

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

O'Leary, Thomas J; Jackson, Sarah; Izard, Rachel M.; Walsh, Neil P.; Coombs, Charlotte; Carswell, Alexander T.; Oliver, Sam; Tang, Jonathan; Fraser, William; Greeves, Julie P.

British Journal of Nutrition

DOI:

10.1017/S0007114523001812

E-pub ahead of print: 21/09/2023

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA): O'Leary, T. J., Jackson, S., Izard, R. M., Walsh, N. P., Coombs, C., Carswell, A. T., Oliver, S., Tang, J., Fraser, W., & Greeves, J. P. (2023). Sex differences in iron status during military training: a prospective cohort study of longitudinal changes and associations with endurance performance and musculoskeletal outcomes. British Journal of Nutrition. Advance online publication. https://doi.org/10.1017/S0007114523001812

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
 - You may not further distribute the material or use it for any profit-making activity or commercial gain
 You may freely distribute the URL identifying the publication in the public portal?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Sex differences in iron status during military training: a prospective cohort study of

longitudinal changes and associations with endurance performance and musculoskeletal

outcomes

Thomas J O'Leary^{1,2}, Sarah Jackson¹, Rachel M Izard³, Neil P Walsh⁴, Charlotte V Coombs¹,

Alexander T Carswell^{5,6}, Samuel J Oliver⁷, Jonathan CY Tang^{5,8}, William D Fraser^{5,8}, Julie P

Greeves^{1,2,5}

¹Army Health and Performance Research, Army Headquarters, Andover, United Kingdom;

²Division of Surgery and Interventional Science, UCL, London, UK; ³ Defence Science and

Technology, Ministry of Defence, Porton Down, United Kingdom; ⁴Faculty of Science,

Liverpool John Moores University, Liverpool, United Kingdom; ⁵Norwich Medical School,

University of East Anglia, Norwich, United Kingdom; ⁶School of Health Sciences, University

of East Anglia, Norwich, United Kingdom; ⁷College of Human Sciences, Bangor University,

Bangor, United Kingdom; 8Norfolk and Norwich University Hospital, Norwich, United

Kingdom

Corresponding author: Julie P Greeves PhD, Army Health and Performance Research, Army

Headquarters, Andover, Hampshire, SP11 8HT,

United Kingdom. Email:

julie.greeves143@mod.gov.uk

Running title: Iron status in military training.

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

1

Abstract

2	This study investigated sex differences in iron status, and associations between iron status and
3	endurance and musculoskeletal outcomes, in military training. 2,277 British Army trainees
4	(581 women) participated. Iron markers and endurance performance (2.4 km run) were
5	measured at the start (week 1) and end (week 13) of training. Whole-body areal body mineral
6	density (aBMD) and markers of bone metabolism were measured at week 1. Injuries during
7	training were recorded. Training decreased haemoglobin in men and women (mean change
8	[95% CI], $-0.1 [-0.2, -0.0]$ and $-0.7 [-0.9, -0.6]$ g·dL ⁻¹ , both p < 0.001), but more so in women to the contract of the contract o
9	(p < 0.001). Ferritin decreased in men and women (-27 [-28 , -23] and -5 [-8 , -1] ug·L, both
10	$p \le 0.001$), but more so in men (p < 0.001). sTfR increased in men and women (2.9 [2.3, 3.6])
11	and 3.8 [2.7, 4.9] nmol·L, both $p < 0.001$), with no difference between sexes ($p = 0.872$). RDW
12	increased in men (0.3 [0.2, 0.4]%, $p < 0.001$), but not women (0.1 [-0.1, 0.2]%, $p = 0.956$).
13	MCV decreased in men $(-1.5 [-1.8, -1.1] \text{ fL}, p < 0.001)$, but not women $(0.4 [-0.4, 1.3] \text{ fL}, p < 0.001)$
14	= 0.087). Lower ferritin was associated with slower 2.4 km run time (p = 0.018), sustaining a
15	lower limb overuse injury (p = 0.048), lower aBMD (p = 0.021), and higher β CTX and P1NP
16	(both $p < 0.001$) controlling for sex. Improving iron stores before training may protect
17	haemoglobin in women and improve endurance and protect against injury.

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

Introduction

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

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 µg·dL⁻¹ for men and women, and anaemia as haemoglobin < 12 g·dL⁻¹ for women and < 13 g·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).

39

40

41

42

43

44

45

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

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

85 Experimental Design

This study was an observational prospective cohort study. These data were secondary analyses as part of a larger study exploring micronutrient deficiencies and health and performance outcomes (23–25). Venous blood samples were drawn at the start (week 1) and end (week 13) of each basic military training course for the analysis of biochemical markers of iron status, vitamin D status, and biochemical markers of bone metabolism. Endurance performance was assessed by a maximal effort 2.4 km run at the same time points. Body mass, height, and wholebody areal bone mineral density measured by dual-energy X-ray absorptiometry (DXA) were recorded at week 1. Participants self-reported their alcohol intake, use of iron supplementation, smoking habits, and stress fracture history using questionnaires at week 1. Week 1 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 $\mu g\cdot L^{\text{-}1}$ with a sensitivity of 8 μg·L⁻¹. βCTX inter-assay CV was < 3% between 200 and 150 μg·L⁻¹ with a sensitivity of 10 ng·L⁻¹. PTH inter-assay CV was < 3.8% between 1.2 and 5000.0 pg·mL⁻¹. Ferritin inter-assay CV was < 4.2% between 0.5 and 2000.0 µg·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·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.

139

140

141

142

143

144

121

122

123

124

125

126

127

128

129

130

131

132

133

134

135

136

137

138

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.
- 151 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 (*ImerTest 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, β CTX, 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.

205

206

195

196

197

198

199

200

201

202

203

204

Results

- 207 Participants
- 208 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).

212

213

- 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,
- 216 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 =
- 219 0.16) and women (p < 0.001, $d_z = 0.76$) with the decrease greater in women. Haemoglobin was

220 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 \times 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, η_p^2 = 0.035). There was a sex × time interaction for RDW (p < 0.001, η_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 = 0.003

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

234 1 (p < 0.001, d = 0.12), but was lower in men than women at week 13 (p < 0.001, d = 0.52).

236 The Effect of Training on Endurance Performance

223

224

225

226

227

228

229

230

231

232

233

235

238

239

240

241

242

243

244

There was no sex \times time interaction for 2.4 km run time (p = 0.125, η_p^2 = 0.003), but training

decreased 2.4 km run time (main effect of time, p < 0.001, η_p^2 = 0.371) and 2.4 km run time

was faster in men than women (main effect of sex, p < 0.001, η_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 245 or RDW and 2.4 km run time at either time-point in men or women ($r \le 0.07$, $p \ge 0.470$). There 246 was a significant negative relationship between log ferritin and 2.4 km run time at week 13 in 247 men only (r = -0.15 [-0.24, -0.07], p < 0.001), but no evidence of an association between log 248 ferritin and 2.4 km run time at week 1 in or at either time-point in women ($r \le 0.05$, $p \ge 0.224$). 249 There was a significant negative association between log sTfR receptor and 2.4 km run time at 250 251 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 ($r \le 1$ 252 253 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 < 0.001254 0.001), and in women at week 1 (r = -0.25 [-0.36, -0.14], p < 0.001) but not week 13 (r =255 -0.10 [-0.28, 0.08], p = 0.976). There was a negative association between ferritin and MCV 256 with 2.4 km run time at week 1 and a positive association between log sTfR and 2.4 km run 257 time at week 1, when controlling for age, sex, body mass index, smoking, and alcohol intake; 258 there was no evidence of association between haemoglobin or RDW with 2.4 km run time at 259 week 1 (Table 3). There were positive associations between change in ferritin, sTfR, and MCV 260 with change in 2.4 km run time when controlling for sex, concentrations of the respective 261 marker at week 1, and 2.4 km run time at week 1; we found no evidence of an association 262 between change in haemoglobin and change in RDW and change in 2.4 km run time (Table 4). 263

264

265

266

267

268

269

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 β CTX 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, β CTX, 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.

The Effect of Training on Iron Status

295

296

297

298

299

300

301

302

303

304

305

306

307

308

309

310

311

312

313

314

315

316

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

317

318

319

Training decreased haemoglobin more in women, but decreased ferritin more in men. A 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).

320

321

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

347

348

349

350

351

352

353

354

355

356

357

358

359

360

361

362

363

364

365

366

367

368

369

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-1alpha-hydroxylase axis (2).

415

416

417

418

419

395

396

397

398

399

400

401

402

403

404

405

406

407

408

409

410

411

412

413

414

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 menstrual 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).

Acknowledgements

- The authors thank Dr Daniel Kashi, Xin Hui Aw Yong, Mark Ward, Claire Potter, and Dr
- Laurel Wentz for their assistance with data collection.

Funding

445 This study was funded by the UK Ministry of Defence (Army). 446 **Conflits of Interest** 447 The authors have no conflicts of interests to declare. 448 449 **Author Contributions** 450 SJ, RMI, NPW, and JPG designed the study. TJO, SJ, NPW, ATC, and SJO collected the data. 451 JCYT and WDF analysed the biochemical samples. TJO produced the manuscript and 452 performed the data analysis. CVC contributed to manuscript writing and data analysis. All 453 authors edited the manuscript and approved the final version. 454 455 456 **Data Availability** All data are available from the corresponding author pending approval of public release from 457 UK Ministry of Defence. 458

References

- 1. Dallman PR. Biochemical Basis for the Manifestations of Iron Deficiency. Annu Rev Nutr. 1986 Jul;6(1):13–40.
- 2. Gaffney-Stomberg E. The Impact of Trace Minerals on Bone Metabolism. Biol Trace Elem Res. 2019 Mar;188(1):26–34.
- 3. Wardle SL, O'Leary TJ, McClung JP, Pasiakos SM, Greeves JP. Feeding female soldiers: Consideration of sex-specific nutrition recommendations to optimise the health and performance of military personnel. Journal of Science and Medicine in Sport. 2021 Oct;24(10):995–1001.
- 4. McClung JP, Marchitelli LJ, Friedl KE, Young AJ. Prevalence of Iron Deficiency and Iron Deficiency Anemia among Three Populations of Female Military Personnel in the US Army. Journal of the American College of Nutrition. 2006;25:64–9.
- 5. McClung JP, Karl JP, Cable SJ, Williams KW, Nindl BC, Young AJ, et al. Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. The American journal of clinical nutrition. 2009 Jul;90(1):124–31.
- 6. McClung JP, Murray-Kolb LE. Iron nutrition and premenopausal women: effects of poor iron status on physical and neuropsychological performance. Annu Rev Nutr. 2013;33:271–88.
- 7. Scientific Advisory Commitee on Nutrition. Iron and Health [Internet]. Scientific Advisory Commitee on Nutrition; [cited 2023 Jan 25]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/339309/SACN Iron and Health Report.pdf
- 8. Merkel D, Moran DS, Yanovich R, Evans RK, Finestone AS, Constantini N, et al. The Association between Hematological and Inflammatory Factors and Stress Fractures among Female Military Recruits. Medicine & Science in Sports & Exercise. 2008 Nov;40(11):S691–7.
- 9. Yanovich R, Merkel D, Israeli E, Evans RK, Erlich T, Moran DS. Anemia, iron deficiency, and stress fractures in female combatants during 16 months. Journal of strength and conditioning research / National Strength & Conditioning Association. 2011 Dec;25(12):3412–21.
- 10. Looker AC. Prevalence of Iron Deficiency in the United States. JAMA. 1997 Mar 26;277(12):973.
- 11. Knapik JJ, Farina EK, Fulgoni VL, Lieberman HR. Clinically diagnosed iron and iodine deficiencies and disorders in the entire population of US military service members from 1997 to 2015. Public Health Nutr. 2021 Aug;24(11):3187–95.
- 12. Harvey LJ, Armah CN, Dainty JR, Foxall RJ, Lewis DJ, Langford NJ, et al. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. Br J Nutr. 2005 Oct;94(4):557–64.

- 13. McClung JP, Karl JP, Cable SJ, Williams KW, Young AJ, Lieberman HR. Longitudinal decrements in iron status during military training in female soldiers. The British journal of nutrition. 2009 Aug;102(4):605–9.
- 14. Martin NM, Conlon CA, Smeele RJM, Mugridge OAR, von Hurst PR, McClung JP, et al. Iron status and associations with physical performance during basic combat training in female New Zealand Army recruits. The British journal of nutrition. 2019 Apr;121(8):887–93.
- 15. Karl JP, Lieberman HR, Cable SJ, Williams KW, Young AJ, McClung JP. Randomized, double-blind, placebo-controlled trial of an iron-fortified food product in female soldiers during military training: relations between iron status, serum hepcidin, and inflammation. The American journal of clinical nutrition. 2010 Jul;92(1):93–100.
- 16. Hennigar SR, Gaffney-Stomberg E, Lutz LJ, Cable SJ, Pasiakos SM, Young AJ, et al. Consumption of a calcium and vitamin D-fortified food product does not affect iron status during initial military training: a randomised, double-blind, placebo-controlled trial. The British journal of nutrition. 2016 Feb 28;115(4):637–43.
- 17. Booth CK, Probert B, Forbes-Ewan C, Coad RA. Australian army recruits in training display symptoms of overtraining. Mil Med. 2006 Nov;171(11):1059–64.
- 18. Moran DS, Heled Y, Arbel Y, Israeli E, Finestone AS, Evans RK, et al. Dietary intake and stress fractures among elite male combat recruits. Journal of the International Society of Sports Nutrition. 2012 Mar 13;9(1):6.
- 19. Yanovich R, Karl JP, Yanovich E, Lutz LJ, Williams KW, Cable SJ, et al. Effects of basic combat training on iron status in male and female soldiers: a comparative study. US Army Med Dep J. 2015 Jun;67–73.
- 20. O'Leary TJ, Saunders SC, McGuire SJ, Venables MC, Izard RM. Sex Differences in Training Loads during British Army Basic Training. Medicine and science in sports and exercise. 2018 Dec;50(12):2565–74.
- 21. O'Leary TJ, Wardle SL, Rawcliffe AJ, Chapman S, Mole J, Greeves JP. Understanding the musculoskeletal injury risk of women in combat: the effect of infantry training and sex on musculoskeletal injury incidence during British Army basic training. BMJ Mil Health. 2020 Feb 27;
- 22. Chapman S, Roberts J, Smith L, Rawcliffe A, Izard R. Sex differences in dietary intake in British Army recruits undergoing phase one training. Journal of the International Society of Sports Nutrition. 2019 Dec 10;16(1):59.
- 23. Carswell AT, Jackson S, Swinton P, O'Leary TJ, Tang JCY, Oliver SJ, et al. Vitamin D Metabolites are Associated with Physical Performance in Young Healthy Adults. Medicine and science in sports and exercise. 2022 Jun 29;
- 24. Kashi DS, Oliver SJ, Wentz LM, Roberts R, Carswell AT, Tang JCY, et al. Vitamin D and the hepatitis B vaccine response: a prospective cohort study and a randomized, placebo-controlled oral vitamin D3 and simulated sunlight supplementation trial in healthy adults. Eur J Nutr. 2021 Feb;60(1):475–91.

- 25. Harrison SE, Oliver SJ, Kashi DS, Carswell AT, Edwards JP, Wentz LM, et al. Influence of Vitamin D Supplementation by Simulated Sunlight or Oral D3 on Respiratory Infection during Military Training. Medicine & Science in Sports & Exercise. 2021 Jul;53(7):1505–16.
- 26. Tang JCY, Nicholls H, Piec I, Washbourne CJ, Dutton JJ, Jackson S, et al. Reference intervals for serum 24,25-dihydroxyvitamin D and the ratio with 25-hydroxyvitamin D established using a newly developed LC-MS/MS method. The Journal of nutritional biochemistry. 2017 Aug;46:21–9.
- 27. Burger SC, Bertram SR, Stewart RI. Assessment of the 2.4 km run as a predictor of aerobic capacity. S Afr Med J. 1990 Sep 15;78(6):327–9.
- 28. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: Past, present and future. Biochimica et Biophysica Acta (BBA) General Subjects. 2010 Aug;1800(8):760–9.
- 29. Sim M, Garvican-Lewis LA, Cox GR, Govus A, McKay AKA, Stellingwerff T, et al. Iron considerations for the athlete: a narrative review. Eur J Appl Physiol. 2019 Jul;119(7):1463–78.
- 30. Badenhorst CE, Goto K, O'Brien WJ, Sims S. Iron status in athletic females, a shift in perspective on an old paradigm. Journal of Sports Sciences. 2021 Jul 18;39(14):1565–75.
- 31. Peeling P, Dawson B, Goodman C, Landers G, Trinder D. Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. Eur J Appl Physiol. 2008 Jul;103(4):381–91.
- 32. Lutz LJ, Gaffney-Stomberg E, Karl JP, Hughes JM, Guerriere KI, McClung JP. Dietary Intake in Relation to Military Dietary Reference Values During Army Basic Combat Training; a Multi-center, Cross-sectional Study. Military Medicine. 2019 Mar 1;184(3–4):e223–30.
- 33. Gifford RM, Reynolds RM, Greeves J, Anderson RA, Woods DR. Reproductive dysfunction and associated pathology in women undergoing military training. J R Army Med Corps. 2017 Oct;163(5):301–10.
- 34. Double RL, Wardle SL, O'Leary TJ, Weaden N, Bailey G, Greeves JP. Hormonal contraceptive prescriptions in the UK Armed Forces. BMJ Mil Health. 2021 Jan 18;
- 35. Beard J, Tobin B. Iron status and exercise. The American Journal of Clinical Nutrition. 2000 Aug 1;72(2):594S-597S.
- 36. Zhu YI, Haas JD. Iron depletion without anemia and physical performance in young women. The American Journal of Clinical Nutrition. 1997 Aug 1;66(2):334–41.
- 37. Burden RJ, Morton K, Richards T, Whyte GP, Pedlar CR. Is iron treatment beneficial in, iron-deficient but non-anaemic (IDNA) endurance athletes? A systematic review and meta-analysis. Br J Sports Med. 2015 Nov;49(21):1389–97.
- 38. Rubeor A, Goojha C, Manning J, White J. Does Iron Supplementation Improve Performance in Iron-Deficient Nonanemic Athletes? Sports Health. 2018;10(5):400–5.

- 39. Pasricha SR, Low M, Thompson J, Farrell A, De-Regil LM. Iron Supplementation Benefits Physical Performance in Women of Reproductive Age: A Systematic Review and Meta-Analysis. The Journal of Nutrition. 2014 Jun;144(6):906–14.
- 40. Blacker SD, Wilkinson DM, Bilzon JL, Rayson MP. Risk factors for training injuries among British Army recruits. Mil Med. 2008 Mar;173(3):278–86.
- 41. Medeiros DM, Plattner A, Jennings D, Stoecker B. Bone morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. J Nutr. 2002 Oct;132(10):3135–41.
- 42. Harris MM, Houtkooper LB, Stanford VA, Parkhill C, Weber JL, Flint-Wagner H, et al. Dietary iron is associated with bone mineral density in healthy postmenopausal women. J Nutr. 2003 Nov;133(11):3598–602.
- 43. Toxqui L, Pérez-Granados AM, Blanco-Rojo R, Wright I, de la Piedra C, Vaquero MP. Low iron status as a factor of increased bone resorption and effects of an iron and vitamin D-fortified skimmed milk on bone remodelling in young Spanish women. Eur J Nutr. 2014 Mar;53(2):441–8.
- 44. Wright I, Blanco-Rojo R, Fernández MC, Toxqui L, Moreno G, Pérez-Granados AM, et al. Bone remodelling is reduced by recovery from iron-deficiency anaemia in premenopausal women. J Physiol Biochem. 2013 Dec;69(4):889–96.

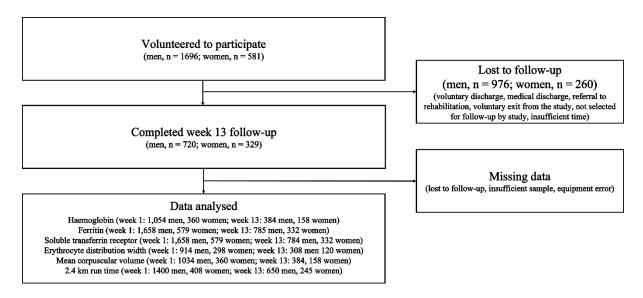


Figure 1. Participant flow through the study.

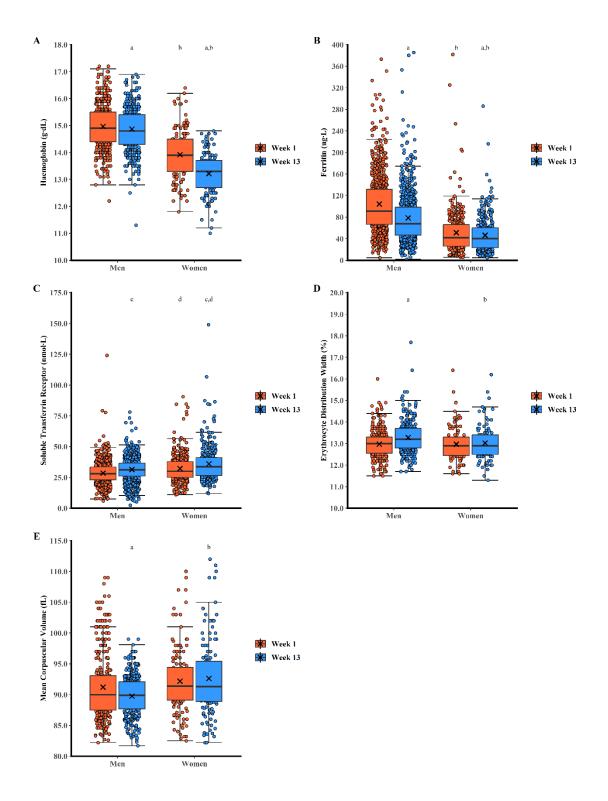


Figure 2. The effect of military training on markers of iron status in men and women. Box plots represent median, interquartile range, and range. Crosses represent mean.

 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

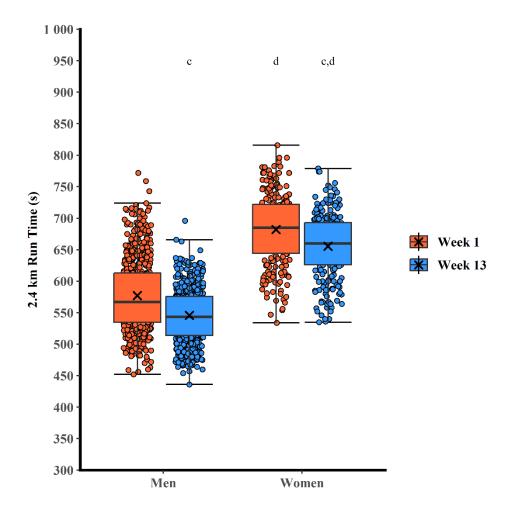


Figure 3. The effect of military training on 2.4 km run time in men and women. Box plots represent median, interquartile range, and range. Crosses represent mean.

 $^a p < 0.05$ 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).

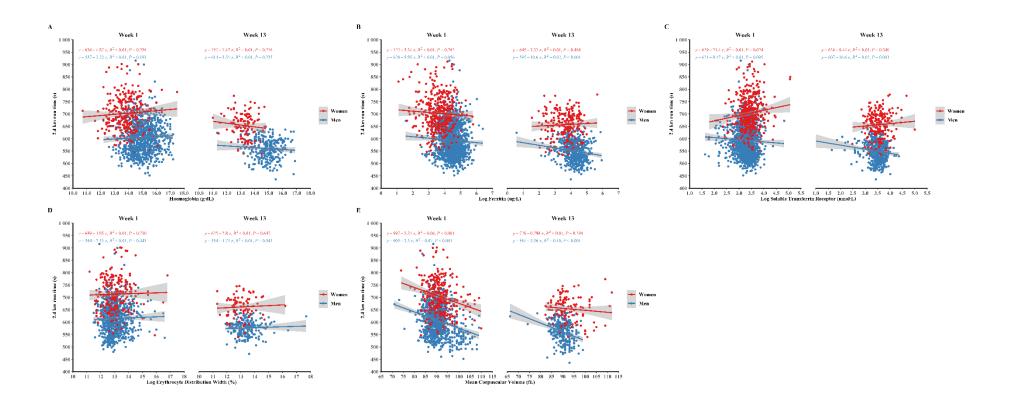


Figure 4. Relationships between markers of iron status and 2.4 km run time at week 1 and week 13 of military training in men and women.

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 \pm 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·m²)	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·dL ⁻¹)	-0.1 [-0.2, -0.0]	-0.7 [-0.9, -0.6]
Ferritin (ug·L ⁻¹)	-27 [-28, -23]	-5 [-8, -1]
Soluble Transferrin Receptor (nmol·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·dL ⁻¹)	1.78 (-2.53, 5.89)	0.433
Ferritin (ug·L ⁻¹)	-0.07 (-0.13, -0.01)	0.018
Soluble Transferrin Receptor (nmol·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·dL ⁻¹)	-4.38 (-8.77, 0.02)	0.051
Change in Ferritin (ug·L ⁻¹)	0.10 (0.03, 0.16)	0.006
Change in Soluble Transferrin Receptor (nmol·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 Ir	Lower Limb Stress Fracture		
Marker of Iron Status*	Odds Ratio (95% CI)	p	Odds Ratio (95% CI)	p
Haemoglobin (g·dL ⁻¹)	1.055 (0.894, 1.246)	0.524	1.114 (0.741, 1.674)	0.603
Ferritin (ug·L ⁻¹)	0.998 (0.995, 1.000)	0.048	0.999 (0.992, 1.004)	0.667
Soluble Transferrin Receptor (nmol·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	·cm ⁻²)	Total 25(OH)D (nmo	l·L ¹)	PTH (pg·mL ¹)		βCTX (μg·L ¹)		P1NP (μg·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·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·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· 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