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Iron status in relation to well-being questionnaire (H10WB) scores in Division I female Collegiate athletes

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Iron Status in Relation to Well-Being Questionnaire (H10WB) Scores in Division I Female Collegiate Athletes

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Abstract

Objective: The purpose of this study was to determine the relationship between iron storage and overall well-being in Division I female collegiate athletes. This was done to determine a cost-effective screening method for iron deficiency.

Design: Retrospective Cohort

Subjects and Settings: 117 Division I female athletes at James Madison University. Subjects were ages 17-22 from different teams (Cross Country, Track & Field, Basketball, Field Hockey, Lacrosse, Volleyball, Golf, Swimming & Diving, Soccer, and Softball). We excluded one subject based on a medical diagnosis.

Main Outcome Measure: Data were retrieved from Electronic Medical Records on subjects who have had ferritin levels tested and completed the Henriques 10-Item Well-being Questionnaire (H10WB) within the same year.

Results: Correlations resulted in no significant relationship between ferritin levels and H10WB total scores with a p-value of 0.141. The data were divided into three groups (A,B,C) based on number of H10WB questionnaires completed. This separation resulted in non-significant relationships. Group A, ferritin 1 and H10WB total (p= 0.070); Group B, ferritin 1 and H10WB total (p=0.819); Group C, ferritin 1 and H10WB total (p= 0.056).

Conclusion: Current data suggest that the symptoms of mood disturbances and changes in an athlete's overall well-being status are not correlated with low ferritin levels. These results suggest the H10WB is not a sufficient tool to predict iron deficiency and iron deficiency anemia; therefore, further research needs to be conducted with larger samples of subjects with iron deficiency to determine an efficacious cost-effective screening tool.

Key Words: iron deficiency, ferritin and athletes, iron deficiency and athletes, ferritin and mental health, female athletes and iron deficiency

Chapter I. Introduction/Review of Literature

The prevalence of iron deficiency in the US population varies depending on age group and gender. According to the 1999-2000 National Health and Nutrition Examination Survey, NHANES, males ages 16-69 had a 2% prevalence for iron deficiency; whereas, females ages 16-19 had a prevalence of 16%, ages 20-49 12% (subject to change within other races), and ages 50-69 9%.[1] The prevalence of iron deficiency anemia was reported at 2% for the whole population with females ages 12-49 at 3%, and ages 50-69 at 3%.[1] Data indicates there is a higher prevalence of iron deficiency in both high level and recreational male and female athletes.[2] In a study with professional athletes from different sports the results found no statistical significant differences in ferritin levels among sports.[3] A study by Auersperger revealed that 71% of female runners had depleted iron stores over a 10 day period.[4] Although statistics suggest there is a higher percentage of iron deficiency and iron deficiency anemia in female athletes, it is still not a common practice to screen these individuals.[2,3] This is partly due to lack of consistent research as well as the cost associated with this testing. Researchers at the University of Wisconsin estimated the median lab fee per draw to test ferritin as US $32.00.[5] Therefore, the current study will be used to identify cost effective screening tools to ensure that sports medicine professionals are testing those who are at high risk for iron deficiency.

Iron Deficiency and Iron Deficiency Anemia

Iron deficiency and iron deficiency anemia have been determined as two distinctive medical diagnoses. Iron deficiency (iron deficiency without anemia, ID) is a decrease in the total iron content in the body. This is characterized by a ferritin level <30ng/L.[6] A decrease solely in ferritin level is not indicative of an individual with anemia, but it is an indication of someone at risk due to the continued depletion of iron stores.[7] Anemia (Iron deficiency anemia, IDA) is a condition that occurs in the event that there is a decrease in the amount of red blood cells or the red blood cells that are present do not contain sufficient hemoglobin.[8] Those diagnosed with IDA were reported to have significantly lower hematocrit, hemoglobin, and serum ferritin than the other groups.[2] These individuals can be characterized by a serum ferritin level < 20 ug/L.[2] When iron deficiency reaches this level there can then be a disruption in erythropoiesis, formation of red blood cells.[6] Anemia can have other causes other than iron deficiency, such as gastrointestinal dysfunctions or abnormality in the red blood cell.[6] The serum transferrin measurement explains the iron-binding capacity in the body and the amount of transferrin in the blood not binding to iron. Serum iron measures the amount of circulating iron in the blood, and serum ferritin measures the amount of stored iron in the body.[9] Milic et. al. observed various metabolic energy demands and iron measurements which represented that testing body iron and serum transferrin are reliable measures to use in observing iron metabolism.[10] Transferrin saturation index is another measurement which can be used when the origin of the decrease in ferritin is unclear. This percentage represents how much of the available transferrin is bound to iron; less than 20% can be classified as iron deficiency.[6] Punnonen et al. also indicated that the combination of the serum transferrin receptor/serum ferritin index and serum ferritin measurements will result in the highest sensitivity and specificity in the diagnosis of iron deficiency.[11] There is extensive research in the relationship between ID/IDA and increased physical activity, specifically in endurance athletes.[3,4,12,13]

Effects of Physical Activity

Individuals who are more active have been reported to have decreased iron storage capacities when compared to those who are sedentary.[8] Many different theories have been examined as the cause of this deficiency in the active population. Hemolysis, which is the loss of red blood cells, is a field of interest for researchers.[14] Although much of the loss of red blood cells in athletes can be contributed to the regulatory trauma of the circulatory system during intense exercise (>1-hr, >75% maximal oxygen uptake), there is data representing the majority of the hemolysis could be due to footstrike.[15] A review done by Alaunyte et al. also states that a possible explanation for this is the adaptive changes in muscles which has higher demands of oxygen in aerobic activities.[16] The increase in exercise and iron deficiency also impacts mitochondrial enzymes in a way that could result in an effect on the relationship between energy demand of exercise and capacity for oxygen transport and utilization.[17] Milic et. al. separated athletes into groups (aerobic, anaerobic, and mixed) according to the energy system required by their sport, female athletes in sports who relied more on mixed sources of energy were at the highest risk for iron deficiency.[10] These results indicate that iron deficiency could involve all types of athletes. Research has also suggested that ferritin levels could be impacted by the dietary intake of the athletes. In a study comparing males and females, the endurance athletes of both sexes did not meet the recommendations of energy intake.[18] Statistically significant correlations were determined between absorbable dietary iron intake and serum ferritin as well as, serum iron in male and female runners (0.639, 0.647 respectively).[19] If these athletes consistently do not meet the Recommended Dietary Allowance (RDA) of energy as well as iron this could result in an iron deficiency. In more recent research there is some discussion on the concept of "hepcidin bursts" which block the route of iron absorption. This will occur during physical activity due to the inflammatory process.[20] Inflammation occurs with any type of physical activity and hepcidin is then produced at an increased rate. Hepcidin manages the iron being released from storage through the use of ferroportin; therefore, increased hepcidin causes ferroportin to respond by obstructing the release of iron into circulation and keeping it within the macrophage system. This mechanism will cause a interference with the transfer from macrophages to erythroblasts, the building blocks of erythrocytes. It will also cause a decrease in absorption of iron and when the this falls below the demand iron stores will begin to deplete potentially causing an iron deficiency.[20] This can be detrimental for competitive athletes due to theories of the impact it has on performance. In a study examining professional athletes, there was no correlation found between training volume and serum ferritin levels.[3] On the other hand, Petkus et al. explained that iron deficiency can lead to hypoxia, causing a decrease in performance specifically among endurance athletes.[21]

Females vs. Males

Surprising data has revealed men could be at risk of excess amounts of iron, which can be detrimental and put at risk for toxicity due to them having significantly higher body iron stores.[22] On the other hand, there is a higher prevalence in females for iron deficiency with or without anemia association.[2] As discussed earlier it is common occurrence that athletes do not meet the RDA for energy intake and iron but specifically females are deficient in this area.[23] A study comparing a group of male and female athletes, 19% of the males were below the RDA for energy intake as opposed to the 63% of female subjects.[12] In this study data was collected from young elite athletes (16.2+/-2.7 years), from 24 different sports, comparing males to females on diet and exercise. In this female population, results represented a sufficient energy intake.[12] In another study of female athletes from karatekas, handball, basketball, and runners; basketball players were the only athletes who reached the minimum iron intake of 15 mg/day compared to the others.[24] This relationship shown between active females and iron status have several possible explanations, some solely physiological.

Different phases of the individual's menstrual cycle could also have an effect on iron status indicator results. In the luteal phase values may be highest and menstrual phase could be lower but, not enough to have any clinical implications.[25] Iron has higher demands in the body for females but, many times it is not then compensated for within the diet. Females are also at greater risk of low iron levels due to decreased dietary iron and blood loss through menstruation.[21,26] Female athletes tend to have a stronger prevalence of menstrual cycle disturbances than non- athletic females.[27] Female athletes sometimes struggle with the irregularity of menses due to their total caloric intake being lower than the RDA.[21] Results from one study represented no differences in baseline ferritin levels between those runners who are amenorrheic and those who are eumenorrheic.[24] Menstrual dysfunction in response to low energy availability has been observed, specifically in exercising women. This has been known as exercise-associated menstrual disturbances (EAMD) and exists in up to 56% of exercising women.[21] EAMD can have many physiological effects such as estrogen deficiency, compromised bone health, increased incidence of stress fractures, and more.[21] This study suggested there was an inverse relationship between menstrual cycle blood loss and iron status.[28] Amenorrhea can decrease the chances of iron deficiency due to the decrease in blood loss.[21] But iron deficiency can lead to complications with reproductive function due to iron demands in a series of phases of follicular development and corpus luteal function which, can then assist in the progression of EAMD in exercising women.[21] Along with EAMD, bone health can also be affected by iron deficiencies.

Low Bone Mineral Density

Iron deficiency can have serious negative implications to bone health and can have an association with osteoporosis.[21] A study done with rats exploring Bone Mineral Density (BMD) and Bone Mineral Content (BMC) of those on an iron deficient diet and the control, revealed an association between an iron-deficient diet and decreased bone mineral density. The results of this study represented that in the rats that were on an iron-deficient diet there was a decrease in BMC and BMD in the femur and lumbar vertebra.[29,30] There has been limited research done on the athletic population but, there was a positive association of iron with a change in BMD in postmenopausal women on hormone replacement therapy.[31] Women with iron intakes of greater than or equal to 20 mg of iron had the highest mean BMD at all bone sites, when compared with those women who had iron intakes of less than 10 mg.[32] In a study of a Chinese population of menopausal women an inverse relationship between phalangeal osteoporosis was found in all females, postmenopausal and premenopausal.[33] In this population of the females, 34.1% had deficient iron intake.[33] When compared to controls, female athletes had higher BMD in the sites of lower limb, lumbar spine, and upper limb sites due to the loads being put on them in their sport. For example, power athletes had greater bone mass than endurance athletes at the lumbar spine.[27] Another possible reason for this decrease in bone mass in endurance athletes could be attributed to increase in menstrual disturbances recorded by these individuals. The combination of mechanical loads depending on the athlete type and hormonal status could be responsible for this.[27] The disruption in the reproductive function due to iron deficiency can also hinder bone health. A study of premenopausal exercising women, in combination with energy deficiency resulted in a decrease in osteoblast activity as well as an increase in osteoclast activity.[34] This change in function is believed to be due to an estrogen deficiency which, can be detrimental to overall bone health. In a study of Female Athlete Triad-related risk variables, the strongest association was seen with bone stress injury and those participating in purposeful activity (≥12 hr/wk), low BMD (z score <-1.0), and low BMI (<21.0 kg/m^2).[35] Some of this research has suggested a possible relationship between EAMD and low BMD is secondary to decreased energy intake or energy deficiency.[36]

Energy Deficiency

Female athletes are a population of large concern when discussing energy deficiency and sport. Energy availability can be evaluated by the result of exercise energy expenditure subtracted from energy intake then divided by lean body mass.[21] Slater et al. found that in a group of recreationally active females, 44.9% were found to be at risk for low energy availability determined by a LEAF-Q Questionnaire.[37] In a study observing young gymnasts it was concluded that there was an increase in energy expenditure and a significant decrease in energy intake by the athletes compared to the control group. Although not a significant difference, there was also a decrease in iron intake (mg/day) by the athletes when compared to the control group.[38] In a study which researchers evaluated the energy intake of amenorrheic runners, eumenorrheic runners and eumenorrheic active controls, no significant difference was revealed in the energy intake of these individuals. Overall their energy intakes were approximately 1700 and 1800 kcal which is below the recommended daily amount.[39] The research is consistent with the relationship between energy deficiency and other concerns such as low bone mineral density, menstrual dysfunction, and more. According to the IOC consensus statement Relative Energy Deficiency in Sport (RED-S) is the new broader term for the clinical syndrome which was formerly known as Female Athlete Triad. This document proposes the idea that the energy deficiency is the dysfunction that leads to other medical concerns.[36] A 2017 review was published discussing the overlap in adverse health conditions between iron deficiency and low energy availability. Low energy availability has been shown to have an effect on the menstrual cycle which can also be referred to as exercise-associated menstrual dysfunction (EAMD), and is present in up to 56% of exercising women.[21] Iron-deficiency anemia can have negative effects on thyroid function which in energy deficient exercising women could advance the subclinical condition of hypothyroidism which can be seen among these individuals.[40] An exacerbation of energy deficiency could be due to metabolic dysfunctions via iron deficient athletes with or without anemia or an increase in energy expenditure.[41] In a study observing bone mass of female athletes, a direct relationship was found between the incidence of bone injuries and the risk factor variables observed. These were Female Athlete Triad-related risk factors such as, low BMD, low BMI, increased activity, dietary restraints, menstrual status, and sport/activity type.[35] Fuel availability may be affected due to the decrease in iron availability then reducing cortisol synthesis. Iron deficiency can also lead to a decrease in Human Growth Hormone secretion which assists with normal functions of the body.[21] The continued physiological mechanisms that are affected by these deficiencies can also lead to changes in mood and overall health.

Overall Well-Being Status

Iron and energy deficiencies can have an effect on mood and overall well-being of the individual. Due to the effects decreased ferritin level has on the body there can then be negative effects seen in mood and behaviors, specifically depressive disorder. The mechanisms in the brain such as the roles in enzymes of oxidation-reduction, electron transport, and synthesis and packaging of neurotransmitters could all result in an altered function that results in a change in mood and behavior.[42] Individuals who suffer from iron deficiency could show signs and symptoms of depression.[42] As described in Bermejo et. al., associated symptoms of ID and IDA are weakness, fatigue, irritability, poor concentration, headache, and intolerance to exercise.[6] These symptoms are usually perceived as being normal until there is an improvement. When compared to the CES-D, used in the study by Onder et. al., these symptoms are also similar to symptoms used to identify depression.[43] There have also been associations with negative mood and lethargic behaviors.[44] Although the subjects in this study did not have an iron deficiency at baseline, following four weeks of IV iron supplementation the distance runners showed a decrease in fatigue and mood disturbances.[45] Research is still in progress, but more studies have revealed evidence to suggest neural functioning and behavioral consequences of iron deficits within the brain are not limited to infants.[46] Gregg Henriques created a questionnaire, Henriques 10-Item Well Being Questionnaire (H10WB), to evaluate individual's overall mental well-being. This questionnaire is based on the Nested Model which contains four domains, the subjective domain, the health and functioning domain, the environmental domain, and the values and ideology domain.[47] Due to the association between depression and anemia, identifying depression-like thoughts or symptoms through this questionnaire could help lead to prevention of ID and IDA. The 10-Item questionnaire can assist with the evaluation of mental well-being of these individuals which, has association with their iron status.

Study Justification

The issue of iron deficiency continues to be linked back to female athletes. This has been shown in studies that observe various types of athletes, in or out of season, their dietary habits, and ferritin levels. It is also a common conclusion that due to their menstrual cycle they require a higher dietary intake but many times they do not compensate properly. The recent research has found relationships between iron deficiency and energy deficiency, menstrual dysfunction, low BMD, cardiac concerns, and more. Some researchers believe there should be more research addressing how nutritional evaluations and gonadal-mediated mechanisms affect bone health as well as, all together its effect on exercising women instead of as individual assessments.[35] It is also suggested that exercising women should be screened for iron deficiency through blood labs. There are contributing factors preventing this being a common practice such as financial complications, lack of resources, and lack of education. Energy status and iron status can be affected by iron deficiency in many different physiological ways such as thyroid function, menstrual dysfunction, and reproductive function.[21] We can conclude from the recent research and as mentioned in Petkus et al. that it is suggested for exercising individuals to be ferritin tested.[12,21] If iron deficiency can be identified early, then treatments can be implemented to prevent the progression of the deficiency. Researchers continue to look at different groups of individuals to determine the best intervention used but, the results have been inconsistent. Tsalis et al. did not see statistically significant changes in serum ferritin levels with supplementation or increased dietary iron intake.[48] On the other hand, Hultén reported that healthy females ages 21-36 without a history of iron deficiency had sufficient dietary iron stores following sufficient iron intake. Sufficient in this study includes a balance of the appropriate amount of heme iron and non-heme iron.[49] In another study comparing three different treatment methods placebo, supplement, and dietary intervention, it was observed that the dietary intervention was most effective and intake of the 50 mg iron supplement was not.[50] Although it was not statistically significant, recreationally active females increased their serum ferritin levels by 5.4% by consuming teff bread (7.0 ± 3.3 mg of iron per day) after a six-week dietary intervention.[51] Although this would be beneficial for all athletes, they may not have the means to monitor this routinely but, there may be other measures which can help identify when someone is at a higher risk of iron deficiency. A study using the serum transferrin receptor/serum ferritin index measurement revealed that there was a higher proportion of individuals with iron deficiencies without anemia.[2] This shows that if athletes are screened and find these individuals with ID, they can then be treated before it progresses to IDA. This study will evaluate the relationship between ferritin levels of Division 1 female athletes and their overall well-being status through the H10WB score.

This relationship can benefit medical professionals to ensure the athletes who are at highest risk for iron deficiency are being screened. Limitations of this study would be that we do not have the menstrual status of these individuals due to the retrospective nature of the data. The data being examined is from individuals that are in different training regimens. There is no analysis of nutritional intake from these subjects. When freshmen are entering college, this questionnaire is sent home; therefore, it could be filled out by a parent in certain situations.

Chapter II. Methodology

Study Design

This will be a retrospective cohort study design. We will be analyzing data which was collected through the James Madison University Sports Medicine Department. We will focus on the relationship between ferritin levels and the amount of iron storage in the body. There will then be an evaluation of the relationship of the Henriques 10-Item Well-being Questionnaire (H10WB) score and ferritin levels.

Subjects

Subjects are all female Division I athletes who participated in JMU varsity sports and have been ferritin tested in the last three years. These subjects were requested by the team physician to receive this testing for many reasons such as, risk of iron deficiency due to sport, risk of anemia, history of anemia, disordered eating, low body mass index, and more. JMU female athletes who have completed both the H10WB and been ferritin tested will be included in this study. All of these measures were taken during their physical examination and will be found in their medical files. Researchers will collect retrospective data such as ferritin levels and Well-Being questionnaire scores. This will be given access due to being an employee of JMU Sports Medicine.

Research Protocol and Measurements

Demographic data will be acquired from the athlete's pre-participation examination which is completed by all athletes in the James Madison University Godwin Hall Athletic Training Room. The incoming freshmen complete this when they come in

August and the returning athletes complete this every April following their initial screening. The following measurements are recorded, menstrual history in the last 3 and 12 months, age, Body Mass Index (BMI), height (m) using a tape measure, weight (kg) using a digital scale, history of eating disorder, history of low Bone Mineral Density (BMD), history of a stress fracture, response to each question in questionnaire, and total score of questionnaire.

Ferritin test results have been obtained by the Sports Medicine Department for the subjects. The participants had blood samples taken at the JMU University Health Center. The blood samples are taken by a nurse at the Health Center who is properly trained. The JMU Health Center then communicated the laboratory results with the JMU sports medicine team physician.

As part of the pre-participation examination all athletes are given the Henriques 10 Item Well-being Scale (H10WB), health and well-being questionnaire (Appendix A) to complete. This contains scaled questions about their mental health and ideas toward well-being.[47] The health and well-being questionnaire will be analyzed based on the subject's answers in the seven-point scale.

The lab results and questionnaires were scanned into a password locked medical documentation database. The hard copies are kept in a folder in a filing cabinet in the sports medicine office, which is locked. The data will be taken from a password protected medical documentation server and saved with a code.

Statistical analysis

The relationship between variables will be identified by correlations. The statistical tests will be completed using IBM's SPSS 24.09 (Armonk, NY). The objectives of the research are to determine the relationship between ferritin levels in female athletes and their Henriques' 10- Item Well-Being questionnaire score. An evaluation of the relationship between ferritin levels and individual questions will be used to determine the importance of certain aspects of the questionnaire. These relationships will be assessed through a correlation to measure the degree of risk an individual may be in for iron deficiency. Data will be evaluated using correlations and simple linear regressions with the variables as ferritin levels and questionnaire total score, then separately with each individual question. The relationship between Ferritin 1 and the total H10WB score among all subject data collected will be assessed through a Pearson's one-tailed correlation to measure the strength of the linear association between ferritin levels and the questionnaire scores. This will then be repeated for Ferritin 2 and H10WB scores. Correlations will also be used to evaluate the strength of the relationship between Ferritin 1 and each question following with Ferritin 2 and each question. Simple linear regressions and multiple regressions will be performed to determine the statistical significance of the linear relationship for those variables that have statistically significant ($p < 0.05$) correlations.

Chapter III: Manuscript

Abstract

Objective: The purpose of this study was to determine the relationship between iron storage and overall well-being in Division I female collegiate athletes. This was done to determine a cost-effective screening method for iron deficiency.

Design: Retrospective Cohort

Subjects and Settings: 117 Division I female athletes at James Madison University. Subjects were ages 17-22 from different teams (Cross Country, Track & Field, Basketball, Field Hockey, Lacrosse, Volleyball, Golf, Swimming & Diving, Soccer, and Softball). We excluded one subject based on a medical diagnosis.

Main Outcome Measure: Data were retrieved from Electronic Medical Records on subjects who have had ferritin levels tested and completed the Henriques 10-Item Well-being Questionnaire (H10WB) within the same year.

Results: Correlations resulted in no significant relationship between ferritin levels and H10WB total scores with a p-value of 0.141. The data were divided into three groups (A,B,C) based on number of H10WB questionnaires completed. This separation resulted in non-significant relationships. Group A, ferritin 1 and H10WB total (p= 0.070); Group B, ferritin 1 and H10WB total (p=0.819); Group C, ferritin 1 and H10WB total (p= 0.056).

Conclusion: Current data suggest that the symptoms of mood disturbances and changes in an athlete's overall well-being status are not correlated with low ferritin levels. These results suggest the H10WB is not a sufficient tool to predict iron deficiency and iron deficiency anemia; therefore, further research needs to be conducted with larger samples of subjects with iron deficiency to determine an efficacious cost-effective screening tool.

Key Words: iron deficiency, ferritin and athletes, iron deficiency and athletes, ferritin and mental health, female athletes and iron deficiency

Introduction

Iron deficiency affects a large number of women in the United States including elite athletes. According to the National Health and Nutrition Examination Survey, NHANES, 1999-2000 females ages 16-19 and ages 20-49 had a prevalence of iron deficiency of 16% and 12% respectively.[1] Those who are more active are seen to have decreased iron storage when compared to those who are sedentary.[2] Even for individuals who are already active (3-4 days/wk), an increase in activity was shown to have an additional decrease in serum iron and serum ferritin for those with decreased iron stores at baseline (ferritin levels <20 mg/L) and normal iron stores (ferritin levels >20 mg/L).[3] According to a study completed at the University of Wisconsin, approximately 30.9% of collegiate female athletes have indicated iron deficiency.[4]

Iron deficiency without anemia (ID) and iron deficiency anemia (IDA) have been determined as two distinctive medical diagnoses. ID is a decrease in the total iron content in the body. A decrease in ferritin levels alone is not indicative of an individual with IDA but it is an indication of someone at risk for developing IDA.[5] IDA is a condition in which there is a decrease in the amount of red blood cells, or the red blood cells that are present do not contain sufficient hemoglobin.[2] When iron deficiency progresses to the level of anemia it can lead to a disruption in erythropoiesis.

Due to this complication, much has been found in the literature regarding the relevance within the athletic population. Parks et. al. evaluated 2749 athletes (56% females) and through blood draws suggested 32.8% had low ferritin, defined as <20 ng/mL.[4] However, if they added subjects who were in the range of 20-35 ng/mL, to account for ID and IDA, there would be more than double the number of subjects in the low group.[4] This higher risk for female athletes has been supported by a variety of factors. For example, hemolysis has been studied to account for this decrease in athletes.[6] The mechanism of hepcidin is another factor that could play a role in this deficiency.[7] During activity the inflammatory process is activated, which includes the release of hepcidin. This release results in ferroportin, inhibiting the release of iron into circulation, impeding the absorption of iron by erythroblasts.[7] A decrease in iron storage has been proven to have negative effects on the body such as exercise-associated menstrual disturbances (EAMD), energy deficiency, decreased bone health, hormone abnormalities, and more.[7,8] Secondly, there has been a documented decrease in overall energy intake compared to the minimum recommendations among athletes.[9,10] Malczewska and colleagues estimated that 63% of female athletes were below the Recommended Dietary Allowance (RDA) for energy intake.[11]

The mechanisms in the brain following a decrease in iron storage – such as the roles of enzymes in oxidation-reduction, electron transport, and synthesis and packaging of neurotransmitters – could all result in an altered function that results in a change in mood and behavior.[12] There has been a suggested association between decreased iron storage with negative moods and lethargic behavior.[13] This suggested relationship increases the inquiry of decreased ferritin levels in female athletes. Some institutions are able to obtain blood draws for many or all of their female athletes, but this could be very costly with an estimation of $32.00 per blood draw at major institutions.[4]

These mechanisms warranted further study of a relationship between low ferritin and decreased overall well-being, which we believe could help healthcare professionals identify a cost-effective way to screen for iron deficiency; therefore, the purpose of this study was to evaluate the relationship between female collegiate athlete iron storage and overall well-being to find a cost-effective screening method.[4] This was done with evaluation of the ferritin levels and a well-being questionnaire.

Methods

This retrospective cohort study evaluated female athletes' ferritin levels in relation with their overall well-being status. This study analyzed data collected through the James Madison University Sports Medicine Department. This study was focused on the relationship between ferritin levels and total scores as well as scores with individual questions on the Henriques 10-Item Well-being Questionnaire (H10WB) (Appendix A).[14]

Subjects

All female JMU varsity athletes (Cross Country, Track & Field, Basketball, Field Hockey, Lacrosse, Volleyball, Golf, Swimming & Diving, Soccer, Softball, and Tennis) who were ferritin tested and completed H10WB questionnaires within a three-year period were included in the study. One individual was excluded due to diagnosis of hereditary hemochromatosis. Athletes were requested by the team physician to receive ferritin testing for many reasons, such as risk of iron deficiency due to sport, risk of anemia, history of anemia, disordered eating, low body mass index, and any others as needed.

Research Protocol and Measurements

All data were obtained from individual Electronic Medical Records (Presagia Izone) taken during their physical examinations. Freshmen pre-participation measures were collected in August. All returning athletes completed measures every April. Data extracted included demographics, (age, sport, height, weight, BMI, menstrual history, history of stress fracture, history of eating disorder, and history of low BMD), ferritin levels, H10WB individual question responses, and H10WB total scores.

Subjects had blood samples taken at the JMU University Health Center. The blood samples were taken by a trained phlebotomist at the University Health Center. The JMU Health Center then communicated the laboratory results with the JMU Sports Medicine team physician. Ferritin test results were obtained by the Sports Medicine Department for the subjects.

As part of the pre-participation examination all athletes are given the H10WB, health and well-being questionnaire to complete. The H10WB contains scaled questions about mental health and ideas toward well-being based on the subject’s answers on a seven-point scale.[14]

Each subject’s ferritin score was matched with the most recent H10WB score (Ferritin 1). Subjects who had multiple ferritin test results compared to one H10WB were identified by (Ferritin 2, Ferritin 3). If a subject then had multiple H10WB responses, data were divided by the initial measurement Group A, secondary Group B, and tertiary Group C.

Statistical analysis

The objectives of the research were to determine the relationship between measured ferritin levels in female athletes and their well-being questionnaire total score. As well as, the relationship between ferritin levels in female athletes and their well-being questionnaire individual question scores. Data were evaluated using correlations, linear regressions, and simple linear regressions. An evaluation of the relationship between ferritin levels and individual questions was used to determine the importance of certain aspects of the questionnaire. The relationship between Ferritin 1 and the total H10WB score among all subject data collected was assessed through a Pearson's one-tailed correlation to measure the strength of the linear association between ferritin levels and the questionnaire scores. This was then repeated for the Ferritin 2 and Ferritin 3 measures. Correlations were used to evaluate the strength of the relationship between Ferritin 1 and individual H10WB questions and Ferritin 2 and individual H10WB questions. Simple linear regression was performed to determine the statistical significance of the linear relationship of the variables with statistically significant ($p < 0.05$) correlations. Multiple regression was not performed due to multi-collinearity within the independent variables. Data were also separated and adjusted to divide the separate data points into Groups A, B, and C representing subjects with more than one H10WB completed. Then Pearson's 1-tailed correlation was performed on Ferritin 1 with H10WB total score as well as Ferritin 2 with H10WB total score for Groups A, B, and C. Correlations were tested between Ferritin 1 and each individual question followed by

Ferritin 2 with each question for Groups A, B, and C. Linear regressions were then performed on these groups for statistically significant correlations. All statistical analyses were completed using IBM's SPSS 24.09 (Armonk, NY).

Results

A total of 117 subjects produced 168 observations (Table 1) due to some having more than one well-being questionnaire score reported. Subjects' ages were 19.5± 2.5 years old with a BMI 26.9 ± 8.8 kg/m^2. As shown in Table 1, 8.5% of these subjects did not have menses within the last three months but did have it within the last 12 months.

The linear regression model used was the predicted ferritin level= $b_0 + b_1$(H10WB total). It was then repeated for each question as predicted ferritin level=$b_0 + b_1$(question 1 score) for those with significant associations. There were 116 observations in Group A, 42 observations in Group B, and 13 observations in Group C. Using the data together the statistics of interest were whether there was a relationship between individual's Ferritin 1 and specific questions within the H10WB. Correlations resulted in a non-significant relationship between ferritin levels and H10WB total scores with a one-tailed p-value = 0.141. The groups were then evaluated separately between Ferritin 1 and Ferritin 2.

Pearson's correlations were used to examine the relationship between Ferritin 1 and H10WB responses in each group. There were significant correlations for the questions regarding health and growth in Group A and for emotional health, autonomy, academic, self-acceptance, and total score in Group C but not Group B, (Table 2). In Group A, with all subjects' Ferritin 1, linear regression determined that the ferritin level in response to the H10WB were statistically significant to predict Well-Being questionnaire questions, specific to health (p=0.017, CI= -10.794, -1.06) and growth (p=0.042, CI= -11.830, -.228) but not for others such as, academics (p=0.077, CI= -9.109, .473) or total (p=0.070, CI= -1.383, .054). The health question accounted for only 5% of the explained variability. In Group B, a linear regression determined that Ferritin 1 did not have a significant relationship for the total Well-Being questionnaire score or with any specific questions. In Group C, a linear regression determined that Ferritin 1 was statistically significant in predicting responses to the question regarding autonomy (p=0.019, CI= 4.68, 42.351) but not for the Well-Being questionnaire total scores (p=0.056, CI=-.137, 8.662) (Table 3).

Pearson's correlations were used to examine relationships between Ferritin 2 and Groups A, B, and C. There were statistically significant correlations between certain questions and the total score of specific groups. Among those in Group A, the question on life was a significant predictor of ferritin levels with a (p-value= 0.032, CI=1.071, 21.763). This question accounted for 14% of the explained variability.

In Group B, a linear regression determined that Ferritin 2 was statistically significant to predict the question regarding emotional health (p=0.004, CI=-19.266, 4.064). The question on emotional health accounted for the most variability at 31.5%. In Group C, a linear regression determined that there was no correlation to the Well-Being questionnaire responses. There were no linear regressions completed for any following ferritin levels due to the low number of observations.

Discussion

The purpose of this study was to determine the relationship between iron storage and overall well-being in Division I female collegiate athletes. This was done to determine a cost-effective screening method to help identify iron deficiency. The results of this study suggested there was not enough evidence to prove there is a significant relationship between iron storage status and overall well-being in this population. There were some statistically significant correlations seen with ferritin levels and the responses to certain questions within the H10WB representing that aspects of mental well-being could be influenced by an iron deficiency, but for overall well-being it appears to not be a good indicator. Data were divided into groups to account for the fact that those in Group A with an irregular test result received a treatment that could have affected their results in B and C. This data separation allowed for data analysis which provided evidence suggesting potential for a relationship of the H10WB and ferritin levels; however, due to the limited number of low ferritin levels specifically in group B following a treatment, this was not able to be shown. Group C also a small sample size specifically with Ferritin 2. The cross country and basketball teams at JMU are tested on a consistent basis due to a higher presence of ID/IDA in these athletes determined by the team physician, according to JMU protocol. The basketball and cross country teams with the highest percentage of subjects in the sample could account for over half of the subjects (54.2%) (Table 1). The deficiency may be identified at an earlier stage and treated, causing subsequent measurements to be within the normal range.

The results of the present study were also due to the fact that these measures happened over time suggesting a possibility in an effect on life changing factors, such as intensity of coursework as the years progress or the introduction of new school workloads for freshman. The relationship between overall well-being and academic achievement has previously been evaluated due to many different variables in life.[15] This could have an impact on well-being responses for the questionnaire as well.

Previous research has determined a relationship between depression symptoms and anemia.[13] The current study has not reflected these results potentially due to the decrease in cases of IDA among this sample. Fordy et. al. reported that poor mood and cognitive functioning in young adults were not associated with low ferritin levels. There were minor statistically significant relationships between cognitive functioning and low ferritin levels for females less than 5 ng/mL but not enough for functional effects.[16] These findings could also suggest that the negative cognitive effects are not seen until the deficiency is at an advanced stage. Depression and poor cognitive function are still of concern in this population but the present sample does not have cases with as severe IDA as in the study by Fordy et. al. There has been additional research in the past on the effects of ID and IDA on performance, with mixed results.[17] Regardless of those results, if it is affecting their cognitive function it will then influence their performance abilities.

The results we obtained were similar to others who have tested these two variables in different ways.[18,19] As the current study demonstrated, Beck et al. also found that there was also no significant relationship between IDA and self-perceived health, well-being, and fatigue. They reported a history of ID, smoking, and having a medical condition accounted for almost one fifth of the variance of the survey. History of ID in some of the subjects could have also had an effect on the results of the present study. Iron deficiency anemia has been related to symptoms of poor mood, lethargy, and problems sustaining attention.[20] The results of this study might have been affected by a majority of subjects who had normal ferritin levels due to close monitoring and treatment. Many of the studies done discussing this association refer to the changes in the body as a result of IDA.[7,21] Although, the research does not address if these physiological changes are happening in the stage of ID. This suggests that more studies need to be done using a larger sample of those with ID and IDA in separate groups. Due to the results that those with ID do not represent these symptoms, it is very important to find a screening tool so we can identify it before individuals are in a detrimental state of IDA.

Results of the current study reflect previous studies and indicates this questionnaire is not a sufficient screening tool for iron deficiency, but may still be an effective tool to use for someone with iron deficiency anemia. The present questionnaire should be tested with a larger sample of female athletes with IDA to help determine if there is a relationship.

The current research reflects the importance of determining the ID before it progresses to IDA. But the practical implication is that there needs to be a screening tool to identify this deficiency. In all of the studies cited blood draws were used. Many programs and settings for athletes such as high school, smaller colleges, recreationally active, etc. will not have the funds to receive continual blood draws. This testing at one time point as well as routine testing can be very costly to a program and not within reach. In the study by Parks et al. evaluation of the costs associated with athlete wide ferritin screenings estimated US $32.00 per lab draw.[4] They determined out of their sample, the cost to identify one anemic athlete was $1332 for females and to identify one iron-deficient athlete was $204 for females. Lower income institutions will not be able to manage this even yearly for their athletes.[4] The importance of a cost-effective and convenient tool to identify athletes at the ID stage would be very beneficial in many aspects of their lives.

Limitations

The questionnaires that were used, for freshman, were completed at home over the summer; therefore, a subject's parents could have completed it. There was already a set sample, no recruiting. There could be some variability due to the timing of the measurements since the ferritin level and questionnaire score were not always at the same time. At the time of reporting the use of the information being for physical exams could have caused some change in the answers. They may have responded differently for a study if they were worried about exposing a medical issue to their sports medicine professional. We also did not have any documentation of menstrual status in order to keep that variable constant among subjects.

Conclusion

This research contributes to the continued literature on ferritin levels and overall health and well-being, which is necessary to help identify iron deficiency in the early stage. Future research should address the relationship of low ferritin to the risk of Female Athlete Triad as well. This research could also be re-evaluated with a larger sample of subjects with iron deficiency anemia. Current research suggests many physiological changes and disturbances to processes in the body secondary to ID or IDA. The weak relationship between the H10WB scores and ferritin levels found in the present study could suggest that mood disturbances and overall well-being are not present until the individual becomes anemic; therefore, continued research to find sufficient ways to detect ID prior to its progression to IDA is necessary. This is important for those settings which do not have the funds or resources to do large screenings for their clients.

Table 1. Demographic information for Division 1 collegiate female athletes including, age, height, weight, BMI, menses history, history of stress fracture, history of eating disorder, history of low BMD, and sport participated

Measurement	n=	Result
Age	168	19.5 ± 2.5
Height(m)	165	1.725 ± .205
Weight(kg)	166	93.65± 49.85
BMI(kg/m^2)	165	26.9 ± 8.8
Menses last 3 months	164	91.5% yes
Menses last 12 months	166	100% yes
Hx of stress fracture	166	18.7% yes
Hx of eating disorder	166	1.8% yes
Hx of low BMD	166	0.6% yes
Sport*	168	10.7% (Track), 35.7% (XC), 18.5% (WBB), 12.5% (FH), 4.8% (Lax), 2.4% (VB), 2.4% (Golf), 3.6% (S&D), 2.4% (Tennis), 1.2% (SB), 6% (Soccer)

*Abbreviations: XC= Cross Country; WBB= Women's Basketball; FH= Field Hockey; Lax= Lacrosse; VB= Volleyball; S&D= Swimming and Diving; SB= Softball

Table 2. Pearson's One-Tailed Correlations (r) with variables of Ferritin 1 and H10WB individual questions and total score between groups A, B, and C in Division I collegiate female athletes

Questions	n=	r	Group A	n=	r	Group B	n=	r	Group C
1-life	116	-.043	.322	42	-.045	.388	13	.360	.113
2- environment	116	-.093	.160	42	.117	.231	13	.330	.135
3- emotional health	116	-.062	.253	42	-.166	.146	13	.486	.046
4- relationship	116	-.143	.063	42	-.246	.059	13	.370	.107
5- autonomy	116	-.163	.040	42	-.213	.088	13	.638	.009
6- self-acceptance	116	-.069	.231	42	-.092	.281	13	.501	.041
7- academic	116	-.165	.038	42	.252	.054	13	-.552	.025
8- health	116	-.221	.009	42	.176	.132	13	.243	.212
9- purpose	116	-.041	.332	42	-.063	.345	13	.260	.195
10- growth	116	-.189	.021	42	-.171	.139	13	.259	.197
Total Score	116	-.169	.035	42	-.036	.409	13	.541	.028

Group A= the initial measurement of the H10WB, Group B= secondary H10WB, Group C= tertiary H10WB, r= correlation coefficient
Shaded= significance set at $p<0.05$

Table 3. Simple Linear Regressions of Ferritin 1 and Ferritin 2 with the Total H10WB Score in Division I collegiate female athletes

Group	**Variables Tested**	**n=**	**p-value**	**CI**	**R^2**	**F-stat**	**T-value**
All data	Ferritin 1- Total H10WB	67	.141	-.994, .143	.013	2.185	-1.478
	Ferritin 2- Total H10WB	65	.724	-1.242, .867	.002	.126	-.355
A	Ferritin 1- Total H10WB	116	.070	-1.383,.054	.029	3.356	-1.832
	Ferritin 2- Total H10WB	32	.194	-.735, 3.482	.054	1.766	1.329
B	Ferritin 1- Total H10WB	41	.819	-1.043, .829	.001	.053	-.231
	Ferritin 2- Total H10WB	23	.101	-2.381, .228	.117	2.927	-1.711
C	Ferritin 1-Total H10WB	12	.056	-.137, 8.662	.293	4.548	2.133
	Ferritin 2- Total H10WB	9	.022	.976, 9.435	.502	8.056	2.838

CI= Confidence Interval, R^2= coefficient of determination, F- stat= ratio of the mean regression sum of squared divided by the mean error sum of squares, T-value= coefficient divided by standard error
Shaded= significance set at $p<0.05$

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Appendix A. The Henriques' 10-Item Well-Being Questionnaire(H10WB)

The H10WB

Age (Yrs):____ Sex: Male / Female

Below are a series of ten statements that describe an attribute associated with your life and functioning and then describe the low and high ends of that attribute. Please read each item carefully, and then circle the appropriate number on the scale ranging from one to seven indicating where you fall on that attribute. Respond to the item based on how you have generally felt during the past month. There are no right or wrong answers, so just answer as honestly as you can.

1. Please rate your overall satisfaction with your **life**. An individual with high life satisfaction feels pleased with most major domains, is at peace with the past, and generally feels fulfilled and content. In contrast, someone with low life satisfaction often wishes things were different, experiences problems in several major areas, and often feels dissatisfied, alienated, or unfulfilled.

1. Very low in life satisfaction
2. Low in life satisfaction
3. Somewhat low in life satisfaction
4. Neutral or sometimes high and sometimes low in life satisfaction
5. Somewhat high in life satisfaction
6. High in life satisfaction
7. Very high in life satisfaction

2. Please rate your sense of mastery over the **environment**, which is the degree to which you feel competent to meet the demands of your situation. Individuals high in environmental mastery feel they have the resources and capacities to cope, adjust and adapt to problems, and are not overwhelmed by stress. Those with a low level of environmental mastery may feel powerless to change aspects of their environment with which they are unsatisfied, feel they lack the resources to cope, and are frequently stressed or overwhelmed.

1. Very low in environmental mastery
2. Low in environmental mastery
3. Somewhat low in environmental mastery
4. Neutral or sometimes high and sometimes low
5. Somewhat high in environmental mastery
6. High in environmental mastery
7. Very high in environmental mastery

3. Please rate your degree of **emotional health**. Someone who is functioning well in this domain is able to experience the full range of emotions, is comfortable with their feelings, and generally feels more positive as opposed to negative emotions (i.e., more joy and excitement relative to frustration and anxiety). In contrast, someone who is having trouble in this domain has difficulty in effectively connecting with their emotions, often feels overwhelmed or afraid of their emotions, and tends to feel more negative than positive emotions.

1. Very low in emotional health
2. Low in emotional health
3. Somewhat low in emotional health
4. Neutral or sometimes high and sometimes low in emotional health
5. Somewhat high in emotional health
6. High in emotional health
7. Very high in emotional health

4. Please rate the overall quality of your **relationship** with others. An individual with positive relationships feels connected, respected, and well-loved. They can share aspects of themselves, experience intimacy, and usually feel secure in their relations. In contrast, individuals with poor relationships often feel unappreciated, disrespected, unloved, disconnected, hostile, rejected, or misunderstood. They tend to feel insecure and sometimes alone or distant from others.

1. Very poor relations with others
2. Poor relations with others
3. Somewhat poor relations with others
4. Neutral or sometimes positive and sometimes negative
5. Somewhat positive relationships with others
6. Positive relations with others
7. Very positive relations with others

5. Please rate your sense of **autonomy**. Individuals with high levels of autonomy are independent, self-reliant, can think for themselves, do not have a strong need to conform, and don’t worry too much about what others think about them. In contrast, individuals low in autonomy feel dependent on others, are constantly worried about the opinions of others, are always looking to others for guidance, and feel strong pressures to conform to others’ desires.

1. Very low in autonomy
2. Low in autonomy
3. Somewhat low in autonomy
4. Neutral or sometimes high and sometimes low
5. Somewhat high in autonomy
6. High in autonomy
7. Very high in autonomy

6. Please rate your levels of **self-acceptance**, which refers to the degree positive attitudes you have about yourself, your past behaviors and the choices that you have made. Someone with high self-acceptance is pleased with who they are and accepting of multiple aspects of themselves, both good and bad. In contrast, individuals with low self-acceptance are often self-critical, confused about their identity, and wish they were different in many respects.

1. Very low in self-acceptance
2. Low in self-acceptance
3. Somewhat low in self-acceptance
4. Neutral or sometimes high and sometimes low
5. Somewhat high in self-acceptance
6. High in self-acceptance
7. Very high in self-acceptance

7. Please rate your levels of satisfaction with your **academic** functioning. This refers to how happy you are with your academic performance, what you are learning and your sense that it is preparing you for a fulfilling career. Individuals highly satisfied with their academic functioning are pleased with the grades they get, enjoy the material they are learning and are hopeful about how this is preparing them for future careers they will find fulfilling. In contrast, those dissatisfied with their academic functioning are struggling to get the grades they desire, are frustrated with either what they are learning or their ability to learn the material and are confused, disappointed or anxious about their future career opportunities.

1. Very low in satisfaction with academic functioning
2. Low in satisfaction with academic functioning
3. Somewhat low in satisfaction with academic functioning
4. Neutral or sometimes high and sometimes low in satisfaction with academic functioning
5. Somewhat high in satisfaction with academic functioning
6. High in satisfaction with academic functioning
7. Very high in satisfaction with academic functioning

8. Please rate your levels of satisfaction with your **health** and fitness. This refers to how happy you are with your bodily health and fitness levels. An individual high in health and fitness does not have chronic health problems, is physically fit, and feels comfortable with their bodies and physical functioning. In contrast, a person who is low in health and fitness experiences chronic health problems, does not have healthy eating, sleeping or exercise patterns, or feels deeply dissatisfied with their bodies or physical functioning.

1. Very low in satisfaction with health and fitness
2. Low in satisfaction with health and fitness
3. Somewhat low in satisfaction with health and fitness
4. Neutral or sometimes high and sometimes low in satisfaction health and fitness
5. Somewhat high in satisfaction with health and fitness
6. High in satisfaction with health and fitness
7. Very high in satisfaction with health and fitness

9. Please rate the level of your sense of **purpose** in life. Individual with a high sense of purpose sees their life has having meaning, they work to make a positive difference in the world, and often feel connected to ideas or social movements larger than themselves. Such individuals have a sense that they know what their life is about. Individuals low in this quality often question if there is a larger purpose, do not feel their life makes sense, and attribute no higher meaning or value to life other than the fulfillment of a series of tasks.

1. Very low in sense of purpose
2. Low in sense of purpose
3. Somewhat low in sense of purpose
4. Neutral or sometimes high and sometimes low
5. Somewhat high in sense of purpose
6. High in sense of purpose
7. Very high in sense of purpose

10. Please rate your level of **personal growth**. Individuals with high levels of personal growth see themselves as changing in a positive direction, moving toward their potential, becoming more mature, increasing their self-knowledge, and learning new skills. Individuals low in personal growth feel no sense of change or development, often feel bored and uninterested in life, and lack a sense of improvement over time.

1. Very low in personal growth
2. Low in personal growth
3. Somewhat low in personal growth
4. Neutral or sometimes high and sometimes low
5. Somewhat high in personal growth
6. High in personal growth
7. Very high in personal growth

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