

Capstone Cover Page

Weber State University Bachelor of Integrated Studies Program

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Project Title: Correlations of Cytokines to Cell Groups and Iron Status Markers in Division 1 Cross-Country Athletes

Brief summary of project: An investigation of iron status in Division 1 Cross-Country athletes. We sought to explore the iron status of cross-country runners because they are at a higher risk of developing an iron deficiency or iron deficient anemia. Correlations between iron status markers, inflammatory cytokines, and immune cell groups were observed. The goal of this project is to gain insight in regards to iron status to provide useful educational information for future athletes to maintain optimal performance, and provide a springboard for future studies.

Area of Emphasis 1: Nutrition Education

Committee Member from that discipline: Dr. David Aguilar-Alvarez

Area of Emphasis 2: Health Promotion

Committee Member from this discipline: Dr. Michael Olpin

Area of Emphasis 3: Chemistry

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Abstract

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Purpose/hypothesis

We investigated how different levels of stored iron (ferritin) within normal ranges may influence immune cell group production and inflammation markers in cross-country runners. In addition, we sought to evaluate the influence of a 3 month season training on iron status markers of cross country athletes.

We hypothesize that in this unique population increases of ferritin will be associated with specific cell groups levels and a better inflammatory profile. Moreover, we expect that athletes will experience iron stores decreases towards the end of the training season.

Methodology

41 collegiate distance runners (Male: 19; Female; 22) were recruited to provide blood samples for analysis before the training season started and at the end of competition 3 months later.

All athletes were introduced to the study under identical baseline activity. Blood was analyzed by complete blood count (CBC). Cytokines were measured using Luminex™ platform. SPSS software was used to analyze the collected data

A podcast/audio recording was produced to promote personal habits to maintain/improve iron status during training.

Results

Significant increases in hemoglobin ($p=.02$), hematocrit ($p=.03$) were found among participants in the high ferritin group when compared to low ferritin group. Participants in the low ferritin group show significant correlation between eosinophils and IL-4. Correlations between eosinophils and IL-5 ($R^2=0.35$) and IL1 β ($R^2=0.42$) were seen in the high ferritin group.

Participants in the high ferritin group show a significant correlation between IL-5 and hemoglobin and hematocrit. Athletes experience an average of 9.2 mg/dL decrease in ferritin levels ($p=0.37$) from pre to post season in contrast with this there was a significant ($p=.003$) increase in hemoglobin levels of 0.4 mg/dL at the end of the season.

Conclusion

Our results suggest that eosinophils may release specific preformed cytokines in response to iron status among male and female collegiate cross-country runners. Therefore, immune cell groups and cytokines may be useful metrics for determining iron status. Furthermore, the health promotion intervention may be useful for these athletes since we observe them to be at increased risk for anemic and non-anemic iron deficiency. Finally, future studies should be performed to determine if immune cell groups and specific cytokines could aid in the detection of iron deficiency anemia, and non-anemic iron deficiency.

Introduction

Endurance athletes are at a higher risk of iron deficiency anemia (Hinton, 2014). Iron is heavily regulated by many biological functions (Knutson, 2010). These biological functions, as well as nearly every other process in the human body, are influenced in part by proteins known as cytokines (Stenken & Poschenrieder, 2015). Of the many processes regulated by cytokines, inflammation of interest in this study. In addition to regulating inflammation, and iron status, cytokines are involved in the stimulation of precursor cell groups (Cameron & Kelvin, 2000-2013). The aim of this study is to observe the correlation between precursor cell groups, cytokines, and iron status in Division 1 Collegiate cross-country runners.

Cytokines

Cytokines are proteins that are involved in many biological processes. These proteins function primarily as intercellular communicators and signal interpretation. Some of signals sent and received by cytokines influence new growth and development, the creation of new blood cells from hematopoietic stem cells, and can range from the immune system response to inflammatory molecules (Cameron & Kelvin, 2000-2013). Inflammatory cytokines can be either pro- or anti-inflammatory. Essentially, cytokines are needed for disease regulation, but are also contributing factors to chronic and auto-immune diseases (Dinarello, 2007).

Long before illness, injury, and disease could be studied at a microscopic level, before the identification of receptors and signaling cascades, pain, fever, localized swelling, and pus were visual indicators of pathology, immune system response and cellular repair (Dinarello, 2007). These intracellular molecules, previously classified as lymphokines and monokines, were

reclassified and renamed cytokines in 1974. The number of known cytokines continues to grow, with well over 60 currently identified. (Cameron & Kelvin, 2000-2013)

Cytokines have been identified as pleiotropic, meaning they have the ability to interact with multiple cells, or pathways, simultaneously, eliciting a variety of physiological responses (Stenken & Poschenrieder, 2015). Dinarello in 2007, stated that not only can cells produce cytokines, but all cells can respond to these proteins, with one exception: red blood cells (RBC). Pro-inflammatory cytokines, which serve as biomarkers for disease, have helped to classify many chronic diseases into auto-immune disorders or chronic inflammation (Dinarello, 2007). Many metabolic disorders and diseases, such as heart disease, obesity and type 2 diabetes, are associated with, or occur because of, chronic increases of pro-inflammatory cytokines (Hotamisligil, 2006) and nearly every disease has some interaction with cytokines (Stenken & Poschenrieder, 2015).

Cross-country

Running has been a necessity for humans for thousands of years. This mode of natural, independent transportation has allowed humans to travel distances over a relatively short amount of time, and helped them survive for eons, through hunting for food, and avoidance of larger predatory animals. Some argue that everything about the human physiology evolved for greater efficiency to run long distances (Mattson, 2012). Mattson, in 2012, listed the design of the shoulders, hips, tendons, sweat glands, and even the limited body hair among the evolutionary traits that suit humans for long distance running. Another evolutionary trait humans possess to

aid in long distance running is the cohesion between the cardiovascular system and metabolism (Mattson, 2012), with the latter being examined in depth further in this text.

As weapons to hunt became more sophisticated to kill prey at longer distances, chasing down food by running became less necessary for survival. Its natural course took running to a militaristic skill that has been utilized by military forces since the time of the ancient Egyptians (Jones, 2016). Running over distances is still a skill today and there are many avenues of official competition. Cross-country races fall under one discipline of distance running.

Not to be mistaken for long distance races generally run on a track, cross-country events take place on natural terrain, with natural obstacles and changes in elevation (IAAF, 2017). In other words, cross-country events are the most basic, natural way for humans to reconnect with history and reinforce human evolution. Official courses are loops of 1750 to 2000 meters (IAAF, 2017), with total event distances of between 4000 and 12000 meters (Tomlinson, 2016). Endurance athletes are at an increased risk for both non-anemic iron deficiency as well as iron deficiency anemia (Hinton, 2014).

Iron Deficiency

The human body needs to maintain a delicate balance of iron due to its vital role in many biological functions, such as oxygen transport and availability in hemoglobin and myoglobin respectively. Of the 4 to 5 grams of iron that is needed, between 1 and 3 milligrams is excreted through the normal human processes. Most of the iron needed for hemoglobin synthesis and replenishment of degraded RBC comes from iron stores (Gozzelino & Arosio, 2016).

While the repletion of excreted iron can be accomplished through dietary means, ironically most individuals become deficient by lacking dietary iron intake (Handelman & Levin, 2008). Many conditions can increase iron needs of an individual; pathological conditions such as cancer or chronic inflammation (Gozzelino & Arosio, 2016). Iron deficiency manifests when iron stores are at a net deficit. The prevalence of iron deficiency is well known and well documented. This micronutrient deficiency is widespread in industrialized societies, effecting more women than men and deemed by the World Health Organization to be an epidemic (World Health Organization, 2017).

The continued depletion of iron stores and severe iron deficiency results in iron deficiency anemia. An estimated 30% of the world's population are anemic, and the majority of cases are resulting from iron deficiency (World Health Organization, 2017). Specific populations, such as endurance athletes, have unique iron requirements and iron losses.

For the endurance athlete, iron deficiency has been shown to have a negative impact on aerobic capacity with an associated decrease of $VO_{2\text{ Max}}$ (Hinton, 2014). Iron deficiency anemia decreases the body's oxygen carrying capacity due to lack of RBC and hemoglobin (Hgb). Negative impacts upon aerobic capacity presents a dismal outcome for endurance athletes (Peeling, Dawson, Goodman, Landers, & Trinder, 2008)

There are various contributors to suboptimal iron status, or an increased iron need, in endurance athletes, and specifically runners. Many of these influences of iron deficiency, as summarized by Peeling et al in 2008, include poor nutrient replenishment from diet, hematuria as a result of constant foot strike, gastrointestinal bleeding and small amounts from sweat; elevation is also a significant factor to consider (Hinton, 2014).

Cytokines also effect iron status. Dietary iron absorption begins in the first portion of the small intestine known as the duodenum. As we will explore further in this text, specific cytokine expression can signal iron storage proteins or iron transport proteins (Koorts, Levay, Becker, & Viljoen, 2011)

Athlete Inflammation and Cytokine Role in Iron Status

Exercised induced cytokine response has been shown to increase pro-inflammatory markers in acute scenarios (Suzuki, et al., 2000). Suzuki, et all, found that after a marathon race, IL-6 had increased among subjects 100-fold. Increased levels of IL-6 have a well-documented positive correlation to hepcidin levels (Suega, 2014). Hepcidin is significant due to the fact that it is the major regulator of iron storage and transport through the iron cycle.

It is important to note that while there is a strong correlation between rising levels of hepcidin and IL-6, hepcidin is not directly signaled by IL-6. IL-6 signals hepatocytes that then secrete hepcidin (Suega, 2014). As hepcidin levels increase, it takes up binding sites of ferroportin, the protein that the body uses to export iron from storage (Knutson, 2010)

Influence of Performance on Cytokines

Many studies have been conducted on both healthy and diseased populations to show decreases in serum inflammatory cytokines after exercise programs. One such study (Stewart, et al., 2007) found significant decreases in serum concentrations of C-Reactive protein (CRP) after a 12-week strength and aerobic training cycle. Healthy active, and inactive, subjects of age

ranges 18-35 and 85-85 were included in the study. No significant change in other inflammatory cytokines (IL-6, TNF- α , IL-1 β) was observed after a 12-week training program, and it was suggested that the exercise induced reduction on inflammatory cytokines is much easier to observe in diseased populations (Stewart, et al., 2007).

Changing when and how cytokines are measured show the positive effect the training can have on inflammation. It is well known that tapering training cycles will improve athletic performance, but one study showed that a taper period of one or three weeks will show a decrease in IL-1 β , IL-6, and tumor necrosis factor (TNF- α) (Farhangimaleki, Zehsaz, & Tiidus, 2009)

Little research has been done on the influence that resistance training has on inflammatory markers, with emphasis being placed on it in recent years. Findings seem to be either conflicting or dependent on the load and intensity of the training program. At higher intensities, there is an exercise-induced increase in IL-6, which is beneficial for long-term inflammatory status. High intensity resistance training programs between 6 and 7 weeks showed a net decrease in IL-6 after the program was finished (Forti, et al., 2017). Forti, et al, conducted another 9-week long resistance training program at lower intensities than previously attempted. The beneficial effects of increased strength and decreased inflammatory markers that were observed in higher intensity programs were not replicated by this study (Forti, et al., 2017).

An interesting correlation between inflammatory cytokines and athletic training and performance is the interaction with mood states. Main et al found that indicators of depression in over-trained individuals increased in parallel with IL-1 β , IL-6, and TNF- α , although whether individuals were chronically depressed was not determined. Higher levels of IL- β has also been

observed in rugby players that express more aggressive moods, such as anger (Pesce, et al., 2013)

Nutrition Interventions for Athletes

Many studies have been done for the treatment of female athletes, due to the fact that they are at a statistically higher risk for iron deficiency (World Health Organization, 2017). Though iron deficiency is prevalent, supplementation is not the recommended approach for non-anemic individuals because overdosing of iron can lead to serious health problems, liver damage being the widest known health problem related to iron poisoning (Robertson & Tenenbein, 2005). In fact, studies suggest that as few as three days of iron supplementation has a point of diminishing returns, stimulating the release of hepcidin to prevent the over absorption of iron (Ishibashi, Maeda, Kamei, & Goto, 2017).

Some studies show a correlation between supplemented iron, athletic performance, and total body iron, suggesting iron-deficient female athletes could benefit from supplementation. Iron deficient, non-anemic female rowers that supplemented 100mg per day of Iron (II) Sulfate (FeSO_4) throughout a six-week training cycle saw a correlation between supplementation and improved athletic performance and an increase in total body iron (Dellavalle & Haas, 2014)

The importance of regularly monitoring easily tested iron status markers for athletes will determine the recommended approach to intervention. Iron status in non-anemic iron deficiency would likely not benefit from iron supplementation; more appropriate interventions would be including food that are high in bioavailable iron, such as high-quality animal sources of protein, iron-enriched cereals and grains (Delimont, Haub, & Lindshield, 2017), beans and legumes, and dark leafy green vegetables (Mayo Clinic Staff, 2016). Some evidence suggests that those with

iron deficiency anemia may also benefit from avoiding tannins such as in wine and tea, although long term effects of tannin consumption on bioavailability of dietary iron have yet to be determined (Delimont, Haub, & Lindshield, 2017).

Important and Well Studied Cytokines

Cytokines have a variety of function and structures. This section will summarize some of the cytokines that relate to, and additional observances, in both healthy and disease states. In order to keep this review as efficient as possible, this section will focus on cytokines that we were able to measure pre-, and post-season. These will be Granulocyte macrophage colony-stimulating factor (GMCSF), IL-5, IL-6, IL-1 β , IL-10, and IL4,

GMCSF

GMCSF is a protein with a primary structure of 144 amino acids that is bound to intracellular membrane (Cell Signaling Technology, Inc., n.d.). Of course, macrophages are one of the main types of cells to express GMCSF, but it also stimulates macrophages and a variety of other precursor cells, which will be discussed further in this text (Cameron & Kelvin, 2000-2013).

Acute exercise increased endothelial progenitor cells and induced endogenous nitric oxide production, but exercise had no effect on GMCSF (Yang, et al., 2007). Airway inflammation, such as in asthmatics subjects, correlates with an increased level of GMCSF and Interleukin-5 (IL-5) (Vignola, et al., 2005).

Both GMCSF and IL-5 are in the same family of cytokines known as common β chain cytokines, due to the fact that contained in their receptor is the β chain CDw131 (Cameron & Kelvin, 2000-2013).

IL-5

Containing 10 fewer amino acids in its primary structure than GMCSF, IL-5 also differs in its location. IL-5 is located outside the cell in the extracellular fluid/space, rather than bound to the cell membrane. This protein aids in hematopoiesis (Lei & Martinez-Moczygemba, 2008), the maturing of B-cells to aid the body's immune system and the differentiation of the precursors known as eosinophils (Cell Signaling Technology, Inc., n.d.; Cameron & Kelvin, 2000-2013). Chronic conditions like asthma occur when these inflammatory cytokines (GMCSF and IL-5) have an anti-apoptotic effect, promoting the survival of damaged cells that otherwise are destroyed through normal biological processes (Vignola, et al., 2005).

IL-6

The most widely studied cytokine that relates to both human performance and iron status is IL-6. IL-6 has both pro-, and anti-inflammatory, interactions; interestingly, other inflammatory cytokines are regulated by IL-6 (Peeling, Dawson, Goodman, Landers, & Trinder, 2008). As previously identified, IL-6 has been identified as a major component in up-regulation of hepcidin. With 212 amino acids making up the structure of IL-6, this cytokine participates in a variety of biological functions; from B-cell, lymphocyte, and monocyte differentiation, to influencing the cells of the central nervous system and hematopoiesis (Cell Signaling Technology, Inc., n.d.). Muscle contraction is a mechanism that signals the release of IL-6 into the blood stream, and a noteworthy effect is that it supports the metabolism of fats and reducing

insulin resistance (Cell Signaling Technology, Inc., n.d.). In certain disease states, such as lupus, higher levels of both IL-6 and hepcidin are observed; also associated with these markers is an inverse association with (Hgb) (Suega, 2014).

Due to the numerous cytokines that have been identified, nomenclature of them is often used to groups of similarly structures of the polypeptides into families. Interleukins have numeric identifiers, because of the growing numbers of identified cytokines that are similar in structure.

IL-1 β

Belonging to the Il-1-like cytokine family, IL-1 β is the longest amino acid chain discussed here, with 269 amino acids in its primary structure (Cell Signaling Technology, Inc., n.d.). Also known as catabolin, IL-1 β is an inflammatory cytokine can contribute to the degradation of connective tissue (Oxford University Press, 2017). IL-1 β , along with other cytokines in the IL-1-like family, are signaled by other inflammation in response to immune system need (Cameron & Kelvin, 2000-2013). Of import to this study is that stimulation of ferritin, or increased iron storage capacity, is directly associated with IL-1 β (Koorts, Levay, Becker, & Viljoen, 2011).

IL-10

IL-10, a protein consisting of 178 amino acids, falls under yet another family of cytokines. Before falling into the Interleukin nomenclature and numeric identifier, it was known as the human cytokine synthesis inhibitory factor (CSIF) because it suppresses inflammatory cytokine production (Cameron & Kelvin, 2000-2013). The acute phase response of IL-10 was observed in Ironman and Half-Ironman competitors by Comassi, et al., 2015. In both athlete

groups, IL-10 increased nearly three to five times that of the baseline before the event to the measurement taken after completing the race (Comassi, et al., 2015).

IL-4

Continuing with the anti-inflammatory cytokines, IL-4 is essential to the production of antibodies, specifically immunoglobulin E (IgE), that regulate allergies (Cameron & Kelvin, 2000-2013). IL-4, also known as B-cell Stimulatory Factor 1, is an activator for resting B-cell (Rabin, Mond, Ohara, & Paul, 1986). This protein, which is 153 amino acids in the primary structure (Cell Signaling Technology, Inc., n.d.), accomplishes IgE production through B-cell stimulation (Cameron & Kelvin, 2000-2013). Iron status mediation by IL-4 is done by signaling the transcription of transferrin (Koorts, Levay, Becker, & Viljoen, 2011).

Eosinophils

Eosinophils are precursor cells originating in the bone marrow and are rarely seen in healthy populations. Many cytokines (over 35) are contained within eosinophils and are often expressed by immune, or allergic, response (Davoine & Lacy, 2014). Of the cytokines previously discussed, GM-CSF, IL-4, IL-5, and IL-6 are stored as pre-formed proteins and transported by eosinophils to cell membranes where they will react with the proper receptor sequence (Davoine & Lacy, 2014; Cameron & Kelvin, 2000-2013). Interestingly, one of the functions of preformed IL-5 from granules of eosinophils is to signal the release of eosinophils (Davoine & Lacy, 2014). Relevant to iron status, specifically in response to excess iron (iron

poisoning), is the release of IL-4 by eosinophils to stimulate liver regeneration/repair (Goh, et al., 2013).

Methods

The participants for the study were forty-one (19M, 22F) NCAA division 1 cross-country athletes, ages 18 to 25 years old, between 125 cm and 195 cm in height, and 40 kg -115kg in weight. The participants came in to the nutrition biochemistry laboratory at Weber State University on two separate occasions, pre-season and post-season, to have their blood drawn with 3 months in between sample collection. The independent variables were season time (pre-post), gender, and ferritin levels. Blood was collected from subjects and analyzed by complete blood count (CBC)(at McKay Dee hospital in Ogden, Utah). An enzymatic spectrophotometer was used to determine the patients' ferritin, hemoglobin, and hematocrit. Inflammatory IL-1 β IL-6, IL10, GM-CSF, IL-5, and IL-4 were measured at baseline the magnetic multiplex panel for Luminex TM platform (at University of Connecticut). IBM SPSS statistics 22 software for windows was used to analyze the collected data. One-way Anova was performed to determine differences in hemoglobin and hematocrit between low and high ferritin male and female groups. Pearson correlation test was used to determine associations between iron biomarkers (ferritin, hemoglobin, and hematocrit), pro-inflammatory cytokines and cell groups (RBC, WBC, platelets, monocytes, neutrophils, and eosinophils).

Health Promotion Intervention

Health promotion is an educational approach to improve healthy behavior in specific populations. For this project, the target audience/population will be the Weber State University Cross-Country team. The goal of the health promotion intervention is for athletes of the WSU

Cross-Country team to value the effect iron status has on athletic performance and know healthy, effective ways to improve iron status if needed.

Due to limited access to team members, an informational podcast was recorded to be presented to the team upon the beginning of the training season. Talking points of the podcast include known effects of endurance sports on iron status, positives and negatives of current blood tests, the benefits and risks of iron supplementation, as well as dietary sources of iron that has a high bioavailability and possible negative dietary interactions with other foods. Some attention was given to inflammation and inflammatory cytokines.

Training factors that contribute to iron loss in endurance athletes, and runners specifically were presented and defined. A brief summary of iron biochemistry as well as current blood tests used to monitor iron status were presented. Suggestions for dietary sources of iron were given.

A key method of health promotion is an outcome assessment. Due to time constraints, we were unable to perform an assessment of how well the athletes synthesized the information into knowledge. Unfortunately, we were unable to assess the perceived value the athletes place on the information presented.

Results

Comparison of mean cytokine differences between high and low ferritin groups.

Significant differences were observed between high ferritin (>40 mg/dL in males, >30 mg/dL in females) and low ferritin subgroup in cytokines (**Figure 1**). Interleukin-1 β was

significantly higher ($p>0.04$) in the high ferritin group, with a Standard Error (SE) of 0.003, when compared to the low ferritin group (SE=0.006). The high ferritin subgroup also had significantly higher ($p>0.05$, SE=0.002) hematopoietic Interleukin-5 when compared to the low ferritin subgroup (SE=0.00067). The low ferritin group (SE=0.00211) had significantly higher IL-4 ($p>0.05$) than when compared to the high ferritin group (SE=0.02358).

Comparison of mean immune cell group differences between high and low ferritin groups.

Eosinophils were significantly higher in the high ferritin (high= 2.276, $p>0.05$, SE=0.2) group when compared to the low ferritin group (low=1.3, SE=0.3, **Figure 2**). Correlations between cytokines and immune cell groups were observed (**Table 1**). A strong correlation between eosinophils and IL-1 β ($R^2=0.42$, $p<0.05$) was observed in the high ferritin group. The correlation between eosinophils and IL-5 ($R^2=0.35$, $p>0.05$) in the high ferritin group was also strong, and significant. Eosinophils and IL-4 had a strong correlation ($R^2=0.75$) in the low ferritin group.

Comparisons between preseason vs post season

Cross country athletes' hemoglobin, hematocrit and ferritin changes from pre-season to post season were observed (**Table 2**). Significant Increases in hemoglobin (15.1 ± 1.3 vs 15.5 ± 1.3 , $p=.003$), and trend increases in hematocrit (44.4 ± 3.9 vs 45.2 ± 3.2 , $p=.062$) were found. In contrast, there was a significant decrease in serum ferritin (68.8 ± 42.4 vs 59.6 ± 32.2 $p=0.37$) levels after a season of competition (Table 2). An observation that it is worth to mention was

that those athletes with higher ferritin at the beginning of the season experience bigger increases on hemoglobin and hematocrit (data not shown).

Discussion

To our knowledge, this is the first study to examine associations between iron status and cytokines over the course of a season of long of cross-country training and competition. The relationships we found between eosinophils, cytokines, and iron status markers show that ferritin levels below 40 mg/dL in men and 30 mg/dL in women (which are well within the range established as sufficient (22-275 mg/dL male, 10-204 mg/dL female) may be associated with decreased iron storage ability and increased attempts to mobilize stored iron. In the high ferritin group, relationships between ferritin, cytokines, and eosinophils show that ferritin levels above 40mg/dL in men and 30mg/dL in women may be associated with an increased iron storage and a decreased need for iron absorption.

In the both high and low ferritin groups, strong correlations between eosinophils and specific cytokines were observed. Previous studies have identified that eosinophils both release cytokines and produced in response to cytokine signaling (Davoine & Lacy, 2014; Cameron & Kelvin, 2000-2013). The strongest correlation between eosinophils and IL-4 was observed in the low ferritin group. IL-4 has been observed to signal an increase in iron uptake (absorption) and mobilization; this is accomplished through transferrin expression (Koorts, Levay, Becker, & Viljoen, 2011). Eosinophils in the high ferritin group had a strong correlation to IL-1 β and IL-5.

Increases in IL-1 β were associated with increases in ferritin, and increases in eosinophils. Pro-inflammatory IL-1 β have been shown to be an influential signaling protein for the production of the iron storage protein ferritin (Koorts, Levay, Becker, & Viljoen, 2011). The

observed correlation between the high ferritin group and increases in IL-1 β supports previous studies that observed IL-1 β down regulates ferroportin by increasing hepcidin levels (Koorts, Levay, Becker, & Viljoen, 2011). Hepcidin works to regulate iron circulation by taking up iron binding sites on ferroportin (Knutson, 2010). As iron storage in the form of ferritin increases, the need to absorb iron decreases inversely to IL-1 β (Gozzelino & Arosio, 2016).

Increases in Interleukin-5 are also associated with increases in ferritin, Hgb, hematocrit, and eosinophils. IL-5 is a pro-inflammatory cytokine that signals hematopoiesis (the production and maturation of blood cells). These increases support previous observations from other studies that IL-5 signals hematopoiesis for blood cell production, which would also correlate to the increases seen in Hgb and hematocrit (Lei & Martinez-Moczygemba, 2008).

Conclusion

Our results suggest that eosinophils may release specific preformed cytokines in response to iron status among male and female collegiate cross-country runners. Therefore, immune cell groups and cytokines may be useful metrics for determining iron status. Furthermore, the health promotion intervention may be useful for these athletes since we observe them to be at increased risk for anemic and non-anemic iron deficiency. Finally, future studies should be performed to determine if immune cell groups and specific cytokines could aid in the detection of iron deficiency anemia, and non-anemic iron deficiency.

Figure 1. Cytokine mean difference between low and high ferritin groups

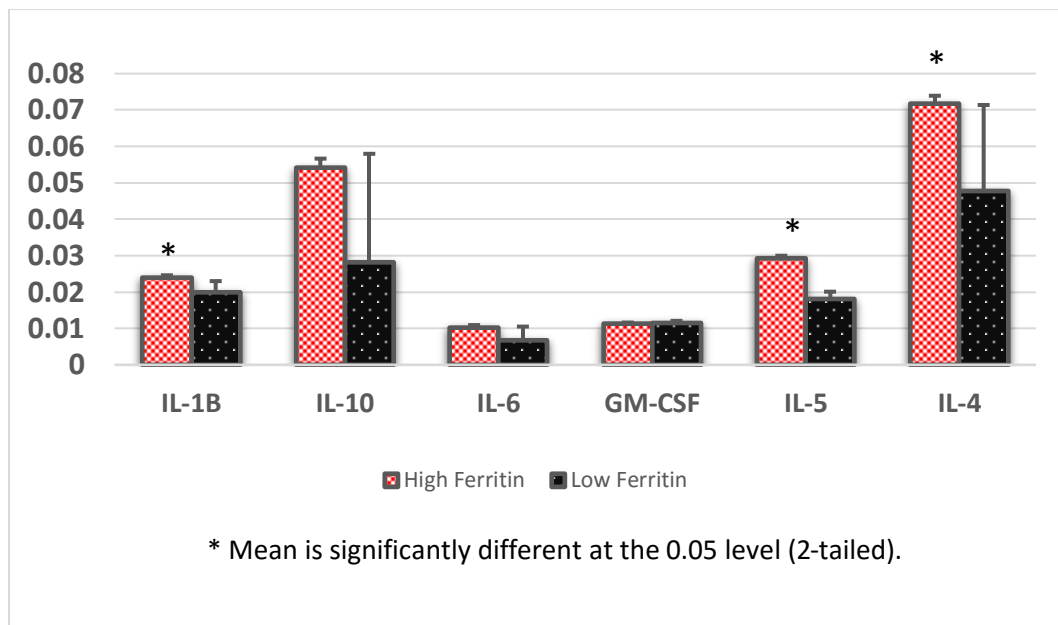


Figure 1. Significant differences were observed between high ferritin (>40 mg/dL in males, >30 mg/dL in females) and low ferritin groups in cytokines (**Figure 1**). Interleukin-1 β was significantly higher (high=0.024, SE=0.003, $p>0.04$) in the high ferritin group when compared to the low ferritin group (low=0.02, SE=0.006). The high ferritin group also had significantly higher (high=0.0293, SE=0.002, $p>0.05$) hematopoietic Interleukin-5 when compared to the low ferritin group (low=0.0181, SE=0.00067). The low ferritin group had significantly higher IL-4 (high=0.0718, SE=0.02358, $p>0.05$) than when compared to the high ferritin group (low=0.0478, SE=0.00211).

Figure 2. Immune cell groups mean difference between low and high ferritin groups

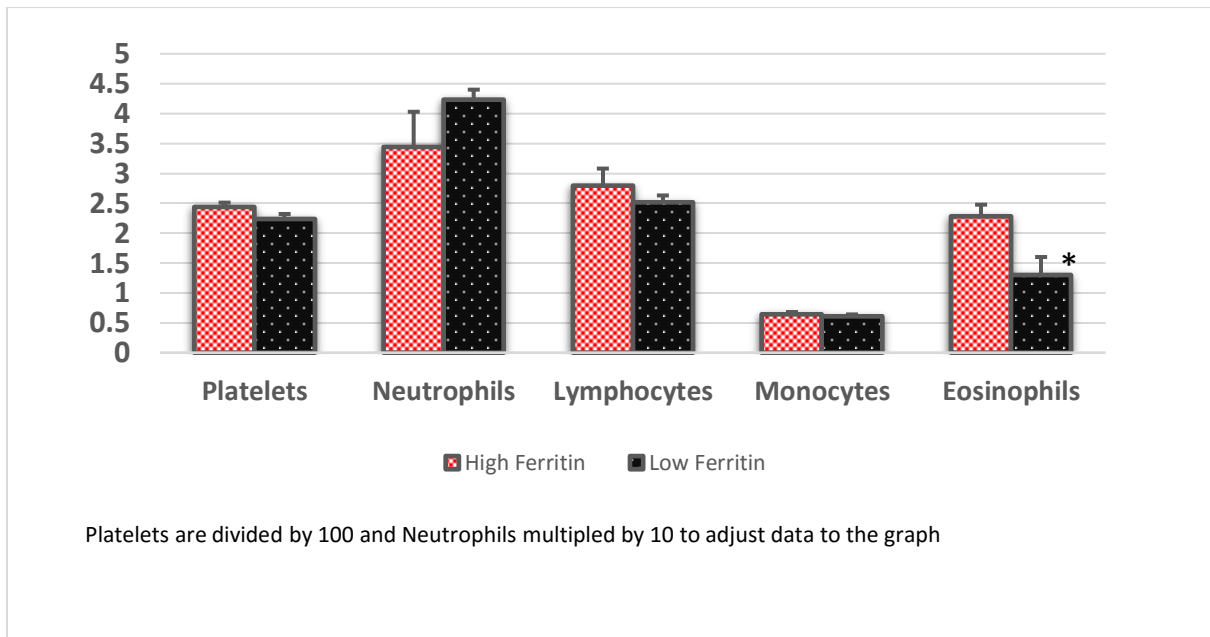


Figure 2. Eosinophils were significantly higher in the high ferritin (high= 2.276, $p>0.05$, SE=0.2) group when compared to the low ferritin group (low=1.3, SE=0.3).

Table 1. Correlations between eosinophils and cytokines.

Eosinophil correlations		
High ferritin group		
	R ²	p-value
IL-5	0.35	0.034
IL-1B	0.42	0.054
Low ferritin group		
	R ²	p-value
IL-4	0.75	0.001

Table 1

Table 1. A strong correlation between eosinophils and IL-1 β ($R^2=0.42$, $p<0.05$) was observed in the high ferritin group. The correlation between eosinophils and IL-5 ($R^2=0.35$, $p>0.05$) in the high ferritin group was also strong and significant. Eosinophils and IL-4 had a strong correlation ($R^2=0.75$) in the low ferritin group.

Table 2 . Comparisons pre and post season

	Pre-season	Post-season
Hemoglobin (mg/dL)	15.1 ± 1.3 ^a	15.5 ± 1.3 ^b
Hematocrit (%)	44.4 ± 3.95 ^a	45.2 ± 3.2 ^a
Ferritin (mg/dL)	68.8 ± 42.4 ^a	59.59 ± 32.1 ^b

¹ Values are means ± SD, n=27; Values in the same row with different superscripts are significantly different at P<0.05

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