SERUM FERRITIN'S RELATIONSHIP TO TRAINING REDUCTION AMONG COLLEGE DISTANCE RUNNERS

by

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DEDICATION

The thesis is dedicated to the wilderness, where man can return to a more natural state.

“Going to the mountains is going home.”

John Muir

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ABSTRACT

Introduction: Iron deficiency (ID), measured as serum ferritin (SF), has been found in 31% and 57% of elite male and female athletes respectively. In distance runners, ID has been found to affect up to 41% of men and 82% of women. There are significant health consequences of ID such as decreased bone mineral density, altered thyroid function, immune suppression, and fatigue. **Purpose**: To determine if there is a relationship between ID and training reductions due to injury, illness, and fatigue in collegiate distance runners. **Hypothesis**: Because ID affects bone health, fatigue, thyroid, and immune function, it is hypothesized that distance runners with ID experience training reductions more frequently than athletes with normal SF levels. **Methods**: A preexisting data set consisting 47 NCAA Division 1 cross country runners' (M=20 years ± 1.69) SF, injury information, fatigue reports, and training volume was analyzed. SF levels were measured preceding the cross-country season in early September and following the season in early December. Athletes supplemented with ferrous sulfate elixir according to the SF level throughout the season. A generalized linear model investigated associations between training reduction, SF, and training volume. A chi-squared test compared ID athletes (≤35 μg/L) and athletes with normal SF (>35 μg/L). Significance was set at ≤ 0.05. **Results**: 57.4% of the athletes were IDNA at some point during the season. The IDNA group was twice as likely to experience a training reduction as the normal iron group ($p < 0.05$). Pre SF ($B = -0.042 \pm 0.0212$), Post SF ($B = 0.033 \pm 0.0169$), and training volume ($B = -0.057 \pm 0.0229$) significantly predicted training reductions ($p \leq 0.05$). **Conclusion:** IDNA was found to be associated with training reductions. Additionally, a higher pre SF value was associated with less training reduction. Athletes' SF should be tested and corrected through supplementation and diet.

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LIST OF ABBREVIATIONS

ID	Iron Deficiency
Hb	Hemoglobin
SF	Serum Ferritin
IDA	Iron Deficient Anemia
IDNA	Iron Deficient Non-anemic
RBC	Red Blood Cells
T_3	Triiodothyronine
T_4	Thyroxine
TSH	Thyroid Stimulating Hormone
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-10	Interleukin-10
NK	Natural Killer Cells
BMD	Bone Mineral Density
Hp	Heptoglobin
sTfR	Soluble Transferrin Receptor
RMR	Resting Metabolic Rate
PICP	Serum Procollagen Type I N-terminal Propeptide
NTx	N-terminal Telopeptide
TNF-α	Tumor Necrosis Factor-alpha

CHAPTER ONE: INTRODUCTION

Iron deficiency (ID) has been a popular subject of study, with respect to health outcomes, due to its high prevalence among the general population, as well as its role in oxygen delivery, bone density, immune regulation and thyroid regulation.[1-4] Athletes are at an increased risk for ID, particularly female distance runners, which has been found to be as high as 82%.[5] Trained distance runners are also at an increased risk of injury, between 31% and 57%.[6-8] The purpose of this study was to investigate the effect of ID on the training reductions caused by injury, illness, and fatigue of college distance runners.

Iron Deficiency

Iron deficiency (ID) is the lack of adequate iron to produce hemoglobin (Hb) for red blood cells to carry oxygen and aid in various cellular functions.[9] Serum ferritin (SF) levels are considered normal between 15 and 150 μg/L for women and 200 μg/L for men.[10] Iron deficient anemia (IDA) is the clinical definition when SF levels are below 12 μg/L. Low SF levels that do not meet the anemic threshold are classified as iron deficient non-anemic (IDNA), which has been identified to have negative effects on fatigue and general health.[11] Peeling et al. suggested three stages for categorizing iron deficiency.[12] Stage one, iron depletion, is SF levels of <35 μg/L indicating depleted iron stores in the spleen, liver, and bone marrow. Stage two, iron deficient erythropoiesis, is SF <20 μg/L which diminishes red blood cell production. Stage three, IDA, is SF levels <12 μg/L which is when hemoglobin production falls.[12]

Prevalence of ID

ID is the most prevalent nutritional deficiency in the world and the second most prevalent nutrient deficiency in the United States.[13,14] ID prevalence is highest among women (approximately 9%) and children.[12] Premenopausal women have an increased risk of ID due to blood loss through the menstrual cycle.[1,13] Up to 16% of premenopausal women have ID.[1,13] Athletes have been found to have higher ID rates than the general public. Across 24 sports, it was reported that 31% and 57% of elite male and female athletes, respectively were IDNA.[15] Kohler et al. also reported 41% of male endurance athletes were IDNA. In highly trained female distance runners, the IDNA rate increased to 82% even when 75% of the athletes reached their dietary recommendation for iron.[5]

Causes of ID

SF is a measure of stored iron used to test for ID.[13] There are a number of reasons that endurance athletes' SF may be low. During the inflammatory response to exercise, iron absorption is decreased.[16] Inflammation stimulates hepcidin release, a regulator of iron entry into circulation, which in turn decreases iron absorption by trapping SF in macrophages.[16,17] Hepcidin release was found be lower in IDNA endurance athletes compared to athletes of normal iron levels.[16,18-20] Peeling et al. found a dose response where a lower SF level decreased normal hepcidin release during inflammation.[16] Hemolysis, the destruction of red blood cells (RBC), increases the strain on iron stores to replace RBCs for oxygen carrying capacity.[21] Exercise induced hemolysis occurs due to increased intramuscular pressure, mechanical load, and foot strike.[21,22] The intramuscular pressure may have a greater impact than foot strike as hemolysis rate is more dependent on speed and intensity than the running surface.[23] Additionally, blood loss due to training

can impact SF levels. Hematuria, blood loss through urine, occurs when exercise intensity decreases renal blood flow via vasoconstriction.[24] Vascular lesions in the upper digestive tract of runners may also lead to gastrointestinal bleeding.[25] Between the low absorption, blood loss, blood damage, and high hemoglobin demand, endurance athletes are at an increased risk of developing ID.[19,21,23-25]

Health Implications of ID

Thyroid function, bone integrity, fatigue, and immune function are all impacted by SF levels.[2-4,26-28] Unexplained fatigue was found to dissipate in women with SF <50 μg/L following iron supplementation.[29] ID associated fatigue may be due to inefficient oxygen transport, but thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), important in metabolism regulation, have been shown to be depressed in IDA and IDNA.[2,28,30] SF specific affects T_3 and T_4 are currently unkown.[2] These alterations in thyroid hormones are potentially important as they can lead to weight gain, impaired lean mass development, affect growth rate, and increase fatigue.[28]

Impaired immune function has also been associated with ID. Hospital patients with IDA have been found to be more susceptible to infections.[31,32] In mice, IDA-related infections have been associated with T-cell lymphocyte dysfunction and a decrease in interleukin-2 (IL-2).[33] Similar immune dysfunction has been seen in ID persons such as a decrease in IL-2 (activates white blood cells) and natural killer cell (NK)(rapid response to infection) activity along with an increase in interleukin-6 (IL-6)(pro and anti-inflammatory) and interleukin-10 (IL-10)(anti-inflammatory).[3,34,35] A decrease in NK activity has been shown in IDNA distance runners compared to distance runners with normal iron levels, which may hinder their immune response to infection.[36]

Iron also plays an important role in bone integrity as an enzymatic cofactor for collagen synthesis.[37] Rat studies have produced most of the findings regarding the effect of iron on bone integrity. When rats were fed diets deficient in iron, decreased bone mass, bone mineral density (BMD), femur strength, bone formation, and bone mineral content have been found, along with an increase in bone fragility.[38-41] Decreased BMD has been associated with dietary iron, most notably when calcium intake was controlled for, proposing that dietary iron impacts BMD independent of calcium.[42-45] In addition, in women receiving hormone replacement therapy (HRT) a direct relationship between iron supplementation and BMD was found.[45] These findings suggest iron plays an important role in bone health. This is particularly important as Bennell and Crossely found stress fractures occurred in 21% of athletes.[6] Risk factors of stress fractures include low bone density, hormonal factors, caloric restriction, and muscle fatigue, of which iron's relationship to these factors cannot be ignored.[46]

Most of the iron research has neglected health outcomes for athletes and instead focused on performance. When looking at competitive track and field athletes, particularly distance runners, injury rates have been reported from 31% to 57%.[6-8] Given the high incidence of injury and ID among athletes and the involvement of iron in important functions, the impact that ID has on endurance athletes is warranted.

Need of the Study

ID has been shown to have negative health and performance implications, thus it is important to measure the extent to which the health and training status of distance athletes are affected by ID.

Purposes of the Study

The purpose of this study is to investigate the relationship of SF levels and training reductions due to injury, illness, and/or fatigue among NCAA Division 1 collegiate distance runners.

Research Hypothesis

Low SF levels will be associated with training reductions due to injury, illness, and/or fatigue.

Operational Definitions

NCAA Division 1 collegiate distance runners: Athletes competing in a NCAA school sponsored cross-country and track and field program.

Iron levels will be measured by a venous blood draw to measure SF levels:

1. <12 µg/L clinical anemia (IDA) – decrease in hemoglobin production.[12]
2. <20 µg/L iron deficient erythropoiesis (IDNA) - diminished red blood cell production.[12]
3. <35 µg/L iron depletion (IDNA) - depleted iron stores in the spleen, liver, and bone marrow.[12]

Significance of the Study

1. Improved health of athletes is not only beneficial for the well-being of the athletes but also for improved performance.
2. This study will determine whether athletes with lower SF levels experience training reductions caused by injury, illness, and/or fatigue than those with normal SF levels.

CHAPTER TWO: LITERATURE REVIEW

Iron Deficiency

Iron deficiency (ID) has been a popular subject of study, with respect to health outcomes, due to its high prevalence among the general population, as well as its role in oxygen delivery, bone density, immune regulation and thyroid regulation.[1-4] ID among athletes, particularly endurance athletes, and performance measures have also been frequently studied. Endurance adaptation requires consistent, uninterrupted training, due to the gradual improvement in mitochondrial density as well as the subsequent decrease in mitochondrial density, stroke volume, and performance within the first week of exercise cessation.[47-49] Injuries, overtraining, fatigue, illness, etc. are all factors that can inhibit training and subsequent performance. Hemolysis, hematuria, and gastric lesions incurred through endurance training put distance runners at an increased risk for ID.[25,50,51] The importance of iron for these athletes is heightened as the requirements for high performance such as maximal oxygen consumption ($\dot{V}O_2$ max), ventilatory threshold, and lactate threshold are particularly influenced by ID as iron is essential for oxygen transport and is a cofactor in mitochondrial energy production.[52] Ironically, while iron status and performance have been studied, there is limited research on the impact of ID on endurance athletes' health and training status. The purpose of this review is to investigate the training effects of ID with respect to endurance athlete's health and training status.

ID Prevalence

ID is the most prevalent nutritional deficiency in the world and the second most prevalent nutrient deficiency in the United States.[13,14] ID prevalence is highest among women and children.[13] Premenopausal women have an increased risk of ID due to blood loss during a menstrual cycle.[13] In premenopausal women, iron deficiency with anemia (IDA, a blood iron concentration of ≤12 μg/L) was identified in 3-5% of the population.[1] Information from the National Health and Nutritional Examination survey from 2003 to 2006 reported ID rates among 3,742 females aged 12-19 and 20-49 to be 15.6% and 15.7%, respectively.[1] In athletes, the iron deficient non-anemic (IDNA, <35 μg/L) prevalence has been found to be higher than the general population.[15] Koehler et al. analyzed 193 elite athletes across 24 sports for IDNA; 31% of male athletes and 57% of female athletes were reported to have IDNA.[15] These percentages increased in male endurance athletes to 41%, but remained nearly the same in female endurance athletes at 54%.[15] Deuster et al. investigated 51 highly trained female distance runners' nutritional intakes and SF levels and found that although 75% of the athletes consumed an adequate amount of dietary iron according to the recommended daily allowance (RDA), 82% were IDNA.[5] These are very high percentages of IDNA compared to the general population and did not appear to be affected by a deficiency of iron within the athlete's diet.

Causes of ID

The increased prevalence of ID in athletes is due to a number of different factors. In 2008, Ostojic and Ahmetovic investigated the effect of training volume on ID. [53] Eighty-four female professional athletes across various sports recorded training hours in conjunction with SF measurements.[53] Of the sports studied, distance runners were found

to have the most training hours per week, at 9.8, and also had the lowest ferritin levels. However, IDNA (<30 μg/L, adjusted to represent the degree of IDNA of participants) correlated poorly with training volume.[53]

Hepcidin

In 2009, Peeling et al. found a decreased hepcidin response in IDNA (<35 μg/L) athletes.[19] IDNA may be classified under different SF levels to reflect the degree of IDNA of the participants.[12,19,53] Hepcidin release is stimulated by inflammation, which in turn decreases SF and gut iron absorption.[17] Peeling et al. speculated that IDNA may decrease hepcidin release, proposing the need for iron overrides the normal response of hepcidin to inflammation.[19] Auersperger et al. investigated the relationship of iron levels and hepcidin in moderately trained females and found that training decreased iron levels and hepcidin after 8 weeks.[20] A follow-up study by Auersperger et al. had 14 female distance runners undergo a 10 week training phase.[18] Seven of the distance runners were already IDNA (<20 μg/L) and 7 were ≥20 μg/L.[18] Following the 10-week training cycle, the number of athletes with IDNA (<20 μg/L) increased from 50% to 71%.[18] Again hepcidin decreased with SF, causing Auersperger et al. to question if iron demand, rather than inflammation, influenced hepcidin release.[18] Peeling et al. revisited the SF and hepcidin relationship with 54 trained male and female endurance athletes.[16] The athletes were separated into 4 groups according to their SF levels.[16] SF levels of the four groups was <30 μg/L, 30-50 μg/L, 51-100 μg/L, and >100 μg/L.[16] An interval session or long, slow distance run was completed followed by a hepcidin measurement.[16] Results showed a significant dose response of hepcidin, increasing incrementally with SF level pre and post exercise.[16] Athletes with optimal SF do not appear to require as much iron

absorption, thus a greater release in hepcidin occurs.[16] None-the-less, hepcidin was still released in all groups, decreasing iron absorption in all of the athletes.[16]

Hemolysis

Exercise induced hemolysis (RBC destruction) has been reported among a range of exercise conditions including swimming, cycling, resistance training, and running.[50,51,54] Selby and Eichner found that muscle contraction during exercise compressed blood vessels which resulted in hemolysis during non-weight bearing activity.[54] However, the weight bearing force incurred through running shows the greatest degree of hemolysis.[50] The damaged RBC's release hemoglobin (Hb) and the associated iron into the plasma where glycoprotein heptoglobin (Hp) "picks up" the remains.[55] A blood profile with increased free Hb and decreased Hp is common among runners.[55] Hansen et al. found decreased Hp levels in recreational runners, comparable to that seen in well-trained distance runners while running on concrete.[56] In 1988, Miller et al. showed heel-strike force to increase the degree of hemolysis, finding that running downhill for an equal amount of time produced greater hemolysis.[57] This finding was supported by Telford et al. while comparing hemolysis between runners and cyclists.[50] Following a one-hour exercise bout at 75% $\dot{V}O_2$max, runners showed a free Hb level up to 4 times greater than the cyclists.[50] The authors concluded that the major cause of hemolysis among runners was heel-strike. However, Peeling et al. had 10 triathletes complete two 10 km training sessions, one on pavement and the other on grass.[23] Following the interval sessions, there was no difference between grass and concrete. A relationship was found between hemolysis and speed, showing a trend towards greater hemolysis during an increased running pace.[23] This study points back to intramuscular

pressure, generated by increased speeds, to have a greater impact on hemolysis than foot-strike.[23] The degree of hemolysis in runners places an increased strain on iron stores, making them more susceptible to ID.[50]

Hematuria

Hematuria (blood in the urine) has also been found to occur with physical activity. Gilligan et al. monitored 49 runners in races from 2.6 to 26.2 miles.[51] Following the strenuous bouts, approximately 25% of the runners were found to have Hb present in their urine.[51] Blacklock found that movement of the bladder, associated with running, hypothesizing that lesions in the interior wall caused hematuria.[58] However, McInnis et al. found hematuria to be associated with increased exercise intensity utilizing an aerobic run, a sprint protocol, aerobic bike, and Wingate protocol.[24] Renal blood flow decreased proportionally to exercise intensity, with no signs of lesions to the bladder.[24] A decrease in plasma flow to the kidneys via vasoconstriction appears to be the cause of hematuria.[24]

Gastric Lesions

Gastric lesions causing gastrointestinal bleeding are also an avenue of iron loss during exercise. Gaudin et al. performed an endoscopic examination before and after a moderately paced long run between 18 and 50km.[25] Six of 7 runners had vascular lesions in the upper digestive tract ranging between congestion to hemorrhage as result of the exercise bout.[25]

Sweat Loss

Iron has also been found in the sweat of cyclists, although sweat may not play a significant role. Waller and Haymes measured iron loss through sweat during an exercise bout and found nearly two times the amount of iron during the first 30 minutes compared

to the second 30 minutes of the session.[59] They concluded that the increase in iron loss during the first 30 minutes may be due to external contamination of cellular debris.[59] A review by Zourdos et al. concluded that iron loss through sweat was minor at best.[52]

ID and Performance

Performance testing in regard to ID is a well-researched area. Studies on performance and physical fitness parameters have produced mixed results, although most studies have found iron supplementation to benefit ID runners.[52] In a classic double blind study, Rowland et al. took 14 iron deficient runners (<20 µg/L) and supplemented half with 975 mg/d of ferrous sulfate with the control group receiving a placebo.[60] Both groups supplemented for 1 month. The ferrous sulfate group increased their SF from 8.7 µg/L to 26.6 µg/L while the placebo group fell from 10.6 µg/L to 8.6 µg/L.[60] Following the supplementation period, 6 of 7 in the treatment group improved their endurance treadmill time while the control group's performance declined.[60] Submaximal, maximal $\dot{V}O_2$, heart rate, or ventilation did not change between groups. Moving into the 2000's, a study took 42 IDNA (≤16 µg/L) women and divided them into ferrous sulfate supplementation and placebo groups.[61] The treatment group took 100 mg of ferrous sulfate for 6 weeks and training took place 5 days/week during the last 4 weeks. Prior to testing there was no difference in 15 km time. Both groups improved their 15 km, decreased their respiratory exchange ratio, and increased work rate.[61] The ferrous sulfate group improved their 15km time by 3.4 minutes on average whereas the placebo group saw improvements of 1.6 minutes. These improvements were partially attributed to their increased SF levels.[61] These results were confirmed by Brownlie et al.[62]

In 2007, Peeling et al. injected 100 mg of ferrous sulfate 5 times over the course of 10 days into a group of 8 IDNA (<35 µg/L) athletes with a control group receiving a placebo injection.[12] The average SF level rose from 19 µg/L on day 1 to 65 µg/L on day 20, and fell to 58 µg/L on day 28 without additional injections.[12] Contrary to expectations, the results yielded no significant improvement over the control group in any parameter including economy, heart rate, blood lactate, submaximal $\dot{V}O_2$ or $\dot{V}O_2$max.[12] These findings go against previous research with oral ferrous sulfate supplementation. The study also included a blood test of soluble transferrin receptor (sTfR, a transporter or iron into cells) which may be a better indicator of performance improvements regarding iron supplementation.[12] The authors noted that although intramuscular iron injections increased SF levels quicker than oral, sTfR did not change as it had during oral supplementation.[12] This may be due to the lower overall dose of iron used in the study or that the athletes were not deficient enough for sTfR to be compromised.[12]

In 2007, Hinton and Sinclair published results from 20 IDNA (≤16 µg/L) recreationally trained mean and women with a 6-week study design very similar to their own study in 2000.[61, 63] The treatment group took oral doses of ferrous sulfate while the control group took a placebo. They found a significant improvement in energy efficiency during a $\dot{V}O_2$max test as well as an increased ventilatory threshold for those who started with lower SF levels.[63] Similar to Peeling et al. findings with ferrous sulfate injections, there was no change in sTfR level which they expected to decrease after iron supplementation. [12,63] The contradictory results found between Peeling et al. and Hinton and Sinclair may be a result of a higher baseline SF level in the Peeling et al. study. [12,63] Hinton and Sinclair also noted that sTfR may remain stable or increase in response to

muscle growth and erythropoiesis which may diminish its response to iron supplementation.[63]

In 2014, Della Valle and Haas investigated performance measures in 40 IDNA college rowers over 6 weeks of either supplementation with oral SF or placebo.[64] Six weeks was found to increase blood SF by 3.0 µg/L ($p=.07$).[64] A 4km time trial revealed that SF supplementation group significantly improved energetic efficiency and energy expenditure whereas the control group did not see an improvement from baseline levels.[64] They also showed a decrease in lactic acid build up in the first half of the time trial and were able to clear lactic acid quicker following the time trial.[64]

Health Consequences of ID

With so much research investigating the performance benefits of adequate SF, there are relatively few studies investigating the health consequences of ID in athletes. There are quite a few health consequences that may occur as a result of ID, although most studies investigate the general population. Chronic fatigue, thyroid and resting metabolic rate (RMR) alterations, bone integrity, and immunosuppression have been associated with ID.[2-4]

Thyroid

Chronic fatigue is often associated with anemia as inefficient oxygen transport may be to blame for the fatigue, but IDA has been shown to affect triiodothyronine (T_3) and thyroxine (T_4).[2] T_3 and T_4, stimulated by thyroid stimulating hormone (TSH), are important for their role in metabolism regulation. ID-related thyroid alterations have been studied particularly in their role as thermoregulators and their effect on RMR. Dillman et al. first showed a decrease in body temperature and T_3 in IDA rats.[65] Following this

finding, Martinez-Torres et al. found a 10% decrease in T_3 among men and women during cold water exposure in IDA individuals; however, this decrease was not statistically significant.[30] They also noted an increase in norepinephrine (NE) and $\dot{V}O_2$ during cold water exposure in the IDA participants and theorized that the elevated $\dot{V}O_2$ increased the metabolic rate to compensate for the drop in body temperature.[30] Beard et al. noticed a discrepancy between the groups in the Martinez-Torres at al. study where the IDA group had a significantly lower body fat.[2, 30] Controlling for body fat, 10 IDA, eight iron depleted (IDP, <30 µg/L), and 12 iron sufficient control participants completed a 12 week iron supplementation protocol of 78 mg of ferrous sulfate daily, again using cold water immersion to investigate thyroid hormones.[2] Prior to supplementation, the IDA group had significantly less T_3 and T_4 and a lower body temperature, a sign of poor TSH, than both the control and IDP groups.[2] Following 12 weeks of iron supplementation, the IDA group was not able to increase SF to a normal level (3.8 ± 2.4 µg/L to 9.7 ± 5.7 µg/L) but increased body temperature during cold water immersion, although their body temperature was still lower than the control group.[2] The IDA group increased their T_3 by 12% after supplementation.[2] However, T_3 and T_4 remained significantly lower in the IDA group than both the IDP and control groups following supplementation, a point Beard et al. attributed to the inability to correct IDA.[2]

A case study published in 2000 followed two female college athletes, a distance runner and swimmer, with IDA over 16 weeks of iron supplementation.[28] Sixteen weeks was sufficient to correct their IDA (5 µg/L to 15 µg/L; 5 µg/L to 20 µg/L), although it was not long enough to return SF to normal levels.[28] One athlete's RMR increased, while the other's decreased. The change in RMR may have been a function of T_4 as a similar

pattern appeared as one athlete's T_4 increased while the other athlete's decreased. It appears that the interaction of iron on thyroid hormones is complex and possibly individualized.[28] The researchers also noted that iron's impact on thyroid hormones is potentially important, as thyroid decrements can impair the development of lean mass, affect growth rate, and increase fatigue.[28]

Bone Integrity

The role of iron in regards to BMD is often overlooked, particularly in an athletic setting, where iron's effect on performance has been heavily researched. Iron contributes to bone health as an enzymatic cofactor of the enzyme prolyl hydroxylase for collagen synthesis, playing an important function in collagen maturation.[37] Most of the information of iron's relationship to BMD comes from rat studies. Medeiros et al. fed rats an iron-deficient diet (5–8 mg/kg of iron per day) over 5 weeks after which the rats developed increased bone fragility and a decrease in bone mass.[41] A similar protocol by Katsumata et al. fed rats a diet deficient in iron, according to the AIN-93 purified diets, for 4 weeks and the rats showed significantly reduced serum osteocalcin (secreted by osteoblasts, increases bone building, bone mineralization and calcium homeostasis).[39] Serum osteocalcin also causes beta cells to release insulin and fat cells to release adiponectin (increases sensitivity to insulin) concentration by 56%, which indicates inhibited osteoblast (bone cell responsible for bone tissue building) function. Bone mineral content, BMD, and femur mechanical strength were also significantly lower in the iron-deficient group, suggesting a decrease in bone formation.[39] Katsumata et al. set up a similar study to clarify previous findings and found significant decrements in bone mineral content, BMD, osteoclast surface area(bone cell responsible for bone tissue

breakdown), and bone formation rate in rats fed an iron-deficient diet.[40] Additionally, insulin-like growth factor-1 (promotes growth in children and adults), osteocalcin concentrations, and serum 1,25 dihydroxycholecalciferol (calcitriol, promotes dietary calcium absorption and calcium release from bone) decreased; suggesting that severe iron-deficiency decreases bone formation.[40] Díaz-Castro et al. measured bone turnover biomarkers among anemic rats fed an iron-deficient diet.[38] Among the significantly affected measures in the iron-deficient rats, serum procollagen type I N-terminal propeptide (PICP) decreased and C-terminal telopeptides of type 1 collagen increased.[38] The decrease in PICP indicates bone mineralization impairment while the C-terminal telopeptides of type 1 collagen are degradation products that decrease type 1 collagen crosslinking, leading to increased bone fragility and weaker collagen fibers.[38]

In humans, Harris et al. found dietary iron to be associated with greater BMD in a cross sectional study of 242 postmenopausal women.[44] Iron had already been associated with high BMD in two other human studies, however, in this particular study calcium was controlled for and iron was still associated with a greater BMD.[42-44] Harris et al. concluded that dietary iron may be more important in bone mineralization than previously thought.[44] In 2005, the effect of iron supplementation on bone health in postmenopausal women again showed iron's importance in bone health.[45] Two-hundred and twenty-eight women receiving hormone replacement therapy (HRT) supplemented with calcium and iron and the effects on the participants bone health were monitored over 1 year. The novel finding was the positive association of SF and BMD.[45] A direct relation of iron supplementation with positive bone mineral density during HRT was also demonstrated.[45] Only one study has been conducted on premenopausal women. One-

hundred and sixty-five women were divided into two ID groups (≤30 μg/L) and an iron sufficient group (>30 μg/L) over 16 weeks.[4] The ID groups either supplemented with iron or iron in addition to vitamin-D fortified skim milk.[4] Toxqui et al. found an inverse relationship between N-terminal telopeptide (NTx), which reflects bone reabsorption, and SF. [4] The ID groups showed increased bone reabsorption, which is the first time iron biomarkers have been related to bone turnover markers in premenopausal women.[4] Thus, both bone reabsorption and remodeling were related to ID.[4]

Immune Suppression

Patients with IDA have been found to be more susceptible to infections.[31,32] Latunde-Dada and Young associated IDA-related infections with T-cell lymphocyte dysfunction and a decrease in Interleukin-2 (IL-2) causing immune system impairment among mice.[33] Sipahi et al. found a decrease in IL-2 among ID children.[34] Following iron supplementation, IL-2 increased in the children to normal values.[34] In 20 adult patients with IDA, Bergman et al. found IL-2 to be decreased as well as an increase in IL-6, IL-10, and tumor necrosis factor-alpha (TNF-α).[3] Considering the important role of IL-2, stimulating natural killer cells (NK) and T-cell lymphocyte proliferation, IDA may contribute to an increased susceptibility to infections.[3] In those with IDNA (12-30 μg/L), Chandra showed untrained individuals had a lower NK cell activity than participants with normal SF levels.[27,31,35] Additionally, Chandra explains that iron is needed by neutrophils (which respond and protect against infection) and lymphocytes (made up of T cells, B cells, and NK cells, important for immune response) for proper function.[35] In patients with ID, aged or degenerated neutrophils are common, although how iron affects this is

still unkown.[35] Lymphocytes appear to require iron for proliferation and function.[66] These immune alterations illuminate the importance of iron's role in immune function.[35,66] Braun et al. investigated NK cell activity and iron status in female collegiate swimmers.[26] Ten swimmers and 9 sedentary participants had their SF and NK cell activity measured late into the swim season. Both groups were IDNA, with the swimmers SF at 20.38 ± 2.73 μg/L and sedentary participants at 16.79 ± 3.51 μg/L.[26] Results showed that the swimmers had higher NK cell activity; however, both groups were considered to be IDNA so the baseline set by the sedentary participants may not be reflective of NK activity among those with normal SF levels.[26] A double blind study investigated female distance runners iron status and immune function during two weeks of intensified training.[67] Twenty-three well trained distance runners completed 2 weeks of normal training to distinguish the typical training volume of each individual.[67] SF and NK cell activity were measured before and after a 2 week increase in training volume, a 40% increase the first week and an additional 40% increase the second week, followed by 8 weeks of recovery training.[67] Runners with SF below 35 μg/L were considered to have low iron scores.[67] Following the two week increase in training volume, the low iron group had significantly decreased NK cells, which is associated with lower NK cell activity.[67] This finding suggests there is some link between immune function and iron status.[67] Following the 8 week recovery phase, in which the low iron group supplemented the SF scores above 35 μg/L, NK cell activity was still depressed.[67] This suggests that the immune system may require longer periods of normal iron stores or that other factors may be involved to alter immune function.[67]

Injury Rates of Distance Runners

Numerous studies have investigated the prevalence and associations of running and injuries. The injury rate among distance runners have been found to be between 19.4-79.3% for lower extremity injuries, and up to 92.4% when all injuries are included.[68] However, competitive distance runners have reported injury rates at 43%, 31%, and 57%.[6-8] Stress fractures have been found in 21% of track and field athletes.[6] Unfortunately, those studies did not find particular causes for the injuries. The majority of studies looking at injury rates among runners are in recreational runners that are older than the typical college athletes.[68] Limited and contradictory evidence associated running injuries with age, gender, height, weight, leg length differences, competition, and running pace.[68] An increased training volume has been found to be a significant predictor of injury in runners, particularly above 64 kilometers/week.[69,70] However, two studies with marathon runners showed a protective effect of knee and foot injuries with increased training volume.[71,72] Previous injury may be the most significant predictor of injury as 4 studies have found an association between injury rate and injury history.[69,70,72,73] These findings are inconclusive relating injury to a common cause, particularly among young, college athletes. With the high incidence of injury and ID, along with the important role iron plays, further investigation into ID is warranted.

CHAPTER THREE: METHODS

Participants

Data from 47 Division 1 distance runners (30 females, 17 males), aged 18-24 years, were analyzed in this cross sectional study. Data were compiled from September through December 2014 and again in September through December 2015. This study was approved by the Institutional Review Board and no experimental intervention took place.

Data Set

The data set included weekly mileage, SF levels, injury type, degree of injury, fatigue symptoms, and degree of fatigue symptoms. These data sets were of particular interest as it has a number of important parameters regarding endurance athletes' health and success with injuries, weekly mileage, and SF levels.

SF was used to assess iron levels as it is the most sensitive test to measure stored iron.[13] Each athlete's SF levels were measured via venous blood samples by the student health clinic and analyzed with a Beckman Dxi 800 immunoassay system in the first week of September and again the second week of December. Participants were instructed not to supplement with iron 24 hours prior to testing.

Injuries and fatigue symptoms were self-reported by athletes to the team's athletic trainers, who diagnosed and treated injuries throughout the training seasons. Athletic trainers referred athletes to a medical doctor for diagnosis if a further diagnosis was warranted. The athletic trainers completed weekly reports on athlete's injury status. Injuries were divided into 2 types: bone or tissue injuries. Injury location, type, and

severity were recorded. Fatigue symptoms were reported as chronic fatigue or the particular sickness an athlete had, such as mononucleosis, influenza, etc. Severity of injury and fatigue symptoms were measured on 5 levels, from 0 to 4 increasing with severity (Table 1).

Table 1. Severity of Injury, Illness, and Fatigue as Ranked by Athletic Trainers

Classification	Severity	Training Load Effect
Level 0	No Injury	Continue at normal training load
Level 1	Minor Injury	No reduction in training load
Level 2	As Tolerated	Continue normal training load if tolerable
Level 3	Limited	Decrease in training load
Level 4	Out	No practice sessions or cross training

During times of injury or fatigue symptoms, athletes may have participated in rehabilitation exercises and/or cross training sessions depending upon the athletic trainers' and coaches' discretion. If an injury was believed to be a tissue injury, but later found to be a bone injury, previous weekly injury reports were edited to reflect the new diagnosis. Mileage was self-reported by athletes on a weekly basis, recorded by the coaches, and used to cross check injury status.

Procedure

Participants supplemented with liquid ferrous sulfate elixir (220 mg/5 ml, Pharmatech, Livonia, MI). A gradual increase in ferrous sulfate elixir occurred over 4 weeks according to the United State Olympic Committee (USOC) recommendations, shown in Table 2. Based on the initial SF measure, athletes were instructed to follow the

weekly recommendations until they reached their designated SF level, at which point they stayed on that regimen until the next testing date.

Table 2. USOC Iron Supplementation Protocol

	Serum Ferritin Level	Orange Juice/Vitamin C	Ferritin Sulfate Elixir
Week 1	Females < 40 μg/L, Males < 50 μg/L	8 oz. OJ + 500 mg Vit. C	5 ml x 2 per day
Week 2		8 oz. OJ + 500 mg Vit. C	10 ml x 2 per day
Week 3	Females < 30 μg/L, Males < 40 μg/L	8 oz. OJ + 500 mg Vit. C	15 ml x 2 per day
Week 4	Females < 20 μg/L, Males < 30 μg/L	8 oz. OJ + 500 mg Vit. C	15 ml x 3 per day

Females below 20 μg/L and males below 30 μg/L followed dosage through week 4 and continued with that dosage. Females below 30 μg/L and males below 40 μg/L followed dosage through week 3 and continued with that dosage. Females below 40 μg/L and males below 50 μg/L followed the week 1 dosage. Athletes with a SF ≥ 40 μg/L and ≥ 50 μg/L, for females and males respectively, supplemented according to week 1 of the USOC iron supplementation protocol to maintain their SF. Following the SF measure in December, athletes adjusted their intake accordingly. Along with the iron supplementation guidelines, participants were given additional informational resources explaining iron supplementation in regards to calcium, which blocks absorption, and vitamin C, which increases absorption.[74]

Data Analysis

SF was analyzed on a continuous scale and was grouped into iron depleted (≤35 μg/L) and normal (>35 μg/L) groups for data analysis. Following supplementation and

retesting, the athletes were separated into two groups based on their SF. If an athlete was IDNA during their pre or post SF, they were placed in the IDNA group. If an athlete was not IDNA during their pre and post SF, then they were placed in the normal iron group (N). Training reduction was categorized as no reduction in training, which corresponded with injury levels 0, 1, and 2 according to the athletic trainer assessment. Level 3 was classified as partial training reduction (training continued but at a reduced volume) and level 4 was classified as complete training reduction (no training). For data analysis, levels 0, 1, and 2 were combined to level 0 reflecting no reduction in training volume. Level 3 (partial training reduction) was classified at level 1 and level 4 (complete training reduction) as level 2. Weekly mileage and injury/fatigue symptom severity were compared to ensure accurate classification of training reduction.

Descriptive statistics were conducted for age, gender, injury incidence, injury type, injury severity, SF level, and weekly mileage. A generalized linear model analysis was used to determine the association between Pre and post SF levels, mileage, and gender as predictors of a reduction in training volume. Using the 2 groups following the post SF test, a chi-squared was used to analyze the difference in training reduction among the groups. SPSS (Version 22, IBM Corp. Armonk, NY) was used for all statistical analyses and statistical significance was set at ≤ 0.05.

CHAPTER FOUR: RESULTS

Data were collected on 17 male and 30 female NCAA Division I collegiate distance runners between the ages of 18 and 24 ($\bar{x}$ ± sd = 20.00 ± 1.69 years) with a mean school year of 2.34 ± 1.23. SF measures and mileage are presented in Table 3. The average pre SF was 37.5 ± 19.0 μg/L and increased to 53.8 ± 24.2 μg/L. Women's pre and post SF, 31.3 ± 14.1 μg/L and 48.4 ± 22.4 μg/L, respectively, were significantly lower than the men's SF (Pre: 49.0 ± 21.5 μg/L, Post: 63.3 ± 24.9 μg/L) (Pre: p=0.001, Post: p=0.04). SF improved by an average of 15.8 ± 22.2 μg/L. There was a wide variation in the SF changes across the study population, with one athlete's SF decreased by 19 μg/L while another's increased by 114 μg/L. Those who supplemented with a higher dose of ferrous sulfate elixir led to a greater SF increase, although there was no significant difference between the lowest (88 mg of elemental iron) and middle (246 mg) supplement groups. However the highest (396 mg) supplement group was significantly different from the lowest (88 mg) supplement group (p=0.002) (see Table 4). Those on the highest supplementation (396 mg) improved SF by 34.2 ± 31.6 μg/L, the second highest (264 mg) improved SF by 16.7 ± 18.1 μg/L and the least amount of supplementation (88 mg) improved by 7.7 ± 14.4 μg/L. Training volume averaged 60.7 ± 17.4 miles/week (53.5 ± 16.3 miles/week for women, 73.5 ± 10.9 miles/week for men) with a minimum of 17 miles/week and a maximum of 92 miles/week.

Table 3. Pre and Post SF Values, SF Change, and Training Volume of the Distance Runners Measured During the Cross Country Season

	N	Mean	Min	Max	Std. Deviation
Pre SF (μg/L)	47	37.5	10	86	19.0
Male	17	49.0	13	86	21.5
Female	30	31.1	10	57	14.1
Post SF (μg/L)	47	53.8	15	131	24.2
Male	17	63.3	32	109	24.9
Female	30	48.4	15	131	22.4
SF change (μg/L)	47	15.8	-19	114	22.2
Volume (miles/wk)	47	60.7	17	92	17.4

Table 4. SF's Response to Supplementation Dose

Iron Supplement	N	Change in SF (μg/L)	Std. Deviation
Group 1 (88 mg)	24	7.7	14.4
Group 2 (264 mg)	13	16.7	18.1
Group 3 (396 mg)	10	34.2	31.6

Of the 47 athletes whose data were analyzed, 11 (23%) experienced a complete reduction in training volume, 16 (34%) experienced a partial reduction in training volume, and 20 (43%) did not experience a reduction in training due to injury, illness, or fatigue (Table 5). Twenty-three athletes had normal SF levels above 35 μg/L and 20 maintained normal SF levels, while three fell into IDNA, at the post SF test. Twenty-four athletes were IDNA at the pre SF test. Fifteen were able to correct their IDNA while nine remained IDNA at the post SF test. Eighteen of the 30 female athletes and nine of the 19

male athletes experienced a reduction in training. The chi-square test performed to examine training reductions between the N group and IDNA group found IDNA to be significantly associated with training reduction, $X^2 = 7.19$, N = 47, p = 0.027. Training reductions are shown in Table 5 and group information is shown in Table 6. In the N group, seven of the 20 (35%) athletes experienced a reduction in training due to injury, illness, or fatigue, while the IDNA group had 20 of 27 athletes who experienced a reduction in training.

Table 5. Training Reductions Experienced by the N and IDNA Groups

	N	No Training Reduction	Training Reduction	Partial Reduction	Full Reduction
N group (>35 μg/L)	20	13 (65.0%)	7 (35.0%)	4 (20.0%)	3 (15.0%)
IDNA group (≤35 μg/L)	27	7 (25.9%)	20 (74.0%)	12 (44.4%)	8 (29.6%)

Table 6. SF and Training Volume Differences Between N and IDNA Groups

	Sex (M, F)	Pre SF (μg/L)	Post SF (μg/L)	SF Change (μg/L)	Training Volume (miles/wk)
N group (>35 μg/L)	11, 9	55.1 ± 13.9	68.1 ± 19.4	11.9 ± 15.1	70.4 ± 11.2
IDNA group (≤35 μg/L)	6, 21	24.5 ± 9.2	43.5 ± 22.1	18.7 ± 26.2	53.6 ± 17.9

A multinomial generalized linear model was run to predict how reductions in training were effected by training volume, gender, pre SF, and post SF (Table 7). Training volume, Pre SF, and Post SF were statistically significant ($p<0.05$), while gender was not. As pre SF decreases, there is a 4.3 % increase in the likelihood of experiencing training reductions due to injury, given all other variables are held constant. As post SF increases, there is a 3.3% increase in the likelihood of experiencing a training

reduction due to injury. As training volume decreases there is a 5.8% increase in the likelihood of experiencing a training reduction due to injury.

Table 7. Parameter Estimates for the Linear Model of Training Reduction Associated with Pre SF, Post SF, and Training Volume.

	Exp(B)	B	Std. Error	df	Sig.
Pre SF	1.043	0.042	0.0212	1	0.049
Post SF	0.967	-0.033	0.0169	1	0.05
Training Volume	1.058	0.057	0.0229	1	0.013

CHAPTER FIVE: DISCUSSION

The purpose of this study was to investigate the relationship between SF and reductions in training due to injury, illness, or fatigue. The hypothesis was that SF would be associated with training reductions. The IDNA group was found to have a significantly higher rate of training reductions (74%) compared to the N group (35%). Pre SF and training volumes were negatively correlated with training reductions, suggesting that a low SF and low training volume were associated with reductions in training. Post SF was positively correlated, suggesting that a relationship between training reductions and a higher post SF, although this may be due to those with low SF dramatically improving their SF.

Women's average SF was significantly lower than men's SF with 70% of women in the IDNA group while only 35% of men were in the IDNA group. The IDNA prevalence among women was expected as women are at a higher risk of ID due to menstrual blood loss.[1,13] However, the generalized linear model did not find any significant associations between sex and training reductions. On average, SF increased by 15.8 ± 22.2 μg/L, which was enough to correct IDNA in 62.5% of the IDNA group. This suggests that the supplementation and dietary recommendations were adequate for some athletes to improve SF levels in 14 weeks. Previous research has found similar improvements in SF, between 13 μg/L and 20 μg/L, with supplementation amounts of 100-200 mg of elemental iron over 4-16 weeks, with most improvement occurring in the first two weeks.[60,75-77] A more aggressive oral supplementation may be effective, as the

group of athletes that supplemented with the greatest amount of ferrous sulfate (396 mg) experienced an improvement in SF of 34.2 μg/L in 14 weeks. Changes in Hb were only found in two studies and did not occur until 8 weeks and 16 weeks from the start of supplementation.[77,78] There was a wide variation in SF change, with a maximum increase of 114 μg/L and a decrease of 19 μg/L, suggesting that some athletes may respond well to increased iron intake. Eight (17%) of the athletes experienced a decrease in SF, which may reflect the increased strain on SF caused by training such as hemolysis, hepcidin release, and hematuria.[19,23,24] Additionally, some of the athletes may not have been compliant with the supplement guidelines or were non-responders, although the literature does not make any mention of non-responders. However, adherence to and the effectiveness of the supplementation protocol is unknown as supplementation and dietary intake compliance were not monitored. The variation in SF improvement is partially due to the graduated supplementation protocol, but may also be affected by supplementation practices and individual variation. Due to variation in SF improvement and for adjusting supplementation dosage, athletes should have SF retested regularly and iron intake monitored to observe supplementation effectiveness for the individual.

The IDNA group experienced more than twice as many partial and complete training reductions (74%) as the N group (35%). Training reduction occurred due to a variety of injuries (stress fractures, muscle strains, etc.), illness (mononucleosis, upper respiratory tract infection, flu, etc.), and fatigue symptoms (elevated resting HR, general perceived fatigue, etc.). Of the 27 IDNA athletes, 74% experienced a reduction in training due to injury, illness, and/or fatigue while 35% of the N group experienced a training reduction. This suggests that the health consequences of ID, such as

compromised bone integrity, immune suppression, and fatigue, may contribute to training reductions in IDNA athletes.[2-4,26,28,31,32,44] As pre SF decreases, a 4.3% increase in the likelihood of a training reduction was found. Although training reductions were expected at a higher rate in the IDNA group, a linear correlation was not expected, as iron depletion occurs at <35 μg/L.[12] However, the cutoff of <35 μg/L for detrimental effects may not explain the whole situation, as unexplained fatigue in women with ≤50 μg/L was found to improve with iron supplementation.[29] Thyroid hormones, bone biomarkers, immune function, and fatigue were not directly measured; therefore the training reductions cannot be directly related to those issues potentially related to low SF.

This is the first study to find an association between SF and training reductions caused by injury, illness, and/or fatigue. Previous studies that have investigated running injuries explored age, gender, height, weight, leg length differences, competition, and running pace found no definite cause.[68] In trained distance runners, a few studies have found previous injury to be the only repeated finding with significance.[69,70,72,73] The variation in injury type and individual athletes presents difficulty in finding a specific cause for running injuries. However, there are a number of reasons ID may effect the health and training of athletes. ID has been found to compromise bone integrity, which is particularly alarming when stress fractures were found in 21% of track and field athletes.[4,7,40,44] Immune suppression, particularly a decrease in NK cells, has also been seen in ID female distance runners undergoing an increase in training volume. Flynn et al. suggested that it may take normal iron stores a while to regain normal immune function as NK cells remained below normal levels 8 weeks following the intervention, although how long this make take is unknown.[67] Decreased thyroid hormones have also

been associated with an increase in training load among female rowers. Baylor and Hackney found 10 of 17 women's TSH and T^3 decreased following intense training, while it has also been shown that ID affects thyroid hormones.[2, 79] The various ways in which ID and IDNA effect bone health, the immune system, and thyroid function, as well as the alarmingly high rate of IDNA among these male and female athletes (35% and 70% respectively) suggests that nutritional deficiencies may play an important role in maintaining health and training. Although bone, immune system, and thyroid biomarkers were not measured in the current study, a number of the injuries, illnesses, and fatigue symptoms have the potential to be a symptom of ID. This is not to say that all injuries are caused by ID, but that ID may create an adverse environment for an athlete to overcome the stress of training. Maintaining training volume may also have a performance benefit as well. Assuming that the training stimulus and rest are appropriate, athletes that are able to maintain their training load should continue to improve whereas an injured, sick, or tired athlete will miss out on the training adaptation until they are healthy enough for such activity.

Interestingly, high post SF levels were associated with a reduction in training. This finding may be due to a number of factors regarding the timing of the post SF measurement. Athletes were re-tested in December, which may have occurred well after the reduction in training occurred. Therefore, SF at the time of injury, illness, or fatigue symptoms was not known. At the same time, athletes had decreased their training load by this time, which may reduce the stress on SF. Additionally, the athletes with low initial SF levels had a higher supplementation dose, causing a five-fold and two-fold increase in SF levels above the lowest and middle supplementation groups (see Table 4), possibly

elevating their post SF levels, although the post SF of the IDNA (43.5 μg/L) was not higher than the post SF of the N group (68.1 μg/L). However, four of the individuals who were IDNA during the pre SF measurement were among the highest during the post SF measurement, particularly the highest post SF value of 131 μg/L, which started at 17 μg/L. Therefore, this finding may be reflective of the greater SF supplementation, and subsequent greater improvement in SF, in the athletes with more pronounced ID.

Furthermore, training volume was negatively associated with training reduction due to injury, suggesting that athletes who trained at a higher volume were injured less often. In recreational runners, increased training volume above 64 kilometers/week was found to be a significant predictor of injury.[69,70] However, in recreational marathon runners, training volume has been found to be protective of injury.[71,72] This association may only show that runners who are healthy are able to run more miles. Weeks of reduced training due to injury, illness, or fatigue were not included when calculating average weekly training volume in an attempt to eliminate the effect of the training reduction on weekly training volume averages.

The use of pre-existing data limited this study and therefore SF was not measured at the time of injury. Additionally, potential ID detriments such as thyroid hormone regulation, bone biomarkers, and immune function were not investigated. Although prescribed, the athlete's supplementation and the dietary recommendations to increase iron intake were not monitored for compliance and do not allow a true measure of the effect of the supplementation protocol. Furthermore, there may be some association that athletes who take great care to remain healthy also follow the supplementary and dietary recommendation more closely.

Conclusions

IDNA and low pre SF levels were associated with training reductions due to injury, illness, and fatigue. This finding does not suggest that every injury, illness, and/or fatigue is cause by ID, but that ID may lower an athlete's resistance to particular injuries or illnesses that may require a reduction in training. Surprisingly, less weekly volume and high post SF levels were associated with training reductions, although the post SF measurement did not occur at the time of injury. Previous research on athlete's iron levels has primarily focused on performance.[60-62] However, research on the health and training status of ID athletes is lacking, as only a couple studies have investigated the effects of ID in athletes.[28,67] SF measurement and iron supplementation, along with dietary instructions to increase iron intake, may decrease the need for injury, illness, and fatigue-related training reductions among collegiate distance runners. Coaches and trainers should have SF levels measured prior to and following a season to monitor SF levels and see how well an athlete responds to iron supplementation. Future research should investigate ID's effect on other groups of athletes, measuring SF at the time of injury, and monitoring corrected IDNA's effect on training. Further investigations into particular biomarkers of bone, immune, and thyroid function in ID athletes may prove beneficial for the health and training of athletes.

Practical Application

Coaches should have athlete's SF tested every 3-4 months to see if they are IDNA (<35 μg/L). Even if athletes are near 35 μg/L, supplementation may be beneficial due to the finding that unexplained fatigue in women with a SF of <50 μg/L improved following supplementation.[29] A good time to test SF would be during preseason or prior to a

competitive season to allow SF time to improve. Prior to testing, athletes should avoid iron supplementation and meals high in iron content. SF testing on an empty stomach is recommended. If an athlete's SF is low, they should supplement and make dietary changes to increase iron intake. The USOC supplementation protocol (see Table 2), as well as dietary recommendations, were effective in maintaining or improving 83% of the athlete's SF in this study. Ferrous sulfate elixir is a liquid form of iron (available over the counter at local pharmacies) which should be taken with orange juice and a 500 mg vitamin C pill to increase absorption.[80] Athletes should avoid consuming calcium near supplementation as it blocks the absorption of iron.[80] Supplementing 30 minutes prior to a meal may be an effective strategy to avoid calcium intake near supplementation. Ferrous sulfate elixir often has an unpleasant taste and may cause teeth discoloration. An effective method of ingesting ferrous sulfate elixir is to mix the supplementation amount with 1-2 ounces of orange juice and swallow with minimal teeth contact, followed by 8 ounces of orange juice and a vitamin c pill. Brushing immediately after may help to avoid teeth discoloration. Iron supplementation may initially cause gastric distress and black stools. Athletes should be retested every 3-4 months to avoid toxicity levels (1,000 μg/L), to remain within the upper limit of the normal SF range (women: 150 μg/L, men: 200 μg/L) and to make individual adjustments to supplementation amount.[81]

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APPENDIX

IRB Approval Letter

BOISE STATE UNIVERSITY
RESEARCH AND ECONOMIC DEVELOPMENT

Date: May 16, 2016

To: Taylor Thompson cc: Shawn Simonson

From: Office of Research Compliance (ORC)

Subject: SB-IRB Notification of Exemption - 103-SB16-100

Serum Ferritin's Relationship to Training Reduction among College Distance Runners

The Boise State University ORC has reviewed your protocol application and has determined that your research is exempt from further IRB review and supervision under 45 CFR 46.101(b).

Protocol Number: 103-SB16-100

Approved: 5/16/2016 Application Received: 5/11/2016

Review: Exempt

Category: 4

This exemption covers any research and data collected under your protocol as of the date of approval indicated above, unless terminated in writing by you, the Principal Investigator, or the Boise State University IRB. All amendments or changes (including personnel changes) to your approved protocol **must** be brought to the attention of the Office of Research Compliance for review and approval before they occur, as these modifications may change your exempt status. Complete and submit a Modification Form indicating any changes to your project.

Annual renewals are not required for exempt protocols. When the research project is completed, please notify our office by submitting a Final Report. The exempt status expires when the research project is completed (closed) or when the review category changes as described above.

All forms are available on the ORC website at http://goo.gl/D2FYTV

Please direct any questions or concerns to ORC at 426-5401 or humansubjects@boisestate.edu.

Thank you and good luck with your research.

Office of Research Compliance