1 Title: Serum ferritin distribution in elite athletes

Abstract

Objectives: It is not uncommon for athletes to be diagnosed with iron deficiency, yet there remains uncertainty whether the prevalence of suboptimal iron status in elite athletes differs from the normal population or warrants routine screening. The purpose of this study is to describe the distribution of serum ferritin (SF) in a cohort of elite athletes.

Design: Retrospective cohort study.

Methods: Electronic health records of 1085 elite adult athletes (570 women, 515 men) from 2012–2017 were examined retrospectively. SF values were compared to published normal population data. The proportion of athletes meeting criterion values for iron deficiency or initiation of treatment was examined.

Results: SF distributions in male athletes were significantly lower than normal males aged 20 to <24 yrs. (χ^2 28.8, $p<0.001$) and aged 24 to <28 yrs. (χ^2 91.9, $p<0.001$). SF status was similar in female athletes and normal women aged 20 to <24 yrs. (χ^2 9.5, $p>0.05$) or aged 24 to <28 yrs. (χ^2 11.5, $p>0.05$). Using 35 ng/ml as the criterion value for stage one iron deficiency, 15% of male athletes and 52% of female athletes displayed suboptimal iron status.

Conclusions: Male athletes have a significantly lower population distribution of SF values as compared to normative data on healthy males, with 15% of male athletes having suboptimal SF status. The distribution of SF values in elite female athletes did not differ from population values, however approximately half women athletes were iron deficient. These data suggest that iron screening should be considered in both male and female athlete populations.

Keywords: iron supplementation, iron deficiency, exercise, sex differences

Practical Implications

- Male elite athletes have significantly lower serum ferritin status than healthy male non-athletes, with up to 15% of elite male athletes meeting suboptimal iron status thresholds.
- Female athletes and non-athletes have a high prevalence of suboptimal iron status.
- Routine screening for iron status should be considered in both male and female athlete populations.

Introduction

The most prevalent nutritional disorder, even in the developed world, is iron deficiency. [1] Athletes are not immune to this condition, and it is not uncommon for athletes to be diagnosed with iron deficiency, even with hemoglobin and hematocrit values that fall within clinically normal population ranges. [2, 3] Athletes may experience both training-mediated iron loss and impaired iron absorption – in excess of the normal, untrained population - through factors including hemolysis, hematuria, sweating, gastrointestinal bleeding, altered dietary regimens, and downstream effects of pro-inflammatory cytokines resulting in hepcidin mediated changes in iron movement and metabolism. [3-6]

Many researchers and clinicians in sports medicine practice believe, based on their experience, that normative blood chemistry values, particularly for serum ferritin (SF), within trained athletes may be substantially different than population norms. [7] This belief is supported, in part, by studies suggesting an increased iron demand with physiological adaptations to training in athletes, and thus higher iron stores may be required in athletes to avoid an impaired training response. [8] Several threshold levels of SF for treatment interventions with athletes appear in the literature, which target higher than normal clinical ranges. [2, 9-11] However, the basis for these recommendations is unclear, as no data exists establishing whether the population distribution of iron stores in elite adult athletes differs from the non-athlete population. In fact, in a literature search for SF values in elite adult athletes, the largest cohort included only 123 men and 174 women [12], too few to draw conclusions about normative values in athletes.

It is important to understand normative iron levels in the elite athlete population, as there is uncertainty as to whether the prevalence of suboptimal iron status in elite athletes warrants routine screening. [13, 14] Guidelines for the periodic screening of elite athletes suggest clinicians consider iron screening in women, where there is a high prevalence of low iron status. [3] However, in male athletes, there is a historical perspective of low yield and lack of perceived benefit for iron screening. [12, 15, 16] While many authors conclude that SF screening is reasonable for both men and women [7, 17, 18], others believe routine iron screening should not be performed.[14, 19] These conflicting recommendations may be better informed through improved data on the normal distribution of iron status in athlete populations.

The purpose of this descriptive study was to determine the distribution of SF measures in a large cohort of elite athletes, for comparison to the normal, non-athletic population. Utilizing data from >1000 athletes training at United States Olympic Training Centers, this data set is unique in its size and the elite athlete nature of the cohort. We hypothesized the distribution of SF measures in elite athletes would differ significantly from non-athletes for both sexes. Ultimately, this data can be used to inform the decision of whether to screen for iron status in elite athlete populations.

Methods

This was a retrospective cohort study reported using STROBE guidelines. [20] A retrospective analysis of de-identified medical records from the United States Olympic Committee (USOC) electronic health record (EHR) (Centricity, GE, Chicago, IL) was performed for all laboratory studies completed on adult athletes over a five-year period from 2012 to 2017. Athletes in the USOC EHR include US athletes training and competing for the Olympics or Paralympics under the USOC Sports Performance Division. Therefore, all athletes were defined as "elite" through objective or subjective criteria used by the USOC and/or the athlete's US national governing bodies for sport. All athletes gave consent for evaluation and treatment. This project was approved by the institutional review board of Southern California University of Health Sciences.

Blood collection occurred at hospitals and clinical laboratories associated with the USOC and US Olympic Training Centers in Colorado Springs, Chula Vista and Lake Placid. Blood was collected in evacuated serum separation tubes and either processed on site (typically <60 min from the time of collection) or transported to regional clinical laboratories, where the samples were typically processed within 12 h of collection. SF was determined by "sandwich" enzyme-linked immunosorbent assay, using commercially available kits (Dimension®, Flex® Reagent Cartridge, Siemens, Malvern, PA; VITROS® Immunodiagnostic Products Ferritin Reagent Pack, Ortho-Clinical Diagnostics, Rochester, NY), which have reported within-calibration coefficients of variation of 1.3–4.1%. Laboratory reliability was not

assessed as part of this study, and standardization and interlaboratory comparability was assumed for the SF assessment.

The EHR database was queried for all laboratory studies performed between 2012-2017 with data analytics software (Tableau, Seattle WA). The specific reason the laboratory blood draw was ordered was not available in the database. However, usual care at USOC Clinics includes the use of laboratory screening of healthy athletes as an assessment of micronutrient status by dietitians, for monitoring of response to training or altitude stimulus by physiologists, and as a health screening or diagnostic tool by sports medicine staff.

Data were converted into comma-separated value format and analyzed using Microsoft Excel and Microsoft R Open (Redmond, WA). Clinical and demographic information included in the query included the SF value on their first study recorded in the database, the athletes' sex and sport, and a randomly generated unique patient identifier.

Distributions of SF results were calculated for each athlete and stratified by sex. For comparisons to normal population values, we utilized the data set of Custer et al. [21] The Custer et al. study represents, to date, the most thorough and largest descriptive investigation of SF to determine "the physiologic range of normalcy and consequently form a basis for a more detailed interpretation of clinical values." From an initial data set of over 900,000 test panels, a subset of >14,000 men and >21,000 women were identified in which 28 laboratory results (exclusive of the SF level) were within limits that approximated conventional reference ranges. Custer et al. stratified the SF data into four-year age bins; therefore, SF distributions from the 20 to <24 yr. old age group (720 men, 1711 women) and 24 to <28 yr. old age group (1085 men, 2175 women) were compared to the elite athlete distributions, as these age ranges best match that of the elite athlete cohort. Additionally, Custer et al. reported data in percentile bins of 2.5%, 15.9%, 25%, 50%, 75%, 84.1%, and 97.5%. Distribution graphs and tables using these percentiles were created for each sex, using the quantile function in Microsoft R, which uses linear interpolation when there is not an exact value in the dataset at the specified percentile. Counts in distribution bins were compared using Pearson's chi-square test for independence.

To facilitate discussion of an appropriate criterion value for SF for commencing treatment, we calculated the percentages of athletes and the normal population who met select thresholds. We selected four threshold values.

1. <12 ng/mL: This SF value has been correlated with depleted bone marrow iron stores [22] and is commonly used as the lower bound of the normal range by clinical testing laboratories. This also is the SF component threshold of stage three iron-deficient anemia, as defined originally by Bothwell et al.[23], and updated by others. [2]
2. <20 ng/mL: This matches the stage two iron-deficient erythropoiesis SF threshold. [2, 23]
3. <35 ng/mL: This matches the stage one iron depletion threshold [2, 23] and the iron deficiency threshold recommended by several other authors for treatment in athletic populations. [24, 25]
4. <50 ng/mL: This matches the threshold recommendation of Custer et al. [21] for men, as well as the SF threshold used for inclusion by many researchers in examining iron treatments for patients presenting with fatigue. [26] This threshold has also been recommended as a minimum level for adult athletes preparing to train at altitude. [18]

Suboptimal iron rates among normal men and women aged 20 to <24 yrs. and 24 to <28 yrs. at 12, 20, 35, and 50 ng/mL were estimated by fitting a second-order polynomial equation for each cohort to log-transformed percentile and SF values from Custer et al. [21] and calculating the proportion of subjects below each threshold. Chi-square tests were used to compare the proportion of each group (athletes, normals 20 to <24y, normals 24 to <28y) at each SF threshold value. The alpha for statistical significance was set at $p<0.05$.

Results

We included results from 1,085 elite athletes (570 women, 53%; 515 men, 47%). A breakdown of athletes by sex and sport can be found in Table 1. Distribution of values for SF by sex, compared to the population data reported by Custer et al., can be seen in Figure 1. In elite athletes, the median SF was 74.0 ng/mL (interquartile range 45.5–112.0 ng/mL) for men and 33.0 ng/mL (interquartile range 30.7–

51.3 ng/mL) for women. In the normal population reported by Custer et al., the median for men age 20 to <24 yrs. (n=720) is 90.2 ng/mL (interquartile range 58.6–131 ng/mL) and for ages 24 to <28 yrs. (n=1085) 105 ng/mL (76.9–172 ng/mL). Similarly, for women aged 20 to <24 yrs. (n=1,711), the published normal population median from Custer et al. was 31.8 ng/mL (interquartile range 18.6 – 52.3 ng/mL) and for women equal to 24 to <28 yrs. (n=2,175) the median was 38.8 ng/mL (22.5–63.4 ng/mL). SF distributions differed between the elite male athletes and both normal men aged 20 to <24 yrs. (χ^2 (7)=28.8, p<0.001) and 24 to <28 (χ^2 (7)=91.9, p<0.001) but not between the elite female athletes and normal women aged 20 to <24 yrs. (χ^2 (7)=9.49, p=0.219) or aged 24 to <28 yrs. (χ^2 (7)=11.5, p=0.118).

SF status at thresholds of 12, 20, 35, and 50 ng/mL in athletes and normal men and women can be found in Table 2, with SF percentiles for each group in Table 3. Elite men athletes had a greater proportion below the 35 ng/mL and 50 ng/mL thresholds compared to normal men aged 20 to <24 yrs. (35 ng/mL: χ^2 (1)=8.19, p=0.004; 50 ng/mL: χ^2 (1)=15.03, p<0.001) and at 20 ng/ml, 35 ng/mL, and 50 ng/mL thresholds compared to normal men aged 24 to <28 yrs. (20 ng/mL: χ^2 (1)=8.53, p=0.003; 35 ng/mL: χ^2 (1)=31.43, p<0.001; 50 ng/mL: χ^2 (1)=58.63, p<0.001). In elite women athletes, there was a greater proportion of athletes below thresholds of 35 ng/mL and 50 ng/mL compared to normal women aged 20 to <24 yrs. (35 ng/mL: χ^2 (1)=4.64, p=0.031; 50 ng/mL: χ^2 (1)=8.55, p=0.003) but not compared to normal women aged 24 to <28 yrs. (35 ng/mL: χ^2 (1)=1.28, p=0.258; 50 ng/mL: χ^2 (1)=2.18, p=0.140).

Discussion

The purpose of this study was to describe SF status in a large cohort of elite athletes and to determine if differences exist in the distribution of SF levels between athletes and the normal, non-athlete population. In our novel population of over 1000 elite US athletes, our data indicate the distribution of SF in elite male athletes was different than the SF distribution within an otherwise normal 20 to <28-yr old US male population, with between 3 and 15% of athletes below the common thresholds of SF <20 ng/ml and <35 ng/ml, respectively. While the SF distribution in elite women athletes was not statistically different from otherwise normal 20 to <28-year old women, the prevalence of iron deficiency (using any

of the commonly utilized criterion levels) is substantial (e.g. ~23% to 52% of the elite athletes displayed SF <20 ng/ml and <35 ng/ml, respectively).

The reason iron status is screened, even in otherwise healthy athletes, is the critical role iron plays in exercise performance. Iron is a core element within hemoglobin, myoglobin, cytochromes and other mitochondrial electron chain proteins important for oxygen utilization.[7, 18] Insufficient iron stores reduce O_2-carrying capacity to working skeletal muscles [27], and there are well established links between iron levels, total hemoglobin mass, maximal oxygen uptake, and aerobic exercise performance. [7] Iron deficiency prevents erythropoiesis in response to erythropoietic stimulating agents, both in clinical populations (e.g. dialysis patients) [28] and athletes training at altitude [24], and interestingly, iron depletion even without anemia worsens exercise performance. [29] It is for these reasons (and perhaps others not listed) that iron screening is often utilized in the diagnosis of athletic performance decline, as well as with athlete screening at routine intervals, even in the absence of athletic performance issues.

Previous studies have examined iron deficiency in youth, collegiate, and elite athletes with outcomes and author opinions both in support [7, 17, 18] and against [14, 19] routine iron screening. By definition, screening tests are used to "determine whether an asymptomatic individual has an undetected disease or condition". [30] In the decision-making process, clinicians must weigh the cost of a diagnostic test (e.g. patient burden, health risk, monetary expense) against the probative value of the test. It has been suggested by Herman [30], writing in the Ethics Journal of the American Medical Association, that the two major objectives of a good screening program are: 1)- detection of disease at a stage when treatment can be more effective than it would be after the patient develops signs and symptoms, and 2)- identification of risk factors that increase the likelihood of developing the disease and use of this knowledge to prevent or lessen the disease by modifying the risk factors. While iron deficiency is not a disease, for the elite athlete, it does affect an important quality of life outcome: Athletic performance. The cost to support an elite athlete can be substantial, from direct financial costs such as athlete salary, coaching and support staff salaries, travel, equipment, and university scholarships, to opportunity costs such as hours of training and time away from family. [13]

Our data demonstrate the distribution of SF levels in elite men athletes is significantly lower than the normal population of 20-28 year old men, a finding that challenges past recommendations that iron screening is not warranted in this population. [14-16] Intense physical training increases iron metabolism, which can shift iron balance towards deficiency [25], so this outcome is not surprising. Utilizing the second criterion of Herman [30], we believe iron screening in athletic cohorts of both sexes is justified, as being an actively training athlete and being a woman are both risk factors for developing iron deficiency. [7, 18] The task then becomes satisfying Herman's first criterion, specifically to determine what SF level is the appropriate threshold for early detection of iron deficiency and treatment intervention.

We believe the data from our athlete population can be used as normative data for clinicians developing screening programs, regardless of the threshold they prefer. This is useful, as a universal criterion level for SF to denote iron deficiency and for instigating iron supplementation in athletes remains a point of debate among researchers and clinicians. [2, 7, 9-11, 18] Ultimately, in the absence of guidelines from established clinical societies or universally accepted position papers, it remains up to the clinician to determine what SF level should trigger treatment interventions for their patients/athletes. For example, in an inquiry to 26 sports medicine facilities in Germany [25], the lower limit of SF for intervention in women athletes ranged from values <15 ng/mL (7% of clinics), 15–25 ng/mL (43% of clinics), 26–35 ng/mL (28% of clinics) to >35 ng/mL (21% of clinics). In the same inquiry, the lower SF limit for intervention in male athletes ranged from <20 ng/mL to >40 ng/mL (21% and 14% of clinics, respectively), with other criterion values between.

To aid the discussion, we determined iron deficiency prevalence rates at four different SF criterion levels suggested in the literature as thresholds for iron supplementation treatment (table 2), from 12 ng/mL to 50 ng/mL. For example, at <35 ng/mL, 82 out of 515 men athletes (15%) were identified as meeting the criteria for stage-one iron deficiency. By comparison, at roughly the same percentile (15.9%) in the normal men population of Custer et al., SF values were significantly different than the athlete cohort (46.7 and 53.5 ng/mL for ages 20 to <24 and 24 to <28 yrs. respectively). Contrary to our hypothesis, the distribution of SF values in our elite female athletes is nearly identical to the normal

population (Figure 1); however, over 50% of elite women athletes met the <35 ng/mL criterion threshold for stage-one iron deficiency. Additionally, SF is an acute phase reactant, which causes SF to increase disproportionately to actual bone marrow iron stores. Therefore, we would anticipate athletes to have some level of training-induced inflammation, which can inflate SF measures and decrease the number of athletes identified with suboptimal iron status.

While the reason for the blood draw was not recorded in the USOC EHR database, in the USOC clinical setting it is common for athlete labs to be ordered as part of training camps or on a routine basis for wellness screening. It is possible that some of the athletes in the population tested may have been unhealthy at the time of the blood draw. Athletes may have had previous blood draws as part of their overall health care or wellness screening and may have been supplemented at the time of the blood draw in this data set. Similarly, dietary intake of iron from food or any iron supplementation routine at the time of blood sampling was not recorded. We did not measure transferrin saturation values, which is one of three criteria (along with SF and hemoglobin concentration) used to categorize the three stages of iron deficiency [2, 23] and may provide the clinician with additive information on which to base treatment decisions.

Conclusion

While it is established that chronic exercise training in athletes places considerable stress on multiple factors affecting iron uptake, storage and loss, it previously has not been established whether the distribution of SF levels in athletes and normal individuals are similar or different. Our SF data from the largest elite athlete cohort to appear in the literature indicates that the SF distribution is different between elite athletes and normal men. While there was no difference in the SF distribution between elite athlete and normal women, a substantial portion of both groups can be considered iron deficient. Our findings suggest that routine iron screening should be recommended in both male and female athlete populations.

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Conflicts of Interest

The authors declare no conflicts of interest. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Figure Legends

Figure 1. (top) Distribution of serum ferritin by sex. N = number of subjects. (bottom) Cumulative probability plots of serum ferritin values in cohorts of men and women athletes and normal subjects of differing age groups. Note log scaling of the X-axis. Solid lines represent least squares regression. Values for normal subjects are taken from Custer et al. [20]