Body composition and training levels in relation to iron deficiency among athletes and other physically active individuals

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2018

Thesis for the degree of Master of Science in Human Nutrition

UNIVERSITY OF ICELAND
SCHOOL OF HEALTH SCIENCES
FACULTY OF FOOD SCIENCE AND NUTRITION

Holdafar og þjálfunarmagn í tengslum við járnskort meðal íþróttafólks og annarra líkamlega virkra einstaklinga

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60 einingar

Júní 2018

Ritgerð til meistaragráðu í næringarfræði

HÁSKÓLI ÍSLANDS
MATVÆLA- OG NÆRINGARFRÆÐIDEILD

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Ritgerð þessi er til meistaragráðu í næringarfræði og er óheimilt að afrita ritgerðina á nokkurn hátt nema með leyfi rétthafa.

Prentun: Háskólaprent

Reykjavík, Ísland 2018

Ágrip

Inngangur: Algengi járnskorts er hærra meðal íþróttafólks en almennings og hefur slíkur skortur í fyrri rannsóknum verið tengdur verri framistöðu í íþróttum. En fremur hefur líkamsfita og líkamsþyngdarstuðull verið tengd auknu algengi járnskorts.

Markmið: 1) Rannsaka tengsl milli hlutfalls líkamsþyngdarstuðuls, líkamsfitu og járnskorts hjá íþróttafólki og líkamlega virkum einstaklingum. 2) Kanna hvort líkur á járnskorti aukist eftir því sem þjálfunarmagn eykst.

Aðferðir: Rannsókn var gerð á gögnum sem fengin voru úr eldri rannsóknum sem framkvæmdar höfðu verið við Íþróttadeild Háskólans í Umeå, Svíþjóð. Um var að ræða 443 þátttakendur, allt unga einstaklinga sem ýmist voru líkamlega virkir eða stunduðu skipulagðar íþróttir; 271 konur og 172 karlar á aldrinum 16 – 38 ára og var gögnunum safnað á árunum 2013 – 2017. Upplýsingar um þjálfunarmagn á viku og blóðgreiningar voru til fyrir hvern einstakling. Einnig voru til niðurstöður úr líkamsþáttamælingum (líkamsþyngdarstuðull og prósent líkamsfitu) fyrir hluta hópsins eða 266 einstaklinga (59%) og voru þær einnig notaðar. Járnskortur var skilgreindur sem ferritin <30 μg/L og hemoglobin ≥130 g/L (karlar) eða ≥120 g/L (konur).

Niðurstöður: Í þessu samsetta þýði voru 30% kvenna með járnskort borið saman við 9% karla (P<0.001) (40% kvenna og 10% karla í minna þýðinu (P<0.001)). Hærri líkamsþyngdarstuðull var tengdur minni líkum á járnskorti (OR: 0.80; 95%CI: 0.68-0.95) en engin tengsl fundust milli hlutfalls líkamsfitu og járnskorts. Þegar litið var á líkanið skipt eftir kyni komu fram minni líkur á járnskorti við aukna þjálfun hjá körlum (OR: 0.821; 95%CI: 0.69-0.98) en ekki hjá konum. Auk þess virðist aukinn aldur hjá konum tengjast minni líkum á járnskorti (OR: 0.88; 95%CI: 0.81-0.95).

Ályktanir: Ljóst er að járnskortur er algengara vandamál meðal íþróttakvenna heldur en íþróttamanna. Það var áhugavert að sjá að bæði aukin þjálfun og hærri líkamsþyngdarstuðull draga úr líkum þess að þjást af járnskorti. Þörf er á frekari rannsóknum á járnskorti meðal líkamlega virkra einstaklinga, gjarnan framsýnar ferilrannsóknir, og rannsaka betur hvort tengsl séu á milli hlutfalls fituvefs og járnskorts hjá íþróttafólki og öðrum líkamlega virkum einstaklingum.

Abstract

Background: Athletes are found to have a higher prevalence of iron deficiency (ID) and previous/early studies have associated that deficiency with decreased athletic performance. Both percentage body fat (%BF) and body mass index (BMI) have been linked to increased prevalence of ID.

Objective: 1) Investigate the association between BMI and %BF with ID among athletes and physically active individuals. 2) Explore whether increased amount of training increases the probability of ID among athletes and physically active individuals.

Methods: A study performed on results from a combination of four cohorts, including blood sample analysis and derived from previous and ongoing research studies at the Sports Medicine Unit, Umeå University, Sweden. This was a group of 443 young elite athletes and/or physically active individuals; 271 females and 172 males, 16 – 38 years of age, recruited in 2013-2017. Information on training hours per week, supplement intake and blood and iron status were available for every subject. Results from body composition measurements (BMI and %BF) were available for 266 (59%) of the subjects. ID was diagnosed as having ferritin <30 μg/L and hemoglobin ≥130 g/L (males) or ≥120 g/L (females).

Results: In the larger sample, 30% of females experienced ID compared to 9% of males (P<0.001) (40% females vs 10% males in the smaller sample (P<0.001)). Higher BMI was associated with decreased risk of experiencing ID (OR: 0.80; 95%CI: 0.68-0.95), but no association was found between %BF and ID. Divided by gender training reduced the likelihood of experiencing ID among males (OR: 0.821; 95%CI: 0.69-0.98) but not females. Additionally, increased age was associated with a reduction in the likelihood of females experiencing ID (OR: 0.88; 95%CI: 0.81-0.95).

Conclusions: It is clear that ID is a common issue among female athletes, more so than in their male counterparts. Interestingly among males, both increased training amounts (hours/week) and higher BMI decreased the likelihood of experiencing ID. Further research is needed on ID among physically active individuals and whether there is a relationship between adipose tissue and ID in athletes and other physically active individuals.

Acknowledgement

The work presented in this thesis was carried out at the Faculty of Health Promotion, Sports and Leisure Studies at the School of Education, University of Iceland, in cooperation with the Faculty of Food Science and Nutrition, University of Iceland, and the Sports Medicine Unit, Umeå University, Sweden. The data collection was conducted at the Sports Medicine Unit in Umeå, and I would like to thank Roger Andersson and Mikael “Knappen” Therell for their contributions. Likewise, my thanks go to all the staff at the Sports Medicine Unit for their unlimited patience and kindness.

I would like to express my deepest gratitude to my supervisors, Professor Anna Sigríður Ólafsdóttir and Associate Professor Michael Svensson, for their guidance and support. I would especially like to thank them for giving me the opportunity to study and live abroad. That experience has expanded my horizons and given me invaluable lessons for the future. It has been a pleasure to learn and work with such (talented) leaders in their fields. I would also like to thank Ingibjörg Kjartansdóttir for her valuable help and advice on statistical analysis of the data and Bonni Yeatman for the great work she did on editing and proofreading. My thanks also go to Professor Bryndís Eva Birgisdóttir for her valued advice and guidance and I wish her all the best for her new exciting platform in Denmark.

Further I would like to extent my gratitude to all of my fellow students for their wonderful company and supportive presence, especially Dagný Ólafsdóttir, Jenný Rut Ragnarsdóttir, Lilja Guðmundsdóttir and Ingunn Erla Ingvarsdóttir.

Last but not least I would like to thank my family, especially my loving girlfriend Elísa Kristinsdóttir for her endless support, patience and faith in me during the entire period of my studies and for sticking with me through it all. My thanks also go to my loving parents, Ragnhildur Hanna Finnbogadóttir and Rúnar Vigfússon Amin for always believing in me, to my grandmother who helped me settle in a new country, my sister for helping me move back home and to my family in law for their countless support and kindness.

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Abbreviations

ACSM	American College of Sports Medicine
ADP	Adenosine diphosphate
AND	Academy of Nutrition and Dietetics
ATP	Adenosine triphosphate
CaO_2-CvO_2	Oxygen content difference
CHr	Reticulocyte hemoglobin content
CO_2	Carbon dioxide
CRP	C-reactive protein
DC	Dietitians of Canada
EPO	Erythropoietin
FADH2	Flavin adenine dinucleotide
Fe	Iron
Fe^{2+}	Ferrous form of Iron
Fe^{3+}	Ferric form of Iron
fL	Femoliters
FPNA1	Ferroportin
g	Gram
Hb	Hemoglobin
ID	Iron deficiency
IDA	Iron deficiency anemia
IL-6	Interleukin 6
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mg	milligram
NADH	Nicotinamide adenine dinucleotide
NUS	Norrlands University Hospital
O_2	Oxygen
PCr	Phosphocreatine
pg	Picograms
RBCs	Red blood cells
sFer	Serum ferritin

sTfR	Serum transferrin receptor
tHb-mass	Total hemoglobin mass
VO_{2max}	Maximal oxygen uptake
WHO	World Health Organization
µg	Microgram
%BF	Percentage body fat

1 Introduction

Iron deficiency (ID), characterized by low storage of ferritin, is one of the most widespread nutrient deficiencies in the world and affects a significant proportion of the population in nearly every country across the globe (1). ID has been reported to be more prevalent among athletes and physically active individuals than among inactive individuals (2, 3). Due to iron's essential role in the oxygen transportation system and the electron transport chain, ID can have profound effects on athletic performance, since exercise performance relies on maximal oxygen carrying capacity (4-6). The most common causes of iron deficiency anemia (IDA) worldwide are; blood loss, the maternal-fetal bridge of iron deficiency (due to pregnancy), malaria and hookworm, as well as low iron intake and malabsorption of iron (7). Athletes are considered to be at more risk due to increased iron loss through hemolysis, sweating and increased hepcidin concentration (the primary regulator of iron homeostasis in the body) due to exercise-induced inflammation, and also in some cases due to gastrointestinal bleeding and urinary blood loss (8, 9).

Research has shown that prevalence of ID is also higher among obese individuals compared with people of normal weight. This has been linked to a similar chronic low-grade inflammation also found in obesity, including hepcidin messenger RNA expression in adipose tissue and greater adipose hepcidin (10-13). The association between BMI and ID has been previously studied (14) but body composition as percentage body fat (%BF) and ID has not been studied among healthy individuals or athletes. One of the reasons for increased risk for ID among athletes is thought to be exercise-induced inflammation, and it is logical to look into the possibility of an existing relationship between %BF and ID among athletes and physically active individuals, on the grounds of inflammation and hepcidin production.

The aim of this thesis is to investigate the association between body mass index, percentage of body fat and ID among young athletes and physically active individuals. Further, to explore whether increased training amounts increase the probability of ID.

2 Review of the literature

This chapter reviews research on iron and its properties, and the source of iron and iron metabolism in human biology. It will also review research on diseases associated with iron deficiency and overload, particularly the connection of some of these disorders to a person's engagement in sports and physical activity. Measurements of iron and body composition will be examined in a theoretical context aiming to demonstrate the importance and innovation of this study.

2.1 Iron and its properties

Iron (Fe) is by mass the most abundant element on Earth. It forms much of Earth's inner and outer core as well as being the fourth most common element in its crust. Iron has the assigned atomic number of 26, taking its place in group 8, period 4 in the periodic table. Categorized as a transitional metal, iron exists in a range of oxidation states, from -2 to +7, although +2 and +3 are the most common. In standard conditions for temperature and pressure, iron is in a solid phase, melting at 1811 K (1538°C) and boiling at 3134 K (2862°C) (15).

Iron plays a vital role in human biology. Existing in the two most common oxidation states, the ferrous form (Fe^{2+}) and the ferric form (Fe^{3+}), iron takes part in a multitude of redox reactions necessary to support basic metabolic functions (16). Given the fact that ferric iron is poorly soluble at physiological pH, and the ability of ferrous iron to reduce oxygen intermediates to harmful free radicals, all organisms have developed binding molecules to transport and store iron, and to control its reactivity (4). Due to these harmful free radicals, iron is not only an essential nutrient but also a powerful toxicant, making iron homeostasis extremely important (4, 16, 17).

2.2 Iron sources

The human body obtains iron in two ways: through the diet as either heme iron or non-heme iron, or from an endogenous source like the breakdown of circulating red blood cells (4, 18). The absorption from the intestines is tightly controlled - women in general absorb 1.0 to 1.5 mg/day (dependent on menstrual blood loss) (17), and men less or an average of 0.5 to 1.0 mg/day.

Table 1. Calculated iron needs based on daily needs and losses for adolescents and adults according to NNR (4).

Age (years)	Weight (kg)	Needs for growth (mg/d)	Basal losses (mg/d)[1]		Menstrual losses (mg/d)[2]		Total iron need (mg/d)			Necessary intake of iron from foods to cover 50%, 90% and 95% of the iron need of groups on a diet for which iron absorption is assumed to be 15% (mg/d)		
	mean		median	median	90%	95%	Median	90%	95%	50%	90%	95%
Boys												
10-13	37.5	0.55	0.53				1.08		1.35	7		9
14-17	57	0.60	0.80				1.40		1.75	9		12
Men 18+	76		1.05				1.05		1.37	7		9
Girls												
10-13[8]	38.5[5]	0.55	0.54				1.09		1.36	7		9
10-13	38.5[5]	0.55	0.54	0.46[3]	1.05	1.69[4]	1.55	2.14[7]	2.78[7]	10	14	19
14-17	53.5[5]	0.30	0.75	0.46[3]	1.05	1.69[4]	1.51	2.1[7]	2.74[7]	10	14	18
Women 18+	62[6]		0.87	0.48[3]	1.35	1.90	1.35[2]	2.22[7]	2.77[7]	9	15	19
Women after menopause	62[6]		0.87				0.87		1.13	6		8

[1] Basal losses are estimated to be 0.014 mg/kg$^{-1/d-1}$

[2]Evaluated from the amount of menstrual blood in ml/28 days. Menstrual losses for girls are assumed to be the same in both age groups. Hemoglobin concentration is calculated as 135g/L and it is assumed that 1 g Hb/L x 3.34 mg iron. Menstrual iron loss (mg) = [blood loss (mL)/28 days x 135 g Hb/L x 3.34 mg iron/g Hb)/(1000 mL/L)].

[3]Calculated with a median blood loss of 28.4 mL for adolescent girls and 30 mL for adult women every 28 days.

[4] Calculated from the equation in the US recommendations derived from a fitted log normal distribution with a Monte Carlo simulation [ln (blood loss) = 3.3183 + 0.6662(SD)].

[5] Children weights 1973-1977.

[6] Mean weight of men and women aged 15-80 years.

[7] Sum on basal losses, need for growth, and 90th and 95th percentiles of menstrual losses, respectively. It is assumed that there is no distribution in values for basal losses and need for growth.

[8] Not menstruating.

In order for the body to maintain iron balance, 90% of the daily iron needs are obtained from recycled red blood cells while the last ten percent of iron, necessary also for healthy growth and pregnancy, is obtained from the diet (18). Heme iron, present in hemoglobin and myoglobin in foods from animal sources is generally more efficiently absorbed if compared to non-heme iron, which is present in foods from both plant and animal sources (18, 19). According to the Nordic Nutrition Recommendations (NNR) (4), the recommended daily intake of iron for the average man and woman of childbearing age is 9 mg/day and 15 mg/day, respectively (Table 1). Average iron intake in the Nordic countries is typically 15 to 20 mg/day, females having a considerably lower intake than males. The average iron intake reported for Swedish adult males is 12.3 mg/d compared to 10.4 mg/d for females (4).

Heme iron represents a relatively small part of the total dietary iron intake even though it has higher bio-availability. It has been suggested that the muscle proteins in meat can explain the reason for a

higher bioavailability of heme iron. The partially digested peptides, cysteine and histidine, bind iron and form structures that are soluble and available for absorption (4). It is a well-known practice in many cultures to enhance the diet with heme iron-rich food products, and for example in the Nordic diet, about 10% of the total iron is heme iron found in meat like lamb, beef, and offal (4, 20). In addition, although currently only used in research, there have been pioneering advances in developing a heme iron concentrate and heme-iron-based supplements and/or fortifiers including heme-iron fortified chocolate, biscuits, and meat pâté (20-22). The amount of absorption of heme iron from the diet is generally about 25% and usually not affected by other food components, although calcium has been reported to decrease bioavailability of both heme and non-heme iron (19).

In the case of non-heme iron, absorption depends not only on the composition of meals as there are many more complexities to consider. Iron absorption can be inhibited by some substances in the food, for example, by the nutrients calcium and manganese (4). Phytates, also known as phytic acid, is the principal storage form of phosphorus in many plants and is the main inhibitor of non-heme iron absorption along with their metabolites, polyphenols. The negative effects of phytates have been shown to be dose-dependent and the amount and type of polyphenols can affect absorption. Coffee for example has a more negative effect than tea or wine, even though they are all polyphenol containing drinks (19). In contrast, ascorbic acid (vitamin C) can enhance the absorption of non-heme iron (4).

2.3 Iron absorption and regulation

There are two main steps involved in the absorption of iron into the blood: absorption of iron from the lumen into the small intestine epithelial cells, and absorption of iron from the epithelial cells into the blood. The amount of ingested iron taken up by the epithelial cells is determined by four major factors: physiologic need for iron, dietary iron intake, bioavailability of the dietary iron, and adaptation. Adaptation here refers to the ability of the mucosal cell to adjust iron absorption to physiologic demands and bioavailability of dietary iron, doing so with the help of hepcidin, the primary regulator of iron homeostasis in the body (17, 18).

The main area of iron absorption is in the upper part of the intestine, the duodenum and proximal jejunum (23). The iron is absorbed by enterocytes located in the duodenal lining in a process called receptor-mediated endocytosis. In order to be absorbed, iron needs to be either part of a protein or in its ferrous form (24). An enzyme on the enterocytes brush border reduces ferric iron to ferrous iron, enabling it to be absorbed (25). Iron is then transported as ferrous iron across the enterocyte´s cell membrane into the cell. Once there, iron can either be stored in the cell as ferritin (and if not used then excluded from the body when the cell dies), or it can move through the cell and into the blood stream, doing so by a very well-regulated pathway.

Iron is excreted from the enterocyte across the basolateral membrane by the transporter protein ferroportin, a process facilitated by the ferroxidase activity of the ceruloplasmin homologue hephaestin. Once in the bloodstream iron circulates either as free iron or attached to transferrin. From the bloodstream, iron travels mainly to two places: the liver for storage, or to the bone marrow for erythropoiesis. In the liver, iron is taken up by liver cells called hepatocytes, with the help of transferrin receptor 1 or 2. Transferrin receptor 2 serves as a sensor of circulating transferrin-bound iron, thus

influencing the expression of hepcidin in the hepatocyte (26). The liver stores iron as ferritin and when needed, it is excreted from the hepatocyte into the blood stream via ferroportin (17).

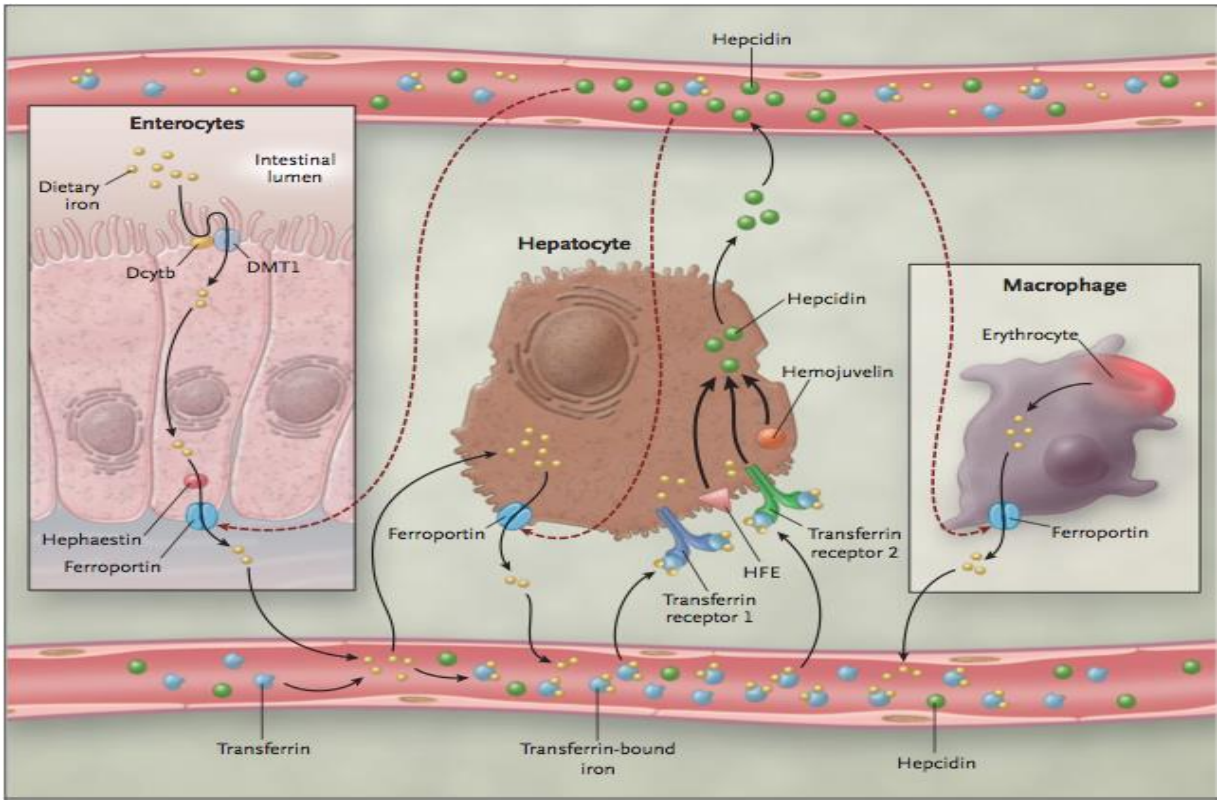

Figure 1. Absorption and regulation of iron *(26).*

Since iron does not have an excretion regulation pathway, dietary intake, intestinal absorption and iron recycling must be finely regulated (27). Hepcidin is the main regulator for iron release into the plasma. This 25-amino acid peptide hormone is mainly produced by the hepatocytes, possibly because of their location astride the portal venous system that transports iron absorbed in the intestine (28).

The production of hepcidin is up-regulated by high concentrations of iron in liver and plasma, inflammation and physical activity, but down-regulated by iron deficiency; erythropoiesis, hypoxia, and endocrine signals (27). This means that hepatocytes produce more hepcidin when iron is plentiful, reducing further iron absorption and release from stores (29, 30). This occurs as the hepcidin produced binds to and down-regulates the iron transporter, ferroportin (FPN1) (31). When iron is scarce, hepatocytes decrease or stop producing hepcidin, thereby increasing iron release into the plasma. Additionally, hepcidin production is suppressed during erythropoiesis, increasing iron available for hemoglobin synthesis. Indeed all situations stimulating erythropoiesis such as bleeding, hemolysis, hypoxia and even erythropoietin (EPO) injection, completely suppresses the synthesis of hepcidin (29, 30). During infections and systemic inflammatory diseases, blood hepcidin concentration and hepcidin production is increased through increase in interleukin-6 (IL-6). Due to this inflammation-related increase, it might limit the availability of iron for erythropoiesis, contributing to anemia-of-inflammation, also known as anemia of chronic disease (30). It has been shown that the regulation of hepcidin

production by inflammatory stimuli is not restricted only to the liver tissue, but also correlates with IL-6 or CRP expressions in adipose tissue (10).

2.4 The role of iron in the human body

The physiological importance of iron is significant. The total amount of iron in the human body is estimated to be approximately 2.3 g in females and 3.8 g in males. In an adult female, iron is distributed as follows: 1.7 g in hemoglobin, 300 mg in the form of ferritin and hemosiderin, 120 mg in myoglobin in muscles, 15 mg in enzymes and 3 mg in transport iron (transferrin) (32). In an adult male, iron is distributed as follows: 2.4 g in hemoglobin, 700 mg depot iron in the form of ferritin, 300 mg in hemosiderin, 150 mg in myoglobin in muscles, 20 mg in enzymes and 4 mg in transferrin (16, 33). In both females and males approximately 20 to 25 mg of iron is metabolized daily through erythrophagocytosis and recycled into new erythrocytes. In addition, small amounts of iron in the body are lost in feces (~0.6 mg/day), urine (<0.1 mg/day) and sweat (<0.3 mg/day). Women of childbearing age experience an average blood loss of approximately 40 mL/cycle (16).

2.4.1 Erythrocytes

As mentioned earlier, most of the iron in the body is present in hemoglobin, a molecule kept inside the erythrocytes (the red blood cells, or RBCs). Erythrocytes are flat, disc-shaped cells indented in the middle on both sides and resemble doughnuts but without the hole. The erythrocyte's main function is to transport O_2 in the blood to different parts of the body. Its bi-concave shape provides a large area for diffusion of O_2 and the thinness allows O_2 to diffuse rapidly between the surface and innermost areas of the cell (17). Erythrocytes do not have a nucleus like most other cells but carry the hemoglobin molecule.

Hemoglobin is found in red blood cells. It is composed of four polypeptide chains, two alpha and two beta chains. The alpha and beta chains have distinctive sequences of amino acids and fold up to form similar three-dimension structures. Each of these sub-units contains a co-factor called a heme group with an iron atom center. This allows the hemoglobin to fully load with four O_2 atoms in the lungs and to unload them in the tissues (34-36). In addition to carrying $O_{2,,}$ hemoglobin can also help transport CO_2 from tissue cells back to the lungs, and serves as a buffer for H^+ from CO_2 to minimize its alteration on the pH of the blood (17). In the mitochondria O_2 is utilized to support oxidative phosphorylation. The maximal rate at which O_2 can be transported from the environment to the mitochondria is physically limited by the Fick equation:

$$\textit{Cardiac Output (CO)} = \frac{\textit{Oxygen Consumption (VO2)}}{\textit{Arteriovenous Oxygen Diff (CaO2 – CvO2)(37)}}$$

This maximal rate is termed the maximal oxygen uptake (VO_{2max}). VO_{2max} is directly related to fitness capacity and an athlete with a higher VO_{2max} is able to perform at a higher level for a longer period of time compared to an athlete with a lower VO_{2max} (38). O_2 transportation is the most significant limiting factor of VO_{2max} in trained endurance athletes, whereas mitochondrial O_2 consumption also limits VO_{2max} in the untrained individuals (39). However, according to Louise Burke (40), the limitations to VO_{2max} are debatable, that is, whether it is due to the delivery of O_2 to the active muscles, or due to the ability of the muscle to use O_2. VO_{2max} is reached by the simultaneous increase in cardiac output and the

arteriovenous oxygen content difference (CaO_2-CvO_2). The ability to increase CaO_2-CvO_2 is dependent mainly on the arterial O_2 content and hemoglobin concentration, the measured hemoglobin concentration being dependent on the total circulation mass of hemoglobin (tHb-mass) and plasma volume (39). Ekblom and coworkers (41) showed that a 13% reduction in hemoglobin concentration lowered VO_{2max} by 10%. It is therefore clear that iron status for athletes is significantly important.

2.4.2 Myoglobin and skeletal muscle characteristics

Myoglobin within the muscles is similar to hemoglobin. It is found in the cytoplasm of the muscle cells, facilitating the diffusion rate of O_2 from capillary red blood cells to the cytoplasm and mitochondria of the muscle cell (17, 42). The human body has three primary systems for energy production: high-energy phosphorylation (ATP and PCr), glycolytic ATP synthesis and oxidative phosphorylation. The skeletal muscle comprises three muscle fiber types with different characteristics; type IIX fast glycolytic, type IIA fast oxidative and type I slow oxidative (17, 43). Type I fibers have a high myoglobin and mitochondria content and a high density of blood capillaries favoring oxygen-uptake and oxidation of fatty acids and carbohydrates, making them extremely suitable for longer endurance training. Type IIA fibers also have a high content of myoglobin and mitochondria and many surrounding blood capillaries, as well as hydrolyzing adenosine triphosphate (ATP) at a high rate and thus having a faster peak contraction velocity. On the other hand, type IIX fibers have a low myoglobin content, less mitochondrion and low density of blood capillaries, thus less endurable than type IIA and type I. Type IIX fibers can hydrolyze ATP at a high rate similar to the IIA fibers but, unlike IIA and I type fibers, the IIX type has a high content of glycogen and is therefore very useful in high power outputs. Type IIA and IIX have higher level of PCr and creatine kinase (CK) and therefore a higher capacity to produce ATP and muscle power. (40, 42).

2.4.3 Erythropoiesis

Because mature erythrocytes do not contain a nucleolus they do not possess the ability to synthesize proteins for repair, growth or division. They survive an average of 120 days and must be replaced by new erythrocytes at the average rate of 2 million to 3 million cells per second (17). This process requires ~ 20 to 40 mg/day of iron which is far more than the average amount absorbed from the diet per day (44). The production of erythrocytes takes place in the red bone marrow, a process termed erythropoiesis. In children, most bones are filled with red bone marrow, but as a person matures yellow bone marrow gradually replaces the red marrow. Adults can only produce red blood cells in certain places such as the sternum, ribs, pelvis and upper ends of the long limb bones (17). In the red blood marrow are stem cells that are the ultimate source for all blood cells i.e. RBCs, leukocytes and platelets. These cells, called erythroid progenitor cells, differentiate into pro-erythroblasts, basophilic erythroblasts, polychromatopilic erythroblasts, orthochromatic erythroblasts, reticulocytes, and mature erythrocytes. The maturation and production of early erythroid progenitor cells depends on EPO (44). Reduced O_2 levels to the kidneys stimulates them to secrete the hormone EPO into the blood stream, stimulating erythropoiesis by the red bone marrow (17, 44).

2.4.4 The electron transport chain

In addition to being vital for O_2 transportation, iron plays an essential role in the electron transport chain in the mitochondrion. The transfer of electrons along the chain is made possible by the change in the oxidation state of iron. In the mitochondrial inner membrane, electrons from NADH and FADH2 will pass from electron donors to electron acceptors (among them being Fe^{3+} and Fe^{2+}), until electrons are passed to O_2, which then is reduced to H_2O. This passage of electrons releases energy used to generate a proton gradient across the mitochondrial membrane. This entire process is called oxidative phosphorylation since ADP is phosphorylated to ATP, which muscles can then use for muscle contractions and motions (36, 42).

2.5 Iron deficiency and iron deficiency anemia

Iron deficiency (ID) can occur in two main forms: functional or absolute. Functional ID is a condition where total body iron stores are normal or increased, but the iron supply to the bone marrow is insufficient. More relevant to the present study is absolute ID, present when total body iron stores are low or drained (27). ID develops through three phases. First, iron stores in the reticuloendothelial cells of the liver, spleen and bone marrow are depleted, which is detected as a fall in serum ferritin and is referred to as iron storage depletion. In the second phase, iron transport is decreased and iron supply to the cell is reduced. This phase is represented by erythropoiesis, characterized as low serum iron, increased total binding capacity and a decrease in transferrin saturation. In the third and last phase of iron deficiency, hemoglobin synthesis falls due to insufficient iron supply, resulting in iron deficiency anemia (IDA) (table 2) (2, 45). Signs and symptoms depend generally on the magnitude of the anemia. In early stages individuals can be entirely asymptomatic (16). Patients may complain of poor mental performance and cold intolerance in addition to commonly reporting weariness and exercise-associated dyspnea (7). These symptoms can have a significant impact on athletic performance both in training and in competition. Patients may also complain of a pins-and-needles sensation in the feet and hands, along with headaches, irritability, dizziness or tinnitus (16).

Table 2. Stages of iron deficiency, tissue effects, hematological signs, and cut-off markers *(2, 45, 46).*

Stage of iron deficiency	Tissue effects	Hematological signs	Cut-off markers
Stage 1 Iron storage depletion	Iron stores in liver, spleen and bone marrow are depleted.	Serum ferritin levels drop.	sFer <30 µg/L
Stage 2 Early functional iron deficiency	Transport of iron decreases, hence iron supply to cells is reduced. Erythropoiesis is impaired.	Low serum iron, increased total binding capacity and a decrease in transferrin saturation.	sFer ≤15 µg/L sTfR>8.5 mg/L Transferrin saturation <16%
Stage 3 Iron deficiency anemia (IDA)	Hemoglobin synthesis drops due to inadequate iron supply.	Hemoglobin levels drop.	Hb<130 g/L (male) Hb<120 g/L (female)

ID is one of the most widespread nutrient deficiencies in the modern world. It affects a significant number of the population in nearly every country across the globe (1). The need for iron is increased during specific life stages and according to gender, as for example in preschool children and menstruating and pregnant women (46). The World Health Organization (47) estimated that in 2011, approximately 43% of children, 38% of pregnant women, 29% of non-pregnant women and 29% of all women of reproductive age, have anemia globally. This analysis estimated that the proportion of all anemias amenable to iron was approximately 50% in women and 42% in children. The major causes of IDA worldwide are blood loss, the maternal-fetal bridge of iron deficiency, malaria, hookworm as well as diet, and malabsorption of iron (7).

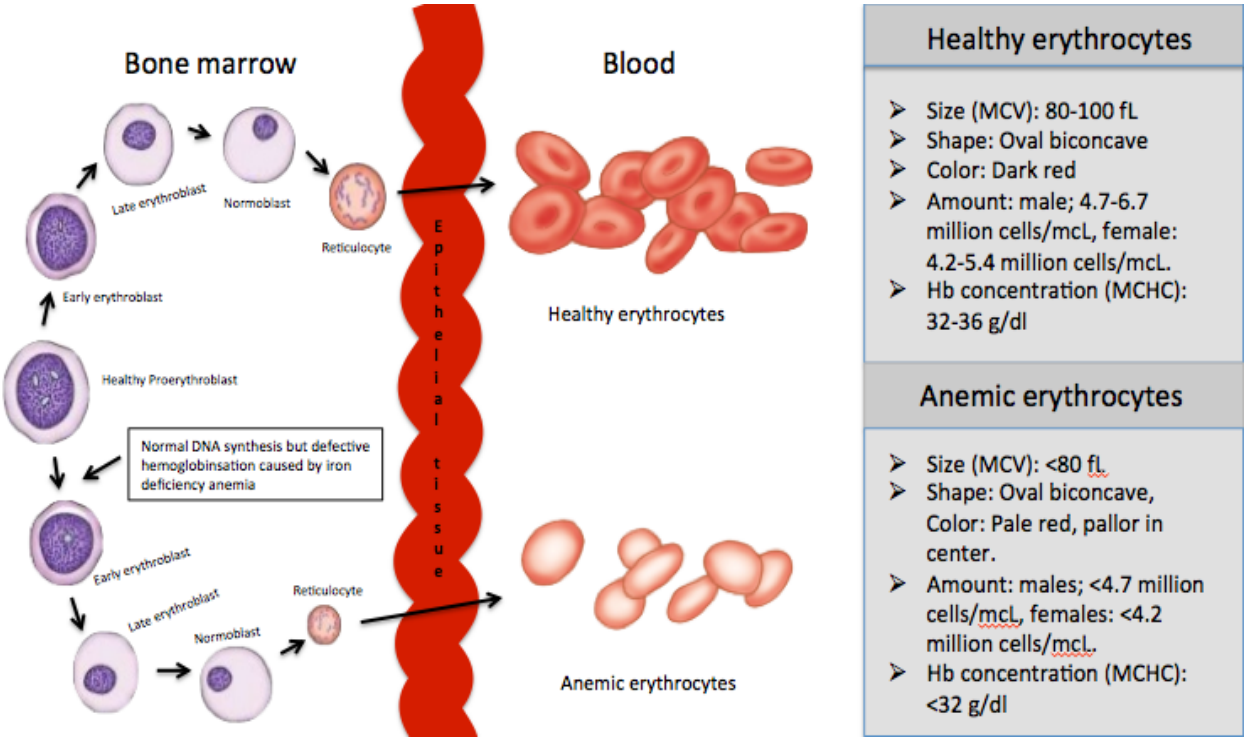

Figure 2. Difference between cell division under normal circumstances and in iron deficiency anemia The difference in size, shape, color, amount and hemoglobin concentration of the erythrocytes is listed. Figure designed from (2, 45, 48-50).

In addition to those at-risk groups, studies have shown that athletes can be at greater risk of ID and IDA than their sedentary counterparts, and more so females than males (8).

Reduced blood hemoglobin concentration is used to diagnose anemia. The threshold is dependent on age, sex, pregnancy, altitude and smoking. A fully-grown man is considered anemic when his hemoglobin concentration is <130 g/L, and a fully-grown woman when her hemoglobin concentration is <120 g/L (see table 3) (27). However, in order to make a definitive diagnosis the results from several tests are necessary as will be described later (see table 4) (51).

Table 3. Hemoglobin concentration thresholds (g/L) used to diagnose anemia *(27)*.

	Healthy	Mild anemia	Moderate anemia	Severe anemia
Boys and girls (0.5-4 years)	≥110	100-109	70-99	<70
Boys and girls (5-11 years)	≥115	110-114	80-109	<80
Boys and girls (12-14 years)	≥120	110-119	80-109	<80
Non-pregnant women and girls (≥15 years)	≥120	110-119	80-109	<80
Pregnant women and girls (≥15 years)	≥110	100-109	70-99	<70
Men and boys (≥15 years)	≥130	110-129	80-109	<80

Another widely used assessment of anemia is hematocrit or packed cell volume. It yields indicators of mean corpuscular volume (MCV) and the number of red blood cells (RBCs) (46).

There is unfortunately no single standard test to assess ID, so to partially overcome that limitation the use of multiple tests is needed (1). Serum ferritin (sFer) reflects iron stores in the liver and is therefore most commonly used to measure iron stores in the body. Individuals are clinically identified as ID with sFer <12 µg/L and hemoglobin levels of >120 g/L (females) or >130 g/L (males), but a basal sFer level of <35 µg/L is often used as a cut-off to begin supplementation. The physiological range of sFer in adult females is 15 to 200 µg/L. Because of this wide variation the lower limit for indication of latent ID in female athletes is not well established. Different studies use sFer in the range of 12 to 35 µg/L to define ID in study participants, making comparison difficult (45, 52, 53). This measurement is limited to the fact that this is an acute phase protein, meaning that sFer levels are increased during inflammation and infection, and after intensive exercise, in pregnancy and with liver damage (2). If inflammation is present, ferritin levels might be normal or even high. In these cases ID can be positively identified by low transferrin saturation (16).

In ID serum iron levels are reduced and transferrin levels (total iron-binding capacity) elevated. Hence a net reduction in transferrin saturation occurs (1). Transferrin saturation <20% indicates ID (2), therefore measuring serum transferrin receptors (sTfR) can test for ID. sTfR levels increase gradually as the supply of iron to the tissues becomes more deficient (1). Measuring sTfR is helpful to investigate the pathophysiology of anemia, quantitatively assessing the absolute rate of erythropoiesis and the adequacy of bone marrow proliferative capacity for any given degree of anemia. Furthermore, this measurement can help monitor the early erythropoietic response to various forms of therapy when hemoglobin changes have not yet occurred (54).

In addition to these tests, ID can be measured using mean corpuscular hemoglobin content (MCH), where low iron levels in red blood cells is reflected by low MCH (28 to 35 pg. being in the normal range) (16). Reticulocyte hemoglobin content (CHr) is a very early indicator of ID representing the iron demand of erythropoiesis, and often measured along with an absolute reticulocyte count which assesses red cell production (2). Table 4 shows the most commonly used parameters to evaluate iron status and detect iron deficiencies.

Table 4. Parameters commonly used to evaluate iron status and detect iron deficiencies. Showing cut-off for normal iron status, ID, and IDA. Data in this table was collected from following sources (2, 27, 45, 46, 52, 54-56).

Parameter	Definition	Units	Cut-off normal	Cut-off ID*	Cut-off IDA*
Hemoglobin (Hb)	Blood hemoglobin concentration	Grams/liter (g/L)	Female: >120 g/L Male: >130 g/L	Female: >120 g/L Male: >130 g/L	Female: < 120 g/L Male: <130 g/L
Ferritin (sFer)	Protein in blood containing iron, represents iron storage	Micrograms/L (µg/L)	>30 µg/L	12-30 µg/L	<12 µg/L
Transferrin saturation	Represents occupied iron-binding sites of transferrin	Percentage (%)	20% - 50%	<20%	<16%
sTfR	Indirect marker to define ID. Not influenced by inflammation and exercise	Milligrams/L (mg/L)	4.0-6.0 mg/L	> 6.0 mg/L	>6.0 mg/L
Reticulocyte count, absolute	Assesses production of red blood cells	10^9/L	20-100 10^9/L	20-100 10^9/L	20-100 10^9/L
C-reactive protein (CRP)	Indicates infection and inflammation	Milligrams/L (mg/L)	<3 mg/L	<3 mg/L	<3 mg/L[a]
Mean cell volume (MCV)	Average volume of an erythrocyte	Femoliters (fL)	80-100 fL	<80 fL	<75 fL
Mean cell hemoglobin (MCH)	Average weight of Hb in the erythrocyte	Picograms (pg)	26-32 pg	<26 pg	<26 pg
Mean cell hemoglobin concentration (MCHC)	Average concentration of Hb in the erythrocyte volume	Grams/deciliter (g/dL)	32-36 g/dL	<32 g/dL	<32 g/dL

[a] CRP levels ≥5 mg/L are considered elevated, suggesting inflammation. If CRP levels are elevated sFer cannot be used to identify ID or IDA.

*In order to clinically diagnose ID or IDA multiple tests need to be performed and the use off one positive parameter should not be used to diagnose ID or IDA.

2.6 Physical activity, exercise and training

Physical activity is commonly defined as any type of work performed by the skeletal muscles that increases energy consumption beyond that which occurs at rest. Physical activity includes exercise in addition to other activities that involve bodily movement (57, 58). Although it includes exercise, physical activity should not be mistaken as ‘exercise’. Exercise is defined as a subgroup of physical activity that is scheduled, structured, repetitive and purposeful - in the sense where the improvement or maintenance of one or multiple components of physical fitness is the objective (57). Training can therefore be defined as the systematic use of exercises to promote bodily fitness and strength (59).

In order for athletes to improve their performance, alterations in training load are necessary. These changes are made at various times during the training period depending on the phase of training. The training load can be altered by changing frequency, duration and intensity of the training (60). Measurements on training load have been defined as being either internal or external. Internal measurements being relative biological stressors such as heart rate, blood lactate and oxygen consumption, and external measurements being power output, speed, acceleration and time-motion

analysis. In order to provide the greatest insight to training stress, both internal and external measures should be performed in combination (61, 62).

2.6.1 Training amount

In the global recommendations on physical activity for health published by the World Health Organization (63), it is recommended that (young) children and adolescent people accumulate at least 60 minutes of moderate-to-vigorous intensity of physical activity per day, while adults are recommended at least 150 minutes of moderate-intensity aerobic physical activity throughout the week, or 75 minutes per week of vigorous-intensity aerobic physical activity. These recommendations are presented with the aim of reducing the prevalence of non-communicable diseases and improving general health in the world population, rather than focusing on performance. The recommendations on physical activity from Sweden and from the NNR for young people and adults are comparable to those published by the World Health Organization (4, 64).

In order to improve performance, athletes are expected to train more hours per week than the recommendation to the population in general. Average weekly training hours can vary between sports, age, gender and athletic level, from beginners to elite. A British study (The TOYA study) on the effect of training at a young age performed on 453 athletes over the course of three years showed that; young athletes age 17-19 years trained on average 10 and up to 13.5 hours per week, gymnasts and swimmers trained 13.5 and 13 hours respectively, while soccer and tennis players trained 10.3 and 10.5 hours per week respectively (65). A study on Serbian female athletes in diverse sports reported training between 8.3 and 9.8 hours per week among athletes aged 19-22. In that study, distance runners had the highest amount of training hours per week and volleyball players the lowest (66).

2.6.2 Training, IL-6 and hepcidin

The innate immune system offers immediate protection against inflammation. It acts through the recruitment of immune cells such as neutrophils, monocytes, and macrophages who prompt the release of cytokines (67).

Interleuking-6 (IL-6) is a cytokine involved in numerous cell-to-cell immunological processes following their release from immune cells (68). Interestingly, studies have consistently shown high amounts of IL-6 being released into the blood stream from contracting skeletal muscles during exercise (69, 70). As discussed earlier (see chapter 2.3), IL-6 increases hepcidin production through the IL-6/STAT3 pathway and thus can have a significant impact on iron absorption (30). Roecker and coworkers (71) found that hepcidin levels were significantly higher 24 hours after a marathon race but went back to baseline levels 72 hours after the race. In a recent study performed on female runners, hepcidin levels were found to be 200% higher after a 120-minute time trial than after a 60-minute time trial, indicating that exercise duration plays a large part in determining the post-exercise hepcidin response (72).

In addition to high production in skeletal muscles, IL-6 gene expression gradually increases in adipose tissue during exercise. The difference being that IL-6 in the muscle is expressed almost only during the time of exercise, whereas the adipose tissue expression is longer lasting and extends over the recovery period (68, 73).

2.7 Iron and athletes

Iron metabolism and its effects on performance in athletes has grown in importance as a topic of research, due to the vital role iron plays in the transport of oxygen and production of energy in the human body (34, 74-76). As mentioned above, ID has been reported to be more frequent among athletes than among more inactive individuals (2, 3). Female athletes are known to be twice as likely to be ID compared to their sedentary counterparts (52). The prevalence of ID in females competing in sports has been reported to range from 25-36% and may vary quite significantly between training seasons from 14-70% (77-79). The prevalence of IDA has been estimated as 10-38% among female athletes competing in variety of sports (80-82). In contrast with these findings, others have reported that regular sporting activity does not increase the prevalence of ID or IDA in amateur or professional female athletes, compared to sedentary females and regardless of previous iron status (33, 78). The clear effects of ID are a reduction in O_2 transport capacity and a decrease at the cellular level of oxidative capacity, thus affecting muscle metabolism (54, 80).

Being less common among males, ID prevalence tested at 15% among elite male basketball players whereas IDA tested only 3% (80). A recent study on prevalence of ID and IDA in collegiate athletes in the United States found that 30.9% of female athletes were ID and 2.2% had IDA, compared to only 2.9% of the male athletes being ID and 1.2% having IDA (83). Another study on the nutritional status of elite female soccer players in Germany showed that 59% of the players had ferritin serum levels under the recommended levels and that 69% had iron intake below the recommended daily allowance (84). To further support these findings, a recent study on female volleyball players showed a significant difference in ferritin levels between junior and elite players, with significantly lower absolute values in the elite group. Authors concluded that the reasons for this may include differences in training load (85). However, iron status seems to be stable and inadequately related to training in male elite soccer players throughout the competitive season (86). A study performed on Japanese collage athletes in different sports showed that 83% of male athletes consumed more iron than the recommended daily requirements while only 4% did not meet the recommended requirements. However, only 15% of the female athletes consumed more iron than recommended requirements while 83% of them did not (87).

This increased risk of ID and IDA in athletes is thought to be due to diet low in iron, hemolysis (foot strike), increased iron losses (gastrointestinal tract, hematuria, and sweat), or altered intestinal iron absorption, including the effects of inflammation due to training (88-91).

Both ID and IDA can have considerable effects on athletic performance. ID is represented as a decline in sFer stores. This decrease can affect compounds associated with muscle metabolism and thus may result in impaired endurance performance such as energy efficiency and endurance time (52). Low hemoglobin concentration present in IDA can lead to insufficient O_2 transport to the working muscles, resulting in the primary mechanism for reduced performance caused by IDA. This reduction in performance is reflected in decreased VO_{2max} and aerobic capacity. IDA is clinically defined as low hemoglobin concentration in blood and is known as absolute anemia. Another type of anemia, so-called pseudo-anemia also known as sports anemia, can develop as the body adapts to regular aerobic exercise. In this condition, the increase in plasma volume occurs more rapidly (first weeks) than the increase in erythrocyte mass, resulting in relatively lower hemoglobin concentration due to dilution.

However, as training stimulates erythropoiesis and results in a marked increase in blood volume, the relatively small decrease in Hb-concentration is negligible (92, 93). The increase in blood volume in elite athletes likely explains the correlation between hemoglobin concentration and endurance capacity (2, 94).

2.7.1 Athletes and their nutritional needs

Athletes and physically active individuals have higher energy requirements than sedentary individuals due to a higher energy expenditure (40). However, recommendations for energy intake varies between sports, recommending a long-distance runner eat differently than a body builder or a ballet dancer. Although there is some difference between sports, the foundation of these recommendations is based on healthy eating and nutritional adequacy (95). Exercise and training strain many of the metabolic pathways in the body. These pathways require micronutrients to function properly and training can result in muscle biochemical adaptation resulting in an increased need for some micronutrients. This is most true in the case of iron, vitamin D and calcium (96). Athletes should be able to fulfill their need for micronutrients if they consume sufficient energy to maintain body weight from a variety of nutrient-rich foods (97).

In the case of iron, it has been calculated that roughly 6 mg of iron is consumed per 1000 kcal. Due to the increased risk for ID, athletes should try to keep their iron above the recommended daily allowance for non-athletes, that is >15 mg for females and >9 mg for men. In fact, female athletes requirement for iron may be increased by ~70% (96). Still, it is important not to exceed the upper level of recommended iron intake of 60 mg (4). Evidence suggests that vitamin D plays a special role in athletes' health, training and performance and could be needed for optimal muscle function (98). There is however no evidence to support vitamin D being an ergogenic aid for athletes. In individualized assessments it is recommended to determine if vitamin D supplements are required due to inadequate intake or low exposure to ultraviolet B light (sunlight) (96). Athletes who restrict their energy intake or avoid dairy products are at risk of a too low calcium intake (96), but calcium is important for bone health and regulation of muscle contraction (99, 100). Every day 1500 mg of calcium is required to optimize bone health in athletes with low energy intake (96).

Studies have shown however that many athletes are not meeting their energy or micronutrient needs. A study of young swimmers showed that both boys and girls in general did not meet their energy intake requirements and that the girls did not meet their iron requirements (101). A Turkish study on adolescent athletes showed that the majority of the participants had an imbalanced and poor nutritional status and that their intake on several micronutrients was low (102).

The use of dietary supplements is common among athletes (103) but they should not be used to compensate for poor food choices. However, a well-balanced diet reinforces the benefits of evidence-based use of supplements. This means that an athlete who has poor iron status is unlikely to get the full benefit of supplements if his or her diet is insufficient (104).

2.7.2 The female athlete

As highlighted earlier, female athletes are considerably more likely to experience ID or IDA than male athletes. The reasons for this gender imbalance are not entirely known.

It is considered the most likely cause for higher prevalence of ID in female athletes is related to diet and menstruation. Due to a number of reasons, female athletes may find it difficult to consume >15 mg/iron per day through the diet (105, 106). These reasons include energy restriction, the avoidance of foods (such as meat products) with highly bioavailable iron and vegetarianism (107) and may also include disordered eating and eating disorders. Menstrual blood loss is the largest route of iron excretion in pre-menopausal females. Thus, heavy menstrual flow may be a big risk factor for poor iron status in female athletes and it has been reported to be highly prevalent among exercising females (108, 109).

However, research indicate that about 66% of female athletes experience menstrual disorders, a condition that can result in serious health issues (110, 111). Menstrual disorders along with eating disorders and osteoporosis are termed the female athlete triad. Poor diet (with or without eating disorders) can alter the activity of the hypothalamic pituitary axis along with hormones involved in menstrual function and bone metabolism, thus making the female athlete syndrome of three interrelated spectrums (112). Each spectrum can have serious irreversible consequences on the athlete's health, including infertility, eating disorders, nutrient deficiencies and low bone mineral density (113). When diagnosing the female athlete triad, it is rare to diagnose all components at once. Female athletes more often evolve symptoms of the syndrome along with non-congruent disease continuums for each component. Unfavorable energy balance is more often the primary disorder in the triad, leading to menstrual dysregulations and low bone mineral density (114).

Treatments most often used to treat the female athlete triad are either pharmacological therapies like hormone replacement therapy or the use of oral contraceptive pills or non-pharmacological therapies, but also include alternations in diet and exercise behaviors. Increased energy intake along with supplements or increased intake of calcium, iron and vitamin D from the diet and/or reduction in energy expenditure have proved efficient (113, 115). Lagowska (116) found that restoring iron status in female athletes using nutritional intervention may improve menstrual function.

2.8 Screening for iron deficiency in athletes

As previously highlighted, prevalence of ID and IDA among athletes is high. Alaunyte and coworkers (117) found that over a third of the female runners participating in their study had sFer levels that indicated ID, using <12 µg/L as the cut-off value. Similarly, Santolo and coworkers (78) found that a third of their amateur female athletes had ID and a fifth had IDA. Sandström and coworkers (118) looked at ID in adolescent female athletes and discovered that ID and IDA were common among female adolescents, both in athletes and in non-athletic persons. This is despite findings that should favor a better iron status in athletes such as higher iron intake and less menstrual bleeding. In a recent study Lenczowska and coworkers (8), found a very high frequency of latent ID (stage 1 and 2) among female adolescent athletes. Sinclair and coworkers found that 29% of recreationally active women and 4% of recreationally active men had ID. These findings stress the importance for female athletes to be monitored at regular intervals for both ID and IDA. Simple parameters such as sFer and transferrin

saturation seem adequate to identify most of the ID in the majority of athletes (8, 78, 117-119). Fallon (120) concluded that due to the effects of ID and IDA on performance, it is reasonable to perform a full blood count and a serum ferritin test on athletes entering elite training programs. According to the literature the ferritin cut-off for athletes 15 years and older should be 30 μg/L, since the iron stores are considered to be low when ferritin levels are 15 to 30 μg/L and equivalent to empty if they are under 15 μg/L (2).

With regard to the prevalence of ID and IDA among athletes, the use of iron supplementation with the intent of both increasing health and the possibility of increasing performance has been studied for many years. Due to the negative effects a compromised iron status can have on health and physical and mental performance, the Academy of Nutrition and Dietetics (AND), Dietitians of Canada (DC) and the American College of Sports Medicine (ACSM) came to the conclusion that a compromised iron status warrants prompt medical intervention and monitoring. Athletes who are ID or are concerned about their iron status should adopt eating strategies that promote an increased intake in iron-rich food sources as the first line of defense. Supplemental recommendations should be personalized, recognizing that targeted supplementation may be implied to treat or prevent ID (96, 121).

2.8.1 Iron supplementation

A few studies have examined the effects of iron supplementation on iron status and performance in both professional and non-professional individuals (see table 5). DellaValle and coworkers conducted a randomized control trial with oral iron supplementation in ID female rowers and showed improvements in energy expenditure (assessed with indirect calorimetry) and energy efficiency compared to the placebo group (88). Hinton and coworkers found significant improvements in time-trials with iron-supplemented ID training females, compared to the placebo-treated group (122). McClung and coworkers (123), investigating female army recruits found improvement in two-mile running times in recruits with IDA after supplementation. However, those with ID did not show any change in performance (123). Similarly, Waldvogel and coworkers (124) found no significant difference in fatigue or aerobic capacity in their study on ID female blood donors after iron supplementation. Garvican and coworkers performed a randomized control trial on 27 highly-trained distance runners. Participants were sorted into four groups according to iron status and supplementation. The runners had either low (sFer <35 μg/L and transferrin saturation <20%) or suboptimal iron status (sFer <65 μg/L) and were supplemented with either oral iron or intravenous iron inserted into a forearm vein. They found that both forms of supplementation increased ferritin levels in all four groups. tHb-mass increased in the low intravenous group and was associated with an increase in VO_{2max} and less run time to exhaustion (125).

Table 5 gives an overview of studies investigating the effects of iron supplementation on iron status and performance in active males and females (both amateur and professionally active people). Also included in the table is one cross-sectional study investigating the effects of ID on female rowers' endurance training and exercise capacity. As seen in table 5, the cut-off markers for ferritin used in these studies range widely, between 16 to 35 μg/L. More research is necessary to define the cut-off marker for ferritin that should be used in future studies on athletes, and whether that cut-off marker should be different from the one used for sedentary individuals.

Iron supplementation can be administered to athletes in two ways: orally or via intramuscular or intravenous injection. The oral option can be limited and restoration of iron storage can take 3-6 months. It has been suggested that the effect of iron supplement treatment on sFer diminishes once the treatment continues for longer than 80 days (126). Time periods of iron supplementation and quantities of iron varies between studies, making it difficult to know the optimal dose of iron that ensures the most effective and time-efficient treatment. Burden and coworkers (127) gave ID elite runners a single 500 mg intravenous iron injection that improved their iron status for at least 4 weeks. DellaValle and coworkers (88, 128) showed that a 100 mg/day of oral iron for 6 weeks improved iron status in ID female rowers. Hinton and Sainclair (129) gave their ID athletes considerably lower doses of oral iron - 30 mg/day for six weeks. The iron supplementation significantly increased the sFer levels compared to the placebo group but had no effects on Hb or hematocrit levels. McClung and coworkers (123) studied the effects of an eight-week, 100mg/day iron supplementation on iron status in ID female soldiers during military training. They found that the supplementation attenuated the reduction in iron status indicators that the military training caused. These studies indicate that iron supplementation can improve iron status in athletes, but that dosage and duration of the supplementation needs to be studied further in order to find an optimal treatment.

However, iron overload can occur in some individuals. This is often associated with genetic factors, more specifically a mutation in the HFE gene (130). A screening of 99.711 racially diverse North-Americans showed that 299 individuals had a mutation in the HFE gene, being most common in Caucasians (131). Local intestinal toxicity has been found to be a side effect of therapeutic iron, which can appear in the range between 50 to 60 mg/d. Therefore a varied iron-rich diet should be adopted as a first resort in preventing iron deficiency, the diet to include iron-rich foods such as dark greens, eggs, red meat, fish, pulses and beans (4). Alaunyte and coworkers (132) performed a cereal-based dietary iron intervention on English female runners. They found promising signs that staple food products can improve dietary iron intake and iron status in female runners. Furthermore, a study on dietary habits of Dutch athletes showed that mean iron intake was below the daily recommended intake, both in athletes who reported taking supplements and in those not using them. This suggests that athletes should be practical about their dietary habits (133).

Table 5. Overview of studies investigating the effects of iron supplementation on iron status and performance in active males and females.

Author, year, study design	Population	Baseline Iron Status	Intervention	Iron status results	Performance results
Hinton, 2000 **(122)**. Randomized ctrl trial.	42 ID females (18-33 yr).	sFer <16 µg/L, Hb >120 mg/L.	Oral, 100 mg/d ferrous sulfate for 6 weeks. Trained 30 min/d, 5 days/week for 4 weeks.	T↑ sFer, ↓sTfR	T and P ↓TT time, RER: T↓TT time greater vs P; T↓%VO_{2max} during TT.
Hinton and Sinclair, 2007 **(129)**. Randomized ctrl trial.	20 ID male and female (18-41 yr).	sFer <16 µg/L, sTfR>8.0 mg/L. Females; Hb>120g/L, male; >130 mg/L.	Oral, 30 mg/d of ferrous sulfate for 6 weeks.	T↑ sFer NS effects on Hb or hematocrit.	T did not decline in VT, ↑ gross energetic efficiency.
McClung, 2009 **(123)**. Randomized ctrl trial.	219 female army recruits, ID, IDA and normal, (20 yr).	ID; sFer < 12 µg/L, transferrin <16% or RBC distribution width >15%. IDA; <120 g/L.	Oral, 100 mg/d ferrous sulfate, 8 weeks.	P↓sFer, ↑sTfR	T ↓ 2 mile run time
Waldvogel, 2012 **(124)**. Randomized ctrl trial.	154 females (18-50 yr) with ID.	sFer ≤ 30 µg/L, Hg ≥ 120 g/L.	Oral, 80 mg/d ferrous sulfate, 4 weeks.	T↑ Hb, sFer compared to P.	NS effect on fatigue or aerobic capacity.
DellaValle, 2012 **(128)**.* Cross-sectional study.	48 (24 ID, 24 normal) female rowers (> 18 yr)	ID; sFer <20 µg/L. Normal >20 µg/L.	No intervention	---	↓ training load/day and VO_{2max}, slower PR in 2-km, first 3600m of a 4-km slower.
Burden, 2014 **(127)**. Randomized ctrl trial.	15 ID male and female runners (20-23 yr)	Females; sFer <30.0 µg/L, Hb >120 mg/L, male; sFer <40 µg/L, Hb >120 mg/L.	Intravenous iron, 500 mg Ferinject. Exercise tests before treatment, within 24 h and 4 weeks after treatment.	T↑ sFer, transferrin, serum iron. NS difference in tHb-mass.	NS difference in VO_{2max}, submaximal blood lactate, running economy, PRE or time to exhaustion.
DellaValle, 2014 **(88)**. Randomized ctrl trial.	40 (17 normal, 23 ID) female rowers (>18 yr)	ID; sFer <20 µg/L, Hb >120 mg/L.	Oral, 100mg/d ferrous sulfate, 8 weeks.	T↑sFer, ↓sTfR	NS effects on TT time and maximal RER. T and P ↑ maximal WR and total EE.
Garvican, 2014 **(125)**. Randomized ctrl trial.	27 male and female distance runners.	Low; sFer <35 µg/L, transferrin <20%. Sub; sFer <65 µg/L. 4 groups; IV-low, IV-sub, Oral-low and Oral-sub.	Oral; 105 mg elemental iron 2x/d in oral-low; 1x/d in oral-sub. IV; 2-4 times Fe carboxymaltose, 6 weeks.	↑sFer, ↑ tHb-mass in IV-low, NS difference in Hb.	↑ VO_{2max}, time to exhaustion in IV-low group.
Brownlie, 2002 **(134)**. Randomized ctrl trial.	49 ID untrained females (18-33 yr)	ID: sFer <16 µg/L, Hb >120 g/L.	Oral, 50 mg $FeSO_4$ 2x/d, 6 weeks.	T↑sFer, sTfR and transferrin saturation. Did not affect [Hb].	↑ VO_{2max} and maximal RER in both groups. Significantly greater VO_{2max} in T group.
Brownlie, 2004 **(135)**. Randomized ctrl trial.	41 ID untrained females (18-33 yr)	ID: sFer <16 µg/L, Hb >120 g/L.	Oral, 50 mg $FeSO_4$ 2x/d, 6 weeks. Trained 5d/week for at least 4 weeks.	Significant effects on serum iron, transferrin saturation, sFer, and hematocrit.	↓time in TT, ↓% of VO_{2max}, ↓□WR when sTfr >8 mg/L.

P: placebo group; T: treatment group; NS: No significant difference; TT: time trial; RER: respiratory exchange ratio; WR: work rate; EE: energy expenditure; PR: personal record, ID; Iron deficiency, IDA; Iron deficiency anemia.

*Investigated the effects of ID on female rower's endurance training and exercise capacity

2.9 Body composition and percentage body fat

The human body consists of water, proteins, minerals and fat. It is often divided into fat mass (FM) and fat-free mass (FFM) (136). A favorable ratio of fat mass versus fat-free mass (that is, low fat mass coupled with high fat-free mass) can decrease the risk of various diseases such as sarcopenia and metabolic syndrome, in addition to improving quality of life (137). Assessments of body composition can be done in a variety of ways. Despite forming a central tenet of medical research for almost a century, there has been no accepted golden standard for body fat assessment with any better than 1% accuracy across a range of body types (138, 139). It is clear that body composition is influenced by various factors including age, gender, race, heredity and stature (136).

One of the more precise methods for body fat assessment is by using x-rays. Dual energy X-ray absorptiometry (DXA) scans individuals using x-rays at two different energy levels. The amount and percentage of adipose tissue, soft tissue and bone mineral density can be calculated based on the restriction in the fluctuation of the x-rays across the fat and fat-free masses (74, 140).

Body mass index (BMI) is a weight-height index that has been used for many years in an attempt to determine the “ideal weight” (relationship between body length and body mass) of an individual, and providing for a measure of ponderosity (138). The validity of BMI as a measure of adipose tissue in non-athletic populations is based on the assumption that, as BMI increases, so does adiposity (141, 142). When using the BMI to measure overweight and obese youth between 15 and 18 years of age, both gender and age has to be considered. In 2000 Cole and coworkers developed international cut-off points for overweight and obesity in young individuals using data from six different countries. These cut-off points are shown in table 6 (143). The international cut-off points for overweight and obesity in adults are however the same for all ages ≥18y and both genders. Adult BMI can be classified into six categories; under-nourished, normal weight, overweight, obesity group one, obesity group two and obesity group three with the BMI cut off-points being <18.5, 18.5 - 24.9, 25 - 29.9, 30 - 34.9, 35 - 39.9, ≥40, respectively (144).

Table 6. International cut off points for BMI for overweight and obesity in young individuals aged 15-18 years, divided by sex (119).

	Body mass index 25 kg/m^2 (overweight)		**Body mass index 30 kg/m^2 (obesity)**	
Age (years)	**Females**	**Males**	**Females**	**Males**
15	23.94	23.29	29.11	28.30
15.5	24.17	23.60	29.29	28.60
16	24.37	23.90	29.43	28.88
16.5	24.54	24.19	29.56	29.14
17	24.70	24.46	29.69	29.41
17.5	24.85	24.73	29.84	29.70
18	25	25	30	30

2.9.1 Body composition measurements on athletes

Body composition is important as a proxy variable for health and athletic performance. Having higher FFM and lower FM is related to improved performance in athletes, and therefore athletes tend to have relatively higher ratio of FFM and lower FM compared to sedentary individuals (137). A desirable percentage of %BF for physically active individuals and sedentary individuals is shown in table 7.

Table 7. Desirable percentage body fat (%BF) for children 15-17 years old and sedentary adults and physically active adults, respectively (121, 122).

Sedentary females	**Undesirable**	**Low**	**Average**	**High**	**Obesity**
15 years		15.7	24.1	29.9	
16 years		15.5	24.3	30.1	
17 years		15.1	24.4	30.4	
18-34 years	<20	20	28	35	>35
35-55 years	<25	25	32	38	>38
55+ years	<25	25	30	35	>35
Sedentary males					
15 years		10.4	15.8	20.7	
16 years		10.1	15.5	20.3	
17 years		9.8	15.4	20.1	
18-34 years	<8	8	13	22	>22
35-55 years	<10	10	18	25	>25
55+ years	<10	10	16	23	>23
Physically active females		**Low**	**Average**	**High**	
18-34 years		16	23	28	
35-55 years		20	27	33	
55+ years		20	27	33	
Physically active males					
18-34 years		5	10	15	
35-55 years		7	11	18	
55+ years		9	12	18	

When using DXA to measure body composition in athletes the main advantage of the technique is that it is fast and convenient, in addition to being minimally influenced by fluctuations in body water content (138). However, this method is thought to have its limitations when scanning athletic populations where height and wide trunk breadth are considered a favorable physique (145). The scanning area is typically 60 - 66 cm wide and 190 -198 cm long, so in order to be able to include subjects taller or wider than the scanning area particular techniques are used, such as excluding the head or feet, scanning with bent knees or joining two partial scans. These techniques can be increasingly prone to error but are considered acceptable (145, 146). Despite these limitations, measuring fat mass in athletes using DXA scans is considered one of the most accurate methods of assessing body composition available today (140, 145, 147-149).

When using BMI to measure body composition in athletes, the association between increased BMI and consequently increased adiposity is greatly reduced and therefore adiposity is consistently overestimated in athletic populations, thus the measure is prone to error (141, 142).

2.9.2 Body composition and ID

Studies have shown that the prevalence of ID is higher among obese individuals than those of normal weight. Studies indicate that this can be explained by the chronic low-grade inflammation, a 2-3-fold elevation in inflammatory cytokines present in obese individuals (11-13, 150). Bekri and coworkers (10) found that hepcidin messenger RNA (mRNA) expressed in adipose tissue of obese patients, correlates with indexes of inflammation such as IL-6 and C-reactive protein. Of these patients, 68% had low transferrin saturation ratio and 24% presented with anemia. Yanoff and coworkers (12) found that adipocyte hepcidin expression positively correlated with body mass index (BMI, kg/m^2) in obese patients with a trend towards a negative association with transferrin saturation, therefore lower bioavailable iron might be related to greater adipose hepcidin. Similarly, a study on individuals undergoing cardiac surgery (BMI 19.7 - 36.8 kg/m^2) demonstrated an increase of hepcidin mRNA expression in adipose tissue after the acute surgical procedure (151). Aeberli and coworkers (152) found that obese children were significantly more likely to be iron deficient compared to normal weight children. No difference was detected in dietary iron intake or bio-availability. The serum hepcidin levels were however significantly increased in the obese group compared to the normal weight group, suggesting hepcidin-mediated reduced iron absorption. Tussing-Humphreys and coworkers (153) found that ID in obese females was mainly a condition of true body iron deficit rather than a maldistribution of iron due to inflammation, although inflammation might affect the condition by hepcidin-mediated inhibition of iron absorption. A recent study showed that iron absorption increases in iron-deficient obese subjects following fat loss after bariatric surgery and that both IL-6 and hepcidin were significantly lower in all subjects after surgery (154). A study on adolescent girls showed an inverse association between BMI and serum ferritin, where overweight adolescents demonstrated an increased prevalence of IDA (14).

To the best of the author's knowledge, no studies have been performed on athletes in relation to adipose tissue, hepcidin and ID.

3 Methods

The data in the present study derives from previous and ongoing research studies on young elite athletes at the Sports Medicine Unit, Umeå University, Sweden. Four subsets of results from blood sample analysis were gathered and analyzed, i.e., young elite athletes from two upper secondary sports schools (in Umeå and Lycksele); from female soccer players in the Swedish national team; and from individuals that perform exercise-training for 5-22 hours per week, from an ongoing research project (ProIron) in Umeå and including information on personal and physical data in all four datasets.

3.1 Participants

The novel dataset used in the present thesis includes competitive athletes and physically active subjects regularly exercising at a minimum of five hours per week who were recruited during the years 2013-2017. The data includes 443 subjects; 271 females and 172 males, 16 - 38 years of age with information on training hours per week and supplement intake as well as blood and iron status. Information on both supplement intake and training hours were obtained with questionnaires, and on the same day as the blood was drawn. The questions on training and supplement intake were comparable in all four cohorts. In addition, from the 433 subjects, results from body composition measurements (BMI and %BF) were measured by Dual-energy X-ray absorptiometry (DXA) and available for 266 individuals (59%) - 122 females and 144 males. Both height and weight were measured using the DXA machine. All participants signed an informed consent form before participating in any of the studies. All studies were approved by the regional ethical committee of northern Sweden (Dnr 2017-372-32M; Dnr 2013-287-31; Dnr 2011-161-31M).

3.1.1 ProIron

Baseline data from initial screening in the ongoing double-blinded randomized control trial “ProIron” was obtained in the latter half of 2017. The data included 155 volunteers; 13 males and 142 females, aged between 16 - 37 years and were recruited mainly from sports teams and also from health centers and local colleges in the vicinity of Umeå University.

3.1.2 Athletes from upper secondary sports school

Participants included were 244 adolescent athletes; 142 males and 102 females aged between 16 - 19 years and studying in Dragonskolan Elite Sports School in Umeå in the autumn/fall of 2015. These subjects were participants in a study conducted during the years 2013 - 2017.

3.1.3 Subtests on athletes

All data used from the subtests performed on both the elite cross-country skiers and on the Swedish national soccer team were recorded in 2014. These subtests were performed on a regular basis as part of training protocols. The data on the upper secondary school cross-country skiers from Lycksele included 32 elite skiers; 17 males and 15 females between 16 - 19 years of age. The data used from the Swedish national soccer team included 12 elite female soccer players between 18 - 26 years.

3.1.4 Criteria used for this study

When composing the database used for this study, all participants that reported taking either iron or ascorbic acid supplements, including all types of multivitamins prior to blood sampling, were excluded. ID was defined as having pFer <30 μg/L and Hb level of ≥130 g/L (males) or ≥120 g/L (females). IDA was defined as having pFer <30 μg/L and Hb level <130 g/L (male) or <120 g/L (female) (2, 155-157).

3.2 Blood measurements

In all four cohorts venous blood samples were collected from the median cubital vein using the BD Vacutainer blood collection system which is a closed vacuum system consisting of a sterile double-ended needle (0,8 x 25mm) with safety valve (Becton, Dickinson and Company, Plymouth, UK) (158). The WHO guidelines on drawing blood was followed in this procedure (159). Blood samples were collected into two types of sample tubes, in all collecting 8 mL of blood from each subject. Four mL of blood was collected into a LH PST II tube containing spray-coated lithium heparin and a gel for plasma separation, and four mL was collected into a spray-coated K2 EDTA tube (used for whole blood hematology determinations and immunohematology testing) (Becton, Dickinson and Company, Plymouth, UK) (160, 161). All blood samples were taken by a professional nurse, a staff member working at the Sports Medicine unit. Blood samples obtained from participants were analyzed in Cobas 8000 using the IRON GEN2 test from Roche (162). Blood parameters measured from the samples included leukocyte, erythrocyte, hemoglobin, hematocrit, thrombocyte, erythrocyte-MCV and plasma ferritin. In addition erythrocyte-MCH was calculated using the equations shown in Appendix II. Blood samples were analyzed in the Clinical Chemistry Laboratory at the University Hospital of Umeå (NUS).

3.3 Statistical analysis

The analysis of participants blood samples was displayed in Microsoft Excel for each blood value used. Those results, in addition to all information regarding participants gathered during recruiting, were transferred to IBM SPSS Statistic version 24 (IBM Corporation, United States), where all the data included in this study was analyzed. Cross tabulation was performed to assess the frequency and percentage of participants grouped by body composition, gender and age. Few participants were defined as low in %BF, thus they were grouped together with the average %BF group. Similarly, underweight and normal weight as defined by BMI were grouped together and overweight and obese individuals together. The Pearson Chi-Square test was used to compare difference between groups. Training hours were defined into three groups, under 8 hours, 8.5 hours to 11 hours and 11.5 hours or more. In the questionnaire the participants were asked to specify the amount of training hours per week within an accuracy of one half-hour.

Associations between blood parameters, body composition variables and training amounts were inspected by Spearman's correlation coefficient. An independent sample T-test was used to compare differences in blood parameters, and age, training and body composition between iron status groups. The Mann-Whitney test was used to compare difference between body composition groups and blood variables, age and training amounts.

The logistic regression model was used to predict the likelihood of iron deficiency based on age, gender, training amount in hours, BMI and %BF. As well the logistic regression was run and divided by gender. Level of significance was set at $P \leq 0.05$.

Due to the fact that few individuals in the combined cohort were diagnosed with IDA (16 females and 3 males), ID and IDA were grouped together in order to strengthen the analyzes. However, the groups were viewed separately when characteristics of the population were examined.

3.4 Author's contribution

My research work on this project was segregated into four stages.

1. Assisting with blood sampling

My work on this project began with assisting the blood sampling for the screening part of the ProIron study. With the principal investigator on the project Michael Svensson, and Roger Anderson the nurse who oversaw the blood sampling, we went to sports clubs and a sports high school to draw blood from volunteers. My assistance involved preparing the kits for blood sampling, labelling the sample tubes before they were sent to the laboratory and doing any other relevant task that came up during our visits.

2. **Recording data from blood samples and personal information**

All data was recorded into Excel sheets. Each study or subtest was recorded in a separate sheet. Results from the screening process from the ProIron study were recorded immediately into an Excel sheet once the results arrived from the laboratory, along with age, gender and training hours per week. The blood sample data from the subtests and the high school athletes had already been recorded into Excel sheets. My work was therefore to match training hours and age to the subject's blood measurements in addition to taking out any additional and irrelevant blood measurements which were not available in the other datasets.

3. Statistical analysis and presentations

I was responsible for importing both blood sample results and other gathered personal information to the program SPSS version 24. I performed an error check to correct any errors that occurred. I performed all appropriate statistical analyses on the dataset with supervision from my supervisors.

4. Writing the thesis.

I wrote this thesis as well as a draft for a scientific paper using the results from the statistical analysis. PubMed, ProQuest and Google Scholar were used in the search for relevant articles for references, in addition to numerous text books. Some references were gathered from citations in other articles. The online search was performed using search words such as: Iron deficiency, Athletes, Iron deficiency anemia, Performance, Training amount, Iron metabolism and Iron supplementation.

It is worth noting that initially the intent of my trip to Umeå was to take part in the ProIron study, an interventional study on athletes. The purpose of the study was to see if iron supplementation with or

without lacto-bacteria would have effects on aerobic capacity and performance in iron-deficient athletes. Due to delays in the screening process it was decided to use existing data and write a theoretical summary that covers both topics.

4 Results

4.1 Results of the second aim

Results related to the primary aim, are presented in the draft article in Appendix I: Body composition in relation to iron deficiency among athletes and physically active individuals. Those are the main results of this thesis.

Results of the secondary aim; to explore whether the probability of ID increases as the amount of training increases, are presented below. Note that tables have consecutive numbering aligned to the chapters of this thesis. These results are presented as a short communication with a brief description of the results, mainly shown in the table at the end of the Results chapter.

4.2 Iron deficiency and training amounts among athletes

As shown in Table 8, 451 participants from the four combined cohorts were included in this part of the study, 283 females and 168 males. Significant difference was found between genders in all blood and iron parameters, except MCH. Female participants were considerably more likely to have ID than their male counterparts, 29.7% compared to 8.9% of the males (P<0.001). Further, IDA was more frequent among females than males, 5.7% and 1.8%, respectively (P<0.001). When comparing training amounts, male participants trained more hours per week compared to the female participants (P<0.001), as shown in table 9.

A Spearman‘s correlation coefficient was used to assess the association between training hours and blood variables (ferritin, hemoglobin, erythrocytes and MCH) in females and males. Preliminary analyzes showed no significant relationship between training hours or any of the blood measurements taken (table 10). When divided into three groups by amount of training (i.e. 5 - 8 hours, 8.5 – 11 hours and 11.5 hours or more), a comparison showed a significant difference in age between all female training groups, but particularly between the females who trained the least and the ones who trained the most. Males training 8 hours or less were older in years than the ones training 11.5 hour or more. When comparing those with normal iron status with those who had ID with or without anemia, both females and males in the ID group were younger, had lower hemoglobin levels, ferritin levels, MCH and MCV. Difference in training hours was only significant between the male groups, indicating that those with ID trained less than those with normal iron status (table 12).

A binomial logistic regression was performed to ascertain the effects of age, gender and training hours on the likelihood that participants had ID. The logistic regression model was statistically significant, $X^2(3) = 48.448$, P ≤0.001. The model explained 15.4% (Nagelkerke R^2) of the variance in ID.

Of the three predictor variables only two were statistically significant - age and gender - while training amounts did not have an impact (table 13). Females had 4.67 (95%CI: 2.59-8.43) times higher odds of experiencing ID compared with men, but increased age was associated with a reduction in the likelihood of experiencing ID. Table 14 shows the same models but divided by gender. The logistic regression models were both statistically significant. The female model $X^2(2) = 11.231$ P ≤0.004 explained 5.5% (Nagelkerke R^2) of the variance in ID, and the male model $X^2(2) = 6.333$ P ≤0.045 explained 7.9% (Nagelkerke R^2) of the variance. The only significant predicting variable was age for females while training hours were significant for males only. In both instances the associations showed a reduction in

the likelihood of experiencing ID, i.e. the likelihood of experiencing ID decreased with age among women and more training hours predicted less likelihood for men to experience ID.

Table 8. Characteristics of the study population according to gender.

	Females (n=283)	Males (n=168)	P-value
Age (years)	18.6 (2.8)	20.9 (5.1)	<0.001*
Training (hours/week)	9.5 (2.4)	11.4 (3.6)	<0.001*
Plasma ferritin (μg/L)	43.3 (24.8)	76.8 (49.3)	<0.001*
Hemoglobin (g/L)	131.9 (8.6)	146.1 (8.5)	<0.001*
MCH (pg)	29.4 (1.7)	29.3 (1.3)	0.420
MCV (fL)	88.2 (4.3)	86.9 (3.7)	<0.001*
Erythrocytes (10^9/L)	4.5 (0.3)	5.0 (0.3)	<0.001*
Iron status			<0.001*
Normal Iron status (%)[a]	183 (64.7)	150 (89.3)	
Iron deficiency (%)[a]	84 (29.7)	15 (8.9)	
Iron deficiency anemia (%)[a]	16 (5.7)	3 (1.8)	

Iron deficiency cut off points are ferritin < 30 μg/L and hemoglobin ≥120 g/L (females) ≥130 g/L (males) and for iron deficiency anemia the cut off points are ferritin <30 μg/L and hemoglobin <120 g/L (females) <130 g/L (males).

Data is displayed as mean and standard deviation (SD)

[a] Are represented as frequency and percent in each group

*Significant difference between gender (P≤0.05) measured with Mann-Whitney test

Table 9. Training amount categorized into groups.

	Females (n=282)	Males (n= 166)	P-value
8 hours or less (%)	91 (32.3)	31 (18.7)	<0.001*
8.5 to 11 hours (%)	131 (46.5)	61 (36.7)	
11.5 hours or more (%)	60 (21.3)	74 (44.6)	

Data is displayed as frequency and percentage

* Significant difference between gender (P≤0.05)

Table 10. Blood parameters in correlation with training amount.

	Training amount (r)	
	Females (n=282)	Males (n=166)
Ferritin (μg/L**)**	-0.006	0.020
Hemoglobin (g/L)	-0.053	0.006
Erythrocytes (10^9/L)	-0.029	0.013
MCH (pg)	-0.054	0.063

Spearman's correlation coefficient (r) was used to define the significance of associations between the variables. The test was non-significant for all variables.

Table 11. Blood parameters and age divided by training amount and gender.

Females	**≤8 hours (n=91)**	**8.5 – 11 hours (n=131)**	**≥11.5 hours (n=60)**
Age (years)	21.8[a,c]	18.6[a,b]	19.9[b,c]
Hemoglobin (g/L)	132.7	131.5	131.4
Ferritin (μ/L)	46.0	40.2	46.0
MCH (pg)	29.5	29.3	29.3
MCV (fL)	88.3	88.3	88.0
Erythrocytes (10^9/L)	4.5	4.5	4.5
Males	**≤8 hours (n=31)**	**8.5 – 11 hours (n=61)**	**≥11.5 hours (n=74)**
Age (years)	21.3[c]	18.4	17.9[c]
Hemoglobin (g/L)	146.1	146.6	145.7
Ferritin (μ/L)	83.0	72.6	74.5
MCH (pg)	29.4	29.4	29.2
MCV (fL)	87.2	87.4	86.4
Erythrocytes (10^9/L)	5.0	5.0	5.0

Data is displayed as mean

[a] Significant difference between the ≤8 hours group and the 8.5-11 hours group (P≤0.05)

[b] Significant difference between the 8.5-11 hours group and the ≥11.5 hours group (P≤0.05)

[c] Significant difference between the ≤8 hours group and the ≥11.5 hours group (P≤0.05)

Table 12. Blood parameters, age and training amount grouped by iron status and gender.

Females	***Normal iron status (n=183)***	***Iron deficiency (n=99)***	***P-value***
Age (years)	20.4	19.0	0.001*
Training (hours/week)[a]	9.5	9.5	0.865
Hemoglobin (g/L)	133.8	128.2	<0.001*
Ferritin (μ/L)	56.0	19.9	<0.001*
MCH (pg)	29.8	28.5	<0.001*
MCV (fL)	88.9	86.9	<0.001*
Erythrocytes (10^9/L)	4.5	4.5	0.830
Males	***Normal iron status (n=148)***	***Iron deficiency (n=18)***	***P-value***
Age (years)	18.8	17.8	0.020*
Training (hours/week)[b]	11.7	10.0	0.032*
Hemoglobin (g/L)	146.8	140.2	0.002*
Ferritin (μ/L)	81.8	22.8	<0.001*
MCH (pg)	29.5	28.1	<0.001*
MCV (fL)	87.8	84.9	0.010*
Erythrocytes (10^9/L)	5.0	5.0	0.973

Data is displayed as mean

*Significant difference between normal iron status and iron deficiency (P≤0.05)

[a] n=181 and 96 for Normal iron status and Iron deficiency, respectively.

[b] n= 142 and 17 for Normal iron status and Iron deficiency, respectively.

Table 13. Logistic regression predicting likelihood of iron deficiency based on age, gender and, training amount (hours/week).

	B	*S.E.*	*Wald*	*P-value*	*Odds Ratio*	*95% C.I.*	
						Lower	*Upper*
Age (years)	-0.11	0.04	11.80	0.001*	0.88	0.81	0.94
Gender	1.54	0.30	26.23	<0.001*	4.67	2.59	8.43
Training)	-0.09	0.05	3.19	0.74	0.92	0.83	1.01
Constant	1.25	0.99	1.58	0.209	3.48		

*Statistically significant variables (P≤0.05)

Gender is for females compared to males

Table 14. Logistic regression predicting likelihood of iron deficiency based on age and training amount (hours/week) divided by gender.

Females	*B*	*SE*	*Wald*	*P-value*	*Odds Ratio*	*95% CI*	
						Lower	*Upper*
Age (years)	-0.13	0.04	9.85	0.002*	0.88	0.81	0.95
Training	-0.03	0.06	0.35	0.555	0.97	0.86	1.08
Constant	2.20	1.07	4.25	0.039	9.05		
Males	***B***	***SE***	***Wald***	***P-value***	***Odds Ratio***	***95% CI***	
						Lower	***Upper***
Age (years)	-0.13	0.11	1.48	0.224	0.879	0.71	1.08
Training	-0.20	0.09	4.56	0.033*	0.821	0.69	0.98
Constant	2.36	2.30	1.05	0.306	10.566		

* Statistically significant variables (P≤0.05)

5 Discussion and conclusion

The results for the first aim related to BMI and %BF and association with ID among athletes and physically active individuals are discussed in Appendix I. The results for the second aim, whether the probability of ID increases as the amount of training increases are discussed below. To simplify the presentation of the data and since the two analyses depend on a different number of participants, the thesis is presented both as a manuscript and a thesis, hence giving separate results for background data and prevalence numbers of ID and IDA.

5.1 Summary of findings

The aim of this study was to explore whether the probability of ID increases as the amount of training increases, in addition to exploring the prevalence of ID among the study population. The results show that in this combined group of four cohorts, females had almost five times higher odds of experiencing ID than males, and that the prevalence of ID among female participants was 29.7% while only 8.9% for males.

The likelihood of sexperiencing ID decreased with age although the association was weak. When the logistic regression model was divided by gender, age remained the only significantly predicting variable for females while training amounts were a significant predictor for males. The likelihood of experiencing ID decreased with age among women and more training amounts predicted less likelihood for men to experience ID, while age did not stay significant in the male model. These findings are inconsistent with what was predicted.

5.2 Discussion

The primary findings of the present study showed that the prevalence of ID among female participants has proven to be considerably higher than among male participants. These results are consistent with a number of previous studies who found the prevalence of ID among physically active females and males to vary between 29-77% and 4-15% respectively. The prevalence of IDA among physically active females and males has been found to be from 10-14% and 2-3% respectively (2, 45, 80, 81, 108, 163, 164). On the basis of these results and those of earlier studies, it is logical to speculate on the cause of this gender difference. As shown in this and earlier studies, the clear difference in the mean ferritin levels between females and males might suggest that the cut-off criteria is wrong for both sexes ID is defined using the same ferritin cut-offs for both genders but the hemoglobin cut-offs are different for females and males. Since males have relatively higher levels of iron in their body, it is rational to suggest that the ferritin cut-off criteria be higher for males, just as it is for hemoglobin.

Interestingly there are some speculations on the difference in steroid hormones between the genders in relation to hepcidin production and iron status. Bachman and coworkers (165) showed that testosterone administration was associated with an increase in hemoglobin levels and hematocrit. They speculate that this increase is due to a suppressing effect of testosterone on hepcidin in addition to an stimulatory effect on erythropoiesis (165). However a large study on males found a significant negative correlation between ferritin and testosterone, i.e., ferritin levels decreased with the rise of testosterone (166). Another cross-sectional study on males also showed a negative correlation between testosterone

and ferritin (167). Future studies that investigate the relationship between testosterone and iron status, and ID and IDA are warranted.

The results of the present study may also be explained by the fact that a vast majority of the male participants took part in either cross-country skiing or ice hockey where it is possible that the mechanical loads of foot strike are considerably less than in sports where running is involved, thus less hemolysis. A study where 10 male triathletes completed two separate sessions of running and cycling showed that foot strike is the main cause of hemolysis during running (168, 169). Telford and coworkers (169) looked into body-size, sex and sports dependency of hematology in trained athletes. They were able to demonstrate that hematologistical status varies between sports. However they were unable to investigate this relationship further, due to variance in BMI and gender between groups of participants in each sport.

Another possible reason for these findings can be that a lower intake of iron-containing foods, possibly due to disordered eating or eating disorders, may be more prevalent among females in addition to higher losses of iron due to menstruation and compared to males.

Furthermore, the present study showed that females trained on average fewer hours per week than males, but when compared between groups based on iron status males with ID trained less than males with normal iron status. When the likelihood of ID was predicted divided by gender, it showed that increased training amounts among males decreased the likelihood of experiencing ID. These result are contradictory to previous studies and a recent study reported increased prevalence of ID among male participants who participated in prolonged strenuous training programs (163). Similarly, the prevalence of ID among adolescent male army recruits increased after six months of training, where half of the new onset anemia resulted from iron deficiency. The other half was due to hemodilution (170).

One possible explanation for the findings in the present study may be that low iron status in ID athletes might begin to affect physical capacity much sooner than ID can be clinically diagnosed. Ferritin cut-offs are based on bone marrow iron microscopy and ferritin has been show to correlate well with bone marrow iron stores (171, 172). Since the bone marrow is a major storage site for iron in the body, this method evaluates body iron stores with high accuracy (173, 174). Recent studies found that during ID the production of erythropoietin is decreased, thus lowering the production of red blood cells and diminishing iron drain from the systemic iron pool. This occurs at the expense of the iron needs of other tissues (175). Hagler and coworkers found that as iron deficiency progressed, preferential utilization of iron occurred both between and within tissue. Iron deficient rats experienced a 35% reduction in hemoglobin and 20-37% reduction in skeletal muscle myoglobin resulting in impaired skeletal muscle oxidative capacity (176). Furthermore, ID has been linked to chronic increase in AMP-activated protein kinase in rat skeletal muscle. This results in a shift from oxidative metabolism in the muscles towards glycolytic metabolism, which reflects in muscle oxidative capacity (177). The results from these studies raise a question as to whether the enzyme activity in muscle fibers may be negatively affected by low iron status much earlier than a measurable decrease in iron status in the bone marrow is found. Thus, skeletal muscle aerobic function might not be a priority over immune function or heart muscle function. Therefore, the clinical cut-off for athletes may likely be higher as a small reduction in iron status may significantly affect athletic performance. In their systematic review Haas and Brownlie (178) found that energy efficiency was negatively affected in ID humans. They found strong effects of both severe IDA

and moderate IDA on aerobic capacity (VO2max) and a strong biological mechanism (reduced oxygen transport associated with anemia) for the effect of IDA on a work capacity/performance (144). Although we are unable to confirm this due to limitations in this study (listed below), a plausible explanation for the difference in training amounts found in this study could be that ID males are more likely to experience from weariness and exercise-associated dyspnea, which are known symptoms of ID (7).

Another possible explanation for the results of the present study can be that males who have a higher training load have increased testosterone levels linked to higher hemoglobin and hematocrit levels, and studies have shown an increase in testosterone levels after training interventions (179, 180). It is possible that male athletes who had a higher training load were also more aware of the importance of their food intake and thus less likely to experience ID. Dietary intake was not investigated in the current study. In a New Zealand study elite athletes had better nutrition knowledge and their dietary habits were more in line with national nutrition guidelines compared with non-elite athletes (181).

No difference in training amount was found between ID females and those with normal iron status. This might be due to the fact that the females in this study trained less than the males, or possibly the sample was simply too small or too homogenous in training load and amount.

When blood parameters, age and amount of training were compared between iron status groups within gender, both ID females and males were one year younger than their counterparts in the normal iron status group, suggesting age to be a protective factor, although weak. However, this does not seem to be the case in other studies. For example in Merkel and coworkers (182) study on adolescents, the prevalence of ID among strenuously trained adolescents to was 24.5%. Rowland and coworkers (183) found that 45% of adolescent female athletes had ID and 17% of adolescent male athletes. These figures are consistent with results obtained on the prevalence of ID among physically active adults as listed above.

5.2.1 Strengths and limitations

Due to the ex post facto design of the performed study, missing information on dietary iron intake and nutrition knowledge are limiting factors in the analysis. In addition, a more detailed classification of the athletes' training such as intensity and type would have been valuable. BMI was calculated directly through the DXA scanner and information on weight and height was not recorded separately, thus calculations on average energy requirements were not performed. The comparability in the female training load is a limiting factor which might mean that the sample did not include females with a high and diverse enough training load. It is worth noting that the data for this study was gathered from four cohorts in the main involving elite athletes in addition to physically active individuals. Thus, the majority of athletes were trained under supervision from a professional coach and/or were enrolled in schools for young elite athletes.

An analysis on inflammation status through C-reactive protein, IL-β, TNF-α and IL-6, which are all associated to an inflammatory mediated raise in ferritin and hepcidin levels, was not performed in the present study. Therefore, an analysis of whether ID is related to increased hepcidin level was not possible which also limits the study. Since hepcidin is known to increase following exercise (due to exercise induced inflammation) it would be interesting and valuable to include measurements on

inflammation markers and hepcidin in future studies to assess whether this might be a mechanism behind the increased prevalence of ID among athletes and physically active individuals. In addition, we did not measure sTfR, a blood marker unaffected by inflammation, and thus could not calculate the ratio of sTfR to ferritin (total body iron), which would have increased strength to /this analysis.

And finally, an inclusion of steroid hormone analysis would give information of the potential role of a steroid hormone-iron axis.

This cohort study was performed in conjunction with an ongoing research project which was delayed. Therefore, previously existing data was used and was valuable in terms of giving an overview of iron status in a diverse sample of athletes both in relation to body composition and training amount. The novelty in this study is, among other things, the examination of the relationship between BMI, %BF and ID in healthy individuals. In the best knowledge of the author this is the first study on the matter of body fat and ID in normal weight individuals. Looking at the relationship between training amount and ID in such a diverse sample contributes valuable information for future studies. The present study clearly shows the difference in the mean ferritin levels between females and males, thus it is bringing attention to and supports a potential review, depending on gender, that the ferritin cut-offs used to diagnose ID should be different.

5.3 Conclusion

It is clear that ID is common among athletes and more so among female athletes. Interestingly the cut-off limit is the same for both genders despite distinct differences in means of ferritin levels, a fact that may be important to consider in association with future studies around this topic. It seems that increased age, even only one year, is a protective factor for ID in female athletes, though none of the female participants were old enough to have experienced menopause. In contrast with previous findings, increased training amounts decreased the likelihood of males experiencing ID while no association was seen in females. Further research is needed to better assess the relationship between training amounts and prevalence of ID among both female and male athletes to enable more exact measurements. Future studies should specify amount, intensity and type of training to determine whether there is a relationship between iron status and training amounts. It would be interesting to look at inflammation markers, both prior to exercise and after in relationship to hepcidin levels and iron status. Diet and nutrition information should or could be included in order to assess iron intake and how it may affect iron status. In order to prevent both ID and IDA among athletes an optimal diet should be used as the first line of defense. Before recommending iron supplementation, efforts should be made to adjust the diet so that intake of iron is increased through iron-rich foods, being mindful of harmful effects that iron supplements can have on the body if the intake is excessive. Thus, supplementation should be the last resort and always in consultation with a physician, nutritionist or other professionals.

6 Future perspectives

This study was designed to investigate the relationship between training amounts and iron status in athletes and physically active individuals and incorporating existing data from earlier studies. Originally the intent was to conduct an intervention study including ID athletes and physically active individuals. The plan was to give subjects iron supplements for 12 weeks. In addition to having them perform submaximal cycling tests to assess work efficiency, aerobic capacity to oxidize fat and carbohydrate, VO2max, lactate threshold and perceived exertion, (Borg) at the beginning and then at four-week intervals over the 12 weeks. Blood analysis would be conducted at the be beginning of each visit. The aim of the study was to assess whether iron stores improved with iron supplementation, in addition to assessing participants endurance performance and whether there was an existing relationship between improved iron status and performance at an interval of four weeks.

More studies are needed on the effects of ID on athlete's health and performance and the mechanism behind an increased prevalence of ID among athletes. Furthermore, studies on the prevalence of ID among Icelandic athletes would be useful, though up until now no studies have been performed. The opportunity to conduct a study on iron status in Icelandic athletes, including information on their dietary habits and iron intake in addition to measurements on hepcidin, inflammation factors and ideally a detailed training diary, would be an exceedingly interesting follow-up project.

7 References

1. World Health Organization. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. 2001
2. Clénin G, Cordes M, Huber A, Schumacher YO, Noack P, Scales J, et al. Iron deficiency in sports–definition, influence on performance and therapy. Swiss Medical Weekly. 2015;145:w14196.doi:https://doi.org/10.4414/smw.2015.14196
3. Nielsen P, Nachtigall D. Iron supplementation in athletes. Sports Medicine 1998;26(4):207-16
4. Nordic Council of Ministers. Nordic nutrition recommendations 2012: integrating nutrition and physical activity: Nordic Council of Ministers; 2014.
5. Ross AC. Modern Nutrition in Health and Disease. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2014.
6. Portal S, Epstein M, Dubnov G. Iron deficiency and anemia in female athletes--causes and risks. Harefuah. 2003;142(10):698-703
7. Miller JL. Iron deficiency anemia: a common and curable disease. Cold Spring Harbor perspectives in medicine. 2013;3(7):a011866.doi:10.1101/cshperspect.a011866
8. Malczewska-Lenczowska J, Orysiak JS, B. , Turowski D, Burkhard-Jagodzińska K, Gajewski J. Reticulocyte and erythrocyte hypochromia markers in detection of iron deficiency in adolescent female athletes. Biology of Sport. 2017;34(2):111.doi:10.5114/biolsport.2017.64584
9. Peeling P, Sim M, Badenhorst CED, Brian. , Govus AD, Abbiss CR, Swinkels DW, et al. Iron status and the acute post-exercise hepcidin response in athletes. PLoS One. 2014;9(3):e93002.doi:https://doi.org/10.1371/journal.pone.0093002
10. Bekri S, Gual P, Anty R, Luciani N, Dahman M, Ramesh BI, et al. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. Gastroenterology. 2006;131(3):788-96.doi:https://doi.org/10.1053/j.gastro.2006.07.007
11. Nead KGH, Jill S. Kaczorowski, Jeffrey M. Auinger, Peggy. Weitzman, Michael. Overweight children and adolescents: a risk group for iron deficiency. Pediatrics. 2004;114(1):104-8
12. Yanoff L, Menzie C, Denkinger B, Sebring N, McHugh T, Remaley A, et al. Inflammation and iron deficiency in the hypoferremia of obesity. International journal of obesity. 2007;31(9):1412-9.doi:doi:10.1038/sj.ijo.0803625
13. Zimmermann M, Zeder C, Muthayya S, Winichagoon P, Chaouki N, Aeberli I, et al. Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. International Journal of Obesity. 2008;32(7):1098-104.doi:doi:10.1038/ijo.2008.43
14. Eftekhari M, Mozaffari-Khosravi H, Shidfar F. The relationship between BMI and iron status in iron-deficient adolescent Iranian girls. Public Health Nutrition. 2009;12(12):2377-81.doi:https://doi.org/10.1017/S1368980009005187
15. Chang R. Chemistry. 11th ed. New York: McGraw-Hill; 2013.
16. A. Catharine Ross BC, Robert J. Cousins, Katherine L. Tucker, Thomas R. Ziegler. Modern Nutrition in Health and Disease. 11th ed. Baltimore: Lippincott Williams & Wilkins, a Walters Kluwer business; 2014.
17. Sherwood L. Human Physiology From Cells to Systems. Seventh Edition ed. Arbogast M, editor. Belmont, CA, USA: Yolanda Cossio; 2010.
18. Roughead ZK, Hunt JR. Adaptation in iron absorption: iron supplementation reduces nonheme-iron but not heme-iron absorption from food. The American Journal of Clinical Nutrition. 2000;72(4):982-9.doi:https://doi.org/10.1093/ajcn/72.4.982

19. Hurrell R, Egli I. Iron bioavailability and dietary reference values. The American Journal of Clinical Nutrition. 2010;91(5):1461-7.doi:https://doi.org/10.3945/ajcn.2010.28674F

20. Hoppe M, Brün B, Larsson MP, Moraeus L, Hulthén L. Heme iron-based dietary intervention for improvement of iron status in young women. Nutrition. 2013;29(1):89-95.doi:https://doi.org/10.1016/j.nut.2012.04.013

21. Navas-Carretero SP-G, Ana M. Sarriá, Beatriz. Vaquero, M Pilar. Iron absorption from meat pate fortified with ferric pyrophosphate in iron-deficient women. Nutrition. 2009;25(1):20-4.doi:https://doi.org/10.1016/j.nut.2008.07.002

22. González-Rosendo G, Polo J, Rodríguez-Jerez JJ, Puga-Díaz R, Reyes-Navarrete EG, Quintero-Gutiérrez AG. Bioavailability of a heme-iron concentrate product added to chocolate biscuit filling in adolescent girls living in a rural area of Mexico. Journal of food science. 2010;75(3).doi:https://doi.org/10.1111/j.1750-3841.2010.01523.x

23. Fuqua BK, Vulpe CD, Anderson GJ. Intestinal iron absorption. Journal of Trace Elements in Medicine and Biology. 2012;26(2):115-9.doi:https://doi.org/10.1016/j.jtemb.2012.03.015

24. Bruce Alberts DB, Karen Hopkin, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter. Essentiak Cell Biology. New York: Garland Science; 2014.

25. McKie AT, Barrow D, Latunde-Dada GOR, Andreas. , Sager G, Mudaly E, Mudaly M, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001;291(5509):1755-9.doi:DOI: 10.1126/science.1057206

26. Fleming RE, Bacon BR. Orchestration of iron homeostasis. The New England Journal of Medicine. 2005;352(17):1741-4

27. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. The Lancet. 2016;387(10021):907-16.doi:https://doi.org/10.1016/S0140-6736(15)60865-0

28. Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2006;290(2):199-203.doi:https://doi.org/10.1152/ajpgi.00412.2005

29. Daher R, Karim Z. Iron metabolism: State of the art. Transfusion Clinique et Biologique. 2017;24(3):115-9.doi:https://doi.org/10.1016/j.tracli.2017.06.015

30. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2012;1823(9):1434-43.doi:https://doi.org/10.1016/j.bbamcr.2012.01.014

31. Zhao N, Zhang A-S, Enns CA. Iron regulation by hepcidin. The Journal of Clinical Investigation. 2013;123(6):2337

32. Hoffbrand AV, Moss PA. Essential haematology: John Wiley & Sons; 2011.

33. Sandström G. Iron deficiency in female adolescent athletes-prevalence, mechanisms and diagnostics2014.

34. Gupta C. Role of iron (Fe) in body. IOSR Journal of Applied Chemistry (IOSR-JAC). 2014;7:38-46

35. William S. Klug MRC, Charlotte A. Spencer, Michael A. Palladino. Concepts of Genetics. 10th ed. California: Pearson Education, Inc.; 2012.

36. Harvey RA, Harvey RA, Ferrier DR. Biochemistry: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2011.

37. Fick A. Fick's first law of diffusion. Annalen der Physik. 1855;170:59

38. Priya KRN, Minal. , Kumari U, Marandi N. Prediction of cardioresiratory fitness by measuring VO2max. International Journal of Computer Engineering and Applications. n.d.

39. Otto JM, Montgomery HER, Toby. Haemoglobin concentration and mass as determinants of exercise performance and of surgical outcome. Extreme Physiology and Medicine. 2013;2(1):33.doi:https://doi.org/10.1186/2046-7648-2-33

40. Burke L, Deakin V. Clinical Sports Nutrition2015.

41. Ekblom B, Goldbarg AN, Gullbring B. Response to exercise after blood loss and reinfusion. Journal of Applied Physiology. 1972;33(2):175-80.doi:https://doi.org/10.1152/jappl.1972.33.2.175

42. Sareen S. Gropper JLS, James, L Groff. Advanced Nutrition and Human Metabolism. 4th ed. Belmont: Peter Marshall; 2005.

43. Deakin LBV. Clinical Sports Nutrition. 5th ed. Australia: McGraw-hill Education; 2015.

44. Papanikolaou GP, Kostas. Systemic iron homeostasis and erythropoiesis. International Union of Biochemistry and Molecular Biology Life. 2017;69(6):399-413.doi:https://doi.org/10.1002/iub.1629

45. Alaunyte I, Stojceska V, Plunkett A. Iron and the female athlete: a review of dietary treatment methods for improving iron status and exercise performance. Journal of the international society of sports nutrition. 2015;12(1):38.doi:https://doi.org/10.1186/s12970-015-0099-2

46. Longo DL, Camaschella C. Iron-deficiency anemia. New England Journal of Medicine. 2015;372(19):1832-43.doi:DOI: 10.1056/NEJMra1401038

47. World Health Organization. The global prevalence of anaemia in 2011. 2015

48. Cercamondi CI, Egli IM, Zeder C, Hurrell RF. Sodium iron EDTA and ascorbic acid, but not polyphenol oxidase treatment, counteract the strong inhibitory effect of polyphenols from brown sorghum on the absorption of fortification iron in young women. British Journal of Nutrition. 2014;111(3):481-9.doi:https://doi.org/10.1017/S0007114513002705

49. ERA055. ZSFG Pearls: Anemia+DVT DDX,SCARS. ucsfmed.wordpress.com: University of California San Francisco; 2017.

50. Porth C. Essentials of pathophysiology: Concepts of altered health states: Lippincott Williams & Wilkins; 2011.

51. Wu AC, Lesperance L, Bernstein H. Screening for iron deficiency. Pediatrics 2016;23(5):171-7

52. DellaValle DM. Iron supplementation for female athletes: effects on iron status and performance outcomes. Current Sports Medicine Reports. 2013;12(4):234-9.doi:10.1249/JSR.0b013e31829a6f6b

53. Miller TL. Endurance Sports Medicine: Springer; 2016.

54. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clinica Chimica Acta. 2003;329(1):9-22.doi:https://doi.org/10.1016/S0009-8981(03)00005-6

55. Iqbal T, Stein J, Sharma N, Kulnigg-Dabsch S, Vel S, Gasche C. Clinical significance of C-reactive protein levels in predicting responsiveness to iron therapy in patients with inflammatory bowel disease and iron deficiency anemia. Digestive Diseases and Sciences. 2015;60(5):1375-81

56. Sarma PR. Clinical Methods The History, Physical, and Laboratory Examinations. 3 ed. Boston1990.

57. World Health Organization. Physical Activity www.who.intn.d. [Available from: http://www.who.int/dietphysicalactivity/pa/en/.

58. Gígja Gunnarsdóttir, Anna Björg Aradóttir, Erlingur Jóhannsson, Jan Triebel, Svandís J Sigurðardóttir, Sveinsson Þ. Ráðleggingar um hreyfingu. In: Lýðheilsustöð, editor.: Lýðheilsustöð; 2008.

59. OED Online. Oxford University Press; 2017. “physical, adj.”.

60. Halson SL. Monitoring training load to understand fatigue in athletes. Sports Medicine. 2014;44(2):139-47

61. Bourdon PC, Cardinale M, Murray A, Gastin P, Kellmann M, Varley MC, et al. Monitoring athlete training loads: consensus statement. International journal of sports physiology and performance. 2017;12(2):2-170

62. McLaren SJ, Macpherson TW, Coutts AJ, Hurst C, Spears IR, Weston M. The relationships between internal and external measures of training load and intensity in team sports: A meta-analysis. Sports Medicine.1-18

63. World Health Organization. Global recommendations on physical activity for health. 2010

64. Physical activity www.folkshalsomyndigheten.se: The Public Health Agency of Sweden; 2016 [Available from: https://www.folkhalsomyndigheten.se/the-public-health-agency-of-sweden/living-conditions-and-lifestyle/physical-activity/.

65. Baxter-Jones AD, Helms PJ. Effects of training at a young age: a review of the training of young athletes (TOYA) study. Pediatric Exercise Science. 1996;8(4):310-27.doi:https://doi.org/10.1123/pes.8.4.310

66. Ostojic S, Ahmetovic Z. Weekly training volume and hematological status in female top-level athletes of different sports. Journal of Sports Medicine and Physical Fitness. 2008;48(3):398

67. Van Dyke TE, Kornman KS. Inflammation and factors that may regulate inflammatory response. Journal of periodontology. 2008;79(8S):1503-7.doi:https://doi.org/10.1902/jop.2008.080239

68. Keller C, Keller P, Marshal S, Pedersen BK. IL-6 gene expression in human adipose tissue in response to exercise–Effect of carbohydrate ingestion. The Journal of Physiology. 2003;550(3):927-31.doi:https://doi.org/10.1113/jphysiol.2003.044883

69. Pedersen B, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: is IL-6 a candidate? Journal of Muscle Research and Cell Motility. 2003;24(2-3):113

70. Pedersen B, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? Proceedings of the Nutrition Society. 2004;63(2):263-7.doi:

https://doi.org/10.1079/PNS2004338

71. Roecker LM-B, R. Brechtel, L. Nemeth, E. Ganz, T. Iron-regulatory protein hepcidin is increased in female athletes after a marathon. European Journal of Applied Physiology. 2005;95(5-6):569-71

72. Newlin MK, Williams S, McNamara T, Tjalsma H, Swinkels DWH, Emily M. The effects of acute exercise bouts on hepcidin in women. International Journal of Sport Nutrition and Exercise Metabolism. 2012;22(2):79-88.doi:https://doi.org/10.1123/ijsnem.22.2.79

73. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen B. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. The Journal of Physiology. 1998;508(3):949-53.doi:https://doi.org/10.1111/j.1469-7793.1998.949bp.x

74. Gropper SS, Smith JL. Advanced nutrition and human metabolism: Cengage Learning; 2005.

75. Harvey RA, Ferrier DR. Biochemistry: Lippincott Williams & Wilkins; 2011.

76. S. W, Klug MR, Cummings CA, Spencer MA, Palladino. Concepts of Genetics. 10 ed: Pearson; 2012.

77. Beard J, Tobin B. Iron status and exercise. The American journal of clinical nutrition. 2000;72(2):594-7.doi:https://doi.org/10.1093/ajcn/72.2.594S

78. Di Santolo M, Stel G, Banfi G, Gonano F, Cauci S. Anemia and iron status in young fertile non-professional female athletes. European Journal of Applied Physiology. 2008;102(6):703-9

79. Malczewska J, Raczynski G, Stupnicki R. Iron status in female endurance athletes and in non-athletes. International Journal of Sport Nutrition and Exercise Metabolism. 2000;10(3):260-76.doi:https://doi.org/10.1123/ijsnem.10.3.260

80. Dubnov G, Constantini NW. Prevalence of iron depletion and anemia in top-level basketball players. International Journal of Sport Nutrition and Exercise Metabolism. 2004;14(1):30-7.doi:https://doi.org/10.1123/ijsnem.14.1.30

81. Dubnov G, Foldes AJ, Mann G, Magazanik A. High prevalence of iron deficiency and anemia in female military recruits. Military Medicine. 2006;171(9):866.doi:https://doi.org/10.7205/MILMED.171.9.866

82. Landahl G, Adolfsson P, Börjesson M, Mannheimer C, Rödjer S. Iron deficiency and anemia: a common problem in female elite soccer players. International Journal of Sport Nutrition and Exercise Metabolism. 2005;15(6):689-94.doi:https://doi.org/10.1123/ijsnem.15.6.689

83. Parks RB, Hetzel SJ, Brooks MA. Iron deficiency and anemia among collegiate athletes: a retrospective chart review. Medicine and Science in Sports and Exercise. 2017;49(8):1711-5.doi:10.1249/MSS.0000000000001259

84. Braun H, von Andrian-Werburg J, Schänzer W, Thevis M. Nutrition Status of Young Elite Female German Football Players. Pediatric Exercise Science. 2017;30(1):157-67.doi:https://doi.org/10.1123/pes.2017-0072

85. Milić R, Dopsaj, M. Possible differences in hematological status between junior and elite female volleyball players. British Journal of Sports Medicine. 2011;45(6):539-.doi:http://dx.doi.org/10.1136/bjsm.2011.084558.17

86. Ostojic S, Ahmetovic Z. Indicators of iron status in elite soccer players during the sports season. International Journal of Laboratory Hematology. 2009;31(4):447-52.doi:https://doi.org/10.1111/j.1751-553X.2008.01064.x

87. Fujii T, Okumura Y, Maeshima E, Okamura K. Dietary Iron Intake and Hemoglobin Concentration in College Athletes in Different Sports. International Journal of Sports and Exercise Medicine. 2015;1:029

88. Dellavalle DM, Haas JD. Iron supplementation improves energetic efficiency in iron-depleted female rowers. Medicine and Science in Sports and Exercise. 2014;46(6):1204-15.doi:10.1249/MSS.0000000000000208

89. Akiboye RD, Sharma DM. Haematuria in Sport: A Review. European Urology Focus. 2018.doi:https://doi.org/10.1016/j.euf.2018.02.008

90. De Oliveira EP, Burini RC. The impact of physical exercise on the gastrointestinal tract. Current Opinion in Clinical Nutrition & Metabolic Care. 2009;12(5):533-8.doi:doi: 10.1097/MCO.0b013e32832e6776

91. DeRuisseau KC, Cheuvront SN, Haymes EM, Sharp RG. Sweat iron and zinc losses during prolonged exercise. International Journal of Sport Nutrition and Exercise Metabolism. 2002;12(4):428-37.doi:https://doi.org/10.1123/ijsnem.12.4.428

92. Heinicke K, Heinicke I, Schmidt W, Wolfarth B. A three-week traditional altitude training increases hemoglobin mass and red cell volume in elite biathlon athletes. International Journal of Sports Medicine. 2005;26(05):350-5

93. Mairbäurl H. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. Frontiers in physiology. 2013;4:332.doi:https://doi.org/10.3389/fphys.2013.00332

94. Suedekum NA, Dimeff RJ. Iron and the Athlete. Current Sports Medicine Reports. 2005;4(4):199-202.doi:10.1097/01.CSMR.0000306207.79809.7f

95. Bingham ME, Borkan ME, Quatromoni PA. Sports nutrition advice for adolescent athletes: A time to focus on food. American Journal of Lifestyle Medicine. 2015;9(6):398-402

96. Thomas DT, Erdman KA, Burke LM. Position of the academy of nutrition and dietetics, dietitians of canada, and the american college of sports medicine: Nutrition and athletic performance. Journal of the Academy of Nutrition and Dietetics. 2016;116(3):501-28.doi:https://doi.org/10.1016/j.jand.2015.12.006

97. Rodriguez NR, DiMarco NM, Langley S. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. Journal of the American Dietetic Association. 2009;109(3):509-27.doi:10.1016/j.jada.2009.01.005

98. Hamilton B. Vitamin D and human skeletal muscle. Scandinavian journal of medicine & science in sports. 2010;20(2):182-90.doi:https://doi.org/10.1111/j.1600-0838.2009.01016.x

99. Flynn A. The role of dietary calcium in bone health. Proceedings of the Nutrition Society. 2003;62(4):851-8.doi:https://doi.org/10.1079/PNS2003301

100. Sherwood L, Kell R, Ward C. Human physiology: from cells to systems Cengage Learning. Belmont, USA. 2010

101. Martínez S, Pasquarelli BN, Romaguera D, Arasa C, Tauler P, Aguiló A. Anthropometric characteristics and nutritional profile of young amateur swimmers. The Journal of Strength & Conditioning Research. 2011;25(4):1126-33

102. Keser A, Binnetoglu FK, Asil E, Babaoglu K. Comparison of Food Consumption and Nutritional Statuses of Athletic Adolescents. The Anthropologist. 2016;25(1-2):60-9.doi:https://doi.org/10.1080/09720073.2016.11892089

103. Huang S-HS, Johnson K, Pipe AL. The use of dietary supplements and medications by Canadian athletes at the Atlanta and Sydney Olympic Games. Clinical Journal of Sport Medicine. 2006;16(1):27-33.doi:doi: 10.1097/01.jsm.0000194766.35443.9c

104. Larson-Meyer DE, Woolf K, Burke LM. Assessment of Nutrient Status in Athletes and the Need for Supplementation. International journal of sport nutrition and exercise metabolism. 2017:1-52.doi:https://doi.org/10.1123/ijsnem.2017-0338

105. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Journal of the American Dietetic Association. 2001;101(3):294-301.doi:https://doi.org/10.1016/S0002-8223(01)00078-5

106. Manore MM. Dietary recommendations and athletic menstrual dysfunction. Sports medicine. 2002;32(14):887-901

107. McClung JP, Gaffney-Stomberg EL, Jane J. Female athletes: a population at risk of vitamin and mineral deficiencies affecting health and performance. Journal of Trace Elements in Medicine and Biology. 2014;28(4):388-92.doi:https://doi.org/10.1016/j.jtemb.2014.06.022

108. Harvey LJ, Armah CN, Dainty JR, Foxall RJ, Lewis DJ, Langford NJ, et al. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. British Journal of Nutrition. 2005;94(4):557-64.doi:https://doi.org/10.1079/BJN20051493

109. Bruinvels G, Burden R, Brown N, Richards T, Pedlar C. The prevalence and impact of heavy menstrual bleeding (menorrhagia) in elite and non-elite athletes. PLoS One. 2016;11(2):e0149881.doi:https://doi.org/10.1371/journal.pone.0149881

110. Nattiv AL, AB. Manore, MM. Sanborn, CF. Sundgot-Borgen, J. Warren, MP. The female athlete triad special communications: position stand. Medicine and Science in Sports and Exercise 2007;39(10):1867-82

111. Quah YV, Poh BK, Ng LO, Noor MI. The female athlete triad among elite Malaysian athletes: prevalence and associated factors. Asia Pacific Journal of Clinical Nutrition. 2009;18(2):200-8.doi:10.6133/apjcn.2009.18.2.08

112. Barrack MT, Ackerman KE, Gibbs JC. Update on the female athlete triad. Current reviews in musculoskeletal medicine. 2013;6(2):195-204.doi:10.1007/s12178-013-9168-9

113. Stand P. The female athlete triad. Medicine and Science in Sports and Exercise. 2007;39(10):1867-82

114. Horn E, Gergen N, McGarry KA. The female athlete triad. Rhode Island Medical Journal. 2014;97(11):18-21

115. Desbrow B, McCormack J, Burke LM, Cox GR, Fallon K, Hislop M, et al. Sports Dietitians Australia position statement: sports nutrition for the adolescent athlete. International Journal of Sport Nutrition and Exercise Metabolism. 2014;24(5):570-84

116. Łagowska K. Does improving hematological iron status restore the menstrual cycle in female athletes? Science & Sports. 2018;33(2):118-22.doi:https://doi.org/10.1016/j.scispo.2017.09.006

117. Alaunyte L, Stojceska V, Plunkett A, Derbyshire E. Dietary iron intervention using a staple food product for improvement of iron status in female runners. Journal of the International Society of Sports Nutrition. 2014;11(1):50.doi:https://doi.org/10.1186/s12970-014-0050-y

118. Sandström G, Börjesson M, Rödjer S. Iron Deficiency in Adolescent Female Athletes—Is Iron Status Affected by Regular Sporting Activity? Clinical Journal of Sport Medicine. 2012;22(6):495-500.doi:doi: 10.1097/JSM.0b013e3182639522

119. Auersperger I, Škof B, Leskošek B, Knap B, Jerin A, Lainscak M. Exercise-induced changes in iron status and hepcidin response in female runners. PloS one. 2013;8(3).doi:https://doi.org/10.1371/journal.pone.0058090

120. Fallon KE. Utility of hematological and iron-related screening in elite athletes. Clinical Journal of Sport Medicine. 2004;14(3):145-52

121. Rodriguez NRD, Nancy M. Langley, Susie. Nutrition and athletic performance. Medicine and Science in Sports and Exercise. 2009;41(3):709-31

122. Hinton PS, Giordano C, Brownlie T, Haas JD. Iron supplementation improves endurance after training in iron-depleted, nonanemic women. Journal of applied physiology. 2000;88(3):1103-11

123. McClung JP, Karl JP, Cable SJ, Williams KW, Nindl BC, Young AJL, Harris R. Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. The American Journal of Clinical Nutrition. 2009;90(1):124-31.doi:https://doi.org/10.3945/ajcn.2009.27774

124. Waldvogel S, Pedrazzini B, Vaucher P, Bize R, Cornuz J, Tissot J-D, et al. Clinical evaluation of iron treatment efficiency among non-anemic but iron-deficient female blood donors: a randomized controlled trial. BMC Medicine. 2012;10(1):8.doi:https://doi.org/10.1186/1741-7015-10-8

125. Garvican LA, Saunders PU, Cardoso T, Macdougall IC, Lobigs LM, Fazakerley R, et al. Intravenous iron supplementation in distance runners with low or suboptimal ferritin. Medicine and science in sports and exercise. 2014;46(2):376-85.doi:10.1249/MSS.0b013e3182a53594

126. Burden RJ, Morton K, Richards T, Whyte GP, Pedlar CR. Is iron treatment beneficial in, iron-deficient but non-anaemic (IDNA) endurance athletes? A systematic review and meta-analysis. British Journal of Sports Medicine. 2015;49(21):1389-97.doi:http://dx.doi.org/10.1136/bjsports-2014-093624

127. Burden RJ, Pollock N, Whyte GP, Richards T, Moore B, Busbridge M, et al. Impact of intravenous iron on aerobic capacity and iron metabolism in elite athletes. Medicine and Science in Sports and Exercise. 2014;47(7):1399-407

128. DellaValle DM, Haas JD. Iron status is associated with endurance performance and training in female rowers. Medicine and Science in Sports and Exercise. 2012;44(8):1552-9.doi:10.1249/MSS.0b013e3182517ceb

129. Hinton P, Sinclair L. Iron supplementation maintains ventilatory threshold and improves energetic efficiency in iron-deficient nonanemic athletes. European Journal of Clinical Nutrition. 2007;61(1):30.doi:doi:10.1038/sj.ejcn.1602479

130. Bacon BR. Hemochromatosis: diagnosis and management. Gastroenterology. 2001;120(3):718-25.doi:https://doi.org/10.1053/gast.2001.21913

131. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. New England Journal of Medicine. 2005;352(17):1769-78

132. Alaunyte I, Stojceska V, Plunkett A, Derbyshire E. Cereal-based dietary iron intervention for improvement of iron status in female runners. The Proceedings of the Nutrition Society. 2015;74(OCE1)

133. Wardenaar F, Brinkmans N, Ceelen I, Van Rooij B, Mensink M, Witkamp R, et al. Micronutrient intakes in 553 Dutch elite and sub-elite athletes: Prevalence of low and high intakes in users and non-users of nutritional supplements. Nutrients. 2017;9(2):142.doi:doi:10.3390/nu9020142

134. Brownlie T, Utermohlen V, Hinton PS, Giordano C, Haas JD. Marginal iron deficiency without anemia impairs aerobic adaptation among previously untrained women. The American Journal of Clinical Nutrition. 2002;75(4):734-42.doi:https://doi.org/10.1093/ajcn/75.4.734

135. Brownlie T, Utermohlen V, Hinton PS, Haas JD. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. The American Journal of Clinical Nutrition. 2004;79(3):437-43.doi:https://doi.org/10.1093/ajcn/79.3.437

136. Forbes GB. Human body composition: growth, aging, nutrition, and activity: Springer Science & Business Media; 2012.

137. Keay DN. British Journal of Sports Medicine Blog [Internet]. www.blogs.bmj.com: British Journal of Sports Medicine 2017. [cited 2018]. Available from: http://blogs.bmj.com/bjsm/2017/12/28/body-composition-health-sports-performance/.

138. Maughan RJ. The Encyclopaedia of Sports Medicine: An IOC Medical Commission Publication, Sports Nutrition: John Wiley & Sons; 2013.

139. Kehayias RRJJ, Dawson-Hughes B, Heymsfield SB. Use of dual-energy x-ray absorptiometry in body-composition studies: not yet a “gold standard”. The American Journal of Clinical Nutrition. 1993;58:589-59l

140. D. Stewart A, Hannan WJ. Prediction of fat and fat-free mass in male athletes using dual X-ray absorptiometry as the reference method. Journal of Sports Sciences. 2000;18(4):263-74.doi:https://doi.org/10.1080/026404100365009

141. Nevill AM, Winter EM, Ingham S, Watts A, Metsios GSS, Arthur D. Adjusting athletes' body mass index to better reflect adiposity in epidemiological research. Journal of Sports Sciences. 2010;28(9):1009-16.doi:https://doi.org/10.1080/02640414.2010.487071

142. Mazić S, Lazović B, Đelić M, Suzić-Lazić J, Aćimović T, Brkić P. Body composition assessment in athletes: A systematic review. Medicinski Pregled. 2014;67(7-8):255-60

143. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. British Medical Journal 2000;320(7244):1240.doi:https://doi.org/10.1136/bmj.320.7244.1240

144. BMI classification WHO :: Global Database on Body Mass Index: World Health Organization; 2006 [Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html.

145. Nana A. Reliability of dual-energy X-ray absorptiometry (DXA) in assessing body composition in elite athletes. 2013

146. Nana AS, Gary J. Stewart, Arthur D. Burke, Louise M. Methodology review: using dual-energy X-ray absorptiometry (DXA) for the assessment of body composition in athletes and active people. International Journal of Sport Nutrition and Exercise Metabolism. 2015;25(2):198-215.doi:https://doi.org/10.1123/ijsnem.2013-0228

147. Bilsborough JC, Greenway K, Opar D, Livingstone S, Cordy J, Coutts AJ. The accuracy and precision of DXA for assessing body composition in team sport athletes. Journal of Sports Sciences. 2014;32(19):1821-8.doi:https://doi.org/10.1080/02640414.2014.926380

148. Matute-Llorente Á, Lozano-Berges G, Gómez-Bruton A, Gómez-Cabello A, González-Agüero A, Vicente-Rodríguez G, et al. Comparison of Body Composition Methods for the Assessment of Body Fat in Adolescent Soccer Players. Medicine and Science in Sports and Exercise 2016;48(5):986-7

149. Santos DA, Gobbo LA, Matias CN, Petroski EL, Gonçalves EM, Cyrino ES, et al. Body composition in taller individuals using DXA: A validation study for athletic and non-athletic populations. Journal of Sports Sciences. 2013;31(4):405-13.doi:https://doi.org/10.1080/02640414.2012.734918

150. Mathur N, Pedersen BK. Exercise as a Mean to Control Low-Grade Systemic Inflammation. Mediators of Inflammation. 2008.doi:http://dx.doi.org/10.1155/2008/109502

151. Vokurka M, Lacinová Z, Kremen J, Kopecký P, Bláha J, Pelinková K, et al. Hepcidin expression in adipose tissue increases during cardiac surgery. Physiological Research. 2010;59(3):393

152. Aeberli I, Hurrell RF, Zimmermann MB. Overweight children have higher circulating hepcidin concentrations and lower iron status but have dietary iron intakes and bioavailability comparable with normal weight children. International Journal of Obesity. 2009;33(10):1111-7.doi:10.1038/ijo.2009.146

153. Tussing-Humphreys LM, Nemeth E, Fantuzzi G, Freels S, Guzman G, Holterman AXL, et al. Elevated systemic hepcidin and iron depletion in obese premenopausal females. Obesity. 2010;18(7):1449-56.doi:https://doi.org/10.1038/oby.2009.319

154. Cepeda-Lopez AC, Allende-Labastida J, Melse-Boonstra A, Osendarp SJ, Herter-Aeberli I, Moretti D, et al. The effects of fat loss after bariatric surgery on inflammation, serum hepcidin, and iron absorption: a prospective 6-mo iron stable isotope study. The American Journal of Clinical Nutrition. 2016;104(4):1030-8.doi:https://doi.org/10.3945/ajcn.115.115592

155. Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. Clinical Chemistry. 2003;49(10):1573-8.doi:10.1373/49.10.1573 Published September 2003

156. The Royal Collage of Pathologists of Australasia. Ferritin www.rcpa.edu.au2015 [cited 2018. Available from: https://www.rcpa.edu.au/Library/Practising-Pathology/RCPA-Manual/Items/Pathology-Tests/F/Ferritin.

157. Peyrin-Biroulet LW, Nicolas. , Cacoub P. Guidelines on the diagnosis and treatment of iron deficiency across indications: a systematic review. The American Journal of Clinical Nutrition. 2015;102(6):1585-94.doi:https://doi.org/10.3945/ajcn.114.103366

158. Becton Dickinson UK L. BD Diagnostics - Preanalytical Systems Product Catalogue 2009/10. In: Becton DUKL, editor. 2009.

159. World Health Organization. WHO guidelines on drawing blood: best practices in phlebotomy. 2010

160. BD Vaucutainer Tube Guide In: Becton DaC, editor. Oakville, Ontario2006.

161. Read about plastic tubes for blood banking. In: Becton DaC, editor. www.bd.com2006.

162. Roche Diagnostics. IRON2, Iron Gen.2 Cobas 8000. In: GmbH RD, editor. Mannheim2015.

163. Epstein D, Borohovitz A, Merdler I, Furman M, Atalli E, Sorkin A, et al. Prevalence of Iron Deficiency and Iron Deficiency Anemia in Strenuously Training Male Army Recruits. Acta Haematologica. 2018;139(3):141-7.doi:https://doi.org/10.1159/000485736

164. Sinclair L, Hinton P. Prevalence of iron deficiency with and without anemia in recreationally active men and women. Journal of the American Dietetic Association. 2005;105(6):975-8.doi:https://doi.org/10.1016/j.jada.2005.03.005

165. Bachman E, Travison TG, Basaria S, Davda MN, Guo WL, Michelle. , Connor Westfall J, et al. Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: evidence for a new erythropoietin/hemoglobin set point. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2013;69(6):725-35.doi:https://doi.org/10.1093/gerona/glt154

166. Liu Z, Ye F, Zhang H, Gao Y, Tan A, Zhang S, et al. The association between the levels of serum ferritin and sex hormones in a large scale of Chinese male population. PloS one. 2013;8(10):75908.doi:https://doi.org/10.1371/journal.pone.0075908

167. Chao K-C, Chang C-C, Chiou H-Y, Chang J-S. Serum ferritin is inversely correlated with testosterone in boys and young male adolescents: a cross-sectional study in Taiwan. PloS one. 2015;10(12):e0144238.doi:https://doi.org/10.1371/journal.pone.0144238

168. Telford RD, Sly GJ, Hahn AG, Cunningham RB, Bryant C, Smith JA. Footstrike is the major cause of hemolysis during running. Journal of Applied Physiology. 2003;94(1):38-42

169. Telford RD, Cunningham RB. Sex, sport, and body-size dependency of hematology in highly trained athletes. Medicine and science in sports and exercise. 1991;23(7):788-94

170. Merkel DH, Michael. Grotto, Itamar. Blum, Dalit. Rachmilewitz, Eliezer. Fibach, Eitan. Epstein, Yoram. Shpilberg, Ofer. Incidence of anemia and iron deficiency in strenuously trained adolescents: results of a longitudinal follow-up study. Journal of Adolescent Health. 2009;45(3):286-91

171. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia. Journal of General Internal Medicine. 1992;7(2):145-53

172. Tabassum H, Sinha AK, Singh T. Correlation of Bone Marrow Iron Storage with Different Types of Anemia. Congestive Heart Failure. 2016;1:2

173. Riley RS, Williams D, Ross M, Zhao S, Chesney A, Clark BD, et al. Bone Marrow Aspirate and Biopsy: A Pathologist's Perspective. II. Interpretation of the Bone Marrow Aspirate and Biopsy. Journal of Clinical Laboratory Analysis. 2009;23:259-307.doi:https://doi.org/10.1002/jcla.20305

174. Garcia-Casal MN, Pasricha SR, Martinez RX, Lopez-Perez L, Peña-Rosas JP. Serum or plasma ferritin concentration as an index of iron deficiency and overload. The Cochrane Library. 2015.doi:10.1002/14651858.CD011817

175. Kim A, Nemeth E. New insights into iron regulation and erythropoiesis. Current Opinion in Hematology. 2015;22(3):199.doi:10.1097/MOH.0000000000000132

176. Hagler L, Askew E, Neville J, Mellick P, Coppes Jr R, Lowder Jr J. Influence of dietary iron deficiency on hemoglobin, myoglobin, their respective reductases, and skeletal muscle mitochondrial respiration. The American Journal of Clinical Nutrition. 1981;34(10):2169-77.doi:https://doi.org/10.1093/ajcn/34.10.2169

177. Merrill JF, Thomson DMH, Shalene E. Hepworth, Squire D. , Willie S, Hancock CR. Iron deficiency causes a shift in AMP-activated protein kinase (AMPK) subunit composition in rat skeletal muscle. Nutrition and Metabolism. 2012;9(1):104.doi:https://doi.org/10.1186/1743-7075-9-104

178. Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. The Journal of Nutrition. 2001;131(2):676-90.doi:https://doi.org/10.1093/jn/131.2.676S

179. Alghadir AH, Gabr SA, Aly FA. The effects of four weeks aerobic training on saliva cortisol and testosterone in young healthy persons. Journal of physical therapy science. 2015;27(7):2029-33

180. Emami A, Nazem MR, Hedayati M, Karami M. Four months of combined and compound morning training improves testosterone/cortisol ratio, adiponectin and insulin resistance in male students. Sport Sciences for Health. 2016;12(2):215-20

181. Harrison J, Hopkins W, MacFarlane D, Worsley A. Nutrition knowledge and dietary habits of elite and non-elite athletes. Australian journal of nutrition and dietetics. 1991

182. Merkel D, Huerta M, Grotto I, Blum D, Rachmilewitz E, Fibach E, et al. Incidence of anemia and iron deficiency in strenuously trained adolescents: results of a longitudinal follow-up study. Journal of Adolescent Health. 2009;45(3):286-91.doi:https://doi.org/10.1016/j.jadohealth.2009.02.003

183. Rowland TW, Black SA, Kelleher JF. Iron deficiency in adolescent endurance athletes. Journal of Adolescent Health Care. 1987;8(4):322-6.doi:https://doi.org/10.1016/0197-0070(87)90002-7

184. Red Blood Cell (RBC) Indices: Definitions and Calculations: LabCE.com; 2001 [Available from: https://www.labce.com/spg579119_red_blood_cell_rbc_indices_definitions_and_calcula.aspx.

8 Appendixes

8.1 Appendix I – Manuscript draft

Body composition in relation to iron deficiency among athletes and physically active individuals

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Key words: Iron deficiency, athletes, body fat percentage, BMI, training

Abstract

Background: Athletes have a higher prevalence of **i**ron deficiency (ID) than their sedentary counterparts but the condition has been associated with decreased athletic performance. High body fat percentage has been related to higher prevalence of ID among overweight and obese individuals.

Objective: To investigate the association between body mass index (BMI) and percentage body fat (%BF) with ID among athletes and physically active individuals. Furthermore, to explore whether higher amount of training increases the probability of ID among athletes and physically active individuals.

Methods: This study was performed at the Sports Medicine unit, Umeå University, Sweden, on young elite athletes and physically active young people. Results from three different cohorts were combined, including 266 subjects; 122 females and 144 males, 15 – 26 years of age who were recruited during the years 2013-2017. Information about body composition measurements (BMI and %BF measured throug DXA scans), training amount (hours per week) and supplement intake measured with questionnaires were available for every subject as well as blood sample analysis on iron status. The study only included individuals who trained on average at least 5 hour per week or more. Furthermore, individuals who reported taking vitamin C supplements or iron supplements were excluded from the analysis.

Results: Both higher BMI and higher training amount were associated with decreased risk of experiencing ID. %BF was not associated with ID. Additionally, females were more likely to experience iron deficiency, but higher age in females was associated with a reduction in the likelihood of experiencing ID, although weak.

Conclusions: It is clear that ID is a common issue among female athletes, more so than among their male counterparts. Surprisingly both increased training amount in males and higher BMI decreased the likelihood of experiencing ID but no association was found between %BF and ID. Further research is needed on ID among athletes and physically active individuals and whether there is a relationship between percentage body fat and ID in this population.

Introduction

Iron deficiency (ID) is one of the most widespread nutrition deficiencies in the world and is diagnosed as iron deficiency anemia (IDA) in its more severe form. In 2011 the World Health Organization estimated that approximately 43% of children and 29% of all females of reproductive age had anemia globally of which approximately 50% in females and 42% in children could be attributed to iron (1, 2). Furthermore, ID has been reported to be more frequent among athletes than in inactive individuals, especially female athletes, who can be twice as susceptible compared to their sedentary counterparts (3-5). ID can have considerable effects on athletic performance, resulting in impaired performance such as energy efficiency and endurance (4). With regards to the prevalence of both ID and IDA among athletes and the effects it can have on performance, especially in females, iron status of athletes should be monitored at regular intervals (6-10) as well as in the general population.

The most common causes of IDA worldwide are blood loss, the maternal-fetal bridge of iron deficiency, malaria, hookworm as well as diet and malabsorption of iron (11). Athletes are considered to be at more risk than less active people due to increased iron loss through hemolysis, sweating, increased hepcidin concentration (the primary regulator of iron homeostasis in the body) due to exercise-induced inflammation and in some cases due to gastrointestinal bleeding and urinary blood loss (9, 12). Hepcidin, the main regulator for iron absorption is produced in skeletal muscles during exercise in addition to being gradually expressed in adipose tissue during exercise (13-18). Interestingly, hepcidin has also been indicated to be expressed in adipose tissue of obese patients correlating with indexes of inflammation (19). Aebeli and coworkers. (20) found that obese children are more likely to be ID compared to normal weight children and that it could potentially be explained by increased hepcidin levels in the obese group. In a recent study, iron absorption increased in iron deficient obese subjects following fat loss after bariatric surgery and hepcidin levels decreased in all subjects after surgery (21). These results indicate that there may be a relationship between body fat, hepcidin levels and iron status. The association between BMI and ID has been studied previously (22), but there are no existing studies on the relationship between percentage body fat (%BF) and prevalence of ID in normal weight individuals. Therefore, it is of special interest to examine whether this relationship exists among athletes, since the prevalence of ID has been found to be particularly high in that population. The aim of this study was to investigate the association between (BMI) and (%BF) with ID among athletes and physically active individuals. Furthermore, to explore whether higher amount of training increases the probability of ID among athletes and physically active individuals.

Methods

Participants were all Swedish competitive athletes or physically active young people, exercising at a minimum of five hours per week. The data for the current study was combined from three previous or ongoing cohorts/projects at the School of Sport Science at Umeå University. These included young elite athletes from an upper secondary sports school in Umeå (Dragonskolan) including data from 224 participants (95 females, 129 males) collected in 2015, young elite cross-country skiers from an upper secondary school in Lycksele including data from 30 participants (14 females, 16 males) collected in

2014 and data collected on twelve professional female soccer players in the Swedish national team from measurements performed at regular intervals.

Thus, measurements were available from 266 subjects; 122 females and 144 males aged between 15 and 26 years. All participants signed an informed consent form before participating in any of the studies.

Measurements

Blood measurements included leukocytes, erythrocytes, hemoglobin, hematocrit, thrombocyte, erythrocyte- mean corpuscular volume (MCV) and plasma ferritin. In addition, erythrocyte- mean corpuscular hemoglobin (MCH) was calculated using the equations; $MCH = Hb\left(\frac{g}{dl}\right)x10 \div RBC(x10^{12}/L)$ (23, 24). To determine ID in samples a cut-off point including both ≤30 μg/L ferritin as well as hemoglobin ≥120 g/L for females and ≥130 g/L for males (3). IDA was determined as having ferritin <30 μg/L as well as hemoglobin <120 g/L for females and ferritin <30 μg/L and hemoglobin <130 g/L for males. Additionally, the data included information on both supplement intake and training amount, measured in hours, obtained with questionnaires, asked same day as the blood was drawn. The questions on training and supplement intake were comparable in all three cohorts. Measurements on height and weight for body mass index (BMI) and %BF measured by Dual-energy X-ray absorptiometry (DXA).

Statistical data and analysis

The study participants were identified as having normal iron status, ID or IDA. As only a few participants classified as being anemic (n=6 females, n=3 males) they were grouped together with ID participants for data analysis.

In addition to the iron status groups, the study population was defined according to gender, age and body composition. BMI cut off points for individuals under 18 years were according to the international cut-offs for BMI developed by Cole et al (25). The BMI cut-offs used for adults (18 - 26 years) were according to the WHO BMI classifications for adults (26). The cut-offs used to group by % BF have been defined by Heyward and coworkers and McCarthy and coworkers (27, 28). It is worth noting that no cut-off points are available for physically active young individuals, thus the cut-offs for young individuals not otherwise specified were used. Cross tabulation was performed to assess the frequency and percentage of participants grouped by gender, age and body composition. Few participants (1 female and 3 males) were defined as low in body fat, thus they were grouped together with the average body fat group. Similarly, underweight and normal weight as defined by BMI were grouped together and overweight and obese individuals together. Pearson Chi-Square test was used to compare differences between groups.

Correlation between blood variables, body composition variables and training amount was found using Spearman's correlation coefficient as the data was not normally distributed. An independent sample T-test was used to compare differences in age, training amount, body composition and blood variables between iron status groups. Mann-Whitney test was used to compare difference between age, training amount, body composition groups and blood variables.

Logistic regression was performed to predict the likelihood of iron deficiency based on age, gender, training amount in hours, BMI and % BF. Additionally, the logistic regression was also run divided by gender. Level of significance was set at P≤0.05. Due to the significant correlation found between BMI and %BF an interaction variable was constructed and put into the logistic regression model. It was nonsignificant and thus not examined further.

Results

Characteristics of the study population according to gender are shown in table 1. Females had on average lower ferritin levels than their male participants (37.3vs. 66.2 μg/L; P=0.001) and thus ID was considerably more common among females, or 40.2% compared to 10.4%, respectively (P<0.001). Additionally, difference was found between the genders in training amount, %BF, hemoglobin and erythrocytes. When participants were divided into groups according to BMI and body fat percentage no statistical difference was found between gender regardless of age (table 2). Spearman's correlation showed a significant association between training amount and ferritin status in females (R=0.195; p=0.20). Additionally, a correlation was found between BMI and body fat percentage in both genders (table 3).

When differences in blood variables, training amount, age and body composition were compared between normal iron status group and ID group, both females and males with normal iron status were on average one year older than those with ID (18.31 vs. 17.04 years for females and 17.44 vs. 16.88 years for males, respectively; P=<0.001 and P=0.019). Additionally, males with normal iron status trained on average more than ID males (11.94 vs. 9.97; P=0.015) (table 4). As shown in table 5, male participants with higher fat percentage were a bit older (17.28 vs. 17.73 years; P=0.025) in addition to having higher ferritin levels (62.19 vs. 80.71) (P=0.019). A significant difference in body fat percentage was found between individuals who were under- or normal weight and those who were overweight or obese, showing that those with higher BMI also had higher body fat percentage (table 6). A significant difference in age was only found for male participants, indicating that those who were defined using BMI as overweight or obese were on average younger (P=0.044).

A binomial logistic regression was performed to ascertain the associations of age, gender, training amount, BMI and, body fat percentage on the likelihood that participants present with iron deficiency. The logistic regression model was statistically significant, $X^2(5)$ = 69,450 P ≤0.001 explaining 34.3% (Nagelkerke R^2) of the variance in iron deficiency, both OR and 95% CI are shown in table 7. As shown in table 7 of the five predictor variables, four of them were statistically significant: age (P=<0.001), gender (P=0.024), training amount (P=0.033) and, BMI (P=0.009). Females were 3.54 times more likely to experience ID, but increased age was associated with a reduction in the likelihood of experiencing ID. Additionally both higher BMI and increased training amount were associated with decresed risk of experiencing iron deficency. Table 8 shows a binomial logistic regression performed dependent on gender. The logistic regression models were both statistically significant. The female model, $X^2(4)$ = 22.574 P ≤0.001 explained 23.3% (Nagelkerke R^2) of the variance in iron deficiency and the male model, $X^2(4)$ = 12.309, P ≤ 0.015 explained 16.2% (Nagelkerke R^2) of the variance, both OR and 95% CI are

shown in table 8. The only statistically significant predicting variable was age for females but it was associated with reduction in the likelihood of experiencing iron deficiency.

Discussion

The results of this study show a positive association between BMI and training amount and decreased risk for ID while no association was found with %BF. Furthermore, the results show that females have a substantially higher prevalence of ID than males, or a staggering 40.2% versus 10.4%.

No association was found between %BF and ID, in contrast, when compared with males with low and average body fat percentage, males who presented with high percentage of body fat also presented with higher ferritin levels. These results contradict other findings who show association between adiposity and poor iron status. However, it should be noted that those studies were not conducted on athletes and adiposity would include higher levels of body fat than is to be expected among highly active individuals. Tussing-Humphreys and coworkers. (29) found that ID was associated with BMI and inflammation in female adolescents. Similarly, a US national health and nutrition examination survey showed that as BMI increased from normal weight to at risk for overweight (85^{th}-94^{th} percentile) and then to overweight so did the prevalence of ID in children and adolescents, with ID being especially common among adolescents (30). Furthermore, studies have also shown that this increased prevalence of ID among overweight and obese individuals is not due to poor dietary iron intake which indicates that the reason for this could be due to the effects of adipose-related inflammation on dietary iron absorption through hepcidin (31, 32).

Interestingly, we found that ID males trained on average fewer hours than males with normal iron status. Haas and Brownlie (33) found in their systematic review that energy efficiency was affected in ID humans. Additionally, they found strong effects of both severe IDA and moderate IDA on aerobic capacity and a strong biological mechanism for the effect of IDA on work capacity. Although we are unable to confirm it due to the limitations in data in this study, a plausible explanation for this difference in training amount could be that ID males are more likely to experience weariness and exercise-associated dyspnea, known symptoms of ID (11). However, no difference in training amount was found between ID females and those with normal iron status. This might be due to the fact that the females in this study trained less than the males and had less variance of training amount.

Ferritin cut-offs are based on bone marrow iron microscopy but ferritin has been show to correlate well with bone marrow iron stores (34, 35). Since the bone marrow is a major storage site for iron in the body this method evaluates body iron stores with high accuracy (36, 37). Recent studies have found that during ID the production of erythropoietin is decreased, thus lowering the production of red blood cells and reducing iron drain from the systemic iron pool. This occurs at the expence of iron needs of other tissues (38). Hagler and coworkers found that during ID preferential utilization of iron occured both between and within tissue. ID rats experienced a 35% reduction in hemoglobin and 20-37% reduction in skeletal muscle myoglobin resulting in impaired skeletal muscle oxidative capacity (39). Furthermore, ID has been linked to chronic increase in AMP-activated protein kinase in rat skeletal muscle. This results in a shift from oxidative metabolism in the muscles towards glycolytic metabolism which reflects in muscle oxidative capacity (40). The results from these studies raise a question whether the enzyme

activity in muscle fibers may be negatively affected by low iron status much earlier than a measurable decrease in iron status in the bone marrow is found. Thus, skeltetal muscle aerobic function might not be a priority over immune function or heart muscle function. Therfore, the clinical cut-off for athletes may likely be higher as a small reduction in iron status may affect athletetes performance to a quite an extent.

The present results show a much higher prevalence of ID among female participants than among male participants. These findings are consistent with a number of studies who have found prevalence of ID and IDA among physically active females to range from 29% to 77% and 10% to 14% respectively. The prevalence of ID and IDA among physically active males has been found to range from 4% to 15% and 2% to 3%, respectively in other studies (3, 41-46). On basis of these results and those of earlier studies it is logical to speculate on the cause of this gender difference. The clear difference in the mean ferritin levels between females and males, as shown in present study as well as in earlier studies, might suggest that the cut-off criteria is wrong for males or females. ID is defined using the same ferritin cut-offs for both genders, but the hemoglobin cut-offs are different for females and males. Since males have relatively higher levels of iron in their body, it is rational to suggest that the ferritin cut-offs criteria be higher for males just as it is for hemoglobin.

When compared between iron status groups, both females and males with ID were found to be younger than their counterparts in the normal iron status group. This is reflected in our finding where age appears to be a protective factor. This does however not seem to be the case in all studies on adolescents. It is however worth noting that the age range in the present study ranged higher than in the following studies. Merkel and coworkers (47) found that the prevalence of ID among strenuosly trained adolescents was 24.5%. Rowland and coworkers (48) found that 45% of adolecent female athletes had ID and 17% of adolecent male athletes. These findings are consistent with results obtained on the prevalence of ID among physically active adults. Nead and coworkers (30) found ID to be more prevalent among adolescents.

Additionally, we found that 39.5% of the older males were classified as having high body fat percentage but only 13.9% of the younger males. This however, is not the case when looking at BMI where 2.3% of the older males are classified as being either overweight or obese while 11.9% of the younger males are presented as being either overweight or obese. This proposes the question whether there are any flaws in the criteria used to classify percentage of body fat in males. The likely explanation to this it that the cut-offs used for older males are specific for physically active persons, but no specific cut-offs are available with regard to physical activity in the younger age group. Thus, the cut-offs for unspecified young males was used which is higher than for the older males, 20% vs 15%, respectively (27, 49).

In order to prevent both ID and IDA among athletes optimal diet should be used as the first line of defence. Before recommending iron supplementation efforts should be made to adjust the diet so that intake of iron increase trough iron rich foods due to the harmful effects that iron can have on the body if the intake is excessive.

Strengths and limitations

This research is not without its limitations. Due to the ex post facto design of our study, information on dietary iron intake was unavailable and accurate data on type of training performed and the intensity of the training load was missing. Additionally, the comparability in the famale training amount is considered a limitating factor which might mean that the sample did not include females with high and diverse enough training amount. We did not have measurements on inflammation markers such as C-reactive protein or IL-6 to assess the states of inflammation nor did we have measurements on hepcidin levels which would have given strength to our analysis. Due to these limitations we were unable to explore whether ID was related to increased hepcidin secretion. Hepcidin is known to be produced in adipose tissue (15, 50) and increases following exercise due to exercise induced inflammation (51). Future studies should include measurements on inflammation markers and hepcidin in order to assess whether this might be the mechanism behind the increased prevalence of ID among athletes and physically active individuals. Therefore, these findings offer a promising area for future focus in this field of research.

Conclusions: The present study show a positive association between BMI and training amount and decreased risk for ID while no association was found with %BF. Additionally, females who are physically active are more likely to experience ID than physically active males. Further research is needed to study the relationship between adipose tissue and ID in athletes and physically active individuals and what mechanisms are potentially involved. More studies are needed on both adipose tissue-related inflammation and exercise-related inflammation and its effects on iron status in athletes and physically active individuals.

Table 1. Characteristics of the study population according to gender.

	Females (n=122)	Males (n=144)	P-value
Age (years)	17.7 (2.02)	17.4 (0.95)	0.991
BMI (kg/m^2)	21.0 (2.52)	21.3 (2.31)	0.258
Percentage body fat (%)	25.9 (4.47)	15.9 (4.13)	<0.001*
Training (hours/week)	10.3 (2.06)	11.6 (3.47)	0.001*
Plasma ferritin (μg/L)	37.3 (21.14)	66.2 (32.13)	<0.001*
Hemoglobin (g/L)	130 (8.32)	146 (8.47)	<0.001*
MCH (pg)	29.0 (1.74)	29.2 (1.30)	0.444
MCV (fL)	88 (4.46)	87 (3.70)	0.081
Erythrocytes (10^9/L)	4.5 (0.30)	5.0 (0.27)	<0.001*
Iron status			
Normal iron status (%)[a]	67 (54.9)	126 (87.5)	<0.001*
Iron deficiency (%)[a]	49 (40.2)	15 (10.4)	
Iron deficiency anemia (%)[a]	6 (4.9)	3 (2.1)	

Iron deficiency cut off points are ferritin < 30 μg/L and hemoglobin ≥120 g/L (females), ≥130 g/L (males) and for iron deficiency anemia the cut off points are ferritin <30 μg/L and hemoglobin <120 g/L (females) <130 g/L (males).

Data is displayed as mean and standard deviation (SD)

[a] Are represented as frequency and (percent) in each group

*Significant difference between gender (P≤ 0.05) measured with Mann-Whitney test

Table 2a. Study population defined by body compositions, gender and age.

	Under 18 years			Adults (18y and older)		
	Female (n=78)	Male (n=101)	P-value	Female (n=44)	Male (n=43)	P-value
Underweight (%)	*Not specified*		0.849	9 (20.5)	3 (7.0)	0.317
Normal weight (%)	68 (87.2)	89 (88.1)		32 (72.7)	39 (90.7)	
Overweight (%)	10 (12.8)	11 (10.9)		3 (6.8)	1 (2.3)	
Obese (%)	0 (0.0)	1 (1.0)		0 (0.0)	0 (0.0)	
Body fat %			0.237			0.052
Low body fat (%)	1 (1.3)	3 (3.0)			*Not specified*	
Average body fat (%)	61 (78.2)	84 (83.2)		35 (79.5)	26 (60.5)	
High body fat (%)	16 (20.5)	14 (13.9)		9 (20.5)	17 (39.5)	

Cut off points made for individuals under 18 years are according to the international cut off point for BMI developed by Cole et al. The BMI cut off points for adults are according to the WHO BMI classification.

Data is displayed as frequency and (percentage)

Pearson Chi-Square test was used to compare difference between groups.

Table 2b. Study population defined by body composition and gender.

	Female	**Male**	**P-value**
Under- and normal weight (%)	109 (89.3)	131 (91.0)	0.656
Overweight and obese (%)	13 (10.7)	13 (9.0)	
Body fat %			0.836
Low and average body fat (%)	97 (79.5)	113 (78.5)	
High body fat (%)	25 (20.5)	31 (21.5)	

Data is displayed as frequency and (percentage)

Pearson Chi-Square test was used to compare difference between groups.

Table 3. Correlation coefficients between blood variables, body composition variables and, training amount and body composition variables and training amount.

	Females (n=122)			Males (n=144)		
	BMI	Body fat%	Training amount	BMI	Body fat%	Training amount
Plasma ferritin (μg/L)	0.08	0.01	0.20*	0.07	0.04	0.12
Hemoglobin (g/L)	-0.04	-0.01	0.01	0.13	-0.02	0.00
Erythrocytes (10^9/L)	0.06	0.04	-0.98	0.09	-0.09	0.02
MCH (pg)	-0.11	-0.04	0.03	0.01	0.01	0.06
BMI (kg/m^2)		0.60*	0.00		0.40*	0.15
Body fat %	0.60*		-0.98	0.40*		-0.11
Training (hours/week)	0.00	-0.10		0.15	-0.11	

*Significance of association between the variables (P≤ 0.05)

Spearman ‘s correlation coefficient was used to define the significance of association between the variables.

Table 4. Blood variables, age, training amount and, body composition divided by iron status.

Females	**Normal (n=67)**	**Iron deficiency (n=55)**	**P-value**
Age (years)	18.3	17.0	<0.001*
Training (hours/week) [a]	10.6	9.9	0.091
Fat percentage	25.7	26.1	0.646
BMI (kg/m^2)	21.3	20.7	0.218
Hemoglobin (g/L)	132	129	0.061
Plasma ferritin (μg/L)	51.4	20.2	<0.001*
MCH (pg)	29.5	28.5	0.003*
MCV (fL)	88	87	0.135
Erythrocytes (10^9/L)	4.5	4.5	0.257
Males	**Normal (n=126)**	**Iron deficiency (n=18)**	**P-value**
Age (years)	17.4	16.9	0.019*
Training (hours/week) [b]	11.9	9.9	0.015*
Fat percentage	15.8	16.7	0.393
BMI (kg/m^2)	21.4	20.6	0.305
Hemoglobin (g/L)	147	140	0.002*
Plasma ferritin (μg/L)	72.4	22.8	<0.001*
MCH (pg)	29.4	28.1	<0.001*
MCV (fL)	87	85	0.014*
Erythrocytes (10^9/L)	5.0	4.9	0.927

Data is displayed as mean

*Significant difference between normal iron status and iron deficiency (P≤0.05)

Independent sample T-test was used to compare difference between groups

[a] n=65 and 53 for normal iron status and iron deficiency, respectively

[b] n=121 and 17 for normal iron status and iron deficiency, respectively

Table 5. Blood variables, age, training amount and BMI divided by percentage body fat (% BF).

Females	**Low and average %BF (n=97)**	**High %BF (n=25)**	**P-value**
Age (years)	17.8	17.4	0.819
Training (hours/week) *	10.5	9.8	0.139
BMI (kg/m^2)	20.5	23.0	<0.001**
Hemoglobin (g/L)	131	129	0.393
Plasma ferritin (μg/L)	36.5	40.6	0.324
MCH (pg)	29.1	28.8	0.710
MCV (fL)	88	87	0.642
Erythrocytes (10^9/L)	4.5	4.5	0.742
Males	**Low and average %BF (n=113)**	**High %BF (n=31)**	**P-value**
Age (years)	17.3	17.7	0.025**
Training (hours/week) *	11.8	11.4	0.605
BMI (kg/m^2)	20.7	23.3	<0.001**
Hemoglobin (g/L)	146	145	0.606
Plasma ferritin (μg/L)	62.2	80.7	0.019**
MCH (pg)	29.2	29.2	0.955
MCV (fL)	87	87	0.857
Erythrocytes (10^9/L)	5.0	5.0	0.221

Data is displayed as mean

*n= 94 and 24 for female low and average fat percentage group and high fat percentage group, respectively; n=109 and 29 for male low and average fat percentage group and high fat percentage group, respectively

Mann-Whitney test was used to compare difference between groups

**Significant difference between groups (P≤0.05)

Table 6. Blood variables, age, training amount and, percentage body fat (%BF) divided by body mass index (BMI)

Females	**Under- and normal weight (n=109)**	**Overweight and obese (n=13)**	**P-value**
Age (years)	17.8	17.0	0.150
Training (hours/week) *	10.4	10.4	0.188
% BF	25.2	32.1	<0.001**
Hemoglobin (g/L)	130	129	0.702
Plasma ferritin (μg/L)	36.7	42.3	0.357
MCH (pg)	29.1	28.7	0.194
MCV (fL)	88	87	0.221
Erythrocytes (10^9/L)	4.5	4.5	1.000
Males	**Under- and normal weight (n=131)**	**Overweight and obese (n=13)**	**P-value**
Age (years)	17.4	16.9	0.044**
Training (hours/week) *	11.7	12.1	0.712
% BF	15.2	23.5	<0.001**
Hemoglobin (g/L)	146	149	0.218
Plasma ferritin (μg/L)	66.1	66.8	0.315
MCH (pg)	29.2	29.4	0.983
MCV (fL)	87	87	0.782
Erythrocytes (10^9/L)	5.0	5.1	0.621

Data is displayed as mean

*n= 106 and 12 for under- and normal weight group and overweight and obese group, respectively; n= 125 for male under- and normal weight group

Mann-Whitney test was used to compare difference between groups

**Significant difference between groups (P≤0.05)

Table 7. Logistic regression predicting likelihood of iron deficiency based on age, gender, training amount (hours/week), BMI and, percentage body fat (%BF).

	B	***SE***	***Wald***	***P-value***	***Odds Ratio***	***95% CI***	
						Lower	***Upper***
Age (years)	-0.60	0.16	13.21	<0.001*	0.55	0.40	0.76
Gender	1.26	0.56	5.08	0.024*	3.54	1.18	10.64
Training	-0.15	0.07	4.53	0.033*	0.86	0.75	0.99
BMI (kg/m^2)	-0.22	0.09	6.73	0.009*	0.80	0.68	0.95
% BF	0.06	0.05	1.75	0.185	1.06	0.97	1.17

*Statistically significant variables (P≤0.05)

Gender is for females compared to males

Table 8. Logistic regression predicting likelihood of iron deficiency based on age, training amount (hour/week), BMI and, percentage body fat (%BF) divided by gender.

Females	***B***	***SE***	***Wald***	***P-value***	***Odds Ratio***	***95% CI***	
						Lower	***Upper***
Age (years)	-0.60	0.19	10.11	0.001*	0.55	0.38	0.80
Training	-0.15	0.10	2.12	0.145	0.86	0.71	1.05
BMI (kg/m^2)	-0.21	0.11	3.50	0.061	0.81	0.65	1.01
% BF	0.04	0.06	0.38	0.536	1.04	0.92	1.17
Males	***B***	***S.E***	***Wald***	***P-value***	***Odds Ratio***	***95% C.I***	
						Lower	***Upper***
Age (years)	-0.59	0.34	3.09	0.079	0.55	0.29	1.07
Training	-0.14	0.10	2.16	0.141	0.87	0.72	1.05
BMI (kg/m^2)	-0.24	0.14	2.93	0.087	0.79	0.60	1.04
% BF	0.10	0.07	1.75	0.186	1.10	0.96	1.27

*Statistically significant (P≤0.05)

References

1. Longo DL, Camaschella C. Iron-deficiency anemia. New England Journal of Medicine. 2015;372(19):1832-43.doi:DOI: 10.1056/NEJMra1401038
2. World Health Organization. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. 2001
3. Clénin G, Cordes M, Huber A, Schumacher YO, Noack P, Scales J, et al. Iron deficiency in sports–definition, influence on performance and therapy. Swiss Medical Weekly. 2015;145:w14196.doi:https://doi.org/10.4414/smw.2015.14196
4. Dellavalle DM, Haas JD. Iron supplementation improves energetic efficiency in iron-depleted female rowers. Medicine and Science in Sports and Exercise. 2014;46(6):1204-15.doi:10.1249/MSS.0000000000000208
5. Nielsen P, Nachtigall D. Iron supplementation in athletes. Sports Medicine 1998;26(4):207-16
6. Alaunyte L, Stojceska V, Plunkett A, Derbyshire E. Dietary iron intervention using a staple food product for improvement of iron status in female runners. Journal of the International Society of Sports Nutrition. 2014;11(1):50.doi:https://doi.org/10.1186/s12970-014-0050-y
7. Di Santolo M, Stel G, Banfi G, Gonano F, Cauci S. Anemia and iron status in young fertile non-professional female athletes. European Journal of Applied Physiology. 2008;102(6):703-9
8. Fallon KE. Utility of hematological and iron-related screening in elite athletes. Clinical Journal of Sport Medicine. 2004;14(3):145-52
9. Malczewska-Lenczowska J, Orysiak JS, B. , Turowski D, Burkhard-Jagodzińska K, Gajewski J. Reticulocyte and erythrocyte hypochromia markers in detection of iron deficiency in adolescent female athletes. Biology of Sport. 2017;34(2):111.doi:10.5114/biolsport.2017.64584
10. Sandström G, Börjesson M, Rödjer S. Iron Deficiency in Adolescent Female Athletes—Is Iron Status Affected by Regular Sporting Activity? Clinical Journal of Sport Medicine. 2012;22(6):495-500.doi:doi: 10.1097/JSM.0b013e3182639522
11. Miller JL. Iron deficiency anemia: a common and curable disease. Cold Spring Harbor perspectives in medicine. 2013;3(7):a011866.doi:10.1101/cshperspect.a011866
12. Peeling P, Sim M, Badenhorst CED, Brian. , Govus AD, Abbiss CR, Swinkels DW, et al. Iron status and the acute post-exercise hepcidin response in athletes. PLoS One. 2014;9(3):e93002.doi:https://doi.org/10.1371/journal.pone.0093002
13. Daher R, Karim Z. Iron metabolism: State of the art. Transfusion Clinique et Biologique. 2017;24(3):115-9.doi:https://doi.org/10.1016/j.tracli.2017.06.015
14. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2012;1823(9):1434-43.doi:https://doi.org/10.1016/j.bbamcr.2012.01.014
15. Keller C, Keller P, Marshal S, Pedersen BK. IL-6 gene expression in human adipose tissue in response to exercise–Effect of carbohydrate ingestion. The Journal of Physiology. 2003;550(3):927-31.doi:https://doi.org/10.1113/jphysiol.2003.044883
16. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen B. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. The Journal of Physiology. 1998;508(3):949-53.doi:https://doi.org/10.1111/j.1469-7793.1998.949bp.x
17. Pedersen B, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: is IL-6 a candidate? Journal of Muscle Research and Cell Motility. 2003;24(2-3):113
18. Pedersen B, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? Proceedings of the Nutrition Society. 2004;63(2):263-7.doi:

https://doi.org/10.1079/PNS2004338

19. Bekri S, Gual P, Anty R, Luciani N, Dahman M, Ramesh BI, et al. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. Gastroenterology. 2006;131(3):788-96.doi:https://doi.org/10.1053/j.gastro.2006.07.007

20. Aeberli I, Hurrell RF, Zimmermann MB. Overweight children have higher circulating hepcidin concentrations and lower iron status but have dietary iron intakes and bioavailability comparable with normal weight children. International Journal of Obesity. 2009;33(10):1111-7.doi:10.1038/ijo.2009.146

21. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. The Lancet. 2016;387(10021):907-16.doi:https://doi.org/10.1016/S0140-6736(15)60865-0

22. Eftekhari M, Mozaffari-Khosravi H, Shidfar F. The relationship between BMI and iron status in iron-deficient adolescent Iranian girls. Public Health Nutrition. 2009;12(12):2377-81.doi:https://doi.org/10.1017/S1368980009005187

23. Red Blood Cell (RBC) Indices: Definitions and Calculations: LabCE.com; 2001 [Available from: https://www.labce.com/spg579119_red_blood_cell_rbc_indices_definitions_and_calcula.aspx.

24. Cook JD. Defining optimal body iron. Proceedings of the Nutrition Society. 1999;58(2):489-95.doi:https://doi.org/10.1017/S0029665199000634

25. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. British Medical Journal 2000;320(7244):1240.doi:https://doi.org/10.1136/bmj.320.7244.1240

26. BMI classification WHO :: Global Database on Body Mass Index: World Health Organization; 2006 [Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html.

27. Heyward VH, Gibson A. Advanced fitness assessment and exercise prescription 7th edition: Human kinetics; 2014.

28. McCarthy H, Cole T, Fry T, Jebb S, Prentice A. Body fat reference curves for children. International journal of obesity. 2006;30(4):598.doi:doi:10.1038/sj.ijo.0803232

29. Tussing-Humphreys LM, Liang H, Nemeth E, Freels S, Braunschweig CA. Excess adiposity, inflammation, and iron-deficiency in female adolescents. Journal of the American Dietetic Association. 2009;109(2):297-302.doi:https://doi.org/10.1016/j.jada.2008.10.044

30. Nead KG, Halterman JS, Kaczorowski JM, Auinger P, Weitzman M. Overweight children and adolescents: a risk group for iron deficiency. Pediatrics. 2004;114(1):104-8

31. Cepeda-Lopez AC, Osendarp SJ, Melse-Boonstra A, Aeberli I, Gonzalez-Salazar F, Feskens E, et al. Sharply higher rates of iron deficiency in obese Mexican women and children are predicted by obesity-related inflammation rather than by differences in dietary iron intake–. The American Journal of Clinical Nutrition. 2011;93(5):975-83.doi:https://doi.org/10.3945/ajcn.110.005439

32. Menzie CMY, Lisa B. Denkinger, Blakeley I. McHugh, Teresa. Sebring, Nancy G. Calis, Karim A. Yanovski, Jack A. Obesity-related hypoferremia is not explained by differences in reported intake of heme and nonheme iron or intake of dietary factors that can affect iron absorption. Journal of the American Dietetic Association. 2008;108(1):145-8.doi: https://doi.org/10.1016/j.jada.2007.10.034

33. Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. The Journal of Nutrition. 2001;131(2):676-90.doi:https://doi.org/10.1093/jn/131.2.676S

34. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia. Journal of General Internal Medicine. 1992;7(2):145-53

35. Tabassum H, Sinha AK, Singh T. Correlation of Bone Marrow Iron Storage with Different Types of Anemia. Congestive Heart Failure. 2016;1:2

36. Riley RS, Williams D, Ross M, Zhao S, Chesney A, Clark BD, et al. Bone Marrow Aspirate and Biopsy: A Pathologist's Perspective. II. Interpretation of the Bone Marrow Aspirate and Biopsy. Journal of Clinical Laboratory Analysis. 2009;23:259-307.doi:https://doi.org/10.1002/jcla.20305

37. Garcia-Casal MN, Pasricha SR, Martinez RX, Lopez-Perez L, Peña-Rosas JP. Serum or plasma ferritin concentration as an index of iron deficiency and overload. The Cochrane Library. 2015.doi:10.1002/14651858.CD011817

38. Kim A, Nemeth E. New insights into iron regulation and erythropoiesis. Current Opinion in Hematology. 2015;22(3):199.doi:10.1097/MOH.0000000000000132

39. Hagler L, Askew E, Neville J, Mellick P, Coppes Jr R, Lowder Jr J. Influence of dietary iron deficiency on hemoglobin, myoglobin, their respective reductases, and skeletal muscle mitochondrial respiration. The American Journal of Clinical Nutrition. 1981;34(10):2169-77.doi:https://doi.org/10.1093/ajcn/34.10.2169

40. Merrill JF, Thomson DMH, Shalene E. Hepworth, Squire D. , Willie S, Hancock CR. Iron deficiency causes a shift in AMP-activated protein kinase (AMPK) subunit composition in rat skeletal muscle. Nutrition and Metabolism. 2012;9(1):104.doi:https://doi.org/10.1186/1743-7075-9-104

41. Alaunyte I, Stojceska V, Plunkett A. Iron and the female athlete: a review of dietary treatment methods for improving iron status and exercise performance. Journal of the international society of sports nutrition. 2015;12(1):38.doi:https://doi.org/10.1186/s12970-015-0099-2

42. Dubnov G, Constantini NW. Prevalence of iron depletion and anemia in top-level basketball players. International Journal of Sport Nutrition and Exercise Metabolism. 2004;14(1):30-7.doi:https://doi.org/10.1123/ijsnem.14.1.30

43. Dubnov G, Foldes AJ, Mann G, Magazanik A. High prevalence of iron deficiency and anemia in female military recruits. Military Medicine. 2006;171(9):866.doi:https://doi.org/10.7205/MILMED.171.9.866

44. Epstein D, Borohovitz A, Merdler I, Furman M, Atalli E, Sorkin A, et al. Prevalence of Iron Deficiency and Iron Deficiency Anemia in Strenuously Training Male Army Recruits. Acta Haematologica. 2018;139(3):141-7.doi:https://doi.org/10.1159/000485736

45. Harvey LJ, Armah CN, Dainty JR, Foxall RJ, Lewis DJ, Langford NJ, et al. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. British Journal of Nutrition. 2005;94(4):557-64.doi:https://doi.org/10.1079/BJN20051493

46. Sinclair L, Hinton P. Prevalence of iron deficiency with and without anemia in recreationally active men and women. Journal of the American Dietetic Association. 2005;105(6):975-8.doi:https://doi.org/10.1016/j.jada.2005.03.005

47. Merkel D, Huerta M, Grotto I, Blum D, Rachmilewitz E, Fibach E, et al. Incidence of anemia and iron deficiency in strenuously trained adolescents: results of a longitudinal follow-up study. Journal of Adolescent Health. 2009;45(3):286-91

48. Rowland TW, Black SA, Kelleher JF. Iron deficiency in adolescent endurance athletes. Journal of Adolescent Health Care. 1987;8(4):322-6.doi:https://doi.org/10.1016/0197-0070(87)90002-7

49. Mazić S, Lazović B, Đelić M, Suzić-Lazić J, Aćimović T, Brkić P. Body composition assessment in athletes: A systematic review. Medicinski Pregled. 2014;67(7-8):255-60

50. Roecker LM-B, R. Brechtel, L. Nemeth, E. Ganz, T. Iron-regulatory protein hepcidin is increased in female athletes after a marathon. European Journal of Applied Physiology. 2005;95(5-6):569-71

51. Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2006;290(2):199-203.doi:https://doi.org/10.1152/ajpgi.00412.2005

8.2 Appendix II – Erythrocyte-MCH equation

Erythrocyte mean cellular hemoglobin content equation (184).

Mean cell hemoglobin (MCH)	Average weight of hemoglobin (Hb) in the RBC	Pigocgrams (pg) or 10^{-12} grams	$\boldsymbol{MCH = \frac{Hb\left(\frac{g}{dL}\right) x\ 10}{RBC\ (\frac{x10^{12}}{L})}}$