Randomised double-blind, placebo-controlled trial of iron supplementation attenuates fatigue and declining iron stores for female officers-in-training

Christine K Booth[1], Julia E Carins[1], Iain K Robertson[2]

Abstract

Background: Physical training by female military trainees can put them at risk of iron deficiency and inferior health and performance.

Purpose: To determine the effect of iron supplementation on iron status, fitness, fatigue, and leisure activities.

Material and Methods: In this randomised, double-blind, placebo-controlled trial, female officers-in-training were randomly allocated to treatment (n = 25, 18 mg iron) or placebo tablets (n = 24). Outcomes were iron status, fitness, fatigue, and leisure activity at baseline, seven weeks and 13 weeks.

Results: Ferritin declined by midpoint in the placebo group (P = 0.001). There was a treatment effect in the second half of the trial in emotional fatigue (-4.2 to -0.6 95% CI, P = 0.04). There was a negative relationship with iron intake for emotional fatigue (OR 0.61; 0.44 to 0.87 95% CI; P = 0.006) and positive for vigour (OR 1.51; 1.08 to 2.11 95%CI; P = 0.016) and small negative association between initial dietary iron intake and initial serum ferritin (0.2 to 9.2 μg/L 95% CI; P = 0.042).

Conclusion: Officer training affects iron status and iron supplementation improves indicators of iron status and emotional fatigue in female officers-in-training.

Conflict of Interest: There was no conflict of interest.

Keywords: Nutrition, iron status, females, military, iron supplement.

Introduction

Low dietary iron intake is common in many developed nations, particularly amongst women. A survey of Australian adults revealed that 50% of women aged 19–24 years had a daily iron intake below the recommended level of 12–16 mg[1]. Iron deficiency might affect 8% of all Australian women[2], with the prevalence of anaemia being approximately 2–5%. Other evidence has shown prevalence of iron deficiency in Australian women in the 15 to 30 year age group as high as 12.5% (as indicated by low serum ferritin)[3].

There is some evidence that iron deficiency (without anaemia) can have a negative effect on physical performance, particularly on endurance[4]. For example, the iron status of a group of British Royal Engineers was positively associated with aerobic fitness (multistage fitness test) during adventurous training in the hot wet tropics[5]. Many other non-specific symptoms such as fatigue, lethargy, weakness, headaches, irritability and dizziness have been associated with iron deficiency[2]. These symptoms all have the potential to affect general health and reduce the quality of life in terms of work, leisure, social activities and family responsibilities.

The proportion of women suffering iron deficiency and anaemia increased after basic officer training in the United States[6,7], so the commencement of physical training by Australian Defence Force (ADF) female officers-in-training may put them at risk of iron deficiency. If they do become iron deficient they may fail to complete training or may fail essential fitness tests. Further, they may suffer non-specific symptoms such as fatigue that may interfere with work duties, social and family life[2]. All these problems increase the likelihood that female officers-in-training will be lost from the ADF.

The recruitment and retention of women in the ADF is a matter of concern with the proportion of women in the ADF as low as 12.8%[8]. Defence has stated that 20% of recruits are women, so the figures suggest that retention may be a problem. Women can bring

valuable skills to the ADF, which in turn cannot afford the risk of losing them due to a preventable nutritional deficiency.

This study aimed to determine whether the iron status of female officers-in-training declines during military training, whether the iron status affects fitness, fatigue, health and leisure activities and whether a low dose daily iron supplement would prevent adverse changes in iron status, fitness, fatigue or leisure activities.

Iron supplementation is not without risk. Increased dietary iron intake is a risk factor for the development of haemochromatosis (iron overload) in subjects homozygous for the C282Y allele of the HFE gene [9]. High doses of iron supplements are associated with gastric upset, the severity and occurrence of which depends upon the formulation of the supplement [10]. Iron is also believed to play a role as catalyst for oxygen free radical-induced tissue damage[11]. A low-dose iron preparation of ferrous gluconate (18 mg elemental iron) was chosen for this study. Guidance states that a daily supplement of 17 mg ferrous iron would not be expected to produce adverse effects in the majority of people, other than those with haemochromatosis[10]. Folic acid was added to the capsules to minimise the risk of folate-deficiency anaemia during the study.

This was an exploratory study because this type of study had not previously been conducted with ADF trainees.

Materials And Methods

Subjects

This study was conducted through the Canberra Area Medical Unit and was approved by the Australian Defence Human Research Ethics Committee (ADHREC protocol 314/02). Written, informed consent was obtained from all participants. First and second year female officer cadets (Army, Navy and Air Force) from the Australian Deference Force Academy (ADFA) and female staff cadets (Army) from the Royal Military College (RMC) participated in the study. The initial date of recruitment for the study was February 2003. On each occasion testing was conducted over two consecutive mornings.

Exclusion criteria included current medical problems, recent blood donation, pregnancy in the previous 12 months, breast-feeding, anaemia (haemoglobin < 120 g/L), iron overload (serum ferritin > 300 μg/L), or a positive Helicobacter pylori antibody test. Participants were asked to refrain from taking supplements containing iron throughout the study.

University contact hours and time spent in organised physical training varied greatly between students and weeks. A typical week for ADFA students consisted of around 20 hours of classes (mainly lectures) with 2–7 hours of organised physical training (PT). PT involved either sporting activities, or military PT such as pack marching, fire and movement, battle training, rock climbing and obstacle courses. The training conducted with RMC students was scheduled in blocks, so students would either be in the field (conducting simulated exercises) or back at base performing predominately PT with a number of lectures. Mean academic class attendance per week over the 13 weeks totalled 12.4 h (range 0–47.5 h) and mean time spent in PT was 10.4 h per week (range 0–42 h) for the two groups.

Study Design

After completion of baseline blood tests, participants were divided into two groups, those above and those below the median serum ferritin concentration for all participants.

Participants within each group were then randomly allocated to either treatment (1 capsule per day, iron supplementation) or placebo (1 capsule per day) by use of an on-line random number generator. The study was conducted in a double blinded fashion, with capsule codes being broken and revealed to the researchers only after all measurements were completed and reported.

All capsules (Gold Coast Laboratories Pty Ltd, Burleigh Junction, Australia), contained 0.5 mg folic acid (range 0.465–0.535 mg). The treatment capsule also contained 18 mg elemental iron as ferrous gluconate (range 16.7–19.4 mg), while the placebo contained glucose. The contents of the capsules were confirmed by an independent accredited laboratory (ConMac Laboratory Services, Bethania, Australia).

Capsules in coded bottles were issued to individual participants at each testing point. Participants were instructed to consume 1 capsule with water each day immediately before eating breakfast. To allow estimation of compliance, participants were required to return their coded bottles containing the remaining capsules at the mid-point, (to obtain a re-issue of treatment/placebo capsules) and at the final point. By recording the number of capsules issued, and the number returned, compliance and iron intake from supplements could be determined.

Outcome measures (iron status, fitness, fatigue and leisure activity) and potential confounding factors (body mass index, dietary iron intake and menstrual bleeding index) were measured at the beginning of the academic semester (baseline), the end of 7 weeks

(mid-point) and again after 13 weeks at the end of the academic semester (final-point). To determine dietary iron consumption, participants completed a food frequency questionnaire (FFQ) (Anti Cancer Council of Victoria, Melbourne, Australia)[12] at baseline and at the final point. To determine any possible affect of menstruation on iron status measures, the initial health screening questionnaire was used to calculate a Menstrual Bleeding Index (MBI). The MBI was calculated using the following formula:

MBI = bleeding length (days) * bleeding intensity (rating)/cycle length (days)

where intensity is a perceived 3-point rating of 1 = light, 2 = medium, and 3 = heavy. This method is a modified version of that used in a previous study on iron status in female endurance athletes[13].

Biological sample collection and analysis

Blood from each participant was collected after an overnight fast by using antecubital venipuncture and collection tubes containing the appropriate anticoagulatant. Serum and whole EDTA blood samples were collected at the three time points. A basic haematological profile (haematocrit, haemoglobin concentration, red and white cell counts and counts of neutrophils, monocytes and lymphocytes) was performed on whole EDTA blood within 5 hr of collection by routine methods (Beckman Coulter MaxM automated analyser, Miami, Florida, USA). Frozen serum and plasma samples were air-freighted to the DSTO-Scottsdale laboratory. Standard commercial methods were used in the analyses: ferritin, soluble transferrin receptor and transferrin (ProSpec autoanalyser, Dade Behring Diagnostics, Marburg, Germany) and serum iron (Cobas Bio clinical analyser, Roche Diagnostics, Germany). H. pylori IgG antibodies were detected in fasted serum at the baseline by use of a commercial enzyme-linked immune assay (DTect ELISA, Diagnostic Technology, Belrose, NSW, under licence from the School of Microbiology and Immunology, University of NSW, Sydney). The percent saturation of transferrin (TS) was calculated as:

TS = [Fe (μmol/L) / (25 x transferrin (g/L))] x 100%

Female officers-in-training with iron deficiency (ID) were identified using the criteria recommended by the Australian Iron Status Advisory Panel (Table 1)[14].

Fitness, fatigue, leisure activity measures

Aerobic capacity and endurance was measured using the multistage fitness test[15]. The tests were completed under the supervision of a physical training instructor during the officer or staff cadets' usual physical training classes. Results were presented as the stage reached before volitional exhaustion (i.e. the higher scores indicate a better fitness level).

The concept of fatigue is ill defined and can constitute symptoms such as tiredness, lethargy, lack of vitality, lack of energy, sleepiness, decreased strength and poor concentration. This suggests that fatigue might be measured by assessing a range of symptom domains[16]. We used the Multidimensional Fatigue Symptom Inventory–short form (MFSI-SF) which recognises the multidimensional nature of fatigue[16]. It is a validated questionnaire that is sensitive enough to detect changes in fatigue over short periods[17]. It comprises 30 items; participants rate their experience of each symptom as not at all, a little, moderately, quite a bit or extremely. The item scores combine to produce five subscales, each producing a score ranging from 0 to 24, measuring different dimensions of fatigue—general fatigue, physical fatigue, emotional fatigue, mental fatigue and vigour. Higher scores on the MFSI-SF subscales other than for vigour are indicative of more fatigue.

*TABLE 1. Stages of iron deficiency**

Measurement	Normal	Stage 1	Stage 2	Stage 3
Ferritin (mg/L)	30–250	<30	<15	<5
Transferrin Saturation (%)	16–49	16–49	<16	<16
Soluble Transferrin Receptor (mg/L)	0.4–1.8	0.4–1.8	>1.8	>1.8
Haemoglobin (g/L)	120–180	120–180	120–180	<120

* Iron deficiency can be classified into three stages depending upon severity: iron depletion (stage 1) with decreased ferritin reflecting loss of iron stores; iron–deficient erythropoiesis (stage 2) indicated by a further decrease in ferritin concentration and/or an increase in soluble transferrin receptor concentration and decreased transferrin saturation; and finally a significant decrease in circulating haemoglobin indicating iron-deficiency anaemia (stage 3)[14].

A Leisure Activity Questionnaire was devised to obtain a score of leisure activity. Participants noted the amount of time they spent on leisure activities, in various categories over the previous three days. The categories were self maintenance, demanding sports, medium level sports, low level sports, social, solitary hobbies, personal improvement, relaxation, sleeping/resting, and purposeful. Each category was assigned an energy rating, based on metabolic equivalent intensity levels[18]. Examples were given in each category, including exercise, social and household activities. From this questionnaire a score representing the level of leisure time activity per day was obtained by multiplying total minutes by an energy rating.

Calculations and statistics

Sample size calculations indicated that 215 female officers-in-training were needed in each group (placebo and treatment) in order to detect a clinically significant (alpha = 0.05) change in ferritin concentrations (i.e.5 μg/L ferritin) with 80% power based on a repeated measures design and an SD of 22.2 μg/L ferritin measured at baseline (n = 76). The total number of female officers-in-training available for our study was around 100.

The change in outcome measures of iron status and fatigue scale scores from the beginning to the end of the study in each treatment group separately, and the difference in means in the intervention compared to placebo groups at each separate measurement time point were estimated using mixed-methods linear regression, corrected for repeated measures and adjusted for age, BMI, alcohol intake, dietary iron intake and menstrual bleeding index. The possibility of violation of the assumptions of linear regression was tested using Cameron & Trivedi's decomposition of information matrix test (to test for heteroskedasticity, skewness and kurtosis of regression residuals) and Ramsay's reset test (to test for missing power values). In a number of cases, such violations were identified. Also, the fatigue scale scores are inherently rank-ordered in nature rather than as continuous interval data. All primary analyses were repeated using repeated-measures mixed-effects ordered logistic regression (a "non-parametric" equivalent of the mixed effects linear regression analysis), which estimates odds ratios as effect sizes (which are not simple to interpret). Despite the assumption violations and the rank-ordered nature of the fatigue scales, the "parametric" and "non-parametric analyses appeared to give equivalent result interpretations. Therefore, the analyses are expressed as means (± standard deviations) for placebo and intervention groups at each measurement time point, mean difference (95% confidence intervals, p-values) between initial and subsequent measurements in the placebo and intervention groups separately, and the mean difference (95% confidence intervals, P-values) between the placebo and intervention groups in those measurements at each time point separately (adjusted for the placebo-intervention difference at the initial time point). The P-values for the fatigue scale result, however, were those derived from the mixed-effects ordered logistic regression analyses. P-values were corrected for multiple comparisons using the Holm method. The effect of missing outcome data points was assessed using multiple imputation. Additional analyses were conducted to test whether the effect of the supplement, if any, varied between the 'high' and 'low' iron status groups (based on initial ferritin concentrations). All statistical analyses were performed using Stata MP2/13.0 (Statacorp LP, College Station TX, USA).

Results

Seventy-seven participants were recruited in the study, of whom five were excluded because of a positive Helicobacter pylori test and one because of anaemia. Seventy-one healthy female officers-in-training commenced the study and forty-nine completed all elements of the study. The age, anthropometric characteristics and other potential measured confounding variables of the participants did not differ between the trial groups, as shown in Table 2. There were no differences in either class hours or PT hours between the treatment and placebo groups.

Although there were no reports of side effects due to taking the capsules, compliance with the daily capsule supplement was not perfect. Placebo and treatment groups had similar compliance and dropout rates. Mean compliance (placebo = 24, treatment = 25) excluding those who ceased entirely, was six days per week or 85% (range 52% –100%) which corresponded to a mean supplementation of 16.8 ± 2.1 mg elemental iron (range 12.8–19.4 mg, n = 25).

Dietary intake

There was no reduction in energy intake in the placebo group. In the intervention group initial energy intake was 10% higher than the placebo then fell below the placebo group by the final-point (Table 3). In particular, the mean daily differences for energy and iron intakes over the second half of the trial were -960 kJ (-1862 to -58 kJ 95% CI, P = 0.07) and -1.9 mg (-3.3 to -0.4 mg 95% CI, P = 0.026), respectively.

*TABLE 2. Initial characteristics of trial subjects**

		Placebo (N=34)	Intervention (N=35)	Comparison		95%CI	P-value
Age	Mean ± SD†	20.0 ± 2.0	20.0 ± 3.1	Mean Diff†	0.1	(-1.2 to 1.3)	>0.90
BMI	Mean ± SD†	23.3 ± 2.2	22.8 ± 2.0	Mean Diff†	-0.5	(-1.5 to 0.5)	0.32
MBI§	Median & IQR‡	0.29 (0.16 to 0.37)	0.29 (0.14 to 0.40)	OR‡	1.02	(0.44 to 2.33)	>0.90
Smoker	N‡	1	2	OR‡	2.00	(0.17 to 23.6)	0.58
Alcohol (g/day)	Median & IQR‡	9.7 (5.6 to 16.7)	8.1 (3.1 to 14.5)	OR‡	0.66	(0.28 to 1.51)	0.32
Blood Donor	N2	2	2	OR‡	0.97	(0.13 to 7.41)	>0.90

*Seventy-one healthy female officers-in-training commenced the study, sixty-nine completed at least two time points and forty-nine completed all elements of the study

†Continuous variables (age and BMI—body mass index) were summarised (mean ± standard deviation) and compared using unadjusted general linear modelling (mean difference; 95% confidence intervals; P-values)

‡ Other variables were summarised (median & inter-quartile range—IQR, or number) and compared using unadjusted ordered logistic regression (odds ratio— OR; 95% confidence intervals; P-values)

§MBI: menstrual bleeding index has a range of 0 to 1.

Iron status

A small negative association was found between initial dietary iron intake and initial serum ferritin (a rise of 4.2mg/day dietary iron—1 SD—was associated with a 4.7μg/L fall in serum ferritin; 0.2 to 9.295% CI; P = 0.042). For female officers-in-training in the placebo group there was evidence for a decline in iron status over the first half of the trial with a mean decline in serum ferritin concentration of 30% at mid-point (mean difference -9.2 μg/L; -14.4 to -4.4 μg/L 95% CI; P = 0.001) and no evidence of recovery in the second half of the trial (Table 4). For female officers-in-training in the treatment group a small mean decline in serum ferritin across the trial was not significant and there was evidence for improved iron status over the second half of the course, with significant mean increase in TS (mean difference = 22.8 %; 12.6 to 33.0 95% CI; P < 0.001) and mean decrease in sTfR concentration (mean difference = -0.27 mg/L; -0.41 to -0.14 mg/L 95% CI; P < 0.001) at the final-point. There was weak evidence for a treatment effect in the second half of the trial with mean improvements in TS (mean difference = 3.2 %; -1.2 to 27.5 % 95% CI; P = 0.14) and sTfR (mean difference -0.13 mg/L; -0.32 to 0.07 mg/L 95% CI; P = 0.2) (Table 4).

For those female officers-in-training who had iron status measured at each testing point, there was no change in the prevalence or severity of iron deficiency across the testing period (Figure 1).

Fatigue, fitness and leisure activity

Female officers-in-training in both groups showed improved fitness over the first half of the trial, which appeared to be lost by the final-point, as measured by the multi-stage fitness test (Figure 2, Table 5). There was no evidence for a treatment effect due to the iron supplement.

Female officers-in-training in the placebo group had a greater initial general fatigue score (GFAT) than those in the treatment group (mean difference = -3.5; -5.6 to -1.3 95% CI; P = 0.006) and across the trial both groups displayed improved general fatigues scores. There was some evidence that female officers-in-training in the placebo group felt less vigorous (VFAT score) by the final-point than at the beginning of the trial (mean difference = -2.0; -3.6 to -0.4 95% CI; P = 0.08). There was no evidence for a treatment effect due to the iron supplement for either GFAT or VFAT. There were no significant changes in either group or treatment effects for physical fatigue (PFAT), mental fatigue (MFAT) or leisure activity (LAC).

There was evidence that treatment with the iron supplement improved emotional fatigue (EFAT) (mean difference = -2.4; -4.2 to -0.6 95% CI; P = 0.04). Female officers-in-training in the treatment group had a mean EFAT score 20% less than female officers-in-training in the placebo group at the final-point (Table 5). There was no evidence of a relationship between improvements in emotional

TABLE 3. Comparison of dietary intake of nutrients during the trial

Intake*		Placebo					Intervention					Placebo vs Intervention		
	Period†	N‡	Mean ± SD	Diff§	95%CI	P-value ||	N‡	Mean ± SD	Diff§	95%CI	P-value ||	Diff||	95%CI	P-value#
Energy	0	34	6,792 ± 2,658				35	7,966 ± 2,657				1,174	(66 to 2,283)	0.11
	2	24	6,901 ± 2,840	109	(-531 to 749)	0.74	25	7,115 ± 2,478	-851	(-1,483 to -219)	0.06	-960	(-1,862 to -58)	0.07
Total	0	34	62.1 ± 29.0				35	73.5 ± 28.3				11.3	(-1.3 to 24.0)	0.24
Fat	2	24	63.0 ± 31.1	0.9	(-6.4 to 8.1)	0.81	25	66.3 ± 28.9	-7.2	(-14.3 to 0.0)	0.12	-8.1	(-18.3 to 2.2)	0.24
Protein	0	34	71.4 ± 31.6				35	86.5 ± 31.6				15.1	(1.0 to 29.3)	0.11
	2	24	71.2 ± 33.1	-0.2	(-7.7 to 7.3)	>0.90	25	76.3 ± 26.7	-10.2	(-17.6 to -2.8)	0.06	-10.1	(-20.6 to 0.5)	0.12
Carbo	0	34	174 ± 62				35	204 ± 62				29	(1 to 57)	0.13
	2	34	180 ± 66	6	(-11 to 23)	>0.90	35	179 ± 62	-24	(-42 to -7)	0.10	-30	(-55 to -6)	0.031
Alcohol	0	34	11.5 ± 16.2				35	8.6 ± 13.1				-2.9	(-9.4 to 3.6)	0.75
	2	24	12.5 ± 13.4	1.0	(-2.1 to 4.0)	0.53	25	6.6 ± 10.4	-2.0	(-4.9 to 1.0)	0.59	-2.9	(-7.2 to 1.3)	0.36
Iron	0	34	9.94 ± 4.17				35	11.86 ± 4.62	0.0			1.92	(-0.10 to 3.95)	0.19
	2	24	10.18 ± 4.47	0.24	(-0.80 to 1.27)	>0.90	25	10.24 ± 4.20	-1.62	(-2.64 to -0.59)	0.10	-1.86	(-3.32 to -0.39)	0.026

* Nutrient intake measures: Energy (kJ/day); Total Fat (g/day); Protein (g/day); Carbohydrate (g/day); Alcohol (g/day); Iron (mg/day)

† Trial period: 0=Start of trial; 2=End of trial (13 weeks)

‡ Seventy-one healthy female officers-in-training commenced the study, sixty-nine completed at least two time points and forty-nine completed all elements of the study

§ Changes in nutrient intakes from the start to the middle and end of the trial were estimated within each treatment group separately; results were expressed as mean ± standard deviation (for illustrative purposes only), and compared formally expressed as difference of means (Diff, 95% confidence intervals, p-values) estimated by repeated-measures random-effects mixed methods linear regression, adjusted for age, body mass index, menstrual bleeding index, alcohol intake, dietary iron intake and blood donation.

|| The differences in nutrient intake between placebo and intervention groups were compared at each time point; results were compared formally expressed as difference of means (Diff, 95% confidence intervals, p-values) estimated by repeated-measures random-effects mixed methods linear regression, adjusted for age, BMI, menstrual bleeding index, alcohol intake, dietary iron intake and blood donation.

P-values were corrected for multiple comparisons using the Holm method.

TABLE 4. Comparison of iron status measures during the trial

Iron status*		Placebo					Intervention					Placebo vs Intervention		
	Period†	N‡	Mean ± SD	Diff§	95%CI	P-value#	N‡	Mean ± SD	Diff§	95%CI	P-value#	Diff‖	95%CI	P-value#
Hb	0	34	136.8 ± 9.5	0.0			35	135.0 ± 7.8	0.0			-1.9	(-5.8 to 2.1)	>0.90
	1	29	135.1 ± 6.2	-1.7	(-4.6 to 1.3)	>0.90	28	135.0 ± 7.7	0.0	(-3.0 to 3.1)	>0.90	1.7	(-2.6 to 6.0)	0.43
	2	24	136.8 ± 8.6	0.0	(-3.2 to 3.1)	>0.90	25	135.8 ± 8.3	0.8	(-2.4 to 4.1)	>0.90	0.9	(-3.7 to 5.4)	>0.90
Ferritin	0	34	27.9 ± 25.7	0.0			35	27.8 ± 19.6	0.0			-0.1	(-10.6 to 10.3)	>0.90
	1	29	18.7 ± 20.3	-9.2	(-14.0 to -4.4)	0.001	27	22.2 ± 12.7	-5.6	(-10.6 to -0.6)	0.14	3.6	(-3.3 to 10.6)	0.62
	2	24	18.4 ± 17.5	-9.5	(-16.0 to -2.9)	0.019	25	25.4 ± 15.8	-2.4	(-9.1 to 4.4)	>0.90	7.1	(-2.4 to 16.6)	0.44
TS	0	34	24.9 ± 11.8	0.0			33	24.6 ± 6.5	0.0			-0.3	(-5.5 to 4.9)	0.90
	1	29	26.4 ± 11.2	1.4	(-4.7 to 7.6)	<0.90	27	31.1 ± 12.0	6.4	(0.2 to 12.7)	0.13	5.0	(-3.8 to 13.8)	0.53
	2	24	34.6 ± 17.1	9.7	(-0.3 to 19.7)	0.18	23	47.4 ± 30.9	22.8	(12.6 to 33.0)	<0.001	13.2	(-1.2 to 27.5)	0.14
sTfR	0	34	1.34 ± 0.40	0.00			35	1.30 ± 0.28	0.00			-0.04	(-0.22 to 0.14)	0.67
	1	29	1.41 ± 0.50	0.07	(-0.05 to 0.19)	0.77	27	1.28 ± 0.36	-0.01	(-0.14 to 0.11)	0.83	-0.09	(-0.26 to 0.09)	0.35
	2	24	1.19 ± 0.38	-0.15	(-0.28 to -0.02)	0.11	25	1.03 ± 0.36	-0.27	(-0.41 to -0.14)	<0.001	-0.13	(-0.32 to 0.07)	0.20

* Iron status measures: Hb = Haemoglobin (g/L); Ferritin = Serum ferritin (µg/L); TS = Transferrin saturation %; sTfR =Soluble transferrin receptor (mg/L)

†Trial period: 0 = Start of trial; 1 = Middle of trial (about 7 weeks); 2 = End of trial (13 weeks)

‡ Seventy-one healthy female officers-in-training commenced the study, sixty-nine completed at least two time points and forty-nine completed all elements of the study

§ Changes in iron status from the start to the middle and start to the end of the trial were estimated within each treatment group separately; results were expressed as mean ± standard deviation (for illustrative purposes only), and compared as a difference of means (Diff, 95% confidence intervals, p-values) estimated by repeated-measures random-effects mixed methods linear regression, adjusted for age, BMI, menstrual bleeding index, alcohol intake, dietary iron intake and blood donation.

‖ The difference between placebo and intervention groups in iron status were compared at each time point; results were compared as a difference of means (Diff, 95% confidence intervals, P-values) estimated by repeated-measures random-effects mixed methods linear regression, adjusted for age, BMI, menstrual bleeding index, alcohol intake, dietary iron intake and blood donation; the estimates for the middle and end of trial periods were corrected for differences between the placebo and intervention groups in initial iron status measures.

P-values were estimated by repeated-measures random-effects mixed methods linear regression, adjusted for age, BMI and oral iron intake, and corrected for multiple comparisons using the Holm method.

TABLE 5. Comparison of effect of iron supplementation on measures of fatigue during the trial

Fatigue		Placebo					Intervention					Placebo vs Intervention		
Scale*	Period†	N‡	Mean ± SD	Diff§	95%CI	P-value‖	N‡	Mean ± SD	Diff§	95%CI	P-value#	Diff‖	95%CI	P-value#
GFAT	0	34	18.3 ± 4.8	0.0			35	14.8 ± 4.3	0.0			-3.5	(-5.6 to -1.3)	0.006
	1	29	17.0 ± 4.9	-1.3	(-2.8 to 0.3)	0.10	28	12.4 ± 3.3	-2.4	(-4.0 to -0.8)	0.011	-1.1	(-3.3 to 1.1)	0.62
	2	24	15.7 ± 4.4	-2.6	(-4.4 to -0.8)	0.009	25	12.9 ± 4.2	-2.0	(-3.8 to -0.2)	0.10	0.6	(-2.0 to 3.2)	>0.90
PFAT	0	34	12.3 ± 3.2	0.0			35	11.5 ± 3.6	0.0			-0.8	(-2.3 to 0.7)	>0.90
	1	29	12.7 ± 3.3	0.5	(-0.7 to 1.7)	0.89	28	11.0 ± 2.7	-0.5	(-1.7 to 0.8)	>0.90	-0.9	(-2.6 to 0.8)	0.89
	2	24	11.8 ± 2.8	-0.4	(-1.8 to 0.9)	0.55	25	11.3 ± 3.8	-0.2	(-1.5 to 1.2)	0.82	0.2	(-1.7 to 2.2)	>0.90
EFAT	0	34	11.5 ± 4.0	0.0			35	11.5 ± 3.7	0.0			0.0	(-1.8 to 1.8)	>0.90
	1	29	11.5 ± 4.7	0.0	(-1.1 to 1.2)	>0.90	28	9.8 ± 3.0	-1.7	(-2.8 to -0.5)	0.033	-1.7	(-3.3 to 0.0)	0.15
	2	24	12.1 ± 3.6	0.6	(-0.6 to 1.8)	>0.90	25	9.7 ± 3.3	-1.8	(-3.0 to -0.5)	0.028	-2.4	(-4.2 to -0.6)	0.040
MFAT	0	34	12.4 ± 4.0	0.0			35	11.1 ± 3.2	0.0			-1.3	(-3.2 to 0.5)	0.46
	1	29	12.7 ± 4.6	0.2	(-0.8 to 1.3)	>0.90	28	10.9 ± 3.7	-0.2	(-1.3 to 0.9)	0.70	-0.5	(-2.0 to 1.1)	>0.90
	2	24	12.2 ± 3.4	-0.2	(-1.6 to 1.1)	0.72	25	10.1 ± 3.2	-1.0	(-2.3 to 0.4)	0.50	-0.7	(-2.6 to 1.2)	>0.90
VFAT	0	34	18.4 ± 3.5	0.0			35	19.9 ± 3.7	0.0			1.5	(-0.3 to 3.3)	0.22
	1	29	17.0 ± 4.0	-1.4	(-2.6 to -0.2)	0.11	28	20.1 ± 4.1	0.2	(-1.0 to 1.5)	>0.90	1.6	(-0.1 to 3.4)	0.21
	2	24	16.4 ± 4.2	-2.0	(-3.6 to -0.4)	0.08	25	19.7 ± 4.5	-0.2	(-1.8 to 1.5)	0.85	1.8	(-0.5 to 4.1)	0.25
FIT	0	32	8.37 ± 1.19	0.00			35	8.72 ± 1.39	0.00			0.36	(-0.20 to 0.91)	0.63
	1	20	8.85 ± 0.92	0.48	(0.20 to 0.77)	0.004	19	9.10 ± 1.45	0.38	(0.08 to 0.68)	0.06	-0.10	(-0.52 to 0.31)	0.62
	2	19	8.34 ± 1.02	-0.03	(-0.39 to 0.34)	0.88	17	8.64 ± 1.64	-0.08	(-0.47 to 0.31)	0.67	-0.06	(-0.60 to 0.49)	>0.90
LAC	0	34	81.9 ± 35.7	0.0			35	80.9 ± 31.6	0.0			-1.0	(-18.3 to 16.4)	>0.90
	1	29	96.0 ± 47.7	14.1	(-0.2 to 28.4)	0.26	28	86.3 ± 43.1	5.4	(-9.3 to 20.2)	>0.90	-8.7	(-29.3 to 11.9)	0.81
	2	24	85.7 ± 31.5	3.8	(-11.5 to 19.2)	>0.90	25	87.6 ± 31.4	6.7	(-8.9 to 22.3)	>0.90	2.9	(-19.1 to 24.9)	>0.90

* Fatigue scales: GFAT = General fatigue; PFAT = Physical fatigue; EFAT = Emotional fatigue; MFAT = Mental fatigue; VFAT = Vigour; FIT = Multi-stage fitness test; LAC = Leisure activities.

† Trial period: 0 = Start of trial; 1 = Middle of trial (about 7 weeks); 2 = End of trial (13 weeks)

‡ Seventy-one healthy female officers-in-training commenced the study, sixty-nine completed at least two time points and forty-nine completed all elements of the study

§ Changes in fatigue scale scores from the start to the middle and end of the trial were estimated within each treatment group separately; results were expressed as mean ± standard deviation (for illustrative purposes only), and compared as a difference of means (Diff, 95% confidence intervals, P-values) estimated by mixed-effects ordered logistic regression, adjusted for age, BMI, menstrual bleeding index, alcohol intake, dietary iron intake and blood donation.

‖ The difference between placebo and intervention groups in the fatigue scale scores were compared at each time point; results were compared as a difference of means (Diff, 95% confidence intervals, P-values) estimated by mixed-effects ordered logistic regression, adjusted for age, BMI, menstrual bleeding index, alcohol intake, dietary iron intake and blood donation; the estimates for the middle and end of trial periods were corrected for differences between the placebo and intervention groups in initial fatigue scale scores.

P-values were estimated by repeated-measures random-effects ordered logistic regression, adjusted for age, BMI and oral iron intake, and corrected for multiple comparisons using the Holm method

FIGURE 1.

Prevalence of iron deficiency. Seventy-seven participants were recruited in the study, of whom five were excluded because of a positive Helicobacter pylori test and one because of anaemia. Seventy-six participants completed the baseline blood testing and forty-nine completed all elements of the study. The frequency distribution includes all female officers in-training; baseline n = 76, midpoint n = 57 and final point n = 49. Stages of iron deficiency were classified as iron deficiency or stage 1, iron deficiency erythropoiesis or stage 2, and iron deficiency anaemia or stage 3, as defined in Table 1. From left to right the bars indicate the numbers of female officers in-training with normal, stage 1, stage 2 and stage 3 iron deficiencies at each test point. Logistic regression analysis was used to compare the proportions of subjects in the treatment and placebo groups who showed a decrease in stages of iron deficiency. In this analysis age was employed as a covariate. There was no evidence for changes in prevalence and severity of iron deficiency between testing points or between treatment (n = 24) and placebo (n = 25) groups.

FIGURE 2.

Vigour as a function of total iron intake (from all sources). Vigour is a dimension of the Multidimensional Fatigue Symptom Inventory–short form. Iron intake had a positive relationship with vigour (OR 1.51; 95%CI 1.08 to 2.11; P = 0.016). The positive association is illustrated here by use of linear regression (Pearson r = 0.24, P = 0.008, 125 observations). The 95% confidence interval for the linear regression fit is indicated as a dotted line.

fatigue and either the initial levels or the changes in the iron status variables. However iron intake had a negative relationship with emotional fatigue (OR 0.61; 95% CI 0.44 to 0.87; P = 0.006) and a positive relationship with vigour (OR 1.51; 95%CI 1.08 to 2.11; P = 0.016, Figure 2).

Correction for missing measurements using multiple imputation analysis (in order to perform an approximation of an intention-to-treat analysis) did not result in any substantial change in the estimates of the effects. There was a minor reduction in the estimate of the intervention on emotional fatigue (e.g. -2.36 95%CI -4.16 to -0.57; P=0.042 to -1.99 95%CI -4.38 to 0.40; P=0.1), compatible with a mild increase in variance due to the substituted imputed values for real but missing values.

Discussion

The major finding of this randomised, double-blind, placebo controlled trial is that low-dose iron supplementation, while not attenuating the decline in iron stores (ferritin), improved other indicators of iron status (TS and sTfR) and emotional fatigue observed during 13 weeks of female officer training. Female officers-in-training with the lowest intakes of dietary iron experienced more emotional fatigue and felt less vigorous.

One possible explanation for the changes in iron status seen in the present study is that iron was mobilised to the tissues at the expense of iron stores (ferritin), most likely in response to a high level of physical activity early in the semester. By the end of the trial some general improvements in iron status were observed for female officers-in-training in the treatment group but not in the placebo group with a small decrease in serum sTfR concentration and an increase in TS, but iron stores (serum ferritin) in both groups remained depressed.

Although there is little evidence for an association between iron deficiency and fatigue, these findings and those of a randomised placebo controlled trial involving Swiss women suggest iron supplementation may improve fatigue for non-anemic women[19]. A previous iron-intervention study of young Australian women[2] found fatigue responded positively to a high dose iron supplement (105 mg elemental iron as ferrous sulphate per day) and to an iron-rich diet. A common finding is that improvements in fatigue do not follow changes in iron status. However these findings and those of the earlier Australian study[2] suggest that fatigue is negatively associated with dietary iron consumption. It may be that symptoms related to iron deficiency resolve more quickly than accompanying improvements in iron status measures[19].

Military studies show that a decline in iron status is a common outcome for males and females after periods of intense physical training[5,7,20,21,22,23]. A definitive mechanism for these observations has not been described. Insufficient dietary iron is part of the cause with other mechanisms including proinflammatory processes[24], gastrointestinal bleeding, increased loss of iron in sweat and exercise-induced haematuria potentially contributing to the outcome[25]. This may not have long-term health or performance issues if iron stores are then replenished by a period of good diet and less physical activity. However, for military women who have been shown to have chronically low iron stores[7,26,27], this study supports the recommendation for iron supplementation to prevent or restore declining iron status. US military

studies have also shown iron supplementation to attenuate the decline in iron status seen in female soldiers during military training[22,23]. However in the case of the more recent US study where the supplement was in the form of an iron-fortified food bar, the improvement was only seen in iron-deficient anemic women[22].

Consistent with the US military studies was the observation that iron supplementation provided to female officers-in-training at concentrations close to the recommended daily intake (Australia: 18 mg/day)[28] did not alter the prevalence or severity of iron-deficiency. It is possible that the recommended daily dietary intake of 18 mg iron is not sufficient to maintain iron status for female officers-in-training[23].

Clearly there is a strong argument for female officers-in-training to improve their dietary iron intake. Furthermore, the poor compliance with iron supplementation demonstrated in this study argues against single-nutrient supplementation as the only strategy to improve the iron status of women in the military[29]. Combined strategies of testing the iron status of female soldiers/officers embarking on intense physical training; ensuring sufficient availability of highly bioavailable iron-rich foods in barracks and field catering, including combat rations; providing low dose iron supplementation during physical training courses, and nutritional education should be implemented to improve the iron status of women in the military. It is likely that these findings and recommendations apply to other young women, particularly college or university students engaged in physical training programs.

Limitations of the study

Thirty percent of baseline participants withdrew from the study. It is unknown as to whether this may have affected the study outcomes. However, based on actual sample size (n = 25) for each group and the SD for baseline ferritin concentrations, the estimated power to find a clinically significant treatment effect of 5 μg/L ferritin was only 20%. To achieve the required sample size (n = 215 for each group) the study would need to be repeated over a number of years, or performed in collaboration with larger military populations. There was a 24% rate of missing data in the follow-up of subjects. Attempted correction for this by multiple imputation did not clarify whether the missing data might have altered the estimates of the effects of intervention, although no such alteration was apparent.

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The authors responsibilities were as follows—CKB and JEC designed and conducted research. IKR analysed data with assistance from CKB. CKB wrote the paper, and had primary responsibility for final content. All authors provided input, read and approved the final manuscript. The authors did not have a conflict of interest.

This study conducted through the Canberra Area Medical Unit was approved by the Australian Defence Human Research Ethics Committee (ADHREC protocol 314/02) and was registered under the Therapeutic Goods Administration Clinical Trail Notification Scheme (trial number 2003/146, protocol number 314/02, iron and folic acid supplement). A copy of the trial protocol can be obtained from the corresponding author. The trial was funded through the Defence Science & Technology Organisation's annual tasking.

Authors' affiliations: [1] Defence Science & Technology Organisation, Land Division, [2] Human Life Sciences, University of Tasmania
Corresponding author: Christine K Booth PhD, email: christine.booth@dsto.defence.gov.au

References

1. Australian Bureau of Statistics. National nutrition survey: nutrient intakes and physical measurements. Canberra Australia: Australian Bureau of Statistics;1998.
2. Patterson AJ, Brown WJ, Roberts DC. Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. J Am Coll Nutr. 2001;20:337-342. doi.10.1080/07315724.2001.10719054.
3. Rangan AM, Aitkin I, Blight GD, et al. Factors affecting iron status in 15 - 30 year old female students. Asia Pacific J Clin Nutr. 1997;6:291-295.

4. Hinton PS, Giordano C, Brownlie T, et al. Iron supplementation improves endurance after training in iron-depleted, non-anemic women. J Appl Physiol. 2000;88:1103-1111.
5. Booth CK, Coad RA, Roberts W. Evaluation of an Australian combat ration pack as a sole nutrition source during 23 days of military adventurous training in the tropics. Nutr Diet. 2004;60:239–247.
6. Cline AD, Patton JF, Tharion WJ, et al. Assessment of the Relationship Between Iron Status, Dietary Intake, Performance, and Mood State of Female Army Officers in a Basic Training Population. U.S Army Research Institute of Environmental Medicine Natick MA, Technical Report T-98, 1998.
7. McClung JP, Marchitelli LJ, Freidl KE, et al. Prevalence of iron deficiency and iron deficiency anemia among three populations of female military personnel in the US Army. J American Coll Nutr 2006;25(1):64-69. doi.10.1080/07315724.2006.10719516.
8. Department of Defence. Defence workplace equity and diversity annual report. Canberra Australia Defence Equity Organisation; 2002.
9. Zoller H, Vogel W. Iron supplementation in athletes--first do no harm. Nutrition. 2004;20:615-619. doi.10.1016/j.nutr.2004.04.006.
10. Expert panel on vitamins and minerals and FSA-UK. Iron, 2003. Internet: http://www.food.gov.uk/multimedia/pdfs/evm_iron.pdf . (accessed June 2013).
11. Lauffer RB. Exercise as prevention: do the health benefits derive in part from lower iron levels? Med Hypotheses. 1991;35:103-107.doi.10.1016/0306-9877(91)90031-S.
12. Hodge A, Patterson AJ, Brown WJ, et al.. The Anti Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation. Aust NZ J Public Health. 2000;24:576-583.doi.10.1111/j.1467-842x.2000.tb00520.x.
13. Malczewska J, Raczynski G, Stupnicki R. Iron status in female endurance athletes and in non-athletes. Int J Sport Nutr Exerc Metab. 2000;10:260-276.
14. Australian Iron Status Advisory Panel, 2002. Internet: www.ironpanel.org.au . (accessed July 2010).
15. Brewer J, Ramsbottom R, Williams C. Multistage fitness test. A progressive shuttle-run test for the prediction of maximum oxygen uptake. Belconnen Australia: Australian Coaching Council; 1988.
16. Stein KD, Martin SC, Hann DM, et al. A multidimensional measure of fatigue for use with cancer patients. Cancer Pract. 1998;6:143-152. doi.10.1046/j.1523-5394.1998.006003143.x.
17. Stein KD, Jacobsen PB, Blanchard CM, et al. Further validation of the Multidimensional Fatigue Symptom Inventory –Short Form. J Pain Manage. 2004;27:14-24.doi.10.1016/j.jpainsymman.2003.06.003.
18. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: An update of activity codes and MET intensities. Med Sci Sports Exerc 2000; 32:S498-S504. doi.10.1097/00005768-2000009001-00009.
19. Verdon F, Burnand B, Fallab Stubi C-L, et al. Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. BMJ 2003;326:1124-1126.doi.10.1136/bmj.326.7399.1124.
20. Booth CK, Coad RA, Forbes-Ewan CH, et al. The physiological and psychological effects of combat ration feeding during a 12-day training exercise in the tropics. Mil Med. 2003;168:63-70.
21. Booth CK, Probert B, Forbes-Ewan CF, et al. Australian Army Recruits in Training Display Symptoms of Overtraining. Mil Med 2006;171:1059-1064.
22. Karl JP, Lieberman HR, Cable SJ, et al. Randomised, 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. Am J Clin Nutr 2010;92:93-100.doi.10.3945/ajcn.2010.29185.
23. McClung JP, Karl JP, Cable SJ, 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. Am J Clin Nutr 2009;90:1-8.doi.10.3945/ajcn.2009.27774.
24. Ganz T, Nemeth E, Hepcidin and iron homeostasis BBA 2012;1823:1434-1443.
25. Ottomana C, Franchini M, Sports anaemia: Facts or fiction? Blood Transfus 2012;10:252-254. DOI 10.2450/2012.0019-12.

26. Constantini N, Dubnov G, Foldes A, et al. High prevalence of iron deficiency and anemia in female l military recruits. Mil Med 2006;171(9):866-869.

27. Booth CK, Coad RA. The 1998 Army recruit health and diet survey. International Rev Armed Forces Medical Services 2004;77(2):100-107.

28. National Health and Medical Research Council. Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes, Executive Summary (2006). Available at: http://www.nhmrc.gov.au/publications/synopses/n35syn.htm. Accessed September 2013.

29. Johnson AE. Iron supplementation and the female soldier. Mil Med 2006;171(4):298-300.