Signature	Date
K.V.Balamurugan (PhD)	________________	______________
Co-Advisor	Signature	Date

As members of the board of Examiners of the MSc. Thesis open defense Examination, we certify that we have read and evaluated the Thesis work prepared by Hailu Gebreslassie Weletatyos and examine the candidate. We recommend that the thesis be accepted as fulfilling the Thesis requirement for the degree of Master of Science in Sport Nutrition.

________________________	________________	______________
Name of Chair Man	Signature	Date
________________________	________________	______________
Name of Internal Examiner	Signature	Date
________________________	________________	______________
Name of External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of its final copy to the council of Graduate Studies (CGS) through the candidates department or school graduate committee (DGC or SGC).

DEDICATION

I dedicated this thesis manuscript to my families and parents. Without their tolerance, understanding, support and most of all love, the completion of the work would have been impossible.

STATEMENT OF THE AUTHOR

I the undersigned, declare that this is my original work and has never been presented for a degree in any other university and all the source of materials used for this thesis has been appropriately acknowledged. This thesis has been submitted in partial fulfillment of the requirement for M.Sc. degree at Haramaya University and is deposited at the university library to be made available to borrowers under rules of the library. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Brief quotations from this thesis are allowed without special permission provided that an accurate acknowledgment of source is made. Requests for permission for extended quotation form or duplicate of this manuscript in whole or in part may be granted by the head of the department/school or dean of the school of graduate studies. In all other instances, however, permission must be obtained from the author.

Name: Hailu Gebreslassie Weletatyos **Signature: ________________**

Place: Haramaya University, Ethiopia

Date of Submission: ______________

BIOGRAPHICAL SKETCH

The author, Hailu Gebreslassie, was born on June 27, 1983 E.C at Adwa town located in Central Zone of Tigray Regional State. He started his elementary education at Hahayle Complete Primary School and attended and completed secondary and preparatory school at Lidya Secondary School and Nigste- Saba Preparatory School respectively. Then, he joined Aksum University in the Department of Sport Science in 2002 E.C and graduated with Bachelor of Science in Sport Science in 2004 E.C. After completion of undergraduate program he was employed as a teacher in teaching physical education in 2005 E.C in Tselemti woreda, North Western Zone of Tigray Regional State. After serving for two years, he joined Mekelle University in Summer Program to study MEd in teaching physical education in 2007 E.C. In 2010 E.C, he was employed as Graduate Assistance in Mekelle University and he joined Haramaya University in the department of sport science to pursue his post graduate studies in Sport Nutrition.

ACKNOWLEDGMENT

First and foremost, I thank the Almighty God who gave me the health, stamina and strength to go through the rigor of graduate study and helped me to finish the research work, thesis write up and lead to successful completion of the study.

I would like to express my deepest gratitude to my advisors Dr. Negussie Bussa and Dr. K.V.Balamurugan for their unreserved effort in advising me to conduct my research and providing me with valuable information and materials. I really appriciated their critical evaluations of the thesis draft and immediate feedback with valuable comments as well as their valuable inputs to accomplish this study work in its present form.

My thank is also extended to ministry of education (MOE) for finance support to carry out this study. My great gratitude also goes to all Mekelle University female sport science students who agreed to participate in the study, Mekelle University staff members and Haramaya University staff members.

I would like to to give special thanks to the laboratory technicians at Mekelle University Ayder Comprensive Specialized Referral Hospital for their technical assistance during the collection of data from the blood test.

My great gratitude also goes to my familiy for their financial, moral and whole support during the ongoing of the research. I would also like to thank all my colleague and my friends who motivated me indeed during the research work.

ACRONYMS AND ABBREVIATIONS

ACSM	American College Of Sport Medicine
BMI	Body Mass Index
BP	Blood Pressure
Bpm	Beat Per Minute
CBC	Complete Blood Count
DBP	Diastolic Blood Pressure
EDTA	Ethylene Diamine Triacetic Acid
Fe	Iron
$Feso_4$	Ferrous Sulfate
H^0	Null Hypothesis
H^A	Alternative Hypothesis
Hb	Hemoglobin
HRERC	Health research ethics review committee
MU	Mekelle University
NCCLS	National Committee Clinical Laboratory Standards
POWSG	Post Workout Supplementation Group
PWSG	Pre Workout Supplementation Group
RBC	Red Blood Cells
RHR	Resting Heart Rate
SBP	Systolic Blood Pressure
WBC	White Blood Cells
WHO	World Health Organization

TABLE OF CONTENTS

TABLE OF CONTENT (Continued*)*

TABLE OF CONTENT (Continued*)*

LIST OF TABLES

Table **Page**

LIST OF TABLES IN THE APPENDIX

Appendix Table **Page**

LIST OF FIGURE IN THE APPENDIX

Figure **Page**

Effect of Pre versus Post Exercise Iron Supplementation and Aerobic Exercise on Hematological, Anthropometric and Physiological Profile of Mekelle University Female Students, Ethiopia

ABSTRACT

Iron is vital for a broad range of metabolic and physiological processes in the human body. This study was aimed to investigate the effect of iron supplementation with aerobic exercise on Hemoglobin, RBC, Hematocrit, Platelet and physiological parameters in both pre and post workout supplementation groups of Mekelle University female Sport Science students. A laboratory test was conducted at Mekelle University Ayder Comprehensive Specialized Referral Hospital to find out the effect of pre versus post workout iron supplementations on Hemoglobin, RBC, Hematocrit and Platelets and physiological parameters. Twenty (10 in each group) students were assigned using simple random sampling technique into pre and post workout supplementation groups. Three hundred mg of ferrous sulfate was used for both pre and post workout supplements groups. Blood samples were collected before and after supplementations to test the hematological and physiological parameters. Trainings were made for 3 months (3days/week, at 50-75% of MHR for 50-65min). The result showed that Hemoglobin significantly increased by 0.21 g/dl ($p<0.05$), RBC increased by 0.069×10^6 µl ($p<0.05$), Hematocrit by 0.045% ($p<0.05$), Platelet by 4.7×10^3($p<0.05$), SBP decreased by 2.2mmHg ($p<0.05$), DBP decreased by 1.4mmHg ($p<0.05$) and RHR reduced by 2.2b/min ($p<0.05$) in the pre workout supplementation group. In the post workout supplementation group Hemoglobin was increased by 0.82g/dl ($p<0.05$), RBC by 0.356 million/cm^3 ($p<0.05$), Hematocrit by 1.87% ($p<0.05$), Platelet by 8.2×10^3 ($p<0.05$), SBP reduced by 4.9mmHg ($p<0.05$), DBP reduced by 5mmHg ($p<0.05$) and RHR decreased by 4.3 b/min ($p<0.05$). It was concluded that, iron supplementation improved the hematological and physiological parameters in post aerobic exercise group than in pre aerobic exercise group.

***Key Words**: Iron Supplementation, Hematology and Physiological Parameters, Aerobic Exercise*

1. INTRODUCTION

1.1. Background of the Study

Iron is vital for a broad range of metabolic and physiological processes in the human body. Minerals are essential in muscle contraction, heart rhythm, nerve impulse conduction, oxygen transport, oxidative phosphorylation, enzyme activation, immune functions, anti oxidant activities, bone health and acid-base balance of the blood (Speich *et al*., 2001).

Nutritional supplements intended for consumption before and after training to improve performance are extremely popular among young and athletes. Training has been shown to increase muscle fiber size and strength in men and women with a concomitant increase in lipolysis and fat oxidation, it is not surprising that performance supplements are consumed in conjunction with resistance training in an attempt to balance the missing substances and improve athletic performance (Kerksick *et al*., 2017).

Such supplements are researched before either separately or in combination with others for their significance on different variables even if iron supplementation is conducted before but as the researcher viewed supplementations are taken before exercise in the previous study as different scholars discussed there is a significant difference between pre and post exercise supplements towards different variables. In a follow-up study by, Spillane *et al*. (2011) untrained men and investigated the same pre-supplements significant. Iron deficiency may reduce the ability of the athlete to train in the right intensity zones. Thus, the training process may be impaired and the resulting adaptation was also be negatively impacted (Randy, 2009).

1.2. Statement of the Problem

There is a strong body of evidence which suggests that exercise affects iron status, although some studies do not support this assertion. Iron plays a critical role in oxygen transport as it is necessary for the formation of Hb, the oxygen transport protein that is critical for aerobic capacity. Iron is also needed for the optimal functioning of many oxidative enzymes affecting the intracellular metabolism (i.e., the electron transport chain and oxidative phosphorylation pathways in the mitochondria). Not only prolonged aerobic exercise but, to some extent, short duration activities (i.e. sprints), may influence the above mechanisms (Deli *et al*., 2013).

Numerous studies have attempted to clarify the effectiveness of enhanced iron intake, either through diet or through supplement consumption, to restore iron status or to enhance physical performance. Yet, no valid conclusions have been drawn. The results of these studies are contradictory as some of them produced positive effects whereas others dispute such effects. An important factor in iron absorption seems to be the previous iron status of the individual. This means that, several iron parameters are seen to be ameliorated following iron supplementation in situations of iron deficiency, whereas this is not always the case for individuals with normal iron status (Crichton *et al.,* 2003).

But in most studies, iron supplementation was given before exercise and iron supplementation following workout is not examined yet. Additionally, such studies are concentrating on the effect of iron in hemoglobin, but it is crucial to see its' significance towards the whole complete blood count parameters (hematocrit, platelet and RBC). That is why the study was interested to examine the effect of Pre versus Post Exercise Iron Supplementation on physiological and hematological profile basically on hemoglobin, RBC count, hematocrit and platelets. Therefore the real gap of the reviewed studies was, they only concentrated in pre workout iron supplementations towards different variables in different subjects.

1.3. Hypothesis

H^A: Iron supplement and exercise would have a significant effect on hematological variables (hemoglobin, hematocrit, RBC and platelet) of Mekelle University sport sciences female students.

H^0: Iron supplement and exercise would not have a significant effect on hematological variables (hemoglobin, hematocrit, RBC and platelet) of Mekelle University sport sciences female students.

H^A: There would be a significant difference among pre exercise iron supplementation and post exercise iron supplementation on physiological (RHR and Blood pressure) variables of Mekelle University sport sciences female students.

H^0: There would not be a significant difference among pre exercise iron supplementation and post exercise iron supplementation on physiological (RHR and Blood pressure) variables of Mekelle University sport sciences female students.

H^A: There might be a significant difference among pre exercise iron supplementation and post exercise iron supplementation on hematological profile (hemoglobin, hematocrit, RBC and platelet) of Mekelle University sport sciences female students.

H^0: There may not be a significant difference among pre exercise iron supplementation and post exercise iron supplementation on hematological profile (hemoglobin, hematocrit, RBC and platelet) of Mekelle University sport sciences female students.

1.4. Scope of the Study

This study focused on the significant differences between Pre and Post Exercise Iron Supplementation and Aerobic Exercise on Hematological, Anthropometric and Physiological Profile of Mekelle University Female Students. It focused also on:

- Mekelle University sport science female students
- Under the age of 19-22 years old
- Physiological, Anthropometric and Hematological parameters using moderate intensity exercise (aerobic exercise for 50-60 minute: rope jumping, tempo run, running on treadmill and jogging)

1.5. Significance of the Study

The main significance of this study was to examine the significant difference between pre and post exercises of iron supplementation using Mekelle University female sport science students. In addition, the study was intended to find out the following importances:-

1. To know the timing of Iron supplementations for all community members.
2. To know the effect of Iron supplementation coupled with moderate intensity aerobic exercise on physiological and hematological variables.
3. To compare the effect of pre versus post supplementations of Iron with moderate intensity on physiological and hematological variables.

4. To know the extent of Iron supplementations deficiency and losses due to birth control and menstrual cycles.

1.6. Objective of the Study

1.6.1. General Objective

To compare the effect of pre versus post iron supplementations coupled with moderate intensity exercise on physiological and hematological profile of Mekelle University female sport sciences students.

1.6.2. Specific Objective

1. To compare the difference between pre and post exercise Iron supplementations with moderate intensity aerobic exercise on physiological variables (SBP, DBP and RHR)

2. To compare the difference between pre and post exercise Iron supplementations with moderate intensity aerobic exercise on hematological status (RBC, hematocrit, Platelet and Hb).

3. To determine the significance of Iron supplementation on physiological and hematological profile of Mekelle University female Sport Science students.

2. REVIEW OF LITERATURE

2.1. Iron

Iron is one of the minerals in the human body. It is one of the components of hemoglobin, the substance in red blood cells that helps blood carry oxygen throughout the body. If you do not have enough iron, your body cannot make hemoglobin, and you may develop anemia. This is known as iron-deficiency anemia, the most common type of anemia (Pena-Rosas and Juan, 2015).

Iron supplements, also known as iron salts and iron pills, are a number of iron formulations used to treat and prevent iron deficiency including iron deficiency anemia. For prevention they are only recommended in those with poor absorption, heavy menstrual periods, pregnancy, hemodialysis, or a diet low in iron. Prevention may also be used in low birth weight babies. They are taken by mouth, injection into a vein, or injection into a muscle. While benefits may be seen in days up to two months may be required until iron levels return to normal (Etxebarri, 2011).

Common side effects include constipation, abdominal pain, dark stools, and diarrhea. Other side effects, which may occur with excessive use, include iron overload and iron toxicity. Ferrous salts used as supplements by mouth include ferrous fumarate, ferrous gluconate, ferrous succinate, and ferrous sulfate. Injectable forms include iron dextran and iron sucrose. They work by providing the iron needed for making red blood cell (Clénin *et at*., 2015).

The body has considerable store of iron, mainly in the liver, bound up in a protein called ferritin. Ferritin serves as buffer against iron deficiency. About 50% of the total body iron is in hemoglobin, 25% in the other heme containing protein (mainly the cytochromes) in the cell of the body, and 25% is in liver ferritin. More over the cycling of iron is very efficient (Brissot *et al.*, 2004; Biruk *et al.*, 2014).

Iron is an indispensable factor for the formation of Hb, the protein responsible for oxygen transport from the respiratory organs to the peripheral tissues. Lack of adequate amounts of iron for the formation of Hb due to iron deficiency, can strongly affect physical work capacity, by reducing oxygen conveyance to the exercising muscles. Iron is also a vital component for the formation of myoglobin, the iron-storage protein within the muscle that regulates the diffusion of oxygen from the erythrocytes to the cytoplasm and on to the mitochondria where it is used as the final acceptor of electrons processed by the respiratory chains producing water and forming energy in the process (Deli *et al.*, 2013).

2.1.1. Iron Supplementation

One of the more common problems that occur with elite level athletes, especially females, involves an excessive loss of iron from training and an inadequate intake of iron in their diets resulting in something called “iron deficiency” and at the extreme level “anaemia”. Although iron supplementation in excess of normal iron stores does not enhance performance, a decrease of the iron stores has been found to decrease an athlete’s performance (Valeska *et al.*, 2018).

An iron deficiency no longer solely can affect aggressive performance, however it might also additionally affect training. Iron deficiency can also minimize the potential of the athlete to educate in the proper intensity zones. Thus, the training process may also be impaired and the resulting adaptation will also be negatively impacted (Valeska *et al.*, 2018).

This review will give a brief introduction of the function iron plays in the production of RBCs and Hb as nicely as other important chemical electricity reactions in the body. Then the paper will define the different stages of iron deficiency, how iron is lost from the body, and the more practical methods for changing iron to help maintain athletic performance (Randy, 2012).

Unfortunately, the body does not manufacture its own supply of iron and thus, the athlete must rely on their diet for their iron source. The body contains about 3-4 grams of iron at any one time. The largest component of iron in the body is found in Hb (60-70% or total iron) and myoglobin in the muscle tissues (10% of total iron). About 30% of the body’s total iron in a healthy young adult male (and about 10% in females) is stored in the form of ***ferritin*** (known as storage iron) located in such areas as the liver, bone marrow, and muscle. From these locations, iron is transported through the body via the blood by ***transferrin (***Randy, 2012) .

For example, ferrous sulfate (FeSO4) contains 20% elemental Fe; thus 100 mg of FeSO4 is equivalent to 20 mg of elemental Fe (close to the RDA of 18 mg/dl). In comparison, ferrous fumarate contains about 33% and ferrous gluconate 12% elemental Fe. The same factors that affect the bioavailability of dietary Fe will affect the absorption of supplemental iron. For example, the absorption of a low moderate dose of oral supplemental iron, such as 100 mg FeSO4, is most efficient when consumed daily with a source of vitamin C (e.g., citrus juice) and less so when consumed with polyphenolic compounds (e.g., coffee and tea) (Cassat *et al.*, 2013).

2.1.2. Post Workout Supplementation

Post-workout supplements are supplements that taken soon after exercise, in order to replace lost nutrients and jump-start the recovery. Such feeding practice contains a mix of amino acids, which are the building blocks of muscle, as well as other ingredients to assist in recovery, rehydration and replace the nutrients that are lost during intense exercise.

Most post workout recovery supplements come within the form of powders that are blended into drain, water, or mixed into shakes designed to be digested and absorbed quickly for right away work Antonio (2013). There is a possibility to find recovery supplements in the form of capsules or pills, which can be more convenient in some cases. The main ingredient in most post-workout supplements is branched chain amino acids (BCAAs). There are 3 amino acids that are considered branched chain amino acids; leucine, isoleucine, and valine. These three amino acids are absolutely essential to the building and preservation of healthy muscles (Troy, 2016).

Other common ingredients in post-workout products include electrolytes, such as whey protein isolates which help for hydration. Both of these ingredients can be taken on their own, but sometimes it is better to have them in one easy package (Troy, 2016).

Post-workout supplements are designed to help body recover after a strenuous workout. They typically contain a blend of ingredients including amino acids, BCAAs, protein, creatine, and more. Each formula is different, but they all aim to achieve a similar goal: faster, better post-workout recovery (Antonio, 2013).

2.1.3. Iron and Female Athletes

Randomized, placebo-controlled supplementation trials of iron-depleted female athletes have appeared that oral press supplementation in dosages of 100-mg FeSO4/dl (roughly 20 mg natural iron) improves iron status and may improve measures of physical execution. It is prescribed that female competitors most at chance of ID be screened at the starting of and amid the preparing season utilizing hemoglobin and serum ferritin, and suitable dietary and/or supplementation recommendations be made to those with compromised iron status (Della *et al*., 2015).

Data from a recent analysis of the National Health and Nutrition Examination Survey (NHANES) 2003 to 2006 show that over 50% of U.S. women were consuming dietary supplements, and 15% of supplement-consuming women aged 19 to 30 years reported taking one containing Fe. These data are comparable to that of female athletes showing that more than 50% use some type of supplement, and those containing Fe are among the most popular. Despite reported Fe supplement use, ID continues to be a problem and has many consequences relevant to athletes (Harvey, 2005).

2.1.4. Iron Metabolism

Iron isn't only critical for the formation of hemoglobin however moreover for different crucial components within the physique (eg., myoglobin, cytochromes, cytochrome oxidase, peroxidase, catalase), it is fundamental to recognize the potential by using which iron is utilized by within the body (Brissot *et al.*, 2004). The human body consists of 3-5mg of iron; most of it is coordinates within the lively center of the oxygen transport proteins hemoglobin (60-70%) and myoglobin (10%). A littler division (2%) is concerned in biological system such as electron transport in mitochondria, antioxidant protein and DNA replication. Approximately 30% of the complete body press in fitness grown-up men (and around 10% in ladies) is stored within the form of ferritin and hemosiderin within the liver, bone marrow and muscle and can be utilized for erythropoiesis when required.The transport of iron in plasma and greater vascular fluid is certain by means of transferring, an iron-binding plasma protein which offers iron to nearby tissues when needed (Brissot *et al.,* 2004).

If active women do require more Fe, it may most likely be for those engaged in weight-bearing activities, which result in greater losses via the gastrointestinal tract and foot-strike hemolysis. Future studies that screen active women for ID and monitor Fe status as well as dietary intake and menstrual status upon recruitment, during training, and throughout the competitive season (or military deployment)may help to substantiate the argument over increased Fe requirements for this population (ACSM, 2013).

2.1.5. Iron Overload

‘‘Iron overload’’ is a term used to describe increased total body Fe stores, with or without organ dysfunction. Consumption of excess Fe (through dietary or supplemental sources) is classified as secondary Fe overload, in contrast to a primary deformity within the control of Fe adjust (e.g., innate hemochromatosis (HHC). Long-term results of Fe overload are vague, but potential results include organ and cellular damage due to oxidative stress and the formation of free radicals (Della *et al*., 2015).

2.1.6. Exercise and Iron Absorption

The results of the study also suggested that exercise causes a blocking effect that inhibits the absorption of iron. Additionally, iron is lost through sweat during workouts, gastrointestinal blood, and menstrual blood in women (Bread, 2000).

The increased incidence of iron deficiency in female endurance athletes is thought to be the result of low dietary iron intake in this population, losses of iron in menstrual blood, sweat iron loss, and gastrointestinal blood loss. But there's another possible reason, which is what this study (from researchers at Florida State) is all about. In brief: exercise produces inflammation (as evidenced by elevated levels of cytokines in the blood); inflammation causes increased production of hepcidin (a hormone produced in the liver); and hepcidin reduces iron levels in the blood (Alex, 2012).

2.2. Blood

Blood is composed of cells and liquid, called plasma, in which they are suspended. The cells are erythrocytes, the leukocyte (white blood cells), and the platelets, which are not complete cells but cell fragments. More than 99% of blood cells are erythrocytes, which carry oxygen (windmair *et al*., 2001).

The hematocrit is the percentage of blood volume that is occupied by erythrocytes. The volume of blood in an average sized person (70kg; 154lbs) is approximately 5.5 L. if we take the hematocrit to be 45% (Maton *et al*., 1993), then

Erythrocyte volume= 0.45x 5.5 L= 2.5 L

Since the volume occupied by leukocytes and platelets is normally negligible, the plasma volume equals the difference between blood volume and erythrocyte volume; therefore, in our average person;

Plasma volume= 5.5 L- 2.5 L= 3.0 L (Widmair *et al*., 2001).

2.2.1. Erythrocytes

The major functions of erythrocytes are to carry oxygen taken in by the lungs and carbon dioxide produced by cells. Erythrocytes contains large amount of the protein hemoglobin with oxygen and to a lesser amount carbon dioxide reversibly combine. Oxygen binds to iron atoms in the hemoglobin molecules (Persons, 2003).

The direct control of erythrocytes production (erythropoiesis) is exerted primarily by hormone called erythropoietin, which secreted in to the blood mainly by particular group of hormone secreting connective tissue cells in the kidney (Maxwell, 2003). Erythropoietin acts on the bone marrow to stimulate the proliferation of erythrocyte progenitor cells and their differentiation in to mature erythrocyte. The total mass of red blood cells in the circulatory system is regulated within narrow limits, so that an adequate number of red blood cells are always available to provide sufficient transport of oxygen from the lungs to the tissues, yet the cells do not become numerous that they impede blood flow (Widmair *et al*., 2003).

2.2.2. Hemoglobin

Synthesis of Hemoglobin is present in blood at concentrations of 13.5 –18.0 g/dl in men and 11.5 –16.0 g/dl in women. Each erythrocyte contains around 200 –300 million molecules of Hemoglobin (Mabaera *et al*., 2008).

Hemoglobin has a complex quaternary structure, and the binding of oxygen occurs via a number of molecular interactions. The 'P50' is important in understanding changes in the position of the oxyhemoglobin dissociation curve when a single form of hemoglobin is considered and it can also be used to compare different forms (Mabaera *et al*., 2008).

Understanding the concepts of the Bohr and Haldane effects is essential in the understanding of gas transfer along the capillaries. Hemoglobin has other functions besides the transport of oxygen; carbon dioxide is carried as carbamino-hemoglobin and hydrogen ions formed in bicarbonate production are buffered by hemoglobin. Hemoglobin has an additional role in the metabolism of nitric oxide. Many abnormalities in hemoglobin exist and can be due to altered structure and production of globin chains, the binding of other ligands, or an abnormal haem–iron complex (Lumb *et al*., 2010).

2.2.3. Hematology and Aerobic Exercise

Hematology is medical term related to blood often begin with *hemo*- or *hemato*- (also spelled *haemo*- and *haemato*-) from the Greek word " *haima"* for "blood". Hematology is that medical specialty which concerns itself with the blood, and the generation of blood in the bone marrow. Hematology studies the red and white blood cells, their relative proportions and general cell health, and the diseases that are caused by imbalances between them, notably leukemia and anemia. Red blood cells carry oxygen from the lungs to the various parts of the body, and white blood cells fight infections. Both are necessary, but they have to be in the body in the right proportions or systems will break down (Dampier *et al.*, 2011).

It is a well-known fact that blood parameters vary in accordance with the stress, duration and type of exercise. There can be changes in the blood values during and after the intensive exercise caused by the differences such as the state of individual training, environmental factors and nutrition (Hürmüz *et al*., 2012).

2.3. Moderate Intensity Exercise

Health guidelines give a prescription for the kind and amount of exercise needed for the best health benefits. Moderate-intensity aerobic exercise is recommended for either 30 minutes a day for five days a week or a total of two hours and 30 minutes per week. This can be as simple as brisk walking. Find out which activities count as moderate exercise and how you can tell if you are in the right zone (Wendy, 2018).

A moderate level of activity noticeably increases your heart rate and breathing rate. You may sweat, but you are still able to carry on a conversation. You can talk, but you can't sing. You know you are exercising compared with doing a daily activity such as walking at an easy pace, but you are not huffing and puffing **(**Garber, 2011).

The Centers for Disease Control (CDC) defines the moderate-intensity heart rate zone as 50 percent to 70 percent of maximum heart rate. Your maximum heart rate varies by age and can be found by using a heart rate zone chart or calculator. To measure your heart rate, you can take your exercise pulse or use a heart rate monitor, heart rate app, or get a wrist-based heart rate from a fitness band or smart watch (Wendy, 2018).

Recently physiological and hematological changes due to different physical activities have received much attention from many corners of the society. Since some exercise sessions have higher intensity, especially at in-season tournaments, the circulatory system and hematological variables may undergo changes that in the long run considerably influencing performance and the result of the competition (Fereshteh *et al*., 2012). Moreover exercise training causes the body to adapt and improve performance over time. One area of ongoing interest is the adaptations that take place in physical fitness and the production of blood as a response to exercise (Dampier *et al*., 2011).

Most studies express that long-term regular exercises make positive contributions to human body. The most important effect of regular exercise is on physical and blood hematology.
When we analyze hematology, the effect of regular exercise on hematology is different. It is stated that these differences depend on the intensity, duration and frequency of exercise as well as physical and physiological conditions of subjects. Furthermore, the severity, duration and frequency of exercise should be well-organized to have similar positive influence on blood biochemistry (Hürmüz *et al*., 2012).

Kinds of Moderate Intensity Exercise

As Christopher (2015) suggested that, there are many activities which are generally counted as moderate-intensity exercise; here are some of the most common ones:

- ✓ Tennis (single)
- ✓ Tempo run
- ✓ Walking two miles in 30 minutes
- ✓ Biking five miles in 30 minutes
- ✓ Swimming laps for 20 minutes
- ✓ Running one and a half miles in 15 minutes
- ✓ Doing water aerobics for 30 minutes
- ✓ Playing volleyball for 45 minutes
- ✓ Playing pick-up basketball for 20 minutes
- ✓ Jumping rope for 15 minutes
- ✓ Walking stairs for 15 minutes

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Mekelle University, Mekelle, Tigray regional state, Ethiopia. Mekelle University is located around 780 kilometers (480 mi) in Mekelle town north of the Ethiopian capital city Addis Ababa. It is found on the latitude of 13.4936° N, and longitude of 39.4657° E with an elevation of 2,254 meters (7,395 ft) above sea level. Administratively, Mekelle is considered a special Zone, which is divided into seven sub-cities (Daniel, 2018).
The specific site of the experiment was Mekelle University main campus (Arid campus), and it was conducted from December 2018 to February 2019.

The Arid Zone Agricultural College was established at the University of Asmara, but was then moved to Agarfa southern Ethiopia in 1990. In 1993, the Arid Zone Agricultural College was moved to Mekelle and started with 42 students in 3 degree programs.

3.2. Study Materials

The study required the following equipment throughout the study in the field. The equipment were stopwatch, pen, paper, cone, treadmill, tennis ball, hematological measurements (CBC counting machine) for hemoglobin, RBC, hematocrit and platelets and sphygmomanometer for BP and RHR, anthropometric measuring calibrated weight and height measurement devices and 300 mg ferrous sulfate and drinking water. The hematological measurements were taken in Mekelle University Ayder Comprehensive Specialized Referral Hospital.

3.3. Operational Definition of Terms

Anthropometric measurement is referring to the measurement of the human individual. An early tool of physical anthropology, it has been used for identification, for the purposes of understanding human physical variation, in paleo-anthropology and in various attempts to correlate physical with racial and psychological traits (Hey, 2007).
Complete blood count (CBC) is a measure of the number of red blood cells, white blood cells, hematocrit and platelets in the blood (Neil, 2013).
Hematocrit is the percentage of red blood cells in the total blood volume (Carissa, 2017).

Hematology is the diagnosis, treatment, and prevention of diseases of the blood and bone marrow as well as the immunologic, hemostatic (blood clotting) and vascular systems (Ananya, 2018).

Hemoglobin is an iron-containing respiratory pigment of vertebrate red blood cells that consists of a globin composed of four subunits each of which is linked to a heme molecule, that functions in oxygen transport to the tissues after conversion to oxygenated form in the gills or lungs, and that assists in carbon dioxide transport back to the gills or lungs after surrender of its oxygen (Korin, 2018).

Iron deficiency is the state in which a body has not enough (or not qualitatively enough) iron to supply its eventual needs (Badal, 2015).

Iron supplementation is an act of Iron addition and also known as iron salts and iron pills, are a number of iron formulations used to treat and prevent iron deficiency including iron deficiency anemia (WHO, 2015).

Platelet: Platelets are tiny blood cells that help your body form clots to stop bleeding (Berry, 2017).

3.4. The Study Design

In this study an informal experimental design (before and after without control group) was used. From the total population of 40 female students under the department of sport sciences, 20 female sport science students were purposively selected for the study. These 20 female students were grouped in to two groups (PWSG and POWSG) by using simple randomization. Both pre exercise iron supplementation group (PWSG) and post exercise iron supplementation group (POWSG) were exposed to the same type of moderate intensity exercise. Both groups were also given 300mg of ferrous sulfate (60mg elemental iron), but PWSG took the supplementation 30 minute prior to exercise and the other POWSG was take the supplementation 30 minute following the exercise. Then, pre test and post tests data were tested using paired T-test at $p \leq 0.05$ for the selected variables.

3.5. Description of Population and Sampling Methods

The source of population was Mekelle University main campus (Arid) sport science students. There were 40 female students in Sport Science Department. Twenty (20) participants were purposively selected from 40 female students for the study. The students were completed medical history questionnaires and used invasive method of medical examination in order to determine the initial amount of blood hemoglobin, hematocrit, platelet and red blood cells. Anthropometric measurement (height, weight and body mass index), physiological measurements (resting heart rate (RHR), and blood pressure (BP)) were measured for pre and post workout supplementation groups.

3.6. Inclusion and Exclusion Criteria

Based on the medical analysis and the sampling techniques twenty female students between 19 – 22 years of Mekelle University sport sciences department who passed the medical history questionnaires were included in the study.

Students who had asthma, hypertension diabetes, chronic throat disease, stroke, osteon-articular diseases and those beyond the age of 19-22 were excluded from the study.

3.7. Type of Data and Data Collection Method

Data collected from the experimental study group through pre- test and post- test was used. The method of data available in the study is quantitative data collection method to collect through the appropriate selected physiological (RHR and BP) and hematological (laboratory) Hematocrit, platelet, HB and RBC count parameters.

3.8. Method and Procedure of Data Collection

The laboratory test was strictly administered and standardized in terms of administration, organization and implementation conditions. Up on entering the laboratory, pre intervention test was made hematological (hemoglobin, RBC, hematocrit and platelet), physiological (blood pressure and resting heart rate) and anthropometric variables (weight, height and BMI). Then the post test was made after the participants completed the intervention period of 12 weeks. The data was recorded by the researcher and laboratory technician. Test protocols that were used to measure the participants' anthropometric, physiological and hematological profile were as follows. The hematological measurements were taken in Mekelle University Ayder comprehensive specialized referral hospital.

3.8.1. Anthropometric Measurement

Body weight measurement: the person stands on scales, which was calibrate for accuracy using weight authenticated by a government department of weights and measures, with minimal movement with hands by their side. Shoes and excess clothing was removed. Kilo gram (Kg) was used as unit of measurement (Andreoli, 2016)

Body height measurement: the measurement was taken by calibrated height measurement digital scale by using meter (M) as unite measurement to determine total body height of the individual.

Body mass index: according to Philips 2006, wrote the body mass index is an estimate of fatness, calculated as body weight (kg) divided by the square of body height in meter (m^2). This was calculated as, BMI= weight÷ height (m^2).

3.8.2. Hematological Analysis

The laboratory test was focused on hematological indices. All hematological measurements were taken at 9:00 AM-11:00 AM two times at the beginning and end of the study. The result of the laboratory (hematological) tests was strictly measured and recorded by the researcher and certified laboratory technician of Mekelle University Ayder comprehensive specialized referral hospital.

Procedures of blood collection for hematological test were;

- ✓ First sterile, dry and plastic syringe of the capacity which required (5ml capacity with 20 gauge needle).
- ✓ Soft tubing tourniquet was applied to the upper of the client to enable the veins to be seen and felt. The tourniquets were applied too tightly or for longer than 2 minutes.

- ✓ Then the client was asked to make a tight fist which makes the vein more prominent. The puncture site was cleaned with 70% ethanol and allows drying.
- ✓ The vein puncture was made with the bevel of the needle directed upwards in the line of the vein. The plunger of the syringe was withdrawn steadily at the speed it's taking the vein to fill.
- ✓ 3ml of blood was collected. The needle and tourniquet was removed completely.
- ✓ Then the client was instructed to open his fist and immediately press the puncture site with a piece of cotton-wool, continue pressing on the puncture site until the bleeding was stopped.
- ✓ The needle was removed from the syringe and the EDTA-anticogulated tube was filled carefully with the collected blood.
- ✓ The blood in an EDTA- anticogulated tube was mixed immediately.
- ✓ Then using appropriate solution and dilution small amount of blood (3ml) was place in a glass counting chamber thereby, counting the cells amount.
- ✓ Using appropriate dilutes and dilutions the number of hemoglobin, hematocrit, platelet and RBC was estimated in this way.

3.8.3. Physiological Measurement

1. Blood Pressure Measurement

Blood pressure was measured with an instrument called a sphygmomanometer**.** First, a cuff was placed around your arm and inflated with a pump until the circulation is cut off. A small valve slowly deflates the cuff, and the doctor measuring blood pressure used a stethoscope, placed over the arm, then the result of SBP, DBP and RHR was recorded from digital sphygmomanometer. Blood pressure was measured in millimeters of mercury (mm Hg) and recorded with the systolic number first, followed by the diastolic number, and RHR at the bottom.(Chris, 2009).

2. Resting Heart Rate Test

Resting heart rate was measured by digital sphygmomanometer and again cheeked at carotid artery in the neck, then using the index and middle finger counted the number of beats for 10 seconds then multiplied the number by 6 then resting heart rate in beat per minute was calculated. To calculate target heart rate first maximum heart rate was calculated by subtracting the mean of the participants' age from 220 then multiplied the result by 50% and 75% to find the target zone since moderate intensity exercise is 50-75% of maximum heart rate (Paolo, 2009).

3.9. Supplementation and Exercise Training Protocol

The pre workout supplementation group was treated with 300 mg of ferrous sulfate per training day before moderate intensity (50%-75% HRmax) aerobic exercise for three days per week on Mondays, Wednesdays, and Fridays. The post workout supplementation group was also treated similarly with the same exercise of 30 minute following the workout for consecutive 12 weeks of three days/week. The aerobic exercise training programs were; Aerobic dance, jumping rope, jogging on treadmill, stationary bike and tempo run. They was performed 6:00 am-7:00 am throughout the study because to minimize the risk of inconvenient time of the students.

3.10. Data Quality Control

To ensure the data quality, all the laboratory test procedures including collection and handling of materials carried out in accordance with standard protocol. The reagents were checked for the expiry dates kept away from contamination and store at favorable temperatures. To insure general safety disposable gloves were used and universal precautions were followed at all times (NCCLS, 2002). Sample collections were made using sterile and disposable materials.

3.11. Method of Data Analysis

Data collected through anthropometric, physiological and hematological parameters before and after iron supplementation with moderate intensity aerobic exercise training was analyzed by using SPSS version 21 statistical soft ware packages. Data analyses included descriptive statistics such as mean and standard deviations and tested at $p \leq 0.05$.

3.12. Research Ethics

The study was conducted under the auspices of Mekelle University research, policies and code of conduct governing research activities and ethical issues. The study was obtained approval from the Health research ethics review committee (HRERC) of Mekelle University Ayder comprehensive specialized referral hospital. The ethical clearance authorized by the concerned authority was submitted to the participants, department, school of graduate studies and other concerned bodies.

4. RESULTS AND DISCUSSIONS

4.1. Demographic Characteristics of the Study Participants

Twenty female sport science students were selected for the study. All selected female sport science students (ten pre exercise supplement group and ten post exercise supplement group) completed the supplement and training periods.

4.2. Anthropometric Test Results

Table 1: Mean effect of anthropometric test of PWSG and POWSG before and after Fe supplements

Groups	Parameters	Before supplementation	After supplementation	MD	Sig
Pre exercise supplement group	Weight (kg)	51.55±5.65	51.4±5.89	0.15	0.839
	Height (m)	1.59±0.079[a]	1.59±0.079[a]	0.00	1.000
	BMI(kg/m^2)	20.25±2.26	20.18±2.17	0.63	0.830
Post exercise supplement group	Weight (kg)	52.85±2.21	52.2±2.57	0.65	0.361
	Height (m)	1.61±0.043[a]	1.61±0.043[a]	0.00	1.000
	BMI(kg/m^2)	20.42±1.41	20.18±1.79	0.24	0.392

a correlation and t cannot be computed because the standard error of the difference is 0.

Mean ±SD in the same column in each parameter are significantly different ($p<0.05$), Height (m)=height in meter, Weight(kg)= weight in kilogram, BMI(kg/m^2)=body mass index in kilogram per square meter

Table 1 show that the mean value of anthropometric characteristics in post workout and pre workout supplementation group before and after iron supplementation and moderate intensity exercise. As indicated in table 1 the mean value of participants' height was 1.59 m in PWSG and 1.61m in POWSG. The mean value of participants' weight of PWSG was 51.55 kg before supplementation and decreased to 51.4 kg after training and supplementation, the mean value of participants weight in POWSG was 52.85 kg before supplementation and aerobic training and decreased to 52.2 kg after training and supplementation and decreased by 0.65 kg.

Significant mean difference in the BMI within each test between the groups was not observed as indicated in table 1. From the table the mean BMI distribution of POWSG was 20.42 kg/m^2 before supplementation and aerobic exercise and after supplement and aerobic the BMI of POWSG was goes to 20.18kg/m^2.

Whereas for PWSG the mean value of BMI distribution was 20.25 kg/m^2 before supplementation and training and 20.18 kg/m^2 after supplementation so as BMI decreased by 0.24 kg/m^2 in POWSG while in PWSG decreased by 0.63 kg/m.

Biruk (2014) stated that, moderate intensity and iron supplementation slightly decrease BMI by 0.20kg/m^2 but the participants have not shown that much significant effect because p value was 0.778 which is >0.05.

4.3. Hematological Test Results

Table 2: Mean effect of hematological test of PWSG and POWSG before and after Fe supplements

Groups	**Parameters**	**Before supplementation**	**After supplementation**	**MD**	**Sig**
Pre exercise supplement group	Hemoglobin	14.49±1.503	14.7260±1.4	0.21*	0.004
	RBC	4.88±0.52	4.95±0.52	0.06*	0.006
	Hematocrit	42.6±3.65	43.1±3.58	0.04*	0.003
	Platelet	289.4±79.6	294.1±79.38	-4.70*	0.006
Post exercise supplement group	Hemoglobin	13.99±1.134	14.81±0.91	0.82*	0.000
	RBC	4.3±0.57	4.66±0.58	0.35*	0.002
	Hematocrit	40.9±2.66	42.8±2.66	1.87*	0.003
	Platelet	309±52.32	317±50.88	-8.2*	0.000

Mean ± SD in the same column in each parameter with different superscripts are significantly different (p<0.05) md=mean difference, hemoglobin in g/dl(gram/deciliter), RBC in μl hematocrit in percentage, platelets in μl .*

There was a significant mean effect of hemoglobin in both pre workout supplement group (PWSG) and post workout supplement group (POWSG). 0.21g/dl mean difference was seen in PWSG that was significant at 0.004 p value where as 0.82 g/dl mean difference was seen in POWSG that was significant at p value 0.000. Therefore the rate of significant was very high in POWSG than of PWSG. And this result was agreed with Researchers at Florida State published a study in the International Journal of Sport Nutrition and Exercise Metabolism that suggests exercise increases inflammation, which increases the production of a hormone called hepcidin, which then reduces iron levels in the blood (Alex, 2012). Therefore the researcher accepted the alternative hypothesis.

There was also a significant mean effect in RBC in both groups.0.06×10^6 µl mean difference was seen in PESG which is significant at p=0.006 and 0.35×10^6 µl mean difference was seen in POWSG at p value 0.002.then the POWSG was more significant than of PWSG.

This result was in agreement with Achebe (2017). Red blood cells carry hemoglobin, an iron-rich protein that attaches to oxygen in the lungs and carries it to tissues throughout the body. Anemia occurs when you do not have enough red blood cells or when your red blood cells do not function properly.

As indicated on the above table 0.04×10^3 µl mean difference was seen in PWSG hematocrit which is significant at 0.003 where as the POWSG mean difference goes to 1.87 that was significant at p=0.003. There was no any significant difference between groups in hematocrit but there was a significant improvement with in groups on the level of hematocrit.

Fujii *et al.* (2014) were concluded that resistance and aerobic exercise decreases iron absorption, whereas the whole body iron content is not affected, and an increase in iron recycling in the body seems to be responsible for this effect.

As the above table indicates that, the number of platelets was increased by mean difference 4.7 which was positively significant at p=0.006 in PWSG where as the platelets in POWSG was increased by mean difference 8.2 at p=0.000. Therefore the increment of platelets was better in POWSG than of PWSG by comparing the level of significant in both groups.

The result was agreed with wardiana . *et al.* (2018) argued that, increase the sensitivity of bone marrow cells to Epo (erythropoietin) , resulting in increased red blood cell production. Levels of other types of blood cells (white blood cells and platelets) were also often increased in this condition.

According to Assefa (2013), the short duration high intensity group had increased from 263.3 x 10^3 μl to 308.8 x 10^3 μl. The mean difference of platelets count for that group was improved by 45.5μl. On the other hand, the mean value of platelets count for long duration low intensity increased from 297 x 10^3 μl to 312.9 x 10^3 μl.

The result of the study was also highly agreed with the result of Özyener and colleagues, They indicated that, short-term and maximal intensity exercise has an effect on platelet parameters. While Ersöz, indicated that sub maximal exercises do not cause a significant increase in platelet numbers. It is indicated that the difference stems come from intensity and duration of the exercise.Therefore the researcher accepted the alternative hypothesis.

4.4. Physiological Parameters Test Results

Table 3: Mean effect of physiological test of PWSG and POWSG before and after Fe supplements

Groups	**Parameters**	**Before supplementation**	**After supplementation**	**MD**	**Sig**
Pre exercise supplement group	SBP	110±9.45	107±9.6	2.200000	0.000
	DBP	71±8.98	69.6±8.72	1.40000	.025
	RHR	70.9±5.38	68.7±5.59	2.20000	.003
Post exercise supplement group	SBP	110±8.56	105±7.69	4.90000	.005
	DBP	83.2±11.68	78.2±10.15	5.00000	.001
	RHR	75.1±9.58	70.8±8.49	4.30000	.000

Mean ±SD in the same columns in each parameter, SBP(mmHg)=systolic blood pressure in millimeter mercury, DBP(mmHg)=diastolic blood pressure in millimeter mercury, RHR(b/m)=resting heart rate in beat per minute.

Table 3, shows that the mean value of physiological parameters in pre and post workout supplements groups.

Post exercise supplement group mean value of blood pressure SBP (systolic blood pressure) and DBP(diastolic blood pressure)was 110/83.2 mmHg before supplement and moderate intensity aerobic training; after supplement and moderate intensity training blood pressure decreased to 105/78.2 mmHg totally the mean difference was decreased by 4.9 (5.4%)mmHg SBP and 5 (4.1%) mmHg in DBP throughout the study period.

And also as indicated in table 3, pre workout supplement group mean value of blood pressure (SBP (systolic blood pressure) and DBP(diastolic blood pressure) was 110/71 mmHg before supplement and moderate intensity aerobic training; after supplement and moderate intensity training blood pressure decreased to 107/69.6mmHg. The total mean difference was decreased by 2.20mmHg in SBP and 1.4mmHg in DBP throughout the study period.

However, statistical significant mean drops of blood pressure (SBP and DBP) were observed in the mean value of post workout supplement group which was significant at($p<0.05$), additionally the blood pressure (SBP and DBP) were shown significant value in the pre workout supplement group at ($p<0.05$). but the rate of significant was much better in POWSG which is $P=0.001$ in DBP and the same improvement observed in both groups SBP. So the researcher accepted the alternative hypothesis.

The result indicated that effective physiological changes have been observed in both post workout supplement group and pre workout supplement group. Loana *et al.* (2008) was in agreement with this result the combination of aerobic exercise and non hem iron decreased both DBP and SBP.

As indicated in the table 3 the significant mean difference in the RHR (resting heart rate) within each test between the groups was observed. From this table the mean RHR distribution of post workout supplementation group of female sport science students was 75.1 b/min before supplementation and moderate intensity exercise training; after supplementation and moderate intensity aerobic training mean RHR of this group was decreased to 70.8b/min at the end of the study period, totally the mean of RHR was decreased by 4.3b/min throughout the study period.

However the mean RHR distribution of pre workout supplementation group of female sport science students of Mekelle University was 70.9 b/min before supplementation with moderate intensity aerobic training, after supplementation and moderate training the mean value of RHR was decreased to 68.7 throughout the training period with in the normal range of adults from 60 to 100 b/min.

The result was in agreement with Edward (2010). They stated that, iron supplementation increases the amount of hemoglobin that carries oxygen and the increment hemoglobin amount leads transport enough oxygen to the working muscles and decreases RHR. Statistically significant mean difference have been observed in post workout and pre workout supplementation group between the measurements at ($p<0.05$).

From this result post workout supplementation group was revealed better mean RHR decrement throughout the study period, while statistically significant mean were observed in the PWSG. But the rate of significant in POWSG (0.000) was better than the significant on PWSG (0.003). Alex (2012) argued that, exercise have an absorption blocking effect by creating inflammation so that iron cannot be easily absorbed from the empty stomach.

5. SUMMERY, CONCLUSION AND RECOMMENDATION

5.1. Summery

This study examined and investigated effect of iron supplementation on increasing blood level (CBC) complete blood count during the supplement taking before workout and taking after workout with moderate intensity aerobic training program among Mekelle university female sport science students. To achieve the purpose of the study, 20 students were selected from Mekelle University. The selected subject's age group ranged from 19-22 years. The subjects were divided into two groups of ten using simple random sampling technique. Both groups were experimental, divided pre workout supplementation group and post workout supplementation. Test was taken twice pre and post test and results were analyzed by paired sample t-test to find out the significant difference among groups and between groups.

The experimental period was 12 weeks. The selected variables were hematological (hemoglobin, RBC, hematocrit and platelet), physiological (SBP, DBP and RHR) and anthropometric (weight, height and body mass index). Major finding of this investigation was better increment of hematological (hemoglobin, RBC, hematocrit and platelet) in post workout supplementation group than of pre workout supplementation group.

In hematological and physiological parameters better significant were observed in the post workout supplement group than of pre workout supplementation group. No significant difference was seen in anthropometric parameters (height, weight and BMI) but a little improvement was observed.

5.2. Conclusion

Based on the major findings of the study, to elevate hemoglobin, RBC, hematocrit and platelet, to improve physiological (systolic blood pressure, diastolic blood pressure and RHR) performance of Mekelle University female sport science students the following conclusion ware drown.

- ✓ Post workout supplementation group developed better improvement in Hemoglobin, RBC, Hematocrit and platelet level than pre workout supplementation group of MU female sport science students.
- ✓ Iron supplementation with moderate intensity aerobic exercise has positive significant effect on hematological and physiological parameters.
- ✓ Post workout supplementation group highly improved physiological (DBP and RHR) than pre workout supplementation group of MU female sport science students.
- ✓ Iron supplement does not shown significant value on both POWSG and PWSG in anthropometric parameters (height, weight and BMI)

5.3. Recommendation

By considering the major findings and conclusions of the study, the following recommendations were made.

- Females should take iron supplementation in addition to their daily meal especially if they were more exposed to exercise because females loss blood due to exercise, delivery and menstrual cycle.
- The sport professionals, sport dietitians, and athletes were highly expected to understand the perfect timing of iron supplementations.
- Iron supplementation must be taken after workout in order to gain the expected change in the expected variables because exercise has a blocking effect in the absorption of the supplement and good for fast recovery.
- Further researches on the role of iron for the development of such parameters like physiological, echocardiography, hematological and performance parameters change is important to conduct.

6. REFERENCES

Achebe, M.M. and Gafter-Gvili, A. 2017. How I treat anemia in pregnancy: iron, cobalamin, and folate. *Blood*, 129(8): 940-949.

Alayash, A.I. 2004. Oxygen therapeutics: medical sciences and sport exercise; national review of drug discover 3:152.

Alex picca., Martone, A.M., Marzetti, E., Calvani, R., Tosato, M., Santoro, L., Di Giorgio, A., Nesci, A., Sisto, A., Santoliquido, A. and Landi, F., 2017. Exercise and protein intake: a synergistic approach against sarcopenia. *BioMed research international, 2017*.

American College of Sports Medicine. 2013. Iron Supplementation of Active Women: Response of Body Iron Status, 12(N4): 236-239.

American Health Assosiesion. 2014 . Understanding Blood Pressure readings.

Antonio, J. and Ciccone, V. 2013. The effects of pre versus post workout supplementation of creatine monohydrate on body composition and strength. *Journal of the International Society of Sports Nutrition*, 10(1): 36.

Assefa, F., 2013. *Effect of Combined Aerobic and Resistance Exercises on Blood Glucose Level and Risk Factors in Male Type II Diabetic Patients Residing in Haramaya University Main Campus and its Surrounding* (Doctoral dissertation, Haramaya University).

Beard, J. 2007. Indicators of the iron status of populations: free erythrocyte protoporphyrin and zinc protoporphyrin; serum and plasma iron, total iron binding capacity and transferrin saturation; and serum transferrin receptor. Assessing the iron status of populations. 2nd ed. Geneva: World Health Organization.

Beard, J.L. 1998. Weekly iron intervention: the case for intermittent iron supplementation. *The American journal of clinical nutrition*, 68(2): 209-212.

Biruk, A., Molla, D. and Sirak, H. 2014. Effects of iron supplement and exercise intensities on the levels of blood hemoglobin among Jimma University male soccer players. *Turkish Journal of Sport and Exercise*, 16(1): 90-94.

Brissot, P., Troadec, M.B. and Loreal, O. 2004. The clinical relevance of new insights in iron transport and metabolism. *Current hematology reports,* 3(2): 107-115.

Brown, J.K., Byers, T., Doyle, C., Courneya, K.S., Demark-Wahnefried, W., Kushi, L.H., McTiernan, A., Rock, C.L., Aziz, N., Bloch, A.S. and Eldridge, B. 2003. Nutrition and physical activity during and after cancer treatment: an American Cancer Society guide for informed choices. *CA: a cancer journal for clinicians,* 53(5): 268-291.

Cassat, J.E. and Skaar, E.P. 2013. Iron in infection and immunity. *Cell host & microbe,* 13(5): 509-519.

Chaudhary, K. and Sukhwal, I. 2016. Impact of nutrition education programme on college going girls engaged in sports activity. *Age,* 18(19): 20-21.

Chimowitz, M.I., Lynn, M.J., Derdeyn, C.P., Turan, T.N., Fiorella, D., Lane, B.F., Janis, L.S., Lutsep, H.L., Barnwell, S.L., Waters, M.F. and Hoh, B.L. 2011. Stenting versus aggressive medical therapy for intracranial arterial stenosis. *New England Journal of Medicine,* 365(11): 993-1003.

Christopher, T. 2015. what does moderate intensity mean. Bone, muscle, and joint team.*BMC Muskuloskeletal disorder*. 16(1): 258

Clénin, G., Cordes, M., Huber, A., Noack, P., Kriemler, S., Schumacher, YO., Scales, J. 2015. "Iron deficiency in sports – definition, influence on performance and therapy".

Crichton, R.R. and Ward, R.J. 2003. An overview of iron metabolism: molecular and cellular criteria for the selection of iron chelators. *Current medicinal chemistry,* 10(12): 997-1004.

Dampier, C., LeBeau, P., Rhee, S., Lieff, S., Kesler, K., Ballas, S., Rogers, Z., Wang, W. and Comprehensive Sickle Cell Centers (CSCC) Clinical Trial Consortium (CTC) Site Investigators. 2011. Health‐related quality of life in adults with sickle cell disease (SCD): A report from the comprehensive sickle cell centers clinical trial consortium. *American journal of hematology,* 86(2): 203-205.

Deakin, V.I.C.K.I. 2006. Iron depletion in athletes. *Clinical sports nutrition, 263-312.*

Deli, C.K., Fatouros, I.G., Koutedakis, Y. and Jamurtas, A.Z. 2013. Iron supplementation and physical performance. In Current Issues in Sports and Exercise Medicine. IntechOpen. 18(4): 134-136.

DellaValle, D.M. and Haas, J.D. 2012. Iron status is associated with endurance performance and training in female rowers. *Medicine and science in sports and exercise,* 44(8):1552-1559.

DellaValle, D.M. 2013. Iron supplementation for female athletes: effects on iron status and performance outcomes. *Current sports medicine reports*, 12(4): 234-239.

Della,V., D., Rousseau, D., Wadsten, S. and Haas, J., 2015. Examining the Relationships Between Dietary Intake, Iron Status, and Physical Performance in Female Collegiate Rowers. *The FASEB Journal*, 29(1): 733-17.

DeRuisseau, K.C., Cheuvront, S.N., Haymes, E.M. and Sharp, R.G. 2002. Sweat iron and zinc losses during prolonged exercise. *International journal of sport nutrition and exercise metabolism*, 12(4): 428-437.

Edward R. 2010. Normal resting heart rate: http://www.mayoclinic.com: accessed on April 12, 2019.

Etxebarria, N. 2011. Biochemistry of Exercise HEMATOLOGY; Hematology or haematology?, 1-39.

Fereshteh, S., A. Seyed, M. Yahya and Kandi. 2012. The Effect of a Maximal Aerobic Exercise Session in the Morning and Afternoon on Certain Hematological Factors in Young Athletes. *Scholars Research Library, Tehran, Iran,* 2703-2707.

Fujii, T., Matsuo, T., Okamura, K. 2014. Effect of resistance exercise on iron observation

Garber, C.E., Blissmer, B., Deschenes, M.R., Franklin, B.A., Lamonte, M.J., Lee, I.M., Nieman, D.C. and Swain, D.P. 2011. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for exercise. *Medicine and science in sports and exercise,* 43(7): 1334 1359.

Gleeson, M., Nieman, D.C. and Pedersen, B.K. 2004. Exercise, nutrition and immune function. *Journal of sports sciences*, 22(1): 115-125.

Grimstad, T., Bjørndal, B., Cacabelos, D., Aasprong, O.G., Janssen, E.A., Omdal, R., Svardal, A., Hausken, T., Bohov, P., Portero-Otin, M. and Pamplona, R. 2012. Dietary supplementation of krill oil attenuates inflammation and oxidative stress in experimental ulcerative colitis in rats. *Scandinavian journal of gastroenterology*, 47(1): 49-58.

Gropper, S.S., Blessing, D., Dunham, K. and Barksdale, J.M. 2006. Iron status of female collegiate athletes involved in different sports. *Biological trace element research*, 109(1): 1-13.

Hallberg, L. and Hulthén, L. 2003. High serum ferritin is not identical to high iron stores. *The American journal of clinical nutrition*, 78(6): 1225-1226.

Harvey, L.J., Armah, C.N., Dainty, J.R., Foxall, R.J., Lewis, D.J., Langford, N.J. and Fairweather-Tait, S.J. 2005. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *British Journal of Nutrition*, 94(4): 557-564.

Haymes, E. 2006. *Sort nutrition, vitamins and trace elements.* New York: CRT/taylor and Francis. 203-16.

Hill, M.R., Iron Supplementation: What is it and how to use it to overcome iron deficiency.

Hürmüz, K. T., Ali, Ö., Ahmet, S., Recep, G., Kadir. and E. Murat. 2012. The effect of acute exercises on blood hematological parameters in handball players. *African Journal of Microbiology Research*, 6(9): 2027-2032.

Hurrell, R. and Egli, I. 2010. Iron bioavailability and dietary reference values. *The American journal of clinical nutrition*, 91(5): 1461S-1467S.

Ioanna Tzoulaki,et al.; July 2008. “British Medical Journal”; Relation of Iron and Red Meat Intake to Blood Pressure: Cross Sectional Epidemiological Study;

Kerksick, C.M., Arent, S., Schoenfeld, B.J., Stout, J.R., Campbell, B., Wilborn, C.D., Taylor, L., Kalman, D., Smith-Ryan, A.E., Kreider, R.B. and Willoughby, D. 2017.

International society of sports nutrition position stand: nutrient timing. *Journal of the International Society of Sports Nutrition*, 14(1): 33.

Koehler, K., Braun, H., Achtzehn, S., Hildebrand, U., Predel, H.G., Mester, J. and Schänzer, W. 2012. Iron status in elite young athletes: gender-dependent influences of diet and exercise. *European journal of applied physiology*, 112(2): 513-523.

Lappin, T. 2003. The cellular biology of erythropoietin receptors. *The Oncologist Supplement*, 8(1): 15-18.

Laughlin, M.J., Barker, J., Bambach, B., Koc, O.N., Rizzieri, D.A., Wagner, J.E., Gerson,S.L., Lazarus, H.M., Cairo, M., Stevens, C.E. and Rubinstein, P. 2001. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *New England Journal of Medicine*, 344(24): 1815-1822.

Lumb, A.B. 2016. *Nunn's applied respiratory physiology eBook*. Elsevier Health Sciences.

Lynch, S. 2004. Indicators of the iron status of populations: red blood cell parameters. Assessing the Iron Status of populations: including litrature review:report of joint world health organization/center of disease control and prevention technical consultation on the assesment of iron status at the population level,Geneva, switzerland. 6-8

Mabaera, R., West, R.J., Conine, S.J., Macari, E.R., Boyd, C.D., Engman, C.A. and Lowrey, C.H. 2008. A cell stress signaling model of fetal hemoglobin induction: what doesn't kill red blood cells may make them stronger. *Experimental hematology*, 36(9): 1057 1072.

Martin, L., Lambeth, A. and Scott, D. 2006. Nutritional practices of national female soccer players: Analysis and recommendations. *Journal of sports science & medicine*, 5(1): 130.

Maxwell, P. 2003. HIF-1: an oxygen response system with special relevance to the kidney. *Journal of the American Society of Nephrology*, 14(11): 2712-2722.

National Committee for Clinical Laboratory Standard (NCCS). 2002. Performance standards for hematological susceptibility test. Approved standard M29-A.

Noseworthy, J. 2006. *Neurological therapeutics: principles and practice.* CRC Press. (CRC Press, Boca Raton (Florida), USA), 23(3):214-218.

Peña-Rosas, J.P., De-Regil, L.M., Dowswell, T. and Viteri, F.E. 2012. Intermittent oral iron supplementation during pregnancy. *The Cochrane database of systematic reviews,* 7, p.CD009997.

Persons, D.A. 2003. Update on gene therapy for hemoglobin disorders. Current opinion in molecular therapeutics, 5(5): 508-516.

Philip, A.W. 2006. *How fats works.* Harvard university, Press Cambridge, London, England. *Jornal of vascular surgery*, 50(1): 54-60

Randy, E. 2012. Perennial questions: on fatigue, on iron, and on anemia. *Current sports medicine reports,* 11(6): 274-275.

Robin, M. (n.d). *Blood Pressure Readings Explained*. Medically reviewed by Judith M., MD., 2018.

Shelmadine, B., Cooke, M., Buford, T., Hudson, G., Redd, L., Leutholtz, B. and Willoughby, D.S. 2009. Effects of 28 days of resistance exercise and consuming a commercially available pre-workout supplement, NO-Shotgun, on body composition, muscle strength and mass, markers of satellite cell activation, and clinical safety markers in males. *Journal of the International Society of Sports Nutrition,* 6(1): 16.

Speich, M., Pineau, A. and Ballereau, F. 2001. Minerals, trace elements and related biological variables in athletes and during physical activity.*Clinica Chimica Acta,* 312(1-2): 1-11

Spillane, M., Schwarz, N., Leddy, S., Correa, T., Minter, M., Longoria, V. and Willoughby, D.S. 2011. Effects of 28 days of resistance exercise while consuming commercially available pre-and post-workout supplements, NO-Shotgun and NO-Synthesize on body composition, muscle strength and mass, markers of protein synthesis, and clinical safety markers in males. *Nutrition & metabolism,* 8(1): 78.

Sureira, T.M., Amancio, O.S. and Braga, J.A.P. 2012. Influence of artistic gymnastics on iron nutritional status and exercise-induced hemolysis in female athletes. *International journalof sport nutrition and exercise metabolism,* 22(4): 243-250.

Temple, N.J. 2012. *The marketing of dietary supplements: profit before health*. In Nutritional Health (435-449). Humana Press, Totowa, (NJ)

Trigg, M.E. 2004. Hematopoietic stem cells. *Pediatric medical journal of Australian*: 113

Troy, Jacobson March 7, 2016 Supplement Overview: Iron for Endurance Athletes.

Valeska, P., Casanova, Adelina R. Aires , Silvana G. Collet , Alexandre Krause Rafael N. Moresco , Guilherme V. Bochi , Aleksandro S. Silva and Marta L.R. Leal. 2018. *Iron supplementation for lambs experimentally infected by Haemonchus contortus: response to anemia and iron store in the bone marrow*, 38(8): 1543-1548.

Volpe, S. 2006. Vitamins minerals and exercise: a practice manual for professionals. *Chicago; journal of America dietetic association,* 97:176

Wardiana, A. and Ningrum, R.A., 2016. The emergence of Biosimilars in Indonesia: guidelines, challenges and prospects. *ANNALES BOGORIENSES-JOURNAL OF TROPICAL GENERAL BOTANY*, *20*(2):37-46.

Wendy, B. 2018. Moderate intensity level exercise. How It Feels and How to Achieve It.

West, J.B. 2008. *Pulmonary pathophysiology: the essentials*. Lippincott Williams & Wilkins. (Philadelphia, USA)

Whiting, S.J. and Barabash, W.A. 2006. Dietary reference intakes for the micronutrients: considerations for physical activity. *Applied physiology, nutrition,& metabolism,* 31(1): 80-85.

Widmaier, P., Eric Hershel, Raff and Kevin T. Strong. 2001. Vanders human physiology the mechanism of body function. 8th ed, the McGrawHill companies: 375-37

Zecca, L., Youdim, M.B., Riederer, P., Connor, J.R. and Crichton, R.R., 2004. Iron, brain ageing and neurodegenerative disorders. *Nature Reviews Neuroscience*, 5(11): 863.

7. APPENDIXES

Appendix A

Medical History Questionnaire

The questionnaire was prepared for research entitled **"Effect of Pre versus Post Exercise Iron Supplementation and Aerobic Exercise on Hematological, Anthropometric and Physiological Profile of Mekelle University Female Students, Ethiopia".** So you are kindly requested to give appropriate information for the following questions regarding to your health status.

I. participant information

Name ______________________

Age______________________

II. Personal Health History

1. Is this your first visit to the exercise program? (if not specify it)

2. Have you ever suffered, from:

A. Disease of Blood (Anemia, Leukemia, Tendency to Hemorrhage; Nose, Teeth and Stool) ______________________

B. Heart Disease (Shortness of Breath, Known Lesion, High Blood Pressure, Chronic Heart Failure and High Heart Rate). __________________________________

C. Lung Disease (Infection, Acute or Chronic Tubercloresis, Asthma, Chronic Obstructive Pulmonary Disease, Bronchitis) ____

D. Disease of Digestive System, Liver, Pancreas_________________________________

E. Disease of Genito-Urinary System (Kidney Stone and Prostate Gland Hypertrophy) ____

F. Metabolic Disorder (Diabetes Mellitus, Disease of Thyroid Gland) ____

G. Disease of Nervous System (Tremor, Fatigue, Depression, Mental Trouble) _________________

H. Chronic Ear, Nose and Throat Diseases ____

I. allergies (asthma, hay fever, and neuron dermatitis)________________________________

J. if any not mentioned above

____

3. Do you take any medicine regularly?

____

4. Have you ever been in the habit of taking drug or alcohol? Is so specify the amount

I have read and understand and have given accurate information regarding to my health.

_____________________________ ___________________ ____________

Name of participant place and date signature

_____________________________ _______________ ___________

Name of investigator place and date signature

Source Adopted from (Biruk, 2014)

Thank you for taking your time to respond!

Medical Examination

Medical checkup will be for the subjects of the specific study. The participants will complete and sign medical history questionnaire. The medical examination will be helpful to check whether the participants are free from any cardiovascular disease, diabetes, hypertension etc… before participating in the study. The medical examination will be taken in Mekelle university Ayder specialized referral hospital.

Appendix B

Participants Consent Form

Researcher's Name: **Hailu Gebreslassie**

Supervisor's Name: 1. **Negussie Bussa (BPharm, PhD)**

2. **KV.Balamurgan (PhD)**

Research Title

Effect of Pre versus Post Exercise Iron Supplementation and Aerobic Exercise on Hematological, Anthropometric and Physiological Profile of Mekelle University Female Students, Ethiopia.

Introduction: before agreeing to participate in this research study, it is important to read the following explanation about the research. This statement describes; the right, purpose, procedures, benefit, risk and precautions of the program. There will be no costs for participating in this research. Also participants will not be paid for their participation in this study.

Right: your participation is voluntary. If you believe you may choose your comfort exercise and do not answer any question that makes you uncomfortable. Also there are alternative procedures available for you, as well as your right to withdraw from the study at any time.

Purpose of the research: the purpose of this study is to fulfill master's degree of sport nutrition. And desire to assess, determine and compare the timing of iron supplementation effect with moderate intensity exercise on hematological and physiological parameters.

Procedure and duration of the study: the study will be conducted at the time from December 2018 – February 2019 and you will give the blood sample of around 3-5cc onetime for interval for 3 days per week. You also spend about 15-20 min without any work after supply and blood extract that start from data collection to end result.

Risk and benefit: the pain that you feel when you give sample is milled and all the apparatus used to drown blood is sterilized and it will not expect to have any contamination. If there will be a problem during supplement and blood extract for laboratory the researcher will responsible for all medical emergency assessment for you. This will takes place with cooperation with Mekelle university Ayder referral hospital.

Benefit: some facilities like soap, packed water and supplement was available from the researcher fund and you will be able to learn the use of mineral (iron) in rising hemoglobin and developing performance efficiency and all students will learn from this expected result about the significance of mineral and appropriate timing of the supplements to significantly enhance performance efficiency and prevent such deficiencies.

Participants' responsibility: participants should report any information regarding his/her individual health status that may affect the safety of exercise tests, or previous experience of unusual feeling with prolonged physical exercise.

Confidentiality: if you consent to participate in this evaluation, your personal information will be kept confidential. Participant's individual score will not be disclosed outside of the testing personnel without each participant's written permission. However the only researcher may review the study data without written consent.

Furthermore, will have do favor to the research to contribute to the greater field of sport sciences.

Questions regarding to the research should be directed to: Hailu Gebreslassie, +251914197252, Dr. Negussie Bussa, +251910275526. And any problem and complain can be addressed to HRERC, Mekelle, Ayder

Agreement: I have read all the information provided on this form and consent to participate in this study.

Name ___________________________ signature and date_________________________

If you do not consent to participate you do not need to sign this form, simply return to the researcher.

Signature of investigator______________________ date_______________________

Appendix C

Training Session Protocols

The aim of the training program was to give 300mg ferrous sulfate and structured moderate intensity training to the subjects of this study in order to examine the effect of iron supplement and moderate intensity exercise on Anthropometric, physiological and Hematological parameters of healthy adult female students. The training was designed based on the scientific principles of sport training. The intensity of exercise for this study was monitored through maximum heart rate and target heart rate range. To estimate subjects' maximum heart rate simply age was subtracted from the number 220. Then, the number of beats per minute at which the heart beating during aerobic exercise or target heart rate was calculated by multiplying MHR to 50%-75%. Example if subject A is 20 years old: MHR for this will be 220-20 = 200 beat per minute. If subject A maximum heart rate is 200, the low end rage 50%MHR would be 100 beat per minute and the high end range 75%MHR would be 160 beat per minute. So, subject moderate intensity exercise based on MHR and target heart rate was range from 100-160 beat per minute.

The supplement 300mg ferrous sulfate was given for pre exercise group 30 minutes prior to exercise and the same amount of supplementation is give 30 minute following the exercise for post exercise supplement group three times per week for consecutive 12 weeks in the form of tablet. Furthermore, detail program protocols for the 3 months experimental study was:

Frequency: 3 days per week (Monday, Wednesday and Friday)

Duration per Session: 50-65 minute

Rest: Active and passive rests depending on intensity and duration of exercise.

Time of Training: always from 6:00 am- 7:00am in the morning.

Intensity: for the first month 50-60%MHR, second month 60%-70% and third month was gradually increase to 70-75% MHR.

Activity for Warm up: Light jogging, slow rope skipping and dynamic stretching of major muscle group

Activity for Main Part: Walking, jogging, rope jumping, tempo run, aerobic dance and treadmill run.

Activity for Cool Down: relaxation exercise and static stretching

1: Aerobic Exercise training program for First month (December, 2018)

Training Day	Mode of Exercise	Time	Repetition	Rest	Duration	Intensity
Monday	Warm up and Dynamic Stretching	5min	1×5	1 min passive rest b/n exercise	50 minute per session	**50-60% MHR**
	Aerobic dance	10min	2×5			
	Treadmill run	15min	1×15			
	Tempo run	15min	3×5			
	Cool down	5min	1×5			
Wednesday	Warm up and Dynamic Stretching	5min	1×5	1 min passive rest b/n exercise	50 minute per session	**50-60% MHR**
	Jogging	5min	1×5			
	Rope jumping	15min	3×5			
	Aerobic endurance activities	20min	1×20			
	Cool down	5min	1×5			
Friday	Warm up and Dynamic Stretching	5min	1×5	1 min passive rest b/n exercise	50 minute per session	**50-60% MHR**
	Rope jumping	10min	2×5			
	Treadmill run	15min	1×15			
	Tempo run	15min	3×5			
	Cool down	5min	1×5			

2: Aerobic Exercise training program for Second month (January, 2019)

Training Day	Mode of Exercise	Time	Repetition	Rest	Duration	Intensity
Monday	Warm up and Dynamic Stretching	5min	1✕5	1 min passive rest b/n exercise	55 minute per session	**60-70% MHR**
	Rope jumping	10min	2✕5			
	Treadmill run	20min	1✕20			
	Tempo run	15min	3✕5			
	Cool down	5min	1✕5			
Wednesday	Warm up and Dynamic Stretching	5min	1✕5	1 min passive rest b/n exercise	55 minute per session	**60-70% MHR**
	Jogging	5min	1✕5			
	Aerobic run	15min	1✕15			
	Aerobic endurance activities	25min	1✕25			
	Cool down	5min	1✕5			
Friday	Warm up and Dynamic Stretching	5min	1✕5	1 min passive rest b/n exercise	55 minute per session	**60-70% MHR**
	Rope jumping	10min	2✕5			
	Treadmill run	15min	1✕15			
	Stationary bicycle	20min	2✕10			
	Cool down	5min	1✕5			

3: Aerobic Exercise training program for Third month (February, 2019)

Training Day	**Mode of Exercise**	**Time**	**Repetition**	**Rest**	**Duration**	**Intensity**
Monday	Warm up and Dynamic Stretching	5min	1×5	2 min passive rest b/n exercise	65 minute per session	**70-75% MHR**
	Rope jumping	15min	3×5			
	Treadmill run at 0 level	20min	1×20			
	Tempo run	20min	2×10			
	Cool down	5min	1×5			
Wednesday	Warm up and Dynamic Stretching	5min	1×5	2 min passive rest b/n exercise	65 minute per session	**70-75% MHR**
	Jogging	5min	1×5			
	Aerobic run	20min	1×20			
	Aerobic endurance activities	30min	1×30			
	Cool down	5min	1×5			
Friday	Warm up and Dynamic Stretching	5min	1×5	2 min passive rest b/n exercise	65 minute per session	**70-75% MHR**
	Rope jumping	15min	3×5			
	Treadmill run	20min	1×20			
	Tempo run	20min	2×10			
	Cool down	5min	1×5			

Appendix D

Descriptive Statistics for Anthropometric Variables

4: Paired sample T-test value for anthropometric characteristics of PWSG

Paired Samples Test

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
weightpretest - weightposttest	.15	2.27364	.71899	-1.47646	1.77646	.209	9	.839
bodymassindexpretest - bodymasindexposttest	.063	.90328	.28564	-.58317	.70917	.221	9	.830

Paired Samples Statistics

Variables	Mean	N	Std. Deviation	Std. Error Mean
weightpretest	51.5500	10	5.65415	1.78800
weightposttest	51.4000	10	5.89161	1.86309
heightpretest	1.5970[a]	10	.07931	.02508
heightposttest	1.5970[a]	10	.07931	.02508
bodymassindexoretest	20.2500	10	2.26286	.71558
bodymasindexposttest	20.1870	10	2.17090	.68650

a. The correlation and t cannot be computed because the standard error of the difference is 0.

5: Paired sample T-test value for anthropometric characteristics of POWSG

Paired Samples Test

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
weightpretest-weightposttest	.65000	2.13503	.67515	-.87731	2.17731	.963	9	.361
bodymassindexpretest-bodymasindexposttest	.24000	.84485	.26717	-.36437	.84437	.898	9	.392

Paired Samples Statistics

Variables	Mean	N	Std. Deviation	Std. Error Mean
weightpretest	52.8500	10	2.21171	.69940
weightposttest	52.2000	10	2.57337	.81377
heightpretest	1.6120[a]	10	.04315	.01365
heightposttest	1.6120[a]	10	.04315	.01365
bodymassindexPretest	20.4200	10	1.41091	.44617
bodymasindexposttest	20.1800	10	1.79493	.56761

a. The correlation and t cannot be computed because the standard error of the difference is 0.

Appendix E

Descriptive Statistics for Hematological Parameters

6: Paired sample T-test value for Hematological Parameters of PWSG

Paired Samples Test

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
hemoglobinposttest - hemoglobinpretest	.23600	.19682	.06224	.09520	.37680	3.792	9	.004
Redbloodcellsposttest - Redbloodcellspretest	.06900	.06100	.01929	.02536	.11264	3.577	9	.006
hematocritposttest - hematocritpretest	.45100	.36088	.11412	.19284	.70916	3.952	9	.003
plateletposttest - plateletpretest	-4.7000	4.2176	1.33375	-7.71715	-1.68285	-3.524	9	0.006

Paired Samples Statistics

Variables	Mean	N	Std. Deviation	Std. Error Mean
hemoglobinposttest	14.7260	10	1.40479	.44423
hemoglobinpretest	14.4900	10	1.50366	.47550
Redbloodcellsposttest	4.9560	10	.52673	.16657
Redbloodcellspretest	4.8870	10	.52368	.16560
hematocritposttest	43.1110	10	3.58898	1.13493
hematocritpretest	42.6600	10	3.64911	1.15395
plateletposttest	289.4000	10	79.6692	25.19136
plateletpretest	294.1000	10	79.38206	25.10531

7: Paired sample T-test value for Hematological Parameters of POWSG

Paired Samples Test

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
hemoglobinposttest - hemoglobinpretest	.82000	.31903	.10088	.59178	1.04822	8.128	9	.000
Redbloodcellsposttest - Redbloodcellspretest	.35600	.26871	.08497	.16378	.54822	4.190	9	.002
hematocritposttest - hematocritpretest	1.87000	1.46746	.46405	.82024	2.91976	4.030	9	.003
plateletposttest - plateletpretest	-8.20000	4.56557	1.44376	-11.46601	-4.93399	-5.680	9	0.000

Paired Samples Statistics

Variables	Mean	N	Std. Deviation	Std. Error Mean
hemoglobinposttest	14.8100	10	.91706	.29000
hemoglobinpretest	13.9900	10	1.13475	.35884
Redbloodcellsposttest	4.6620	10	.58009	.18344
Redbloodcellspretest	4.3060	10	.57668	.18236
hematocritposttest	42.8000	10	2.66542	.84288
hematocritpretest	40.9300	10	2.78011	.87915
plateletposttest	317.6000	10	50.88779	16.09213
plateletpretest	309.1000	10	52.32898	16.54788

Appendix F

Descriptive Statistics for Physiological Parameters

8: Paired sample T-test value for Physiological Parameters of PWSG

Paired Samples Test

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
SBP pretest – SBPposttest	--2.200	1.13529	0.35901	-3.012	-1.3878	-6.12	9	**0.000**
DBPpretest – DBPposttest	1.40000	1.64655	.52068	.22213	2.57787	2.689	9	.025
RHRpretest - RHRposttest	2.20000	1.75119	.55377	.94727	3.45273	3.973	9	.003

Paired Samples Statistics

Variables	Mean	N	Std. Deviation	Std. Error Mean
SBP Pretest	110.0000	10	9.45163	2.98887
SBP Posttest	107.8000	10	9.6009	3.03608
DBP Pretest	71.0000	10	8.98146	2.84019
DBP Posttest	69.6000	10	8.72035	2.75762
RHR Pretest	70.9000	10	5.38413	1.70261
RHR Posttest	68.7000	10	5.59861	1.77044

9: Paired sample T-test value for Physiological Parameters of POWSG

Paired Samples Test

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
SBP pretest – SBPposttest	4.90000	4.25441	1.34536	1.85658	7.94342	3.642	9	.005
DBPpretest – DBPposttest	5.00000	3.46410	1.09545	2.52193	7.47807	4.564	9	.001
RHRpretest - RHRposttest	4.30000	1.82878	.57831	2.99177	5.60823	7.435	9	.000

Paired Samples Statistics

	Mean	N	Std. Deviation	Std. Error Mean
SBP Pretest	110.0000	10	8.56349	2.70801
SBP Posttest	105.1000	10	7.69488	2.43333
DBP Pretest	83.2000	10	11.68855	3.69624
DBP Posttest	78.2000	10	10.15218	3.21040
RHR Pretest	75.1000	10	9.58529	3.03113
RHR Posttest	70.8000	10	8.49575	2.68659

Appendix G

Standard Reference Value

10: Standard Reference Value for Hematological Level

No	Sex	Hb	RBC	Hematocrit	WBC	Platelets
1	Male	13.5-17.5 grams/dL	: 4.32-5.72 trillion cells/L	38.8-50.0 percent	3.5-10.5 billion cells/L	150-450 billion/L
2	Female	12.0-15.5 grams/dL	3.90-5.03 trillion cells/L	34.9-44.5 percent	3.5-10.5 billion cells/L	150-450 billion/L

L = liter, mcL = microliter, dL = deciliter , RBC= Red blood cells Hb= Hemoglobin WBC= White blood cell. Source (WHO, 2015)

11: Standard Reference Value for Hemoglobin Level Based on Sex and Age

sex	Age	Interval level	Mean
Both	Birth	13.5- 24 g/dl	16.5g/dl
Both	<1 month	10.0 to 20.0 g/dl	13.9 g/dl
Both	1-2 month	10.0 to 18.0 g/dl	11.2 g/dl
Both	2-6 month	9.5 to 14.0 g/dl	12.6 g/dl
Both	0.5-2 years	10.5 to 13.5 g/dl	12.0 g/dl
Both	2 to 6 years	11.5 to 13.5 g/dl	12.5 g/dl
Both	6-12 years	11.5 to 15.5 g/dl	13.5g/dl
females			
	12-18 years	12.0 to 16.0 g/dl	14.0 g/dl
	>18 years	12.1 to 15.1 g/dl	14.0g/dl
Males			
	12-18 years	13.0 to 16.0 g/dl	14.5 g/dl
	>18 years	13.6 to 17.7 g/dl	15.5 g/dl

g/dl= gram per deciliter Hb= hemoglobin **Source**: (Brian, 2014)

12: Standard Reference Value for Blood Pressure Classification

Stage	**Systolic BP**	**Diastolic BP**	**Unit**
Normal	<120	<80	Mm Hg
Elevated	120-129	<80	Mm Hg
Stage 1	130-139	80-89	Mm Hg
Stage 2	>140	>90	Mm Hg
Hypertensive crisis	>180	>120	Mm Hg

Bp= blood pressure   Mm= millimeter   Hg= mercury   Mm Hg= millimeter mercury

SOURCE Robin M., "n,,d" reviewed by Judith, 2018

13: Standard Reference Value for Body Mass Index

BMI(Kg/m^2)	Category
<18.5	Under weight
18.5-24.9	Normal weight
25-29.9	Over weight
30-35	Moderately obese (class I)
36-40	Severely obese (class II)
>40	Morbid

BMI= Body Mass Index

Source: (Andreoli, 2016)

Appendix H

Data Recording Sheet

14: Data Recording Sheet for Hematological, Physiological and Anthropometrical Variables

Name of Group: ____________________________

Participants' Code: _________________________

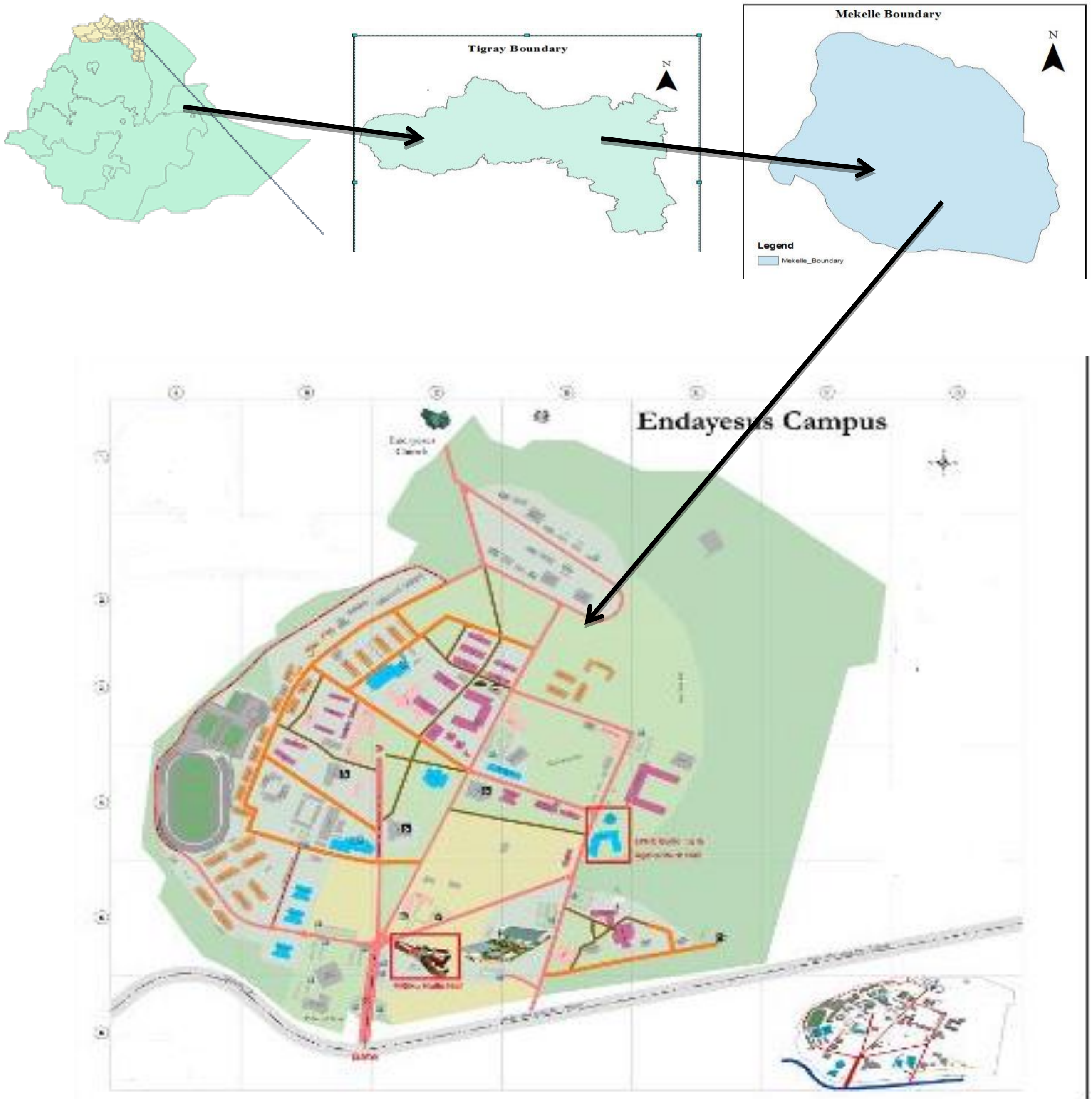
Age in Years: _________________________________

NO	**PARAMETERS**	**UNITE**	**TESTED DATA**	
			PRE TEST	**POST TEST**
1.	Hemoglobin	g/dl		
2.	Red Blood Cells	Number		
3.	Hematocrit	Percent		
4.	Platelet	Number		
5.	Systolic Blood Pressure	MmHg		
6.	Diastolic Blood Pressure	MmHg		
7.	Resting heart rate	b/min		
8.	Body Weight	Kg		
9.	Height	M		
10.	Body Mass Index	Kg/m^2		

Participants were coded in number whereas groups were coded as PWSG and POWSG.

Appendix I

Map of the Study Area



Appendix J

Approval for Ethical Clearance

Mekelle University
College of Health Sciences
Health Research Ethics Review Committee (HRERC)

To: Hailu Gebreslassie
Principal Investigator
Mekelle

Date: 21/12/2018

RE: Notification of Expedited Approval
ERC 1559/2018

Protocol: *Effect of pre versus post exercise iron supplement and moderate intensity exercise on hematological, anthropometric and physiological profile: the case of Mekelle Undergraduate female sport sciences students.*

Dear local PI:

This is your notification that your above referenced study has received **EXPEDITED APPROVAL** on December, 21, 2018. This ethics review approval will expire on December, 20, 2019.

The research study cited above has been reviewed and it has been determined that it meets the criteria for expedited review. The HRERC will be apprised of this decision at its monthly meeting.

The PI should comply with national and international scientific and ethical guidelines. Any reportable events (serious adverse events, breaches of confidentiality, protocol deviation or protocol violations) or issues resulting from this study should be reported immediately to the HRERC. Any amendments (changes to any portion of this research protocol including but not limited to protocol or informed consent changes) must have HRERC approval before being implemented.

All correspondences and inquires concerning this research protocol must include the ERC number, the name of the PI and the protocol title.

Sincerely,

CC:
- Sport Science
- CARD

CHS

College of Health Sciences Mekelle University Institutional Ethics Review Committee

Commencing health research without approval is unethical!