

Body Fat Percentage and Blood Donation are the Strongest Determinants of Iron Stores in Premenopausal Women Joining the New Zealand Army

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ABSTRACT

Introduction:

Suboptimal iron status is an issue for women joining the military because of its association with impaired aerobic performance, yet no studies have investigated dietary and non-dietary determinants of iron status simultaneously in this population. The purpose of this study was to explore associations between iron stores, dietary patterns (DPs), and potential non-dietary determinants of iron status in premenopausal women at the commencement of basic military training (BMT) in the New Zealand Army.

Methods:

During week 1 of BMT, demographic, body composition, lifestyle, medical history, and dietary data were measured as potential determinants of serum ferritin (SF) in 101 participants. Following univariate analysis, age, body fat percentage, previous blood donation, at least 6 h of exercise per week that raised the heart rate, and a vegetarian DP were analyzed using a multiple linear regression model.

Results:

An increase in body fat percentage was associated with increased SF ($P < .009$), although blood donation in the past year decreased SF ($P < .011$) compared to those participants who did not donate blood. There was no association between SF and a vegetarian DP or hours of exercise per week. The model explained 17.5% of the variance in SF at the commencement of BMT.

Conclusion:

Body fat percentage and blood donation in the past year were the strongest determinants of iron stores in healthy premenopausal women commencing BMT. It is recommended that women joining the New Zealand Army are provided information to maintain or improve their iron status based on these findings. This includes clinical screening of iron status, advice for women considering blood donation, and dietary advice regarding total energy requirements and iron bioavailability.

INTRODUCTION

Iron is a nutritionally essential trace mineral enabling a number of biochemical pathways critical in physical performance, such as oxygen transport and energy metabolism.¹ In developed countries, premenopausal women are particularly at risk of poor iron status because of the imbalance between iron absorption and menstrual blood loss.²

Dietary and non-dietary factors have been identified as determinants of serum ferritin (SF), a marker of iron status. In premenopausal women living in New Zealand, the reported intake of meat³ and meat/fish/poultry⁴ was significantly lower in participants with suboptimal iron status. In addition, suboptimal iron status has been associated with recent blood donation and duration of menstrual period,^{4,5} history of nose bleeds and low body mass index (BMI),⁴ and Asian ethnicity and previous iron deficiency.⁵ Although most studies investigating associations between dietary intake and iron status have focused on individual foods and nutrients, dietary pattern (DP) analysis is considered both an alternate and complementary approach.⁶

The iron status of physically active premenopausal women, including military personnel, is further compromised by exercise-induced iron losses and reduced iron absorption.⁷ Iron deficiency, with and without anemia, has been reported in women at the beginning of basic military training (BMT) in New Zealand,⁸ the USA,^{9,10} and Israel.¹¹ Suboptimal iron status is associated with impaired aerobic fitness in female

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The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official views or opinions of the New Zealand Defence Force.

doi:<https://doi.org/10.1093/milmed/usad023>

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recruits,^{8,12} and this relationship is operationally relevant as aerobic fitness is associated with injury risk¹³ and completion of physically demanding occupational tasks¹⁴ within the military environment.

One previous study of females in the IDF reported that the mean dietary iron intake was not significantly different between participants with normal iron stores, iron deficiency, or iron deficiency anemia on the commencement of BMT.¹¹ Despite the prevalence of suboptimal iron status in female military recruits, no further studies have been published, to the best of the authors' knowledge. The purpose of this study is therefore to explore associations between iron stores, DPs, and potential non-dietary determinants in premenopausal women at the commencement of BMT in the New Zealand Army.

METHODS

All female recruits ($n = 108$) who enlisted in the New Zealand Army between February 2014 and March 2016 were eligible and invited to participate. A total of 106 female recruits volunteered and provided written, informed consent.

The study was conducted at Waiouru Military Camp, where all New Zealand Army recruits undertake BMT, a 16-week residential course designed to prepare recruits for the demands of service as a soldier. During week 1 of BMT, blood samples and body composition measurements were completed, and participants self-reported data on demographics, lifestyle behavior, medical history, and frequency of food intake.

A fasted venipuncture blood sample of 18 mL in total was collected between 06:00 and 07:30 AM. No muscle damaging exercise was performed for at least 2 days before the assessment. Hemoglobin was analyzed using a Sysmex XT-2000i automated hematology analyzer (Sysmex Corporation, Kobe, Hyogo Prefecture, Japan). The cobas[®] 6000 (Roche Diagnostics, Indianapolis, IN, USA) analyzed SF (electrochemiluminescence immunoassay, e601) and C-reactive protein (CRP) (via the two-point end method with an immunoturbidimetric assay, c501). All biomarkers were analyzed at Medlab, Whanganui (International Accreditation New Zealand), with both internal and external quality controls. The reduction of SF is clinically useful for detecting iron deficiency in healthy individuals; however, as an acute-phase reactant protein, SF is elevated in response to inflammation and infection, masking any potential deficiency. Measuring CRP, a marker of inflammation, can counter this limitation of SF, and in this study, participants with CRP > 10 mg/L were excluded from the analyses.

A questionnaire was administered via SurveyMonkey (Momentive Inc., San Mateo, USA) to determine demographics, lifestyle behavior, and medical history at week 1. Using the concept of prioritized ethnic groups, self-identification with multiple ethnicities was prioritized into a single group in the following order: Māori, Pacific, and New Zealand European.¹⁷ Height was measured by a trained researcher. Body mass (kg) and body fat percentage were measured via

bioelectrical impedance analysis (InBody230, Biospace Co. Ltd., Seoul, Korea). Body mass index was calculated as mass (kg)/height (m^2).

A non-quantitative iron food frequency questionnaire (FeFFQ)¹⁸ was administered online via SurveyMonkey at week 1. The FeFFQ was designed to determine consumption patterns of foods and food groupings that contain iron or factors affecting iron bioavailability during the 4 weeks before BMT commencing. In a validation study, the FeFFQ was compared with a food record, which showed that the FeFFQ was reproducible and reasonably valid for determining iron-related food groups and DPs.¹⁸ Each of the 143 food variables in the FeFFQ was asked as an independent question with nine response options, ranging from never to four or more times per day. Responses were converted into frequencies of intake per week based on a 28-day month.

All statistical analyses were performed using IBM SPSS Statistics, Version 28.0 (Armonk, NY: IBM Corp). The Kolmogorov–Smirnov and Shapiro–Wilk tests and box plots were used to assess the data for normality. Descriptive statistics are presented as mean \pm standard deviation for normally distributed data, median (25th, 75th percentile) for non-normally distributed data, and number of participants (%) for each group with categorical data. A $P < .05$ was considered statistically significant throughout the results. A power calculation was conducted using G*Power Version 3.1.9.4¹⁹ to determine the minimum sample size required to test the study hypothesis. A sample size of 92 participants was required to achieve 80% power to detect a medium effect size at 5% significance for five independent variables.

DPs from the FeFFQ were identified using factor analysis, which aggregates food groupings based on the degree to which they correlated with one another.⁶ Orthogonal varimax rotation facilitated the analysis. Food variables consumed on average ≥ 2.0 times per week were entered into the factor analysis to determine DPs based on consideration that consumption frequency of at least once every 4 days will likely affect iron status.¹⁸ Excluding water, the 27 food variables selected accounted for 45.8% of consumption frequency per week. The Kaiser–Meyer–Olkin test (a measure of sampling adequacy) and the Bartlett's Test of Sphericity (tests the null hypothesis that the correlation matrix is an identity matrix) were conducted to ensure acceptability to proceed with factor analysis. Three factors (DPs) were identified based on eigenvalues > 1, scree plots, and interpretability of the DPs. A factor score was created for each participant using the regression method. The DP names are based on interpretation of the food variables within each factor with a loading ≥ 0.3 .

Univariate regression analysis was performed to identify potential determinants associated with SF. The predictor variables were entered as continuous or categorical variables. Age, BMI, body fat percentage, age of menarche, duration of period, number of heavy days during period, and the three DPs were entered as continuous variables. A dummy categorical variable was created and entered for menstrual

cycle, type of contraception, and ethnicity, with 21-35 days, no contraception, and New Zealand European as the reference categories, respectively. Education level, smoking status, weekly hours of exercise, current use of contraception, dietary supplement use in the previous 6 months, history of poor iron status, previous treatment for poor iron status, donating blood at least once in the previous 12 months, and history of nose bleeds were entered as dichotomous categorical variables.

Predictor variables with a univariate $P < .05$ (age, body fat percentage, blood donation, ≥ 6 h of exercise per week, and the vegetarian DP) were then entered into a multiple linear regression model. Assumptions for the regression model were defined as a Durbin-Watson statistic of 1.5-2.5 for autocorrelation of residuals, a variance inflating factor < 5 for assessment of multicollinearity, and a satisfactory normal P-P plot of regression standardized residual.

RESULTS

Of the 106 female recruits who volunteered to participate, data for five were excluded: Four had a CRP > 10 mg/L and one had FeFFQ data, which were considered abnormal. For the remaining 101 participants included, their characteristics are summarized in Table I.

Based on self-reported data, 94% of participants characterized their eating pattern as including “a variety of all foods, including animal products.” The remaining 6% described not eating red meat, though still eating chicken and/or fish. All participants using contraception reported using a hormonal contraceptive, in the form of an oral contraceptive pill, injection, implant, or intrauterine device. The oral contraceptive pill was used by 67% of contraceptive users.

Based on the factor analysis, Table II presents the factor loadings for each DP and the mean weekly consumption frequency for each food variable consumed ≥ 2.0 times per week on average during the 4 weeks before BMT. Three DPs were identified as “vegetarian,” “meat and vegetables,” and “bread and beverages,” which explained 33.5% of the variance in food intake scores.

Potential determinants of SF during week 1 of BMT were entered into a multiple linear regression model for analysis. Table III illustrates that body fat percentage and donating blood at least once in the previous 12 months were significant determinants of SF. The model explained 17.5% of the variance in SF.

DISCUSSION

The main findings of this study are that body fat percentage and blood donation in the previous 12 months were the strongest predictors of SF at the commencement of BMT. A 1% increase in body fat significantly increased SF and donating blood in the past year significantly decreased SF compared to those participants who did not donate blood. There was no significant association between SF and a vegetarian DP or hours of exercise per week.

TABLE I. Characteristics of Participants During Week 1 of Basic Military Training ($N = 101$)

Characteristics	Measure
	Median (25,75 percentile)
Age (years)	18 (18, 20)
	Mean \pm SD
BMI (kg/m ²)	24.0 \pm 2.9
Body fat (%)	27.1 \pm 5.6
SF (μ g/L)	53.5 \pm 33.7
Hb (g/L)	139.7 \pm 9.2
Age of menarche (years) ^a	13.0 \pm 1.4
Average duration of menstrual period (days) ^b	4.9 \pm 1.0
Average number of heavy days during menstrual period ^b	1.9 \pm 0.9
	<i>n</i> (%)
Current contraception use ^c	63 (62)
Dietary supplement use in previous 6 months ^c	46 (46)
History of poor iron status ^c	26 (26)
Previous treatment for poor iron status ^c	20 (20)
Blood donation in previous 12 months ^c	8 (8)
History of nose bleeds ^c	23 (23)
Smoker ^{c,d}	10 (10)
Ethnicity	
NZ European	60 (59)
Māori	31 (31)
Pacific	10 (10)
Education level ^e	
Non-tertiary	83 (82)
Tertiary	18 (18)
Menstrual cycle	
<21 days	10 (10)
21-35 days	63 (63)
36-90 days	6 (6)
Irregular	21 (21)
Exercise hours per week ^f	
<6	60 (59)
≥ 6	41 (41)

^a $n = 100$, one participant self-reported they had not reached menarche.
^b $n = 79$, excludes 21 participants with irregular periods and one participant who had not reached menarche.
^c“Yes” responses presented for dichotomous variables.
^dParticipants were categorized as smokers if they responded, “yes, every-day” or “yes, occasionally,” or non-smokers if they responded, “never” or “I used to”.
^eTertiary education included a tertiary certificate, diploma, or degree.
^fExercise at an intensity that elevated heart rate over the past 4 weeks. Cutoff was set at 6 h to ensure as equal distribution of participants as possible.
 Abbreviations: BMI, body mass index; Hb, hemoglobin; NZ, New Zealand; SD, standard deviation; SF, serum ferritin.

Body fat percentage was the strongest determinant of iron stores in this study with a significant positive association. However, no association with BMI was observed at the univariate level. Previous studies in healthy premenopausal women have reported contradictory findings regarding associations between iron status and measures of overweight and obesity. Cepeda-Lopez et al.²⁰ reported a significant positive association between SF and body fat percentage, although

TABLE II. Factor Loadings for Each Food Variable for the Three Dietary Patterns Identified for the 4 Weeks Before Basic Military Training

Food variables from the FeFFQ	Factor loadings			Consumption frequency per week ^a
	DP 1: Vegetarian	DP 2: Meat and vegetables	DP 3: Bread and beverages	
Beef	-0.327	0.436	-0.102	2.26 (1.96, 2.57)
Chicken	-0.074	0.443	-0.246	2.76 (2.16, 3.36)
Eggs	0.719	-0.047	-0.037	2.57 (2.02, 3.12)
Cheese	0.514	-0.016	0.152	2.40 (1.91, 2.89)
Milk, as a drink	0.384	-0.062	0.321	3.76 (2.59, 4.93)
Milk, added to drinks	0.097	-0.059	0.475	6.58 (4.93, 8.22)
Milk, added to food	0.327	0.065	0.292	5.89 (4.61, 7.16)
Yogurt	0.596	-0.118	-0.01	2.32 (1.60, 3.04)
Apples	0.613	0.161	-0.242	3.28 (2.50, 4.06)
Bananas	0.417	0.167	-0.156	3.26 (2.34, 4.18)
Citrus fruit	0.419	0.296	-0.25	2.81 (1.83, 3.79)
Stone fruit	0.611	-0.057	-0.177	2.29 (1.36, 3.22)
Potatoes	0.158	0.063	0.279	2.92 (2.57, 3.27)
Broccoli	0.188	0.72	-0.018	2.39 (1.99, 2.80)
Carrots	0.016	0.68	-0.021	3.03 (2.36, 3.71)
Lettuce	0.468	0.395	0.123	3.29 (2.77, 3.81)
Onions, leeks, and celery	0.01	0.587	0.144	2.42 (1.98, 2.86)
Tomatoes	0.272	0.338	0.164	2.56 (2.06, 3.07)
Peas	0.131	0.706	0.17	2.26 (1.80, 2.72)
White bread and rolls	-0.004	-0.165	0.443	2.86 (1.93, 3.78)
Whole meal or grain bread	0.033	0.295	0.359	2.78 (2.17, 3.39)
Butter, margarine	-0.136	0.001	0.505	5.07 (3.85, 6.29)
Cooking oil	-0.097	0.332	0.68	3.46 (2.60, 4.31)
Sugar	-0.265	0.065	0.615	5.14 (3.50, 6.78)
Coffee	-0.12	-0.067	0.505	2.75 (1.61, 3.90)
Herbal tea, fruit tea	0.387	0.123	0.102	2.51 (1.44, 3.58)
Fruit or vegetable juices	0.158	0.132	0.548	2.00 (1.15, 2.85)

Kaiser–Meyer–Olkin = 0.576, *P* < .001.

^aConsumption frequency reported as mean (95% CI).

Factor loadings >0.3 are presented in bold.

Abbreviations: CI, confidence interval; DP, dietary pattern; FeFFQ, iron food frequency questionnaire.

Lim et al.³ observed no association. A significant positive association has been reported between SF and BMI,^{4,21} although others have observed no association.^{3,22,23} Excess body fat triggers low-grade inflammation, increasing ferritin synthesis, and falsely elevating SF.²⁴ However, in this non-obese cohort, where participants with a CRP > 10 mg/L were excluded, a higher body fat percentage appears to be protective. A lower body fat percentage associated with decreasing SF may be because of a reduced intake of total energy compromising dietary iron intake. It may also be because of higher physical activity levels that reduce iron absorption, through the stimulation of hepcidin, the iron metabolism regulator.²⁵

Blood donation in the previous 12 months had a significant negative association with SF in this study. This strengthens earlier studies of premenopausal women living in New Zealand where blood donation in the previous 4 months⁴ and past year⁵ was the strongest determinant of suboptimal iron status (SF < 20 µg/L). During the standard donation of 450-500 mL of blood, nearly 250 mg of iron is lost.²⁶ For blood donors, strategies to mitigate the risk of

iron deficiency include pre-donation measurement of SF,²⁷ extending the inter-donation interval, and post-donation iron supplementation.^{26,28}

The association between SF and exercise ≥ 6 h per week did not achieve statistical significance in this study. This was likely the result of a limited sample size. However, the cumulative effects of exercise-induced iron losses and/or reduced iron absorption and iron recycling remain a concern for physically active women. The losses include gastrointestinal bleeding, hemolysis, hematuria, and sweat.⁷ The reduced absorption and recycling from increased hepcidin expression are likely because of the exercise-induced inflammatory response.²⁹ It is therefore recommended that heavy training loads are considered a relevant determinant of iron status in premenopausal women both joining and serving in the military.

The significant association between SF and the vegetarian DP at the univariate level in this study was lost when analyzed alongside non-dietary risk factors for iron status. This finding is similar to a previous study in premenopausal New Zealand women where the odds of suboptimal iron status

TABLE III. Multiple Linear Regression Model to Identify Determinants of Serum Ferritin Concentration on Commencement of Basic Military Training

	β^a	95% CI for β^a	β^b	P value
Age	1.991	-0.893, 4.874	0.130	.174
Body fat (%)	1.524	0.394, 2.655	0.254	.009
Blood donation ^c	-30.202	-53.180, -7.225	-0.243	.011
≥6 h exercise per week ^d	-12.367	-25.226, 0.492	-0.181	.059
Vegetarian DP	-3.379	-9.973, 3.216	-0.100	.312

F(5, 95) = 5.244, $P < .001$, $R^2 = 0.216$, Adjusted $R^2 = 0.175$.

^aUnstandardized coefficients: express the change in SF ($\mu\text{g/L}$) associated with a 1-unit change in the predictor variables.

^bStandardized coefficients: express the number of SD SF will change with a 1-SD change in the predictor variables.

^cParticipants had donated blood at least once in the previous 12 months.

^dExercise at an intensity that elevated heart rate.

Age, body fat percentage, and vegetarian DP were entered as continuous variables. Blood donation and exercising ≥ 6 h per week were entered as dichotomous variables.

Abbreviations: CI, confidence interval; DP, dietary pattern; SD, standard deviation.

(SF $< 20 \mu\text{g/L}$) were reduced for those following a “meat and vegetable” DP and increased for those following a “milk and yogurt” DP.³⁰ However, in the context of non-dietary determinants, previous blood donation, being Asian, and a history of iron deficiency were stronger predictors of suboptimal iron status than DPs.⁵

Although the vegetarian DP loaded negatively for beef and chicken, the lack of association in the multiple linear regression model may be because of only six participants describing no red meat consumption and no participants describing their eating pattern as excluding all sources of animal flesh. Four of the six participants who excluded red meat had an SF $< 35 \mu\text{g/L}$. Experimental studies generally show a positive association between animal flesh consumption and iron status.³¹⁻³³ This supports a role for meat intake in the prevention of iron deficiency for premenopausal women.

In this study, no association was found between SF and menstruation. In eumenorrheic women, 30-50 mL of blood is lost per menstrual cycle^{34,35} and low menstrual blood loss has been reported to protect against suboptimal iron status.^{4,36,37} This current finding may be because of almost 70% of participants having a regular cycle, ≥ 21 days, and the average duration of a period, 4.9 days. Previous studies have demonstrated that menstruation ≥ 5 days significantly increased the risk of SF $< 15 \mu\text{g/L}$.³⁸ In addition, menstruation > 65 days per year (approximately ≥ 5 days per month) was associated with a 2.5 times increased risk of SF $< 20 \mu\text{g/L}$ compared to women with fewer menstruating days per year.³⁹ Conversely, no significant associations were reported between SF and menstrual cycle length, period duration, or heavy bleeding days in women in Spain⁴⁰ and period length in women in New Zealand.³ The effect of menstrual blood loss on SF in this study may have been mitigated by the high use of hormonal

contraception. Although previous studies have found no association between SF and contraception type,^{3,40} it has been suggested that the protective effect of oral contraceptive pill use on iron status is explained by reduced menstrual blood loss.^{36,40}

A major strength of this study is the inclusion of multiple non-dietary factors associated with iron status in premenopausal women. Given the prevalence of poor iron status previously described in women joining the military,⁸⁻¹⁰ examining all possible risk factors that may contribute is beneficial for guiding future education, screening tools, and clinical guidelines.

Common limitations of factor analysis studies are the necessary, but subjective decisions made in determining DPs.⁶ These decisions included the number of input variables, food groupings, model selection, rotation method, number of factors to retain, and interpretation of the results, including factor loadings. As a result of these decisions and different participant characteristics regarding age, ethnicity, education, and occupation in this study compared to the validation study,¹⁸ the DPs described differ. However, the FeFFQ was designed to identify iron-related DPs and validated for use in premenopausal women using New Zealand-based foods.¹⁸ Given that people consume a variety of foods that contain a range of nutrients, there has been a shift to DP analysis in nutritional epidemiologic studies. This may present a clearer picture than the role of a single food or nutrient.⁴¹ A further limitation of this study is the non-quantitative FeFFQ used as the primary method for collecting dietary data. Quantity, timing, and combinations of foods consumed within a meal were therefore not assessed. This information would have facilitated a better understanding of the total energy intake and the mechanisms driving the relationship between body fat percentage and iron stores.

CONCLUSION

These findings demonstrate that body fat percentage and blood donation in the past year were the strongest determinants of iron stores in a population of healthy premenopausal women commencing BMT. This highlights the importance of a prevention-focused approach, before the commencement of military training, which includes non-dietary determinants of iron stores. In addition to recommending clinical screening of iron status and prescribed treatment with iron supplementation if indicated, it is suggested that women joining the New Zealand Army are provided advice to maintain or improve their iron status. Although DPs did not influence iron stores in this study, it remains prudent that advice regarding total energy requirements and iron bioavailability also be provided.

ACKNOWLEDGMENTS

The authors would like to thank the recruits who volunteered to participate in this study, the Command staff and instructors at The Army Depot, and the medical staff at the Waiouru Defence Health Centre, Waiouru Military

Camp. We would like to acknowledge the following New Zealand Army Medical Officers for their professional support during the data collection, Drs Ali Riniker, Malcolm Joblin, Katia Hayes, and Kate Stanbridge.

FUNDING

This study was funded by the New Zealand Army, with a financial contribution toward undertaking the conduct from the School of Sport, Exercise and Nutrition, Massey University. N.M.M. was the recipient of the New Zealand Dietetic Association Neige Todhunter Award.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author.

CLINICAL TRIAL REGISTRATION

Not applicable.

ETHICS COMMITTEE APPROVAL (HUMAN PARTICIPANTS)

This study was approved by Massey University Human Ethics Committee: Southern A (Application 13/85) and adhered to Defence Force Order 3 (New Zealand), prescribing conduct of personnel research.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

Not applicable.

INSTITUTIONAL CLEARANCE

Institutional clearance approved.

INDIVIDUAL AUTHOR CONTRIBUTION STATEMENT

N.M.M. was the principal investigator and wrote the manuscript; N.M.M., K.L.B., C.A.C., and P.R.v.H. contributed to the study design; N.M.M., R.J.M.S. and O.A.R.M. conducted the study, including participant briefings and data collection; N.M.M. and K.L.B. completed the data analyses; and N.M.M., K.L.B., C.A.C., and P.R.v.H. contributed to the interpretation of the findings. All authors read, revised, and approved the final version of the manuscript.

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