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Sex differences in iron status during military training: a prospective cohort study of longitudinal changes and associations with endurance performance and musculoskeletal outcomes

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Key Words: Bone; Endurance; Musculoskeletal Injury; Nutrition.

Abstract

This study investigated sex differences in iron status, and associations between iron status and endurance and musculoskeletal outcomes, in military training. 2,277 British Army trainees (581 women) participated. Iron markers and endurance performance (2.4 km run) were measured at the start (week 1) and end (week 13) of training. Whole-body areal body mineral density (aBMD) and markers of bone metabolism were measured at week 1. Injuries during training were recorded. Training decreased haemoglobin in men and women (mean change [95% CI], −0.1 [−0.2, −0.0] and −0.7 [−0.9, −0.6] $g \cdot dL^{-1}$, both $p < 0.001$), but more so in women ($p < 0.001$). Ferritin decreased in men and women (−27 [−28, −23] and −5 [−8, −1] ug·L, both $p \leq 0.001$), but more so in men ($p < 0.001$). sTfR increased in men and women (2.9 [2.3, 3.6] and 3.8 [2.7, 4.9] nmol·L, both $p < 0.001$), with no difference between sexes ($p = 0.872$). RDW increased in men (0.3 [0.2, 0.4]%, $p < 0.001$), but not women (0.1 [−0.1, 0.2]%, $p = 0.956$). MCV decreased in men (−1.5 [−1.8, −1.1] fL, $p < 0.001$), but not women (0.4 [−0.4, 1.3] fL, $p = 0.087$). Lower ferritin was associated with slower 2.4 km run time ($p = 0.018$), sustaining a lower limb overuse injury ($p = 0.048$), lower aBMD ($p = 0.021$), and higher βCTX and P1NP (both $p < 0.001$) controlling for sex. Improving iron stores before training may protect haemoglobin in women and improve endurance and protect against injury.

Key Words: Bone; Endurance; Musculoskeletal Injury; Nutrition.

Introduction

Iron is a trace element that contributes to normal physiological function by incorporation into enzymes—including those involved in energetic metabolic pathways—and proteins involved in oxygen transport—including haemoglobin (> 65% total body iron) (1). Iron is also a mineral component of bone and contributes to the synthesis of collagen (2,3), a significant component of musculoskeletal tissues. Iron status is determined by the measurement of a combination of blood biochemical markers including ferritin, transferrin saturation, soluble transferrin receptor (sTfR), erythrocyte distribution width (RDW), mean corpuscular volume (MCV), and haemoglobin (4–7). Iron deficiency is defined as low iron stores before haemoglobin levels are affected and iron deficiency anaemia is defined as low iron stores and low haemoglobin (1,6). There are several criteria for defining iron deficiency (low iron stores), which complicates our interpretation of iron deficiency and its effect on health and performance. The World Health Organization define depleted iron stores as ferritin < 15 $\mu g \cdot dL^{-1}$ for men and women, and anaemia as haemoglobin < 12 $g \cdot dL^{-1}$ for women and < 13 $g \cdot dL^{-1}$ for men (7). Suboptimal iron status leads to poorer endurance and cognitive performance, lethargy and fatigue, and impaired aerobic adaptations to training (6), and may contribute to poorer musculoskeletal outcomes including poorer vitamin D status (2), lower bone mineral density (2,3), and increased injury risk (8,9).

There are well described differences in iron status between men and women; more women than men have iron deficiency and iron deficiency anaemia in the general (7,10) and military (11) populations. Premenopausal women are at high risk of iron deficiency due to menstrual blood loss and insufficient dietary iron intake to meet these increased iron requirements from menstrual bleeding (6,12). Therefore, poor iron status may play a more important role in the health and performance of women than men, particularly in environments where iron intake

and iron stores are challenged (3). Basic military training diminishes iron status in women (5,13–15) with better iron status associated with better endurance performance (13,14). Similar observations have been made in men (16–18), but there are little data comparing the effect of military training in men and women. A study of US Army basic training found military training degraded iron status to a greater extent in women compared with men (19), but these data were on a relative small sample and no study has studied sex differences in the effect of training on iron status in a UK Armed Forces population or examined the association between iron status and measures of musculoskeletal health. Women are now fully integrated into all roles in the UK and US Armed Forces and operate alongside men in the most arduous roles. Understanding sex differences in iron status is essential in optimising health and performance of both sexes. Women experience higher physical demands (20), more musculoskeletal injuries (21), and consume less iron despite higher daily requirements (22) than men in military training. Iron status in male and female British Army recruits and it's association with endurance performance and musculoskeletal outcomes will provide important insight into methods to protect health and performance.

The primary aim of this study was to examine sex differences in changes in iron status during British Army basic military training. The secondary aims were to explore associations between markers of iron status and endurance performance, musculoskeletal injury incidence, areal bone mineral density, vitamin D status, and biochemical markers of bone metabolism. We hypothesised that iron status deteriorates during military training to a greater extent in women than men. We also hypothesised that markers of iron status would be associated with endurance performance and musculoskeletal outcomes.

Methods

71 *Participants*

72 The study was advertised to new British Army trainees from April 2013 to July 2017 during
73 week one of their basic training courses. Participants were recruited from three British Army
74 training populations: male infantry recruits at Infantry Training Centre, Catterick; standard
75 entry (non-infantry and non-officer) female recruits at Army Training Centre, Pirbright; and
76 male and female officer cadets at Royal Military Academy, Sandhurst, providing a
77 representative sample of all individuals commencing British Army basic training. All
78 participants passed an initial military medical assessment and were confirmed to be injury free
79 and not have any medical condition that precluded military service. This study was conducted
80 according to the guidelines laid down in the Declaration of Helsinki and all procedures were
81 approved by the Ministry of Defence Research Ethics Committee (ref: 165/Gen/10). Each
82 participant had the study procedures and risks fully explained verbally and in writing. Written
83 informed consent was obtained from all participants.

84

85 *Experimental Design*

86 This study was an observational prospective cohort study. These data were secondary analyses
87 as part of a larger study exploring micronutrient deficiencies and health and performance
88 outcomes (23–25). Venous blood samples were drawn at the start (week 1) and end (week 13)
89 of each basic military training course for the analysis of biochemical markers of iron status,
90 vitamin D status, and biochemical markers of bone metabolism. Endurance performance was
91 assessed by a maximal effort 2.4 km run at the same time points. Body mass, height, and whole-
92 body areal bone mineral density measured by dual-energy X-ray absorptiometry (DXA) were
93 recorded at week 1. Participants self-reported their alcohol intake, use of iron supplementation,
94 smoking habits, and stress fracture history using questionnaires at week 1. Week 1
95 measurements were made following the initial medical assessment and before military training

commenced. Male infantry recruits completed the 26-week British Army infantry basic training course or the 28-week British Army parachute regiment course. Standard entry female recruits completed the 14-week British Army soldier basic military training course. The officer cadets completed the 44-week officer commissioning course. The first 14 weeks of each British Army basic military training course is similar between training sites and intended to develop basic military skills and physical fitness. The 14-week training programme involves periods of aerobic endurance training, strength and conditioning, military-specific fitness training (obstacle course, circuit training), military drill, progressive loaded marching, and basic military skills (field exercise, weapon handling). Week 14 involves a decrease in typical military activities and an increase in the administrative burden as trainees prepare to complete the basic training component of their course; post-training measurements were, therefore, taken in week 13. Participants' medical records were accessed to obtain a record of clinician-diagnosed lower limb overuse injuries and lower limb stress fractures (including hip/pelvis) during the first 14 weeks of training; lower limb stress fractures were recorded separately from lower limb overuse injuries.

Blood Collection and Biochemical Analyses

A venous blood sample was collected either in the morning (~0900 to 1100 h) after breakfast (0600 to 0700 h), or early afternoon (~1300 to 1500 h) after lunch (1200 to 1300h). Follow-up measurements were taken at approximately the same time of day. Venous blood was withdrawn from a vein in the antecubital fossa and collected in serum and EDTA BD Vacutainer® tubes (Becton Dickinson, New Jersey, USA). Serum samples were left to clot for 1 hour at room temperature. Haemoglobin, RDW, and MCV were measured in EDTA whole blood within 30 min of collection using the COULTER A^{C}·T diff 2 Analyzer (Beckman Coulter, California, USA). Blood samples were centrifuged at 1500 *g* and 4°C for 10 min before serum and plasma

were separated into universal tubes and stored at −80°C until analysis. Plasma procollagen type 1 N-terminal propeptide (PINP), c-telopeptide cross-links of type 1 collagen (βCTX), intact parathyroid hormone (PTH), and serum ferritin were analysed by electro-chemiluminescence immunoassays (ECLIA) on the COBAS c601 (Roche Diagnostics, Mannheim, Germany) platform. PINP inter-assay coefficient of variation (CV) was < 3% between 20 and 600 $\mu g \cdot L^{-1}$ with a sensitivity of 8 $\mu g \cdot L^{-1}$. βCTX inter-assay CV was < 3% between 200 and 150 $\mu g \cdot L^{-1}$ with a sensitivity of 10 $ng \cdot L^{-1}$. PTH inter-assay CV was < 3.8% between 1.2 and 5000.0 $pg \cdot mL^{-1}$. Ferritin inter-assay CV was < 4.2% between 0.5 and 2000.0 $\mu g \cdot L^{-1}$. Serum sTfR was measured by immunoturbidimetric assays performed on the COBAS c501 analyser (Roche Diagnostics, Mannheim, Germany). sTfR inter-assay CV was < 6.0% between 5.9 and 472.0 $nmol \cdot L^{-1}$. Serum samples were analysed for total 25-hydroxyvitamin-D (25(OH)D) by liquid chromatography tandem mass spectrometry (26). The 25(OH)D3 and 25(OH)D2 assays were calibrated using the National Institute of Science and Technology standard reference material SRM972a. Total 25(OH)D was calculated from the sum of 25(OH)D3 and 25(OH)D2. Total 25(OH)D inter-assay CV was < 8.5% between 0.1 and 200.0 $nmol.L^{-1}$. All biochemical analyses (excluding haemoglobin, RDW, and MCV analyses) were undertaken by the Good Clinical Laboratory Practice and Vitamin D Exernal Quality Assessment Scheme (DEQAS) certified Bioanalytical Facility at the University of East Anglia, Norwich, UK.

Endurance Performance

Endurance performance was assessed as the time to complete a maximal effort 2.4 km run on a standardised running course at each training site. Participants completed an 800 m warm-up and completion time was recorded to the nearest second. The time to complete a 2.4 km run is indicative of maximal aerobic capacity (27) and is a military field test assessed during selection,

training, and throughout a military career. All participants were accustomed to performing this test from selection, before commencing military training.

Whole-Body Areal Bone Mineral Density

Whole-body areal bone mineral density was assessed by DXA (Lunar iDXA, GE Healthcare, Buckinghamshire, UK), with men wearing underwear and women wearing light clothing. Scans were not performed on men and women at the Royal Military Academy, Sandhurst due to lack of scanner availability at that site.

Statistical Analyses

These data were secondary analyses (23–25) and so no a priori sample size was calcualed for iron markers. The lowest number of follow-up measurements was for RDW for women (120 observations). Based on a two group sample size of 240, the smallest effect size we could detect was a partial eta-squared (η_p^2) of 0.01 with a power of 80% and alpha of 0.05. All data were analysed using the R programming language (*v.*4.2.2). Distribution of the demographic and anthropometric data was checked using frequency distribution histograms. Participant demographics and anthropometrics at week 1 were compared between men and women with independent samples *t*-tests or a Welch's *t*-test for groups with unequal variances. The number of men and women using iron supplements was compared using a chi-squared test. Linear mixed effect models with restricted maximum likelihood estimation were used to compare changes in markers of iron status (haemoglobin, ferritin, sTfR, RDW, and MCV) and 2.4 km run time in men and women during training (*lme4 package v.*1.1.31). Sex (men *vs* women), time (week 1 *vs* week 13), and their interaction were included as fixed effects to examine sex differences. Random intercepts were assigned to each participant to account for within-participant correlation for repeated measures. Significance of the fixed effects from each model

were determined with Sattherwaite degrees of freedom (*lmerTest package v*.3.1.3). Variance and normality of the residuals for each model were checked visually by plotting the residuals against the fitted values and from Q-Q plots. Data were log transformed for models where the residuals seriously violated these assumptions. P values were corrected with the Holm-Bonferroni method (n = 5 p values for five iron status outcomes). In the event of a significant interaction, pairwise comparisons with Holm-Bonferroni corrections and Kerward-Roger degrees of freedom were used on the linear mixed effects model to identify differences between time points within each sex and differences between each sex within each time point (*emmeans package v*.1.8.3). Pooled data were used for main effects when there was no significant interaction. Effect sizes are presented as η_p^2 for main and interaction effects, Cohen's d for between-group comparisons, and paired Cohen's d for within-group paired comparisons (*effectsize package v*.0.8.2).

Simple linear regression was used to test the associations between each marker of iron status and 2.4 km run time at both week 1 and week 13 in men and women separately. P values were corrected with the Holm-Bonferroni method (n = 5 p values for five iron status outcomes at each time point). Multiple linear regression was used to test the association between each marker of iron status and 2.4 km run time at week 1 controlling for age, sex, body mass index, smoking, and alcohol intake (23). Multiple linear regression was used to test the association between absolute change in each marker of iron status with absolute change in run time controlling for that respective iron marker and run time at week 1. Binary logistic regression was performed to assess the association between injury (lower limb overuse injury or lower limb stress fracture) and each marker of iron status controlling for sex, BMI, 2.4 km run time, total 25(OH)D, smoking, stress fracture history, and Army training course (infantry, standard entry, or officer). Army training type was included to account for differences in training site,

equipment, and other practices that may contribute to differences in musculoskeletal injury incidence. Multiple linear regression was used to test the association between each marker of iron status and musculoskeletal outcomes (whole-body areal bone mineral density, total 25(OH)D, PTH, βCTX, and P1NP) controlling for sex and body mass index. Each marker of iron status was entered separately into each multiple linear regression and binary logistic regression model (five models per outcome). Variance and normality of the residuals for simple and multiple linear models were checked visually by plotting the residuals against the fitted values and from Q-Q plots. Data were log transformed for models where the residuals seriously violated these assumptions. Figures were drawn in the *ggplot2 package* (*v.*3.4.0). Significance was accepted as $p \leq 0.05$.

Results

Participants

2,277 British Army trainees (1,696 men and 581 women, Table 1) volunteered to participate. Men were heavier ($p < 0.001$) and taller ($p < 0.001$) than women, but age was not different between sexes ($p = 0.933$); more women than men took an iron supplement ($p = 0.023$) (Table 1). A total of 1,049 (720 men and 329 women) completed week 13 testing (Figure 1).

The Effect of Training on Iron Status

Biochemical markers of iron status are presented in Figure 2 with mean absolute changes presented in Table 2. Examination of the residuals showed that ferritin, sTfR, and MCV errors, had heteroscedasticity and long-tailed distributions and so results are reported for log transformed data. There was a sex × time interaction for haemoglobin ($p < 0.001$, $\eta_p^2 = 0.094$). Post-hoc pairwise comparisons revealed that haemoglobin decreased in men ($p < 0.001$, $d_z = 0.16$) and women ($p < 0.001$, $d_z = 0.76$) with the decrease greater in women. Haemoglobin was

higher in men than women at week 1 ($p < 0.001$, $d = 1.25$) and week 13 ($p < 0.001$, $d = 2.06$). There was a sex × time interaction for log ferritin ($p < 0.001$, $\eta_p^2 = 0.042$). Post-hoc pairwise comparisons revealed that log ferritin decreased in men ($p < 0.001$, $d_z = 0.74$) and women ($p = 0.001$, $d_z = 0.15$) with a greater decrease in men. Log ferritin was higher in men than women at week 1 ($p < 0.001$, $d = 1.33$) and week 13 ($p < 0.001$, $d = 0.96$). There was no sex × time interaction for log sTfR ($p = 0.873$, $\eta_p^2 < 0.001$), but training increased log sTfR (main effect of time, $p < 0.001$, $\eta_p^2 = 0.062$) and log sTfR was higher in women than men (main effect of sex, $p < 0.001$, $\eta_p^2 = 0.035$). There was a sex × time interaction for RDW ($p < 0.001$, $\eta_p^2 = 0.026$). Post-hoc pairwise comparisons revealed that RDW increased in men ($p < 0.001$, $d_z = 0.48$), but not women ($p = 0.956$, $d_z = 0.08$). RDW was not different between men and women at week 1 ($p = 0.194$, $d = 0.09$), but was higher in men than women at week 13 ($p = 0.003$, $d = 0.31$). There was a sex × time interaction for log MCV ($p < 0.001$, $\eta_p^2 = 0.031$). Post-hoc pairwise comparisons revealed that log MCV decreased in men ($p < 0.001$, $d_z = 0.40$), but not women ($p = 0.087$, $d_z = 0.08$). Log MCV was not different between men and women at week 1 ($p < 0.001$, $d = 0.12$), but was lower in men than women at week 13 ($p < 0.001$, $d = 0.52$).

The Effect of Training on Endurance Performance

There was no sex × time interaction for 2.4 km run time ($p = 0.125$, $\eta_p^2 = 0.003$), but training decreased 2.4 km run time (main effect of time, $p < 0.001$, $\eta_p^2 = 0.371$) and 2.4 km run time was faster in men than women (main effect of sex, $p < 0.001$, $\eta_p^2 = 0.431$) (Figure 3, Table 2).

Associations Between Iron Status and Endurance Performance

Simple linear regressions between markers of iron status and 2.4 km run time are presented in Figure 4. Examination of the residuals showed that ferritin and sTfR errors had heteroscedasticity and long-tailed distributions and so simple linear regression results are

reported for log transformed data. There was no evidence of associations between haemoglobin or RDW and 2.4 km run time at either time-point in men or women ($r \leq 0.07$, $p \geq 0.470$). There was a significant negative relationship between log ferritin and 2.4 km run time at week 13 in men only ($r = -0.15$ [−0.24, −0.07], $p < 0.001$), but no evidence of an association between log ferritin and 2.4 km run time at week 1 in or at either time-point in women ($r \leq 0.05$, $p \geq 0.224$). There was a significant negative association between log sTfR receptor and 2.4 km run time at week 13 in men only ($r = -0.13$ [−0.21, −0.05], $p = 0.009$), but no evidence of an association between log sTfR and 2.4 km run time at week 1 in men or at either time-point in women ($r \leq 0.11$, $p \geq 0.096$). There were negative associations between MCV and 2.4 km run time in men at week 1 ($r = -0.26$ [−0.32, −0.20], $p < 0.001$) and week 13 ($r = -0.31$ [−0.41, −0.20], $p < 0.001$), and in women at week 1 ($r = -0.25$ [−0.36, −0.14], $p < 0.001$) but not week 13 ($r = -0.10$ [−0.28, 0.08], $p = 0.976$). There was a negative association between ferritin and MCV with 2.4 km run time at week 1 and a positive association between log sTfR and 2.4 km run time at week 1, when controlling for age, sex, body mass index, smoking, and alcohol intake; there was no evidence of association between haemoglobin or RDW with 2.4 km run time at week 1 (Table 3). There were positive associations between change in ferritin, sTfR, and MCV with change in 2.4 km run time when controlling for sex, concentrations of the respective marker at week 1, and 2.4 km run time at week 1; we found no evidence of an association between change in haemoglobin and change in RDW and change in 2.4 km run time (Table 4).

Associations Between Iron Status and Musculoskeletal Injury Incidence

Associations between markers of iron status and injury incidence controlling for sex, body mass index, 2.4 km run time, total 25(OH)D, smoking status, previous stress fracture, and training course can be seen in Table 5. The incidence of at least one lower limb overuse injury was 24.1% for men and 34.0% for women (26.5% for both sexes combined) and for lower limb

stress fractures was 2.9% for men and 2.5% for women (2.9% for both sexes combined). There was no evidence of associations between haemoglobin, sTfR, RDW, or MCV at week 1 and developing a lower limb overuse injury or a stress fracture. Lower ferritin at week 1 was associated with developing a lower limb overuse injury, but there was no evidence of ferritin being associated with developing a lower limb stress fracture.

Association Between Iron Status and Bone Mineral Density and Markers of Bone Metabolism

Associations between markers of iron status and areal bone mineral density and markers of bone metabolism controlling for sex and body mass index can be seen in Table 6. There was no evidence of haemoglobin or RDW being associated with areal bone mineral density or markers of bone metabolism at week 1. Higher ferritin was associated with higher areal bone mineral density and lower βCTX and P1NP. Higher sTfR was associated with higher P1NP and higher PTH. Higher MCV was associated with higher total 25(OH)D and lower PTH, βCTX, and P1NP.

Discussion

Training resulted in widespread and sex-specific changes in markers of iron status, indicative of poorer iron status at the end of training in both men and women. Lower iron stores (lower ferritin) was associated with poorer endurance performance, higher musculoskeletal injury incidence, lower whole-body areal bone mineral density, and higher biochemical markers of bone metabolism. Therefore, these data have important implications for managing the health and performance of men and women in the British Army. The large sample size also provides novel insight into sex differences in iron metabolism and associations with musculoskeletal outcomes in young adults.

295 *The Effect of Training on Iron Status*

296 Training resulted in widespread disturbances to iron status; haemoglobin and ferritin decreased,
297 and sTfR increased in men and women. Training also increased RDW and decreased MCV—
298 red blood cell size characteristics—but only in men. These changes in iron markers are all
299 indicative of poorer iron status at the end of military training (13). Haemoglobin is the most
300 abundant of the iron containing [heme-] proteins and is vital for oxygen transport (1). Ferritin
301 reflects iron stores in the liver, spleen, and bone marrow (1), but can be impacted by
302 inflammation, acute phase response, and pathologies, although very few conditions other than
303 iron deficiency decrease ferritin (28). Previous studies of women in basic military training have
304 shown training decreased ferritin and increased sTfR, similar to our data, but increased
305 haemoglobin and RDW (5,13–15). Conversely, we observed a decrease in haemoglobin in both
306 sexes and an increase in RDW in men only. There is evidence of decreased haemoglobin in
307 men and mixed samples of men and women after basic military training courses (16–18),
308 supporting our findings. The data from our study and these previous studies show military
309 training results in poorer iron status, consistent with other data showing poorer iron status in
310 athletes compared with non-athletes (29,30). There are fewer military studies directly
311 comparing men and women, but US Army basic military training decreased ferritin and
312 increased sTfR more in women than men (19); our study builds on these findings by exploring
313 sex differences with a larger sample size and by providing the first data in a UK population
314 undergoing a different military training program. Differences between our study findings and
315 these previous studies could be due to differences in demographics of participants, training
316 length, training modalities, and sample size.

317

318 Training decreased haemoglobin more in women, but decreased ferritin more in men. A
319 decrease in haemoglobin could be due to plasma volume expansion, but the changes in other

markers of iron status support the mechanism is depletion of iron (13). Complete depletion of iron stores can occur before haemoglobin is decreased with low haemoglobin a late phase of iron deficiency (1). Women had lower iron stores (lower ferritin) than men on entry to military training. These lower iron stores could explain why women were more susceptible to developing decreases in haemoglobin, whereas men experienced greater decreases in iron stores (decreases in ferritin), protecting haemoglobin. Decreased MCV in men supports evidence of impaired erythropoiesis, but this decrease in cell size appears due to attenuated macrocytosis (MCV > 100 fL) rather than the development of microcytosis (MCV < 80 fL) with all men with macrocytosis at week 1 in the normal range by week 13. These sex differences could be due to differences in iron status before training rather than any sex difference in response to training. However, several factors may increase the risk of developing iron deficiencies in basic military training for women. The physical demands of military training are typically higher for women than men (20) and so exercise-induced iron losses could be greater for women. Basic military training may increase iron losses through gastrointestinal bleeding, sweat loss, haematuria, haemolysis from ground impact forces and eccentric muscle contraction, and increased inflammation and hepcidin (5,6,13,29–31). Hepcidin is a key regulator of iron status and inhibits iron absorption (31) with the increase in hepcidin with exercise dependent on initial iron status and therefore potentially sex (29). There is also evidence that women consume less iron than men in basic military training despite higher daily requirements (19,22,32). Menstrual bleeding is a primary cause of iron loss in women (12), although the effect of basic military training on menstrual blood loss is not clear (33) and a large proportion of Servicewomen take hormonal contraceptives that stop menstrual bleeding (34). Finally, sex differences in circulating sex steroid concentrations may contribute to sex differences in iron status; sex steroids play a role in erythropoiesis and may influence iron metabolism, with the female sex steroids potentially influencing hepcidin (29).

Associations Between Iron Status and Endurance Performance

Higher ferritin concentrations, higher MCV, and lower sTfR were associated with a faster 2.4 km run time at week 1 in multiple linear regressions controlling for sex and other factors known to influence endurance performance. The 2.4 km run is used as a test of aerobic fitness on entry and in-service in the British Army and is related to maximal oxygen uptake (27), although other factors such as submaximal running economy likely contribute to performance. Low ferritin and high sTfR are early signs of iron deficiency and these data show that better iron stores are associated with better endurance performance; although, the confidence intervals around the coefficients were wide and so the exact estimates are not clear. Visual examination of the sTfR data by sex and week of training (Figure 4) suggests that the sTfR and 2.4 km run time is sex dependent, although this interaction was not formally tested. Iron deficiency and iron deficiency anaemia have different physiological effects with implications for performance; tissue oxidative capacity is impaired with iron deficiency with oxygen carrying capacity reduced with iron deficiency anaemia (6,35). We observed no relationship between haemoglobin and run time, but few people had low haemoglobin so performance may not be impacted at the haemoglobin concentrations observed in our study. Higher levels of tissue iron and increased activity of iron-dependent oxidative enzymes could have contributed to better endurance performance (35), although other mechanisms like less subjective lethargy may contribute (29). Higher sTfR was associated with slower 2.4 km run time (14), and higher RDW and lower haemoglobin were associated with slower 3.2 km run time (13) in basic military training in other nations. These studies did not find evidence of an association between run time and ferritin, but they did not control for other factors known to influence performance. Laboratory studies show ferritin is positively associated with maximal oxygen uptake independently of haemoglobin in women (36). Iron supplementation increases ferritin and

maximal oxygen uptake in those with low ferritin and normal haemoglobin (37), but not all studies show endurance performance improvements when ferritin is increased (31,38). Iron supplementation improved 3.2 km run time in female military recruits, but only those with iron deficiency anaemia (5), with the efficacy of iron supplementation on improving performance likely dependent on starting iron status (39).

The largest improvements in 2.4 km run time were associated with the largest decreases in ferritin, sTfR, and MCV. There is some evidence that better iron status contributes to better training adaptations (6), but these data show greater degradation of iron stores contribute to better aerobic training adaptations. A possible explanation is that those experiencing the highest training load improved performance the most and also had the largest decreases in ferritin. The decrease in MCV was largely due to attenuated macrocytosis (men with MCV >100 fL, Figure 2E) rather than microcytosis and it is not clear if attenuated macrocytosis is beneficial for performance. These data are in contrast to a previous military study showing bigger increases in sTfR change were associated with poorer improvements in 3.2 km run time in women during basic military training (13). The magnitude of the association between change in markers of iron status and change in endurance performance is not clear due to the wide confidence intervals around the coefficients.

Associations Between Iron Status and Musculoskeletal Outcomes

Lower ferritin at the start of training was associated with developing a lower limb overuse injury during training, but the effect was small and there was no evidence of an association between iron status and developing a lower limb stress fracture. Previous military studies have shown no difference in iron status between men who develop a stress fracture and those who do not (18), but there is evidence of an increased prevalence of iron deficiencies in women with

stress fractures than controls (8,9). Low aerobic capacity is a risk factor for musculoskeletal injuries (40) and was associated with iron status, however, we controlled for 2.4 km run time in our analyses and so better fitness is unlikely to explain why better iron stores were associated with lower injury incidence. Iron is a mineral component of bone and contributes to the synthesis of collagen (2,3), a significant component of musculoskeletal tissues. Therefore, iron deficiency could contribute to musculoskeletal injuries through the degradation of musculoskeletal tissues. We observed a positive association between ferritin and whole-body areal bone mineral density—in agreement with studies in animals (41) and dietary intary intake studies in postmenopausal women (42)—supporting a role for iron status in musculoskeletal health. Lower ferritin and MCV were associated with higher rates of bone turnover—higher βCTX and P1NP—which may contribute to the lower whole-body areal bone mineral density. Similar associations between iron markers and bone metabolism have been shown previously (43), and treatment of iron deficiency with iron supplements decreased markers of bone resorption and formation (44). Lower areal bone mineral density and higher markers of bone metabolism with lower ferritin could be due to the effects of reduced iron availability on enzyme activity involved in collagen synthesis, and altered osteoblasts and osteoclasts activity due to reduced prolyl hydroxylase activity and altered cell signalling (2). Higher sTfR was associated with higher PTH and higher MCV was associated with higher total 25(OH)D and lower PTH, so the mechanism could be through the effects of iron deficiency on the PTH-1-alpha-hydroxylase axis (2).

Limitations

This study did not measure circulating concentrations of hepcidin or transferrin, which could have helped further explain some of our findings. Men and women also completed different training courses; however, the first 14 weeks of all basic military training courses are

programmed to be similar. We did not control for plasma volume in our analysis of haemoglobin; however, our other markers of iron status support a decline in iron status as a mechanism for decreased haemoglobin. We did not control for physical activity before the blood sample and it is not clear if acute changes in inflammation influenced our results. The length of our study was also only several months and longer periods of study may be required to detect changes in iron status and for the clinical effects of iron depletion to manifest. Finally, we did not measure iron intake or menstrual bleeding or menstrtual status during training, which may explain the changes we observed in women.

Conclusions

British Army basic training resulted in widespread disturbances to iron status, indicative of poorer iron status at the end of training in both men and women. Men had a larger decrease in iron stores (early-stage iron deficiency) whereas women had a larger decrease in haemoglobin (late stage iron deficiency). Better iron stores were associated with better endurance performance, lower incidence risk of musculoskeletal injury, higher bone mineral density, and lower circulating concentrations of markers of bone metabolism. Increasing iron intake should be a consideration before and during military training to protect health and performance for men and women, but is particularly important for women to reduce the risk of developing iron deficiency anaemia (3).

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446

447 **Conflits of Interest**

448 The authors have no conflicts of interests to declare.

449

450 **Author Contributions**

451 SJ, RMI, NPW, and JPG designed the study. TJO, SJ, NPW, ATC, and SJO collected the data.
452 JCYT and WDF analysed the biochemical samples. TJO produced the manuscript and
453 performed the data analysis. CVC contributed to manuscript writing and data analysis. All
454 authors edited the manuscript and approved the final version.

455

456 **Data Availability**

457 All data are available from the corresponding author pending approval of public release from
458 UK Ministry of Defence.

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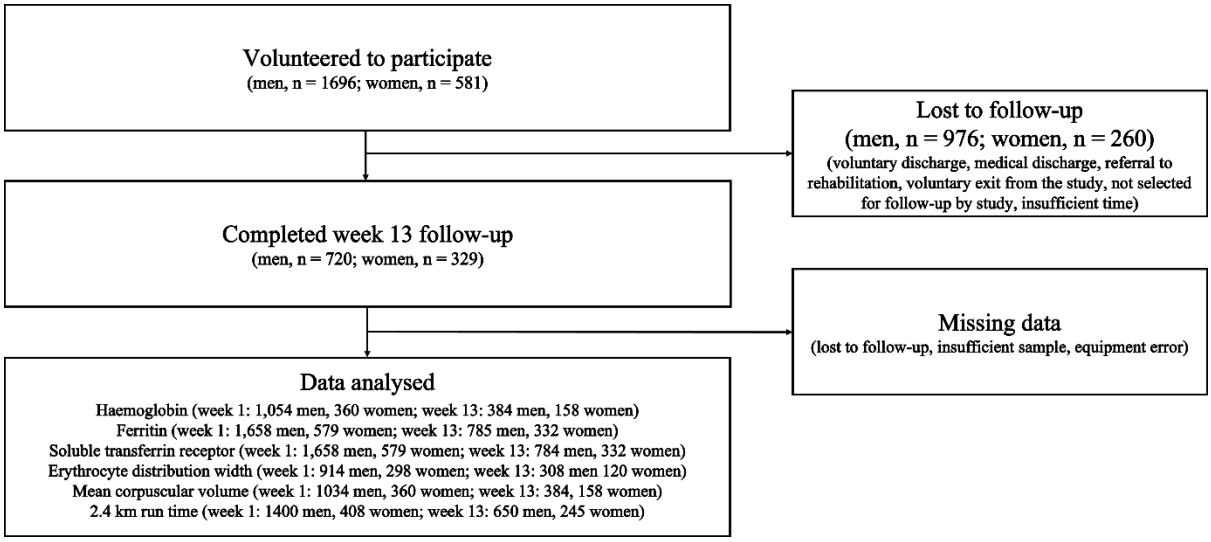

Figure 1. Participant flow through the study.

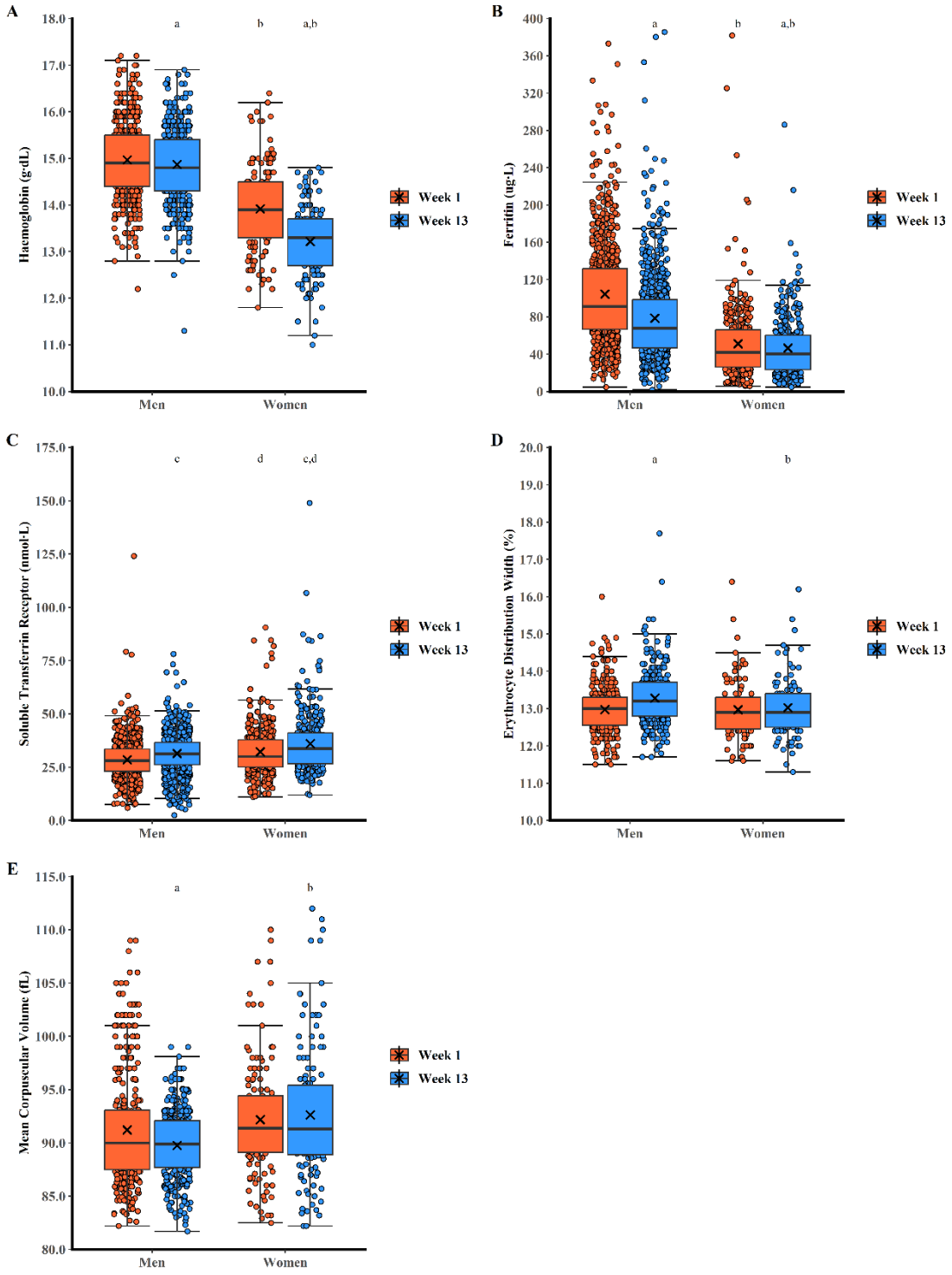

Figure 2. The effect of military training on markers of iron status in men and women. Box plots represent median, interquartile range, and range. Crosses represent mean.

[a]$p < 0.05$ vs week 1 (within sex); [b]$p < 0.05$ vs men at same time-point; [c]$p < 0.05$ vs week 1 (main effect of training); [d]$p < 0.05$ vs men (main effect of sex).

Data are truncated at 80.0 fL for mean corpuscular volume for clarity; three men had a value < 80.0 fL at week 1 and week 13

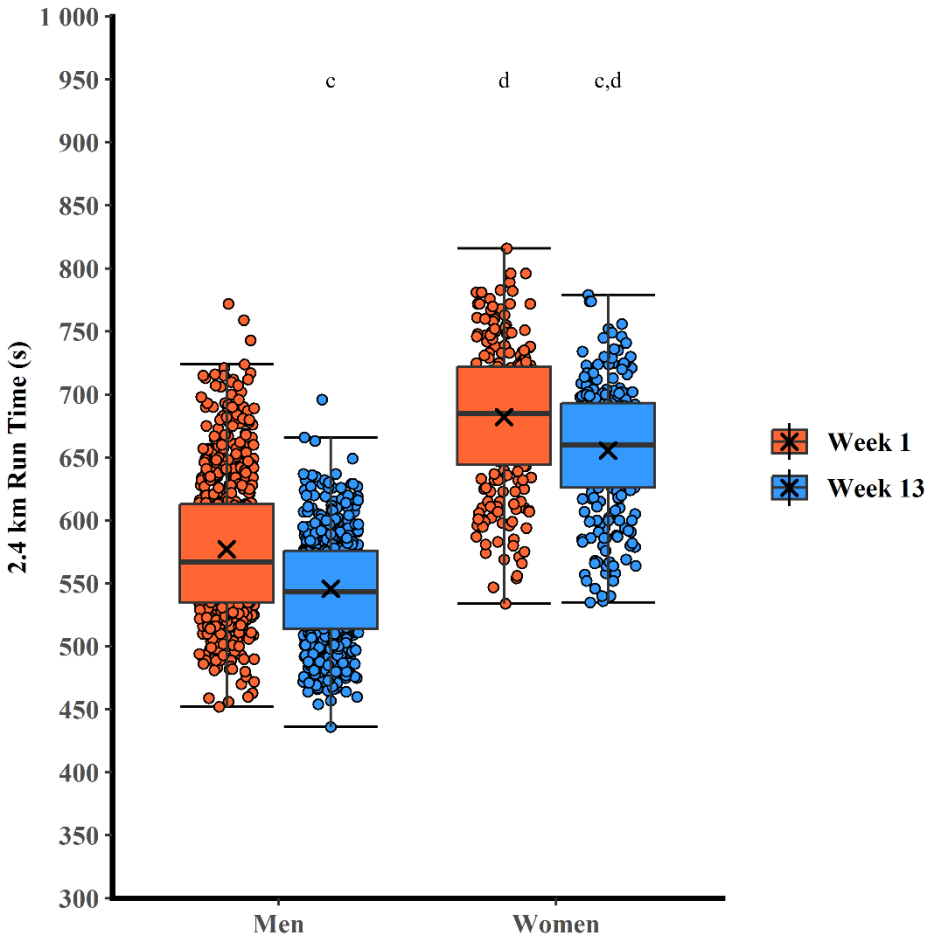

Figure 3. The effect of military training on 2.4 km run time in men and women. Box plots represent median, interquartile range, and range. Crosses represent mean.

[a]$p < 0.05$ vs pre-training (within sex); [b]$p < 0.05$ vs men at same time-point; [c]$p < 0.05$ vs pre-training (main effect of training); [d]$p < 0.05$ vs men (main effect of sex).

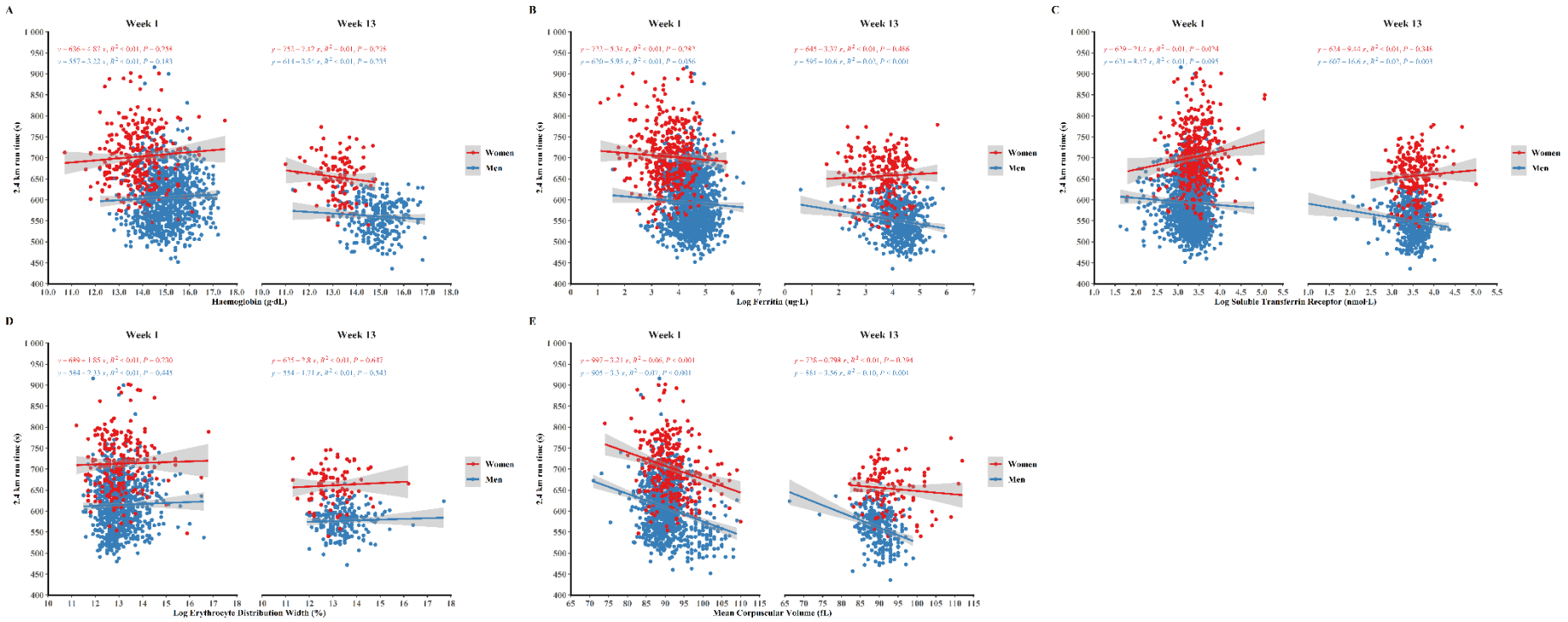

Figure 4. Relationships between markers of iron status and 2.4 km run time at week 1 and week 13 of military training in men and women.

P values are unadjusted for multiple comparisons. Adjusted p values are presented within the text.

Table 1. Participant demographics and anthropometrics. Data are n (%) or mean ± SD.

	Men (n = 1,696)	Women (n = 581)
Training Type		
Infantry	1,293 (76%)	0 (0%)
Standard Entry	0 (0%)	475 (82%)
Officer	403 (24%)	106 (18%)
Age (years)	22 ± 8	22 ± 3
Body Mass (kg)	76.2 ± 9.9	64.8 ± 7.9[a]
Height (m)	1.78 ± 0.06	1.65 ± 0.06[a]
Body Mass Index ($kg \cdot m^2$)	24.1 ± 2.6	23.7 ± 2.3
Taking Iron Supplements	62 (3.8%)	30 (5.4%)[a]

Missing data: Age, 41 men and 4 women; Body Mass, 46 men and 14 women; Height, 46 men and 12 women; Body Mass Index, 46 men and 16 women; Taking Iron Supplements, 61 men and 29 women.

[a]$p < 0.05$ vs men

Table 2. Mean absolute change [95% confidence intervals] in markers of iron status and 2.4 km run time from week 1 to week 13 of military training in men and women.

	Men	Women
Haemoglobin ($g \cdot dL^{-1}$)	−0.1 [−0.2, −0.0]	−0.7 [−0.9, −0.6]
Ferritin ($ug \cdot L^{-1}$)	−27 [−28, −23]	−5 [−8, −1]
Soluble Transferrin Receptor ($nmol \cdot L^{-1}$)	2.9 [2.3, 3.6]	3.8 [2.7, 4.9]
Erythrocyte Distribution Width (%)	0.3 [0.2, 0.4]	0.1 [−0.1, 0.2]
Mean Corpuscular Volume (fL)	−1.5 [−1.8, −1.1]	0.4 [−0.4, 1.3]
2.4 km run time (s)	−31 [−34, −39]	−27 [−31, −22]

Table 3. Association between markers of iron status and 2.4 km run time (s) at week 1.

Marker of Iron Status*	Coefficient (95% CI)	p
Haemoglobin ($g \cdot dL^{-1}$)	1.78 (−2.53, 5.89)	0.433
Ferritin ($ug \cdot L^{-1}$)	−0.07 (−0.13, −0.01)	**0.018**
Soluble Transferrin Receptor ($nmol \cdot L^{-1}$)	0.33 (0.02, 0.64)	**0.036**
Erythrocyte Distribution Width (%)	0.79 (−4.35, 5.94)	0.762
Mean Corpuscular Volume (fL)	−3.21 (−3.94, −2.47)	**<0.001**

*Controlling for age, sex, body mass index, smoking status, and alcohol intake.

Table 4. Association between changes in markers of iron status and changes in 2.4 km run time (s) from week 1 to week 13.

Marker of Iron Status*	Coefficient (95% CI)	p
Change in Haemoglobin ($g \cdot dL^{-1}$)	−4.38 (−8.77, 0.02)	0.051
Change in Ferritin ($ug \cdot L^{-1}$)	0.10 (0.03, 0.16)	**0.006**
Change in Soluble Transferrin Receptor ($nmol \cdot L^{-1}$)	0.23 (0.02, 0.43)	**0.030**
Change in Erythrocyte Distribution Width (%)	3.60 (-1.65, 8.84)	0.178
Change in Mean Corpuscular Volume (fL)	1.20 (0.40, 2.00)	**0.003**

*Controlling for sex, week 1 2.4 km run time, and week 1 marker of iron status.

Table 5. Associations between markers of iron status at week 1 and injury incidence.

	Lower Limb Overuse Injury		Lower Limb Stress Fracture	
Marker of Iron Status*	Odds Ratio (95% CI)	p	Odds Ratio (95% CI)	p
Haemoglobin ($g \cdot dL^{-1}$)	1.055 (0.894, 1.246)	0.524	1.114 (0.741, 1.674)	0.603
Ferritin ($ug \cdot L^{-1}$)	0.998 (0.995, 1.000)	**0.048**	0.999 (0.992, 1.004)	0.667
Soluble Transferrin Receptor ($nmol \cdot L^{-1}$)	1.008 (0.997, 1.019)	0.143	1.005 (0.977, 1.027)	0.662
Erythrocyte Distribution Width (%)	1.074 (0.877, 1.310)	0.486	0.915 (0.544, 1.457)	0.723
Mean Corpusucular Volume (fL)	0.988 (0.951, 1.027)	0.545	1.046 (0.944, 1.158)	0.390

*Controlling for sex, body mass index, 2.4 km run time, total 25(OH)D, smoking status, previous stress fracture, and training course.

Table 6. Associations between markers of iron status and bone mineral density and markers of bone metabolism at week 1.

	Whole-Body aBMD ($g \cdot cm^{-2}$)		Total 25(OH)D ($nmol \cdot L^{-1}$)		PTH ($pg \cdot mL^{-1}$)		βCTX ($\mu g \cdot L^{-1}$)		P1NP ($\mu g \cdot L^{-1}$)	
Marker of Iron Status*	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p
Haemoglobin ($g \cdot dL^{-1}$)	−0.003 (−0.010, 0.003)	0.289	−0.085 (-1.723, 1.553)	0.919	0.010 (−0.060, 0.080)	0.774	0.002 (−0.010, 0.013)	0.783	−0.740 (-3.078, 1.599)	0.535
Ferritin ($ug \cdot L^{-1}$)	0.000 (0.000, 0.000)[a]	**0.021**	−0.023 (−0.047, 0.001)	0.059	−0.001 (−0.002, 0.000)	0.077	0.000 (−0.001, 0.000)[b]	**<0.001**	−0.152 (−0.182, −0.123)	**<0.001**
Soluble Transferrin Receptor ($nmol \cdot L^{-1}$)	0.000 (0.000, 0.001)	0.540	0.105 (−0.013, 0.224)	0.081	0.013 (0.008, 0.018)	**<0.001**	0.000 (−0.001, 0.001)	0.830	0.166 (0.017, 0.316)	**0.029**
Erythrocyte Distribution Width (%)	−0.007 (−0.014, 0.001)	0.097	1.706 (−0.374, 3.786)	0.108	0.043 (−0.044, 0.129)	0.332	−0.001 (−0.015, 0.013)	0.888	1.289 (−1.741, 4.319)	0.404
Mean Corpusucular Volume (fL)	0.000 (−0.002, 0.001)	0.773	0.595 (0.300, 0.891)	**<0.001**	−0.021 (−0.033, −0.008)	**0.002**	−0.006 (−0.008, −0.004)	**<0.001**	−1.250 (−1.671, −0.825)	**<0.001**

*Controlling for sex and body mass index.

aBMD, areal bone mineral density; βCTX, beta C-telopeptide cross-links of type 1 collagen; PINP, procollagen I N-terminal propeptide; PTH, parathyroid hormone; total 25(OH)D, total 25-hydroxyvitamin D.

a, coefficient = 0.0003

b, coefficient = −0.0005