Nutrition related health of female recruits in the New Zealand Army

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Nutritional Science

at Massey University, Albany
New Zealand

Nicola Marie Martin

2023

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

Abstract

Background: Basic training for military recruits is a physically demanding course that is generally a sudden change from an individual's habitual lifestyle. Female recruits have physiological disadvantages in comparison to males, which contribute to lower aerobic fitness and higher risk of musculoskeletal injuries. Nutrition related health, including iron and vitamin D status, dietary intake and body composition is essential to support the health and physical fitness of female recruits. However, little is known about how these nutrition related health factors change during completion of basic training, their determinants or associations with measures of physical fitness in female recruits in the New Zealand Army.

Objectives: The objectives of this research in females undertaking 16-weeks of basic training in the New Zealand Army were to 1) characterise iron status in female recruits during basic training and investigate associations with physical fitness; 2) investigate associations between iron stores, dietary patterns and non-dietary determinants of iron stores in female recruits at the commencement of basic training; 3) characterise vitamin D status in female recruits during basic training and investigate potential determinants at the commencement of basic training; and 4) characterise body composition, physical fitness and dietary intake during basic training and investigate associations between these three factors in female recruits.

Methods: Data were collected at weeks 1 and 16 of basic training from female recruits who enlisted in the New Zealand Army between February 2014 and March 2016. Demographic, lifestyle and medical history information were self-reported via an online questionnaire at week 1. Dietary intake data from a food frequency questionnaire (FFQ) administered online and body composition measurements were collected at weeks 1 and 16. Body composition was determined by the InBody$_{230}$ bioelectrical impedance analyser and included body mass (BM), skeletal muscle mass, fat mass (FM), fat free mass (FFM), total body water and percent body fat (%BF). Height and body mass index (BMI) were also determined. Biochemical data were assessed at weeks 1 and 16 and included serum ferritin (SF), transferrin saturation (TS), soluble transferrin receptor (sTfR), erythrocyte distribution width (RDW), haemoglobin (Hb) and serum 25-hydroxyvitamin D (25(OH)D). A 2.4km run, push-ups and curl-ups were performed at weeks 1 and 8 to assess physical fitness. Changes during basic training in iron status, 25(OH)D, body composition, physical fitness and dietary intake were investigated using paired t-tests. To explore dietary determinants of iron stores at the commencement of basic training, dietary patterns (DPs) from the FFQ were identified using factor analysis. The DPs were then examined alongside potential non-dietary determinants of iron stores. Following univariate analysis, age, %BF, previous blood donation, ≥6-hours of exercise per week and a vegetarian DP were analysed using a multiple linear regression model. To explore vitamin D status, changes in 25(OH)D were characterised by ethnicity and season. Following univariate analysis, age, BMI, ethnicity, season, exercise and SF were analysed as potential determinants of 25(OH)D at the commencement of basic training using a hierarchical linear regression model. Associations between physical fitness and iron status indicators and %BF were investigated using Pearson's correlation coefficients. Associations between frequency intake of food categories and %BF were explored using the rho-Spearman's correlation.

Results: Of the 108 female recruits invited to take part in this research, 106 volunteered to participate. During basic training, the mean ± standard deviation (SD) changes for iron status indicators were that SF decreased (56.6 ± 33.7 to 38.4 ± 23.8µg/L, $P<0.001$), TS decreased (38.8 ± 13.9 to 34.4 ± 11.5%, $P=0.014$), sTfR increased (1.21 ± 0.27 to 1.39 ± 0.35mg/L, $P<0.001$), RDW increased (12.8 ± 0.6 to 13.2 ± 0.7%, $P<0.001$) and Hb increased (140.6 ± 7.5 to 142.9 ± 7.9g/L, $P=0{\cdot}009$). At week 16, sTfR was positively associated ($r=0.29$, $P=0.012$) and TS was negatively associated ($r=-0.32$, $P=0.005$) with the week 8 run time. There were no significant associations between iron status and push-ups or curl-ups. Serum ferritin was positively associated with %BF ($P<0.009$) and negatively associated with blood donation in the past year ($P<0.011$), explaining 17.5% of the variance in SF. There was no association between SF and DPs in the multiple linear regression model. From week 1 to week 16, the mean ± SD for 25(OH)D was 102.5 ± 33.6 to 67.4 ± 22.6nmol/L ($P<0.001$) for basic training commenced in summer and 67.4 ± 21.5 to 73.8 ± 18.9nmol/L ($P=0.033$) for basic training commenced in winter. Overall, more than two-thirds of participants had suboptimal vitamin D status (<75nmol/L) at the end of basic training, regardless of the season training commenced. Increasing age and BMI, being of Pacific or Māori ethnicity and commencing basic training in winter were negatively associated with 25(OH)D. Collectively these determinants explained 45.0% of the variance in 25(OH)D at the commencement of basic training. From week 1 to week 16, the mean ± SD change for FM was -3.8 ± 3.6kg, %BF was -5.5 ± 3.7% and FFM was 3.8 ± 1.8kg (all $P<0.001$). There was no change in BM or BMI. All measures of physical fitness improved during basic training ($P<0.001$). There was a significant increase in frequency intake of protein, grains, fats, discretionary items and beverages (all $P<0.001$). A higher %BF at week 1 was positively associated with the 2.4km run time and negatively associated with push-ups at both weeks 1 and 8 (all $P<0.05$).

Conclusions: Overall, 16-weeks of basic training in the New Zealand Army provides adequate nutrition to support training-induced adaptations in physical fitness and body composition of most female recruits. Optimal %BF and FFM were associated with improved physical fitness, while BMI is an unreliable measure of body composition in this physically active cohort. However, storage and functional iron parameters indicated a decline in iron status and 25(OH)D indicated a decline in vitamin D status in female recruits during basic training. Diminished tissue iron status was associated with impaired aerobic fitness. A lower %BF and blood donation in the past year were the strongest determinants of reduced iron stores while wintertime and being of Pacific or Māori ethnicity were the strongest determinants of reduced 25(OH)D at the commencement of basic training. Therefore, while positive changes in nutrition related health are occurring during basic training, several factors are negatively impacting the iron and vitamin D status of female recruits, both before and during basic training. These factors are limiting the potential of female recruits to achieve optimal health and physical fitness. Delivering education, clinical screening and early supplementation of iron and vitamin D are recommended strategies to counter suboptimal iron and vitamin D status and enhance the success of female recruits during basic training in the New Zealand Army.

Acknowledgements

I would first like to thank the female recruits who bravely volunteered to participate in this research. Soon after arrival at Waiouru Military Camp you agreed to endure greater time constraints on your own training and recovery to support data collection. This was done in the hope the females following you will have a more equitable start line for basic training. I am deeply grateful to you all.

To my supervisors, Associate Professor Kathryn Beck, Professor Cathryn Conlon and Professor Pamela von Hurst, thank you. I would like to acknowledge your patience, perseverance and commitment to this research. I thank you for your flexibility as I navigated conversion from Masters, parental leave and military deployments. Each of you has been a calm, guiding influence throughout all aspects of the research. Thank you for enabling me to communicate the nutrition related health challenges faced by female recruits in the New Zealand Army.

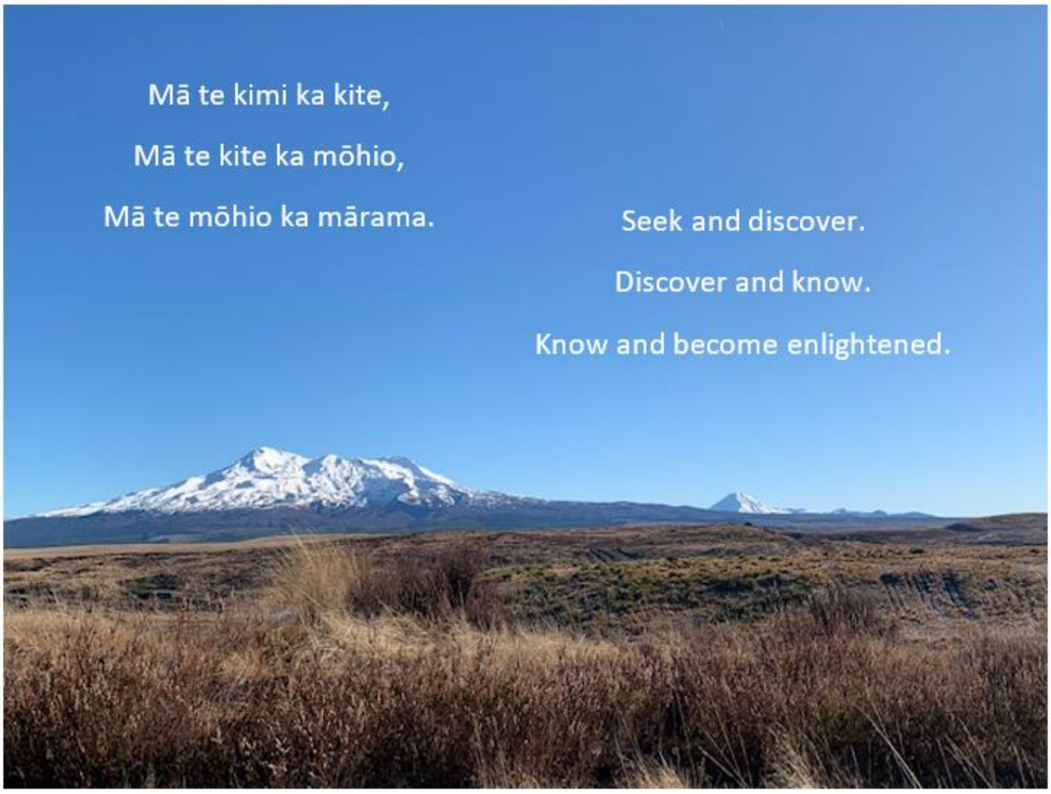
This research simply wouldn’t exist without Rebecca Smeele and Owen Mugridge. Thank you both for your enthusiasm and support over 2.5-years of data collection. From the early mornings to the sunset runs up Waitangi and late nights in between, I am grateful to both of you for your work ethic, commitment and friendship. There was blood, sweat and tears…and even some from the recruits! Rebecca, it’s been a joy to watch you carve out your own career as a female in the Royal New Zealand Navy – Bravo Zulu!

I would like to acknowledge the command staff and instructors at The Army Depot, support staff at Training and Doctrine Command and the medical staff at the Waiouru Defence Health Centre. Thank you for your commitment to the research and flexibility to accommodate the research team over an extended duration.

To Dr James McClung of the United States Army Research Institute of Environmental Medicine, I express my gratitude. Thank you for your encouragement and guidance early in the research. I am also grateful for the international collaborative opportunities you provided me.

Approval for the research described in this thesis was obtained from the Massey University Human Ethics Committee (Southern A). As a recipient of the Neige Todhunter Award, I am grateful to Dietitians New Zealand for your financial support.

The final thank you is reserved for my family. To my children Nieve and Elliott, thank you for understanding the time commitment required to complete this PhD. I look forward to more weekends spent mountain biking. To my partner Charles, thank you for your unwavering support, which enabled me to accomplish this milestone. This achievement belongs to us all.



Waiouru Military Training Area
📷 Nicola Martin

Table of contents

List of tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of figures

Chapter 1

Chapter 2

Chapter 5

Abbreviations

%BF	Percent body fat
$1,24,25(OH)_3D$	1,24,25-trihydroxyvitamin D
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
$24,25(OH)_2D$	24(R),25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
4DDR	4-day diet record
7-DHC	7-dehydrocholesterol
AI	Adequate Intake
AUS	Australia
BIA	Bioelectrical impedance analysis
BM	Body mass
BMI	Body mass index
BT	Basic training
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
CV	Coefficient of variation
DBP	Vitamin D-binding protein
DcytB	Duodenal cytochrome B
DLW	Doubly labelled water
DMT1	Divalent metal transporter 1
DP	Dietary pattern
DXA	Dual energy x-ray absorptiometry
EAR	Estimated Average Requirement
EE	Energy expenditure
EI	Energy intake
Fe^{2+}	Ferrous form
Fe^{3+}	Ferric form
FeFFQ	Iron food frequency questionnaire
FFM	Fat free mass
FFQ	Food frequency questionnaire
FGF23	Fibroblast growth factor 23
FM	Fat mass
Hb	Haemoglobin
HRG1	Haem-responsive gene 1
IDA	Iron deficiency anaemia
IDNA	Iron deficiency non-anaemia
IL-6	Interleukin-6
IOM	Institute of Medicine

LBM	Lean body mass
LCFT	Land combat fitness test
LC-MS/MS	Liquid chromatography-tandem-mass spectrometry
LM	Lean mass
MBL	Menstrual blood loss
MDRI	Military Dietary Reference Intakes
MDRV	Military Dietary Reference Values
MELAA	Middle Eastern/Latin American/African
MRDI	Military Recommended Dietary Intakes
MRI	Magnetic resonance imaging
n	Number
NCEA	National Certificate of Educational Achievement
NZHS	New Zealand Health Survey
OCP	Oral contraceptive pill
OR	Odds ratio
PTH	Parathyroid hormone
RDI	Recommended Dietary Intake
RDW	Erythrocyte distribution width
RXR	Retinoid X receptor
SARS-CoV-2, or Covid-19	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SF	Serum ferritin
sFe	Serum iron
SMM	Skeletal muscle mass
STEAP3	Six-transmembrane epithelial antigen of the prostate 3
sTfR	Soluble transferrin receptor
sTfR:F	Soluble transferrin receptor:ferritin index
TBW	Total body water
Tf	Transferrin
TfR1	Transferrin receptor 1
TIBC	Total iron binding capacity
TS	Transferrin saturation
UK	United Kingdom
US	United States
UVB	Ultraviolet beta
VDR	Vitamin D receptor
VDREs	Vitamin D response elements
Vitamin D_2	Ergocalciferol
Vitamin D_3	Cholecalciferol
VO_2max	Maximal aerobic capacity
ZnPP	Zinc protoporphyrin

Terminology

The following terms are used in this thesis as described:

Term	Description
Basic training	The first training course following enlistment to any international military, in any branch: Navy, Air Force, Army, or Marines. It does not include officer cadets.
Recruit	A trainee who has not yet passed basic training.
Females and Women	Both terms are used throughout this thesis. An individual's biological and physical sex according to reproductive organs and functions classifies them as 'female'. An individual's classification as a 'woman' is based on their personal identity according to social and cultural constructs. There is no intention to offend individuals who may identify with one term and not the other.

Publications and conference presentations

Publications related to this thesis	
2023	Martin, N. M., von Hurst, P. R., Conlon, C. A., Smeele, R. J. M., Mugridge, O. A. R., & Beck, K. L. (2023). Body fat percentage and blood donation are the strongest determinants of iron stores in premenopausal women joining the New Zealand Army. *Military Medicine*. https://doi.org/10.1093/milmed/usad023
2019	Martin, N. M., Conlon, C. A., Smeele, R. J. M., Mugridge, O. A. R., von Hurst, P. R., McClung, J. P., & Beck, K. L. (2019). Iron status and associations with physical performance during basic combat training in female New Zealand Army recruits. *British Journal of Nutrition*, 1-7. https://doi.org/10.1017/s0007114519000199
Manuscripts related to this thesis accepted for publication	
2023	Determinants and longitudinal changes of serum 25-hydroxyvitamin D during basic training in female recruits in the New Zealand Army. Accepted by The Journal of Sport and Exercise Science.
Conference presentations and abstracts related to this thesis	
Annual Meeting of the Nutrition Society of New Zealand, Auckland, New Zealand, 28-30 November 2018	Martin, N., Conlon, C., Smeele, R., Mugridge, O., Hurst, P. v., McClung, J., & Beck, K. (2019). Iron status and associations with physical performance in female New Zealand Army recruits. *Proceedings*, 8(1), 17. https://www.mdpi.com/2504-3900/8/1/17
4th International Congress on Soldiers' Physical Performance, Melbourne, Australia, 28 November-01 December 2017	Martin, N., Beck, K., Conlon, C., Smeele, R., Mugridge, O., McClung, J., & von Hurst, P. (2017). Iron status and associations with aerobic performance and stress fracture risk during initial military training. *Journal of Science and Medicine in Sport*, 20, S164-S165. https://doi.org/https://doi.org/10.1016/j.jsams.2017.09.562
Collaboration and presentation concurrent to this thesis	
2021	Karl, J. P., Margolis, L. M., Fallowfield, J. L., Child, R. B., Martin, N. M., & McClung, J. P. (2021). Military nutrition research: contemporary issues, state of the science and future directions. *European Journal of Sport Science*, 1-12. https://doi.org/10.1080/17461391.2021.1930192
4th International Congress on Soldiers' Physical Performance, Melbourne, Australia, 28 November-01 December 2017	Martin, N. (2017). Performance nutrition: the New Zealand Defence Force perspective. *Journal of Science and Medicine in Sport*, 20, S143-S144. https://doi.org/https://doi.org/10.1016/j.jsams.2017.09.515

Research contribution by PhD candidate

Stage	Task	Contribution
Study preparation	Study design	• Co-designed in collaboration with supervisors, based on the literature and the candidates' personal experience as both a female and health practitioner in the New Zealand Army
	Ethical approval	• Attained ethical approval through the Massey University Human Ethics Committee
	New Zealand Defence Force approval	• Attained required approval to conduct personnel research within the New Zealand Defence Force
	Funding	• Secured funding for the study conduct through the New Zealand Army
	Participant forms	• Developed the information sheet and individual consent form
	Questionnaires	• Adapted previous questionnaires designed at Massey University and incorporated blood loss questions from Heath et al., (2001)[a] to design the health and lifestyle questionnaire • Set-up questionnaires within SurveyMonkey (Momentive Inc., San Mateo, United States)
	Standard operating procedures	• Developed standard operating procedures for each measure (e.g., body composition, blood collection and questionnaire administration) and the overall study conduct for both week 1 and week 16 (e.g., participant and staff briefings and timings of data collection)
	Resources	• Coordinated availability of all resources (e.g., bio-electrical impedance analysis unit, iPads and computer software)
Administration	Point of contact	• Main point of contact throughout all stages
	Liaison	• Communicated with all internal and external parties involved to coordinate timings, access resources, minimise disruptions to recruit training and collate external data (e.g., physical fitness tests)
	Participant documentation	• Maintained protection of all documentation in a secure New Zealand Defence Force location
	Physical fitness tests	• Collated results of all physical fitness tests conducted during week 1 and week 8
Conduct of study at week 1 only	Participant information and consent	• Coordinated all participant information sessions • Collated all participant consent forms
Conduct of study at week 1 and week 16	Blood sampling	• Coordinated all blood sampling and laboratory assessments

Stage	Task	Contribution
	Body composition measurements	• Coordinated and co-conducted (alongside Owen Mugridge and Rebecca Smeele) all body composition measurements: height, body mass, bio-electrical impedance analysis
	Questionnaires	• Coordinated and co-conducted (alongside Rebecca Smeele) administration of all questionnaires via SurveyMonkey (i.e., health and lifestyle questionnaire and food frequency questionnaire)
Data management	SurveyMonkey	• Exported all data as Microsoft Excel spreadsheets to secure New Zealand Defence Force drive
	Data cleaning and transformation	• Identified incorrect, duplicate or incomplete data and remedied this as necessary within the dataset • Formatted and organised multiple datasets for compatibility with SPSS • Coded data as required and maintained a record of rules, definitions, and inclusion and exclusion criteria
Data analysis	SPSS	• Analysed all data using SPSS • Interpreted all results
Publications	Manuscripts	• Main author of all manuscripts • Drafted all manuscripts and completed necessary revisions following review by co-authors • Completed all administration for submission of manuscripts to peer-reviewed journals, including minor revisions as required

[a] Heath, A. L., Skeaff, C. M., Williams, S., & Gibson, R. S. (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutrition*, *4*(2), 197-206.

Research contribution by manuscript co-authors

Associate Professor Kathryn Beck Primary supervisor, Massey University	Co-designed study, contributed to and supported funding and ethics applications, contributed to the development of the health and lifestyle questionnaire, confirmed use of the food frequency questionnaire, supported problem solving throughout the study conduct, assisted with the statistical analysis of data, assisted with interpretation of the results, revised and approved all manuscripts (chapters 3-6)
Professor Cathryn Conlon Secondary supervisor, Massey University	Co-designed study, contributed to the ethics application, supported problem solving throughout the study conduct, assisted with interpretation of the results, revised and approved all manuscripts (chapters 3-6)
Professor Pamela von Hurst Secondary supervisor, Massey University	Co-designed study, supported problem solving throughout the study conduct, assisted with the interpretation of the results, revised and approved all manuscripts (chapters 3-6)
Rebecca Smeele New Zealand Registered Dietitian Royal New Zealand Navy	Supported administrative and liaison tasks throughout the study period, conducted all participant information sessions, co-conducted body composition measurements and administration of the questionnaires, revised and approved all manuscripts (chapters 3-6)
Owen Mugridge Research Trials Manager, Massey University	Coordinated all technology requirements for the study conduct, co-conducted all body composition measurements and administration of the questionnaires, supported data management with secure storage within a Massey University drive, revised and approved all manuscripts (chapters 3-6)
Dr James McClung Chief, Military Nutrition Division, United States Army Research Institute of Environmental Medicine	Provided guidance with the study concept and specifically assisted with the interpretation of the results, revision and approval of chapter 3

Chapter 1 Introduction

1.1 Introduction

In 2014, the New Zealand Ministry of Defence released a report, ‘Maximising opportunities for Military Women in the New Zealand Defence Force’. The report recognised that improving the representation of military women would benefit operational capability. To achieve this, a key recommendation from the report was to expand systems to increase women’s retention (Ministry of Defence, 2014). As of 31 October 2022, 15% of regular force personnel in the New Zealand Army were female. All soldiers commence their career in the New Zealand Army undertaking basic training at Waiouru Military Camp, located in the central North Island. The gender-integrated residential course is 16-weeks and designed to prepare recruits for the demands of service as a soldier. Training is dominated by arduous physical military tasks. Due to a range of factors, up to 45% of female recruits are reported to attrite annually, compared to approximately 20% of male recruits (unpublished data, Defence Human Resources, New Zealand Defence Force, November 2022). The report recognised that small improvements in retention make a big difference in total representation (Ministry of Defence, 2014). Therefore, a targeted effort to improve retention of women during basic training in the New Zealand Army has the potential to make a substantial contribution to improving operational capability.

Nutrition related health is one system where expansion could provide strategies and solutions to increase women’s retention during basic training. Physiological sex differences in body composition typically result in females having lower physical fitness and a higher risk of injury during basic training than their male counterparts (Knapik et al., 2001a; Nindl et al., 2016). These risk factors are associated with attrition in female recruits (Ministry of Defence, 2016). Optimal nutrition is essential to support health, physical performance and prevent injury. While studies have investigated the nutritional status of female recruits internationally, it is important to investigate these research findings in the New Zealand Army. This will account for differences in ethnicity, geographical location, dietary intake, baseline physical fitness and the physical demands of basic training that may differ in duration, intensity and functional outputs. In addition, the highly structured nature of basic training provides a standardised environment for variables such as sedentary behaviour, sleep, social connection, access to health care, dietary intake, sun exposure, and clothing coverage. This provides a unique opportunity to assess the impact of basic training on female recruits.

Iron is a nutritionally essential trace mineral that enables a number of biochemical pathways critical in physical performance, such as oxygen transport and energy metabolism (Suedekum & Dimeff, 2005). Iron deficiency, with and without anaemia, has been reported in females at the commencement of basic training in the United States (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2006) and Israel (Dubnov et al., 2006; Israeli et al., 2008). In addition, iron status has been reported to significantly decline in females during basic training in the United States Army (McClung et al., 2009a; McClung et al., 2009b). The association between iron deficiency anaemia and impaired aerobic fitness has been clearly demonstrated in female recruits (McClung et al., 2009b; Myhre et al., 2015). However, the effects of diminished iron status on physical fitness in female recruits remains unclear, particularly when biomarkers fall outside of the clinical diagnostic parameters. Physical fitness is an operationally relevant measure of military readiness, which in turn impacts attrition in women. Given the physiological sex differences females are already burdened with, it is paramount to identify first, if iron deficiency is prevalent in female recruits in the New Zealand Army and second, if diminished iron status further compromises their physical fitness. As previous studies have been limited to measuring changes in iron status over 9-weeks, it is important that further studies reflect changes that are closer to the 120-day (17-weeks) lifespan of erythrocytes. These findings may support initiatives for clinical screening and supplementation that prevent a decline in iron status.

Despite the prevalence of suboptimal iron status in female recruits internationally, few studies have explored the determinants in this physically active military population. In premenopausal women living in developed countries, inadequate dietary iron intake and increased iron losses through menstruation and blood donation have been identified as significant contributors to suboptimal iron status (Beck et al., 2014; Beck et al., 2013; Heath et al., 2001). The iron status of physically active women, including military recruits, is further compromised by exercise-induced iron losses (McClung et al., 2014). In addition, reduced iron absorption and iron recycling from increased hepcidin expression, likely due to the exercise-induced inflammatory response (Newlin et al., 2012) can further diminish iron status.

Most studies investigating associations between dietary intake and iron status have focused on individual foods and nutrients. In female recruits in the Israel Defense Forces, there was no relationship between dietary iron intake and iron status at the commencement of basic training (Israeli et al., 2008). However, people consume a variety of foods that contain a range of nutrients. Therefore, the association between dietary patterns (DPs), that consider the whole diet, and iron status may present a clearer picture than the role of a single food or nutrient (Hu 2002). To date, no studies have investigated associations between DPs and iron status in female recruits. This presents a novel opportunity, with a potentially powerful outcome of providing dietary recommendations to the target population that focus on a total diet approach. The importance of DPs must also be considered in the context of non-dietary determinants of iron status, such as menstruation, blood donation and physical activity. No studies have simultaneously explored associations between iron status and dietary and non-dietary determinants in female recruits. Examining these determinants collectively may support targeted prevention of suboptimal iron status.

In addition to iron, vitamin D has been identified as a micronutrient of interest for female military personnel, including recruits. Evidence for the role of vitamin D in skeletal health is robust. Suboptimal vitamin D status has also been associated with impaired aerobic fitness (Carswell et al., 2018). Serum 25-hydroxyvitamin D (25(OH)D) concentration ≥75nmol/L has been recommended as the optimal target to achieve maximum musculoskeletal benefits from vitamin D (Holick et al., 2011). Previous studies in the United States Army have indicated that most female recruits have suboptimal vitamin D status at the end of basic training, if not also throughout (Andersen et al., 2010; Lutz et al., 2012). However, no studies have investigated vitamin D status in a New Zealand military population. Numerous environmental, genetic and lifestyle factors influence the vitamin D status of adults. Ethnicity is consistently a strong individual predictor of 25(OH)D concentration (Bertrand et al., 2012; Knight et al., 2017; Liu et al., 2018; Nessvi et al., 2011). In adult women living in New Zealand, the prevalence of 25(OH)D <50nmol/L is 28.5% for the total population (including New Zealand Europeans), 39.0% for Māori and 53.2% for Pacific (Ministry of Health, 2012). It is therefore necessary to establish a situational awareness of vitamin D status in diverse ethnic groups and geographical locations that differ from cohorts in the United States. Investigating vitamin D status at training locations approaching 40° is recommended to measure the effects of more extreme environments on vitamin D status in female recruits. These findings may inform the development and implementation of timely strategies that prevent suboptimal vitamin D status.

Basic training is a demanding course that is generally a sudden change from an individual's habitual lifestyle. Adequate dietary intake is required to both fuel physical training and enhance body composition adaptations, resulting from physical training (Thomas et al., 2016). Optimal body composition, in turn, is associated with functional outcomes in the military, such as improved physical performance (Pierce et al., 2017) and reduced musculoskeletal injuries (Jones et al., 2017). These positive outcomes can reduce attrition in military recruits (Knapik et al., 2001b). Although body composition, physical fitness and dietary intake are three inter-related factors, previous studies in female recruits have focused on either one or two factors only. Body mass index has been suggested as an imprecise measure for body composition in this population (Foulis et al., 2021; Margolis et al., 2012). However, few studies have explored associations between percent body fat and physical fitness that may offer greater specificity for determining optimal body composition in female recruits. In addition, few studies have incorporated measures of muscular endurance (e.g., push-ups and curl-ups) alongside aerobic fitness (e.g., running). Therefore, exploring the changes in body composition, physical fitness and habitual dietary intake simultaneously in female recruits during basic training and the associations between these three factors is a novel investigation. Early identification of high-risk groups in terms of suboptimal dietary intake, body composition and/or physical fitness can support targeted education and interventions at the appropriate time. These findings may also inform potential changes in the delivery of physical training and nutrition services if required.

In summary, exploring the nutrition related health of female recruits is an opportunity to identify factors contributing to attrition during basic training. These findings may subsequently be utilised to develop strategies and solutions that improve the health and physical performance of female recruits. The likely increase in retention as a result has the potential to make a substantial contribution to improving operational capability in the New Zealand Army and wider New Zealand Defence Force.

1.2 Study aims and objectives

The aim of this research is to establish a situational awareness of iron and vitamin D status, dietary intake and body composition in female recruits in the New Zealand Army; determinants; and associations with physical fitness during 16-weeks of basic training.

The objectives of this research are to:

- Characterise iron status in female recruits during basic training and investigate associations with physical fitness.
- Investigate associations between iron stores, dietary patterns and potential non-dietary determinants in female recruits at the commencement of basic training.

- Characterise vitamin D status in female recruits during basic training and investigate potential determinants at the commencement of basic training.
- Characterise body composition, physical fitness and dietary intake during basic training and investigate associations between these three factors in female recruits.

1.3 Study hypotheses

Hypothesis 1: Indicators of iron status will be diminished during basic training.

Hypothesis 2: Diminishing iron status will be associated with impaired aerobic fitness.

Hypothesis 3: Certain dietary patterns will be significant predictors of iron stores at the commencement of basic training.

Hypothesis 4: Blood donation, a history of iron deficiency and menstrual blood loss will be significant non-dietary predictors of iron stores at the commencement of basic training.

Hypothesis 5: Vitamin D status will improve during basic training conducted through winter and spring and diminish during basic training conducted through summer and autumn.

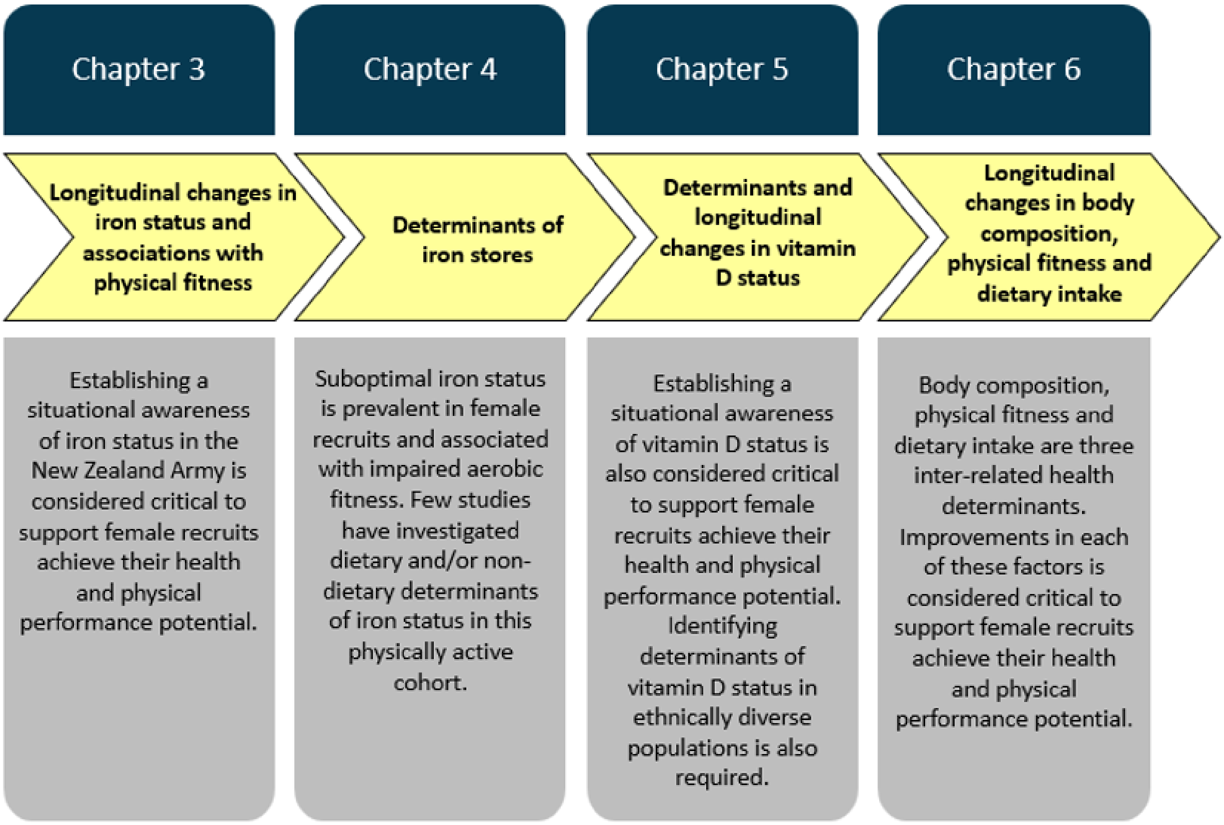
Hypothesis 6: Vitamin D status of Māori and Pacific female recruits will be lower than New Zealand European recruits during basic training.

Hypothesis 7: Positive changes in body composition and physical fitness will occur during basic training.

Hypothesis 8: A higher percent body fat will be associated with impaired aerobic fitness.

1.4 Structure of thesis

This thesis begins with a review of the literature (chapter two), which focuses on iron, vitamin D, physical fitness, body composition and dietary intake of females and provides a situational awareness of their impact on the health and physical fitness of female recruits in the New Zealand Army. The following four chapters represent the four manuscripts prepared for this thesis by publication. As each chapter is written in the format of a manuscript suitable for submission to a peer-reviewed journal, there may be repetition throughout this thesis. The first manuscript (chapter three) characterises iron status in female recruits during basic training and investigates associations with physical fitness. The second manuscript (chapter four) investigates associations between iron stores, DPs and potential non-dietary determinants in female recruits at the commencement of basic training. The third manuscript (chapter five) characterises vitamin D status in female recruits during basic training and investigates potential determinants at the commencement of basic training. The fourth manuscript (chapter six) characterises body composition, physical fitness and dietary intake during basic training and investigates associations between these three factors in female recruits. Figure 1.1 illustrates how the manuscript chapters are connected and Table 1.1 outlines the methodology tests conducted during basic training. The final chapter includes a discussion, conclusion and recommendations (chapter seven). Included are a discussion of the key findings from each study, methodological strengths and limitations, future research suggestions and recommendations to optimise the health and performance of female recruits as they commence their careers as soldiers in the New Zealand Army. Finally, supporting information relating to the study conduct is provided in the appendices (chapter eight).



Chapter 3

Longitudinal changes in iron status and associations with physical fitness

Establishing a situational awareness of iron status in the New Zealand Army is considered critical to support female recruits achieve their health and physical performance potential.

Chapter 4

Determinants of iron stores

Suboptimal iron status is prevalent in female recruits and associated with impaired aerobic fitness. Few studies have investigated dietary and/or non-dietary determinants of iron status in this physically active cohort.

Chapter 5

Determinants and longitudinal changes in vitamin D status

Establishing a situational awareness of vitamin D status is also considered critical to support female recruits achieve their health and physical performance potential. Identifying determinants of vitamin D status in ethnically diverse populations is also required.

Chapter 6

Longitudinal changes in body composition, physical fitness and dietary intake

Body composition, physical fitness and dietary intake are three inter-related health determinants. Improvements in each of these factors is considered critical to support female recruits achieve their health and physical performance potential.

Figure 1.1. Connection between manuscript chapters

Table 1.1. Methodology tests conducted during basic training

Tests conducted			Week 1	Week 8	Week 16
Fasted blood sampling	Complete blood count	Haemoglobin	✓		✓
	Iron studies	Serum ferritin Serum iron Transferrin Transferrin saturation Erythrocyte distribution width Total iron binding capacity	✓		✓
	Soluble transferrin receptor		✓		✓
	Vitamin D – 25-hydroxyvitamin D		✓		✓
	C-reactive protein		✓		✓
Body composition	Height		✓		
	Bioelectrical impedance analysis	Body mass Fat mass Fat free mass Skeletal muscle mass Total body water Percent body fat	✓		✓
	Body mass index		✓		✓
Questionnaires	Health and lifestyle		✓		✓
	Iron food frequency		✓		✓
Physical fitness test	Timed 2.4-kilometre run Maximum continuous push-ups Maximum continuous curl-ups		✓	✓	

1.5 References

Andersen, N. E., Karl, J. P., Cable, S. J., Williams, K. W., Rood, J. C., Young, A. J., Lieberman, H. R., & McClung, J. P. (2010). Vitamin D status in female military personnel during combat training. *Journal of the International Society of Sports Nutrition*, *7*, 38. https://doi.org/10.1186/1550-2783-7-38

Beck, K. L., Conlon, C. A., Kruger, R., Heath, A. L., Matthys, C., Coad, J., Jones, B., & Stonehouse, W. (2014). Blood donation, being Asian, and a history of iron deficiency are stronger predictors of iron deficiency than dietary patterns in premenopausal women. *Biomed Res Int*, *2014*, 652860. https://doi.org/10.1155/2014/652860

Beck, K. L., Kruger, R., Conlon, C. A., Heath, A. L., Matthys, C., Coad, J., & Stonehouse, W. (2013). Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand. *European Journal of Nutrition*, *52*(2), 467-476. https://doi.org/10.1007/s00394-012-0348-y

Bertrand, K. A., Giovannucci, E., Liu, Y., Malspeis, S., Eliassen, A. H., Wu, K., Holmes, M. D., Laden, F., & Feskanich, D. (2012). Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three US cohorts. *British Journal of Nutrition*, *108*(10), 1889-1896. https://doi.org/10.1017/s0007114511007409

Carswell, A. T., Oliver, S. J., Wentz, L. M., Kashi, D. S., Roberts, R., Tang, J. C. Y., Izard, R. M., Jackson, S., Allan, D., Rhodes, L. E., Fraser, W. D., Greeves, J. P., & Walsh, N. P. (2018). Influence of vitamin D supplementation by sunlight or oral D3 on exercise performance. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/mss.0000000000001721

Dubnov, G., Foldes, A. J., Mann, G., Magazanik, A., Siderer, M., & Constantini, N. (2006). High prevalence of iron deficiency and anemia in female military recruits. *Military Medicine*, *171*(9), 866-869.

Foulis, S. A., Hughes, J. M., Walker, L. A., Guerriere, K. I., Taylor, K. M., Proctor, S. P., & Friedl, K. E. (2021). Body mass does not reflect the body composition changes in response to similar physical training in young women and men. *International Journal of Obesity (2005)*, *45*(3), 659-665. https://doi.org/10.1038/s41366-020-00730-0

Heath, A. L., Skeaff, C. M., Williams, S., & Gibson, R. S. (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutrition*, *4*(2), 197-206.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., & Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism*, *96*(7), 1911-1930. https://doi.org/10.1210/jc.2011-0385

Israeli, E., Merkel, D., Constantini, N., Yanovich, R., Evans, R. K., Shahar, D., & Moran, D. S. (2008). Iron deficiency and the role of nutrition among female military recruits. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S685-690. https://doi.org/10.1249/MSS.0b013e31818946ae

Jones, B. H., Hauret, K. G., Dye, S. K., Hauschild, V. D., Rossi, S. P., Richardson, M. D., & Friedl, K. E. (2017). Impact of physical fitness and body composition on injury risk among active young adults: a study of Army trainees. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S17-s22. https://doi.org/10.1016/j.jsams.2017.09.015

Karl, J. P., Lieberman, H. R., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2010). 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. *American Journal of Clinical Nutrition*, *92*(1), 93-100. https://doi.org/10.3945/ajcn.2010.29185

Knapik, J. J., Canham-Chervak, M., Hauret, K., Hoedebecke, E., Laurin, M. J., & Cuthie, J. (2001a). Discharges during U.S. Army basic training: injury rates and risk factors. *Military Medicine*, *166*(7), 641-647.

Knapik, J. J., Sharp, M. A., Canham-Chervak, M., Hauret, K., Patton, J. F., & Jones, B. H. (2001b). Risk factors for training-related injuries among men and women in basic combat training. *Medicine and Science in Sports and Exercise*, *33*(6), 946-954.

Knight, J. A., Wong, J., Cole, D. E. C., Lee, T. K., & Parra, E. J. (2017). Predictors of 25-hydroxyvitamin D concentration measured at multiple time points in a multiethnic population. *American Journal of Epidemiology*, *186*(10), 1180-1193. https://doi.org/10.1093/aje/kwx180

Liu, X., Baylin, A., & Levy, P. D. (2018). Vitamin D deficiency and insufficiency among US adults: prevalence, predictors and clinical implications. *British Journal of Nutrition*, *119*(8), 928-936. https://doi.org/10.1017/s0007114518000491

Lutz, L. J., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Vitamin D status, dietary intake, and bone turnover in female soldiers during military training: a longitudinal study. *Journal of the International Society of Sports Nutrition*, *9*(1), 38. https://doi.org/10.1186/1550-2783-9-38

Margolis, L. M., Pasiakos, S. M., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Differential effects of military training on fat-free mass and plasma amino acid adaptations in men and women. *Nutrients*, *4*(12), 2035-2046. https://doi.org/10.3390/nu4122035

McClung, J. P., Gaffney-Stomberg, E., & Lee, J. J. (2014). Female athletes: a population at risk of vitamin and mineral deficiencies affecting health and performance. *Journal of Trace Elements in Medicine and Biology*, *28*(4), 388-392. https://doi.org/10.1016/j.jtemb.2014.06.022

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Nindl, B. C., Young, A. J., & Lieberman, H. R. (2009a). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *American Journal of Clinical Nutrition*, *90*(1), 124-131. https://doi.org/10.3945/ajcn.2009.27774

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Young, A. J., & Lieberman, H. R. (2009b). Longitudinal decrements in iron status during military training in female soldiers. *British Journal of Nutrition*, *102*(4), 605-609. https://doi.org/10.1017/s0007114509220873

McClung, J. P., Marchitelli, L. J., Friedl, K. E., & Young, A. J. (2006). 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*, *25*(1), 64-69.

Ministry of Defence. (2014). *Maximising opportunities for military women in the New Zealand Defence Force*. Wellington: Ministry of Defence.

Ministry of Defence. (2016). *Interim report on the health risks to women in ground close combat roles*. Hampshire: Department of Manning (Army)

Ministry of Health. (2012). *Vitamin D status of New Zealand adults: findings from the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health

Nessvi, S., Johansson, L., Jopson, J., Stewart, A., Reeder, A., McKenzie, R., & Scragg, R. K. (2011). Association of 25-hydroxyvitamin D3 levels in adult New Zealanders with ethnicity, skin color and self-reported skin sensitivity to sun exposure. *Photochemistry and Photobiology*, *87*(5), 1173-1178. https://doi.org/10.1111/j.1751-1097.2011.00956.x

Newlin, M. K., Williams, S., McNamara, T., Tjalsma, H., Swinkels, D. W., & Haymes, E. M. (2012). The effects of acute exercise bouts on hepcidin in women. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(2), 79-88.

Nindl, B. C., Jones, B. H., Van Arsdale, S. J., Kelly, K., & Kraemer, W. J. (2016). Operational physical performance and fitness in military women: physiological, musculoskeletal injury, and optimized physical training considerations for successfully integrating women into combat-centric military occupations. *Military Medicine*, *181*(1 Suppl), 50-62. https://doi.org/10.7205/milmed-d-15-00382

Pierce, J. R., DeGroot, D. W., Grier, T. L., Hauret, K. G., Nindl, B. C., East, W. B., McGurk, M. S., & Jones, B. H. (2017). Body mass index predicts selected physical fitness attributes but is not associated with performance on military relevant tasks in U.S. Army soldiers. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S79-s84. https://doi.org/10.1016/j.jsams.2017.08.021

Suedekum, N. A., & Dimeff, R. J. (2005). Iron and the athlete. *Current Sports Medicine Reports*, *4*(4), 199-202. https://doi.org/10.1097/01.csmr.0000306207.79809.7f

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine joint position statement. Nutrition and athletic performance. *Medicine and Science in Sports and Exercise*, *48*(3), 543-568. https://doi.org/10.1249/mss.0000000000000852

Chapter 2 Literature review

2.1 Introduction

This literature review begins with an overview of basic training in the New Zealand Army, followed by nutrition related health challenges concerning female recruits. Specifically, this review will explore iron metabolism, iron status indicators, and stages of iron deficiency to understand iron status in female recruits and longitudinal changes. In addition, associations between iron status and functional outcomes, such as physical fitness will be investigated. Dietary and non-dietary determinants of iron status will be explored to improve understanding of the potential causes of poor iron status, particularly in a young, physically active population. This review will then explore assessment of vitamin D status, including the controversy around terminology, serum 25-hydroxyvitamin D (25(OH)D) targets, and vitamin D requirements. Determinants of vitamin D status will also be examined. Various dietary assessment tools and methods for determining habitual dietary intake will then be explored, including a review of dietary pattern (DP) analysis. Finally, the changes in physical fitness, body composition, and dietary intake experienced by female recruits during basic training will be examined. This will be supported by a review of body composition assessment methods, with a particular emphasis on the validity of suitable methods for field-based settings; the demands of physical fitness during basic training; and the nutrient requirements of female recruits.

2.2 Basic training in the New Zealand Army

In the New Zealand Army, basic training is a 16-week course designed to take an individual from a civilian to self-disciplined soldier, prepared for further professional training. While recruits pass the required aptitude and physical tests during the recruiting phase, basic training places recruits under controlled stress to ensure they are suitable for a career in the New Zealand Army. Physical activity is embedded in many of the military tasks undertaken during basic training and is supported by regular formal physical training sessions. On completion of basic training, all roles in the New Zealand Army, including combat and special forces, are open to females.

Basic training is a residential course and recruits live in shared barrack accommodation for the duration. All food is provided and typically consists of three main meals and additional snacks. Military dining hall menus and operational ration packs adhere to the New Zealand Defence Force Catering Nutrition Standards. Although these standards are based on the Australian Military Recommended Dietary Intakes (MRDIs) for recruits in both garrison and field settings, the nutrition related health of recruits is not routinely monitored. It is therefore difficult to determine the adequacy of recruits' dietary intake. International literature has identified total energy, protein, carbohydrate, iron, calcium and vitamin D as nutrients of particular concern for the health and physical performance of female recruits.

2.3 Iron

2.3.1 Iron metabolism

Iron is a nutritionally essential trace mineral. The iron content of healthy adults is approximately 3-5g, dependent on diet, sex and health status (Anderson & Frazer, 2017; Daher et al., 2017). Haemoglobin (Hb) within red blood cells contains 60-70% of the body's iron and a further 10% is found in myoglobin. The main role of the protein, Hb, is to transport oxygen from the lungs to the tissues. The life span of Hb equates roughly to the 120-day life of the red blood cell in circulation. Myoglobin is a protein in muscle, responsible for transporting and storing oxygen for use during muscle contraction. The iron containing enzymes, including cytochromes of electron transfer, contain approximately 1% of the body's iron content (Dallman, 1986). The remaining 20-30% of the body's iron is found in the major iron storage compounds, ferritin and haemosiderin, primarily located in the liver, reticuloendothelial cells and erythroid precursors of the bone marrow. Haemosiderin is a degraded form of ferritin. Finally, less than 0.2% of body's iron content is in the plasma transport pool, with the majority bound to transferrin (Tf) (Dallman, 1986).

Iron exists in two valency states: the reduced ferrous form (Fe^{2+}) and the oxidised ferric form (Fe^{3+}) (Prentice, 2017). As it readily accepts or donates electrons, free iron (when it is not bound to protein or other organic molecules) is highly reactive and toxic, making it an efficient catalyst for electron transfer and free-radical reactions (Coffey & Ganz, 2017). Iron is an essential nutrient that is typically very beneficial; however, it can cause oxidant damage if not regulated appropriately (Prentice, 2017).

Iron absorption occurs in the proximal small intestine, the duodenum and upper jejunum. Approximately 2mg is absorbed daily from the diet. This is balanced by normal iron losses of 1-

2mg/day, mainly from desquamation of skin, sloughing of intestinal epithelial cells and blood loss (Wallace, 2016). As the body has no controlled mechanism to excrete excess iron, the body's iron content is balanced by regulating dietary iron absorption (Coffey & Ganz, 2017).

Dietary iron is classified as either haem or non-haem iron. Most haem iron is derived from animal sources and comes from the digestion of Hb and myoglobin. Non-haem iron is found in animal tissue and is the only form found in plants (Anderson & Frazer, 2017). There are alternate pathways for the uptake of haem and non-haem iron into the intestinal enterocytes (Ganz, 2013). Most non-haem iron in the diet is in the ferric form and its absorption requires it to be reduced to the ferrous form by the membrane bound ferric reductase, duodenal cytochrome B (DcytB). This is co-located on the apical brush border membrane of intestinal epithelial cells with the proton-dependent ferrous iron transporter, divalent metal transporter 1 (DMT1) (McKie et al., 2001). Haem iron absorption has not yet been precisely defined; however, once taken up by the enterocyte the haem molecule is catabolised by haem oxygenase to release iron (Daher et al., 2017).

Regulation of iron absorption primarily occurs at the basolateral membrane of the intestinal enterocytes, where release of iron into the systemic circulation occurs via the only known iron exporter, ferroportin (Donovan et al., 2005; McKie et al., 2000). In the intestine, the release of iron requires it to be re-oxidised to ferric iron by a membrane bound ferroxidase, hephaestin, before it can bind with Tf, the iron binding protein in plasma (Vulpe et al., 1999). The release of iron from other cell types, such as macrophages and hepatocytes also require ferroportin and re-oxidation is assisted by another ferroxidase, ceruloplasmin (Harris et al., 1999; Osaki et al., 1966). If iron is not immediately required by the body, it is retained within the enterocytes or macrophages in ferritin. In enterocytes, this will quickly be lost from the body as these cells turn-over every 2-5-days (Anderson & Frazer, 2017; Ganz, 2013).

Transferrin is responsible for distributing iron to all organs, including bone marrow to produce Hb in red blood cells and the liver for storage as ferritin (Daher et al., 2017). Transferrin receptor 1 (TfR1) is located on the cell's surface and is responsible for the uptake of Tf bound iron through endocytosis. The endosomes are then acidified, allowing the release of iron from Tf (Qian & Tang, 1995). The ferrireductase, six-transmembrane epithelial antigen of the prostate 3 (STEAP3) then reduces ferric iron to the ferrous form for export to the cytoplasm of cells from the endosome via DMT1 (Ohgami et al., 2005). Finally, apotransferrin, still bound to TfR1, is recycled back to the cell surface, and released back into circulation (Daher et al., 2017).

The iron released from senescent red blood cells is a major source of iron that can be recycled to produce new red blood cells in bone marrow (Wallace, 2016). Erythrocytes at the end of their lifespan undergo phagocytosis by macrophages of the reticuloendothelial system and are digested in the phagolysosome during this process (Korolnek & Hamza, 2015). Haem is transported across the phagolysosomal membrane via haem-responsive gene 1 (HRG1) and is then metabolised by haem oxygenase in the cytosol to release its iron (Poss & Tonegawa, 1997; White et al., 2013).

Iron resulting from the breakdown of erythrocytes can either be stored temporarily in ferritin within the macrophage or transported back across the plasma membrane to the systemic circulation via ferroportin, which is also on the surface of macrophages (Daher et al., 2017; Wallace, 2016). It is estimated that 20-25mg/day of iron is recycled within the body (Hentze et al., 2004), contributing about 90% of the iron required for erythropoiesis (Coad & Conlon, 2011). The regulation of iron metabolism is illustrated in Figure 2.1.

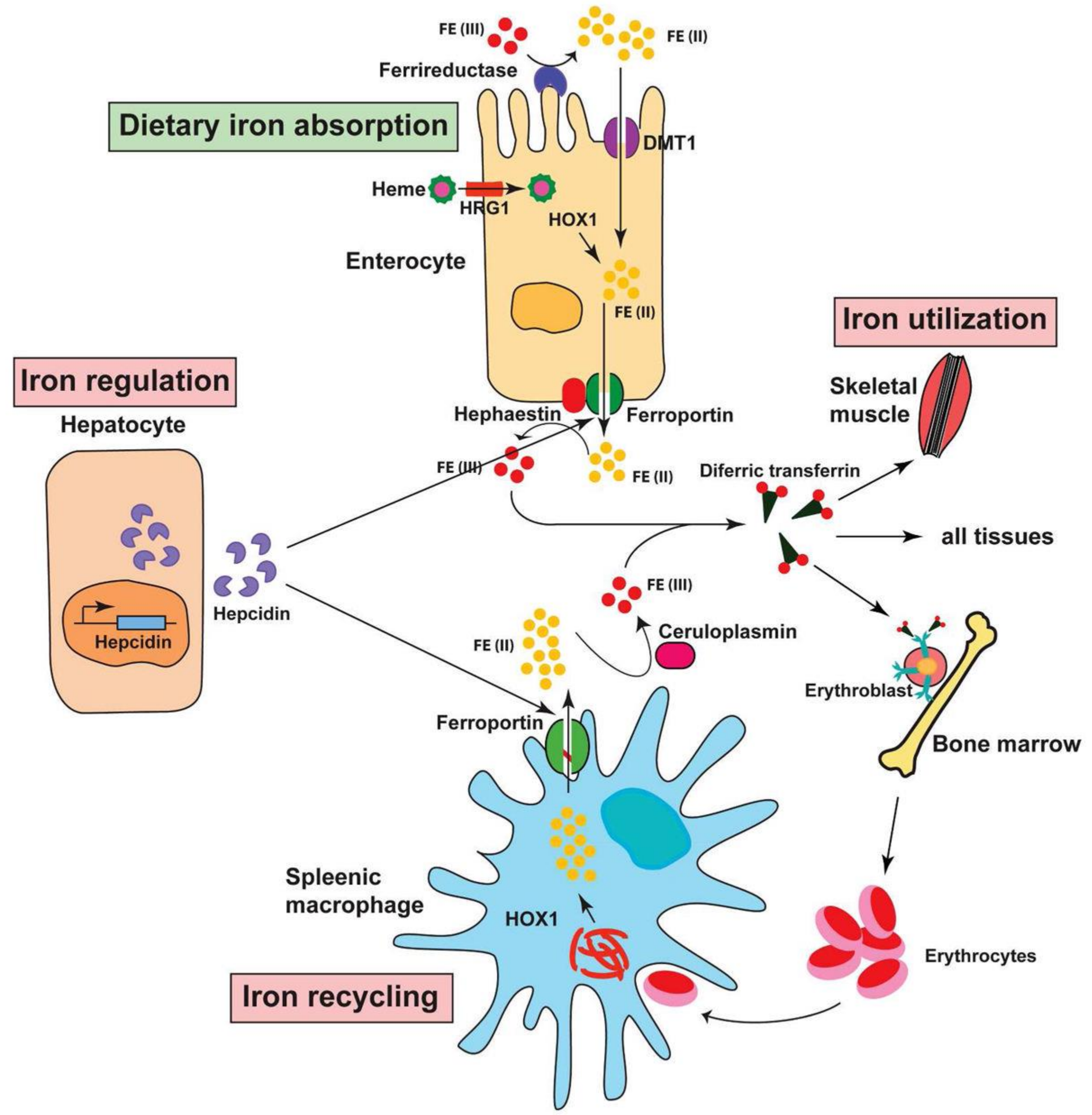


Figure 2.1. Regulation of iron metabolism

Reprinted with permission from Pantopoulos, K., Porwal, S. K., Tartakoff, A., & Devireddy, L. (2012). Mechanisms of mammalian iron homeostasis. *Biochemistry*, *51*(29), 5705-5724. Copyright (2012) American Chemical Society.

Iron homeostasis is maintained through the regulation of absorption, transport, storage and mobilisation of cellular iron to avoid either insufficient or excess iron supply (Daher et al., 2017). This primarily involves hepcidin and ferroportin (Wallace, 2016). Hepcidin is a liver-derived peptide responding to the body’s demand for iron (Krause et al., 2000; Park et al., 2001; Pigeon et al., 2001).

The liver in turn regulates the expression of hepcidin, distributing it to bind with ferroportin, where hepcidin subsequently reduces cellular iron export by internalising and degrading ferroportin (Nemeth et al., 2004). This occurs specifically in macrophages; while in intestinal cells, hepcidin internalises and degrades DMT1 (Brasse-Lagnel et al., 2011; Chaston et al., 2008).

When the body's iron demands are high, hepcidin expression is suppressed, allowing for increased dietary iron absorption and iron recycling from macrophages. This allows more iron to be available for erythropoiesis (Ganz & Nemeth, 2006) and occurs during iron deficiency, hypoxia, haemolysis and following blood loss. It functions as an internal protective mechanism. Conversely, iron overload and inflammation stimulate hepcidin expression, blocking iron's release into circulation (Daher et al., 2017).

2.3.2 Assessment of iron status

Iron deficiency is a condition in which iron stores are depleted, limiting the availability of iron to the systemic circulation and compromising the supply of iron to functional tissue (World Health Organization, 2001). Classification of iron deficiency is traditionally based on laboratory biomarkers and it is important not to rely on single test results (World Health Organization, 2001). Diagnosis is challenging in the context of inflammation (Camaschella, 2015a).

2.3.2.1 Indicators of iron status

- Haemoglobin is an iron-containing protein in red blood cells, which carries oxygen from the lungs to body tissues. Low Hb can indicate iron deficiency anaemia in conjunction with certain iron indicators to improve specificity (World Health Organization and Centers for Disease Control and Prevention, 2007).

- Serum ferritin (SF) indicates the iron stores in the body and its reduction has been identified as the optimal indicator for detecting iron deficiency in the absence of infection or inflammation. As an acute-phase protein, SF is elevated in response to any inflammatory process (World Health Organization, 2001).

- Transferrin saturation (TS) is a measure of how saturated the globular protein Tf is with iron molecules being transported to the cellular transferrin receptor sites (Daher et al., 2017; World Health Organization and Centers for Disease Control and Prevention, 2007). Transferrin saturation is therefore a measure of iron available for erythropoiesis, independent of iron stores and is reduced during iron deficiency. It is susceptible to diurnal and post-prandial variations and may decrease in inflammation (Camaschella, 2017).

- Erythrocyte distribution width (RDW) is a measure of the range in the volume and size of erythrocytes. It is elevated in iron deficiency and may contribute to the overall assessment; however, the change occurs late due to the long lifespan of red blood cells (Camaschella, 2015b, 2017).

- Soluble transferrin receptor (sTfR) closely reflects iron stores and increases during iron deficiency as it is cleaved from the reticulocyte membrane (Punnonen et al., 1997). It is a sensitive indicator of functional or tissue iron deficiency and is not affected by inflammation (Gibson, 2005). It can therefore help distinguish between anaemia that is caused by iron deficiency and that caused by inflammation or chronic disease (World Health Organization, 2001).

- Zinc protoporphyrin (ZnPP) indicates the adequacy of iron supply to bone marrow for production of red blood cells. Zinc is substituted for iron in red blood cell protoporphyrin when iron stores are diminished and circulating iron in bone marrow is depleted (Lynch, 2012). It is not a specific measure for iron deficiency and should be used in combination with other indicators of iron status.

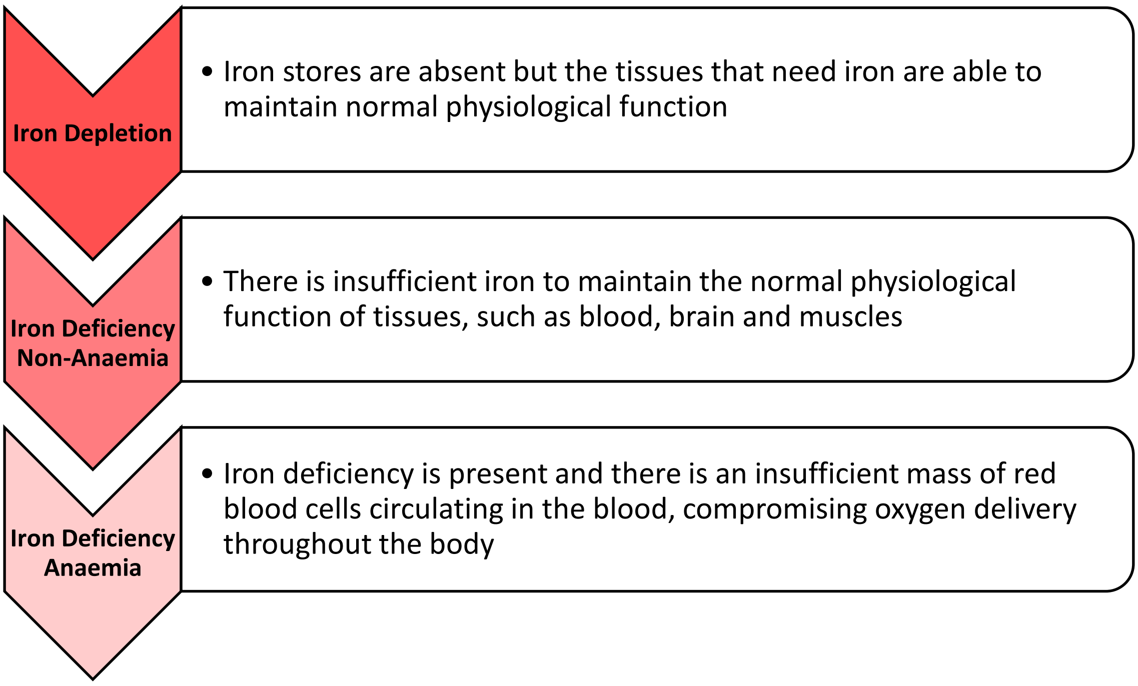
- C-reactive protein (CRP) is an acute phase protein that is elevated with infection and inflammation. It should be measured concurrently to identify inflammation and help interpret data on SF (World Health Organization and Centers for Disease Control and Prevention, 2007).

- Serum hepcidin is a measure of the concentration of hepcidin in blood and is decreased in iron deficiency. It can be upregulated with recent exercise and inflammation or infection (Galesloot et al., 2011). Serum hepcidin is primarily only measured in research at present and has not yet entered routine laboratory testing (Camaschella, 2017).

Due to the limitations of each test, multi-variable models are recommended when defining iron deficiency to avoid misclassifications (Looker et al., 1995; World Health Organization and Centers for Disease Control and Prevention, 2007). As several indicators are also acute-phase reactants to inflammatory cytokines, caution must be exercised when interpreting study results.

2.3.2.2 Stages of iron deficiency

Iron deficiency is assessed on a continuum. This is commonly considered to occur over three stages, referred to as i) iron depletion, ii) iron deficiency non-anaemia and iii) iron deficiency anaemia, as described in Figure 2.2. While SF<15µg/L is considered reflective of depleted iron stores for adults, this cut-off has not been universally adopted and SF ranging from 12-30µg/L has been used in the literature (World Health Organization, 2020). There are no well-defined diagnostic criteria for iron deficiency non-anaemia. The lower limit of clinical reference ranges is often 10-20µg/L (Soppi, 2018). Other indicators of iron status, including TS, RDW, sTfR and ZnPP are useful in the assessment of iron deficiency non-anaemia. Iron deficiency anaemia is characterised by reduced production of erythrocytes and when Hb is two standard deviations below the mean for a normal population of the same age, sex, and altitude. In females, this equates to Hb<120g/L (World Health Organization, 2001).



- Iron stores are absent but the tissues that need iron are able to maintain normal physiological function
- There is insufficient iron to maintain the normal physiological function of tissues, such as blood, brain and muscles
- Iron deficiency is present and there is an insufficient mass of red blood cells circulating in the blood, compromising oxygen delivery throughout the body

Figure 2.2. Stages of iron deficiency
(World Health Organization and Centers for Disease Control and Prevention, 2007)

2.3.3 Iron requirements

The recommended dietary intake (RDI) for iron is 18mg/day for women aged 19-50-years living in New Zealand, which is based on estimates of menstrual losses and basal losses through faeces, urine, sweat and skin (Department of Health and Ageing and Ministry of Health, 2006). Considering the additional mechanisms of iron loss associated with exercise, it is likely those participating in regular, intense physical activity require higher iron requirements than the general population. Currently, no athlete specific recommendations exist. Milman (2019) reported that most women living in European countries do not meet the country specific recommended intakes for dietary iron. A median or mean dietary iron intake of <10mg/day was reported in Belgium, Bosnia, Denmark, Hungary, Italy, Northern Ireland, Serbia, Scotland, Sweden, Switzerland, United Kingdom and Wales (Milman, 2019).

2.3.4 Determinants of iron status in non-pregnant premenopausal women living in developed countries

Multiple factors affect iron status and can cause iron deficiency. Table 2.1 highlights the key factors that affect iron status in healthy and physically active non-pregnant, premenopausal women living in developed countries. Simultaneous occurrence of these factors is also common in individuals and can confound the risk (Camaschella, 2015a). To maintain iron balance, absorbed dietary iron must match the body's iron losses, any increased iron requirements and compensate for any decreased iron absorption (Alleyne et al., 2008; Hurrell & Egli, 2010).

The iron status of an individual is the dominant influence over the absorption of iron, particularly non-haem iron. Using SF as an indicator of iron status, there is an inverse association with iron absorption as a protective feedback mechanism (Hurrell & Egli, 2010). The individual's iron status also affects the bioavailability, with iron deficiency enhancing absorption (Miret et al., 2003).

Table 2.1. Causes of iron deficiency in non-pregnant, physically active premenopausal women living in developed countries

Increased iron loss	Menstrual blood loss Blood donation Non-steroidal anti-inflammatory drugs Gastro-intestinal bleeding Haematuria Sweating Haemolysis
Decreased dietary iron intake	Diets with poor iron bioavailability, including low intakes of meat, fish, and chicken, vegetarian, or vegan diets Food insecurity Low energy intake
Decreased iron absorption from the gut	Inflammatory response to exercise and obesity, which stimulates hepcidin The presence of dietary inhibitors of iron absorption
Increased iron requirements	Rapid growth (adolescence)

(Alleyne et al., 2008; Camaschella, 2015a, Hurrell & Egli, 2010)

2.3.4.1 Dietary determinants

<u>Dietary iron bioavailability</u>

Iron bioavailability (how much dietary iron is absorbed and utilised for healthy body functions) is more important than total dietary iron intake in determining iron absorption and iron status (Hallberg & Hulthén, 2000). While haem iron only contributes 10-15% towards total iron intake in an omnivorous diet, due to its tight sequestration within a protoporphyrin ring (Anderson & Frazer, 2017), its absorption rate is 15-35% (Carpenter & Mahoney, 1992). In contrast, most non-haem iron is not tightly sequestered. Although non-haem iron is commonly the largest contributor of dietary iron intake, its absorption rate is typically less than haem iron and is affected by the presence of dietary inhibitors and enhancers of iron absorption (Hurrell & Egli, 2010). Depending on these factors, approximately 2-20% of non-haem iron from a single meal is absorbed (Beard & Tobin, 2000).

Meat, fish, and poultry / haem iron

Cheng et al. (2013) investigated the effects of a high and low red meat diet with approximately 300g and 80g of lean meat per day, respectively for 12-months. Following the intervention period, SF in the high red meat group was significantly greater (*P*=0.021). In a cross-over study design where participants adhered to a non-vegetarian diet of 184g meat (beef and chicken) per day and a lacto-ovo-vegetarian diet for 8-weeks each, non-haem iron absorption from the lacto-ovo-vegetarian diet was 1.1% compared to 3.8% from the non-vegetarian diet (*P*<0.01), however no substantial changes in biomarkers of iron status were observed (Hunt & Roughead, 1999). Tetens et al. (2007) reported a meat-based diet (median daily meat/fish intake of 152g) maintained SF and Hb over a 20-week study period, while a vegetable-based diet (median daily meat/fish intake of 31g) resulted in a significant reduction in SF (*P*<0.001) and Hb (*P*<0.003). As the total dietary iron content was similar between the two groups, Tetens et al. (2007) highlighted the importance of iron bioavailability for the maintenance of iron status. In a 16-week randomised controlled trial by Blanton (2013) participants consumed 85g of a beef or non-beef (chicken, turkey, pork, and ham) protein lunch three times weekly. At the end of the intervention, the beef group had significantly greater SF and Hb than the non-beef group (*P*<0.0001).

Observational studies in premenopausal women in New Zealand have reported intake of meat (Lim et al., 2020) and meat/fish/poultry (Heath et al., 2001a) as the only dietary factors associated with iron stores. Lim et al. (2020) reported that participants with iron sufficiency (SF≥30µg/L and Hb≥120g/L) consumed meat 9.2 times per week compared to 6.3 times for participants with iron insufficiency (SF<30µg/L and Hb<120g/L or ≥120g/L) (*P*=0.006). Heath et al. (2001a) reported that participants with iron sufficiency (SF≥20µg/L and Hb≥120g/L) consumed 111g of meat/fish/poultry per day compared to 86g for participants with iron insufficiency (SF<20µg/L and Hb≥120g/L) (*P*<0.01). However, when meat intake was entered into a multiple linear regression analysis, it was only associated with SF in participants of New Zealand European ethnicity (Lim et al., 2020).

Overall, the literature for premenopausal women supports a role for animal flesh consumption in the improvement and maintenance of iron status. While experimental studies differed in intervention type and duration, the intake of meat, fish and poultry is generally associated with significant improvements in iron status.

Enhancers and inhibitors of non-haem iron

Iron bioavailability can be positively or negatively influenced by the presence of nutritional components naturally occurring in food. Ascorbic acid (Lynch & Cook, 1980) and unidentified factors within meat, fish and poultry (Cook & Monsen, 1976) are known to enhance non-haem iron absorption within a mixed meal. Conversely, phytates (Iqbal et al., 1994), polyphenols (Hurrell et al., 1999) and calcium (Hallberg et al., 1991) are considered inhibitors of iron absorption. Calcium is unique as it is thought to negatively affect absorption of both non-haem and haem iron (Hallberg et al., 1991).

Two randomised controlled trials in New Zealand have investigated dietary approaches to treat suboptimal iron status in premenopausal women. Heath et al. (2001b) reported that SF in the supplement and dietary intervention groups increased by 59% ($P<0.001$) and 26% ($P=0.068$), respectively over 16-weeks in comparison to the placebo group. Suboptimal iron status was defined as SF<20µg/L and Hb>120g/L. The dietary intervention group received individual dietary advice by a registered dietitian encouraging an increased intake of iron-rich foods, increased intake of non-haem iron absorption enhancers, decreased intake of non-haem iron absorption inhibitors, modified timing of non-haem enhancers and inhibitors and cooking with a cast-iron pan. The supplement group were requested to take one tablet daily, containing 50mg of elemental iron, with a meal. Beck et al. (2011) reported that adding kiwifruit, rich in ascorbic acid, lutein and zeaxanthin to an iron-fortified breakfast cereal can improve the iron status of women with SF≤25µg/L and Hb≥115g/L, over 16-weeks. This finding was compared to participants adding banana, with negligible amounts of the non-haem iron absorption enhancers present in kiwifruit. For iron status indicators in the kiwifruit group compared to the banana group, SF increased (*P*<0.001) and sTfR decreased (*P*<0.001).

Most observational studies report no association between iron status and daily ascorbic acid intake (Asakura et al., 2009; Blanco-Rojo et al., 2011; Heath et al., 2001a; Leonard et al., 2014; Pynaert et al., 2009; Ramakrishnan et al., 2002; Rangan et al., 1997; Young et al., 2018) or intake of fruits and vegetables, rich in ascorbic acid (Asakura et al., 2009; Blanco-Rojo et al., 2014; Péneau et al., 2008; Young et al., 2018). In young healthy Australian women, no association was observed between SF and baked beans, soybeans, other beans, iron fortified breakfast cereals, rice, pasta, bread or daily dietary fibre intake (Leonard et al., 2014). Exploring iron deficiency, Young et al. (2018) reported no association with grains and cereals in young Australian women, defining suboptimal iron status as SF<20µg/L, while Asakura et al. (2009) reported no association with dietary fibre, phytate or cereals in young Japanese women, defining suboptimal iron status as SF<12µg/L. No association was also reported between SF and phytate (Heath et al., 2001a; Ramakrishnan et al., 2002) or dietary fibre (Blanco-Rojo et al., 2011). Conversely, Péneau et al. (2008) reported a positive association between SF and fibre-poor fruits and vegetables (*P*<0.013). The authors suggested that in a diet consisting of a range of fruits and vegetables, the enhancing and inhibiting effects of ascorbic acid and fibre, respectively, counteract each other, resulting in no meaningful change to iron status.

In women aged 20-23-years in six countries across Europe, a weak, inverse association between dietary calcium intake and SF was reported, regardless of whether calcium was consumed simultaneously with iron or not (van de Vijver et al., 1999). A high calcium intake (approximately 1200mg compared to 400mg) was associated with a 3-fold increase in risk of SF<20µg/L in 15-30-year-olds in Australia (Rangan et al., 1997). More recently, calcium in drinking water was reported to have a weak, inverse association with iron status (Rigas et al., 2018). In contrast, other studies have observed no association between iron status and calcium (Asakura et al., 2009; Blanco-Rojo et al., 2011; Heath et al., 2001a; Leonard et al., 2014; Pynaert et al., 2009; Young et al., 2018) or dairy products (Asakura et al., 2009; Blanco-Rojo et al., 2014; Pynaert et al., 2009; Rigas et al., 2014; Young et al., 2018).

Most studies have reported no association between iron status and polyphenol intake (Cade et al., 2005; Ramakrishnan et al., 2002) or tea and coffee intake (Galan et al., 1998; Heath et al., 2001a; Mennen et al., 2007; Rigas et al., 2014). However, tea intake has been negatively associated with SF concentration (Pynaert et al., 2009) and simultaneous consumption of tea has decreased non-haem iron absorption (Ahmad Fuzi et al., 2017).

Studies demonstrating the manipulative effects of dietary components to enhance or inhibit iron absorption have primarily been conducted in single meal studies. While experimental studies show a role for ascorbic acid improving iron status in premenopausal women, most observational studies have reported no association between iron status and ascorbic acid or fruits and vegetables. In addition, no association has generally been observed with phytate or dietary fibre, and polyphenols or tea and coffee. In the Iron and Health report, released by the United Kingdom's Scientific Advisory Committee on Nutrition (2010) it is suggested that these effects do not substantially influence iron status in the context of long-term, well-balanced diets. Dietary interventions that focus on a range of approaches to enhance iron bioavailability appear more effective than interventions that focus on individual foods or nutrients.

2.3.4.2 Dietary patterns and associations with iron status

Most studies investigating associations between dietary intake and iron status have focused on individual foods and nutrients. However, given that people consume a variety of foods that contain a range of nutrients, the association between DPs and iron status may present a clearer picture than the role of a single food or nutrient (Hu 2002). In a healthy population of premenopausal New Zealand women (*n*=375), the risk of suboptimal iron status (SF<20µg/L and Hb<120g/L or ≥120g/L) was reduced for those following a 'meat and vegetable' DP (OR = 0.589, 95% CI = 0.422, 0.820, *P*=0.002) and increased for those following a 'milk and yoghurt' DP (OR = 1.502, 95% CI = 1.153, 1.957, *P*=0.003) (Beck et al., 2013). The 'meat and vegetable' DP was categorised by a greater frequency of intake of beef, chicken, capsicum, broccoli, carrots, and lettuce. The 'milk and yoghurt' DP was categorised by a greater frequency of intake of milk as a drink, milk added to food and yoghurt. While the evidence for calcium's negative effect on iron absorption remains weaker than other iron inhibitors, the authors stated the pattern's association with an increased risk of suboptimal iron status remained after consumption frequency of meat, poultry and fish were controlled for. This suggests an independent effect of the 'milk and yoghurt' DP on iron status, rather than the result of replacing meat with dairy, as previously suggested by Heath et al. (2001a).

In this same study population group, non-dietary factors, including blood donation, being Asian and a history of iron deficiency were stronger predictors of suboptimal iron status than DPs. A change in one standard deviation in the factor score above the mean for the 'milk and yoghurt' DP increased the risk of suboptimal iron status (OR = 1.44, 95% CI = 1.08, 1.93, *P*=0.012) with similar odds to an additional day of menstrual blood loss (MBL) (OR = 1.38, 95% CI = 1.12, 1.68, *P*=0.002). While following a 'meat and vegetable' DP decreased the odds of suboptimal iron status, this was only significant in women with children (OR = 0.17, 95% CI = 0.08, 0.39, *P*<0.001).

2.3.4.3 Non-dietary determinants

This review will explore associations between iron status and blood loss and the inflammatory response to exercise and obesity. The review will be limited to non-pregnant, premenopausal women living in developed countries.

Blood donation

During the standard donation of 450-500mL of blood, an estimated 200-250mg of iron is lost (Kiss et al., 2015; Reddy et al., 2020). Beck et al. (2014) reported that blood donation in the previous year increased the odds of suboptimal iron status (SF<20µg/L) by six-fold, compared to a non-donor (*P*<0.001). Similarly, Pynaert et al. (2009) observed blood donation within the previous year was significantly associated with reduced SF compared to women who had not donated in the previous year. Blood donation was the strongest determinant of iron stores for both studies (Beck et al., 2014; Pynaert et al., 2009). While Heath et al. (2001a) reported blood donation within the previous 4-12-months was not statistically significant compared to women who did not donate blood, donation within the previous 4-months was the strongest risk factor for SF<20µg/L.

The REDS-II Donor Iron Status Evaluation study in the United States observed that donation frequency is the strongest predictor of iron status. Women with an inter-donation interval less than 19-weeks were at greater risk of SF<12µg/L compared to women with at least 26-weeks since their last blood donation (Cable et al., 2012). Similarly, in a large British study, women had significant reductions in Hb and SF following a decrease in time between donations from 16-weeks to 14- and 12-weeks (Di Angelantonio et al., 2017). In the ongoing epidemiologic cohort study, the Danish Blood Donor Study, Rigas et al. (2014) reported a higher number of blood donations per year increased the risk of SF<15µg/L (OR = 1.45, 95% CI = 1.33, 1.59, *P*<0.01) in premenopausal women. Rigas et al. (2014) also reported that among high frequency donors (≥9 times in 3-years) premenopausal women were particularly at risk for SF<15µg/L with a prevalence of 38.7%, compared to 9.0% in men and 21.7% in postmenopausal women.

For blood donors, strategies to mitigate the risk of iron deficiency include pre-donation measurement of SF (Dijkstra et al., 2019), extending the inter-donation interval and post-donation iron supplementation (Kiss et al., 2015; Rigas et al., 2019). Merkel and Moran (2018) recommend that military recruits avoid blood donation during strenuous training periods, such as basic training.

Menstruation

In eumenorrhoeic women, 30-50mL of blood is lost per menstrual cycle (Dasharathy et al., 2012; Sriprasert et al., 2017). Harvey et al. (2005) observed MBL was the most significant predictor of SF, explaining 11.5% of the unique variance (*P*<0.001) in women in the United Kingdom, while dietary group (red meat, poultry/fish and lacto-ovo-vegetarian) explained only 6.7% (*P*<0.04). In support of these findings, Blanco-Rojo et al. (2014) and Heath et al. (2001a) both reported that low MBL may protect from iron deficiency. Rigas et al. (2014) observed that menstruation ≥5-days increased the risk of SF<15µg/L (OR = 1.56, 95% CI = 1.36, 1.80, *P*<0.01). Beck et al. (2014) observed that each additional day of menstruation increased the risk of SF<20µg/L (OR = 1.38, 95% CI = 1.12, 1.68, *P*=0.002) and that the mean duration of menstruation was 5.4 ± 1.4 days for participants with SF<20µg/L compared to 4.9 ± 1.4 days in participants with sufficient iron stores. Rangan et al. (1997) reported that in 15-30-year-olds, menstruation for >65-days per year (approximately ≥5-days per month) was associated with a 2.5 times increased risk of SF<20µg/L compared to women with fewer menstruating days per year. In contrast, no significant association was reported between SF and menstrual cycle length, period duration or heavy bleeding days in women following vegetarian diets in Spain (Gallego-Narbón et al., 2019) and period length in women in New Zealand (Lim et al., 2020).

Contraceptive use

It is generally accepted that oral contraceptive pill (OCP) use reduces MBL in women. After adjusting for confounders, hormonal contraceptive use (including OCP and hormonal intrauterine devices) was positively associated with SF (*P*<0.001) compared to non-hormonal intrauterine device use in women in Belgium (Pynaert et al., 2009). The protective association of hormonal contraceptives was of a similar magnitude to the negative association with blood donation in the previous year. Harvey et al. (2005) reported that MBL was significantly lower in OCP users compared to those using other contraceptive forms, regardless of dietary intake (*P*<0.001). Conversely, Heath et al. (2001a) found there was no independent association between OCP use and iron deficiency when blood loss volume and duration were controlled for. This suggests the protective effect of OCP use on iron deficiency is explained by reduced MBL, supported by Gallego-Narbón et al. (2019) reporting hormonal contraceptive use was negatively associated with menstrual period length (*P*=0.014) and heavy bleeding days (*P*=0.021).

In contrast, Young et al. (2018) reported that 32.3% of young Australian women (*n*=264) were OCP users and there was no significant difference in OCP use between participants with iron deficiency, iron deficiency non-anaemia or iron replete status. These findings are supported by no significant association between SF and contraception use or type in women following vegetarian diets in Spain (Gallego-Narbón et al., 2019) and contraception type in women in New Zealand (Lim et al., 2020).

Obesity

Inflammation related to adiposity that increases circulating hepcidin and decreases iron absorption is considered the primary link between obesity and iron deficiency (Ellulu et al., 2017; Tussing-Humphreys et al., 2012). Previous studies have reported positive associations between body mass index (BMI) and SF (Milman et al., 2000; Milman & Kirchhoff, 1999; Pynaert et al., 2009). Hu et al. (2017) observed BMI was positively associated with SF (*P*<0.001) in adults in China. However, following adjustment for CRP as a biomarker for inflammation, the association remained. The authors suggested another potential mechanism may be responsible for the association. Conversely, Gallego-Narbón et al. (2019) observed no association between BMI or percent body fat (%BF) and SF. While Cepeda-Lopez et al. (2015) also observed no association between BMI and SF, %BF was positively associated with SF (*P*=0.04).

Exercise

During exercise, blood flow to the gastrointestinal tract is reduced to allow increased blood flow to the body's working muscles and skin. Epithelial cells of the gastrointestinal tract may subsequently be deprived of oxygen, resulting in necrosis and gastrointestinal bleeding (Peters et al., 2001). Haemolysis, the destruction of red blood cells, has been shown to occur with exercise (Miller et al., 1988; Telford et al., 2003). Foot-strike has been identified as the main contributor to haemolysis and is likely affected by the training surface and intensity (Miller et al., 1988). Exercise-induced haematuria, presence of blood in the urine, can occur and foot-strike and bladder trauma are major causes (Shephard, 2016). Bladder trauma has been attributed to high-impact exercise causing bleeding as a result of microscopic lesions of the interior wall (Blacklock, 1977). Sweat is an avenue by which the body may lose iron and the sweating mechanism is stimulated with exercise to assist thermoregulation. For individuals engaging in regular physical activity of high-intensity, high-impact, long duration and/or high frequency, the cumulative effect of these losses may negatively affect iron status (Hinton, 2014; Peeling et al., 2008).

Exercise causes inflammation in the body (Roecker et al., 2005) with the concentration of hepcidin peaking 3-6-hours post-exercise, typically following an increase in IL-6 activity (Newlin et al., 2012; Peeling et al., 2009; Peeling et al., 2017; Peeling et al., 2014; Sim et al., 2012). It has therefore been proposed that during this transient elevation in hepcidin expression post-exercise, dietary iron absorption and iron recycling from macrophages may temporarily be reduced (Peeling, 2010).

Consequently, during periods of heavy training, with regular and successive inflammatory responses, this reduction in iron absorption and recycling during hepcidin expression may further compromise an individual's iron status and contribute to iron deficiency.

Hepcidin

Hepcidin is emerging as a determinant of iron status (Lim et al., 2020; Lim et al., 2016). Hepcidin was the only predictor significantly associated with SF for all ethnicities in a study of premenopausal women in New Zealand (Lim et al., 2020). In premenopausal women in Australia, hepcidin was also reported to be the strongest predictor of SF. Only 20% ($P<0.001$) of the variance in SF was explained by diet, blood donation and MBL; however, this increased to 65% ($P<0.001$) when hepcidin was considered (Lim et al., 2016).

In summary, multiple non-dietary factors appear to negatively affect iron status in premenopausal women. Blood loss through blood donation and menstruation appear well established as strong determinants of suboptimal iron stores. The relationship between obesity and iron status is less clear, complicated by adiposity-related inflammation as the likely mechanism of action. Exercise-induced iron losses and reduced iron absorption are likely occurring simultaneously in physically active individuals and may exacerbate the risk of suboptimal iron status from more overt blood loss.

2.3.5 Iron status in non-pregnant premenopausal women living in developed countries

Iron deficiency is the most common and widespread nutrient deficiency in the world and remains significantly prevalent in developed countries (World Health Organization, 2022). Worldwide, one in three premenopausal women were anaemic in 2011 and iron deficiency is thought to contribute to approximately half of the global burden of anaemia (World Health Organization, 2015).

2.3.5.1 Iron status in New Zealand

The 2008/09 New Zealand Adult Nutrition Survey (University of Otago and Ministry of Health, 2011) reported the prevalence of iron deficiency non-anaemia (SF<12µg/L and ZnPP>60µg/mol) in young women was 10.6% for 15-18-year-olds and 5.2% for 19-30-year-olds, while the prevalence of iron deficiency anaemia (SF<12µg/L, ZnPP>60µg/mol, and Hb<120g/L) was 5.2% for 15-18-year-olds and 1.2% for 19–30-year-olds. Observational studies investigating the iron status of young women in New Zealand are summarised in Table 2.2. Comparisons between the studies are difficult due to varying cut-offs used for determining iron status.

Table 2.2. Observational studies investigating the iron status of premenopausal women in New Zealand

Author (Year)	Characteristics of participants	Iron status classification	Indicator cut-offs	Prevalence (%)
Schaaf et al. (2000)	*n*=896 14-21-years High school students Auckland 46.5% Pacific[a]	Iron deficiency	≥2 of: SF<12µg/L, TS<14%, or RDW>14.5%	10
		Iron deficiency anaemia	≥2 of: SF<12µg/L, TS<14%, or RDW>14.5%, and Hb<120g/L	9
Heath et al. (2001a)	*n*=384 18-40-years Dunedin 95% New Zealand European[b]	Mild iron deficiency	SF<20µg/L Hb≥120g/L	23
		Iron deficiency	SF<12µg/L	4
		Iron deficiency anaemia	SF<12µg/L and Hb<120g/L	2
Beck et al. (2013)	*n*=375 18-44-years Auckland 75% New Zealand European[b]	Suboptimal iron status	SF<20µg/L and Hb<120g/L or ≥120g/L	19
		Iron deficiency anaemia	SF <20µg/L and Hb<120g/L	5

[a] Participants excluded with CRP>6mg/L.
[b] Participants excluded with CRP>10mg/L.

2.3.5.2 Iron status of physically active premenopausal women

The prevalence of both iron deficiency non-anaemia and iron deficiency anaemia has widely been reported to be higher in physically active women and competitive athletes when compared to the general population (DellaValle & Haas, 2011; Dubnov & Constantini, 2004; Landahl et al., 2005; Parks et al., 2017; Sinclair & Hinton, 2005). Table 2.3 presents a summary of observational studies investigating iron status in physically active women in comparison to age-matched controls. These studies indicate the prevalence of iron deficiency anaemia is greater in athletes than controls. While the prevalence of iron deficiency non-anaemia is similar between athletes and controls, that appears only the case when SF is used as a single marker of iron status. In the absence of an inflammatory marker, such as CRP, SF alone is an unreliable measure of iron status in a physically active population at risk of inflammation. When other indicators, such as TS and sTfR are utilised, it appears athletes have progressed beyond iron depletion to functional or tissue iron deficiency, more indicative of iron deficiency non-anaemia. Comparisons between studies are challenging due to wide-ranging cut-offs for iron status indicators, age range, recreational versus competitive training status, type of physical activity, variable dietary intake and lack of data on MBL.

Table 2.3. Observational studies investigating iron status in physically active women in comparison to age-matched controls

Author (Year) Country	Characteristics of participants	Iron status classification	Indicator cut-offs	Prevalence (%)	
				Athletes	**Control**
Malczewska et al. (2000) Poland	16-20-years Competitive college endurance athletes *n*=126, Mean exercise: 15hrs/week Sports: rowing, swimming, running, X-country skiing, modern pentathlon Control *n*=52, Low to moderate activity	Iron deficiency non-anaemia	SF<20µg/L	26	50
Di Santolo et al. (2008) Italy	23.5 ± 4.7 years Recreational athletes *n*=70, Mean exercise: 11.1 ± 2.6 hrs/week Sports: volleyball, soccer, martial arts, skiing, cycling, others Control *n*=121, Mean exercise: 0.7 ± 0.9 hrs/week	Iron deficiency non-anaemia	SF<12µg/L SF<20µg/L SF<30µg/L	27 50 64	30 53 69
		Iron deficiency anaemia	Hb<120g/L and SF<12µg/L	9	6
Woolf et al. (2009) United States	Competitive college athletes *n*=28, 20.0 ± 2.0 years, Training: ≥12 hrs/week Sports: swimming, volleyball, tennis, X-country runners, softball, basketball, hockey Control *n*=28, 24.0 ± 3.0 years, Training: ≤2.0 hrs/week	Iron deficiency non-anaemia	SF<12µg/L TS<16% sTfR>8.0mg/L sTfR:F>4.5	21 29 25 50	18 14 3 18
		Iron deficiency anaemia	Hb<120g/L	7	0
Sandstrom et al. (2012) Sweden	Competitive high school athletes *n*=57, 16.8 ± 0.9 years, Training: 8-15 hrs/week Sports: handball, soccer, tennis, golf, skating, wrestling Control *n*=92, 17.1 ± 0.9 years, Training: ≤2.0 hrs/week	Iron deficiency non-anaemia	Hb>120g/L and SF<16µg/L	52	48
		Iron deficiency anaemia	Hb<120g/L	9	3

SF, serum ferritin; Hb, haemoglobin; TS, transferrin; sTfR, soluble transferrin receptor; sTfR:F, soluble transferrin receptor:ferritin index.

2.3.5.3 Iron status during basic training

Iron status at the beginning of basic training only has been investigated in female recruits in the Israel Defense Forces. Dubnov et al. (2006) reported 15% of women recruited for active military duty had iron deficiency non-anaemia (SF<12µg/L and TS<15%) and 7% had iron deficiency anaemia (SF<12µg/L, TS<15% and Hb<120g/L). A total of 77% had a SF<20µg/L, defined as iron depletion. Using the same iron status definitions, Israeli et al. (2008) reported a higher prevalence of iron deficiency non-anaemia in combatant recruits (29.8%) and non-combatant recruits (27.2%) and iron deficiency anaemia in combatant recruits (12.8%) and non-combatant recruits (17.4%) at the beginning of basic training.

Female recruits participate in heavy and sustained physical activity during basic training. In a cross-sectional study, McClung et al. (2006) defined iron deficiency non-anaemia by the presence of at least two of the following three abnormal indicators: SF<12µg/L, TS<16% or RDW>15%. Iron deficiency anaemia was defined by the presence of at least two of these abnormal indicators and Hb<120g/L. In these female recruits completing 8-weeks of basic training, iron deficiency non-anaemia increased from 13.4% at the beginning to 32.8% at the end and iron deficiency anaemia increased from 5.8% to 20.9%. All iron status indicators measured (SF, TS, RDW and Hb) were significantly ($P<0.05$) diminished during basic training. A limitation of this study by McClung et al. (2006) is that due to the cross-sectional design, the difference in iron status between the two separate groups studied at the beginning and end of basic training may be the result of iron-related variables other than basic training. However, a longitudinal study subsequently confirmed a significant decline ($P\leq0.01$) in iron status indicators (SF, TS, RDW and sTfR) during basic training. Haemoglobin also had a significant change ($P\leq0.01$), but in a positive direction (McClung et al., 2009a). Using the same iron status definitions described above for McClung et al. (2006), the prevalence of iron deficiency non-anaemia in female recruits receiving the placebo capsules in an iron supplementation trial increased from 17%

at the beginning of basic training to 28% at the end (McClung et al., 2009b). There was a reduction in the prevalence of iron deficiency anaemia in the placebo group from 21% to 16%. While longitudinal changes in iron status have been investigated in female recruits, they have been limited to the United States and 9-weeks duration (McClung et al., 2009b; McClung et al., 2009a; McClung et al., 2006).

2.3.6 Iron status and physical fitness in non-pregnant premenopausal women

Given the increased prevalence of iron deficiency non-anaemia and iron deficiency anaemia in physically active women and the diminishing iron status in female recruits during basic training it is prudent to investigate associations between iron status and functional outcomes, such as physical fitness.

2.3.6.1 Iron deficiency anaemia and aerobic fitness

Iron deficiency anaemia, characterised by low Hb concentration, reduces oxygen delivery to working muscles, decreasing aerobic capacity (Garvican et al., 2011). This is the primary mechanism for reduced aerobic fitness due to iron deficiency anaemia (Woodson et al., 1978), which has been well documented to cause fatigue and decrease maximal and submaximal work capacity (Celsing et al., 1986; Celsing & Ekblom, 1986; Gardner et al., 1977; Lukaski et al., 1991; Tufts et al., 1985).

2.3.6.2 Iron deficiency non-anaemia and aerobic fitness

Associations between iron deficiency non-anaemia and aerobic fitness are not as well described as iron deficiency anaemia. In previously untrained women, an association between sTfR and aerobic fitness was identified during time trials on a cycle ergometer (Brownlie et al., 2002; Brownlie et al., 2004; Hinton et al., 2000). Improvements in sTfR following iron supplementation were significantly associated with improvements in relative maximal aerobic capacity (Brownlie et al., 2002) and percent of maximal aerobic capacity utilised during the performance assessments (Brownlie et al., 2004; Hinton et al., 2000). Brownlie et al. (2002) observed an association between TS and aerobic capacity, particularly in subjects with an elevated concentration of sTfR. In addition, Brownlie et al. (2002) and Hinton et al. (2000) demonstrated that improvements in iron status following iron supplementation increased oxidative capacity at the tissue level. This subsequently improved maximal work capacity and endurance performance, particularly when the concentration of sTfR approached the cut-off values used for identifying iron deficiency. These findings suggest that indicators of tissue-iron deficiency, such as sTfR and TS, are more closely associated with aerobic fitness compared with SF. Brownlie et al. (2004) proposed that tissue iron deficiency impairs the ability of mitochondria and myoglobin to adapt in response to aerobic training. Measures of sTfR and TS can therefore be used to distinguish between iron depletion and functional iron deficiency, which appears detrimental to physiological adaptations in endurance capacity.

In contrast, other studies have observed no beneficial effects of iron supplementation on aerobic fitness measures, despite significant improvements in iron stores when compared to participants receiving a placebo (Burden et al., 2015; Peeling et al., 2007). Key differences exist between these two studies and those reporting performance benefits. Burden et al. (2015) used SF<30µg/L and Peeling et al. (2007) used SF<35µg/L as the cut-offs for defining iron deficiency non-anaemia and supplemented for 4-weeks. Hinton et al. (2000), Brownlie et al. (2002) and Brownlie et al. (2004) used SF<16µg/L and supplemented for 6-weeks. Studies have observed that participants with the lowest baseline iron status demonstrate the greatest improvements in measures of aerobic fitness (Brownlie et al., 2002; Brownlie et al., 2004; DellaValle & Haas, 2014; Hinton et al., 2000; Hinton & Sinclair, 2007). It is therefore possible that the baseline iron status of the study groups investigated by Burden et al. (2015) and Peeling et al. (2007) were not depleted enough to illicit performance improvements. Both Burden et al. (2015) and Peeling et al. (2007) incorporated physical tests to exhaustion to measure endurance, which are not reflective of how an athlete typically completes a physical task (time to completion) and are highly dependent on subject motivation.

Multiple variables within these studies limit the ability to reach a conclusion on the association between iron deficiency non-anaemia and aerobic fitness. These include, definitions of iron deficiency non-anaemia, baseline iron status, supplementation route/dose/frequency and consumption advice, measures of aerobic fitness, exercise training during the study, baseline training status, study duration, dietary intake, menstrual cycle and diurnal variation for blood collection and performance measures.

2.3.6.3 Iron deficiency non-anaemia and anaerobic fitness

In elite netballers with SF<40µg/L and Hb>125g/L, there was no effect of a short course of iron supplementation on vertical jump, cycle ergometer sprint test or multi-stage shuttle run performance. This was despite mean SF increasing from 22 to 61µg/L (Blee et al., 1999). It has been suggested that the predominantly anaerobic power-based tests were not sensitive or specific enough to demonstrate the benefits of iron supplementation. In addition, tasks that primarily utilise the aerobic energy system would more likely be associated with performance improvements (Peeling et al., 2008).

2.3.7 Iron status and physical fitness in females during basic training

In female recruits in the United States Army, McClung et al. (2009a) observed that all iron status indicators, excluding Hb, declined during basic training. At the beginning and end of 9-weeks of basic training, Hb and RDW were negatively and positively associated with 3.2km running time ($P \leq 0.05$), respectively. The change in sTfR throughout basic training was positively associated with 3.2km running time performed at the end of basic training ($P \leq 0.05$). The association between Hb and run time was due to the well described effect of reduced oxygen delivery to tissue on aerobic performance. The strongest association between sTfR and aerobic fitness was observed for individuals with elevated sTfR approaching the cut-off values for iron deficiency.

In a further study of female recruits in the United States Army, McClung et al. (2009b) stratified 3.2km run time at the end of 8-weeks of basic training by iron status group at baseline: iron-normal, iron deficiency non-anaemia and iron deficiency anaemia. The iron deficiency anaemia group had significantly slower run times ($P<0.05$). There was also a between-group difference for run time between the placebo and iron supplemented groups, in the participants with iron deficiency anaemia at baseline ($P\leq0.001$) (McClung et al., 2009b). This indicated that female recruits enlisting with iron deficiency anaemia, who received iron supplementation during basic training, ran faster than those females who did not receive supplementation. Both studies had a relatively short duration with respect to assessing changes in iron status indicators and were limited to assessment of running performance alone.

Female recruits entering basic training in the United States Air Force had their physical fitness assessed at the beginning and end of the 8-week course by a 2.4km timed run, followed by 1-minute of maximum push-ups and 1-minute of maximum sit-ups. Despite supplementing trainees identified as having iron deficiency anaemia (Hb<120g/L) those trainees had slower run times and completed fewer push-ups and sit-ups than their non-anaemic counterparts ($P\leq0.001$). This level of significance was observed at both the baseline and end time points (Myhre et al., 2015). Limitations of this study include the lack of follow-up of changes in Hb and no assessment of iron deficiency non-anaemia and its effects on physical fitness.

2.3.8 Conclusion

The iron status of physically active premenopausal women is compromised by both iron losses and reduced iron absorption induced by exercise (McClung et al., 2014). Iron deficiency non-anaemia and iron deficiency anaemia have been reported in female recruits at the beginning of basic training, with the prevalence increasing throughout basic training (McClung et al., 2009b; McClung et al., 2009a; McClung et al., 2006). No studies have established a situational awareness of iron status in female recruits in the New Zealand Army. In addition, no studies have measured changes in iron status beyond 9-weeks, to reflect changes that are closer to the 120-day lifespan of erythrocytes.

The association between impaired aerobic fitness and diminished Hb has been clearly demonstrated in female recruits (McClung et al., 2009a; Myhre et al., 2015). In addition, iron status indicators of functional or tissue iron deficiency, such as diminished sTfR and RDW have been associated with impaired aerobic fitness (McClung et al., 2009a). However, the effects of diminished iron status on physical fitness in female recruits, particularly with more subtle changes in iron status that fall outside of the clinical diagnostic parameters, remains unclear.

Multiple dietary and non-dietary factors have been identified as determinants of iron status in premenopausal women. Despite the prevalence of suboptimal iron status in female recruits, no studies have simultaneously explored associations between iron stores, dietary and non-dietary determinants.

2.4 Vitamin D

2.4.1 Vitamin D sources

Vitamin D is a fat-soluble vitamin, which in its active form is a secosteroid hormone to a wide range of biological processes (Dahlquist et al., 2015). There are two different sources of vitamin D for humans. First, endogenous production of vitamin D_3 (cholecalciferol) occurs when 7-dehydrocholesterol (7-DHC) in the skin absorbs ultraviolet beta (UVB) radiation in the spectrum of 280-315nm (Holick, 2017). This forms previtamin D_3, which is then rapidly isomerised to the thermodynamically stable vitamin D_3 (Holick, 2011). Vitamin D_3 is then ejected out of the plasma membrane into the extracellular space and diffuses into the dermal capillaries (Holick et al., 2007).

Sun exposure is the predominant source of vitamin D_3 for most people (Holick, 2017; Looker et al., 2011; Wacker & Holick, 2013). The exogenous supply of vitamin D from dietary sources, including fortified foods and dietary supplements, comes in two forms. The plant-based vitamin D_2 (ergocalciferol) is produced by UVB irradiation of the ergosterol in plants and fungi, such as yeast and sun-dried mushrooms (Bikle, 2014). It has also been used in the fortification of some foods, such as milk (Holick, 2017). Small quantities of the more bioavailable animal-based source, vitamin D_3, naturally occur in fatty fish (e.g., salmon, sardines, mackerel), cod liver oil and egg yolks (Holick, 2011). Dietary sources of vitamin D are predominantly absorbed into the lymphatic system from the small intestine via chylomicrons, which then enter the venous blood supply (Pludowski et al., 2018). As both the D_2 and D_3 forms are activated, bind to the vitamin D receptor (VDR) and function in the same way (Dirks et al., 2018), this review will focus on the metabolism and mechanism of action of vitamin D_3.

2.4.2 Vitamin D metabolism

Vitamin D, bound to a vitamin D-binding protein (DBP), is transported in the bloodstream to the liver where it is hydroxylated to 25(OH)D by several vitamin D-25-hydroxylases (CYP2R1, CYP27A1, CYP2C11, CYP2J3, CYP3A4) (Charoenngam et al., 2021; Dirks et al., 2018; Haddad et al., 1993). The biologically inert 25(OH)D then continues to the kidneys where it is hydroxylated to the active hormone, 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) by CYP27B1 (1α-hydroxylase) (Bikle, 2014; Dirks et al., 2018).

Although the kidneys are the main source of 1,25$(OH)_2$D, a number of extra-renal tissues also express the CYP27B1 enzyme to produce 1,25$(OH)_2$D. These include, but are not limited to, skin, breast, lungs, intestine, prostate, thyroid, placenta and cells of the immune system (Bikle, 2014). This local production is likely responsible for the non-skeletal health benefits reported for vitamin D (Holick, 2011).

To avoid excess 1,25$(OH)_2$D, both 25(OH)D and 1,25$(OH)_2$D can be degraded to 1,24,25-trihydroxyvitamin D (1,24,25$(OH)_3$D) and 24(R),25-dihydroxyvitamin D (24,25$(OH)_2$D) by further hydroxylation from CPY24A1 (24-hydroxylase) (Bikle, 2014; Dahlquist et al., 2015; Dirks et al., 2018). This metabolic process is tightly regulated in the kidneys by parathyroid hormone (PTH) as a signal of calcium status, fibroblast growth factor 23 (FGF23) as a signal of phosphate status and the direct negative feedback by 1,25$(OH)_2$D itself to stimulate or suppress the two cytochrome P450 enzymes, CYP27B1 and CYP24A1. This in turn controls the expression of 1,25$(OH)_2$D for appropriate calcium homeostasis, bone metabolism and gene modulation in virtually every tissue (Dirks et al., 2018; Henry, 2011). Figure 2.3 illustrates the synthesis and metabolism of vitamin D.

The active hormonal form, $1,25(OH)_2D$, then interacts with vitamin D receptors (VDR), which are in almost every tissue in the body. The VDR forms a heterodimer with the retinoid X receptor (RXR). This heterodimer in turn binds to the DNA sequences in target genes, referred to as vitamin D response elements (VDREs), to activate or suppress transcription of the target genes (Dahlquist et al., 2015; Khammissa et al., 2018).

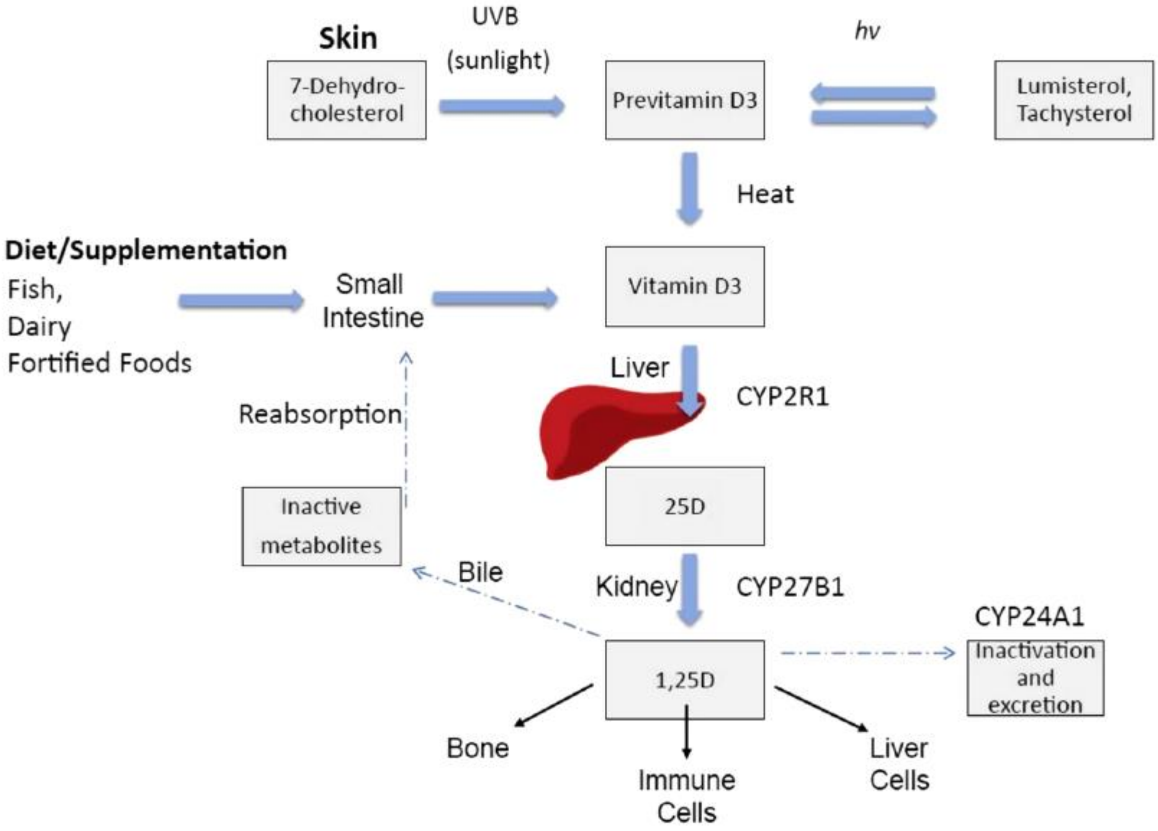


Figure 2.3. Vitamin D synthesis and metabolism
Reprinted with permission from Keane, J. T., Elangovan, H., Stokes, R. A., & Gunton, J. E. (2018). Vitamin D and the liver-correlation or cause? *Nutrients*, *10*(4).

2.4.3 Health consequences of vitamin D deficiency

Evidence for the role of vitamin D in skeletal health is robust. The biologically active form of vitamin D, $1,25(OH)_2D$ stimulates intestinal absorption of dietary calcium and phosphorus. This maintains serum calcium and phosphorous homeostasis, essential for bone mineralisation (DeLuca, 2004) and neuromuscular function (Holick et al., 2011). Vitamin D sufficiency enhances absorption of calcium by 30-40% and phosphorous by 80% (Holick et al., 2011). Vitamin D is also required for bone remodelling by osteoblasts and osteoclasts (Institute of Medicine, 2011). Vitamin D deficiency contributes to rickets in children and osteomalacia and osteoporosis in adults (Institute of Medicine, 2011).

The non-skeletal benefits of vitamin D remain controversial. As most tissues and cells in the body have a vitamin D receptor, the secosteroid hormone may have wide-ranging physiological effects. Vitamin D deficiency has been associated with chronic diseases, such as asthma, cancer, diabetes, multiple sclerosis; as well as autoimmune, cardiovascular, inflammatory and skin diseases (Holick et al., 2011; Zittermann, 2003). There is also a strong association with muscle strength and obesity (Holick et al., 2011). Recent evidence suggests that vitamin D sufficiency is associated with reduced risk and severity of acute respiratory infections (Martineau et al., 2017), including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, or Covid-19) (Kaufman et al., 2020; Meltzer et al., 2020). This is possibly explained by the regulatory role of vitamin D on both acquired and innate immunity (Bae et al., 2022). Large, randomised controlled trials of sufficient duration and standardised methodology are required to conclusively demonstrate the ability of vitamin D to prevent and treat these diseases.

2.4.4 Assessment of vitamin D status

Serum 25(OH)D is the vitamin D metabolite routinely used to assess overall vitamin D status (Zittermann, 2003). It reflects the amount of vitamin D from both the diet and skin absorption. However, there is no consensus on optimal levels or the use and definition of the terms, deficient, adequate, insufficient, sufficient, optimal and suboptimal. The inconsistencies result from differences in functional endpoints (e.g., skeletal health versus general health) and a lack of standardised biochemical analysis for 25(OH)D (Dawson-Hughes et al., 2005).

2.4.4.1 Biochemical assays for vitamin D status

Measures of 25(OH)D in the literature should be interpreted with care, taking into consideration the type of assay, use of automated methods and the year of analysis (Institute of Medicine, 2011). Liquid chromatography-tandem-mass spectrometry (LC-MS/MS) has been proposed as the ‘gold standard’ for assays due to its potential for higher specificity, sensitivity and reproducibility (Farrell et al., 2012; Institute of Medicine, 2011). However, when compared with immunoassays, LC-MS/MS have produced significantly higher 25(OH)D concentrations (de Koning et al., 2013; Farrell et al., 2012; Lai et al., 2012) and according to Atef (2018) LC-MS/MS results can vary and require an experienced analyst.

2.4.4.2 Serum 25-hydroxyvitamin D targets

The United States Institute of Medicine (IOM) based their serum 25(OH)D concentration target of ≥50nmol/L on the strong body of evidence supporting the classical role of vitamin D in promoting bone health (Institute of Medicine, 2011). The Endocrine Society (a global community of physicians and scientists) agrees with a 25(OH)D ≥50nmol/L to prevent rickets, osteomalacia and osteoporosis (Holick et al., 2011). Whilst both groups have defined concentrations below this as vitamin D deficiency, the Endocrine Society have classified vitamin D insufficiency as a 25(OH)D of 50-74nmol/L in their clinical practice guideline. They state that levels above this are required to achieve maximum musculoskeletal benefits from vitamin D. The guideline further states that additional non-skeletal health benefits may be achieved with 25(OH)D ≥75nmol/L (Holick et al., 2011). In contrast, the IOM report concludes there is no additional benefit to achieving 25(OH)D concentrations of 75nmol/L when compared to 50nmol/L (Institute of Medicine, 2011). In the United Kingdom, 25(OH)D of 25nmol/L is defined as the lower limit of adequacy based on the increased risk of rickets and osteomalacia below this value (Scientific Advisory Committee on Nutrition, 2016).

In New Zealand, the Adequate Intake (AI) for vitamin D in the Nutrient Reference Values is based on the amount required to maintain 25(OH)D ≥27.5nmol/L (Department of Health and Ageing and Ministry of Health, 2006). However, several position statements from Australasia have subsequently defined vitamin D adequacy as ≥50nmol/L (Ministry of Health and Cancer Society of New Zealand, 2012; Nowson et al., 2012; The Royal College of Pathologists of Australasia, 2013). These position statements further acknowledge that the 25(OH)D target should be achieved at the end of winter, with Nowson et al. (2012) suggesting the vitamin D target at the end of summer may need to be 10-20nmol/L higher to allow for a seasonal decrease during winter.

The accuracy of clinical targets in the absence of assay standardisation is limited (Atef, 2018). For this literature review, unless otherwise stated, vitamin D deficiency, insufficiency and sufficiency will be defined as 25(OH)D <50, 50-74 and ≥75nmol/L, respectively. Suboptimal vitamin D status is defined as 25(OH)D <75nmol/L.

2.4.4.3 Vitamin D toxicity

Hypercalcaemia is the main concern with excessive vitamin D. Toxicity is not possible from excess sun exposure because once formed, previtamin D_3 absorbs UVB radiation and converts it to a variety of photoproducts with no biological action on calcium metabolism (Holick, 2011). The only cause of toxicity is excessively high oral ingestion of vitamin D for an extended period, likely from manufacturing errors in dietary supplements and fortified food (Araki et al., 2011; Jacobus et al., 1992; Kara et al., 2014; Koutkia et al., 2001). Vitamin D hypersensitivity is very rare (Vieth, 1999).

There is no consensus on a safe upper limit for the concentration of 25(OH)D. The IOM do not recommend treatment to concentrations above 125nmol/L due to the unknown long-term effects (Institute of Medicine, 2011). However, the Endocrine Society guidelines state that 25(OH)D concentrations up to 250nmol/L are not associated with toxicity and that vitamin D toxicity is usually only observed in children and adults with 25(OH)D ≥375nmol/L (Holick et al., 2011). Studies in large community-based settings have confirmed the Endocrine Society's statements (Dudenkov et al., 2015; Kimball et al., 2017; Pérez-Barrios et al., 2016).

2.4.5 Vitamin D requirements

Dietary recommendations assume there will be minimal sun exposure and are set at the level identified as the lowest dietary intake of vitamin D associated with achieving the 25(OH)D target. Table 2.4 displays a selection of international 25(OH)D targets and dietary recommendations for young adults. The AI for dietary vitamin D is 200IU from birth to 50-years for those living in New Zealand and Australia (Department of Health and Ageing and Ministry of Health, 2006). These recommendations have not been updated since the IOM recognised their earlier recommendation of 200IU as inadequate for wider protection of the healthy population against skeletal disorders beyond prevention of rickets (Pludowski et al., 2018) or since several Australasian agencies have recommended higher 25(OH)D targets (Ministry of Health and Cancer Society of New Zealand, 2012; Nowson et al., 2012; The Royal College of Pathologists of Australasia, 2013).

Table 2.4. Serum 25-hydroxyvitamin D targets and dietary vitamin D recommendations for young adults

Organisation (Year)	Country	25(OH)D target (nmol/L)	Age (Years)	Dietary vitamin D recommendations (IU/day)
Department of Health and Ageing and Ministry of Health (2006)	Australia / New Zealand	≥27.5	≤50	200[a]
Institute of Medicine (2011)	United States	≥50	1-70	600[b]
Endocrine Society (Holick et al., 2011)	United States	≥75	1-18 ≥19	600–1000[c] 1500–2000[c]
Scientific Advisory Committee on Nutrition (2016)	United Kingdom	≥25	≥4	400[d]
European Food Safety Authority (2016)	Agency of the European Union	≥50	≥1	600[a]

25(OH)D, 25-hydroxyvitamin D.

[a] Adequate intake.

[b] Recommended Dietary Allowance.

[c] Recommendations that would likely provide all non-skeletal health benefits of vitamin D.

[d] Recommended Nutrient Intake.

2.4.6 Determinants of vitamin D status in adults

Comparisons among studies investigating the determinants of vitamin D status are difficult due to the diversity of study populations, controversy about what cut-offs should be used, seasonality and factors adjusted for in the final model (Holick et al., 2011; Liu et al., 2010; Liu et al., 2018). Prediction models have significant unexplained variability. This is likely the result of methodological errors in measuring predictor variables and 25(OH)D, both between assays and between laboratories (Black et al., 2015). In addition, information regarding other determinants of vitamin D status is often lacking. This includes genetic factors and absolute UV exposure (Bertrand et al., 2012). Table 2.5 illustrates efforts to develop models for 25(OH)D that are applicable to a general adult population.

Table 2.5. Large scale studies investigating individual predictors of 25-hydroxyvitamin D with multiple linear regression models

Study Author (Year)	Country	Characteristics of participants	Individual predictors of 25(OH)D in final model, *P*<0.05	Predicted variance in 25(OH)D from final model (%)
National Health and Nutrition Examination Survey (2001-2010) (Liu et al., 2018)	United States	*n*=26,010 Women and Men 45.6-years (95% CI 45.1, 46.2)	Age, ethnicity, obesity, alcohol intake, season, physical activity, dietary vitamin D	Looked at associations only
Independent Study in Metropolitan Toronto[a] (Knight et al., 2017)	Canada	*n*=309 Women and Men 19-59-years	Sex, ethnicity, month of blood draw, genotype, supplementary vitamin D, sunbed/sunlamp use, sun exposure, dietary vitamin D, outdoor time, alcohol intake	46
The AusD Study (Kimlin et al., 2014)	Australia	*n*=1,002 Women and Men 48.2 ± 15.7 years	Latitude, season, age, melanin density, BMI, physical activity, supplementary vitamin D, clothing cover, sun exposure	40
European Prospective Investigation into Cancer and Nutrition (Kuhn et al., 2014)	Germany (sub-cohort)	*n*=2,100 Women and Men 50.9 ± 8.6 years	Sex, month of blood draw, waist circumference, alcohol intake, smoking status, hormone use, supplementary vitamin D, dietary vitamin D, physical activity, outdoor activity, genotype	33
The Ausimmune Study[b] (Lucas et al., 2013)	Australia	*n*=382 Women and Men 18-61-years	Sun exposure, latitude, clothing cover, wearing of sunglasses, melanin density, sex, anthropometry, genotype	54
Nurses' Health Study (Bertrand et al., 2012)	United States	*n*=2079 Women 42-69-years	Ethnicity, UVB flux, dietary vitamin D, supplementary vitamin D, BMI, physical activity, hormone use, alcohol intake, season of blood draw	33
Nurses' Health Study II (Bertrand et al., 2012)	United States	*n*=1497 Women 32-52-years	Ethnicity, dietary vitamin D, supplementary vitamin D, BMI, physical activity, alcohol intake, season of blood draw	25
Health Professionals Follow-up Study (Bertrand et al., 2012)	United States	*n*=911 Men 46-81-years	Ethnicity, UVB flux, dietary vitamin D, supplementary vitamin D, BMI, physical activity, season of blood draw	28

Study Author (Year)	Country	Characteristics of participants	Individual predictors of 25(OH)D in final model, *P*<0.05	Predicted variance in 25(OH)D from final model (%)
Framingham Offspring Study (Liu et al., 2010)	United States	*n*=805 Women and Men 54.2 ± 9.7 years	Sex, BMI, energy intake, smoking, total vitamin D intake, month of blood draw	26
Women's Health Initiative (Millen, 2010)	United States	n=2472 Women 50-79-years	Month of blood draw, total vitamin D intake waist circumference, physical activity, ethnicity, solar radiation, age	21

UVB, ultraviolet beta; BMI, body mass index.

[a] Results based on single visit at baseline.

[b] Results presented are for the Caucasian group only.

2.4.6.1 Sun exposure

Ultraviolet B radiation is the major determinant of 25(OH)D. Ambient ultraviolet radiation can be assessed through measures such as season, latitude, altitude and cloud cover. However, individual UVB exposure from sunlight is difficult to measure as behaviours, such as time spent outdoors, shade seeking behaviours, clothing coverage and sun protection will influence the absolute dose of UVB received (Knight et al., 2017). Recreational physical activity time, as a proxy for time spent outdoors, has been shown to be a significant predictor of 25(OH)D (Bertrand et al., 2012; Kimlin et al., 2014; Liu et al., 2018; Lucas et al., 2013; Millen, 2010). While season is consistently a strong individual predictor of 25(OH)D in multivariable regression models, it only featured as the largest contributor in the German sub-cohort of the European Prospective Investigation into Cancer and Nutrition, where they did not measure ethnicity (Kuhn et al., 2014).

In a large adult cohort (*n*=21,987) living in New Zealand, 30-35% of participants were reported to have 25(OH)D <50nmol/L during late summer and early autumn, while 63% of participants were predicted to have 25(OH)D <50nmol/L in the winter and spring months (Bolland et al., 2008). The authors predicted that women in New Zealand aged 18-50-years require a minimum 25(OH)D concentration in late summer of 73nmol/L in February and 71nmol/L in March to maintain >50nmol/L throughout the year. These findings were applied to a threshold of 80nmol/L year-round and estimated that individuals require 25(OH)D of 90-105nmol/L at the end of summer. A limitation of this study by Bolland et al. (2008) is the sample is based on individuals with a recorded 25(OH)D medical laboratory measurement. It is likely these participants were at higher risk of vitamin D deficiency for a range of reasons and supplementation use is unknown.

There are several environmental determinants of how much UV radiation reaches the Earth’s surface. Studies have shown a decrease in average 25(OH)D with increasing latitude in countries such as

Australia and New Zealand (Kimlin et al., 2014; Lucas et al., 2013; Nessvi et al., 2011). Latitude of residence contributed 20% to the explained variance of the AusD Study and impacted on the predictive ability of the model (Kimlin et al., 2014). In addition, exposure to UV radiation increases with less cloud coverage, depleted ozone cover, sunlight reaching the Earth from directly overhead rather than at an oblique angle, less air pollution, high altitude and reflectivity of the Earth's surface, such as snow.

Higher levels of melanin pigmentation may lower 25(OH)D in darker-skinned individuals. The melanin absorbs sunlight, reducing its ability to trigger endogenous vitamin D production (Holick, 2007). There is limited research from New Zealand regarding vitamin D status and skin colour in adults. Nessvi et al. (2011) investigated New Zealand adults aged 18-85-years (*n*=503) and found skin colour did not show as much variation in determining 25(OH)D concentration as ethnicity (Nessvi et al., 2011). There is currently no standard measurement of skin colour across ethnic groups in New Zealand and most studies considering the differences in health outcomes have used ethnicity as a self-identifying variable. This creates challenges when determining the relationship between skin colour and ethnicity (Callister et al., 2011).

Clothing inhibits the absorption of UVB radiation and subsequently the production of previtamin D and 25(OH)D (Matsuoka et al., 1992; Salih, 2004). Clothing cover contributed to 27% of the explained variance in the AusD study and for every 10% decrease in clothing cover, 25(OH)D increased by 5.2nmol/L, with no change in the duration of sun exposure required (Kimlin et al., 2014).

2.4.6.2 Ethnicity

Ethnicity is consistently a strong individual predictor of 25(OH)D in multivariable regression models. When compared with white study participants, African American (Bertrand et al., 2012; Chan et al., 2010; Herrick et al., 2019; Liu et al., 2018; Millen, 2010), Hispanic (Bertrand et al., 2012; Herrick et al., 2019; Liu et al., 2018; Millen, 2010), Pacific and Māori (Nessvi et al., 2011) and Asian (Bertrand et al., 2012; Herrick et al., 2019; Knight et al., 2017; Nessvi et al., 2011) participants have lower 25(OH)D concentrations. A strong association between ethnicity and 25(OH)D remained after melanin content (Knight et al., 2017) and skin type (Chan et al., 2010) was accounted for. Ethnicity is likely related to many other factors that can reduce vitamin D status, both non-modifiable, such as increasing latitude; and modifiable, including less physical activity, low quality diets and tobacco smoking (Knight et al., 2017; Liu et al., 2018; Nessvi et al., 2011).

2.4.6.3 Dietary intake

Vitamin D supplement use typically explains a higher proportion of the variation in 25(OH)D than food sources alone. It remains a relatively modest contributor in comparison to other predictors (Kuhn et al., 2014; Liu et al., 2010) but can have a major impact on 25(OH)D at intakes of at least 1000IU/day (Knight et al., 2017). As foods rich in vitamin D may not be consumed every day, it should be noted that their consumption may be missed by some dietary assessment methods. This particularly applies to those methods investigating food intake during less than 1-week (Scientific Advisory Committee on Nutrition, 2016). This may explain the relatively weak association between dietary intake and 25(OH)D concentration.

There is currently no data available to assess the usual dietary intake of vitamin D in the New Zealand population. Studies from countries with both voluntary and mandatory fortification of food staples (Ahmed et al., 2021; Dunlop et al., 2022; Herrick et al., 2019; Kiely & Black, 2012; Lips et al., 2019; Spiro & Buttriss, 2014) estimate mean intakes of vitamin D remain below the conservative RDI for young adults in New Zealand and Australia of 200IU/day (Department of Health and Ageing and Ministry of Health, 2006). In New Zealand, few foods are fortified with vitamin D and dietary supplements are likely to be the most effective method for individuals to increase their intake.

2.4.6.4 Age

Advancing age has been associated with an increased risk of vitamin D deficiency. After adjustment for other potential factors, older adults (≥60-years) were 63% ($P<0.0001$) more likely to have vitamin D deficiency and 46% ($P<0.0001$) more likely to have vitamin D insufficiency than young adults (18-39-years) (Liu et al., 2018). Possible causes for this increase are that ageing diminishes the capacity of the skin to produce vitamin D_3 and reduced outdoor activity limiting sun exposure (MacLaughlin & Holick, 1985). However, recent studies have reported a shift towards a higher prevalence of vitamin D deficiency and insufficiency within the 20-to-40-year age range (Herrick et al., 2019; Horton-French et al., 2021; Malacova et al., 2019; Wang et al., 2020). Possible explanations include more time spent indoors due to computer use at work and home, greater use of sunscreen and other sun-protective behaviours, and lower use of vitamin D supplements.

2.4.6.5 Sex

Vitamin D inadequacy affects both females and males. Large studies with nationally representative samples have reported no sex differences for the prevalence of vitamin D deficiency or insufficiency (Herrick et al., 2019; Malacova et al., 2019). While other studies have observed lower vitamin D status in females compared to males, these were in adults with a mean age ≥50-years and are not representative of the whole population (Kuhn et al., 2014; Liu et al., 2010).

2.4.6.6 Body composition

Epidemiologic studies have demonstrated a significant inverse association between BMI and 25(OH)D (Bertrand et al., 2012; Liu et al., 2010). The potential mechanisms include sequestration of vitamin D in fat cells, reduced outdoor activity or an effect of vitamin D on adipogenesis (Kuhn et al., 2014; Wortsman et al., 2000). None of the epidemiologic studies reviewed in Table 2.5 included %BF in their final multivariable models. Kuhn et al. (2014) suggested that waist circumference, rather than BMI, was included in their final multivariable model as 25(OH)D concentrations have a stronger inverse association with visceral adipose tissue compared to subcutaneous and overall measures of adiposity. This suggestion is consistent with results from imaging studies using computed tomography (CT) (Cheng et al., 2009; Sulistyoningrum et al., 2012).

2.4.7 Vitamin D status of adults living in New Zealand

Table 2.6 presents the vitamin D status of New Zealanders aged 15-years and over from the most recent New Zealand Adult Nutrition Survey in 2008/09 (Ministry of Health, 2012). Vitamin D status was highest for the total population (including New Zealand Europeans), followed by adults identifying as Māori and then Pacific.

Table 2.6. Vitamin D status of women aged 15-years and over in New Zealand

Ethnic group	Mean annual 25(OH)D concentration (nmol/L)[a]	Prevalence of vitamin D deficiency (25(OH)D <25nmol/L) (%)[a]	Prevalence of being below recommended 25(OH)D concentration (25-49nmol/L) (%)[a]
Total	62.4 (60.3-64.5)	5.4 (4.1-7.0)	28.5 (25.5-31.6)
Māori	57.2 (52.7-61.8)	6.7 (3.9-11.3)	39.0 (33.1-45.3)
Pacific	46.0 (42.5-49.4)	9.9 (5.6-17.0)	53.2 (45.7-60.6)

(Ministry of Health, 2012)
25(OH)D, 25-hydroxyvitamin D.
[a] 95% confidence intervals are presented in brackets.

In the total adult population, it was reported that after adjusting for age, gender and ethnicity, those classified as obese according to BMI had a significantly lower mean serum 25(OH)D than those who were underweight or within the normal range. Dividing New Zealand into three regions based on latitude, the mean 25(OH)D was 65.1, 62.6 and 60.5nmol/L in the northern (lowest latitude), central and southern (highest latitude) regions, respectively. Mean concentrations were significantly lower for people living in the central and southern regions compared with the northern region after adjusting for age, gender and ethnicity; however, there was no significant difference in the prevalence of vitamin D deficiency between the three regions (Ministry of Health, 2012).

The prevalence of vitamin D deficiency was highest during the months of August, September and October. Seasonally this reflects late winter and early spring in New Zealand. This is likely due to reduced sunlight hours, less sun exposure and a gradual loss in accumulated vitamin D concentrations from summer. This seasonal vitamin D deficiency was also affected by latitude as after adjusting for age, gender and ethnicity, people living in the southern region were 3.1 times more likely to have vitamin D deficiency during August to October than people living in the northern region (Ministry of Health, 2012).

2.4.8 Vitamin D status during basic training

Studies in military recruits have shown variable changes in vitamin D status during basic training. This is dependent on the season training commenced, ethnicity, baseline 25(OH)D concentration and duration of training. Table 2.7 illustrates the mean 25(OH)D concentration of recruits in the United States undertaking basic training.

Baseline vitamin D status was lower in recruits that commenced basic training in the winter as compared to summer (Andersen et al., 2010; Gaffney-Stomberg et al., 2019; Lutz et al., 2012). In recruits commencing training in winter, 25(OH)D increased over 12-weeks of training (*P*<0.01) yet failed to reach vitamin D sufficiency (Gaffney-Stomberg et al., 2019). Although no measures of vitamin D status were provided at the commencement of 9-weeks of basic training in winter, Lutz et al. (2012) reported the prevalence of suboptimal vitamin D at completion was 64% and 92% for white and non-white participants, respectively (Lutz et al., 2012). For recruits that commenced training at the end of summer, Andersen et al. (2010) observed a significant decrease in 25(OH)D during 9-weeks of training. At the commencement of basic training, 57% of participants had suboptimal vitamin D status and this increased to 75% at the end of training. Carswell et al. (2018) investigated vitamin D status in a longitudinal study of female British Army recruits undertaking 12-weeks of basic training. Although no specific data is available on mean concentrations of 25(OH)D, Carswell et al. (2018) reported that during winter only 36% of women had 25(OH)D ≥50nmol/L. This high level of deficiency occurred in a predominantly (95%) Caucasian study population.

It has been proposed that excess adipose tissue sequesters vitamin D (Wortsman et al., 2000). Gaffney-Stomberg et al. (2019) stated that the observed increase in 25(OH)D in recruits who commenced training in winter was likely due to the release from endogenous fat stores, as participants completing basic training during winter lost more body fat than recruits who commenced training in summer. Lutz et al. (2012) reported a positive association between 25(OH)D and %BF at the beginning of basic training in non-white recruits (*P*<0.05).

Collectively these studies indicate that regardless of ethnicity or season, most female recruits have suboptimal vitamin D status at the end of basic training, if not also for the duration. This may result from limited UVB absorption through skin exposure to produce vitamin D, due to latitude, season, sun exposure and clothing. Despite much of basic training occurring outdoors during daylight hours, recruits are required to wear full coverage military uniform and encouraged to use sunscreen and seek shade cover when appropriate.

Table 2.7. Studies investigating vitamin D status during basic training

Author (Year) Country Basic Training	Study design	Characteristics of participants	Location and month commenced	Time	Mean 25(OH)D (nmol/L)		
Gaffney-Stomberg et al. (2019) United States Marines 12-weeks	Randomised, double-blind, placebo-controlled trial	Placebo group *n*=76 39 males 37 females 18.9 ± 1.6 years	Parris Island, South Carolina, United States Latitude = 34°N July (summer) and February (winter)		**Total**	**Summer (*n*=37)**	**Winter (*n*=39)**
				Pre-BT	74.8 ± 27.3	87.3 ± 21.3	64.3 ± 28.0
				Post-BT	74.0 ± 17.5	81.0 ± 15.3[a]	69.5 ± 17.8[a]
Lutz et al. (2012) United States Army 9-weeks	Longitudinal, observational study	*n*=71 Females only 23.1 ± 0.7 years	Fort Jackson, South Carolina, United States Latitude = 34°N February (winter)		**Total**	**White (*n*=45)**	**Non-white (*n*=26)**
				Baseline	64.1 ± 3.8	77.0 ± 3.5	41.7 ± 4.6[c]
				Week 3	60.4 ± 2.9	70.6 ± 3.5[b]	42.6 ± 4.6[c]
				Week 6	60.7 ± 2.6	68.6 ± 3.5[b]	47.8 ± 4.6[c]
				Week 9	63.2 ± 2.6	70.5 ± 3.5	50.6 ± 4.6[d]
Andersen et al. (2010) United States Army 9-weeks	Subset of a Randomised, placebo-controlled trial	*n*=74 Females only 23.1 ± 0.7 years	Fort Jackson, South Carolina, United States Latitude = 34°N August (summer)		**Total**		
				Pre-BT	72.9 ± 30.0		
				Post-BT	63.3 ± 19.8[b]		

25(OH)D, 25-hydroxyvitamin D; BT, basic training.

[a] Different from baseline within group, *P*<0.01.

[b] Different from baseline within group, *P*<0.05.

[c] Different from white participants at the same time point, *P*<0.05.

[d] Different from week 3, *P*<0.05.

2.4.9 Vitamin D status and functional outcomes during basic training

Observational studies in recruits suggest an association between reduced 25(OH)D and an increased risk of stress fractures (Burgi et al., 2011; Davey et al., 2016; Ruohola et al., 2006), longer recovery time from stress fractures (Richards & Wright, 2018), impaired aerobic fitness (Carswell et al., 2018) and increased incidence of upper respiratory tract infections (Harrison et al., 2021; Laaksi et al., 2007) in otherwise healthy recruits. The incidence of medically diagnosed stress fractures during basic training has been reported up to 5% for males and 18% for females (Jones et al., 1993; Knapik et al., 2012; Moran et al., 2008) with the mean rehabilitation time up to 21-weeks (Richards & Wright, 2018; Wood et al., 2014). In addition, up to 60% of those who sustain a stress fracture attrite from training (Friedl et al., 2008). Injury and illness during basic training has considerable personal and organisational impact with delays to complete training, medical and rehabilitation costs, and potential discharge from military service.

2.4.10 Conclusion

Diminished vitamin D status is a concern for all military personnel. Previous international studies have reported that most female recruits have suboptimal vitamin D status at the end of basic training (Andersen et al., 2010; Carswell et al., 2018; Gaffney-Stomberg et al., 2019; Lutz et al., 2012). This decline is likely influenced by a lack of casual sun exposure, based on environmental factors, such as latitude and season. Ethnicity and dietary intake may also contribute to diminishing vitamin D status in female recruits. No studies have investigated the vitamin D status of female recruits in the New Zealand Army, with training conducted at a latitude approaching 40° and across all seasons.

2.5 Dietary assessment methods for determining habitual dietary intake

2.5.1 Dietary assessment tools

Dietary assessment tools are an integral component of nutrition research. Assessment tools that capture long-term dietary intake are the most appropriate for research at the population or sub-group level as they better reflect habitual diets. These can then be investigated for associations with health outcomes. The appropriate dietary assessment tool is dependent on the research question, study design and sample size (Bailey, 2021). Table 2.8 provides an overview of three traditional dietary assessment tools.

All self-reported dietary assessment tools are subject to both random and systematic measurement errors. In addition, all measurements of dietary intake rely on the accuracy and thorough representation of foods in composition databases to convert intake to energy and nutrients (Bailey, 2021). Large within person day-to-day variations in dietary intake is the usual random error observed, which can be reduced by increasing the number of days recorded (Gibson, 2005). Systematic errors include a tendency for ‘healthy foods’ to be overreported and ‘unhealthy foods’ to be underreported; while some participants may find knowledge of meal composition or portion size recall difficult, which may be linked to factors, such as age, gender and interviewer bias (Bailey, 2021; Gibson, 2005).

Using a combination of dietary assessment tools has been shown to increase the precision of estimating habitual dietary intake (Freedman et al., 2018). Innovative statistical methods have been developed to combine dietary data from a food frequency questionnaire (FFQ) and multiple 24-hour diet recalls from the same individual (Conrad & Nöthlings, 2017). These methods assume that repeated 24-hour recalls provide data on consumption probability and quantity, while the FFQ can provide information on less frequently consumed foods. In a review of studies in large-scale settings, Conrad and Nöthlings (2017) suggest this integrated approach for estimating habitual intake is promising; however, validation with biomarkers is warranted. In addition, consideration needs to be given to the balance of participant burden, practicality and accuracy (Carroll et al., 2012).

Table 2.8. Common dietary assessment tools

Dietary assessment method	Characteristics	Advantages	Disadvantages
Food frequency questionnaire	• Captures habitual dietary intake retrospectively over a specified time, ranging from weeks up to 1-year • Participants select their typical frequency of consumption from a pre-determined list • A comprehensive FFQ may include up to 200 pre-determined items • Generally used in studies with large sample sizes • Can be quantitative, semi-quantitative or qualitative	• Reflects habitual dietary intake • Low participant and researcher burden • Can be administered online to remove the requirement for data entry • Suitable for epidemiological studies	• Does not indicate time consumed, food preparation or cooking method, or other foods consumed simultaneously • Qualitative FFQs do not assess the amount of food consumed • Participant literacy is required • Limited by participant's memory, reducing accuracy • FFQs may be confusing to participants, resulting in response errors and missing data • Seasonal variability can skew food and nutrient intake
Diet recall	• Captures dietary intake, usually from the previous day or 24-hours • Open-ended questions delivered by a trained interviewer • Multiple administrations of the diet recall on non-consecutive days are required to improve data accuracy • Estimates of macronutrients are generally more stable than micronutrients	• Researcher-led interview improves accuracy and minimises response errors and missing data • Recorded food items are not pre-determined and therefore accurately reflect dietary intake • Brands, food preparation and cooking methods included to improve accuracy of nutrients consumed • Timing of eating occasion included • Participant literacy is not required	• The number of items reported by a study population may be >1000, making food variable compression and analysis difficult • Trained interviewer required • Possible interviewer bias • Large day-to-day variations may occur between days of the week, e.g., weekday vs weekend • Habitual intake unlikely to be reflected during such a short assessment period • Seasonal variability can skew food and nutrient intake

Dietary assessment method	Characteristics	Advantages	Disadvantages
Diet record	• Participants record dietary intake at the time of consumption over a specified time, usually 3-7-days • Portion sizes can be weighed and measured or estimated	• Recorded food items are not pre-determined and therefore accurately reflect dietary intake • Quantity and volume are recorded • No interviewer required • No recall biases • Brands, food preparation, and cooking methods are included to improve accuracy of nutrient consumption • Timing of eating occasion included	• The number of items reported by a study population may be >1000, making variable compression and analysis difficult • High participant burden • Participant literacy is required • Training participants is required for enhanced accuracy of reporting • The act of recording may alter the participant away from usual intake, for both ease of recording and a desire to report foods perceived as healthy • Seasonal variability can skew food and nutrient intake

(Bailey, 2021; Schulz et al., 2021; Shim et al., 2014)
FFQ, food frequency questionnaire.

2.5.2 Analysis of dietary data

The traditional method for investigating associations between diet and health outcomes is to explore the effects of individual foods and nutrients. While isolated nutrients are associated with health outcomes, such as the role of folic acid in neural tube defects, several limitations to the traditional method exist. These include failing to account for interactions between nutrients, collinearity and the inability to detect small effects from single nutrients; the effect of dietary components acting in combination over longer periods of time, that accurately reflect meals composed of different foods and nutrients; the challenge to translate findings from research on individual foods and nutrients into meaningful dietary recommendations; and the deficiencies of food composition data regarding nutrient and phytochemical content (Hu, 2002; Newby & Tucker, 2004).

Dietary pattern analysis considers the cumulative and interactive effects among dietary components. This reflects the complexity of the whole diet in the real world. In addition to overcoming the limitations of investigating individual foods and nutrients, DPs may confer extra benefits. These include the presence of unidentified dietary components with positive or negative effects on health outcomes; focusing on a holistic, rather than reductionist approach to diet; and the ability for a DP itself to have a stronger association with a health outcome than any of the individual foods or nutrients that contribute to it (Hodge & Bassett, 2016).

2.5.2.1 Dietary patterns

Examining the role of DPs on health outcomes is now well-established as both an alternative and complementary approach to the traditional method within nutrition epidemiology research (Hu, 2002; Newby & Tucker, 2004). Analysis of DPs is usually conducted in two main ways, *a priori* and *a* *posteriori*, although combinations of approaches are increasingly being used (e.g., reduced rank regression).

A priori method

In the theoretical or investigator-driven approach to DP analysis, known as *a priori*, food variables are grouped according to set criteria for nutritional health (Newby & Tucker, 2004). *A priori* DPs are typically based on a dietary index created by an agency or research group to measure diet quality. The indices are built upon current nutrition knowledge and generally include food variables that represent dietary guidelines or recommendations considered most beneficial for health outcomes (Zhao et al., 2021). The index variables are usually scored and provide an overall measure of diet quality (Hodge & Bassett, 2016; Newby & Tucker, 2004).

Challenges arise when guidelines or recommendations do not have scientific consensus. Although various diet quality indices tend to promote the intake of fruits, vegetables and whole grains, different food variables may be included or omitted, or different weightings applied to variables. This may lead to results that are not comparable between studies (Hodge & Bassett, 2016; Newby & Tucker, 2004). A further disadvantage is that the indices are based on pre-determined food variables, which may not account for the overall dietary intake of a study population (Zhao et al., 2021).

A posteriori method

In the empirical or data-driven approach to DP analysis, known as *a posteriori*, statistical techniques generate DPs from collected dietary data, which are subsequently evaluated (Newby & Tucker, 2004). Data are usually collected from FFQs, but also 24-hour diet recalls and diet records. A strength of this method is that it is not based on a pre-determined concept of a healthful pattern (Newby & Tucker, 2004). Limitations to *a posteriori* DPs include difficulty comparing studies where the patterns are not similar or summarising studies that have similar labels for DPs, e.g., ‘traditional’, but may vary considerably in food composition. In addition, creating DPs does not overcome deficiencies in the original dietary assessment data (Hodge & Bassett, 2016).

The primary methods for determining *a posteriori* DPs are factor analysis and cluster analysis (Sauvageot et al., 2017). In factor analysis, data are reduced into DPs based on correlations between food variables. Factor analysis and principal component analysis are based on similar mathematical concepts (Zhao et al., 2021) and therefore discussed as factor analysis in this review. In cluster analysis, data are reduced into DPs based on individual differences in mean intake (Newby & Tucker, 2004).

In factor analysis, each participant is allocated a score for all derived factors (dietary patterns). The score is a continuous variable. The advantage of this is a greater understanding of which food variables are consumed in combination and the associations between DPs and health outcomes (Zhao et al., 2021). While this makes it difficult to interpret an individual's specific DP, it may increase statistical power in comparison to cluster analysis, particularly for detecting associations between dietary intake and health outcomes in small samples (Slattery, 2010). A further limitation of factor analysis is the retained factors can only partially explain the total variance of the food variables (Zhao et al., 2021).

In cluster analysis, participants are separated into one cluster (categorical groups) based on similarities between diets. Compared to factor analysis, individual DPs are easier to interpret (Sauvageot et al., 2017) and clusters can be compared directly (Kim et al., 2015). The disadvantages of cluster analysis include the inability to accommodate nuance in DPs. As each individual is clustered categorically (Zhao et al., 2021), no established consensus on the number of clusters for analysis (Kim et al., 2015) and the unequal sample size of clusters will limit statistical power in analysis (Thorpe et al., 2016).

Factor analysis methodology

Food frequency questionnaires remain the most common source of dietary data for factor analysis of DPs (Schulz et al., 2021). Most studies collapse the original measured dietary data into a smaller number of input variables for entry into the factor analysis. It is possible to enter all individual food items into a factor analysis. However, a smaller number of input variables will reduce the complexity of data and explain a greater percentage of the variance in dietary intake (Newby & Tucker, 2004). The number of input variables reported in the literature ranges from 25 (Naja et al., 2018) to 99 (Hamer et al., 2010). The input variables are commonly based on food groups (e.g., vegetables) macronutrient content (e.g., high protein), micronutrient content (e.g., fruits high in vitamin C), dietary use (e.g., milk consumed in beverages) or nutrient profile (e.g., processed meat) (Newby & Tucker, 2004; Schulz et al., 2021). In addition, only food variables with a specific consumption frequency (Beck et al., 2012) or minimum number of participants (Schulz et al., 2021) have been selected for input in factor analysis. Any differences in selection of input variables will affect the DPs derived.

Common limitations of factor analysis studies include necessary but subjective decisions made in determining DPs (Edefonti et al., 2020). These decisions include the number of input variables, food groupings, model selection, rotation method, number of factors to retain and interpretation of the results, including factor loadings and the arbitrary labelling of DPs (Edefonti et al., 2009; Hu, 2002; Newby & Tucker, 2004).

2.5.2.2 Validity and reproducibility of food frequency questionnaires

It is important to test the validity (the ability of a tool to accurately measure what the researcher intended to measure conceptually) and reproducibility (the ability of a tool to obtain consistent results when used repeatedly) of newly developed dietary assessment tools such as FFQs in the population for which they are intended to be used. Beck et al. (2012) developed and investigated the relative validity and reproducibility of an iron food frequency questionnaire (FeFFQ) designed specifically to identify iron-related DPs in premenopausal women living in New Zealand. Participants (*n*=115) completed the FeFFQ twice, 1-month apart, and a weighed 4-day diet record (4DDR) following completion of the first FeFFQ. The authors reported good validity for the FeFFQ, compared to the 4DDR and high reproducibility between the two FeFFQs for determining frequency consumption of food variables and identifying iron-related DPs.

2.5.3 Conclusion

Several tools are available to assess dietary intake. Food frequency questionnaires are advantageous in terms of reducing participant burden, while collecting data that reflects habitual dietary intake over a longer period. Caution is required with FFQs regarding the reliance on memory of food intake over time, lack of information on meal combinations and being restricted to a defined list of foods (Bailey, 2021). Dietary patterns reflect the complexity of the whole diet, rather than a reductionist approach, which focuses on individual foods and nutrients. A data-driven approach to DP analysis supports this method. It represents what individuals consumed over the assessment period, rather than conforming to a pre-determined concept of healthful dietary intake. Despite DPs being considered a complementary and alternative approach to examining the role of diet on health outcomes, no studies in female recruits have investigated associations between iron status and DPs.

2.6 Physical fitness during basic training

2.6.1 Characteristics of physical fitness during basic training

Soldiers are required to complete physical training and military tasks across a spectrum of fitness domains that include aerobic, power, muscular endurance and muscular strength (Vaara et al., 2022). As recruits prepare for their role as soldiers, basic training includes formal physical training that covers aerobic fitness, circuit training, strength and conditioning, obstacle courses and swimming; and military tasks that cover marching, manual weapon and equipment handling, repetitive lifting, prolonged load carriage, construction work and casualty extraction (Nindl et al., 2016). While formal physical training sessions are typically conducted at a moderate to high intensity, the large training load experienced by recruits undertaking basic training is generally the result of performing a high volume of low and moderate intensity physical activity over a long duration (Jurvelin et al., 2020; Simpson et al., 2013). Knapik et al. (2007) reported that United States Army recruits performed over 16,000 steps per day, on average, almost 12km. This increased during field-based training to more than 22,000 steps per day, on average, over 16km. In studies of basic training, physical fitness is often characterised by aerobic fitness. This is typically measured by running time over distances varying from 1.6-3.2km or distance achieved in 12 minutes, also known as The Cooper Test (Cooper, 1968).

2.6.2 Physical fitness changes in females during military training

A systematic review of changes in physical fitness from the beginning to end of military training (not specifically basic training) found that across seven studies the median absolute improvement for females was 73 seconds for the 2.4km run. Comparisons are difficult due to the limited information regarding course details, such as track, sealed road or off-road and flat or hills. In six studies that reviewed push-ups, significant improvements were observed in five of nine female groups, with the overall median improvement 70.6%. However, a wide range of results were observed from no improvement to substantial improvement greater than 100%. Seven studies investigated the maximum number of sit-ups performed, with all female groups improving by a median of 53.2% (Varley-Campbell et al., 2018). Again, comparisons are difficult due to pre-enlistment fitness standards, varying durations of training and the training environment, such as altitude and terrain. In addition, the physical fitness test protocols vary widely in terms of what individual tests are completed, the conduct of each test, the order, rest time between components, whether the tests are conducted indoors or outdoors and acceptable weather conditions for testing. No studies internationally have performed the 2.4km run, push-ups and curl-ups together in a similar protocol to the New Zealand Army.

Looking specifically at the 2.4km run in female army recruits, two studies have reported changes from the beginning to end of basic training. During 14-weeks of basic training in the British Army, Richmond et al. (2012) reported that 16 women improved their mean ± SD run time from 767 ± 42 to 687 ± 34 seconds ($P<0.001$), representing a 10% improvement. During an earlier study in the British Army over 12-weeks of basic training, Blacker et al. (2009) reported that 7 women in a mixed-gender platoon improved their mean ± SD run time from 754 ± 51 to 681 ± 29 seconds ($P<0.05$), representing a 10% improvement, while nine women in a female only platoon improved their mean ± SD run time from 739 ± 49 to 689 ± 50 seconds ($P<0.05$), representing a 7% improvement.

2.6.3 Energy expenditure during basic training

The elevated energy demands during basic training are primarily due to a training programme that keeps recruits engaged for up to 16-hours per day. Doubly labelled water (DLW) is the gold standard technique for determining energy expenditure (EE) in a field-based environment (Westerterp, 2017), such as military training. Although it does not quantify the type, intensity or duration of physical activity undertaken, DLW provides an accurate measure of average EE over the period monitored. A summary of studies investigating EE during basic training using DLW is presented in Table 2.9.

Table 2.9. Absolute energy expenditure measured by doubly labelled water during basic training

Author (Year)	Basic training	Characteristics of participants	DLW monitoring	Energy expenditure (MJ/day)[a]	
				Females	Males
Blacker et al. (2009)	British Army 12-weeks	7 females 18.1 ± 2.2 years 7 males 22.6 ± 4.2 years	10-days each across weeks 1-2 and 9-10	12.4 ± 1.1 & 12.5 ± 0.6	15.2 ± 1.5 & 14.8 ± 1.4
Richmond et al. (2012)	British Army 14-weeks	10 females 18.6 ± 1.9 years[b] 9 males 18.9 ± 1.6 years[b]	10-days each across weeks 1-2 and 13-14	12.5 ± 1.6 & 13.5 ± 1.9	17.4 ± 2.6 & 18.2 ± 2.0
O'Leary et al. (2018)[c]	British Army 14-weeks	17 females 16 males	10-days each across weeks 1-2 and 12-13	11.9 ± 1.4 & 14.2 ± 1.4	16.8 ± 2.6 & 17.8 ± 2.3
Proctor et al. (2018)[c]	US Army 10-weeks	14 females 30 males 21.0 ± 2.8 years	5.5-days each during weeks 5 and 10	14.3 ± 1.5 & 13.9 ± 2.1	17.9 ± 1.9 & 17.1 ± 2.1

DLW, doubly labelled water.

[a] Mean ± SD.

[b] Based on initial cohort, n=30.

[c] Energy expenditure converted from kcal/day in original source using factor of 4.184.

Energy expenditure appears partly determined by body mass (BM). Richmond et al. (2012) reported the association between EE and BM for females was r=0.75 (P<0.01) and males was r=0.50 (P<0.01). O'Leary et al. (2018) also reported an association between EE and BM of r=0.62 for females and r=0.63 for males, which strengthened with lean body mass (LBM) to r=0.75 for females and r=0.67 for males. Understanding EE during basic training is essential to ensure energy intake (EI) supports the health and performance of recruits.

2.6.4 Conclusion

Basic training is physically demanding with physical training and military tasks spanning the spectrum of fitness domains, including aerobic, power, muscular endurance and muscular strength. Previous studies have investigated the changes in physical fitness of female recruits during basic training; however, comparisons are difficult due to variations in pre-enlistment fitness standards, training duration, training environment and physical fitness test protocols. Improvements in physical fitness, as measured by faster 2.4km run times (Blacker et al., 2009; Richmond et al., 2012) and a greater number of push-ups and sit-ups (Bell et al., 2000; Knapik et al., 2005; Knapik et al., 2003; Yanovich et al., 2008) have consistently been observed in female recruits. No studies have established a situational awareness of physical fitness changes in female recruits in the New Zealand Army.

2.7 Body composition

2.7.1 Body composition assessment methods

Body composition can be assessed by a variety of methods – reference, laboratory and field. Reference methods are the most accurate for body composition assessment and include cadaver dissection, computed tomography (CT) and magnetic resonance imaging (MRI). While these techniques have limited feasibility for assessing individuals, they are the criterion against which other techniques are compared. Laboratory methods are now widely established as the standard for research and clinical settings. These include the gold standard multi-compartment methods of hydrodensitometry (underwater weighing) and air displacement plethysmography (BODPOD). Although the primary function of dual energy x-ray absorptiometry (DXA) is to measure bone mineral density, body composition is now considered its secondary function. This method is viewed as a laboratory reference standard, against which field techniques are compared. Field methods are the most accessible to employ; however, their validity varies widely. These include bioelectrical impedance analysis (BIA), skinfolds and BMI (Ackland et al., 2012; Duren et al., 2008). The choice of body composition method is often determined by the available technology and requirement to minimise participant burden. Therefore, accessible field-based assessment methods, alongside a comparison with DXA, are summarised in Table 2.10.

Table 2.10. Common body composition assessment methods

Body composition assessment method	Characteristics	Advantages	Disadvantages
Dual energy x-ray absorptiometry (Ackland et al., 2012; Albanese et al., 2003; Duren et al., 2008)	• Full body x-ray scan • Three compartment model measures fat mass, lean mass and bone mineral mass • Considered the reference standard in research and clinical settings	• Precise and accurate, when compared to gold-standard measures • Relatively quick	• Large, expensive and not portable • Requires a stable, temperature-controlled setting • Requires trained operators • Low-dose radiation exposure • Caution against frequency of use
Bioelectrical impedance analysis (Ackland et al., 2012; Duren et al., 2008; Sergi et al., 2017)	• A small electrical current is sent through the body and resistance to the current's flow (impedance) is measured and used to estimate total body water • Proprietary algorithms based on physical characteristics, such as sex, age and height are then used to estimate fat mass and fat free mass • Multi-frequency devices allow for regional analyses (arms, trunk, legs)	• Portable, quick, and non-invasive • Relatively inexpensive • Minimal operator training required • No radiation exposure	• Validity of measures varies significantly • Assumes constant hydration status and therefore requires strict adherence to avoidance of food and fluids prior to measurement • Caution required when applying algorithms outside specific validation population
Skinfolds (Ackland et al., 2012; Duren et al., 2008)	• Measure thickness of subcutaneous fat at various body sites • Measures can be analysed individually at each site or multiple sites can be summed together • Measurements are used in age and sex specific equations • Equations specific to the assessed population are required to improve accuracy	• Inexpensive and highly portable • Reliable scores with trained practitioner	• Requires trained practitioner • Inter-practitioner variability • Limited utility in overweight and obese participants due to calliper range and difficulty holding large skinfold • Uncomfortable for individual • Time-consuming and labour intensive for the practitioner

Body mass index (Ackland et al., 2012; Duren et al., 2008; Ministry of Health, 2018)	• Widely used indirect measure of body fatness • Index of weight adjusted for height • Calculated as body mass (kg)/height (m)^2 ▪ Underweight: <18.5kg/m^2 ▪ Normal weight: 18.5-24.9kg/m^2 ▪ Overweight: 25.0-29.9kg/m^2 ▪ Obese: ≥30.0kg/m^2	• Simple, inexpensive and non-invasive • Provides a useful population-level indicator of overweight and obesity • Standard measure used internationally to allow comparisons across regions, ethnicity and population sub-groups	• Does not distinguish between weight associated with fat, muscle and bone • Does not identify distribution of fat • Measures excess weight rather than excess fat • Limitations for heavily muscular individuals and highly trained athletes • May not be equally valid across age, sex and ethnic groups • Should not be used as a diagnostic tool at the individual level

2.7.2 Comparison of multi-frequency bioelectrical impedance analysis against dual energy x-ray absorptiometry

Numerous studies have compared the validity of multi-frequency BIA measurements for body composition with DXA in healthy adults (Anderson et al., 2012; Day et al., 2018; Garcia et al., 2021; Shafer et al., 2009). While these studies suggest BIA may be a valid method for assessing body composition in field-based research settings, variable methodology between studies limits comparisons. These variables include BIA devices, frequencies, proprietary algorithms, fasting period and restriction on exercise prior to testing, hydration status and time of day. In addition, participant characteristics differ in terms of age, sex, ethnicity, body weight and health status (Sergi et al., 2017). It is therefore recommended that each BIA model be independently validated against accepted reference methods, such as DXA.

2.7.3 Reliability and validity of InBody$_{230}$

The InBody$_{230}$ (Biospace Co. Ltd., Seoul, Korea) is a bioelectrical impedance analyser designed for portability. The InBody$_{230}$ takes 10 impedance measurements with two frequencies (20 and 100kHz) compared to the research grade models, the InBody$_{720}$ and InBody$_{770}$, which both take 30 impedance measurements with 6 frequencies (1, 5, 50, 250, 500, and 1000kHz). Measurements are performed in each segment (left arm, right arm, trunk, left leg, right leg) with the InBody$_{230}$. When compared, all three models were deemed reliable, with the InBody$_{720}$ and InBody$_{770}$ adding minimal benefit over the InBody$_{230}$ for assessment of fat mass (FM), fat free mass (FFM) and %BF (McLester et al., 2020). A study by von Hurst et al. (2016) also reported the InBody$_{230}$ demonstrated excellent reliability with <0.2% difference in repeat measurements and very small 95% confidence intervals.

In healthy adults (*n*=145, 94 females, 51 males, mean ± SD for age: 44.6 ± 20.0 years and BMI: 24.5 ± 3.8kg/m^2) Karelis et al. (2013) reported no significant differences in measures for FM, FFM and %BF between the InBody$_{230}$ and DXA for females, males or the total group. In addition, strong correlations were reported between the two methods for FM, FFM and %BF for the total group ($P<0.01$) and females alone ($P<0.01$) (Karelis et al., 2013). Conversely, von Hurst et al. (2016) reported the InBody$_{230}$ underestimated %BF compared to DXA by 1.6% in the total group of healthy adults (*n*=166, 85 females, 81 males, mean age: 38.9, 95% CI 36.9, 40.9 years and BMI: 25.5, 95% CI 25.1, 26.1kg/m^2) and 1.4% for females alone, with limits of agreement ranging from -5.12 to 8.45%. This finding was supported by McLester et al. (2020) who reported the InBody$_{230}$ underestimated %BF by 2.7% and FM by 1.4%, and overestimated FFM by 4.5% compared to DXA (all $P<0.001$) in healthy adult females (*n*=36, mean ± SD age: 26.8 ± 8 years). In conclusion, the InBody$_{230}$ may be valid in research and population settings, but not at the individual level (von Hurst et al., 2016).

2.7.4 Body composition of females living in New Zealand

The BMI of adults in New Zealand is available from the New Zealand Health Survey (NZHS) 2020/21, a large, nationally representative random sample (Ministry of Health, 2021). Using BMI, in females aged 15-24-years, 4.0% were classified as underweight (<18.5kg/m^2), 42.9% as healthy (18.5-24.95kg/m^2), 26.7% as overweight (25-29.9kg/m^2) and 26.3% as obese (>30kg/m^2). A total of 35.9% of all adult females were classified as obese in the NZHS 2020/21, which continues an upward trend from 21% in 1997 (Ministry of Health, 2021). Although the aetiology of obesity is complex, the environment in New Zealand has become increasingly obesogenic with the wider availability of cheap energy-dense and nutrient-poor foods, alongside a reduction in physical activity (Swinburn, 2008). In a cohort of post-menarche, premenopausal New Zealand women (*n*=378) aged 16-45-years with representation from Māori, Pacific and New Zealand European ethnic groups, the mean ± SD for age was 31.0 ± 0.4 years, for BMI was 27.1 ± 0.3kg/m^2 and for %BF was 34.0 ± 0.4% (Jayasinghe et al., 2019).

2.7.5 Assessment of body composition in the military

Optimal body composition, described as healthy %BF and high FFM (Foulis et al., 2021a) has been associated with several military relevant outcomes, such as improved physical performance (Pierce et al., 2017), reduced musculoskeletal injuries (Jones et al., 2017) and fewer premature discharges from military service (Knapik et al., 2001).

While BMI and %BF are correlated, the large interindividual variations for age, sex and ethnicity combined with an inability to distinguish between weight associated with fat, muscle and bone, makes BMI an imprecise measure for body composition in military personnel (Foulis et al., 2021b). There is currently no consensus on optimal %BF within the military. However, %BF alone may also not sufficiently describe optimal body composition. Recently, Foulis et al. (2021b) observed that in male and female United States Army recruits, a low BMI was indicative of lower-than-expected FFM, while %BF was considered normal. Foulis et al. (2021b) subsequently suggested replacing BMI screening with assessment of lean mass and %BF based on sex-adjusted standards, in combination with physical performance tests that demonstrate strength capability.

2.7.5.1 Body composition assessment for military enlistment

Body composition standards are common in many militaries as part of broad medical requirements for enlistment. Despite the limitations of BMI as an indirect measure of body fat at the individual level, BMI is consistently used as a screening tool for military personnel. As a result, few studies have explored %BF in female recruits. Table 2.11 displays the body composition standards for enlistment in the New Zealand Defence Force and key international partners.

Table 2.11. International body composition standards for military enlistment

Military (Year)	Body mass index (kg/m^2)		Percent body fat (%)	
New Zealand Defence Force (New Zealand Defence Force, 2022)	18-30	Applicants with BMI 31-33 may be eligible following additional assessments		
Australian Defence Force (Defence Jobs, 2022)	18.5-32.9			
British Army (Ministry of Defence, 2022)	<30	Applicants with BMI 30-32 may be eligible following additional assessments		
United States Army (U.S. Department of Defense, 2022)	• Weight for height table with minimum and maximum weight for age categories • The maximum weight equates approximately to a BMI of 26kg/m^2 for females and 27.5kg/m^2 for males		≤30[a] ≤32[b]	• Applicants exceeding the weight for height standard can undergo further screening to estimate %BF, based on body circumference measures of neck, waist and hip (for females)

[a] Females 17-20-years.

[b] Females 21-27-years.

2.7.6 Associations between body composition and physical fitness in females enlisting in the military

Body composition data and physical fitness outcomes were reviewed in female recruits voluntarily enlisting in the Finnish Defence Forces between 2005 and 2015. At entry to basic training, both BM and BMI were inversely associated with the distance covered in the 12-minute running test and the muscle fitness index, based on the results of a standing long jump, push-ups and sit-ups (both $P<0.001$). This indicates that heavier females had lower physical fitness (Santtila et al., 2018). A systematic review examining changes in the physical fitness of United States Army recruits between 1975 and 2013 showed that running performance over 3.2km declined, possibly associated with the increase in BM over the same period (Knapik et al., 2017). No further literature specifically investigates relationships between body composition and physical fitness in female recruits during basic training.

2.7.7 Associations between percent body fat and physical fitness in military personnel

In male Finnish conscripts (*n*=140) increased %BF (measured via DXA) was the strongest predictor of reduced aerobic fitness (as measured by the distance covered in a 12-minute running test) and muscular strength (as measured by standing long jump distance, sit-ups, push-ups, pull-ups and back extensions). Muscular strength was also associated with bone mineral density. Body mass index was not associated with physical fitness in the multivariable models (Mattila et al., 2007). Female soldiers in the United States Army (*n*=629) who met the %BF standards described in Table 2.11 performed significantly better in the 3.2km run and sit-ups compared to soldiers that did not meet the standards. There was no significant difference for push-ups. The mean ± SD for age was 26.3 ± 5.9 years (Anderson et al., 2014). Pletcher et al. (2022) suggested that a lower %BF decreases the amount of work required to move the body.

2.7.8 Body composition changes in females during basic training

Multiple studies have investigated body composition changes in females during basic training. Comparisons between studies are difficult due to training duration, training regimens, EE, dietary intake, assessment methods of body composition, measures of body composition reported and typically low sample sizes. Excluding the mixed gender platoon investigated by Blacker et al. (2009), the study by Lieberman et al. (2008) and the study by Evans et al. (2008) that did not record BM at the end of basic training, all other studies displayed in Table 2.12 observed no change in BM. This is due to a significant reduction in %BF in all studies, except Richmond et al. (2012), alongside a significant increase in lean mass (LM) (Etzion-Daniel et al., 2008; Evans et al., 2008; Foulis et al., 2021a) or FFM (Lieberman et al., 2008; Margolis et al., 2012; Nindl et al., 2011; Richmond et al., 2012). These positive body composition changes highlight the caution required when interpreting BM and therefore BMI data in this population. For those studies reporting a significant loss in %BF, an average of 1.1-1.8% of baseline body fat was lost per week (Blacker et al., 2009; Foulis et al., 2021a; Lieberman et al., 2008; Margolis et al., 2012; Proctor et al., 2018).

2.7.9 Sex differences in body composition and injury risk during basic training

In comparison to males, age-matched females are typically shorter, have smaller BM, more FM, less FFM, fewer type II muscle fibres and less bone mass (Nindl et al., 2016). As a result of these physiological sex differences, females typically have lower aerobic fitness (Nindl et al., 2016) and increased risk of musculoskeletal injury (Knapik et al., 2001) than males. A recent meta-analysis of 12 studies investigating rates of all injuries during basic training reported females had a higher incidence of injury compared to males in all studies, with a total RR = 2.10, 95% CI = 1.89, 2.33 (Schram et al., 2022). Most of these studies were conducted in United States military recruits, with one study each from the British (Blacker et al., 2008) and Irish (Kerr, 2004) armies.

The physiological sex differences have been suggested as the cause of greater injury risk in females (Gemmell, 2002). However, when aerobic fitness is controlled for, sex is no longer a risk factor. Studies have demonstrated that injury risk is similar between females and males with the same absolute aerobic fitness (Bell et al., 2000; Jones & Knapik, 1999).

Table 2.12. Body composition changes in females during basic training

Author (Year)	Basic training	Characteristics of participants	Assessment method	Body composition			*P* value
				Measure	Time 1	Time 2	
Foulis et al. (2021a)	United States Army 10-weeks	*n*=573 20.0 ± 4.0 years	Dual energy x-ray absorptiometry		**Week 1**	**Week 9**	
				BM[a]	62.6 ± 8.8	62.7 ± 8.0	
				%BF	32.4 ± 5.2	28.4 ± 4.3	<0.05
				LM	41.2 ± 5.6	43.8 ± 5.5	<0.05
Proctor et al. (2018)	United States Army 10-weeks	*n*=14 18+ years	Electronic scale and 3-site skinfolds		**Week 1**	**Week 10**	
				BM	63.4 ± 11.3	65.2 ± 10.0	
				%BF	19.8 ± 3.9	17.7 ± 2.9	<0.001
Richmond et al. (2012)	British Army 14-weeks	*n*=10 18.6 ± 1.9 years	Electronic scale and TBW assessed by DLW		**Week 1**	**Week 14**	
				BM	57.2 ± 9.3	57.9 ± 8.4	
				%BF	27.3 ± 5.5	27.0 ± 4.9	
				FFM	41.3 ± 5.6	42.2 ± 5.6	<0.05
Margolis et al. (2012)	United States Army 10-weeks	*n*=91 23.0 ± 6.0 years	Electronic scale and 3-site skinfolds		**Week 0**	**Week 9**	
				BM	66.3 ± 8.3	66.4 ± 7.4	
				%BF	26.6 ± 5.6	22.8 ± 5.1	<0.05
				FFM	48.2 ± 4.8	51.0 ± 5.3	<0.05
Nindl et al. (2011)	United States Army 8-weeks	*n* =77 21.0 ± 5.0 years	Electronic scale and 3-site skinfolds		**Week 1**	**Week 8**	
				BM	62.7 ± 8.5	62.4 ± 6.8	
				FM	17.3 ± 5.4	14.2 ± 3.4	≤0.05
				FFM	45.4 ± 4.8	48.2 ± 5.0	≤0.05
Blacker et al. (2009)	British Army 12-weeks	*n*=7[b] 18.1 ± 2.2 years	Bioelectrical impedance analysis		**Week 1**	**Week 11**	
				BM	58.7 ± 7.1	61.1 ± 5.6	<0.05
				%BF	22 ± 4	19 ± 3	<0.05
		n=9[c] 19.8 ± 4.2 years			**Week 1**	**Week 11**	
				BM	59.6 ± 6.6	59.0 ± 5.0	
				%BF	23 ± 5	18 ± 3	<0.05
Evans et al. (2008)	Israel Defense Forces 16-weeks	*n*=153 19.0 ± 1.0 years	Electronic scale and 4-site skinfolds		**Week 1**	**Week 16**	
				BM	60.9 ± 10.2		
				%BF	30.7 ± 4.9	29.0 ± 4.5	<0.002
				LM	41.9 ± 5.3	43.8 ± 5.0	<0.002

Author (Year)	Basic training	Characteristics of participants	Assessment method	Body composition			*P* value
				Measure	Time 1	Time 2	
Etzion-Daniel et al. (2008)	Israel Defense Forces 16-weeks	*n*=92 and 83[d] 18-19-years	Electronic scale and 4-site skinfolds		**Week 0**	**Week 16**	
				BM	61.0 ± 10.4	62.3 ± 9.9	
				%BF	33.7 ± 3.6	31.8 ± 3.8	<0.05
				LBM	40.1 ± 5.4	42.2 ± 5.4	<0.05
Lieberman et al. (2008)	United States Marine Corps 13-weeks	*n* =50 19.7 ± 2.1 years	Dual energy x-ray absorptiometry		**Week 1**	**Week 12**	
				BM	63.9 ± 0.8	61.7 ± 0.7	<0.001
				%BF	30.2 ± 0.7	23.7 ± 0.7	<0.001
				FFM	41.7 ± 0.5	44.1 ± 0.5	<0.001

BM, body mass (kg); %BF, body fat percentage, LM, lean mass (kg); TBW, total body water; DLW, doubly labelled water; FFM, fat free mass; FM, fat mass; LBM, lean body mass.

[a] *n*=502.

[b] Mixed gender platoon.

[c] Female only platoon.

[d] *n*= 92 at week 0 and 83 at week 16.

2.7.10 Conclusion

Optimal body composition, described as healthy %BF and high FFM, has been associated with several military relevant outcomes. Positive changes in body composition, with significant reductions in %BF and increases in FFM have been observed previously in females during basic training (Etzion-Daniel et al., 2008; Foulis et al., 2021a; Margolis et al., 2012; Nindl et al., 2011). These changes have negated any change in BM and BMI, suggesting BMI may be an imprecise measure for body composition in military personnel. No studies have established a situational awareness of body composition changes in female recruits in the New Zealand Army and BMI is currently the only assessment tool utilised. In addition, no studies have explored associations between %BF and physical fitness in female recruits. The InBody$_{230}$ analyser has demonstrated excellent reliability and may be valid in research and population settings.

2.8 Dietary intake

2.8.1 Dietary intake of young women in the New Zealand population

Based on data for women aged 19-30-years from the most recent New Zealand Adult Nutrition Survey (University of Otago and Ministry of Health, 2011), the mean (95% CI) proportion of EI from protein was 15.8% (15.0-16.6), carbohydrate 49.3% (47.9-50.7) and fat 33.0% (31.6-34.3). An estimated 68.4% consumed inadequate dietary calcium intakes. Dietary intake of vitamin D was not presented due to insufficient food composition data. Table 2.13 presents dietary intake data from the New Zealand Adult Nutrition Survey.

Table 2.13. Daily dietary intake of young women in the total population in New Zealand

Energy/nutrient	15-18-years		19-30-years	
	Median daily intake	95% CI	Median daily intake	95% CI
Energy (kJ)	7635	7253-8017	8245	7653-8837
Carbohydrate (g)	231	220-242	239	221-257
Protein (g)	67	63-71	72	67-77
Fat (g)	68	64-72	71	65-77
Iron (mg)	9.1	8.5-9.7	10.2	9.3-11.1
Calcium (mg)	682	630-734	704	630-778

(University of Otago and Ministry of Health, 2011)
CI, confidence interval.

2.8.2 Nutrient requirements of females during basic training

Optimal nutrition is essential to support health and physical performance in female recruits, whilst fuelling training, promoting recovery and enhancing training adaptations, such as gains in skeletal muscle mass (Thomas et al., 2016). Adequate energy and protein intake are required to facilitate skeletal muscle adaptations to physical training. Carbohydrate has a proven role as a key fuel substrate to support muscular work across a range of intensities and duration, with prolonged and/or intermittent activity (Thomas et al., 2016). Iron, calcium and vitamin D have been identified as micronutrients of importance to protect the musculoskeletal health of female military personnel.

Various militaries have specified energy and nutrient requirements to support military feeding in training, garrison and operational settings. The requirements are typically adapted from recommended intakes and dietary guidelines for the general population and literature for both military personnel and athletes. Table 2.14 displays the recommended intake of energy and key nutrients for females undertaking basic training from the United States Military Dietary Reference Intakes (MDRIs) (Headquarters Departments of the Army et al., 2017), United Kingdom Military Dietary Reference Values (MDRVs) (Scientific Advisory Committee on Nutrition, 2017) and Australian MRDIs (Forbes-Ewan, 2009).

Recently, a group of military nutrition researchers from the United States and United Kingdom (Wardle et al., 2021) suggested the following dietary recommendations as best practice for female military personnel to support bone and muscle health:

- Consume at least 20g of high-quality protein in an energy-sufficient state to optimally stimulate muscle protein synthesis following whole-body resistance exercise.
- Consume 4-8g/kg/day carbohydrate and 1.5-2.0g/kg/day protein to preserve performance and muscle mass during energy deficit.
- Consume 18mg/day dietary iron.
- Consume at least 15µg/day vitamin D and 1000mg/day calcium.

Table 2.14. Military recommendations for energy and key nutrients for females during basic training

Energy and nutrients	US MDRI[a]	UK MDRV[b,c]	AUS MRDI[d]
Energy kJ/day	11,300	13,100	11,500
Protein %EI (g/day)	10 – 35 (70 – 235)	12 – 15 (95 – 115)	13 – 18 (90 – 125)
Carbohydrate %EI (g/day)	50 – 55 (335 – 365)	50 – 60 (385 – 465)	54 – 59 (365 – 400)
Fat %EI (g/day)	25 – 30 (80 – 95)	28 – 35 (100 – 125)	23 – 33 (75 – 105)
Iron (mg/day)	18	14.8	18
Calcium (mg/day)	1000[e]	700[e]	1000[f]
Vitamin D (IU/day)	600	400	200

(Forbes-Ewan, 2009; Headquarters Departments of the Army et al., 2017; Scientific Advisory Committee on Nutrition, 2017)

US MDRI, United States Military Dietary Reference Intakes; UK MDRV, United Kingdom Military Dietary Reference Values; AUS MRDI, Australian Military Recommended Dietary Intakes; EI, energy intake.

[a] Energy based on heavy activity level for reference female of 69kg aged 19+ years.

[b] Energy is the median Estimated Average Requirement (EAR) based on physical activity level of the reference female aged 18-30-years, body mass 60.0kg, height 1.65m undertaking basic training in the British Army. The 25th and 75th percentile for energy is 12,100 and 13,800 kJ/day, respectively.

[c] The MDRVs consider the recommended micronutrient intakes for the general UK population adequate for military personnel. Iron, calcium and vitamin D values are based on adults aged 19-50-years (Public Health England, 2016).

[d] Energy is based on the physical activity level of females aged 19-30 years undertaking basic training in the Australian Army.

[e] Recommended intake for calcium for personnel aged 15-18-years is 800mg/day.

[f] Recommended intake for calcium for personnel aged 17-18-years is 1300mg/day.

Notes:

1. Alcohol is excluded from macronutrient recommendations as it is not available during basic training.
2. The g/day value is rounded up to the nearest 5g.
3. Values displayed in the table may differ from the source documents as conversion factors were used to create consistency across reports.
    a) Atwater factors of 17kJ/g for carbohydrate and protein and 37kJ/g for fat.
    b) Conversion factor of 4.184 from kcal to kJ.
    c) Vitamin D expressed as μg/L was converted to IU by a factor of 40.

2.8.3 Dietary intake of females during basic training

Female recruits may experience challenges consuming adequate energy and nutrients to meet the demands of heavy physical training due to psychological and environmental stressors. Within the garrison environment these include, but are not limited to, rigid meal timings, limited window for eating (commonly 20 minutes), restricted access to food outside meal timings, limited and repetitive food choices, suboptimal dietary behaviours and anxiety related to upcoming training. During field-based training, stressors may include the weather, time available to prepare meals, reluctance to carry the additional weight of food items and limited and potentially undesirable choices from operational ration packs (Baker et al., 2020; O'Leary et al., 2020).

Studies that investigated dietary intake in females during basic training (Table 2.15) report substantial underconsumption of daily EI, on average, in comparison to MDRIs, MDRVs and MRDIs (Chapman et al., 2019; Etzion-Daniel et al., 2008; Herzman-Harari et al., 2013; Margolis et al., 2012). This energy deficiency likely contributes to recommended intakes of macro- and micro-nutrients consistently not being met, particularly for carbohydrate, protein, iron, calcium and vitamin D (Chapman et al., 2019; Etzion-Daniel et al., 2008; Herzman-Harari et al., 2013; Lutz et al., 2019; Margolis et al., 2012). Prolonged energy deficiency may increase the risk of low energy availability, leading to impaired physiological functioning and performance (Mountjoy et al., 2018). The poor intake of dietary iron is concerning as it may be compounded by declines in iron status with high training volumes over a long duration, as previously seen in female recruits (Israeli et al., 2008; McClung et al., 2009b; McClung et al., 2006). While this section has focused on the dietary intake of macronutrients, iron, calcium and vitamin D, females have also been reported to under-consume vitamin A, vitamin E, magnesium, potassium, folate, copper, zinc, α-linolenic acid and dietary fibre (Chapman et al., 2019; Etzion-Daniel et al., 2008; Herzman-Harari et al., 2013; Lutz et al., 2019).

Studies that assessed dietary intake before commencing basic training and again during basic training observed that despite increased energy demands for recruits, EI intake was not increased to meet these requirements. Two studies from Israel reported a significant decrease in EI from baseline to the end of basic training (Etzion-Daniel et al., 2008; Herzman-Harari et al., 2013). In female recruits in the United States Army there was no change in EI (Margolis et al., 2012). The low EI observed by Herzman-Harari et al. (2013) during the final 2-months of basic training was despite a nutrition education programme delivered to recruits during that period.

Table 2.15. Dietary intake of females during basic training

Author (Year) Design Participants	Dietary assessment	Time	Energy (kJ/day)	Carbohydrate (g)	Protein (g)	Fat (g)	Calcium (mg)	Iron (mg)	Vitamin D (µg)
Chapman et al. (2019)[a] British Army Observational cross-sectional study *n*=28 21.4 ± 3.0 years	Researcher-led food weighing at meals, participants completed food diaries and collected waste for additional snacks	Average over 8-days during week 10	9,234 ± 2,448	243 ± 79 45% EI 3.7g/kg BM	83 ± 18 15% EI 1.3g/kg BM	100 ± 27 40% EI	699 ± 287	7 ± 2	1 ± 1
Lutz et al. (2019)[b] United States Army Cross-sectional multi-centre study *n*=280 22.0 ± 5.0 years	110-item semi-quantitative FFQ	Estimated foods consumed during 9-weeks of basic training		53 (49, 57)	15 (13, 17)	34 (30, 37)	748 (569, 1094)	14 (11, 18)	3 (2, 6)

Author (Year) Design Participants	Dietary assessment	Time	Energy (kJ/day)	Carbohydrate (g)	Protein (g)	Fat (g)	Calcium (mg)	Iron (mg)	Vitamin D (µg)
Margolis et al. (2012)[a] US Army Longitudinal study *n*=67 23.0 ± 6.0 years[c]	Semi-quantitative FFQ	Weeks 0 and 9 to assess habitual dietary intake before basic training and changes during training	**Week 0** 7,632 ± 4,243	222 ± 125 49% EI 3.5g/kg BM	69 ± 38 15% EI 1.1g/kg BM	73 ± 46 35% EI			
			Week 9 7,485 ± 2,565	240 ± 84 55% EI 3.6g/kg BM	68 ± 23 15% EI 1.0g/kg BM	66 ± 26 33% EI			
Herzman-Harari et al. (2013)[d] Israel Defense Forces Longitudinal study *n*=44 preinduction *n*=43 2-months *n*=38 post-basic training[e] 18.8 ± 0.1 years	FFQ	Preinduction assessed the previous year before enlistment, 2-months assessed the first half of basic training, and post-basic training (4-months) assessed the second half of basic training	**Preinduction** 9,958 ± 469	323 ± 17 54% EI	97 ± 5 16% EI	79 ± 4 30% EI	926 ± 59	14 ± 1	4 ± 0.5
			2-months[f] 7,088 ± 527	218 ± 19 51% EI	62 ± 5 15% EI	65 ± 5 35% EI	520 ± 51	9 ± 1	1 ± 0.1
			Post-BT[f] 8,017 ± 498	257 ± 20 54% EI	76 ± 5 16% EI	66 ± 4 31% EI	632 ± 43	12 ± 1	2 ± 0.3

Author (Year) Design Participants	Dietary assessment	Time	Energy (kJ/day)	Carbohydrate (g)	Protein (g)	Fat (g)	Calcium (mg)	Iron (mg)	Vitamin D (µg)
Etzion-Daniel et al. (2008)[a] Israel Defense Forces Longitudinal study *n*=92 preinduction *n*=83 post-basic training 18.8 ± 0.1 years	126-item semi-quantitative FFQ	Preinduction assessed the previous year before enlistment and post-basic training (4-months later) assessed the previous 2-months during basic training	**Preinduction** 8,339 ± 3,079	258 ± 95 53% EI 4.2g/kg BM	73 ± 37 15% EI 1.2g/kg BM	76 ± 32 34% EI	702 ± 360	14 ± 6	3 ± 3
			Post-BT[g] 7,100 ± 2,402	260 ± 94 63% EI 4.2g/kg BM	66 ± 23 16% EI 1.1g/kg BM	76 ± 32 34% EI	580 ± 224	13 ± 5	2 ± 1

EI, energy intake; BM, body mass; FFQ, food frequency questionnaire; BT, basic training.

[a] Absolute daily nutrient intake, mean ± standard deviation; proportion of energy intake, %; relative daily nutrient intake, g/kg of body mass.

[b] Estimated intake median (25th, 75th percentile) as a proportion of energy intake for carbohydrate, protein and fat, %.

[c] Based on *n*=91 from original group.

[d] Absolute daily nutrient intake, mean ± standard error; proportion of energy intake, %.

[e] Between the 2-months and post-basic training assessment periods, a nutrition intervention programme with 15 education sessions was delivered.

[f] Energy, carbohydrate, protein, fat, calcium, iron and vitamin D significantly different between time points, *P*<0.05.

[g] Energy, protein, calcium, iron and vitamin D significantly different between time points, *P*<0.05.

Note:

4. Values displayed in the table may differ from the source documents as conversion factors were used to create consistency across studies.
    d) Atwater factors of 17kJ/g for carbohydrate and protein and 37kJ/g for fat.
    e) Conversion factor of 4.184 from kcal to kJ.
    f) Vitamin D expressed in IU was converted to µg by dividing by a factor of 40.

2.8.4 Dietary assessment methods in the military

In a recent scoping review, the dietary assessment tools used to measure the whole diet in both military and veteran populations were investigated (Collins et al., 2020). Of the 89 studies examined, 85% included one dietary assessment method (*n*=76) and 15% included multiple methods (*n*=13). Food frequency questionnaires were the most common method and featured in 45% (*n*=40) of studies. Dietary intake was primarily compared to country-based dietary guidelines, while comparison to military dietary guidelines was minimal. Nutrition research in the military has historically focused on investigating individual foods and nutrients and few studies have examined DPs. Nakayama et al. (2019) investigated DPs via reduced rank regression, based on dietary intake data from a quantitative validated FFQ. Recruits (*n*=401) from across the United States Army, Air Force and Marines were assessed with the objective to assess whether a DP rich in calcium, potassium and protein before basic training is positively associated with bone health indexes of the tibia confirmed. This was achieved through a higher dietary intake of milk, yoghurt and vegetables and lower dietary intake of oils, refined grains and added sugars. In 805 Belgian soldiers, three DPs ('meat', 'healthy' and 'sweet') determined by factor analysis were not associated with an increased BMI after a 5-year follow-up (Mullie & Clarys, 2016). As the participants were all male, white, aged 20-55-years and living freely in the community, they were not representative of female recruits undertaking basic training.

2.8.5 Associations between dietary intake and body composition or physical fitness in females during basic training

No studies have investigated associations between dietary intake and body composition or physical fitness in females during basic training. In studies that reported dietary intake and BM, two studies observed no change in BM during basic training (Etzion-Daniel et al., 2008; Margolis et al., 2012) and one study reported an average increase of 1.9 ± 0.4kg over 16-weeks (Herzman-Harari et al., 2013). Despite all studies reporting an EI substantially lower than recommended, the maintenance of BM throughout basic training suggests energy balance was achieved. This may be the result of underreporting in semi-quantitative FFQs, in comparison to more accurate dietary assessment methods, such as weighed food records (Bailey, 2021). Further research is required to determine how changes in habitual dietary intake may be associated with body composition adaptations and physical fitness during basic training.

2.8.6 Conclusion

Optimal dietary intake is a critical component of military readiness. Female recruits may experience challenges consuming adequate energy and nutrients to meet the demands of heavy physical training. Studies investigating changes in dietary intake of female recruits during basic training have reported that EI is not increased to meet higher energy demands recommended by the MDRIs (Headquarters Departments of the Army et al., 2017). This has likely contributed to recommended intakes of key nutrients not being met (Etzion-Daniel et al., 2008; Herzman-Harari et al., 2013; Margolis et al., 2012). No studies have established a situational awareness of changes in dietary intake in female recruits in the New Zealand Army or associations between habitual dietary intake and body composition or physical fitness during basic training.

2.9 Summary of literature review

This literature review focused on nutrition related health challenges concerning female recruits, including iron, vitamin D, physical fitness, body composition and habitual dietary intake. Associations between physical fitness and suboptimal iron status are not well described, particularly iron status that falls outside the parameters for iron deficiency anaemia. Previous studies investigating changes in iron status in female recruits have been limited due to relatively short durations, assessment of aerobic fitness alone and no inclusion of inflammatory biomarkers. Further research is required to investigate changes in iron status over a longer training duration and associations between iron status and physical fitness in female recruits. In addition, simultaneous investigation of dietary and non-dietary determinants of iron stores should be explored in females enlisting in the military. Suboptimal vitamin D status is associated with musculoskeletal health and performance in military personnel. Previous studies exploring vitamin D status and longitudinal changes in female recruits have been limited in duration and location. Further research to confirm these findings in female recruits that are ethnically diverse and training at geographical locations not previously studied are required. Investigating determinants of vitamin D status should also be examined. Physical fitness, body composition and dietary intake are inter-related factors in optimising the health and performance of military personnel. Previous studies have not simultaneously investigated changes in these three factors during basic training, which typically elicits a sudden change from an individual’s habitual lifestyle. Further research is therefore warranted to not only describe the changes observed in physical fitness, body composition and dietary intake, but also associations between these three factors. In conclusion, while research regarding the nutrition related health of female recruits exists, numerous questions remain regarding the determinants of nutritional status, changes during basic training and associations with functional outcomes. This is particularly relevant in recruit populations outside of the United States and United Kingdom.

2.10 References

Ackland, T. R., Lohman, T. G., Sundgot-Borgen, J., Maughan, R. J., Meyer, N. L., Stewart, A. D., & Müller, W. (2012). Current status of body composition assessment in sport: review and position statement on behalf of the ad hoc research working group on body composition health and performance, under the auspices of the I.O.C. Medical Commission. *Sports Medicine*, *42*(3), 227-249. https://doi.org/10.2165/11597140-000000000-00000

Ahmad Fuzi, S. F., Koller, D., Bruggraber, S., Pereira, D. I., Dainty, J. R., & Mushtaq, S. (2017). A 1-h time interval between a meal containing iron and consumption of tea attenuates the inhibitory effects on iron absorption: a controlled trial in a cohort of healthy UK women using a stable iron isotope. *The American Journal of Clinical Nutrition*, *106*(6), 1413-1421. https://doi.org/10.3945/ajcn.117.161364

Ahmed, M., Praneet Ng, A., & L'Abbe, M. R. (2021). Nutrient intakes of Canadian adults: results from the Canadian Community Health Survey (CCHS)-2015 Public Use Microdata File. *American Journal of Clinical Nutrition*, *114*(3), 1131-1140. https://doi.org/10.1093/ajcn/nqab143

Albanese, C. V., Diessel, E., & Genant, H. K. (2003). Clinical applications of body composition measurements using DXA. *Journal of Clinical Densitometry*, *6*(2), 75-85. https://doi.org/10.1385/jcd:6:2:75

Alleyne, M., Horne, M. K., & Miller, J. L. (2008). Individualized treatment for iron-deficiency anemia in adults. *American Journal of Medicine*, *121*(11), 943-948. https://doi.org/10.1016/j.amjmed.2008.07.012

Andersen, N. E., Karl, J. P., Cable, S. J., Williams, K. W., Rood, J. C., Young, A. J., Lieberman, H. R., & McClung, J. P. (2010). Vitamin D status in female military personnel during combat training. *Journal of the International Society of Sports Nutrition*, *7*, 38. https://doi.org/10.1186/1550-2783-7-38

Anderson, G. J., & Frazer, D. M. (2017). Current understanding of iron homeostasis. *American Journal of Clinical Nutrition*, S1559-s1566. https://doi.org/10.3945/ajcn.117.155804

Anderson, L. J., Erceg, D. N., & Schroeder, E. T. (2012). Utility of multifrequency bioelectrical impedance compared with dual-energy x-ray absorptiometry for assessment of total and regional body composition varies between men and women. *Nutrition Research*, *32*(7), 479-485. https://doi.org/10.1016/j.nutres.2012.05.009

Anderson, M. K., Grier, T., Canham-Chervak, M., Bushman, T. T., & Jones, B. H. (2014). Risk factors associated with higher body fat in US Army female soldiers. *U.S. Army Medical Department Journal*, 75-82.

Araki, T., Holick, M. F., Alfonso, B. D., Charlap, E., Romero, C. M., Rizk, D., & Newman, L. G. (2011). Vitamin D intoxication with severe hypercalcemia due to manufacturing and labeling errors of two dietary supplements made in the United States. *Journal of Clinical Endocrinology and Metabolism*, *96*(12), 3603-3608. https://doi.org/10.1210/jc.2011-1443

Asakura, K., Sasaki, S., Murakami, K., Takahashi, Y., Uenishi, K., Yamakawa, M., Nishiwaki, Y., Kikuchi, Y., & Takebayashi, T. (2009). Iron intake does not significantly correlate with iron deficiency among young Japanese women: a cross-sectional study. *Public Health Nutrition*, *12*(9), 1373-1383. https://doi.org/10.1017/s1368980008004072

Atef, S. H. (2018). Vitamin D assays in clinical laboratory: past, present and future challenges. *Journal of Steroid Biochemistry and Molecular Biology*, *175*, 136-137. https://doi.org/10.1016/j.jsbmb.2017.02.011

Bae, J. H., Choe, H. J., Holick, M. F., & Lim, S. (2022). Association of vitamin D status with COVID-19 and its severity : vitamin D and COVID-19: a narrative review. *Reviews in Endocrine & Metabolic Disorders*, 1-21. https://doi.org/10.1007/s11154-021-09705-6

Bailey, R. L. (2021). Overview of dietary assessment methods for measuring intakes of foods, beverages, and dietary supplements in research studies. *Current Opinion in Biotechnology*, *70*, 91-96. https://doi.org/10.1016/j.copbio.2021.02.007

Baker, B. A., Cooke, M. B., Belski, R., & Carins, J. E. (2020). The influence of training on new army recruits' energy and macronutrient intakes and performance: a systematic literature review. *Journal of the Academy of Nutrition and Dietetics*, *120*(10), 1687-1705. https://doi.org/10.1016/j.jand.2020.06.004

Beard, J., & Tobin, B. (2000). Iron status and exercise. *American Journal of Clinical Nutrition*, *72*(2 Suppl), 594s-597s. http://ajcn.nutrition.org/content/72/2/594s.full.pdf

Beck, K., Conlon, C. A., Kruger, R., Coad, J., & Stonehouse, W. (2011). Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial. *British Journal of Nutrition*, *105*(1), 101-109. https://doi.org/10.1017/s0007114510003144

Beck, K. L., Conlon, C. A., Kruger, R., Heath, A. L., Matthys, C., Coad, J., Jones, B., & Stonehouse, W. (2014). Blood donation, being Asian, and a history of iron deficiency are stronger predictors of iron deficiency than dietary patterns in premenopausal women. *Biomed Res Int*, *2014*, 652860. https://doi.org/10.1155/2014/652860

Beck, K. L., Kruger, R., Conlon, C. A., Heath, A. L., Coad, J., Matthys, C., Jones, B., & Stonehouse, W. (2012). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women. *Journal of the Academy of Nutrition and Dietetics*, *112*(8), 1177-1187. https://doi.org/10.1016/j.jand.2012.05.012

Beck, K. L., Kruger, R., Conlon, C. A., Heath, A. L., Matthys, C., Coad, J., & Stonehouse, W. (2013). Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand. *European Journal of Nutrition*, *52*(2), 467-476. https://doi.org/10.1007/s00394-012-0348-y

Bell, N. S., Mangione, T. W., Hemenway, D., Amoroso, P. J., & Jones, B. H. (2000). High injury rates among female army trainees: a function of gender? *American Journal of Preventive Medicine*, *18*(3 Suppl), 141-146.

Bertrand, K. A., Giovannucci, E., Liu, Y., Malspeis, S., Eliassen, A. H., Wu, K., Holmes, M. D., Laden, F., & Feskanich, D. (2012). Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three US cohorts. *British Journal of Nutrition*, *108*(10), 1889-1896. https://doi.org/10.1017/s0007114511007409

Bikle, D. D. (2014). Vitamin D metabolism, mechanism of action, and clinical applications. *Chemistry and Biology*, *21*(3), 319-329. https://doi.org/10.1016/j.chembiol.2013.12.016

Black, L. J., Anderson, D., Clarke, M. W., Ponsonby, A. L., & Lucas, R. M. (2015). Analytical bias in the measurement of serum 25-hydroxyvitamin D concentrations impairs assessment of vitamin D status in clinical and research settings. *PloS One*, *10*(8), e0135478. https://doi.org/10.1371/journal.pone.0135478

Blacker, S. D., Wilkinson, D. M., Bilzon, J. L., & Rayson, M. P. (2008). Risk factors for training injuries among British Army recruits. *Military Medicine*, *173*(3), 278-286.

Blacker, S. D., Wilkinson, D. M., & Rayson, M. P. (2009). Gender differences in the physical demands of British Army recruit training. *Military Medicine*, *174*(8), 811-816. https://doi.org/10.7205/milmed-d-01-3708

Blacklock, N. J. (1977). Bladder trauma in the long-distance runner: "10,000 metres haematuria". *British Journal of Urology*, *49*(2), 129-132.

Blanco-Rojo, R., Baeza-Richer, C., López-Parra, A. M., Pérez-Granados, A. M., Brichs, A., Bertoncini, S., Buil, A., Arroyo-Pardo, E., Soria, J. M., & Vaquero, M. P. (2011). Four variants in transferrin and HFE genes as potential markers of iron deficiency anaemia risk: an association study in menstruating women. *Nutrition & Metabolism*, *8*, 69. https://doi.org/10.1186/1743-7075-8-69

Blanco-Rojo, R., Toxqui, L., López-Parra, A. M., Baeza-Richer, C., Pérez-Granados, A. M., Arroyo-Pardo, E., & Vaquero, M. P. (2014). Influence of diet, menstruation and genetic factors on iron status: a cross-sectional study in Spanish women of childbearing age. *International Journal of Molecular Sciences*, *15*(3), 4077-4087. https://doi.org/10.3390/ijms15034077

Blanton, C. (2013). Improvements in iron status and cognitive function in young women consuming beef or non-beef lunches. *Nutrients*, *6*(1), 90-110. https://doi.org/10.3390/nu6010090

Blee, T., Goodman, C., Dawson, B., & Stapff, A. (1999). The effect of intramuscular iron injections on serum ferritin levels and physical performance in elite netballers. *Journal of Science and Medicine in Sport*, *2*(4), 311-321.

Bolland, M. J., Chiu, W. W., Davidson, J. S., Grey, A., Bacon, C., Gamble, G. D., & Reid, I. R. (2008). The effects of seasonal variation of 25-hydroxyvitamin D on diagnosis of vitamin D insufficiency. *New Zealand Medical Journal*, *121*(1286), 63-74.

Brasse-Lagnel, C., Karim, Z., Letteron, P., Bekri, S., Bado, A., & Beaumont, C. (2011). Intestinal DMT1 cotransporter is down-regulated by hepcidin via proteasome internalization and degradation. *Gastroenterology*, *140*(4), 1261-1271.e1261. https://doi.org/10.1053/j.gastro.2010.12.037

Brownlie, T. t., Utermohlen, V., Hinton, P. S., Giordano, C., & Haas, J. D. (2002). Marginal iron deficiency without anemia impairs aerobic adaptation among previously untrained women. *American Journal of Clinical Nutrition*, *75*(4), 734-742. http://ajcn.nutrition.org/content/75/4/734.full.pdf

Brownlie, T. t., Utermohlen, V., Hinton, P. S., & Haas, J. D. (2004). Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. *American Journal of Clinical Nutrition*, *79*(3), 437-443. http://ajcn.nutrition.org/content/79/3/437.full.pdf

Burden, R. J., Pollock, N., Whyte, G. P., Richards, T., Moore, B., Busbridge, M., Srai, S. K., Otto, J., & Pedlar, C. R. (2015). Effect of intravenous iron on aerobic capacity and iron metabolism in elite athletes. *Medicine and Science in Sports and Exercise*, *47*(7), 1399-1407. https://doi.org/10.1249/mss.0000000000000568

Burgi, A. A., Gorham, E. D., Garland, C. F., Mohr, S. B., Garland, F. C., Zeng, K., Thompson, K., & Lappe, J. M. (2011). High serum 25-hydroxyvitamin D is associated with a low incidence of stress fractures. *Journal of Bone and Mineral Research*, *26*(10), 2371-2377. https://doi.org/10.1002/jbmr.451

Cable, R. G., Glynn, S. A., Kiss, J. E., Mast, A. E., Steele, W. R., Murphy, E. L., Wright, D. J., Sacher, R. A., Gottschall, J. L., Tobler, L. H., & Simon, T. L. (2012). Iron deficiency in blood donors: the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion*, *52*(4), 702-711. https://doi.org/10.1111/j.1537-2995.2011.03401.x

Cade, J. E., Moreton, J. A., O'Hara, B., Greenwood, D. C., Moor, J., Burley, V. J., Kukalizch, K., Bishop, D. T., & Worwood, M. (2005). Diet and genetic factors associated with iron status in middle-aged women. *American Journal of Clinical Nutrition*, *82*(4), 813-820. https://doi.org/10.1093/ajcn/82.4.813

Callister, P., Galtry, J., & Didham, R. (2011). The risks and benefits of sun exposure: should skin colour or ethnicity be the main variable for communicating health promotion messages in New Zealand? *Ethnicity and Health*, *16*(1), 57-71. https://doi.org/10.1080/13557858.2010.527925

Camaschella, C. (2015a). Iron-deficiency anemia. *New England Journal of Medicine*, *372*(19), 1832-1843. https://doi.org/10.1056/NEJMra1401038

Camaschella, C. (2015b). Iron deficiency: new insights into diagnosis and treatment. *Hematology: The Education Program of the American Society of Hematology*, *2015*, 8-13. https://doi.org/10.1182/asheducation-2015.1.8

Camaschella, C. (2017). New insights into iron deficiency and iron deficiency anemia. *Blood Reviews*, *31*(4), 225-233. https://doi.org/10.1016/j.blre.2017.02.004

Carpenter, C. E., & Mahoney, A. W. (1992). Contributions of heme and nonheme iron to human nutrition. *Critical Reviews in Food Science and Nutrition*, *31*(4), 333-367. https://doi.org/10.1080/10408399209527576

Carroll, R. J., Midthune, D., Subar, A. F., Shumakovich, M., Freedman, L. S., Thompson, F. E., & Kipnis, V. (2012). Taking advantage of the strengths of 2 different dietary assessment instruments to improve intake estimates for nutritional epidemiology. *American Journal of Epidemiology*, *175*(4), 340-347. https://doi.org/10.1093/aje/kwr317

Carswell, A. T., Oliver, S. J., Wentz, L. M., Kashi, D. S., Roberts, R., Tang, J. C. Y., Izard, R. M., Jackson, S., Allan, D., Rhodes, L. E., Fraser, W. D., Greeves, J. P., & Walsh, N. P. (2018). Influence of vitamin D supplementation by sunlight or oral D3 on exercise performance. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/mss.0000000000001721

Celsing, F., Blomstrand, E., Werner, B., Pihlstedt, P., & Ekblom, B. (1986). Effects of iron deficiency on endurance and muscle enzyme activity in man. *Medicine and Science in Sports and Exercise*, *18*(2), 156-161.

Celsing, F., & Ekblom, B. (1986). Anemia causes a relative decrease in blood lactate concentration during exercise. *European Journal of Applied Physiology and Occupational Physiology*, *55*(1), 74-78.

Cepeda-Lopez, A. C., Melse-Boonstra, A., Zimmermann, M. B., & Herter-Aeberli, I. (2015). In overweight and obese women, dietary iron absorption is reduced and the enhancement of iron absorption by ascorbic acid is one-half that in normal-weight women. *American Journal of Clinical Nutrition*, *102*(6), 1389-1397. https://doi.org/10.3945/ajcn.114.099218

Chan, J., Jaceldo-Siegl, K., & Fraser, G. E. (2010). Determinants of serum 25 hydroxyvitamin D levels in a nationwide cohort of blacks and non-Hispanic whites. *Cancer Causes and Control*, *21*(4), 501-511. https://doi.org/10.1007/s10552-009-9481-1

Chapman, S., Roberts, J., Smith, L., Rawcliffe, A., & Izard, R. (2019). Sex differences in dietary intake in British Army recruits undergoing phase one training. *Journal of the International Society of Sports Nutrition*, *16*(1), 59. https://doi.org/10.1186/s12970-019-0327-2

Charoenngam, N., Shirvani, A., & Holick, M. F. (2021). Vitamin D and Its potential benefit for the COVID-19 pandemic. *Endocrine Practice*, *27*(5), 484-493. https://doi.org/10.1016/j.eprac.2021.03.006

Chaston, T., Chung, B., Mascarenhas, M., Marks, J., Patel, B., Srai, S. K., & Sharp, P. (2008). Evidence for differential effects of hepcidin in macrophages and intestinal epithelial cells. *Gut*, *57*(3), 374-382. https://doi.org/10.1136/gut.2007.131722

Cheng, H. L., Griffin, H. J., Bryant, C. E., Rooney, K. B., Steinbeck, K. S., & O'Connor, H. T. (2013). Impact of diet and weight loss on iron and zinc status in overweight and obese young women. *Asia Pacific Journal of Clinical Nutrition*, *22*(4), 574-582. https://doi.org/10.6133/apjcn.2013.22.4.08

Cheng, S., Massaro, J. M., Fox, C. S., Larson, M. G., Keyes, M. J., McCabe, E. L., Robins, S. J., O'Donnell, C. J., Hoffmann, U., Jacques, P. F., Booth, S. L., Vasan, R. S., Wolf, M., & Wang, T. J. (2009). Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. *Diabetes*, *59*(1), 242-248. https://doi.org/10.2337/db09-1011

Coad, J., & Conlon, C. (2011). Iron deficiency in women: assessment, causes and consequences. *Current Opinion in Clinical Nutrition and Metabolic Care*, *14*(6), 625-634. https://doi.org/10.1097/MCO.0b013e32834be6fd

Coffey, R., & Ganz, T. (2017). Iron homeostasis: an anthropocentric perspective. *Journal of Biological Chemistry*, *292*(31), 12727-12734. https://doi.org/10.1074/jbc.R117.781823

Collins, R. A., Baker, B., Coyle, D. H., Rollo, M. E., & Burrows, T. L. (2020). Dietary assessment methods in military and veteran populations: a scoping review. *Nutrients*, *12*(3). https://doi.org/10.3390/nu12030769

Conrad, J., & Nöthlings, U. (2017). Innovative approaches to estimate individual usual dietary intake in large-scale epidemiological studies. *Proceedings of the Nutrition Society*, *76*(3), 213-219. https://doi.org/10.1017/s0029665116003025

Cook, J. D., & Monsen, E. R. (1976). Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. *American Journal of Clinical Nutrition*, *29*(8), 859-867. https://doi.org/10.1093/ajcn/29.8.859

Cooper, K. H. (1968). A means of assessing maximal oxygen intake. Correlation between field and treadmill testing. *JAMA*, *203*(3), 201-204.

Daher, R., Manceau, H., & Karim, Z. (2017). Iron metabolism and the role of the iron-regulating hormone hepcidin in health and disease. *Presse Medicale*, *46*(12 Pt 2), e272-e278. https://doi.org/10.1016/j.lpm.2017.10.006

Dahlquist, D. T., Dieter, B. P., & Koehle, M. S. (2015). Plausible ergogenic effects of vitamin D on athletic performance and recovery. *Journal of the International Society of Sports Nutrition*, *12*, 33. https://doi.org/10.1186/s12970-015-0093-8

Dallman, P. R. (1986). Biochemical basis for the manifestations of iron deficiency. *Annual Review of Nutrition*, *6*, 13-40. https://doi.org/10.1146/annurev.nu.06.070186.000305

Dasharathy, S. S., Mumford, S. L., Pollack, A. Z., Perkins, N. J., Mattison, D. R., Wactawski-Wende, J., & Schisterman, E. F. (2012). Menstrual bleeding patterns among regularly menstruating women. *American Journal of Epidemiology*, *175*(6), 536-545. https://doi.org/10.1093/aje/kwr356

Davey, T., Lanham-New, S. A., Shaw, A. M., Hale, B., Cobley, R., Berry, J. L., Roch, M., Allsopp, A. J., & Fallowfield, J. L. (2016). Low serum 25-hydroxyvitamin D is associated with increased risk of stress fracture during Royal Marine recruit training. *Osteoporosis International*, *27*(1), 171-179. https://doi.org/10.1007/s00198-015-3228-5

Dawson-Hughes, B., Heaney, R. P., Holick, M. F., Lips, P., Meunier, P. J., & Vieth, R. (2005). Estimates of optimal vitamin D status. *Osteoporosis International*, *16*(7), 713-716. https://doi.org/10.1007/s00198-005-1867-7

Day, K., Kwok, A., Evans, A., Mata, F., Verdejo-Garcia, A., Hart, K., Ward, L. C., & Truby, H. (2018). Comparison of a bioelectrical impedance device against the reference method dual energy x-ray absorptiometry and anthropometry for the evaluation of body composition in adults. *Nutrients*, *10*(10). https://doi.org/10.3390/nu10101469

de Koning, L., Al-Turkmani, M. R., Berg, A. H., Shkreta, A., Law, T., & Kellogg, M. D. (2013). Variation in clinical vitamin D status by DiaSorin Liaison and LC-MS/MS in the presence of elevated 25-OH vitamin D2. *Clinica Chimica Acta*, *415*, 54-58. https://doi.org/10.1016/j.cca.2012.09.002

Defence Jobs. (2022). *Defence Jobs Australia - Health & fitness*. Retrieved 22 September 2022 from https://www.defencejobs.gov.au/joining/can-i-join/health-and-fitness

DellaValle, D. M., & Haas, J. D. (2011). Impact of iron depletion without anemia on performance in trained endurance athletes at the beginning of a training season: a study of female collegiate rowers. *International Journal of Sport Nutrition and Exercise Metabolism*, *21*(6), 501-506.

DellaValle, D. M., & Haas, J. D. (2014). Iron supplementation improves energetic efficiency in iron-depleted female rowers. *Medicine and Science in Sports and Exercise*, *46*(6), 1204-1215. https://doi.org/10.1249/mss.0000000000000208

DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. *American Journal of Clinical Nutrition*, *80*(6 Suppl), 1689s-1696s. https://doi.org/10.1093/ajcn/80.6.1689S

Department of Health and Ageing and Ministry of Health. (2006). *Nutrient Reference Values for Australia and New Zealand: including Recommended Dietary Intakes*. Canberra: Commonwealth of Australia.

Di Angelantonio, E., Thompson, S. G., Kaptoge, S., Moore, C., Walker, M., Armitage, J., Ouwehand, W. H., Roberts, D. J., & Danesh, J. (2017). Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): a randomised trial of 45 000 donors. *Lancet*, *390*(10110), 2360-2371. https://doi.org/10.1016/s0140-6736(17)31928-1

Di Santolo, M., Stel, G., Banfi, G., Gonano, F., & Cauci, S. (2008). Anemia and iron status in young fertile non-professional female athletes. *European Journal of Applied Physiology*, *102*(6), 703-709. https://doi.org/10.1007/s00421-007-0647-9

Dijkstra, A., van den Hurk, K., Bilo, H. J. G., Slingerland, R. J., & Vos, M. J. (2019). Repeat whole blood donors with a ferritin level of 30 µg/L or less show functional iron depletion. *Transfusion*, *59*(1), 21-25. https://doi.org/10.1111/trf.14935

Dirks, N. F., Ackermans, M. T., Lips, P., de Jongh, R. T., Vervloet, M. G., de Jonge, R., & Heijboer, A. C. (2018). The when, what & how of measuring vitamin D metabolism in clinical medicine. *Nutrients*, *10*(4). https://doi.org/10.3390/nu10040482

Donovan, A., Lima, C. A., Pinkus, J. L., Pinkus, G. S., Zon, L. I., Robine, S., & Andrews, N. C. (2005). The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metabolism*, *1*(3), 191-200. https://doi.org/10.1016/j.cmet.2005.01.003

Dubnov, G., & Constantini, N. W. (2004). Prevalence of iron depletion and anemia in top-level basketball players. *International Journal of Sport Nutrition and Exercise Metabolism*, *14*(1), 30-37.

Dubnov, G., Foldes, A. J., Mann, G., Magazanik, A., Siderer, M., & Constantini, N. (2006). High prevalence of iron deficiency and anemia in female military recruits. *Military Medicine*, *171*(9), 866-869.

Dudenkov, D. V., Yawn, B. P., Oberhelman, S. S., Fischer, P. R., Singh, R. J., Cha, S. S., Maxson, J. A., Quigg, S. M., & Thacher, T. D. (2015). Changing incidence of serum 25-hydroxyvitamin D values above 50 ng/mL: a 10-year population-based study. *Mayo Clinic Proceedings*, *90*(5), 577-586. https://doi.org/10.1016/j.mayocp.2015.02.012

Dunlop, E., Boorman, J. L., Hambridge, T. L., McNeill, J., James, A. P., Kiely, M., Nowson, C. A., Rangan, A., Cunningham, J., Adorno, P., Atyeo, P., & Black, L. J. (2022). Evidence of low vitamin D intakes in the Australian population points to a need for data-driven nutrition policy for improving population vitamin D status. *Journal of Human Nutrition and Dietetics*. https://doi.org/10.1111/jhn.13002

Duren, D. L., Sherwood, R. J., Czerwinski, S. A., Lee, M., Choh, A. C., Siervogel, R. M., & Cameron Chumlea, W. (2008). Body composition methods: comparisons and interpretation. *Journal of Diabetes Science and Technology*, *2*(6), 1139-1146. https://doi.org/10.1177/193229680800200623

Edefonti, V., De Vito, R., Dalmartello, M., Patel, L., Salvatori, A., & Ferraroni, M. (2020). Reproducibility and validity of a posteriori dietary patterns: a systematic review. *Advances in Nutrition*, *11*(2), 293-326. https://doi.org/10.1093/advances/nmz097

Edefonti, V., Randi, G., La Vecchia, C., Ferraroni, M., & Decarli, A. (2009). Dietary patterns and breast cancer: a review with focus on methodological issues. *Nutrition Reviews*, *67*(6), 297-314. https://doi.org/10.1111/j.1753-4887.2009.00203.x

EFSA Panel on Dietetic Products, Nutrition and Allergies. (2016). Scientific opinion on dietary reference values for vitamin D. *EFSA Journal*, *14(10):4547*. https://doi.org/https://doi.org/10.2903/j.efsa.2016.4547

Ellulu, M. S., Patimah, I., Khaza'ai, H., Rahmat, A., & Abed, Y. (2017). Obesity and inflammation: the linking mechanism and the complications. *Archives of Medical Science*, *13*(4), 851-863. https://doi.org/10.5114/aoms.2016.58928

Etzion-Daniel, Y., Constantini, N., Finestone, A. S., Shahar, D. R., Israeli, E., Yanovich, R., & Moran, D. S. (2008). Nutrition consumption of female combat recruits in army basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S677-684. https://doi.org/10.1249/MSS.0b013e3181894603

Evans, R. K., Antczak, A. J., Lester, M., Yanovich, R., Israeli, E., & Moran, D. S. (2008). Effects of a 4-month recruit training program on markers of bone metabolism. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S660-670. https://doi.org/10.1249/MSS.0b013e318189422b

Farrell, C. J., Martin, S., McWhinney, B., Straub, I., Williams, P., & Herrmann, M. (2012). State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. *Clinical Chemistry*, *58*(3), 531-542. https://doi.org/10.1373/clinchem.2011.172155

Forbes-Ewan, C. (2009). *Australian Defence Force nutritional requirements in the 21st Century (Version 1)*. Retrieved 22 September 2022 from https://apps.dtic.mil/sti/citations/ADA502859

Foulis, S. A., Hughes, J. M., Spiering, B. A., Walker, L. A., Guerriere, K. I., Taylor, K. M., Proctor, S. P., & Friedl, K. E. (2021b). US Army basic combat training alters the relationship between body mass index and per cent body fat. *BMJ Mil Health*. https://doi.org/10.1136/bmjmilitary-2021-001936

Foulis, S. A., Hughes, J. M., Walker, L. A., Guerriere, K. I., Taylor, K. M., Proctor, S. P., & Friedl, K. E. (2021a). Body mass does not reflect the body composition changes in response to similar physical training in young women and men. *International Journal of Obesity (2005)*, *45*(3), 659-665. https://doi.org/10.1038/s41366-020-00730-0

Freedman, L. S., Midthune, D., Arab, L., Prentice, R. L., Subar, A. F., Willett, W., Neuhouser, M. L., Tinker, L. F., & Kipnis, V. (2018). Combining a food frequency questionnaire with 24-hour recalls to increase the precision of estimation of usual dietary intakes-evidence from the Validation Studies Pooling Project. *American Journal of Epidemiology*, *187*(10), 2227-2232. https://doi.org/10.1093/aje/kwy126

Friedl, K. E., Evans, R. K., & Moran, D. S. (2008). Stress fracture and military medical readiness: bridging basic and applied research. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S609-622. https://doi.org/10.1249/MSS.0b013e3181892d53

Gaffney-Stomberg, E., Nakayama, A. T., Guerriere, K. I., Lutz, L. J., Walker, L. A., Staab, J. S., Scott, J. M., Gasier, H. G., & McClung, J. P. (2019). Calcium and vitamin D supplementation and bone health in Marine recruits: effect of season. *Bone*, *123*, 224-233. https://doi.org/10.1016/j.bone.2019.03.021

Galan, P., Yoon, H. C., Preziosi, P., Viteri, F., Valeix, P., Fieux, B., Briançon, S., Malvy, D., Roussel, A. M., Favier, A., & Hercberg, S. (1998). Determining factors in the iron status of adult women in the SU.VI.MAX study. SUpplementation en VItamines et Minéraux AntioXydants. *European Journal of Clinical Nutrition*, *52*(6), 383-388. https://doi.org/10.1038/sj.ejcn.1600561

Galesloot, T. E., Vermeulen, S. H., Geurts-Moespot, A. J., Klaver, S. M., Kroot, J. J., van Tienoven, D., Wetzels, J. F., Kiemeney, L. A., Sweep, F. C., den Heijer, M., & Swinkels, D. W. (2011). Serum hepcidin: reference ranges and biochemical correlates in the general population. *Blood*, *117*(25), e218-225. https://doi.org/10.1182/blood-2011-02-337907

Gallego-Narbón, A., Zapatera, B., & Vaquero, M. P. (2019). Physiological and dietary determinants of iron status in Spanish vegetarians. *Nutrients*, *11*(8). https://doi.org/10.3390/nu11081734

Ganz, T. (2013). Systemic iron homeostasis. *Physiological Reviews*, *93*(4), 1721-1741. https://doi.org/10.1152/physrev.00008.2013

Ganz, T., & Nemeth, E. (2006). Iron imports. IV. Hepcidin and regulation of body iron metabolism. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, *290*(2), G199-203. https://doi.org/10.1152/ajpgi.00412.2005

Garcia, J. R., Manimaleth, R., Czartoryski, P., Napolitano, P., Watters, H., Weber, C., Alvarez-Beaton, A., Nieto, A. C., Patel, A., Peacock, C., Banks, J., Tartar, J., & Antonio, J. (2021). A comparative study of body composition assessment techniques: DXA and InBody 270. *Journal of Exercise and Nutrition, 3(3). Retrieved from https://journalofexerciseandnutrition.com/index.php/JEN/article/view/65*.

Gardner, G. W., Edgerton, V. R., Senewiratne, B., Barnard, R. J., & Ohira, Y. (1977). Physical work capacity and metabolic stress in subjects with iron deficiency anemia. *American Journal of Clinical Nutrition*, *30*(6), 910-917.

Garvican, L. A., Lobigs, L., Telford, R., Fallon, K., & Gore, C. J. (2011). Haemoglobin mass in an anaemic female endurance runner before and after iron supplementation. *International Journal of Sports Physiology and Performance*, *6*(1), 137-140.

Gemmell, I. M. (2002). Injuries among female army recruits: a conflict of legislation. *Journal of the Royal Society of Medicine*, *95*(1), 23-27. https://doi.org/10.1177/014107680209500107

Gibson, R. S. (2005). *Principles of nutritional assessment.* New York: Oxford University Press.

Haddad, J. G., Matsuoka, L. Y., Hollis, B. W., Hu, Y. Z., & Wortsman, J. (1993). Human plasma transport of vitamin D after its endogenous synthesis. *Journal of Clinical Investigation*, *91*(6), 2552-2555. https://doi.org/10.1172/jci116492

Hallberg, L., Brune, M., Erlandsson, M., Sandberg, A. S., & Rossander-Hulten, L. (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *American Journal of Clinical Nutrition*, *53*(1), 112-119.

Hallberg, L., & Hulthén, L. (2000). Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *American Journal of Clinical Nutrition*, *71*(5), 1147-1160. https://doi.org/10.1093/ajcn/71.5.1147

Hamer, M., McNaughton, S. A., Bates, C. J., & Mishra, G. D. (2010). Dietary patterns, assessed from a weighed food record, and survival among elderly participants from the United Kingdom. *European Journal of Clinical Nutrition*, *64*(8), 853-861. https://doi.org/10.1038/ejcn.2010.93

Harris, Z. L., Durley, A. P., Man, T. K., & Gitlin, J. D. (1999). Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(19), 10812-10817.

Harrison, S. E., Oliver, S. J., Kashi, D. S., Carswell, A. T., Edwards, J. P., Wentz, L. M., Roberts, R., Tang, J. C. Y., Izard, R. M., Jackson, S., Allan, D., Rhodes, L. E., Fraser, W. D., Greeves, J. P., & Walsh, N. P. (2021). Influence of vitamin D supplementation by simulated sunlight or oral D3 on respiratory infection during military training. *Medicine and Science in Sports and Exercise*, *53*(7), 1505-1516. https://doi.org/10.1249/mss.0000000000002604

Harvey, L. J., Armah, C. N., Dainty, J. R., Foxall, R. J., John Lewis, D., Langford, N. J., & Fairweather-Tait, S. J. (2005). Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *British Journal of Nutrition*, *94*(4), 557-564.

Headquarters Departments of the Army, the Navy, & the Air Force Washington DC. (2017). *Army regulation 40-25, Nutrition and menu standards for human performance optimization*. 3 January 2017.

Heath, A. L., Skeaff, C. M., O'Brien, S. M., Williams, S. M., & Gibson, R. S. (2001b). Can dietary treatment of non-anemic iron deficiency improve iron status? *Journal of the American College of Nutrition*, *20*(5), 477-484. https://doi.org/10.1080/07315724.2001.10719056

Heath, A. L., Skeaff, C. M., Williams, S., & Gibson, R. S. (2001a). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutrition*, *4*(2), 197-206.

Henry, H. L. (2011). Regulation of vitamin D metabolism. *Best Practice & Research: Clinical Endocrinology & Metabolism*, *25*(4), 531-541. https://doi.org/10.1016/j.beem.2011.05.003

Hentze, M. W., Muckenthaler, M. U., & Andrews, N. C. (2004). Balancing acts: molecular control of mammalian iron metabolism. *Cell*, *117*(3), 285-297.

Herrick, K. A., Storandt, R. J., Afful, J., Pfeiffer, C. M., Schleicher, R. L., Gahche, J. J., & Potischman, N. (2019). Vitamin D status in the United States, 2011-2014. *American Journal of Clinical Nutrition*, *110*(1), 150-157. https://doi.org/10.1093/ajcn/nqz037

Herzman-Harari, S., Constantini, N., Mann, G., Lencovsky, Z., & Stark, A. H. (2013). Nutrition knowledge, attitudes, and behaviors of Israeli female combat recruits participating in a nutrition education program. *Military Medicine*, *178*(5), 517-522. https://doi.org/10.7205/milmed-d-12-00439

Hinton, P. S. (2014). Iron and the endurance athlete. *Applied Physiology, Nutrition, and Metabolism. Physiologie Appliquée, Nutrition et Métabolisme*, *39*(9), 1012-1018. https://doi.org/10.1139/apnm-2014-0147

Hinton, P. S., Giordano, C., Brownlie, T., & Haas, J. D. (2000). Iron supplementation improves endurance after training in iron-depleted, nonanemic women. *J Appl Physiol (1985)*, *88*(3), 1103-1111. https://doi.org/10.1152/jappl.2000.88.3.1103

Hinton, P. S., & Sinclair, L. M. (2007). Iron supplementation maintains ventilatory threshold and improves energetic efficiency in iron-deficient nonanemic athletes. *European Journal of Clinical Nutrition*, *61*(1), 30-39. https://doi.org/10.1038/sj.ejcn.1602479

Hodge, A., & Bassett, J. (2016). What can we learn from dietary pattern analysis? *Public Health Nutrition*, *19*(2), 191-194. https://doi.org/10.1017/s1368980015003730

Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine*, *357*(3), 266-281. https://doi.org/10.1056/NEJMra070553

Holick, M. F. (2011). Vitamin D: evolutionary, physiological and health perspectives. *Current Drug Targets*, *12*(1), 4-18.

Holick, M. F. (2017). The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. *Reviews in Endocrine & Metabolic Disorders*, *18*(2), 153-165. https://doi.org/10.1007/s11154-017-9424-1

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., & Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism*, *96*(7), 1911-1930. https://doi.org/10.1210/jc.2011-0385

Holick, M. F., Chen, T. C., Lu, Z., & Sauter, E. (2007). Vitamin D and skin physiology: a D-lightful story. *Journal of Bone and Mineral Research*, *22 Suppl 2*, V28-33. https://doi.org/10.1359/jbmr.07s211

Horton-French, K., Dunlop, E., Lucas, R. M., Pereira, G., & Black, L. J. (2021). Prevalence and predictors of vitamin D deficiency in a nationally representative sample of Australian adolescents and young adults. *European Journal of Clinical Nutrition*, *75*(11), 1627-1636.

Hu, F. B. (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Current Opinion in Lipidology*, *13*(1), 3-9. https://doi.org/10.1097/00041433-200202000-00002

Hu, P. J., Ley, S. H., Bhupathiraju, S. N., Li, Y., & Wang, D. D. (2017). Associations of dietary, lifestyle, and sociodemographic factors with iron status in Chinese adults: a cross-sectional study in the China Health and Nutrition Survey. *American Journal of Clinical Nutrition*, *105*(2), 503-512. https://doi.org/10.3945/ajcn.116.136861

Hunt, J. R., & Roughead, Z. K. (1999). Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. *American Journal of Clinical Nutrition*, *69*(5), 944-952. https://doi.org/10.1093/ajcn/69.5.944

Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *American Journal of Clinical Nutrition*, *91*(5), 1461s-1467s. https://doi.org/10.3945/ajcn.2010.28674F

Hurrell, R. F., Reddy, M., & Cook, J. D. (1999). Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition*, *81*(4), 289-295.

Institute of Medicine. (2011). *Dietary Reference Intakes for calcium and vitamin D.* The National Academies Press: Washington, DC, USA

Iqbal, T. H., Lewis, K. O., & Cooper, B. T. (1994). Phytase activity in the human and rat small intestine. *Gut*, *35*(9), 1233-1236. https://doi.org/10.1136/gut.35.9.1233

Israeli, E., Merkel, D., Constantini, N., Yanovich, R., Evans, R. K., Shahar, D., & Moran, D. S. (2008). Iron deficiency and the role of nutrition among female military recruits. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S685-690. https://doi.org/10.1249/MSS.0b013e31818946ae

Jacobus, C. H., Holick, M. F., Shao, Q., Chen, T. C., Holm, I. A., Kolodny, J. M., Fuleihan, G. E., & Seely, E. W. (1992). Hypervitaminosis D associated with drinking milk. *New England Journal of Medicine*, *326*(18), 1173-1177. https://doi.org/10.1056/nejm199204303261801

Jayasinghe, S. N., Breier, B. H., McNaughton, S. A., Russell, A. P., Della Gatta, P. A., Mason, S., Stonehouse, W., Walsh, D. C. I., & Kruger, R. (2019). Dietary patterns in New Zealand women: evaluating differences in body composition and metabolic biomarkers. *Nutrients*, *11*(7). https://doi.org/10.3390/nu11071643

Jones, B. H., Bovee, M. W., Harris, J. M., 3rd, & Cowan, D. N. (1993). Intrinsic risk factors for exercise-related injuries among male and female army trainees. *American Journal of Sports Medicine*, *21*(5), 705-710. https://doi.org/10.1177/036354659302100512

Jones, B. H., Hauret, K. G., Dye, S. K., Hauschild, V. D., Rossi, S. P., Richardson, M. D., & Friedl, K. E. (2017). Impact of physical fitness and body composition on injury risk among active young adults: a study of Army trainees. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S17-s22. https://doi.org/10.1016/j.jsams.2017.09.015

Jones, B. H., & Knapik, J. J. (1999). Physical training and exercise-related injuries. Surveillance, research and injury prevention in military populations. *Sports Medicine*, *27*(2), 111-125.

Jurvelin, H., Tanskanen-Tervo, M., Kinnunen, H., Santtila, M., & Kyröläinen, H. (2020). Training load and energy expenditure during military basic training period. *Medicine and Science in Sports and Exercise*, *52*(1), 86-93. https://doi.org/10.1249/mss.0000000000002092

Kara, C., Gunindi, F., Ustyol, A., & Aydin, M. (2014). Vitamin D intoxication due to an erroneously manufactured dietary supplement in seven children. *Pediatrics*, *133*(1), e240-244. https://doi.org/10.1542/peds.2013-0711

Karelis, A. D., Chamberland, G., Aubertin-Leheudre, M., & Duval, C. (2013). Validation of a portable bioelectrical impedance analyzer for the assessment of body composition. *Applied Physiology, Nutrition, and Metabolism. Physiologie Appliquée, Nutrition et Métabolisme*, *38*(1), 27-32. https://doi.org/10.1139/apnm-2012-0129

Kaufman, H. W., Niles, J. K., Kroll, M. H., Bi, C., & Holick, M. F. (2020). SARS-CoV-2 positivity rates associated with circulating 25-hydroxyvitamin D levels. *PloS One*, *15*(9), e0239252. https://doi.org/10.1371/journal.pone.0239252

Keane, J. T., Elangovan, H., Stokes, R. A., & Gunton, J. E. (2018). Vitamin D and the liver-correlation or cause? *Nutrients*, *10*(4). https://doi.org/10.3390/nu10040496

Kerr, G. M. (2004). Injuries sustained by recruits during basic training in Irish Army. *Irish Medical Journal*, *97*(3), 80-81.

Khammissa, R. A. G., Fourie, J., Motswaledi, M. H., Ballyram, R., Lemmer, J., & Feller, L. (2018). The biological activities of vitamin D and its receptor in relation to calcium and bone homeostasis, cancer, immune and cardiovascular systems, skin biology, and oral health. *Biomed Res Int*, *2018*, 9276380. https://doi.org/10.1155/2018/9276380

Kiely, M., & Black, L. J. (2012). Dietary strategies to maintain adequacy of circulating 25-hydroxyvitamin D concentrations. *Scandinavian Journal of Clinical and Laboratory Investigation. Supplement*, *243*, 14-23. https://doi.org/10.3109/00365513.2012.681893

Kim, J., Yu, A., Choi, B. Y., Nam, J. H., Kim, M. K., Oh, D. H., & Yang, Y. J. (2015). Dietary patterns derived by cluster analysis are associated with cognitive function among Korean older adults. *Nutrients*, *7*(6), 4154-4169. https://doi.org/10.3390/nu7064154

Kimball, S. M., Mirhosseini, N., & Holick, M. F. (2017). Evaluation of vitamin D3 intakes up to 15,000 international units/day and serum 25-hydroxyvitamin D concentrations up to 300 nmol/L on calcium metabolism in a community setting. *Dermato-Endocrinology*, *9*(1), e1300213. https://doi.org/10.1080/19381980.2017.1300213

Kimlin, M. G., Lucas, R. M., Harrison, S. L., van der Mei, I., Armstrong, B. K., Whiteman, D. C., Kricker, A., Nowak, M., Brodie, A. M., & Sun, J. (2014). The contributions of solar ultraviolet radiation exposure and other determinants to serum 25-hydroxyvitamin D concentrations in Australian adults: the AusD Study. *American Journal of Epidemiology*, *179*(7), 864-874. https://doi.org/10.1093/aje/kwt446

Kiss, J. E., Brambilla, D., Glynn, S. A., Mast, A. E., Spencer, B. R., Stone, M., Kleinman, S. H., & Cable, R. G. (2015). Oral iron supplementation after blood donation: a randomized clinical trial. *JAMA*, *313*(6), 575-583. https://doi.org/10.1001/jama.2015.119

Knapik, J., Darakjy, S., Scott, S. J., Hauret, K. G., Canada, S., Marin, R., Rieger, W., & Jones, B. H. (2005). Evaluation of a standardized physical training program for basic combat training. *Journal of Strength and Conditioning Research*, *19*(2), 246-253. https://doi.org/10.1519/16324.1

Knapik, J., Montain, S. J., McGraw, S., Grier, T., Ely, M., & Jones, B. H. (2012). Stress fracture risk factors in basic combat training. *International Journal of Sports Medicine*, *33*(11), 940-946. https://doi.org/10.1055/s-0032-1311583

Knapik, J. J., Darakjy, S., Hauret, K. G., Canada, S., Marin, R., & Jones, B. H. (2007). Ambulatory physical activity during United States Army basic combat training. *International Journal of Sports Medicine*, *28*(2), 106-115. https://doi.org/10.1055/s-2006-924147

Knapik, J. J., Hauret, K. G., Arnold, S., Canham-Chervak, M., Mansfield, A. J., Hoedebecke, E. L., & McMillian, D. (2003). Injury and fitness outcomes during implementation of physical readiness training. *International Journal of Sports Medicine*, *24*(5), 372-381. https://doi.org/10.1055/s-2003-40710

Knapik, J. J., Sharp, M. A., Canham-Chervak, M., Hauret, K., Patton, J. F., & Jones, B. H. (2001). Risk factors for training-related injuries among men and women in basic combat training. *Medicine and Science in Sports and Exercise*, *33*(6), 946-954.

Knapik, J. J., Sharp, M. A., & Steelman, R. A. (2017). Secular trends in the physical fitness of United States Army recruits on entry to service, 1975-2013. *Journal of Strength and Conditioning Research*, *31*(7), 2030-2052. https://doi.org/10.1519/jsc.0000000000001928

Knight, J. A., Wong, J., Cole, D. E. C., Lee, T. K., & Parra, E. J. (2017). Predictors of 25-hydroxyvitamin D concentration measured at multiple time points in a multiethnic population. *American Journal of Epidemiology*, *186*(10), 1180-1193. https://doi.org/10.1093/aje/kwx180

Korolnek, T., & Hamza, I. (2015). Macrophages and iron trafficking at the birth and death of red cells. *Blood*, *125*(19), 2893-2897. https://doi.org/10.1182/blood-2014-12-567776

Koutkia, P., Chen, T. C., & Holick, M. F. (2001). Vitamin D intoxication associated with an over-the-counter supplement. *New England Journal of Medicine*, *345*(1), 66-67. https://doi.org/10.1056/nejm200107053450115

Krause, A., Neitz, S., Magert, H. J., Schulz, A., Forssmann, W. G., Schulz-Knappe, P., & Adermann, K. (2000). LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Letters*, *480*(2-3), 147-150.

Kuhn, T., Kaaks, R., Teucher, B., Hirche, F., Dierkes, J., Weikert, C., Katzke, V., Boeing, H., Stangl, G. I., & Buijsse, B. (2014). Dietary, lifestyle, and genetic determinants of vitamin D status: a cross-sectional analysis from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany study. *European Journal of Nutrition*, *53*(3), 731-741. https://doi.org/10.1007/s00394-013-0577-8

Laaksi, I., Ruohola, J. P., Tuohimaa, P., Auvinen, A., Haataja, R., Pihlajamaki, H., & Ylikomi, T. (2007). An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. *American Journal of Clinical Nutrition*, *86*(3), 714-717. https://doi.org/10.1093/ajcn/86.3.714

Lai, J. K., Lucas, R. M., Banks, E., & Ponsonby, A. L. (2012). Variability in vitamin D assays impairs clinical assessment of vitamin D status. *Internal Medicine Journal*, *42*(1), 43-50. https://doi.org/10.1111/j.1445-5994.2011.02471.x

Landahl, G., Adolfsson, P., Borjesson, M., Mannheimer, C., & Rodjer, S. (2005). Iron deficiency and anemia: a common problem in female elite soccer players. *International Journal of Sport Nutrition and Exercise Metabolism*, *15*(6), 689-694.

Leonard, A. J., Chalmers, K. A., Collins, C. E., & Patterson, A. J. (2014). The effect of nutrition knowledge and dietary iron intake on iron status in young women. *Appetite*, *81*, 225-231. https://doi.org/10.1016/j.appet.2014.06.021

Lieberman, H. R., Kellogg, M. D., & Bathalon, G. P. (2008). Female marine recruit training: mood, body composition, and biochemical changes. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S671-676. https://doi.org/10.1249/MSS.0b013e31818943b3

Lim, K., Beck, K. L., Von Hurst, P. R., Rutherfurd-Markwick, K. J., & Badenhorst, C. E. (2020). Iron deficiency and risk factors in pre-menopausal females living in Auckland, New Zealand. *Asia Pacific Journal of Clinical Nutrition*, *29*(3), 638-647. https://doi.org/10.6133/apjcn.202009_29(3).0024

Lim, K. H., Booth, A. O., Nowson, C. A., Szymlek-Gay, E. A., Irving, D. O., & Riddell, L. J. (2016). Hepcidin is a better predictor of iron stores in premenopausal women than blood loss or dietary intake. *Nutrients*, *8*(9). https://doi.org/10.3390/nu8090540

Lips, P., Cashman, K. D., Lamberg-Allardt, C., Bischoff-Ferrari, H. A., Obermayer-Pietsch, B., Bianchi, M. L., Stepan, J., El-Hajj Fuleihan, G., & Bouillon, R. (2019). Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: a position statement of the European Calcified Tissue Society. *European Journal of Endocrinology of the European Federation of Endocrine Societies*, *180*(4), P23-p54. https://doi.org/10.1530/eje-18-0736

Liu, E., Meigs, J. B., Pittas, A. G., Economos, C. D., McKeown, N. M., Booth, S. L., & Jacques, P. F. (2010). Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. *American Journal of Clinical Nutrition*, *91*(6), 1627-1633. https://doi.org/10.3945/ajcn.2009.28441

Liu, X., Baylin, A., & Levy, P. D. (2018). Vitamin D deficiency and insufficiency among US adults: prevalence, predictors and clinical implications. *British Journal of Nutrition*, *119*(8), 928-936. https://doi.org/10.1017/s0007114518000491

Looker, A. C., Gunter, E. W., & Johnson, C. L. (1995). Methods to assess iron status in various NHANES surveys. *Nutrition Reviews*, *53*(9), 246-254.

Looker, A. C., Johnson, C. L., Lacher, D. A., Pfeiffer, C. M., Schleicher, R. L., & Sempos, C. T. (2011). Vitamin D status: United States, 2001-2006. *NCHS Data Brief*(59), 1-8.

Lucas, R. M., Ponsonby, A. L., Dear, K., Valery, P. C., Taylor, B., van der Mei, I., McMichael, A. J., Pender, M. P., Chapman, C., Coulthard, A., Kilpatrick, T. J., Stankovich, J., Williams, D., & Dwyer, T. (2013). Vitamin D status: multifactorial contribution of environment, genes and other factors in healthy Australian adults across a latitude gradient. *Journal of Steroid Biochemistry and Molecular Biology*, *136*, 300-308. https://doi.org/10.1016/j.jsbmb.2013.01.011

Lukaski, H. C., Hall, C. B., & Siders, W. A. (1991). Altered metabolic response of iron-deficient women during graded, maximal exercise. *European Journal of Applied Physiology and Occupational Physiology*, *63*(2), 140-145.

Lutz, L. J., Gaffney-Stomberg, E., Karl, J. P., Hughes, J. M., Guerriere, K. I., & McClung, J. P. (2019). Dietary intake in relation to military dietary reference values during army basic combat training; a multi-center, cross-sectional study. *Military Medicine*, *184*(3-4), e223-e230. https://doi.org/10.1093/milmed/usy153

Lutz, L. J., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Vitamin D status, dietary intake, and bone turnover in female soldiers during military training: a longitudinal study. *Journal of the International Society of Sports Nutrition*, *9*(1), 38. https://doi.org/10.1186/1550-2783-9-38

Lynch, S. (2012). *The rationale for selecting and standardizing iron status indicators. In: Report: priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15-17 September 2010*. World Health Organization.

Lynch, S. R., & Cook, J. D. (1980). Interaction of vitamin C and iron. *Annals of the New York Academy of Sciences*, *355*(1), 32-44. https://doi.org/https://doi.org/10.1111/j.1749-6632.1980.tb21325.x

MacLaughlin, J., & Holick, M. F. (1985). Aging decreases the capacity of human skin to produce vitamin D3. *Journal of Clinical Investigation*, *76*(4), 1536-1538. https://doi.org/10.1172/jci112134

Malacova, E., Cheang, P. R., Dunlop, E., Sherriff, J. L., Lucas, R. M., Daly, R. M., Nowson, C. A., & Black, L. J. (2019). Prevalence and predictors of vitamin D deficiency in a nationally representative sample of adults participating in the 2011-2013 Australian Health Survey. *British Journal of Nutrition*, *121*(8), 894-904. https://doi.org/10.1017/s0007114519000151

Malczewska, J., Raczynski, G., & Stupnicki, R. (2000). Iron status in female endurance athletes and in non-athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *10*(3), 260-276.

Margolis, L. M., Pasiakos, S. M., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Differential effects of military training on fat-free mass and plasma amino acid adaptations in men and women. *Nutrients*, *4*(12), 2035-2046. https://doi.org/10.3390/nu4122035

Martineau, A. R., Jolliffe, D. A., Hooper, R. L., Greenberg, L., Aloia, J. F., Bergman, P., Dubnov-Raz, G., Esposito, S., Ganmaa, D., Ginde, A. A., Goodall, E. C., Grant, C. C., Griffiths, C. J., Janssens, W., Laaksi, I., Manaseki-Holland, S., Mauger, D., Murdoch, D. R., Neale, R., . . . Camargo, C. A., Jr. (2017). Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ*, *356*, i6583. https://doi.org/10.1136/bmj.i6583

Matsuoka, L. Y., Wortsman, J., Dannenberg, M. J., Hollis, B. W., Lu, Z., & Holick, M. F. (1992). Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D3. *Journal of Clinical Endocrinology and Metabolism*, *75*(4), 1099-1103. https://doi.org/10.1210/jcem.75.4.1328275

Mattila, V. M., Tallroth, K., Marttinen, M., & Pihlajamaki, H. (2007). Physical fitness and performance. Body composition by DEXA and its association with physical fitness in 140 conscripts. *Medicine and Science in Sports and Exercise*, *39*(12), 2242-2247. https://doi.org/10.1249/mss.0b013e318155a813

McClung, J. P., Gaffney-Stomberg, E., & Lee, J. J. (2014). Female athletes: a population at risk of vitamin and mineral deficiencies affecting health and performance. *Journal of Trace Elements in Medicine and Biology*, *28*(4), 388-392. https://doi.org/10.1016/j.jtemb.2014.06.022

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Nindl, B. C., Young, A. J., & Lieberman, H. R. (2009b). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *American Journal of Clinical Nutrition*, *90*(1), 124-131. https://doi.org/10.3945/ajcn.2009.27774

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Young, A. J., & Lieberman, H. R. (2009a). Longitudinal decrements in iron status during military training in female soldiers. *British Journal of Nutrition*, *102*(4), 605-609. https://doi.org/10.1017/s0007114509220873

McClung, J. P., Marchitelli, L. J., Friedl, K. E., & Young, A. J. (2006). 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*, *25*(1), 64-69.

McKie, A. T., Barrow, D., Latunde-Dada, G. O., Rolfs, A., Sager, G., Mudaly, E., Mudaly, M., Richardson, C., Barlow, D., Bomford, A., Peters, T. J., Raja, K. B., Shirali, S., Hediger, M. A., Farzaneh, F., & Simpson, R. J. (2001). An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science*, *291*(5509), 1755-1759. https://doi.org/10.1126/science.1057206

McKie, A. T., Marciani, P., Rolfs, A., Brennan, K., Wehr, K., Barrow, D., Miret, S., Bomford, A., Peters, T. J., Farzaneh, F., Hediger, M. A., Hentze, M. W., & Simpson, R. J. (2000). A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Molecular Cell*, *5*(2), 299-309.

McLester, C. N., Nickerson, B. S., Kliszczewicz, B. M., & McLester, J. R. (2020). Reliability and agreement of various InBody body composition analyzers as compared to dual-energy x-ray absorptiometry in healthy men and women. *Journal of Clinical Densitometry*, *23*(3), 443-450. https://doi.org/10.1016/j.jocd.2018.10.008

Meltzer, D. O., Best, T. J., Zhang, H., Vokes, T., Arora, V., & Solway, J. (2020). Association of vitamin D status and other clinical characteristics with COVID-19 test results. *JAMA Netw Open*, *3*(9), e2019722. https://doi.org/10.1001/jamanetworkopen.2020.19722

Mennen, L., Hirvonen, T., Arnault, N., Bertrais, S., Galan, P., & Hercberg, S. (2007). Consumption of black, green and herbal tea and iron status in French adults. *European Journal of Clinical Nutrition*, *61*(10), 1174-1179. https://doi.org/10.1038/sj.ejcn.1602634

Merkel, D., & Moran, D. S. (2018). Coping with iron deficiency and iron deficiency anemia in combat recruits. *Acta Haematologica*, *139*(3), 166-167. https://doi.org/10.1159/000487387

Millen, A. E., Wactawski-Wende, J., Pettinger, M., Mclamed, M. L., Tylavsky F. A., Liu S., Robbins J., LaCroix A. Z., LeBoff M. S., Jackson R. D. (2010). Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: the Women's Health Initiative calcium plus vitamin D clinical trial. *American Journal of Clinical Nutrition*, *91*(5), 1324-1355. https://doi.org/10.3945/ajcn.2009.28908

Miller, B. J., Pate, R. R., & Burgess, W. (1988). Foot impact force and intravascular hemolysis during distance running. *International Journal of Sports Medicine*, *9*(1), 56-60. https://doi.org/10.1055/s-2007-1024979

Milman, N., Byg, K. E., & Ovesen, L. (2000). Iron status in Danes 1994. II: Prevalence of iron deficiency and iron overload in 1319 Danish women aged 40-70 years. Influence of blood donation, alcohol intake and iron supplementation. *Annals of Hematology*, *79*(11), 612-621. https://doi.org/10.1007/s002770000209

Milman, N., & Kirchhoff, M. (1999). Relationship between serum ferritin and risk factors for ischaemic heart disease in 2235 Danes aged 30-60 years. *Journal of Internal Medicine*, *245*(5), 423-433. https://doi.org/10.1046/j.1365-2796.1999.00465.x

Milman, N. T. (2019). Dietary iron intake in women of reproductive age in Europe: a review of 49 studies from 29 countries in the period 1993-2015. *Journal of Nutrition and Metabolism*, *2019*, 7631306. https://doi.org/10.1155/2019/7631306

Ministry of Defence. (2022). *Fitness - British Army Jobs*. Retrieved 22 September 2022 from https://apply.army.mod.uk/how-to-join/can-i-join/fitness

Ministry of Health. (2012). *Vitamin D status of New Zealand adults: findings from the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health

Ministry of Health. (2018). *Body size*. Retrieved 24 September 2022 from https://www.health.govt.nz/our-work/populations/maori-health/tatau-kahukura-maori-health-statistics/nga-tauwehe-tupono-me-te-marumaru-risk-and-protective-factors/body-size

Ministry of Health. (2021). *New Zealand Health Survey annual data explorer*. Retrieved 22 September 2022 from https://minhealthnz.shinyapps.io/nz-health-survey-2020-21-annual-data-explorer/_w_4f868c95/#!/

Ministry of Health and Cancer Society of New Zealand. (2012). *Consensus statement on vitamin D and sun exposure in New Zealand.* Wellington: Ministry of Health.

Miret, S., Simpson, R. J., & McKie, A. T. (2003). Physiology and molecular biology of dietary iron absorption. *Annual Review of Nutrition*, *23*, 283-301. https://doi.org/10.1146/annurev.nutr.23.011702.073139

Moran, D. S., Israeli, E., Evans, R. K., Yanovich, R., Constantini, N., Shabshin, N., Merkel, D., Luria, O., Erlich, T., Laor, A., & Finestone, A. (2008). Prediction model for stress fracture in young female recruits during basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S636-644. https://doi.org/10.1249/MSS.0b013e3181893164

Mountjoy, M., Sundgot-Borgen, J. K., Burke, L. M., Ackerman, K. E., Blauwet, C., Constantini, N., Lebrun, C., Lundy, B., Melin, A. K., Meyer, N. L., Sherman, R. T., Tenforde, A. S., Klungland Torstveit, M., & Budgett, R. (2018). IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. *British Journal of Sports Medicine*, *52*(11), 687-697. https://doi.org/10.1136/bjsports-2018-099193

Mullie, P., & Clarys, P. (2016). Relation between dietary pattern analysis (principal component analysis) and body mass index: a 5-year follow-up study in a Belgian military population. *Journal of the Royal Army Medical Corps*, *162*(1), 23-29. https://doi.org/10.1136/jramc-2014-000356

Myhre, K. E., Webber, B. J., Cropper, T. L., Tchandja, J. N., Ahrendt, D. M., Dillon, C. A., Haas, R. W., Guy, S. L., Pawlak, M. T., & Federinko, S. P. (2015). Prevalence and impact of anemia on basic trainees in the US Air Force. *Sports Med Open*, *2*, 23. https://doi.org/10.1186/s40798-016-0047-y

Naja, F., Jomaa, L., Itani, L., Zidek, J., El Labban, S., Sibai, A. M., & Hwalla, N. (2018). Environmental footprints of food consumption and dietary patterns among Lebanese adults: a cross-sectional study. *Nutrition Journal*, *17*(1), 85. https://doi.org/10.1186/s12937-018-0393-3

Nakayama, A. T., Lutz, L. J., Hruby, A., Karl, J. P., McClung, J. P., & Gaffney-Stomberg, E. (2019). A dietary pattern rich in calcium, potassium, and protein is associated with tibia bone mineral content and strength in young adults entering initial military training. *American Journal of Clinical Nutrition*, *109*(1), 186-196. https://doi.org/10.1093/ajcn/nqy199

Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., Ganz, T., & Kaplan, J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, *306*(5704), 2090-2093. https://doi.org/10.1126/science.1104742

Nessvi, S., Johansson, L., Jopson, J., Stewart, A., Reeder, A., McKenzie, R., & Scragg, R. K. (2011). Association of 25-hydroxyvitamin D3 levels in adult New Zealanders with ethnicity, skin color and self-reported skin sensitivity to sun exposure. *Photochemistry and Photobiology*, *87*(5), 1173-1178. https://doi.org/10.1111/j.1751-1097.2011.00956.x

New Zealand Defence Force. (2022). *Medical & Health Defence Careers*. Retrieved 22 September 2022 from https://www.defencecareers.mil.nz/defence-careers/how-do-i-join/medical-and-health/

Newby, P. K., & Tucker, K. L. (2004). Empirically derived eating patterns using factor or cluster analysis: a review. *Nutrition Reviews*, *62*(5), 177-203. https://doi.org/10.1301/nr.2004.may.177-203

Newlin, M. K., Williams, S., McNamara, T., Tjalsma, H., Swinkels, D. W., & Haymes, E. M. (2012). The effects of acute exercise bouts on hepcidin in women. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(2), 79-88.

Nindl, B. C., Jones, B. H., Van Arsdale, S. J., Kelly, K., & Kraemer, W. J. (2016). Operational physical performance and fitness in military women: physiological, musculoskeletal injury, and optimized physical training considerations for successfully integrating women into combat-centric military occupations. *Military Medicine*, *181*(1 Suppl), 50-62. https://doi.org/10.7205/milmed-d-15-00382

Nindl, B. C., McClung, J. P., Miller, J. K., Karl, J. P., Pierce, J. R., Scofield, D. E., Young, A. J., & Lieberman, H. R. (2011). Bioavailable IGF-I is associated with fat-free mass gains after physical training in women. *Medicine and Science in Sports and Exercise*, *43*(5), 793-799. https://doi.org/10.1249/MSS.0b013e31820065ea

Nowson, C. A., McGrath, J. J., Ebeling, P. R., Haikerwal, A., Daly, R. M., Sanders, K. M., Seibel, M. J., & Mason, R. S. (2012). Vitamin D and health in adults in Australia and New Zealand: a position statement. *Medical Journal of Australia*, *196*(11), 686-687.

O'Leary, T. J., Saunders, S. C., McGuire, S. J., Venables, M. C., & Izard, R. M. (2018). Sex differences in training loads during British Army basic training. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/mss.0000000000001716

O'Leary, T. J., Wardle, S. L., & Greeves, J. P. (2020). Energy deficiency in soldiers: the risk of the athlete triad and relative energy deficiency in sport syndromes in the military. *Front Nutr*, *7*, 142. https://doi.org/10.3389/fnut.2020.00142

Ohgami, R. S., Campagna, D. R., Greer, E. L., Antiochos, B., McDonald, A., Chen, J., Sharp, J. J., Fujiwara, Y., Barker, J. E., & Fleming, M. D. (2005). Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. *Nature Genetics*, *37*(11), 1264-1269. https://doi.org/10.1038/ng1658

Osaki, S., Johnson, D. A., & Frieden, E. (1966). The possible significance of the ferrous oxidase activity of ceruloplasmin in normal human serum. *Journal of Biological Chemistry*, *241*(12), 2746-2751.

Pantopoulos, K., Porwal, S. K., Tartakoff, A., & Devireddy, L. (2012). Mechanisms of mammalian iron homeostasis. *Biochemistry*, *51*(29), 5705-5724. https://doi.org/10.1021/bi300752r

Park, C. H., Valore, E. V., Waring, A. J., & Ganz, T. (2001). Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *Journal of Biological Chemistry*, *276*(11), 7806-7810. https://doi.org/10.1074/jbc.M008922200

Parks, R. B., Hetzel, S. J., & Brooks, M. A. (2017). Iron deficiency and anemia among collegiate athletes: a retrospective chart review. *Medicine and Science in Sports and Exercise*, *49*(8), 1711-1715. https://doi.org/10.1249/mss.0000000000001259

Peeling, P. (2010). Exercise as a mediator of hepcidin activity in athletes. *European Journal of Applied Physiology*, *110*(5), 877-883. https://doi.org/10.1007/s00421-010-1594-4

Peeling, P., Blee, T., Goodman, C., Dawson, B., Claydon, G., Beilby, J., & Prins, A. (2007). Effect of iron injections on aerobic-exercise performance of iron-depleted female athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *17*(3), 221-231.

Peeling, P., Dawson, B., Goodman, C., Landers, G., & Trinder, D. (2008). Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. *European Journal of Applied Physiology*, *103*(4), 381-391. https://doi.org/10.1007/s00421-008-0726-6

Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E. T., Swinkels, D. W., & Trinder, D. (2009). Cumulative effects of consecutive running sessions on hemolysis, inflammation and hepcidin activity. *European Journal of Applied Physiology*, *106*(1), 51-59. https://doi.org/10.1007/s00421-009-0988-7

Peeling, P., McKay, A. K. A., Pyne, D. B., Guelfi, K. J., McCormick, R. H., Laarakkers, C. M., Swinkels, D. W., Garvican-Lewis, L. A., Ross, M. L. R., Sharma, A. P., Leckey, J. J., & Burke, L. M. (2017). Factors influencing the post-exercise hepcidin-25 response in elite athletes. *European Journal of Applied Physiology*, *117*(6), 1233-1239. https://doi.org/10.1007/s00421-017-3611-3

Peeling, P., Sim, M., Badenhorst, C. E., Dawson, B., Govus, A. D., Abbiss, C. R., Swinkels, D. W., & Trinder, D. (2014). Iron status and the acute post-exercise hepcidin response in athletes. *PloS One*, *9*(3), e93002. https://doi.org/10.1371/journal.pone.0093002

Péneau, S., Dauchet, L., Vergnaud, A. C., Estaquio, C., Kesse-Guyot, E., Bertrais, S., Latino-Martel, P., Hercberg, S., & Galan, P. (2008). Relationship between iron status and dietary fruit and vegetables based on their vitamin C and fiber content. *American Journal of Clinical Nutrition*, *87*(5), 1298-1305. https://doi.org/10.1093/ajcn/87.5.1298

Pérez-Barrios, C., Hernández-Álvarez, E., Blanco-Navarro, I., Pérez-Sacristán, B., & Granado-Lorencio, F. (2016). Prevalence of hypercalcemia related to hypervitaminosis D in clinical practice. *Clinical Nutrition*, *35*(6), 1354-1358. https://doi.org/10.1016/j.clnu.2016.02.017

Peters, H. P., De Vries, W. R., Vanberge-Henegouwen, G. P., & Akkermans, L. M. (2001). Potential benefits and hazards of physical activity and exercise on the gastrointestinal tract. *Gut*, *48*(3), 435-439. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1760153/pdf/v048p00435.pdf

Pierce, J. R., DeGroot, D. W., Grier, T. L., Hauret, K. G., Nindl, B. C., East, W. B., McGurk, M. S., & Jones, B. H. (2017). Body mass index predicts selected physical fitness attributes but is not associated with performance on military relevant tasks in U.S. Army Soldiers. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S79-s84. https://doi.org/10.1016/j.jsams.2017.08.021

Pigeon, C., Ilyin, G., Courselaud, B., Leroyer, P., Turlin, B., Brissot, P., & Loreal, O. (2001). A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *Journal of Biological Chemistry*, *276*(11), 7811-7819. https://doi.org/10.1074/jbc.M008923200

Pletcher, E. R., Lovalekar, M., Coleman, L. C., Beals, K., Nindl, B. C., & Allison, K. F. (2022). Decreased percent body fat but not body mass is associated with better performance on combat fitness test in male and female Marines. *Journal of Strength and Conditioning Research*. https://doi.org/10.1519/jsc.0000000000004335

Pludowski, P., Holick, M. F., Grant, W. B., Konstantynowicz, J., Mascarenhas, M. R., Haq, A., Povoroznyuk, V., Balatska, N., Barbosa, A. P., Karonova, T., Rudenka, E., Misiorowski, W., Zakharova, I., Rudenka, A., Lukaszkiewicz, J., Marcinowska-Suchowierska, E., Laszcz, N., Abramowicz, P., Bhattoa, H. P., & Wimalawansa, S. J. (2018). Vitamin D supplementation guidelines. *Journal of Steroid Biochemistry and Molecular Biology*, *175*, 125-135. https://doi.org/10.1016/j.jsbmb.2017.01.021

Poss, K. D., & Tonegawa, S. (1997). Heme oxygenase 1 is required for mammalian iron reutilization. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(20), 10919-10924.

Prentice, A. M. (2017). Clinical implications of new insights into hepcidin-mediated regulation of iron absorption and metabolism. *Annals of Nutrition and Metabolism*, *71 Suppl 3*, 40-48. https://doi.org/10.1159/000480743

Proctor, S. P., Scarpaci, M. M., Maule, A. L., Heaton, K. J., Taylor, K., Haven, C. C., Rood, J., Ospina, M., & Calafat, A. M. (2018). Role of body composition and physical activity on permethrin urinary biomarker concentrations while wearing treated military uniforms. *Toxicology Letters*, *299*, 210-217. https://doi.org/10.1016/j.toxlet.2018.10.001

Public Health England. (2016). *Government dietary recommendations, Government recommendations for energy and nutrients for males and females aged 1 – 18 years and 19+ years*. London, UK: Public Health England.

Punnonen, K., Irjala, K., & Rajamaki, A. (1997). Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood*, *89*(3), 1052-1057.

Pynaert, I., De Bacquer, D., Matthys, C., Delanghe, J., Temmerman, M., De Backer, G., & De Henauw, S. (2009). Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutrition*, *12*(10), 1775-1782. https://doi.org/10.1017/s1368980008004369

Qian, Z. M., & Tang, P. L. (1995). Mechanisms of iron uptake by mammalian cells. *Biochimica et Biophysica Acta*, *1269*(3), 205-214.

Ramakrishnan, U., Frith-Terhune, A., Cogswell, M., & Kettel Khan, L. (2002). Dietary intake does not account for differences in low iron stores among Mexican American and non-Hispanic white women: Third National Health and Nutrition Examination Survey, 1988-1994. *Journal of Nutrition*, *132*(5), 996-1001. https://doi.org/10.1093/jn/132.5.996

Rangan, A. M., Aitkin, I., Blight, G. D., & Binns, C. W. (1997). Factors affecting iron status in 15-30 year old female students. *Asia Pacific Journal of Clinical Nutrition*, *6*(4), 291-295.

Reddy, K. V., Shastry, S., Raturi, M., & Baliga, B. P. (2020). Impact of regular whole-blood donation on body iron stores. *Transfusion Medicine and Hemotherapy. Offizielles Organ der Deutschen Gesellschaft für Transfusionsmedizin und Immunhämatologie*, *47*(1), 75-79. https://doi.org/10.1159/000499768

Richards, T., & Wright, C. (2018). British Army recruits with low serum vitamin D take longer to recover from stress fractures. *Journal of the Royal Army Medical Corps*. https://doi.org/10.1136/jramc-2018-000983

Richmond, V. L., Carter, J. M., Wilkinson, D. M., Homer, F. E., Rayson, M. P., Wright, A., & Bilzon, J. L. (2012). Comparison of the physical demands of single-sex training for male and female recruits in the British Army. *Military Medicine*, *177*(6), 709-715. https://doi.org/10.7205/milmed-d-11-00416

Rigas, A. S., Ejsing, B. H., Sørensen, E., Pedersen, O. B., Hjalgrim, H., Erikstrup, C., & Ullum, H. (2018). Calcium in drinking water: effect on iron stores in Danish blood donors-results from the Danish Blood Donor Study. *Transfusion*, *58*(6), 1468-1473. https://doi.org/10.1111/trf.14600

Rigas, A. S., Pedersen, O. B., Magnussen, K., Erikstrup, C., & Ullum, H. (2019). Iron deficiency among blood donors: experience from the Danish Blood Donor Study and from the Copenhagen ferritin monitoring scheme. *Transfusion Medicine*, *29 Suppl 1*, 23-27. https://doi.org/10.1111/tme.12477

Rigas, A. S., Sørensen, C. J., Pedersen, O. B., Petersen, M. S., Thørner, L. W., Kotzé, S., Sørensen, E., Magnussen, K., Rostgaard, K., Erikstrup, C., & Ullum, H. (2014). Predictors of iron levels in 14,737 Danish blood donors: results from the Danish Blood Donor Study. *Transfusion*, *54*(3 Pt 2), 789-796. https://doi.org/10.1111/trf.12518

Roecker, L., Meier-Buttermilch, R., Brechtel, L., Nemeth, E., & Ganz, T. (2005). Iron-regulatory protein hepcidin is increased in female athletes after a marathon. *European Journal of Applied Physiology*, *95*(5-6), 569-571. https://doi.org/10.1007/s00421-005-0055-y

Ruohola, J. P., Laaksi, I., Ylikomi, T., Haataja, R., Mattila, V. M., Sahi, T., Tuohimaa, P., & Pihlajamaki, H. (2006). Association between serum 25(OH)D concentrations and bone stress fractures in Finnish young men. *Journal of Bone and Mineral Research*, *21*(9), 1483-1488. https://doi.org/10.1359/jbmr.060607

Salih, F. M. (2004). Effect of clothing varieties on solar photosynthesis of previtamin D3: an in vitro study. *Photodermatology, Photoimmunology and Photomedicine*, *20*(1), 53-58.

Sandstrom, G., Borjesson, M., & Rodjer, S. (2012). Iron deficiency in adolescent female athletes - is iron status affected by regular sporting activity? *Clinical Journal of Sport Medicine*, *22*(6), 495-500. https://doi.org/10.1097/JSM.0b013e3182639522

Santtila, M., Pihlainen, K., Koski, H., Ojanen, T., & Kyrolainen, H. (2018). Physical fitness and body anthropometrics profiles of the female recruits entering to voluntary military service. *Military Medicine*. https://doi.org/10.1093/milmed/usy145

Sauvageot, N., Schritz, A., Leite, S., Alkerwi, A., Stranges, S., Zannad, F., Streel, S., Hoge, A., Donneau, A. F., Albert, A., & Guillaume, M. (2017). Stability-based validation of dietary patterns obtained by cluster analysis. *Nutrition Journal*, *16*(1), 4. https://doi.org/10.1186/s12937-017-0226-9

Schaaf, D., Scragg, R., Metcalf, P., Grant, C., & Buchanan, J. (2000). Prevalence of iron deficiency in Auckland high school students. *New Zealand Medical Journal*, *113*(1116), 347-350.

Schram, B., Canetti, E., Orr, R., & Pope, R. (2022). Injury rates in female and male military personnel: a systematic review and meta-analysis. *BMC Women's Health*, *22*(1), 310. https://doi.org/10.1186/s12905-022-01899-4

Schulz, C. A., Oluwagbemigun, K., & Nöthlings, U. (2021). Advances in dietary pattern analysis in nutritional epidemiology. *European Journal of Nutrition*, *60*(8), 4115-4130. https://doi.org/10.1007/s00394-021-02545-9

Scientific Advisory Committee on Nutrition. (2010). *Iron and health*. https://www.gov.uk/government/publications/sacn-iron-and-health-report

Scientific Advisory Committee on Nutrition. (2016). *Vitamin D and health*. https://www.gov.uk/government/publications/sacn-vitamin-d-and-health-report

Scientific Advisory Committee on Nutrition. (2017). *SACN Statement on Military Dietary Reference Values for energy*. London, UK: Scientific Advisory Committee on Nutrition.

Sergi, G., De Rui, M., Stubbs, B., Veronese, N., & Manzato, E. (2017). Measurement of lean body mass using bioelectrical impedance analysis: a consideration of the pros and cons. *Aging Clinical and Experimental Research*, *29*(4), 591-597. https://doi.org/10.1007/s40520-016-0622-6

Shafer, K. J., Siders, W. A., Johnson, L. K., & Lukaski, H. C. (2009). Validity of segmental multiple-frequency bioelectrical impedance analysis to estimate body composition of adults across a range of body mass indexes. *Nutrition*, *25*(1), 25-32. https://doi.org/10.1016/j.nut.2008.07.004

Shephard, R. J. (2016). Exercise proteinuria and hematuria: current knowledge and future directions. *Journal of Sports Medicine and Physical Finess*, *56*(9), 1060-1076.

Shim, J. S., Oh, K., & Kim, H. C. (2014). Dietary assessment methods in epidemiologic studies. *Epidemiol Health*, *36*, e2014009. https://doi.org/10.4178/epih/e2014009

Sim, M., Dawson, B., Landers, G., Wiegerinck, E. T., Swinkels, D. W., Townsend, M. A., Trinder, D., & Peeling, P. (2012). The effects of carbohydrate ingestion during endurance running on post-exercise inflammation and hepcidin levels. *European Journal of Applied Physiology*, *112*(5), 1889-1898. https://doi.org/10.1007/s00421-011-2156-0

Simpson, K., Redmond, J. E., Cohen, B. S., Hendrickson, N. R., Spiering, B. A., Steelman, R., Knapik, J. J., & Sharp, M. A. (2013). Quantification of physical activity performed during US Army basic combat training. *U.S. Army Medical Department Journal*, 55-65.

Sinclair, L. M., & Hinton, P. S. (2005). Prevalence of iron deficiency with and without anemia in recreationally active men and women. *Journal of the American Dietetic Association*, *105*(6), 975-978. https://doi.org/10.1016/j.jada.2005.03.005

Slattery, M. L. (2010). Analysis of dietary patterns in epidemiological research. *Applied Physiology, Nutrition, and Metabolism. Physiologie Appliquée, Nutrition et Métabolisme*, *35*(2), 207-210. https://doi.org/10.1139/h10-006

Soppi, E. T. (2018). Iron deficiency without anemia - a clinical challenge. *Clin Case Rep*, *6*(6), 1082-1086. https://doi.org/10.1002/ccr3.1529

Spiro, A., & Buttriss, J. L. (2014). Vitamin D: an overview of vitamin D status and intake in Europe. *Nutrition Bulletin*, *39*(4), 322-350. https://doi.org/10.1111/nbu.12108

Sriprasert, I., Pakrashi, T., Kimble, T., & Archer, D. F. (2017). Heavy menstrual bleeding diagnosis and medical management. *Contracept Reprod Med*, *2*, 20. https://doi.org/10.1186/s40834-017-0047-4

Sulistyoningrum, D. C., Green, T. J., Lear, S. A., & Devlin, A. M. (2012). Ethnic-specific differences in vitamin D status is associated with adiposity. *PloS One*, *7*(8), e43159. https://doi.org/10.1371/journal.pone.0043159

Swinburn, B. A. (2008). Obesity prevention: the role of policies, laws and regulations. *Aust New Zealand Health Policy*, *5*, 12. https://doi.org/10.1186/1743-8462-5-12

Telford, R. D., Sly, G. J., Hahn, A. G., Cunningham, R. B., Bryant, C., & Smith, J. A. (2003). Footstrike is the major cause of hemolysis during running. *J Appl Physiol (1985)*, *94*(1), 38-42. https://doi.org/10.1152/japplphysiol.00631.2001

Tetens, I., Bendtsen, K. M., Henriksen, M., Ersbøll, A. K., & Milman, N. (2007). The impact of a meat- versus a vegetable-based diet on iron status in women of childbearing age with small iron stores. *European Journal of Nutrition*, *46*(8), 439-445. https://doi.org/10.1007/s00394-007-0683-6

The Royal College of Pathologists of Australasia. (2013). *Position statement: use and interpretation of vitamin D testing*. Surry Hills, NSW: The Royal College of Pathologists of Australasia

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine joint position statement. Nutrition and athletic performance. *Medicine and Science in Sports and Exercise*, *48*(3), 543-568. https://doi.org/10.1249/mss.0000000000000852

Thorpe, M. G., Milte, C. M., Crawford, D., & McNaughton, S. A. (2016). A comparison of the dietary patterns derived by principal component analysis and cluster analysis in older Australians. *The International Journal of Behavioral Nutrition and Physical Activity*, *13*, 30. https://doi.org/10.1186/s12966-016-0353-2

Tufts, D. A., Haas, J. D., Beard, J. L., & Spielvogel, H. (1985). Distribution of hemoglobin and functional consequences of anemia in adult males at high altitude. *American Journal of Clinical Nutrition*, *42*(1), 1-11.

Tussing-Humphreys, L., Pusatcioglu, C., Nemeth, E., & Braunschweig, C. (2012). Rethinking iron regulation and assessment in iron deficiency, anemia of chronic disease, and obesity:

introducing hepcidin. *Journal of the Academy of Nutrition and Dietetics*, *112*(3), 391-400. https://doi.org/10.1016/j.jada.2011.08.038

U.S. Department of Defense. (2022). *DoD Instruction 1308.3, DoD physical fitness/body composition program*. Office of the Under Secretary of Defense for Personnel and Readiness.

University of Otago and Ministry of Health. (2011). *A focus on nutrition: key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.

Vaara, J. P., Groeller, H., Drain, J., Kyröläinen, H., Pihlainen, K., Ojanen, T., Connaboy, C., Santtila, M., Agostinelli, P., & Nindl, B. C. (2022). Physical training considerations for optimizing performance in essential military tasks. *Eur J Sport Sci*, *22*(1), 43-57. https://doi.org/10.1080/17461391.2021.1930193

van de Vijver, L. P., Kardinaal, A. F., Charzewska, J., Rotily, M., Charles, P., Maggiolini, M., Ando, S., Väänänen, K., Wajszczyk, B., Heikkinen, J., Deloraine, A., & Schaafsma, G. (1999). Calcium intake is weakly but consistently negatively associated with iron status in girls and women in six European countries. *Journal of Nutrition*, *129*(5), 963-968. https://doi.org/10.1093/jn/129.5.963

Varley-Campbell, J., Cooper, C., Wilkerson, D., Wardle, S., Greeves, J., & Lorenc, T. (2018). Sex-specific changes in physical performance following military training: a systematic review. *Sports Medicine*, *48*(11), 2623-2640. https://doi.org/10.1007/s40279-018-0983-4

Vieth, R. (1999). Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *American Journal of Clinical Nutrition*, *69*(5), 842-856. https://doi.org/10.1093/ajcn/69.5.842

von Hurst, P. R., Walsh, D. C. I., Conlon, C. A., Ingram, M., Kruger, R., & Stonehouse, W. (2016). Validity and reliability of bioelectrical impedance analysis to estimate body fat percentage against air displacement plethysmography and dual-energy x-ray absorptiometry [Article]. *Nutrition and Dietetics*, *73*(2), 197-204. https://doi.org/10.1111/1747-0080.12172

Vulpe, C. D., Kuo, Y. M., Murphy, T. L., Cowley, L., Askwith, C., Libina, N., Gitschier, J., & Anderson, G. J. (1999). Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nature Genetics*, *21*(2), 195-199. https://doi.org/10.1038/5979

Wacker, M., & Holick, M. F. (2013). Sunlight and vitamin D: a global perspective for health. *Dermato-Endocrinology*, *5*(1), 51-108. https://doi.org/10.4161/derm.24494

Wallace, D. F. (2016). The regulation of iron absorption and homeostasis. *Clinical Biochemist*, *37*(2), 51-62. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5198508/pdf/cbr-37-51.pdf

Wang, L. K., Hung, K. C., Lin, Y. T., Chang, Y. J., Wu, Z. F., Ho, C. H., & Chen, J. Y. (2020). Age, gender and season are good predictors of vitamin D status independent of body mass index in office workers in a subtropical region. *Nutrients*, *12*(9). https://doi.org/10.3390/nu12092719

Wardle, S. L., O'Leary, T. J., McClung, J. P., Pasiakos, S. M., & Greeves, J. P. (2021). 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*, *24*(10), 995-1001. https://doi.org/10.1016/j.jsams.2021.08.011

Westerterp, K. R. (2017). Doubly labelled water assessment of energy expenditure: principle, practice, and promise. *European Journal of Applied Physiology*, *117*(7), 1277-1285. https://doi.org/10.1007/s00421-017-3641-x

White, C., Yuan, X., Schmidt, P. J., Bresciani, E., Samuel, T. K., Campagna, D., Hall, C., Bishop, K., Calicchio, M. L., Lapierre, A., Ward, D. M., Liu, P., Fleming, M. D., & Hamza, I. (2013). HRG1 is essential for heme transport from the phagolysosome of macrophages during erythrophagocytosis. *Cell Metabolism*, *17*(2), 261-270. https://doi.org/10.1016/j.cmet.2013.01.005

Wood, A. M., Hales, R., Keenan, A., Moss, A., Chapman, M., Davey, T., & Nelstrop, A. (2014). Incidence and time to return to training for stress fractures during military basic training. *J Sports Med (Hindawi Publ Corp)*, *2014*, 282980. https://doi.org/10.1155/2014/282980

Woodson, R. D., Wills, R. E., & Lenfant, C. (1978). Effect of acute and established anemia on O_2 transport at rest, submaximal and maximal work. *J Appl Physiol Respir Environ Exerc Physiol*, *44*(1), 36-43. https://doi.org/10.1152/jappl.1978.44.1.36

Woolf, K., St Thomas, M. M., Hahn, N., Vaughan, L. A., Carlson, A. G., & Hinton, P. (2009). Iron status in highly active and sedentary young women. *International Journal of Sport Nutrition and Exercise Metabolism*, *19*(5), 519-535.

World Health Organization. (2001). *Iron deficiency anaemia: assessment, prevention and control; a guide for programme managers*. Geneva: World Health Organization.

World Health Organization. (2015). *The global prevalence of anaemia in 2011*. Geneva: World Health Organization.

World Health Organization. (2020). *WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations.* Geneva: World Health Organization.

World Health Organization. (2022). *Micronutrient deficiencies: iron deficiency anaemia*. Retrieved 30 September 2022 from https://apps.who.int/nutrition/topics/ida/en/

World Health Organization and Centers for Disease Control and Prevention. (2007). *Assessing the iron status of populations: second edition; including literature reviews*. Geneva: World Health Organization.

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. (2000). Decreased bioavailability of vitamin D in obesity. *American Journal of Clinical Nutrition*, *72*(3), 690-693. https://doi.org/10.1093/ajcn/72.3.690

Yanovich, R., Evans, R., Israeli, E., Constantini, N., Sharvit, N., Merkel, D., Epstein, Y., & Moran, D. S. (2008). Differences in physical fitness of male and female recruits in gender-integrated army basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S654-659. https://doi.org/10.1249/MSS.0b013e3181893f30

Young, I., Parker, H. M., Rangan, A., Prvan, T., Cook, R. L., Donges, C. E., Steinbeck, K. S., O'Dwyer, N. J., Cheng, H. L., Franklin, J. L., & O'Connor, H. T. (2018). Association between haem and non-haem iron intake and serum ferritin in healthy young women. *Nutrients*, *10*(1). https://doi.org/10.3390/nu10010081

Zhao, J., Li, Z., Gao, Q., Zhao, H., Chen, S., Huang, L., Wang, W., & Wang, T. (2021). A review of statistical methods for dietary pattern analysis. *Nutrition Journal*, *20*(1), 37. https://doi.org/10.1186/s12937-021-00692-7

Zittermann, A. (2003). Vitamin D in preventive medicine: are we ignoring the evidence? *British Journal of Nutrition*, *89*(5), 552-572. https://doi.org/10.1079/bjn2003837

Chapter 3 Iron status and associations with physical fitness during basic training in female recruits in the New Zealand Army

Establishing a situational awareness of iron status in female recruits is considered critical in support of their health and physical performance. This includes identifying the effects of diminished iron status on physical fitness, particularly with more subtle changes in iron status, which fall outside of the clinical diagnostic parameters. The aim of this study was to characterise iron status and associations with physical fitness in female recruits in the New Zealand Army during 16-weeks of basic training.

Data published:

Martin, N. M., Conlon, C. A., Smeele, R. J. M., Mugridge, O. A. R., von Hurst, P. R., McClung, J. P., & Beck, K. L. (2019). Iron status and associations with physical performance during basic combat training in female New Zealand Army recruits. *British Journal of Nutrition*, 1-7.

3.1 Abstract

Background: Female recruits participate in heavy and sustained physical activity. Iron plays a critical role in physical performance, through oxygen transport and energy metabolism. Decreases in iron status have been reported in females during basic training periods up to 9-weeks.

Objective: The purpose of this study was to characterise iron status and associations with physical fitness in female recruits in the New Zealand Army during 16-weeks of basic training.

Methods: Haemoglobin (Hb), serum ferritin (SF), soluble transferrin receptor (sTfR), transferrin saturation (TS) and erythrocyte distribution width (RDW) were assessed in 76 participants at weeks 1 and 16 of basic training. Participants with iron deficiency non-anaemia (SF<12µg/L and Hb≥120g/L) or iron deficiency anaemia (SF<12µg/L and Hb<120g/L) at week 1 or C-reactive protein >10mg/L at weeks 1 or 16 were excluded. A timed 2.4km run followed by maximum push-ups and curl-ups were performed at weeks 1 and 8. Paired *t*-tests investigated changes in iron status and Pearson's correlation coefficients evaluated associations between iron status and physical fitness.

Results: From week 1 to week 16, mean ± SD for SF (56.6 ± 33.7 to 38.4 ± 23.8µg/L, $P<0.001$) and TS (38.8 ± 13.9 to 34.4 ± 11.5%, $P=0.014$) decreased, while sTfR (1.21 ± 0.27 to 1.39 ± 0.35mg/L, $P<0.001$) and RDW (12.8 ± 0.6 to 13.2 ± 0.7%, $P<0.001$) increased. Mean ± SD for Hb (140.6 ± 7.5 to 142.9 ± 7.9g/L, $P=0.009$) increased during basic training. At week 16, sTfR was positively ($r=0.29$, $P=0.012$) and TS inversely associated ($r=-0.32$, $P=0.005$) with the week 8 run time.

Conclusion: Storage and functional iron parameters indicated a decline in iron status in female recruits during basic training. Associations between tissue-iron indicators and run time suggest impaired aerobic fitness. Optimal iron status appears paramount for enabling success in female recruits.

3.2 Introduction

Iron is important for physically active women, including military women, due to its essential role in maintaining physical and neuropsychological performance (McClung & Murray-Kolb, 2013). Premenopausal women are particularly at risk of poor iron status due to inadequate dietary intake of iron and menstrual blood loss (Harvey et al., 2005; Heath et al., 2001). Exercise may further contribute to negative iron balance (Peeling et al., 2008; Shephard, 2016).

The prevalence of both iron deficiency non-anaemia and iron deficiency anaemia is widely reported to be higher in physically active women and competitive athletes when compared to the general population (DellaValle & Haas, 2011; Dubnov & Constantini, 2004; Landahl et al., 2005; Parks et al., 2017; Sinclair & Hinton, 2005). Following basic training, the prevalence of iron deficiency non-anaemia has been shown to increase in female recruits (McClung et al., 2009a; McClung et al., 2006). The effect of iron deficiency anaemia on reduced aerobic fitness is well-documented (Celsing et al., 1986; Gardner et al., 1977; Tufts et al., 1985). Although the effect of iron deficiency non-anaemia is not as well described, it has been reported to impair aerobic adaptation and endurance capacity in women (Brownlie et al., 2002; Brownlie et al., 2004; McClung et al., 2009b).

While all military personnel are exposed to cognitive and physical challenges, the changes in iron status that women typically incur during intensive periods of physical exertion could impact their response to these challenges (McClung et al., 2006). Basic training provides a unique setting to investigate iron status. Not only do all recruits perform the same type of physical training, but the environment is regulated for variables such as sleep, dietary intake, sedentary behaviour, altitude and access to health care. Previous studies investigating the iron status of female recruits have been limited to 9-weeks and measuring running performance as a marker of physical fitness during training (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2009b; McClung et al., 2006). As these studies were all based in the United States, it is important to confirm these findings in other populations that may differ due to age, ethnicity, dietary intake, physical fitness and variations in the conduct of basic training.

There is a global drive within militaries internationally to enhance the roles and opportunities for female personnel. Identifying the effects of diminished iron status on physical fitness, particularly with more subtle changes in iron status that fall outside of the clinical diagnostic parameters, has been identified as an important area for future research (Anderson & Frazer, 2017; McClung & Murray-Kolb, 2013). Therefore, the aim of this study was to characterise iron status in female recruits in the New Zealand Army during basic training and associations with physical fitness.

3.3 Methods

All female recruits who enlisted in the New Zealand Army from February 2014 to March 2016 were eligible and invited to participate in this longitudinal cohort study. The study was conducted at Waiouru Military Camp, located at 792m above sea level in the central North Island of New Zealand. It is the only location for the 16-weeks of basic training for all New Zealand Army recruits. This study was approved by the Massey University Human Ethics Committee: Southern A (Application – 13/85). Researchers also adhered to Defence Force Order 3 (New Zealand Defence Force, 2020), which prescribes the policy relating to the conduct and approval of personnel research. All participants provided informed and voluntary consent.

Body composition measurements and blood collection for iron status indicators were carried out in a fasted state during weeks 1 and 16 of basic training. Age and ethnicity were collected at week 1 using a questionnaire. Physical fitness was assessed at weeks 1 and 8. A timed 2.4km run was conducted as a measure of aerobic fitness, followed by maximum push-ups as a measure of muscular endurance of the upper body and maximum curl-ups as a measure of core muscular endurance.

Basic training involves aerobic activities, such as prolonged standing and marching in formation; tactical marching with load carriage; distance running and obstacle courses; as well as muscle strength training that includes own-body weight exercises, lifting, load carriage and climbing. Organised physical training sessions are conducted 3-4 times per week for a period of approximately 1-hour. Other military activities involve the fundamentals of weapons training, first aid, navigation, classroom instruction and military field training. Basic training is a residential course and recruits live in shared barrack accommodation. Recruits consume three meals per day from a range of dining options, including self-selected meals from a military dining hall, bag lunches and operational ration packs. Additional snacks are provided, and recruits are not permitted to consume any dietary supplements unless prescribed by a medical doctor. All menus for the dining halls and packed rations meet the Nutrient Reference Values for Australia and New Zealand (Department of Health and Ageing and Ministry of Health, 2006).

This study used the concept of total response ethnicity (University of Otago and Ministry of Health, 2011b) which classifies participants in all ethnic groups they identify with. Therefore, participants may appear in more than one ethnic group and the sum of the ethnic groups may be greater than the number of participants.

For body composition measurements, participants were asked to wear light clothing and remove any jewellery. All measurements were conducted by a trained researcher and determined at weeks 1 and 16, excluding height, which was measured at week 1 only using a SECA 213 portable stadiometer (German Healthcare Export Group, Bonn, Germany). Body mass (BM) was measured using the

InBody$_{230}$ bioelectrical impedance analyser (Biospace Co. Ltd., Seoul, Korea). Body mass index (BMI) was calculated as BM (kg)/height (m)^2.

A venepuncture blood sample of 18ml in total was collected between 0600 and 0730hrs at both weeks 1 and 16. No participants exercised in the 8-hours prior to blood sampling. Haemoglobin (Hb) and erythrocyte distribution width (RDW) were analysed using a Sysmex XT-2000i automated haematology analyser (Sysmex Corporation, Kobe, Hyogo Prefecture, Japan). The Cobas® 6000 (Roche Diagnostics, Indianapolis, IN, United States) was used to analyse serum ferritin (SF) (electrochemiluminescence immunoassay, e601); serum iron (sFe) (two-point end method with a colourimetric assay, c501); and transferrin (Tf) and C-reactive protein (CRP) (both using the two-point end method with an immunoturbidimetric assay, c501). For SF, at control levels of 1.1, 12.3, 20.5 and 392µg/L the within-assay coefficient of variation (CV) was 12.4, 3.8, 4.1 and 21.0%, respectively, and the between-assay CV was 23.4, 6.4, 8.1 and 4.3%, respectively. For sFe, at 11.3 and 54.5µmol/L, the within-assay CV was 1.3 and 0.8%, respectively, while the between-assay CV at 11.8 and 55.1µmol/L was 1.8 and 1.3%, respectively. For Tf at 1.27 and 2.63g/L, the within-assay CV was 1.2 and 1.5%, respectively, while the between-assay CV at 2.14 and 2.96g/L was 0.06 and 0.08%, respectively. Total iron binding capacity (TIBC) was calculated by multiplying Tf by 22.8. Transferrin saturation (TS) was calculated by dividing sFe by TIBC and multiplying by 100. Soluble transferrin receptor (sTfR) was analysed using the BN II System (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, United States) using N-point nephelometry with monoclonal antibodies. At control levels of 0.71 and 1.47mg/L for sTfR, the within-assay CV was 1.4 and 1.5%, respectively, and the between-assay CV was 4.2 and 3.8%, respectively. With this immunoassay, iron deficiency for sTfR is indicated when values exceed 1.76mg/L.

The sTfR was analysed at Canterbury Health Laboratories while all other biomarkers were analysed at Medlab Whanganui. All assays were run with the appropriate standards and both internal and external quality control processes. In the absence of an external quality control programme for sTfR, samples were exchanged with another New Zealand laboratory to analyse precision. Both laboratories are accredited with International Accreditation New Zealand. Participants were excluded if they had iron deficiency non anaemia (SF<12µg/L and Hb≥120g/L) or iron deficiency anaemia (SF<12µg/L and Hb<120g/L) at week 1, CRP>10mg/L at weeks 1 or 16 or if they were prescribed iron supplementation during the study period.

A timed 2.4km run, followed by maximum push-ups and curl-ups are the three components of the base physical fitness standard for New Zealand Army personnel. All recruits must qualify at the minimum standard during basic training. For women aged 16-24-years, this is completion of the run in 740 seconds, 14 push-ups and 50 curl-ups. The test components were conducted at weeks 1 and 8 as part of the standard basic training programme.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 (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 either mean values ± standard deviation (SD) or median values (25th, 75th percentile). Changes in iron status indicators and body composition measures were investigated using paired *t*-tests. Independent *t*-tests investigated any difference in iron status indicators or body composition measures between those participants that finished basic training and those that did not. Associations between iron status indicators and physical fitness were evaluated using Pearson's correlation coefficients. A *P* value less than 0.05 was considered statistically significant.

As the sample size was determined by the feasibility of recruitment, effect sizes are reported and classified as small = 0.1 (accounts for 1% of total variance), medium = 0.3 (accounts for 9% of total variance) and large = 0.5 (accounts for 25% of total variance). Effect sizes are calculated as: $r = \surd t^2 / (t^2 + \mathrm{df})$ where '*t*' is the test statistic and 'df' is the degrees of freedom (Field, 2013).

3.4 Results

Of the 108 female recruits invited to take part in this study, 106 volunteered to participate. Data for 16 were excluded: seven had iron deficiency non-anaemia and three had iron deficiency anaemia at week 1, and four participants at week 1 and two at week 16 had a CRP>10mg/L. A further 14 participants did not finish basic training: 10 were removed from their initial course due to lower limb injuries, including four stress fractures; one due to mental health; and three participants self-withdrew. Table 3.1 displays age, ethnicity and body composition measures for the 76 participants included in the analysis. There was no significant difference in BM, BMI or iron status indicators at week 1 between those participants that finished basic training and the 14 participants that did not.

Table 3.1. Characteristics of study participants at week 1 and week 16 of basic training (*n*=76)

Characteristics	Week 1	Week 16	***P* value**[a]
Ethnicity[b]			
New Zealand European	58		
Māori	22		
Pacific	16		
Age (Years)[c]	18 (18, 19)		
Height (cm)[d]	165.1 ± 5.5		
Weight (kg)[d]	65.8 ± 9.5	65.5 ± 7.4	0.568
Body mass index (kg/m^2)[d]	24.1 ± 2.9	24.0 ± 1.9	0.583

[a] *P* value determined using paired *t*-tests.
[b] Total number of participants in all ethnic groups they identify with (*n*).
[c] Median (25th, 75th percentile).
[d] Mean ± standard deviation.

Table 3.2 highlights the changes in iron status indicators during basic training. Serum ferritin and TS decreased (*r*=0.67, *P*<0.001 and *r*=0.28, *P*=0.014, respectively) while sTfR (*r*=0.63) and RDW (*r*=0.62) increased (both *P*<0.001), all indicating reduced iron status. Haemoglobin increased (*r*=0.30, *P*=0.009) during basic training. From week 1 to 16, the mean SF decreased by 32.2%, sTfR increased by 14.9% and TS decreased by 11.3%. At the end of basic training, 11.8% of participants had a sTfR greater than the clinical cut-off of 1.76mg/L, compared with 3.9% at week 1. Nine percent of participants with normal iron status at week 1 had iron deficiency non-anaemia at week 16 and a further 3% had iron deficiency anaemia.

Table 3.2. Change in iron status indicators during basic training (*n*=76)

Iron status indicators	Week 1[a]	Week 16[a]	*P* value[b]
Haemoglobin (g/L)	140.6 ± 7.5	142.9 ± 7.9	0.009
Serum ferritin (μg/L)	56.6 ± 33.7	38.4 ± 23.8	<0.001
Soluble transferrin receptor (mg/L)[c]	1.21 ± 0.27	1.39 ± 0.35	<0.001
Transferrin saturation (%)	38.8 ± 13.9	34.4 ± 11.5	0.014
Erythrocyte distribution width (%)	12.8 ± 0.6	13.2 ± 0.7	<0.001

[a] Mean ± standard deviation.
[b] *P* value determined using paired *t*-tests.
[c] Soluble transferrin receptor is typically reported to 2 decimal points for clinical significance.

Due to injuries, only 69 of 76 participants completed the physical fitness test at week 1. All 76 participants completed the physical fitness test at week 8. The range for run times was 600-858 seconds at week 1 and 560-791 seconds at week 8. Pearson's correlation coefficient was used to analyse the association between iron status indicators and physical fitness during basic training. At week 16, sTfR was positively associated (*r*=0.29, *P*=0.012) and TS inversely associated (*r*=-0.32, *P*=0.005) with the 2.4km run time at week 8. There was no significant association between iron status indicators and the number of push-ups or curl-ups performed.

3.5 Discussion

This is the first study to investigate the iron status of women in the New Zealand Army and associations with operationally relevant functional outcomes. All iron status indicators (excluding Hb) suggested a decline in iron status during basic training for female recruits. Secondary findings were that reduced iron status, as indicated by increased sTfR and decreased TS, were associated with impaired running performance, while there was no significant association between iron status and push-ups or curl-ups. These findings are important in the military training environment because of the associations between aerobic fitness and injury risk (Jones et al., 2017) and the ability to carry out physically demanding occupational tasks (Hauschild et al., 2017).

The significant changes in iron status indicators observed in this study reflect those found in previous studies of females undergoing basic training (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2009b; McClung et al., 2006). The 32% reduction in SF from baseline is similar to the 34% decrease in females in the United States Army during 8-weeks of basic training (McClung et al., 2009b). It is also similar to the 33% decrease observed in elite female weight-bearing athletes during the first 4-6-weeks of a training season (Ashenden et al., 1998). Serum ferritin was the largest biomarker change during this study and may indicate that iron stores are the most sensitive to declines in iron status due to military training. This finding supports the importance of SF as a biomarker for the early identification of iron deficiency, as despite the longer duration of this study, decreases in SF of a greater magnitude were not observed.

The significant elevation in Hb observed in this study has been reported previously for female recruits following basic training over 8-9-weeks (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2009b). Increased Hb, coupled with reduced SF, may indicate the mobilisation of iron away from storage proteins (ferritin) to produce erythrocytes and maintain oxygen delivery (McClung et al., 2009a). Alongside instructions not to consume water before blood sample collection, the use of a dynamic panel of iron biomarkers provides a further control for hydration state, indicating true biological changes in iron status. This study did not extend beyond the 120-day life span of red blood cells to help understand what may happen to the iron status of female recruits over that duration. This is of interest, as following completion of basic training, soldiers will likely proceed immediately on to advanced training courses.

Significant changes in sTfR and TS from week 1 to week 16 indicate a reduction in iron status in this study and were the only indicators associated with impaired aerobic fitness. The finding that diminished sTfR at week 16 was associated with impaired aerobic fitness at week 8 supports McClung et al. (2009b) who demonstrated that the change in sTfR during basic training was associated with slower 3.2km run times. Work in previously untrained premenopausal women also identified a relationship between sTfR and aerobic performance during time trials on a cycle ergometer (Brownlie et al., 2002; Brownlie et al., 2004; Hinton et al., 2000). Decreases in sTfR following iron supplementation were significantly associated with improvements in relative maximal aerobic capacity (VO_2max) (Brownlie et al., 2002) and $\%VO_2max$ used during work (Brownlie et al., 2004; Hinton et al., 2000). Impaired TS at week 16 was also associated with reduced aerobic fitness at week 8 in this study. Brownlie et al. (2002) reported an association between TS and aerobic capacity, particularly in subjects who had an elevated sTfR. Hinton et al. (2000) and Brownlie et al. (2002) demonstrated that improvements in iron status following supplementation can increase oxidative capacity at the tissue level to improve maximal work capacity and endurance performance, at least when sTfR approaches the cut-off values used for iron deficiency.

These findings suggest that indicators of tissue-iron deficiency, such as sTfR and TS, are more closely associated with aerobic fitness, compared with SF (stored iron). Brownlie et al. (2004) proposed that tissue-iron deficiency impairs the ability of mitochondria and myoglobin to adapt in response to aerobic training. Soluble transferrin receptor and TS can therefore be used to distinguish between iron depletion and functional iron deficiency, which impairs the adaptation in endurance capacity as a result of aerobic training. In this study, diminished tissue-iron status likely impaired the ability of female recruits to carry out typical military tasks, which are often aerobically demanding (Hauschild et al., 2017).

The association between impaired iron status and reduced aerobic fitness is typically strongest in those with iron deficiency anaemia (Celsing et al., 1986; Gardner et al., 1977; Tufts et al., 1985), while iron deficiency non-anaemia has been reported to impair aerobic adaptation and endurance capacity in women (Brownlie et al., 2002; Brownlie et al., 2004; McClung et al., 2009b). After excluding participants with iron deficiency non-anaemia and iron deficiency anaemia, this study indicated that the relationship with aerobic fitness remained in physically active women with clinically normal iron status.

Run times are a validated measure of aerobic fitness (Hauschild et al., 2017) and slower run times indicate reduced aerobic fitness. This is strongly and consistently associated with a higher risk of injuries among all recruits in both the United States and United Kingdom (Blacker et al., 2008; Hall, 2017; Jones et al., 2017; Jones & Hauschild, 2015; Nindl et al., 2016). Push-ups and curl-ups are a measure of muscular endurance. A recent systematic review demonstrated a strong association between push-ups and the performance of common military tasks. However, push-ups have not been shown to be as strongly or as consistently associated with the risk of injury, compared to measures of aerobic fitness (Lisman et al., 2017).

Excluding participants with an elevated CRP, 7% had iron deficiency non-anaemia and 3% had iron deficiency anaemia at week 1. This is lower than the 10.6% for iron deficiency non-anaemia and 5.2% for iron deficiency anaemia in females aged 15-18-years living in New Zealand (University of Otago and Ministry of Health, 2011a). The prevalence of iron deficiency at the commencement of basic training was also lower than previously reported in female recruits in the United States (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2006) and Israel (Dubnov et al., 2006; Israeli et al., 2008). While all studies used a SF cut-off of 12μg/L, the United States based studies used a 3-variable model to identify participants with iron deficiency. Participants were categorised with iron deficiency if they presented with at least two of the following three abnormal iron status indicators: SF<12μg/L, TS<16%

and RDW>15%. The participants were then classified with iron deficiency non-anaemia if Hb≥120g/L or iron deficiency anaemia if Hb<120g/L (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2006). Although this study used SF as a single indicator for iron deficiency non-anaemia, all subjects with a CRP>10mg/L at weeks 1 or 16 were excluded from the analysis (Zimmermann & Hurrell, 2007). This mitigated against false SF elevations, in response to inflammation or infection (World Health Organization, 2017). Although not reported in the results, all participants with iron deficiency non-anaemia and iron deficiency anaemia at the end of basic training in this study had at least two and four diminished iron biomarkers, respectively.

Several factors are likely to contribute to the decrements in iron status demonstrated in this study. Increased iron demand may result from higher rates of erythrocyte production and whole-body iron turnover during periods of more intense physical activity (Beard & Tobin, 2000). The development of lean body mass, as would be expected during basic training, is a potentially iron demanding process that may contribute to the depletion of iron status. Increased iron losses, over and above menstrual blood loss, have been well described in athletes; particularly those involved in exercise that is high impact, weight-bearing and of a long duration (Telford et al., 2003), as encountered during basic training. These exercise-induced losses include gastrointestinal bleeding, haemolysis due to repetitive foot-strike, haematuria and sweat (Peeling et al., 2008; Shephard, 2016).

Physical training may also reduce iron status through the actions of the liver-derived peptide, hepcidin, to inhibit iron absorption (Nemeth et al., 2004; Park et al., 2001). Physical training stimulates the production of pro-inflammatory cytokines, including interleukin-6 (IL-6), increasing hepcidin expression (Roecker et al., 2005). Hepcidin concentration has been shown to peak 3-6-hours post-exercise, typically following a peak in IL-6 activity (Newlin et al., 2012; Peeling et al., 2017; Peeling et al., 2014; Sim et al., 2012). It has therefore been proposed that during this transient elevation in hepcidin expression post-exercise, dietary iron absorption and iron recycling from macrophages may temporarily be reduced (Peeling, 2010). Consequently, during periods of heavy training, with regular and successive inflammatory responses, consistent with the nature of basic training, this reduction in iron absorption and recycling during hepcidin expression may further compromise an individual's iron status. Iron deficiency will likely occur if dietary iron intake is not sufficiently increased to match the iron demand, iron losses or blockage of iron absorption.

Duration is a strength of this study. Iron status indicators were measured at a 15-week interval. No previous studies in females undergoing basic training have investigated iron status beyond 9-weeks. These findings therefore reflect changes in iron status that are much closer to the 120-day life span of red blood cells (Dallman, 1986). A further strength is the measurement of CRP, as an inflammatory biomarker, and exclusion of participants with a CRP>10mg/L at either week 1 or 16 (Zimmermann & Hurrell, 2007). Of the 108 participants available for this study, 106 volunteered to participate. The results therefore accurately reflect the characteristics of female recruits joining the New Zealand Army during this study period.

A limitation of this study is that the follow-up physical fitness tests were not conducted at the same time as the iron status biomarkers, creating challenges interpreting and comparing the results. The volunteers who presented with iron deficiency at week 1 were provided iron supplementation and excluded from further investigation. This ruled out the ability to explore whether iron status indicators in the clinically deficient range at the commencement of basic training were associated with finishing the course. This study was further limited by no analysis of the contribution of dietary intake or blood loss towards understanding the potential causes of diminished iron status during basic training.
Future studies should attempt to explore these potential determinants during basic training, in conjunction with iron status indicators and inflammatory biomarkers. It is recommended that iron status in female soldiers be monitored following basic training and throughout advanced training to understand the impact on Hb beyond 120-days, and any further consequences for physical fitness.

Establishing a consensus amongst clinicians and researchers for iron status indicators and their cut-off values that infer a negative effect on aerobic fitness should be prioritised. In physically active premenopausal women, including military women, consideration should be given to expected declines in iron status during intensive training periods.

In conclusion, all iron status indicators (excluding Hb) were diminished during basic training. These findings suggest that tissue-iron status in particular is reduced in female recruits and may impair aerobic fitness. This has been strongly associated with the risk of injuries in recruits and is fundamental to carrying out mission critical tasks. Maintaining or improving iron status may help optimise the physical fitness of female recruits. Initiatives for future consideration include iron screening at appropriate times throughout the career of females in the military, iron supplementation as appropriate, nutrition education regarding iron bioavailability and ensuring that military dining halls provide foods that promote iron absorption.

3.6 References

Anderson, G. J., & Frazer, D. M. (2017). Current understanding of iron homeostasis. *American Journal of Clinical Nutrition*, S1559-s1566. https://doi.org/10.3945/ajcn.117.155804

Ashenden, M. J., Martin, D. T., Dobson, G. P., Mackintosh, C., & Hahn, A. G. (1998). Serum ferritin and anemia in trained female athletes. *International Journal of Sport Nutrition*, *8*(3), 223-229.

Beard, J., & Tobin, B. (2000). Iron status and exercise. *American Journal of Clinical Nutrition*, *72*(2 Suppl), 594s-597s. http://ajcn.nutrition.org/content/72/2/594s.full.pdf

Blacker, S. D., Wilkinson, D. M., Bilzon, J. L., & Rayson, M. P. (2008). Risk factors for training injuries among British Army recruits. *Military Medicine*, *173*(3), 278-286.

Brownlie, T. t., Utermohlen, V., Hinton, P. S., Giordano, C., & Haas, J. D. (2002). Marginal iron deficiency without anemia impairs aerobic adaptation among previously untrained women. *American Journal of Clinical Nutrition*, *75*(4), 734-742. http://ajcn.nutrition.org/content/75/4/734.full.pdf

Brownlie, T. t., Utermohlen, V., Hinton, P. S., & Haas, J. D. (2004). Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. *American Journal of Clinical Nutrition*, *79*(3), 437-443. http://ajcn.nutrition.org/content/79/3/437.full.pdf

Celsing, F., Blomstrand, E., Werner, B., Pihlstedt, P., & Ekblom, B. (1986). Effects of iron deficiency on endurance and muscle enzyme activity in man. *Medicine and Science in Sports and Exercise*, *18*(2), 156-161.

Dallman, P. R. (1986). Biochemical basis for the manifestations of iron deficiency. *Annual Review of Nutrition*, *6*, 13-40. https://doi.org/10.1146/annurev.nu.06.070186.000305

DellaValle, D. M., & Haas, J. D. (2011). Impact of iron depletion without anemia on performance in trained endurance athletes at the beginning of a training season: a study of female collegiate rowers. *International Journal of Sport Nutrition and Exercise Metabolism*, *21*(6), 501-506.

Department of Health and Ageing and Ministry of Health. (2006). *Nutrient Reference Values for Australia and New Zealand: including Recommended Dietary Intakes*. Canberra: Commonwealth of Australia.

Dubnov, G., & Constantini, N. W. (2004). Prevalence of iron depletion and anemia in top-level basketball players. *International Journal of Sport Nutrition and Exercise Metabolism*, *14*(1), 30-37.

Dubnov, G., Foldes, A. J., Mann, G., Magazanik, A., Siderer, M., & Constantini, N. (2006). High prevalence of iron deficiency and anemia in female military recruits. *Military Medicine*, *171*(9), 866-869.

Field, A. (2013). *Discovering statistics using IBM SPSS statistics* (4th Edition ed.). SAGE Publications.

Gardner, G. W., Edgerton, V. R., Senewiratne, B., Barnard, R. J., & Ohira, Y. (1977). Physical work capacity and metabolic stress in subjects with iron deficiency anemia. *American Journal of Clinical Nutrition*, *30*(6), 910-917.

Hall, L. J. (2017). Relationship between 1.5-mile run time, injury risk and training outcome in British Army recruits. *Journal of the Royal Army Medical Corps*, *163*(6), 376-382. https://doi.org/10.1136/jramc-2016-000756

Harvey, L. J., Armah, C. N., Dainty, J. R., Foxall, R. J., John Lewis, D., Langford, N. J., & Fairweather-Tait, S. J. (2005). Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *British Journal of Nutrition*, *94*(4), 557-564.

Hauschild, V. D., DeGroot, D. W., Hall, S. M., Grier, T. L., Deaver, K. D., Hauret, K. G., & Jones, B. H. (2017). Fitness tests and occupational tasks of military interest: a systematic review of correlations. *Occupational and Environmental Medicine*, *74*(2), 144-153. https://doi.org/10.1136/oemed-2016-103684

Heath, A. L., Skeaff, C. M., Williams, S., & Gibson, R. S. (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutrition*, *4*(2), 197-206.

Hinton, P. S., Giordano, C., Brownlie, T., & Haas, J. D. (2000). Iron supplementation improves endurance after training in iron-depleted, nonanemic women. *J Appl Physiol (1985)*, *88*(3), 1103-1111. https://doi.org/10.1152/jappl.2000.88.3.1103

Israeli, E., Merkel, D., Constantini, N., Yanovich, R., Evans, R. K., Shahar, D., & Moran, D. S. (2008). Iron deficiency and the role of nutrition among female military recruits. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S685-690. https://doi.org/10.1249/MSS.0b013e31818946ae

Jones, B. H., Hauret, K. G., Dye, S. K., Hauschild, V. D., Rossi, S. P., Richardson, M. D., & Friedl, K. E. (2017). Impact of physical fitness and body composition on injury risk among active young adults: a study of Army trainees. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S17-s22. https://doi.org/10.1016/j.jsams.2017.09.015

Jones, B. H., & Hauschild, V. D. (2015). Physical training, fitness, and injuries: lessons learned from military studies. *Journal of Strength and Conditioning Research*, *29 Suppl 11*, S57-64. https://doi.org/10.1519/jsc.0000000000001115

Karl, J. P., Lieberman, H. R., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2010). 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. *American Journal of Clinical Nutrition*, *92*(1), 93-100. https://doi.org/10.3945/ajcn.2010.29185

Landahl, G., Adolfsson, P., Borjesson, M., Mannheimer, C., & Rodjer, S. (2005). Iron deficiency and anemia: a common problem in female elite soccer players. *International Journal of Sport Nutrition and Exercise Metabolism*, *15*(6), 689-694.

Lisman, P. J., de la Motte, S. J., Gribbin, T. C., Jaffin, D. P., Murphy, K., & Deuster, P. A. (2017). A systematic review of the association between physical fitness and musculoskeletal injury risk: Part 1-cardiorespiratory endurance. *Journal of Strength and Conditioning Research*, *31*(6), 1744-1757. https://doi.org/10.1519/jsc.0000000000001855

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Nindl, B. C., Young, A. J., & Lieberman, H. R. (2009a). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *American Journal of Clinical Nutrition*, *90*(1), 124-131. https://doi.org/10.3945/ajcn.2009.27774

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Young, A. J., & Lieberman, H. R. (2009b). Longitudinal decrements in iron status during military training in female soldiers. *British Journal of Nutrition*, *102*(4), 605-609. https://doi.org/10.1017/s0007114509220873

McClung, J. P., Marchitelli, L. J., Friedl, K. E., & Young, A. J. (2006). 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*, *25*(1), 64-69.

McClung, J. P., & Murray-Kolb, L. E. (2013). Iron nutrition and premenopausal women: effects of poor iron status on physical and neuropsychological performance. *Annual Review of Nutrition*, *33*, 271-288. https://doi.org/10.1146/annurev-nutr-071812-161205

Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., Ganz, T., & Kaplan, J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, *306*(5704), 2090-2093. https://doi.org/10.1126/science.1104742

New Zealand Defence Force. (19 November 2020). *Defence Force Order 3: New Zealand Defence Force Human Resource Manual: Version 17/12 - AL 16*. Wellington, New Zealand.

Newlin, M. K., Williams, S., McNamara, T., Tjalsma, H., Swinkels, D. W., & Haymes, E. M. (2012). The effects of acute exercise bouts on hepcidin in women. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(2), 79-88.

Nindl, B. C., Jones, B. H., Van Arsdale, S. J., Kelly, K., & Kraemer, W. J. (2016). Operational physical performance and fitness in military women: physiological, musculoskeletal injury, and optimized physical training considerations for successfully integrating women into combat-centric military occupations. *Military Medicine*, *181*(1 Suppl), 50-62. https://doi.org/10.7205/milmed-d-15-00382

Park, C. H., Valore, E. V., Waring, A. J., & Ganz, T. (2001). Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *Journal of Biological Chemistry*, *276*(11), 7806-7810. https://doi.org/10.1074/jbc.M008922200

Parks, R. B., Hetzel, S. J., & Brooks, M. A. (2017). Iron deficiency and anemia among collegiate athletes: a retrospective chart review. *Medicine and Science in Sports and Exercise*, *49*(8), 1711-1715. https://doi.org/10.1249/mss.0000000000001259

Peeling, P. (2010). Exercise as a mediator of hepcidin activity in athletes. *European Journal of Applied Physiology*, *110*(5), 877-883. https://doi.org/10.1007/s00421-010-1594-4

Peeling, P., Dawson, B., Goodman, C., Landers, G., & Trinder, D. (2008). Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. *European Journal of Applied Physiology*, *103*(4), 381-391. https://doi.org/10.1007/s00421-008-0726-6

Peeling, P., McKay, A. K. A., Pyne, D. B., Guelfi, K. J., McCormick, R. H., Laarakkers, C. M., Swinkels, D. W., Garvican-Lewis, L. A., Ross, M. L. R., Sharma, A. P., Leckey, J. J., & Burke, L. M. (2017). Factors influencing the post-exercise hepcidin-25 response in elite athletes. *European Journal of Applied Physiology*, *117*(6), 1233-1239. https://doi.org/10.1007/s00421-017-3611-3

Peeling, P., Sim, M., Badenhorst, C. E., Dawson, B., Govus, A. D., Abbiss, C. R., Swinkels, D. W., & Trinder, D. (2014). Iron status and the acute post-exercise hepcidin response in athletes. *PloS One*, *9*(3), e93002. https://doi.org/10.1371/journal.pone.0093002

Roecker, L., Meier-Buttermilch, R., Brechtel, L., Nemeth, E., & Ganz, T. (2005). Iron-regulatory protein hepcidin is increased in female athletes after a marathon. *European Journal of Applied Physiology*, *95*(5-6), 569-571. https://doi.org/10.1007/s00421-005-0055-y

Shephard, R. J. (2016). Exercise proteinuria and hematuria: current knowledge and future directions. *Journal of Sports Medicine and Physical Finess*, *56*(9), 1060-1076.

Sim, M., Dawson, B., Landers, G., Wiegerinck, E. T., Swinkels, D. W., Townsend, M. A., Trinder, D., & Peeling, P. (2012). The effects of carbohydrate ingestion during endurance running on post-exercise inflammation and hepcidin levels. *European Journal of Applied Physiology*, *112*(5), 1889-1898. https://doi.org/10.1007/s00421-011-2156-0

Sinclair, L. M., & Hinton, P. S. (2005). Prevalence of iron deficiency with and without anemia in recreationally active men and women. *Journal of the American Dietetic Association*, *105*(6), 975-978. https://doi.org/10.1016/j.jada.2005.03.005

Telford, R. D., Sly, G. J., Hahn, A. G., Cunningham, R. B., Bryant, C., & Smith, J. A. (2003). Footstrike is the major cause of hemolysis during running. *J Appl Physiol (1985)*, *94*(1), 38-42. https://doi.org/10.1152/japplphysiol.00631.2001

Tufts, D. A., Haas, J. D., Beard, J. L., & Spielvogel, H. (1985). Distribution of hemoglobin and functional consequences of anemia in adult males at high altitude. *American Journal of Clinical Nutrition*, *42*(1), 1-11.

University of Otago and Ministry of Health. (2011a). *A focus on nutrition: key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.

University of Otago and Ministry of Health. (2011b). *Methodology report for the 2008/09 New Zealand Adult Nutrition Survey*.

World Health Organization. (2017). *Nutritional anaemias: tools for effective prevention and control*. Geneva: World Health Organization.

Zimmermann, M. B., & Hurrell, R. F. (2007). Nutritional iron deficiency. *Lancet*, *370*(9586), 511-520. https://doi.org/10.1016/s0140-6736(07)61235-5

Chapter 4 Percent body fat and blood donation are the strongest determinants of iron stores in females joining the New Zealand Army

Suboptimal iron status is prevalent in female recruits and associations with impaired aerobic fitness are consistently reported. However, few studies have investigated dietary and/or non-dietary determinants of iron status in this physically active cohort of premenopausal women. While most studies investigating associations between dietary intake and iron status have focused on individual foods and nutrients, dietary patterns reflect the complexity of the whole diet. Dietary pattern analysis is therefore considered alternate and complementary to the reductionist approach. It is recommended that dietary determinants are investigated in the context of non-dietary determinants. The aim of this study was to explore associations between iron stores, dietary patterns and potential non-dietary determinants in premenopausal women at commencement of basic training in the New Zealand Army.

Data published:

Martin, N. M., von Hurst, P. R., Conlon, C. A., Smeele, R. J. M., Mugridge, O. A. R., & Beck, K. L. (2023). Body fat percentage and blood donation are the strongest determinants of iron stores in premenopausal women joining the New Zealand Army. *Military Medicine*.

4.1 Abstract

Background: Suboptimal iron status is an issue for women joining the military due to its association with impaired aerobic fitness, yet no studies have investigated dietary and non-dietary determinants of iron status simultaneously in this population.

Objective: The purpose of this study was to explore associations between iron stores, dietary patterns and potential non-dietary determinants of iron status in premenopausal women at the commencement of basic training in the New Zealand Army.

Methods: During week 1 of basic training, demographic, body composition, lifestyle, medical history and dietary data were measured as potential determinants of serum ferritin (SF), as a marker of iron stores, in 101 participants. Following univariate analysis, age, percent body fat (%), previous blood donation, ≥6-hours of exercise per week that raised the heart rate and a vegetarian dietary pattern (DP) were analysed using a multiple linear regression model.

Results: An increase in %BF was associated with increased SF ($P<0.009$), while blood donation in the past year decreased SF ($P<0.011$) compared to those that did not donate blood. There was no significant 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 basic training.

Conclusion: Percent body fat and blood donation in the past year were the strongest determinants of iron stores in healthy premenopausal women commencing basic training. It is recommended 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.

4.2 Introduction

Iron is a nutritionally essential trace mineral enabling several biochemical pathways critical in physical performance, such as oxygen transport and energy metabolism (Suedekum & Dimeff, 2005). In developed countries, premenopausal women are particularly at risk of suboptimal iron status due to the imbalance between iron absorption and menstrual blood loss (Johnson-Wimbley & Graham, 2011).

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, Lim et al. (2020) and Heath et al. (2001) reported intake of meat and meat/fish/poultry were 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 (Beck et al., 2014; Heath et al., 2001), history of nose bleeds and low body mass index (BMI) (Heath et al., 2001), and Asian ethnicity and previous iron deficiency (Beck et al., 2014). 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 (Newby & Tucker, 2004).

The iron status of physically active premenopausal women, including military personnel, is further compromised by exercise-induced iron losses and reduced iron absorption (McClung et al., 2014). Iron deficiency, with and without anaemia, has been reported in women at the beginning of basic training in the United States (McClung et al., 2009a; McClung et al., 2006) and Israel (Dubnov et al., 2006; Israeli et al., 2008). Suboptimal iron status is associated with impaired aerobic fitness in female recruits (McClung et al., 2009b; Myhre et al., 2015) and this relationship is operationally relevant as aerobic fitness is associated with injury risk (Jones et al., 2017) and completion of physically demanding occupational tasks (Hauschild et al., 2017) within the military environment.

One previous study of female recruits in the Israel Defense Forces reported that mean dietary iron intake was not significantly different between participants with normal iron stores, iron deficiency or iron deficiency anaemia at the commencement of basic training (Israeli et al., 2008). Despite the prevalence of suboptimal iron status in female 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 basic training in the New Zealand Army.

4.3 Methods

This study received ethical approval from the Massey University Human Ethics Committee: Southern A (Application 13/85) and adhered to Defence Force Order 3 (New Zealand Defence Force, 2020), prescribing the conduct of personnel research. 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. This is the location all New Zealand Army recruits undertake basic training, a 16-week residential course designed to prepare recruits for the demands of service as a soldier. During week 1 of basic training, blood samples and body composition measurements were completed and participants self-reported data on demographics, lifestyle behaviour, medical history and frequency of food intake.

A fasted venepuncture blood sample of 18ml in total was collected between 0600 and 0730hrs. No muscle damaging exercise was performed for at least 2-days before the assessment. Haemoglobin (Hb) was analysed using a Sysmex XT-2000i automated haematology analyser (Sysmex Corporation, Kobe, Hyogo Prefecture, Japan). The Cobas® 6000 (Roche Diagnostics, Indianapolis, IN, United States) analysed SF (electrochemiluminescence immunoassay, e601) and C-reactive protein (CRP) (via the two-point end method with an immunoturbidimetric assay, c501). All biomarkers were analysed at Medlab Whanganui (International Accreditation New Zealand) with both internal and external quality control. 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>10mg/L were excluded from the analyses (Zimmermann & Hurrell, 2007).

A questionnaire was administered via SurveyMonkey (Momentive Inc., San Mateo, United States) to determine demographics, lifestyle behaviour and medical history at week 1. Blood loss questions related to blood donation, nose bleeds and menstruation were adapted from Heath et al. (2001). Using the concept of prioritised ethnic groups, self-identification with multiple ethnicities was prioritised into a single group in the following order: Māori, Pacific and New Zealand European (Ministry of Health, 2017). Height was measured by a trained researcher. Body mass (BM) and percent body fat (%BF) were measured using the InBody_{230} bioelectrical impedance analyser (Biospace Co. Ltd., Seoul, Korea). Body mass index was calculated as BM (kg)/height (m^2).

A non-quantitative iron food frequency questionnaire (FeFFQ) (Beck et al., 2012) 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 basic training commenced. In a validation study, the FeFFQ was compared with a food record, which showed the FeFFQ was reproducible and reasonably valid for determining iron related food groups and DPs (Beck et al., 2012). Each of the 143 food variables in the FeFFQ were 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 (e.g., 1-3 times per month was converted to 0.5 times per week and 2-3 times per day was converted to 17.5 times per week).

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 values ± standard deviation (SD) for normally distributed data, median values (25th, 75th percentile) for non-normally distributed data and number of participants (%) for each group with categorical data. A $P<0.05$ was considered statistically significant throughout the results. A power calculation was conducted using G*Power Version 3.1.9.4 (Faul et al., 2007) 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.

Dietary patterns from the FeFFQ were identified using factor analysis, which aggregates food groupings based on the degree to which they correlated with one another (Newby & Tucker, 2004). 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. This was based on the consideration that consumption frequency of at least once every four days will likely affect iron status (Beck et al., 2012). Excluding water, the 27 food variables selected accounted for 45.8% of the 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 (Field, 2013). Three factors (dietary patterns) 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, %BF, 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 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 $P<0.05$ (age, %BF, blood donation, ≥6-hours 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 standardised residual.

4.4 Results

Of the 106 female recruits who volunteered to participate, data for five were excluded: four had a CRP>10mg/L and one had FeFFQ data with implausible intakes. The remaining 101 participants were included and their characteristics are summarised in Table 4.1.

Based on self-reported data, 94% of participants characterised their eating pattern as including ‘a variety of all foods, including animal products’. The remaining 6% described not eating red meat, while 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.

Table 4.1. Characteristics of participants during week 1 of basic training (*n*=101)

Characteristics		**Measure**
		Median (25th, 75th percentile)
Age (years)		18 (18, 20)
		Mean ± SD
Body mass index (kg/m^2)		24.0 ± 2.9
Percent body fat (%)		27.1 ± 5.6
Serum ferritin (µg/L)		53.5 ± 33.7
Haemoglobin (g/L)		139.7 ± 9.2
Age of menarche (years)[a]		13.0 ± 1.4
Average duration of menstrual period (days)[b]		4.9 ± 1.0
Average number of heavy days during menstrual period[b]		1.9 ± 0.9
		***n* (%)**
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)

SD, standard deviation; NZ, New Zealand.

[a] *n*=100, one participant self-reported they had not reached menarche.

[b] *n*=79, excludes 21 participants with irregular periods and one participant that had not reached menarche.

[c] 'Yes' responses presented for dichotomous variables.

[d] Participants were categorised as smokers if they responded, 'yes, everyday' or 'yes, occasionally' or non-smokers if they responded, 'never' or 'I used to'.

[e] Tertiary education included a tertiary certificate, diploma or degree.

[f] Exercise at an intensity that raised the heart rate over the past 4-weeks. Cut-off was set at 6-hours to ensure as equal distribution of participants as possible.

Based on the factor analysis, Table 4.2 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 basic training. Three DPs were identified: ‘vegetarian’, ‘meat and vegetables’ and ‘bread and beverages’, which explained 33.5% of the variance in food intake scores. The vegetarian DP was negatively associated with SF (*P*=0.023).

Table 4.2. Factor loadings for each food variable for the three dietary patterns identified for the four weeks before basic training (*n*=101)

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)
Yoghurt	**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, 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)
Wholemeal 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)

FeFFQ, iron food frequency questionnaire; DP, dietary pattern.
Kaiser-Meyer-Olkin = 0.576, *p*<0.001.
[a] Consumption frequency reported as mean (95% confidence interval).
Factor loadings ≥0.3 are presented in bold.

Potential determinants of SF during week 1 of basic training were entered into a multiple linear regression model for analysis. Table 4.3 illustrates that %BF 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.

Table 4.3. Multiple linear regression model to identify determinants of serum ferritin concentration on commencement of basic training (*n*=101)

Potential determinants	β[a]	95% CI for β[a]	β[b]	*P* value
Age	1.991	-0.893, 4.874	0.130	0.174
Percent body fat (%)	1.524	0.394, 2.655	0.254	0.009
Blood donation[c]	-30.202	-53.180, -7.225	-0.243	0.011
≥6-hours exercise per week[d]	-12.367	-25.226, 0.492	-0.181	0.059
Vegetarian dietary pattern	-3.379	-9.973, 3.216	-0.100	0.312

CI, confidence interval.

F(5, 95)=5.244, $p<0.001$, $R^2 = 0.216$, Adjusted $R^2 = 0.175$.

[a] Unstandardised coefficients: express the change in SF (µg/L) associated with a 1-unit change in the predictor variables.

[b] Standardised coefficients: express the number of standard deviations (SD) SF will change with a 1-SD change in the predictor variables.

[c] Participants had donated blood at least once in the previous 12-months.

[d] Exercise at an intensity that raised the heart rate.

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

4.5 Discussion

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

Percent body fat 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 stores and measures of overweight and obesity. Cepeda-Lopez et al. (2015) reported a significant positive association between SF and %BF, while Lim et al. (2020) observed no association. A significant positive association has been reported between SF and BMI (Heath et al., 2001; Pynaert et al., 2009), while others have observed no association (Lim et al., 2020; Lim et al., 2016; Young et al., 2018). Excess body fat triggers low-grade inflammation, increasing ferritin synthesis and falsely elevating SF (Coimbra et al., 2013). However, in this non-obese cohort, where participants with a CRP>10mg/L were excluded, a higher %BF appears to be protective. A lower %BF associated with decreasing SF may be due to a reduced intake of total energy compromising dietary iron intake. It may also be due to higher physical activity levels that reduce iron absorption, through the stimulation of hepcidin, the iron metabolism regulator (Nemeth et al., 2004).

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-months (Heath et al., 2001) and 12-months (Beck et al., 2014) were the strongest determinants of suboptimal iron status (SF<20 µg/L). During the standard donation of 450-500mL of blood, nearly 250mg of iron is lost (Kiss et al., 2015; Reddy et al., 2020). For blood donors, strategies to mitigate the risk of iron deficiency include pre-donation measurement of SF (Dijkstra et al., 2019), extending the inter-donation interval and post-donation iron supplementation (Kiss et al., 2015; Rigas et al., 2019).

The association between SF and exercise ≥6-hours 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, haemolysis, haematuria and sweat (McClung et al., 2014). The reduced absorption and recycling from increased hepcidin expression are likely due to the exercise-induced inflammatory response (Newlin et al., 2012). 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 analysed 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 (SF<20µg/L) were reduced for those following a ‘meat and vegetable’ DP and increased for those following a ‘milk and yoghurt’ DP (Beck et al., 2013). 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 (Beck et al., 2014).

While the vegetarian DP loaded negatively for beef and chicken, the lack of association in the multiple linear regression model may be due to 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 that excluded red meat had a SF<35µg/L. Experimental studies generally show a positive association between animal flesh consumption and iron status (Blanton, 2013; Cheng et al., 2013; Tetens et al., 2007). 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 eumenorrhoeic women, 30-50mL of blood is lost per menstrual cycle (Dasharathy et al., 2012; Sriprasert et al., 2017) and low menstrual blood loss has been reported to protect against suboptimal iron status (Blanco-Rojo et al., 2014; Harvey et al., 2005; Heath et al., 2001). This current finding may be due to almost 70% of participants having a regular cycle, ≥21-days and the average duration of a period, 4.9-days. Rigas et al. (2014) demonstrated that menstruation ≥5.0-days significantly increased the risk of SF<15μg/L. In addition, menstruation >65-days per year (approximately ≥5.0-days per month) was associated with a 2.5 times increased risk of SF<20μg/L compared to women with fewer menstruating days per year (Rangan et al., 1997). Conversely, no significant associations were reported between SF and menstrual cycle length, period duration or heavy bleeding days in women in Spain (Gallego-Narbón et al., 2019) and period length in women in New Zealand (Lim et al., 2020). The effect of menstrual blood loss on SF in this study may have been mitigated by the high use of hormonal contraception. While Gallego-Narbón et al. (2019) and Lim et al. (2020) found no association between SF and contraception type, previous studies have suggested the protective effect of oral contraceptive pill use on iron status is explained by reduced menstrual blood loss (Gallego-Narbón et al., 2019; Harvey et al., 2005).

A strength of this study is the inclusion of multiple non-dietary factors associated with iron status in premenopausal women. Given the prevalence of suboptimal iron status previously described in women joining the military (McClung et al., 2009a; McClung et al., 2006), 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 include necessary, but subjective decisions made in determining DPs (Newby & Tucker, 2004). 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, 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 (Beck et al., 2012). 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 (Cespedes & Hu, 2015). 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 %BF and iron stores.

In conclusion, these findings demonstrate that %BF and blood donation in the past year were the strongest determinants of iron stores in a population of healthy premenopausal women commencing basic training. This highlights the importance of a prevention-focused approach, before commencement of military training, that 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. While DPs did not influence iron stores in this study, it remains prudent that advice regarding total energy requirements and iron bioavailability also be provided.

4.6 References

Beck, K. L., Conlon, C. A., Kruger, R., Heath, A. L., Matthys, C., Coad, J., Jones, B., & Stonehouse, W. (2014). Blood donation, being Asian, and a history of iron deficiency are stronger predictors of iron deficiency than dietary patterns in premenopausal women. *Biomed Res Int*, *2014*, 652860. https://doi.org/10.1155/2014/652860

Beck, K. L., Kruger, R., Conlon, C. A., Heath, A. L., Coad, J., Matthys, C., Jones, B., & Stonehouse, W. (2012). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women. *Journal of the Academy of Nutrition and Dietetics*, *112*(8), 1177-1187. https://doi.org/10.1016/j.jand.2012.05.012

Beck, K. L., Kruger, R., Conlon, C. A., Heath, A. L., Matthys, C., Coad, J., & Stonehouse, W. (2013). Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand. *European Journal of Nutrition*, *52*(2), 467-476. https://doi.org/10.1007/s00394-012-0348-y

Blanco-Rojo, R., Toxqui, L., López-Parra, A. M., Baeza-Richer, C., Pérez-Granados, A. M., Arroyo-Pardo, E., & Vaquero, M. P. (2014). Influence of diet, menstruation and genetic factors on iron status: a cross-sectional study in Spanish women of childbearing age. *International Journal of Molecular Sciences*, *15*(3), 4077-4087. https://doi.org/10.3390/ijms15034077

Blanton, C. (2013). Improvements in iron status and cognitive function in young women consuming beef or non-beef lunches. *Nutrients*, *6*(1), 90-110. https://doi.org/10.3390/nu6010090

Cepeda-Lopez, A. C., Melse-Boonstra, A., Zimmermann, M. B., & Herter-Aeberli, I. (2015). In overweight and obese women, dietary iron absorption is reduced and the enhancement of iron absorption by ascorbic acid is one-half that in normal-weight women. *American Journal of Clinical Nutrition*, *102*(6), 1389-1397. https://doi.org/10.3945/ajcn.114.099218

Cespedes, E. M., & Hu, F. B. (2015). Dietary patterns: from nutritional epidemiologic analysis to national guidelines. *American Journal of Clinical Nutrition*, *101*(5), 899-900. https://doi.org/10.3945/ajcn.115.110213

Cheng, H. L., Griffin, H. J., Bryant, C. E., Rooney, K. B., Steinbeck, K. S., & O'Connor, H. T. (2013). Impact of diet and weight loss on iron and zinc status in overweight and obese young women. *Asia Pacific Journal of Clinical Nutrition*, *22*(4), 574-582. https://doi.org/10.6133/apjcn.2013.22.4.08

Coimbra, S., Catarino, C., & Santos-Silva, A. (2013). The role of adipocytes in the modulation of iron metabolism in obesity. *Obesity Reviews*, *14*(10), 771-779. https://doi.org/10.1111/obr.12057

Dasharathy, S. S., Mumford, S. L., Pollack, A. Z., Perkins, N. J., Mattison, D. R., Wactawski-Wende, J., & Schisterman, E. F. (2012). Menstrual bleeding patterns among regularly menstruating women. *American Journal of Epidemiology*, *175*(6), 536-545. https://doi.org/10.1093/aje/kwr356

Dijkstra, A., van den Hurk, K., Bilo, H. J. G., Slingerland, R. J., & Vos, M. J. (2019). Repeat whole blood donors with a ferritin level of 30 µg/L or less show functional iron depletion. *Transfusion*, *59*(1), 21-25. https://doi.org/10.1111/trf.14935

Dubnov, G., Foldes, A. J., Mann, G., Magazanik, A., Siderer, M., & Constantini, N. (2006). High prevalence of iron deficiency and anemia in female military recruits. *Military Medicine*, *171*(9), 866-869.

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39(2), 175-191. https://doi.org/10.3758/BF03193146

Field, A. (2013). *Discovering statistics using IBM SPSS statistics* (4th Edition ed.). SAGE Publications.

Gallego-Narbón, A., Zapatera, B., & Vaquero, M. P. (2019). Physiological and dietary determinants of iron status in Spanish vegetarians. *Nutrients*, *11*(8). https://doi.org/10.3390/nu11081734

Harvey, L. J., Armah, C. N., Dainty, J. R., Foxall, R. J., John Lewis, D., Langford, N. J., & Fairweather-Tait, S. J. (2005). Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *British Journal of Nutrition*, *94*(4), 557-564.

Hauschild, V. D., DeGroot, D. W., Hall, S. M., Grier, T. L., Deaver, K. D., Hauret, K. G., & Jones, B. H. (2017). Fitness tests and occupational tasks of military interest: a systematic review of correlations. *Occupational and Environmental Medicine*, *74*(2), 144-153. https://doi.org/10.1136/oemed-2016-103684

Heath, A. L., Skeaff, C. M., Williams, S., & Gibson, R. S. (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutrition*, *4*(2), 197-206.

Israeli, E., Merkel, D., Constantini, N., Yanovich, R., Evans, R. K., Shahar, D., & Moran, D. S. (2008). Iron deficiency and the role of nutrition among female military recruits. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S685-690. https://doi.org/10.1249/MSS.0b013e31818946ae

Johnson-Wimbley, T. D., & Graham, D. Y. (2011). Diagnosis and management of iron deficiency anemia in the 21st century. *Therapeutic Advances in Gastroenterology*, *4*(3), 177-184. https://doi.org/10.1177/1756283x11398736

Jones, B. H., Hauret, K. G., Dye, S. K., Hauschild, V. D., Rossi, S. P., Richardson, M. D., & Friedl, K. E. (2017). Impact of physical fitness and body composition on injury risk among active young adults: a study of army trainees. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S17-s22. https://doi.org/10.1016/j.jsams.2017.09.015

Kiss, J. E., Brambilla, D., Glynn, S. A., Mast, A. E., Spencer, B. R., Stone, M., Kleinman, S. H., & Cable, R. G. (2015). Oral iron supplementation after blood donation: a randomized clinical trial. *JAMA*, *313*(6), 575-583. https://doi.org/10.1001/jama.2015.119

Lim, K., Beck, K. L., Von Hurst, P. R., Rutherfurd-Markwick, K. J., & Badenhorst, C. E. (2020). Iron deficiency and risk factors in pre-menopausal females living in Auckland, New Zealand. *Asia Pacific Journal of Clinical Nutrition*, *29*(3), 638-647. https://doi.org/10.6133/apjcn.202009_29(3).0024

Lim, K. H., Booth, A. O., Nowson, C. A., Szymlek-Gay, E. A., Irving, D. O., & Riddell, L. J. (2016). Hepcidin is a better predictor of iron stores in premenopausal women than blood loss or dietary intake. *Nutrients*, *8*(9). https://doi.org/10.3390/nu8090540

McClung, J. P., Gaffney-Stomberg, E., & Lee, J. J. (2014). Female athletes: a population at risk of vitamin and mineral deficiencies affecting health and performance. *Journal of Trace Elements in Medicine and Biology*, *28*(4), 388-392. https://doi.org/10.1016/j.jtemb.2014.06.022

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Nindl, B. C., Young, A. J., & Lieberman, H. R. (2009a). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *American Journal of Clinical Nutrition*, *90*(1), 124-131. https://doi.org/10.3945/ajcn.2009.27774

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Young, A. J., & Lieberman, H. R. (2009b). Longitudinal decrements in iron status during military training in female soldiers. *British Journal of Nutrition*, *102*(4), 605-609. https://doi.org/10.1017/s0007114509220873

McClung, J. P., Marchitelli, L. J., Friedl, K. E., & Young, A. J. (2006). 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*, *25*(1), 64-69.

Ministry of Health. (2017). *HISO 10001:2017 Ethnicity data protocols.* Wellington: Ministry of Health.

Myhre, K. E., Webber, B. J., Cropper, T. L., Tchandja, J. N., Ahrendt, D. M., Dillon, C. A., Haas, R. W., Guy, S. L., Pawlak, M. T., & Federinko, S. P. (2015). Prevalence and impact of anemia on basic trainees in the US Air Force. *Sports Med Open*, *2*, 23. https://doi.org/10.1186/s40798-016-0047-y

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K., & Ganz, T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *Journal of Clinical Investigation*, *113*(9), 1271-1276. https://doi.org/10.1172/jci20945

New Zealand Defence Force. (19 November 2020). *Defence Force Order 3: New Zealand Defence Force Human Resource Manual: Version 17/12 – AL 16*. Wellington, New Zealand.

Newby, P. K., & Tucker, K. L. (2004). Empirically derived eating patterns using factor or cluster analysis: a review. *Nutrition Reviews*, *62*(5), 177-203. https://doi.org/10.1301/nr.2004.may.177-203

Newlin, M. K., Williams, S., McNamara, T., Tjalsma, H., Swinkels, D. W., & Haymes, E. M. (2012). The effects of acute exercise bouts on hepcidin in women. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(2), 79-88.

Pynaert, I., De Bacquer, D., Matthys, C., Delanghe, J., Temmerman, M., De Backer, G., & De Henauw, S. (2009). Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutrition*, *12*(10), 1775-1782. https://doi.org/10.1017/s1368980008004369

Rangan, A. M., Aitkin, I., Blight, G. D., & Binns, C. W. (1997). Factors affecting iron status in 15-30 year old female students. *Asia Pacific Journal of Clinical Nutrition*, *6*(4), 291-295.

Reddy, K. V., Shastry, S., Raturi, M., & Baliga, B. P. (2020). Impact of regular whole-blood donation on body iron stores. *Transfusion Medicine and Hemotherapy. Offizielles Organ der Deutschen Gesellschaft für Transfusionsmedizin und Immunhämatologie*, *47*(1), 75-79. https://doi.org/10.1159/000499768

Rigas, A. S., Pedersen, O. B., Magnussen, K., Erikstrup, C., & Ullum, H. (2019). Iron deficiency among blood donors: experience from the Danish Blood Donor Study and from the Copenhagen ferritin monitoring scheme. *Transfusion Medicine*, *29 Suppl 1*, 23-27. https://doi.org/10.1111/tme.12477

Rigas, A. S., Sørensen, C. J., Pedersen, O. B., Petersen, M. S., Thørner, L. W., Kotzé, S., Sørensen, E., Magnussen, K., Rostgaard, K., Erikstrup, C., & Ullum, H. (2014). Predictors of iron levels in 14,737 Danish blood donors: results from the Danish Blood Donor Study. *Transfusion*, *54*(3 Pt 2), 789-796. https://doi.org/10.1111/trf.12518

Sriprasert, I., Pakrashi, T., Kimble, T., & Archer, D. F. (2017). Heavy menstrual bleeding diagnosis and medical management. *Contracept Reprod Med*, *2*, 20. https://doi.org/10.1186/s40834-017-0047-4

Suedekum, N. A., & Dimeff, R. J. (2005). Iron and the athlete. *Current Sports Medicine Reports*, *4*(4), 199-202. https://doi.org/10.1097/01.csmr.0000306207.79809.7f

Tetens, I., Bendtsen, K. M., Henriksen, M., Ersbøll, A. K., & Milman, N. (2007). The impact of a meat- versus a vegetable-based diet on iron status in women of childbearing age with small iron stores. *European Journal of Nutrition*, *46*(8), 439-445. https://doi.org/10.1007/s00394-007-0683-6

Young, I., Parker, H. M., Rangan, A., Prvan, T., Cook, R. L., Donges, C. E., Steinbeck, K. S., O'Dwyer, N. J., Cheng, H. L., Franklin, J. L., & O'Connor, H. T. (2018). Association between haem and non-haem iron intake and serum ferritin in healthy young women. *Nutrients*, *10*(1). https://doi.org/10.3390/nu10010081

Zimmermann, M. B., & Hurrell, R. F. (2007). Nutritional iron deficiency. *Lancet*, *370*(9586), 511-520. https://doi.org/10.1016/s0140-6736(07)61235-5

Chapter 5 Determinants and longitudinal changes of serum 25-hydroxyvitamin D during basic training in female recruits in the New Zealand Army

Alongside iron, establishing a situational awareness of vitamin D status in female recruits is considered critical in support of their health and physical performance. This is primarily due to the role of vitamin D in musculoskeletal health. Identifying potential determinants of vitamin D status in ethnically diverse populations and varied geographical locations is also required. The aim of this study was to describe the vitamin D status of female recruits in the New Zealand Army during basic training and to investigate potential determinants at the commencement of basic training.

Data accepted for publication:

Accepted and awaiting publication in The Journal of Sport and Exercise Science.

5.1 Abstract

Background: Diminished vitamin D status negatively impacts military readiness. Most female recruits have been reported to have suboptimal vitamin D status at the end of basic training. Casual sun exposure and ethnicity are likely key determinants of vitamin D status in recruits.

Objective: The purpose of this study was to describe vitamin D status during basic training and identify determinants of serum 25-hydroxyvitamin D (25(OH)D) in female recruits in the New Zealand Army.

Methods: Serum 25(OH)D was measured during week 1 and week 16 of basic training in 87 participants, and further characterised by ethnicity and season. Following univariate analysis, age, body mass index (BMI), ethnicity, season, exercise and serum ferritin were analysed as determinants of 25(OH)D at week 1 using a hierarchical linear regression model.

Results: From week 1 to week 16, mean ± SD 25(OH)D was 102.5 ± 33.6 to 67.4 ± 22.6nmol/L (P<0.001) for basic training commenced in summer and 67.4 ± 21.5 to 73.8 ± 18.9nmol/L (P=0.033) for basic training commenced in winter. Regardless of the season basic training commenced, less than one-third of participants overall had sufficient (≥75nmol/L) vitamin D status at the end of basic training. Age, BMI, ethnicity and season explained 45.0% of the variance in 25(OH)D at week 1.

Conclusion: Wintertime and being of Pacific or Māori ethnicity were the strongest negative determinants of 25(OH)D at the commencement of basic training. These results suggest seasonal ultraviolet beta (UVB) exposure is a major determinant of 25(OH)D and Pacific and Māori ethnic groups are particularly at risk of suboptimal vitamin D status. Education, screening and early supplementation are recommended to prevent suboptimal vitamin D status during basic training.

5.2 Introduction

Diminished vitamin D status is a concern for all military personnel. Observational studies in military recruits suggest an association between reduced serum 25-hydroxyvitamin D (25(OH)D) concentration and increased risk of stress fractures (Burgi et al., 2011; Davey et al., 2016; Ruohola et al., 2006), longer recovery time from stress fractures (Richards & Wright, 2018), impaired aerobic fitness (Carswell et al., 2018) and increased incidence of upper respiratory tract infections (Harrison et al., 2021; Laaksi et al., 2007) in otherwise healthy recruits. Injury and illness during basic training have considerable personal and organisational impacts, with delays in completing training, medical and rehabilitation costs, and potential discharge from military service.

Evidence for the role of vitamin D in skeletal health is robust. The biologically active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) stimulates intestinal absorption of dietary calcium and phosphorus. This maintains homeostasis of serum concentrations, essential for bone mineralisation (DeLuca, 2004) and neuromuscular function (Holick et al., 2011). Vitamin D status has therefore been identified as a modifiable risk factor in reducing the burden of musculoskeletal injuries during basic training. This is particularly relevant for female recruits, where the incidence of stress fractures has been reported up to three times higher than males (Jones et al., 1993; Knapik et al., 2012; Moran et al., 2008).

Most female recruits have insufficient vitamin D status at the end of basic training (Andersen et al., 2010; Carswell et al., 2018; Gaffney-Stomberg et al., 2019; Lutz et al., 2012). This decline is likely influenced by a lack of casual sun exposure, the predominant source of vitamin D for most people. In New Zealand, higher latitudes have been shown to decrease vitamin D status (Ministry of Health, 2012). However, no studies have investigated vitamin D status in a New Zealand military population. In female recruits in the United States, 25(OH)D at the beginning and the end of basic training has been associated with ethnicity (Andersen et al., 2010; Lutz et al., 2012) and the season training commenced (Gaffney-Stomberg et al., 2019). Inadequate dietary vitamin D intake may also contribute to diminishing 25(OH)D (Lutz et al., 2018; Lutz et al., 2012). It is important to confirm these findings in populations outside of the United States that are ethnically diverse. Latitude and seasonal changes may also confer differences in outcomes that are not transferable between populations. In addition, multiple other factors, including body composition, physical activity and smoking status have been reported as significant predictors of 25(OH)D in large scale studies (Kuhn et al., 2014; Liu et al., 2010; Millen, 2010). The objectives of this longitudinal study were therefore to describe the vitamin D status of female recruits in the New Zealand Army during basic training and to investigate potential determinants at the commencement of basic training.

5.3 Methods

New Zealand Army recruits undertake 16-weeks of basic training at Waiouru Military Camp, latitude 39°S and 792m above sea level. All female recruits enlisted in the New Zealand Army from February 2014 to March 2016 were eligible and invited to participate in this longitudinal cohort study. All procedures involving human subjects were approved by the Massey University Human Ethics Committee: Southern A (Application – 13/85). Investigators also adhered to Defence Force Order 3 (New Zealand Defence Force, 2020), which prescribes the policy relating to the conduct and approval of personnel research. Volunteers provided informed and written consent. The sample size was determined by the feasibility of recruitment.

Data were collected over nine intakes of basic training, categorised by the season training started. Summer intakes (*n*=5) commenced in February or early March (end of summer) and ended in May (late autumn) or June (early winter). Winter intakes (*n*=4) started in July or August (winter) and ended in October or November (spring). Serum 25(OH)D was measured during weeks 1 and 16 of basic training. Body composition, demographic and lifestyle data were collected at week 1.

Basic training is a 16-week, residential course designed to take a person from a civilian to a competent and self-disciplined soldier. Core military skills covered include weapon training, first aid, navigation, drill, fieldcraft and military law. Long periods of training occur outdoors. All meals, snacks and beverages are provided. Dietary supplements are not permitted unless prescribed by a medical doctor. Recruits primarily wore a combat uniform, consisting of long trousers, a long-sleeve shirt, boots and a wide-brimmed hat.

A fasted venepuncture blood sample of 18ml was collected between 0600 and 0730hrs at weeks 1 and 16. No participants exercised in the 8-hours prior to blood sampling. Serum 25(OH)D was analysed using a Sciex 4000 QTRAP liquid chromatography-tandem-mass spectrometer (LC-MS/MS) (AB Sciex LLC, Framingham, MA, United States) by Canterbury Health Laboratories. Samples were analysed using deuterated 25(OH)D as an internal standard. External quality control was provided by the Vitamin D External Quality Assessment Scheme. Inter-assay coefficients of variation (CV) ranged from 6% at 25(OH)D ≥150nmol/L to 12% at <25nmol/L. The LC-MS/MS is the proposed gold standard for assays and enhances comparability between studies (Alexandridou et al., 2021). There is emerging evidence of a relationship between vitamin D and iron status, particularly through the effects of vitamin D on hepcidin (Shoemaker et al., 2022), the iron metabolism regulator (Nemeth et al., 2004). Inflammation increases hepcidin expression (Daher et al., 2017). The Cobas® 6000 (Roche Diagnostics, Indianapolis, IN, United States) was used to analyse serum ferritin (SF) as a marker of iron status, using the electrochemiluminescence immunoassay, e601 and C-reactive protein (CRP) as a marker of inflammation, using the two-point end method with an immunoturbidimetric assay, c501. Medlab Whanganui analysed SF and CRP. Both laboratories are accredited with International Accreditation New Zealand. Using 25(OH)D, vitamin D status was categorised as deficient (<50nmol/L), insufficient

(50-74nmol/L) or sufficient (≥75nmol/L) (Holick et al., 2011). All concentrations <75nmol/L were referred to as suboptimal.

In New Zealand, the concept of prioritised ethnicity is the most common methodology used to classify ethnic groups in the health and disability sector (Ministry of Health, 2017). In addition, this method is particularly favoured for regression models (Boven et al., 2020). Using the concept of prioritised ethnicity, participants could self-identify with multiple ethnic groups. Responses were then prioritised into a single ethnic group in the following order: Māori, Pacific and New Zealand European (Ministry of Health, 2017).

Body composition measures were determined at week 1. Prior to all measures, the participants fasted overnight, performed no physical activity for at least 8-hours prior, urinated prior to testing, wore shorts and a t-shirt, and removed all jewellery. Height (m) was measured using a SECA 213 portable stadiometer (German Healthcare Export Group, Bonn, Germany) by a trained researcher. Body mass (kg) and percent body fat (%BF) were measured via bioelectrical impedance analysis using the InBody$_{230}$ (Biospace Co. Ltd., Seoul, Korea). Body mass index (BMI) was calculated as body mass (kg)/height (m)^2.

A questionnaire was administered in week 1 via SurveyMonkey (Momentive Inc., San Mateo, United States). Smoking status was determined by the question, 'do you smoke cigarettes?'. Responses of 'never' and 'I used to' were categorised as 'no'. Responses of 'yes, everyday' and 'yes, occasionally' were categorised as 'yes'. Education level was investigated by the question, 'what is the highest level of education you have received?'. Responses included the New Zealand National Certificate of Educational Achievement (NCEA). The options were 'NCEA Level 1', 'NCEA Level 2', 'NCEA Level 3', 'tertiary certificate/diploma' and 'tertiary degree'. Tertiary certificate/diploma and degree were collapsed to tertiary qualification. The NCEA levels of 1, 2 and 3 are equivalent to study in grades 10, 11 and 12, respectively at a secondary school in the United States. Weekly exercise habits were determined by the question, 'in the past 4-weeks, how many hours per week did you spend exercising at an intensity that raised your heart rate?'. Response options were grouped as '<3.0-hours', '3.0-5.9-hours', '6.0-8.9-hours' and '≥9.0-hours'.

All statistical analyses were performed using IBM SPSS Statistics, Version 28.0 (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test and box plots were used to assess the data for normality. A *P* value <0.05 was considered statistically significant. Descriptive statistics are presented as mean value ± standard deviation (SD) or median value (25th, 75th percentile). Changes in 25(OH)D were investigated using paired *t*-tests. Independent *t*-tests investigated any difference in 25(OH)D between participants that finished basic training and participants that did not. Comparison of mean values of 25(OH)D between ethnicity groups at week 1 and week 16 were investigated using one-way analysis of variance (ANOVA). A subsequent Tukey HSD test identified significant differences between the groups.

Simple linear regression analysis was performed to identify potential determinants associated with 25(OH)D at week 1. These determinants included age, BMI, %BF, SF and CRP, entered as continuous variables; season and smoking status, entered as binary variables; and dummy categorical variables were created and entered for ethnicity and exercise, with New Zealand European and <3.0-hours the reference categories, respectively. Variables with a univariate *P*<0.20 (age, BMI, ethnicity, season, exercise and SF) were then entered into a hierarchical linear regression analysis. This value was chosen as univariate *P* values ≥0.20 were considered unlikely to contribute any unique variance to a model containing other potential determinants of 25(OH)D. At each step, the variables were controlled for those at the same level and the levels above. Assumptions for the regression model were defined as a Durbin-Watson statistic between 1.5 and 2.5 for autocorrelation of residuals, a Variance Inflating Factor <5 for assessment of multicollinearity and a satisfactory normal P-P plot of regression standardised residual.

5.4 Results

In this study, 106 female recruits voluntarily participated from a total of 108 invited recruits. Eighteen participants did not finish basic training: 14 incurred injuries (including five medically diagnosed stress fractures) and were removed from their initial course, and four participants self-withdrew. One participant was excluded due to supplementation with oral vitamin D3 during basic training. Following these exclusions, 87 participants were included in the analysis. One participant consumed a vitamin D supplement before basic training commenced; however, their vitamin D status was deficient at week 1. Therefore, their supplement use did not impact on further analysis and they were included in the results presented. Table 5.1 describes the characteristics of study participants at the commencement of basic training.

Overall, the mean ± SD 25(OH)D declined during basic training from 92.0 ± 34.4nmol/L at week 1 to 69.3 ± 21.7nmol/L at week 16, *P*<0.001. There was no significant difference in 25(OH)D at week 1 between those participants that finished basic training and the 18 participants that did not. From week 1 to week 16, mean ± SD 25(OH)D was 105.2 ± 33.7 to 76.3 ± 17.4nmol/L (*P*<0.001) for New Zealand Europeans, 78.2 ± 28.2 to 65.5 ± 24.5nmol/L (*P*=0.002) for Māori and 60.8 ± 13.5 to 43.7 ± 9.7nmol/L (*P*=0.460) for Pacific. Figure 5.1 and Table 5.2 display ethnic differences in serum 25(OH)D and vitamin D status, respectively.

Table 5.1. Characteristics of participants at week 1 of basic training (*n*=87)

Characteristics	*n* (%)	Mean ± SD	Median (25th, 75th percentile)
Age (years)			18 (18, 20)
Height (cm)		165.1 ± 5.4	
Body mass (kg)		65.5 ± 9.3	
Body mass index (kg/m^2)		24.0 ± 2.8	
Percent body fat (%)		26.8 ± 5.4	
Ethnicity			
New Zealand European	51 (58.6)		
Māori	26 (29.9)		
Pacific	10 (11.5)		
Season			
Summer	61 (70.1)		
Winter	26 (29.9)		
Education			
NCEA Level 1	3 (3.4)		
NCEA Level 2	25 (28.7)		
NCEA Level 3	45 (51.7)		
Tertiary Qualification	14 (16.1)		
Smoker			
Yes	10 (11.5)		
No	77 (88.5)		
Exercise hours per week[a]			
<3.0	13 (14.9)		
3.0 – 5.9	38 (43.7)		
6.0 – 8.9	23 (26.4)		
≥9.0	13 (14.9)		

SD, standard deviation; NCEA, National Certificate of Educational Achievement.

[a] Exercise at an intensity that raised the heart rate.

Across seasons, from week 1 to week 16, the mean ± SD 25(OH)D was 102.5 ± 33.6 to 67.4 ± 22.6nmol/L (*P*<0.001) for basic training commenced in summer (*n*=61) and 67.4 ± 21.5 to 73.8 ± 18.9nmol/L (*P*=0.033) for basic training commenced in winter (*n*=26). See Table 5.2 for further analysis. Unequal group sizes in the winter intakes prevented further analysis of 25(OH)D by season and ethnicity.

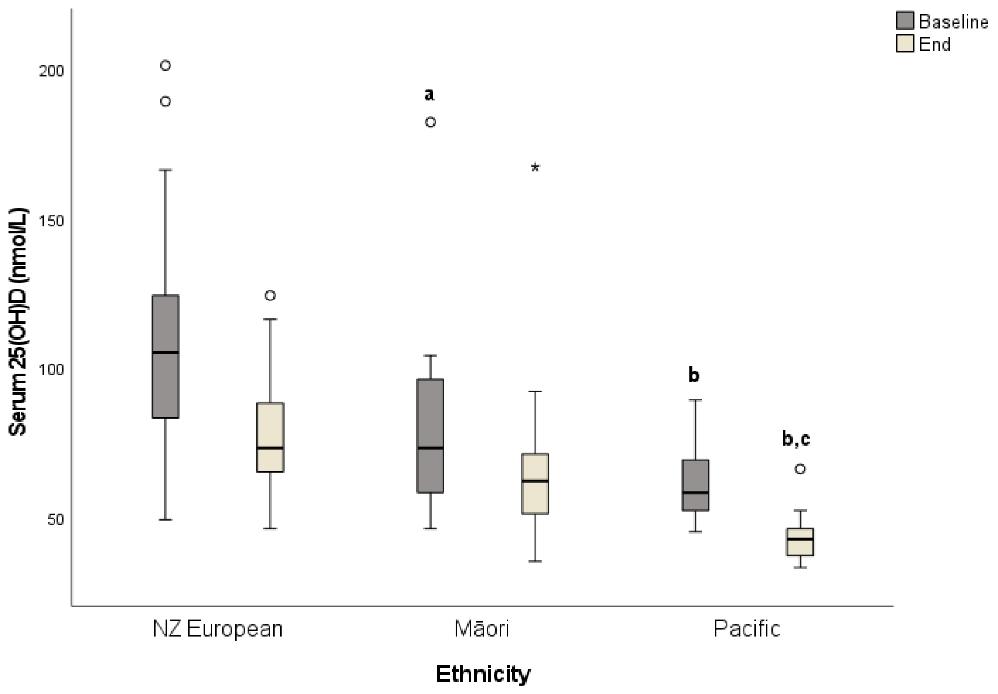


Figure 5.1. Boxplot of serum 25-hydroxyvitamin D at week 1 (baseline) and week 16 (end) of basic training by ethnicity

25(OH)D, serum 25-hydroxyvitamin D; NZ, New Zealand. Total (*n*=87), NZ European (*n*=51), Māori (*n*=26), Pacific (*n*=10). Boxes represent the middle 50th percentile and vertical lines extend to the 10th and 90th percentiles. Median values are marked by a line within each box. Values above the 90th percentile are identified by circles if they are mild outliers (interquartile range x 1.5) or stars if they are extreme outliers (interquartile range x 3.0). A one-way ANOVA with Tukey post-hoc analysis determined the effects of time and ethnicity on serum 25(OH)D. Significant differences between mean values within baseline or end time frames and between ethnicities are represented as a) P=0.001 with New Zealand European, b) P<0.001 with New Zealand European and c) P<0.001 with Māori.

The hierarchical linear regression model for predicting 25(OH)D at week 1 is illustrated in Table 5.3. Age, ethnicity and season were significant determinants of 25(OH)D at the commencement of basic training, accounting for 7.8%, 17.1% and 20.8% of the unique variance, respectively. Excluding the non-significant exercise and SF variables, the final model explained 45.0% of the variance in 25(OH)D.

Table 5.2. Vitamin D status at week 1 and week 16 of basic training by ethnicity and season of intake (*n*=87)

Time	Ethnicity/ Season	Sufficient[a]		Insufficient[a]		Deficient[a]	
		n	%	*n*	%	*n*	%
Week 1	**Total**	54	62.1	27	31.0	6	6.9
	NZ European	41	80.4	9	17.6	1	2.0
	Māori	12	46.2	11	42.3	3	11.5
	Pacific	1	10.0	7	70.0	2	20.0
Week 16	**Total**	28	32.2	44	50.6	15	17.2
	NZ European	24	47.0	26	51.0	1	2.0
	Māori	4	15.4	16	61.5	6	23.1
	Pacific	0	0	2	20.0	8	80.0
Week 1	**Summer**	47	77.0	14	23.0	0	0
Week 16		18	29.5	30	49.2	13	21.3
Week 1	**Winter**	7	26.9	13	50.0	6	23.1
Week 16		10	38.5	14	53.8	2	7.7

NZ, New Zealand.

[a] Serum 25-hydroxyvitamin D (25(OH)D) ≥75nmol/L (sufficient), 50-74nmol/L (insufficient) and <50nmol/L (deficient).

Table 5.3. Hierarchical linear regression model for determinants of serum 25-hydroxyvitamin D at commencement of basic training (*n*=87)

Step	Independent variable		B[a]	95% CI for B	β[b]	*P* value
1	**Age (years)**		-4.428	-7.708, -1.148	-0.280	0.009
2	**Age (years)**		-4.388	-7.644, -1.132	-0.277	0.009
	Body mass index (kg/m^2)		-1.931	-4.461, 0.598	-0.157	0.133
3	**Age (years)**		-3.059	-6.162, 0.044	-0.193	0.053
	Body mass index (kg/m^2)		-1.172	-3.509, 1.166	-0.095	0.322
	Ethnicity	NZ European	Reference			
		Māori	-22.303	-37.385, -7.220	-0.298	0.004
		Pacific	-41.124	-62.152, -20.096	-0.383	<0.001
4	**Age (years)**		-2.376	-5.024, 0.273	-0.150	0.078
	Body mass index (kg/m^2)		-0.159	-2.178, 1.859	-0.013	0.876
	Ethnicity	NZ European	Reference			
		Māori	-21.714	-34.538, -8.890	-0.290	0.001
		Pacific	-46.275	-64.242, -28.307	-0.431	<0.001
	Season	Summer	Reference			
		Winter	-34.927	-47.119, -22.736	-0.467	<0.001

CI, confidence interval; NZ, New Zealand.

F(5, 81)=15.054, $p < 0.001$, $R^2 = 0.482$, adjusted $R^2 = 0.450$.

[a] Unstandardised coefficients.

[b] Standardised coefficients.

Age and body mass index were entered as continuous variables. Ethnicity and season were entered as categorical variables.

5.5 Discussion

This is the first study to investigate the vitamin D status of females in the New Zealand Army and potential vitamin D determinants. During basic training, 25(OH)D declined significantly for participants that commenced training in summer and increased significantly for those that commenced training in winter. Despite the increase observed for the winter intakes, two-thirds of all participants had suboptimal vitamin D status at the end of basic training, regardless of the season they commenced training. For Pacific and Māori, 100% and 85% of participants had suboptimal vitamin D status following 16-weeks of basic training, respectively. Wintertime and being of Pacific or Māori ethnicity were the strongest determinants and inversely associated with 25(OH)D at the commencement of training, when age and body composition were controlled for. This model explained 45.0% of the variance in 25(OH)D at the commencement of basic training.

The results of this study reflect the findings from the 2008/09 New Zealand Adult Nutrition Survey, in which the prevalence of vitamin D deficiency was highest during late winter and early spring (Ministry of Health, 2012). As the major determinant of 25(OH)D is ultraviolet beta (UVB) exposure, New Zealand’s distance from the equator, lower angle of the sun, greater cloud coverage and increased clothing cover during these months; coupled with a gradual loss in accumulated 25(OH)D from summer will have contributed to the seasonal variation (Livesey et al., 2007; Ministry of Health, 2012). The fact that much of basic training occurs outdoors during daylight hours suggests some casual sun exposure during spring supported positive changes in 25(OH)D for winter recruits, rather than further decline as suggested by the New Zealand population data. The findings in this study are similar to those in females and males completing 12-weeks of basic training in the United States Marines without vitamin D supplementation. For participants that commenced training in winter, a significant mean 25(OH)D increase ($P<0.05$) was also reported during basic training. However, the mean 25(OH)D concentration at the end of training failed to reach vitamin D sufficiency (Gaffney-Stomberg et al., 2019).

New Zealand Europeans had the highest vitamin D status, followed by Māori, then Pacific female recruits at all time points throughout this study. This reflects the situation within the New Zealand population, where the mean annual 25(OH)D for women aged 15-years and over was 62.4, 57.2 and 46.0nmol/L for the total population sample, Māori and Pacific, respectively (Ministry of Health, 2012). Ethnicity is consistently a strong individual predictor of 25(OH)D in multivariable regression models (Bertrand et al., 2012; Knight et al., 2017; Liu et al., 2018; Nessvi et al., 2011). Ethnicity is likely related to many other factors that can reduce vitamin D status, both non-modifiable, such as genetics, skin colour and latitude, and modifiable, including physical activity levels, clothing cover, diet quality and tobacco smoking (Knight et al., 2017; Liu et al., 2018; Nessvi et al., 2011). Higher levels of melanin may reduce 25(OH)D in individuals with darker skin pigmentation, including those of Pacific and Māori ethnicity. Melanin absorbs sunlight, reducing its ability to trigger endogenous vitamin D production (Holick, 2007).

For basic training that commenced in summer and winter, the prevalence of suboptimal vitamin D at the end was 71% and 62%, respectively in this study. These results support the findings of two studies in female recruits following completion of basic training in the United States Army at a training location of 34°N (Andersen et al., 2010; Lutz et al., 2012). For participants that commenced basic training at the end of summer, 75% had suboptimal vitamin D status at the end of training (Andersen et al., 2010). For participants that commenced basic training at the end of winter, the prevalence of suboptimal vitamin D status at the end of training was 64% and 92% for white and non-white participants, respectively (Lutz et al., 2012). Collectively these studies indicate that regardless of ethnicity, most female recruits have suboptimal vitamin D status at the end of basic training, if not also throughout basic training.

It has been proposed that excess adipose tissue sequesters vitamin D (Wortsman et al., 2000). In this study there was no significant association between 25(OH)D and BMI or %BF at the commencement of basic training. While Lutz et al. (2012) also reported no significant association with body composition in the total study population of female recruits in the United States Army, there was a positive association between baseline 25(OH)D and %BF in non-white participants (Lutz et al., 2012). Gaffney-Stomberg et al. (2019) stated that the observed increase in 25(OH)D in recruits who began training in the winter was likely due to the release from endogenous fat stores. This was based on the finding that participants completing basic training during winter lost more body fat than those undertaking training during summer.

The incidence of medically diagnosed stress fractures during basic training has been reported up to 5% for males and 18% for females (Jones et al., 1993; Knapik et al., 2012; Moran et al., 2008). Up to 60% of those who sustain a stress fracture attrite from training (Friedl et al., 2008). Vitamin D status is a modifiable risk factor for stress fractures (Abbott et al., 2022; Bishop et al., 2020). Serum 25(OH)D ≥75nmol/L has been linked with lower stress fracture rates in male recruits (Davey et al., 2016; Ruohola et al., 2006). In female recruits in the United States Navy, there was a significant dose-response relationship of higher 25(OH)D with lower stress fracture risk, particularly of the tibia and fibula (Burgi et al., 2011). Females in the highest quintile of 25(OH)D (≥100nmol/L) had a lower stress fracture risk than those in the lowest quintile (<50nmol/L): OR = 0.51, 95% CI = 0.34-0.76, *P*≤0.01 (Burgi et al., 2011).

In this study, two-thirds of participants overall had suboptimal vitamin D status at the end of basic training. In the five participants that incurred medically diagnosed stress fractures and did not finish basic training, they all had suboptimal 25(OH)D at week 1 (range 44-68nmol/L). It is therefore worth considering screening and supplementing recruits with 25(OH)D <75nmol/L at the commencement of basic training to reduce stress fracture risk. Given it may take 2-3-months for vitamin D supplementation to improve status – education, screening and supplementation during the recruiting phase may be required to ensure a protective effect.

Comparisons between studies in the general population to investigate factors affecting vitamin D status are difficult due to the diversity of participants, controversy regarding 25(OH)D cut-offs, seasonality and factors adjusted for in the final model (Holick et al., 2011; Liu et al., 2010; Liu et al., 2018). Prediction models in adult representative samples have significant unexplained variability regarding the factors that affect vitamin D status (Bertrand et al., 2012; Kimlin et al., 2014; Knight et al., 2017; Kuhn et al., 2014; Liu et al., 2010; Lucas et al., 2013; Millen, 2010). This is likely the result of methodological errors in measuring predictor variables, serum 25(OH)D (Black et al., 2015) and lack of information regarding genetic factors and UVB exposure that considers clothing, cloud cover and the use of sunscreen (Bertrand et al., 2012). With minimal UVB exposure, dietary intake of vitamin D can contribute to achieving 25(OH)D targets. Dietary intake of vitamin D was not assessed in this study and at present, there is no data available to assess the usual intake in the New Zealand population. However, few foods are naturally rich sources or fortified with vitamin D in New Zealand. Dietary supplements are therefore likely to be the most effective method for individuals to increase their intake of vitamin D.

There are several strengths associated with this study. No previous investigations in female recruits have explored changes in 25(OH)D beyond 12-weeks. The extended duration of these results may be applicable to other female military populations with longer or consecutive training. A further strength is the location of 39°S. Previous research has encouraged studies in military personnel to be conducted at training locations approaching or greater than latitudes of 40° to measure the effects of more extreme environments on vitamin D status. While blood samples were collected at varying months across three years, the stable environment during basic training, regarding climatic conditions and the level of skin exposure is a strength.

A limitation of this study is that participants were not asked to describe their UVB exposure prior to basic training in terms of time spent outdoors for work, recreation or physical activity; the region they lived in; and sun protection behaviours. While weekly exercise duration of a moderate to high intensity was measured, participants were not asked the frequency, time of day, or whether the exercise occurred indoors or outdoors. These additional questions would have contributed to a greater understanding of participants' UVB exposure prior to basic training. In addition, there was no examination of dietary intake as a potential determinant of 25(OH)D. Although diet is a relatively modest contributor to vitamin D status compared to other predictors, it has contributed significantly towards the explained variance of 25(OH)D in multivariable regression models (Bertrand et al., 2012; Knight et al., 2017; Kuhn et al., 2014; Liu et al., 2018). However, these studies have primarily been conducted in North America and Europe where the fortification of food staples is common. In Australia, where the food supply and vitamin D fortification levels are similar to New Zealand, studies have not identified dietary vitamin D intake as a significant contributor to the explained variance in 25(OH)D (Kimlin et al., 2014; Lucas et al., 2013). Assessment of skin colour/type in this study may have provided more specificity than ethnicity, particularly between Māori and Pacific groups. However, studies have indicated that a strong association between ethnicity and 25(OH)D remained after melanin content (Knight et al., 2017) and skin type (Chan et al., 2010) were accounted for. While no participants in the current study identified as Indian, Middle Eastern or African, Bolland et al. (2008) demonstrated that these ethnic groups in New Zealand have lower mean 25(OH)D than Māori or Pacific groups and are at risk of vitamin D deficiency. It is recommended a low threshold for vitamin D supplementation is applied to recruits identifying as Māori, Pacific, Indian, Middle Eastern or African.

Future research should confirm these findings in an ethnically diverse population of male recruits in the New Zealand Army to ensure recommendations are targeted appropriately. In addition, the feasibility of education, screening and supplementation during the recruiting phase is worth investigating. The impact of this early preventative approach could be assessed through the effect on vitamin D status and risk of musculoskeletal injuries during basic training. Establishing a consensus amongst clinicians and researchers for 25(OH)D sufficiency that promotes optimal musculoskeletal health, as well as general health, should be prioritised. This is particularly relevant in high-risk populations, such as military personnel with intense training periods and operational demands.

In conclusion, this study has demonstrated that 25(OH)D declined significantly for female recruits that commenced basic training in summer and increased significantly for those that commenced basic training in winter. Regardless of the season training commenced, two-thirds of participants overall had suboptimal vitamin D status at the end of 16-weeks of basic training. Seasonal UVB exposure was identified as a major determinant of 25(OH)D, and Pacific and Māori ethnic groups are particularly at risk of suboptimal vitamin D status. It is recommended that health practitioners working with recruits focus on preventing suboptimal vitamin D status. This can be achieved through education, screening and supplementation, before or at the commencement of basic training.

5.6 References

Abbott, A., Wang, C., Stamm, M., & Mulcahey, M. K. (2022). Part II: Risk factors for stress fractures in female military recruits. *Military Medicine*. https://doi.org/10.1093/milmed/usac033

Alexandridou, A., Schorr, P., Stokes, C. S., & Volmer, D. A. (2021). Analysis of vitamin D metabolic markers by mass spectrometry: recent progress regarding the “gold standard” method and integration into clinical practice. *Mass Spectrometry Reviews*. https://doi.org/10.1002/mas.21768

Andersen, N. E., Karl, J. P., Cable, S. J., Williams, K. W., Rood, J. C., Young, A. J., Lieberman, H. R., & McClung, J. P. (2010). Vitamin D status in female military personnel during combat training. *Journal of the International Society of Sports Nutrition*, *7*, 38. https://doi.org/10.1186/1550-2783-7-38

Bertrand, K. A., Giovannucci, E., Liu, Y., Malspeis, S., Eliassen, A. H., Wu, K., Holmes, M. D., Laden, F., & Feskanich, D. (2012). Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three US cohorts. *British Journal of Nutrition*, *108*(10), 1889-1896. https://doi.org/10.1017/s0007114511007409

Bishop, M. E., Ahlmen, A., Rosendorf, J., Erickson, B. J., & Cohen, S. (2020). Bone stress injuries in female athletes. *Annals of Joint*, *6*. https://aoj.amegroups.com/article/view/5928

Black, L. J., Anderson, D., Clarke, M. W., Ponsonby, A. L., & Lucas, R. M. (2015). Analytical bias in the measurement of serum 25-hydroxyvitamin D concentrations impairs assessment of vitamin D status in clinical and research settings. *PloS One*, *10*(8), e0135478. https://doi.org/10.1371/journal.pone.0135478

Bolland, M. J., Chiu, W. W., Davidson, J. S., Grey, A., Bacon, C., Gamble, G. D., & Reid, I. R. (2008). The effects of seasonal variation of 25-hydroxyvitamin D on diagnosis of vitamin D insufficiency. *New Zealand Medical Journal*, *121*(1286), 63-74.

Boven, N., Exeter, D., Sporle, A., & Shackleton, N. (2020). The implications of different ethnicity categorisation methods for understanding outcomes and developing policy in New Zealand. *Kōtuitui: New Zealand Journal of Social Sciences Online*, *15*(1), 123-139. https://doi.org/10.1080/1177083X.2019.1657912

Burgi, A. A., Gorham, E. D., Garland, C. F., Mohr, S. B., Garland, F. C., Zeng, K., Thompson, K., & Lappe, J. M. (2011). High serum 25-hydroxyvitamin D is associated with a low incidence of stress fractures. *Journal of Bone and Mineral Research*, *26*(10), 2371-2377. https://doi.org/10.1002/jbmr.451

Carswell, A. T., Oliver, S. J., Wentz, L. M., Kashi, D. S., Roberts, R., Tang, J. C. Y., Izard, R. M., Jackson, S., Allan, D., Rhodes, L. E., Fraser, W. D., Greeves, J. P., & Walsh, N. P. (2018). Influence of vitamin D supplementation by sunlight or oral D3 on exercise performance. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/mss.0000000000001721

Chan, J., Jaceldo-Siegl, K., & Fraser, G. E. (2010). Determinants of serum 25 hydroxyvitamin D levels in a nationwide cohort of blacks and non-Hispanic whites. *Cancer Causes and Control*, *21*(4), 501-511. https://doi.org/10.1007/s10552-009-9481-1

Daher, R., Manceau, H., & Karim, Z. (2017). Iron metabolism and the role of the iron-regulating hormone hepcidin in health and disease. *Presse Medicale*, *46*(12 Pt 2), e272-e278. https://doi.org/10.1016/j.lpm.2017.10.006

Davey, T., Lanham-New, S. A., Shaw, A. M., Hale, B., Cobley, R., Berry, J. L., Roch, M., Allsopp, A. J., & Fallowfield, J. L. (2016). Low serum 25-hydroxyvitamin D is associated with increased risk of stress fracture during Royal Marine recruit training. *Osteoporosis International*, *27*(1), 171-179. https://doi.org/10.1007/s00198-015-3228-5

DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. *American Journal of Clinical Nutrition*, *80*(6 Suppl), 1689s-1696s. https://doi.org/10.1093/ajcn/80.6.1689S

Friedl, K. E., Evans, R. K., & Moran, D. S. (2008). Stress fracture and military medical readiness: bridging basic and applied research. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S609-622. https://doi.org/10.1249/MSS.0b013e3181892d53

Gaffney-Stomberg, E., Nakayama, A. T., Guerriere, K. I., Lutz, L. J., Walker, L. A., Staab, J. S., Scott, J. M., Gasier, H. G., & McClung, J. P. (2019). Calcium and vitamin D supplementation and bone health in Marine recruits: effect of season. *Bone*, *123*, 224-233. https://doi.org/10.1016/j.bone.2019.03.021

Harrison, S. E., Oliver, S. J., Kashi, D. S., Carswell, A. T., Edwards, J. P., Wentz, L. M., Roberts, R., Tang, J. C. Y., Izard, R. M., Jackson, S., Allan, D., Rhodes, L. E., Fraser, W. D., Greeves, J. P., & Walsh, N. P. (2021). Influence of vitamin D supplementation by simulated sunlight or oral D3 on respiratory infection during military training. *Medicine and Science in Sports and Exercise*, *53*(7), 1505-1516. https://doi.org/10.1249/mss.0000000000002604

Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine*, *357*(3), 266-281. https://doi.org/10.1056/NEJMra070553

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., & Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism*, *96*(7), 1911-1930. https://doi.org/10.1210/jc.2011-0385

Jones, B. H., Bovee, M. W., Harris, J. M., 3rd, & Cowan, D. N. (1993). Intrinsic risk factors for exercise-related injuries among male and female army trainees. *American Journal of Sports Medicine*, *21*(5), 705-710. https://doi.org/10.1177/036354659302100512

Kimlin, M. G., Lucas, R. M., Harrison, S. L., van der Mei, I., Armstrong, B. K., Whiteman, D. C., Kricker, A., Nowak, M., Brodie, A. M., & Sun, J. (2014). The contributions of solar ultraviolet radiation exposure and other determinants to serum 25-hydroxyvitamin D concentrations in Australian adults: the AusD Study. *American Journal of Epidemiology*, *179*(7), 864-874. https://doi.org/10.1093/aje/kwt446

Knapik, J., Montain, S. J., McGraw, S., Grier, T., Ely, M., & Jones, B. H. (2012). Stress fracture risk factors in basic combat training. *International Journal of Sports Medicine*, *33*(11), 940-946. https://doi.org/10.1055/s-0032-1311583

Knight, J. A., Wong, J., Cole, D. E. C., Lee, T. K., & Parra, E. J. (2017). Predictors of 25-hydroxyvitamin D concentration measured at multiple time points in a multiethnic population. *American Journal of Epidemiology*, *186*(10), 1180-1193. https://doi.org/10.1093/aje/kwx180

Kuhn, T., Kaaks, R., Teucher, B., Hirche, F., Dierkes, J., Weikert, C., Katzke, V., Boeing, H., Stangl, G. I., & Buijsse, B. (2014). Dietary, lifestyle, and genetic determinants of vitamin D status: a cross-sectional analysis from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany study. *European Journal of Nutrition*, *53*(3), 731-741. https://doi.org/10.1007/s00394-013-0577-8

Laaksi, I., Ruohola, J. P., Tuohimaa, P., Auvinen, A., Haataja, R., Pihlajamaki, H., & Ylikomi, T. (2007). An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. *American Journal of Clinical Nutrition*, *86*(3), 714-717. https://doi.org/10.1093/ajcn/86.3.714

Liu, E., Meigs, J. B., Pittas, A. G., Economos, C. D., McKeown, N. M., Booth, S. L., & Jacques, P. F. (2010). Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. *American Journal of Clinical Nutrition*, *91*(6), 1627-1633. https://doi.org/10.3945/ajcn.2009.28441

Liu, X., Baylin, A., & Levy, P. D. (2018). Vitamin D deficiency and insufficiency among US adults: prevalence, predictors and clinical implications. *British Journal of Nutrition*, *119*(8), 928-936. https://doi.org/10.1017/s0007114518000491

Livesey, J., Elder, P., Ellis, M. J., McKenzie, R., Liley, B., & Florkowski, C. (2007). Seasonal variation in vitamin D levels in the Canterbury, New Zealand population in relation to available UV radiation. *New Zealand Medical Journal*, *120*(1262), U2733.

Lucas, R. M., Ponsonby, A. L., Dear, K., Valery, P. C., Taylor, B., van der Mei, I., McMichael, A. J., Pender, M. P., Chapman, C., Coulthard, A., Kilpatrick, T. J., Stankovich, J., Williams, D., & Dwyer, T. (2013). Vitamin D status: multifactorial contribution of environment, genes and other factors in healthy Australian adults across a latitude gradient. *Journal of Steroid Biochemistry and Molecular Biology*, *136*, 300-308. https://doi.org/10.1016/j.jsbmb.2013.01.011

Lutz, L. J., Gaffney-Stomberg, E., Karl, J. P., Hughes, J. M., Guerriere, K. I., & McClung, J. P. (2018). Dietary intake in relation to military dietary reference values during army basic combat training; a multi-center, cross-sectional study. *Military Medicine*. https://doi.org/10.1093/milmed/usy153

Lutz, L. J., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Vitamin D status, dietary intake, and bone turnover in female soldiers during military training: a longitudinal study. *Journal of the International Society of Sports Nutrition*, *9*(1), 38. https://doi.org/10.1186/1550-2783-9-38

Millen, A. E., Wactawski-Wende, J., Pettinger, M., Mclamed, M. L., Tylavsky F. A., Liu S., Robbins J., LaCroix A. Z., LeBoff M. S., Jackson R. D. (2010). Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: the Women's Health Initiative calcium plus vitamin D clinical trial. *American Journal of Clinical Nutrition*, *91*(5), 1324-1355. https://doi.org/10.3945/ajcn.2009.28908

Ministry of Health. (2012). *Vitamin D status of New Zealand adults: findings from the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health

Ministry of Health. (2017). *HISO 10001:2017 Ethnicity data protocols.* Wellington: Ministry of Health.

Moran, D. S., Israeli, E., Evans, R. K., Yanovich, R., Constantini, N., Shabshin, N., Merkel, D., Luria, O., Erlich, T., Laor, A., & Finestone, A. (2008). Prediction model for stress fracture in young female recruits during basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S636-644. https://doi.org/10.1249/MSS.0b013e3181893164

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K., & Ganz, T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *Journal of Clinical Investigation*, *113*(9), 1271-1276. https://doi.org/10.1172/jci20945

Nessvi, S., Johansson, L., Jopson, J., Stewart, A., Reeder, A., McKenzie, R., & Scragg, R. K. (2011). Association of 25-hydroxyvitamin D3 levels in adult New Zealanders with ethnicity, skin color and self-reported skin sensitivity to sun exposure. *Photochemistry and Photobiology*, *87*(5), 1173-1178. https://doi.org/10.1111/j.1751-1097.2011.00956.x

New Zealand Defence Force. (19 November 2020). *Defence Force Order 3: New Zealand Defence Force Human Resource Manual: Version 17/12 – AL 16*. Wellington, New Zealand.

Richards, T., & Wright, C. (2018). British Army recruits with low serum vitamin D take longer to recover from stress fractures. *Journal of the Royal Army Medical Corps*. https://doi.org/10.1136/jramc-2018-000983

Ruohola, J. P., Laaksi, I., Ylikomi, T., Haataja, R., Mattila, V. M., Sahi, T., Tuohimaa, P., & Pihlajamaki, H. (2006). Association between serum 25(OH)D concentrations and bone stress fractures in Finnish young men. *Journal of Bone and Mineral Research*, *21*(9), 1483-1488. https://doi.org/10.1359/jbmr.060607

Shoemaker, M. E., Salmon, O. F., Smith, C. M., Duarte-Gardea, M. O., & Cramer, J. T. (2022). Influences of vitamin D and iron status on skeletal muscle health: a narrative review. *Nutrients*, *14*(13). https://doi.org/10.3390/nu14132717

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. (2000). Decreased bioavailability of vitamin D in obesity. *American Journal of Clinical Nutrition*, *72*(3), 690-693. https://doi.org/10.1093/ajcn/72.3.690

Chapter 6 Longitudinal changes in body composition, physical fitness and dietary intake in female recruits during 16-weeks of basic training in the New Zealand Army

Body composition, physical fitness and dietary intake are three inter-related health determinants. Improvements in each of these factors has the potential to substantially enhance functional outcomes in female recruits. Therefore, establishing a situational awareness of changes in these three factors can inform positive developments in the delivery of physical training and nutrition services if required. The aim of this study was to describe changes in body composition, physical fitness and dietary intake during basic training and associations between these three factors in female recruits in the New Zealand Army.

6.1 Abstract

Background: Basic training is a demanding course that is generally a sudden change from an individual's habitual lifestyle. Positive changes in dietary intake, physical fitness and body composition are associated with improvements in health, physical performance and musculoskeletal injury risk in military personnel.

Objective: The purpose of this study was to describe changes in body composition, physical fitness and dietary intake during basic training and associations between these three factors in female recruits in the New Zealand Army.

Methods: Body composition (*n*=88) and dietary intake (*n*=83) were both determined at weeks 1 and 16, while physical fitness tests (2.4km run, maximum push-ups and maximum curl-ups) (*n*=82) were conducted during weeks 1 and 8. Changes in the three factors during basic training were investigated using paired *t*-tests. Associations between percent body fat (%BF) and physical fitness were evaluated using Pearson's correlation coefficients.

Results: The mean ± SD change during basic training was -3.8 ± 3.6kg for fat mass, -5.5 ± 3.7% for %BF and 3.8 ± 1.8kg for fat free mass (FFM) (all $P<0.001$), but no change in body mass or body mass index (BMI) was observed. All measures of physical fitness improved during basic training ($P<0.001$). A higher %BF at week 1 was positively associated with the 2.4km run time and negatively associated with push-ups at both weeks 1 and 8 (all $P<0.05$). There was a significant increase in the frequency intake of protein, grains, fats, discretionary items and beverages (all $P<0.001$).

Conclusion: The New Zealand Army 16-week basic training provides adequate nutrition to support training-induced adaptations in physical fitness and body composition of most female recruits. Optimal %BF and FFM are associated with improved physical fitness, while BMI is an unreliable measure of body composition in this cohort of physically active young women.

6.2 Introduction

Basic training in the New Zealand Army is a rigorous course designed to prepare recruits for the demands of service as a soldier. Physical training and military tasks completed span the spectrum of fitness domains, including aerobic, power, muscular endurance and muscular strength (Vaara et al., 2022). Optimal body composition, described as healthy percent body fat (%BF) and high fat free mass (FFM) (Foulis et al., 2021) has been associated with several military relevant outcomes, such as improved physical performance (Pierce et al., 2017), reduced musculoskeletal injuries (Jones et al., 2017) and fewer premature discharges from military service (Knapik et al., 2001). As a result of physiological sex differences, females typically have lower aerobic fitness (Nindl et al., 2016) and increased risk of musculoskeletal injury (Knapik et al., 2001) than males. It has been suggested that improvements in physical fitness and body composition can mitigate the negative impact of these sex differences (Nindl et al., 2016).

Previous studies have investigated changes in body composition, physical fitness and dietary intake of female recruits during basic training. Positive changes in body composition, with significant reductions in %BF and increases in FFM have been observed (Etzion-Daniel et al., 2008; Foulis et al., 2021; Margolis et al., 2012; Nindl et al., 2011). These changes have negated any shift in body mass (BM) and therefore, body mass index (BMI), suggesting BMI may be an imprecise measure for body composition in military personnel. Improvements in physical fitness have consistently been observed, as measured by faster 2.4km run times (Blacker et al., 2009; Richmond et al., 2012) and a greater number of push-ups and sit-ups (Bell et al., 2000; Knapik et al., 2005; Knapik et al., 2003; Yanovich et al., 2008). Studies investigating changes in dietary intake have reported energy intake is not increased to meet higher energy demands recommended by the United States Military Dietary Reference Intakes (MDRIs) (Headquarters Departments of the Army et al., 2017). This has likely contributed to recommended intakes of nutrients not being met, particularly for carbohydrate, protein, iron, vitamin D and calcium

(Etzion-Daniel et al., 2008; Herzman-Harari et al., 2013; Margolis et al., 2012). To the best of the author's knowledge, no studies have explored the changes in body composition, physical fitness and dietary intake concurrently in female recruits over the period of basic training and the associations between them.

The highly structured nature of basic training with the provision of appropriate nutrition alongside restricted access to ultra-processed food and alcohol creates an environment capable of inducing positive changes in body composition and physical fitness. The objective of this study is therefore to describe the changes in body composition, physical fitness and dietary intake from the beginning to end of basic training and investigate any associations between these three factors in female recruits in the New Zealand Army.

6.3 Methods

All female recruits (*n*=108) who commenced basic training in the New Zealand Army from February 2014 to March 2016, over nine different intakes, were eligible and invited to participate in this longitudinal study. Following a briefing session during week 1 of basic training where the study conduct was explained verbally and in writing, 106 female recruits voluntarily provided informed and written consent. The sample size was determined by the feasibility of recruitment. This study was approved by the Massey University Human Ethics Committee: Southern A (Application 13/85) and researchers adhered to Defence Force Order 3 (New Zealand Defence Force, 2020) regarding the conduct and approval of personnel research. Body composition was measured during weeks 1 and 16, physical fitness tests were conducted during weeks 1 and 8 (as routinely scheduled in basic training) and frequency of food intake was self-reported during weeks 1 and 16.

All recruits in the New Zealand Army undertake basic training at Waiouru Military Camp, located in the central North Island, 792m above sea level. The gender-integrated residential course is 16-weeks. A typical training day can extend to 16-hours of scheduled activity. Training is dominated by physical military tasks, such as formation marching, tactical marching with load carriage, manual weapon and equipment handling, repetitive lifting and construction work. In addition, formal physical training sessions are conducted 3-4 times per week for approximately 1-hour. Physical training is progressive in nature and includes aerobic fitness, circuit training, strength and conditioning, obstacle courses and swimming. All meals and snacks are provided and recruits are not permitted to consume any dietary supplements unless prescribed by a medical doctor. Access to other food is not available. Within the garrison environment, three main meals are provided that include those self-selected from the military dining hall and bag lunches on some days. A daily recruit snack pack is provided consisting of 3-4 food items for in-between meals, such as muesli bars, beef jerky, cheese and crackers, fruit and yoghurt. During field exercises, recruits are provided operational ration packs with a wide range of pre-packaged foods, including meat-based meals, muesli, canned cheese, dried fruit and chocolate. A large variety of beverages are provided daily, including water, fruit juice, cordial and hot beverages. All military dining hall menus adhere to the New Zealand Defence Force Catering Nutrition Standards.

During week 1, recruits completed a questionnaire online via SurveyMonkey (Momentive Inc., San Mateo, United States) to determine demographics, such as age and ethnicity. Recruits self-reported the ethnic groups they identified with. If multiple ethnic groups were selected, responses were prioritised into a single ethnicity in the following order: Māori, Pacific and New Zealand European (Ministry of Health, 2017).

Body composition measures were determined midweek of weeks 1 and 16 by a trained researcher between 0600-0730hrs, using identical methodology at both time points. Participants fasted overnight, performed no physical activity for at least 8-hours prior, urinated prior to testing, wore light clothing and removed all jewellery. Height was measured in week 1 using a SECA 213 portable stadiometer (German Healthcare Export Group, Bonn, Germany). Bioelectrical impedance analysis (BIA) was performed using the InBody$_{230}$ (Biospace Co. Ltd., Seoul, Korea), a vertical, eight-point model with both footpads and handles containing two electrodes. Participants were instructed to stand upright, positioning their bare feet on the footpads and hands on the handles, which were slightly abducted. The BIA first measured BM. Following data input of height, sex and age, a small electrical current sent through the body measured resistance to the current's flow. The InBody$_{230}$ takes 10 impedance measurements with two frequencies (20 and 100kHz). Based on the manufacturer's algorithms, in-built software estimated skeletal muscle mass (SMM), FM, total body water (TBW) and %BF. Fat free mass was calculated as BM minus FM. The same BIA unit was used for all measures. Body mass index was calculated as BM (kg)/height (m)^2. There is currently no consensus on optimal %BF within the military. In the United States Army, personnel who exceed weight for height standards can undergo further screening to estimate %BF, based on body circumference measures. The maximum allowable %BF for females aged 17-20-years is 30% (U.S. Department of Defense, 2022).

The base physical fitness test of the New Zealand Army includes a timed 2.4km run, followed by maximum continuous push-ups and curl-ups. The test was conducted at weeks 1 and 8. The run, as a measure of aerobic fitness, was conducted on a sealed, flat road. Push-ups, as a measure of upper body muscular endurance were performed on a solid gymnasium floor. Curl-ups, as a measure of core muscular endurance, were performed on an exercise mat on a gymnasium floor. The protocol for the run and push-ups is described by Edgar et al. (2020). The curl-up protocol is adapted from the partial sit-up test with 8cm wide tape, used by the American College of Sports Medicine (Heyward 2002). The minimum pass for females aged 16-24-years is 740 seconds for the run, 14 push-ups, and 50 curl-ups. The tests were performed as part of the standard basic training programme. Maximum individual effort was assessed for each component by a New Zealand Army physical training instructor and results were provided to the research team.

A non-quantitative validated food frequency questionnaire (FFQ) (Beck et al., 2012) with 143 food items was administered via SurveyMonkey at weeks 1 and 16. A registered dietitian was present during completion of the FFQ to answer any relevant questions. Frequency intake for each food item 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 (e.g., 1-3 times per month was converted to 0.5 times per week and 2-3 times per day was converted to 17.5 times per week). The FFQ administered during weeks 1 and 16 determined intake frequency during the 4-weeks before basic training commenced and intake during 4-weeks of basic training (weeks 12-15), respectively. The total food items were categorised into food variables, under nine food categories, as described in Appendix C in a supplementary table.

Statistical analysis was 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 values ± standard deviation (SD), median values (25th, 75th percentile) or mean (95% confidence intervals). Changes in body composition, physical fitness and frequency of food intake were investigated using paired *t*-tests. Independent *t*-tests investigated any difference in body composition and physical fitness at week 1 between those participants that finished basic training and participants that did not. Independent *t*-tests also explored differences in body composition and physical fitness for participants with %BF ≤30 or >30 at week 1, based on the standard in the United States Army (U.S. Department of Defense, 2022). Pearson's correlation coefficients were used to investigate associations between %BF at week 1 and physical fitness at weeks 1 and 8, and %BF at week 1 and change in %BF during basic training. The rho-Spearman's correlation investigated associations between total weekly intake frequency of food categories and %BF at weeks 1 and 16. A *P* value less than 0.05 was considered statistically significant.

6.4 Results

A total of 106 female recruits volunteered to participate in this study from the 108 that commenced basic training during this study period. A total of 21 participants did not complete their initial basic training; however, there was varying completion of data collection. Full body composition data were available for 88 participants. Physical fitness analysis excluded 14 female recruits exempt from completing the fitness test in week 1 due to an acute injury and a further 10 who were withdrawn from basic training prior to the week 8 fitness test (*n*=82). Dietary intake analysis excluded the 21 participants that did not complete basic training and a further two participants with FFQ data with implausible intakes (*n*=83). There were no significant differences in body composition or physical fitness at week 1 between the 85 participants that finished basic training and the 21 participants that did not. For the 88 recruits, the median age (25th, 75th percentile) was 18 (18, 20) years, with 59.1% New Zealand European, 29.5% Māori and 11.4% Pacific.

Table 6.1 presents changes in body composition measures from week 1 to week 16 of basic training. At week 1, six participants had a BMI between 18.5-19.9kg/m^2 and one participant had a BMI ≥30kg/m^2. At week 16, all participants had a BMI between 20.0-29.9kg/m^2. There was no change in BM for the total cohort. The mean change in %BF was -5.5 ± 3.7%, FM was -3.8 ± 3.6kg and FFM was 3.8 ± 1.8kg (all *P*<0.001). Percent body fat was reduced in 93% of participants. There was a positive association between initial %BF and change in %BF during basic training (*r*=0.717, *P*<0.001). At week 1, 26 participants had a %BF >30, which was reduced to one participant at week 16 (30.8%). The mean change in %BF from week 1 to week 16 for participants with a %BF at week 1 ≤30 (*n*=62) was 24.0 ± 3.6 to 19.9 ± 3.1, -4.1% (*P*<0.001) and >30 (*n*=26) was 33.4 ± 2.2 to 24.7 ± 3.0, -8.7% (*P*<0.001).

Table 6.1. Change in body composition of female recruits during basic training (*n*=88)

Measures	Week 1		Week 16		*P* value[a]
	Mean ± SD	Range	Mean ± SD	Range	
Height (cm)	165.2 ± 5.5	153.0 – 183.0			
BM (kg)	65.5 ± 9.3	48.7 – 85.3	65.5 ± 7.2	47.9 – 88.7	0.977
BMI (kg/m^2)	24.0 ± 2.8	18.9 – 31.3	24.0 ± 1.9	20.2 – 29.4	0.984
FM (kg)	17.9 ± 5.5	7.9 – 31.3	14.1 ± 3.4	6.9 – 23.4	<0.001
FFM (kg)	47.6 ± 5.3	38.7 – 63.2	51.5 ± 5.1	40.2 – 68.9	<0.001
SMM (kg)	26.5 ± 3.2	20.9 – 35.9	29.0 ± 3.1	22.2 – 39.2	<0.001
TBW (kg)	34.9 ± 3.8	28.3 – 46.2	37.7 ± 3.7	29.5 – 50.3	<0.001
%BF	26.8 ± 5.4	15.9 – 37.5	21.3 ± 3.8	12.6 – 30.8	<0.001

SD, standard deviation; BM, body mass; BMI, body mass index; FM, fat mass; FFM, fat free mass; SMM, skeletal muscle mass; TBW, total body water; %BF, percent body fat.

[a] *P* value determined using paired *t*-tests.

Table 6.2 presents the results of the physical fitness tests conducted at weeks 1 and 8. For the 80 participants with combined body composition and physical fitness data, %BF at week 1 was positively associated with the 2.4km run time at week 1 (*r*=0.463, *P*<0.001) and week 8 (*r*=0.286, *P*=0.010) and negatively associated with the maximum continuous number of push-ups at week 1 (*r*=-0.255, *P*=0.022) and week 8 (*r*=-0.372, *P*<0.001). There was no significant association between %BF at week 1 and curl-ups performed at either time point. Participants with a %BF at week 1 ≤30 (*n*=59) had significantly faster 2.4km run times than participants with a %BF >30 (*n*=21) at week 1 (711 ± 54 v 769 ± 49 seconds, *P*<0.001) and week 8 (665 ± 48 v 694 ± 32 seconds, *P*=0.012). The mean improvement in the 2.4km run time was 46 ± 37 seconds for participants with %BF ≤30 and 76 ± 45 seconds for participants with %BF >30 (*P*=0.005).

Table 6.2. Change in physical fitness of female recruits during basic training (*n*=82)

Physical Fitness Tests	Week 1[a]	Week 8[a]	*P* value[b]
2.4km run (seconds)	726 ± 59	672 ± 46	<0.001
Push-ups[c]	14 ± 8	24 ± 8	<0.001
Curl-ups[c,d]	98 ± 26	115 ± 12	<0.001

[a] Mean ± standard deviation.

[b] *P* value determined using paired *t*-tests.

[c] Maximum continuous number.

[d] Maximum score recorded is 115.

The weekly intake frequency of food variables during the 4-weeks before commencing basic training and weeks 12-15 of basic training are displayed in Table 6.3. The greatest increases in frequency of intake were for ‘potato and kūmara’, ‘beef’, ‘other unprocessed meat’, ‘nuts and seeds’, ‘soy and legumes’, ‘wholegrain bread’, ‘rice’, ‘porridge’, ‘muesli’, ‘butter and margarine’, ‘sweet biscuits’, ‘muesli bars’, ‘ice cream’, ‘added sugar and syrups’ and ‘other beverages’. The greatest decreases in frequency of intake were for ‘other fruits’, ‘fish and shellfish’, ‘cream’, ‘potato crisps’, ‘chocolate’ and ‘alcohol’. No participants reported following a diet for cultural, religious or health reasons. There were no significant associations between the nine food categories (vegetables, fruit, protein, grains, dairy, fats, discretionary items, beverages and alcohol) and %BF at either week 1 or week 16.

Table 6.3. Change in weekly intake frequency of food variables in female recruits during basic training (*n*=83)

Food variables	**Weekly intake frequency**[a]		**Mean change**	***P* value**[d]
	Before basic training[b]	**During basic training**[c]		
Vegetables				
Potato and kūmara[e]	4.63 (3.92, 5.34)	8.30 (6.80, 9.80)	3.67	<0.001
Green leafy vegetables	5.95 (4.86, 7.04)	4.11 (2.94, 5.28)	-1.84	0.006
Other vegetables	24.52 (21.22, 27.82)	26.99 (22.99, 30.99)	2.47	0.262
Sub-total	*35.10 (30.61, 39.60)*	*39.40 (34.20, 44.61)*	*4.30*	*0.154*
Fruits				
Apples	3.28 (2.50, 4.06)	4.47 (3.28, 5.66)	1.19	0.016
Bananas	3.26 (2.34, 4.18)	3.73 (2.64, 4.81)	0.47	0.424
Citrus fruits	2.81 (1.83, 3.79)	3.16 (2.41, 3.92)	0.35	0.547
Other fruits	11.77 (8.57, 14.96)	3.63 (2.46, 4.79)	-8.14	<0.001
Canned and dried fruits	1.67 (0.85, 2.50)	2.14 (1.61, 2.68)	0.47	0.34
Sub-total	*22.79 (17.85, 27.72)*	*17.13 (13.90, 20.35)*	*-5.66*	*0.03*
Protein				
Beef	2.26 (1.96, 2.57)	3.65 (2.93, 4.37)	1.39	<0.001
Chicken	2.76 (2.16, 3.36)	3.72 (3.05, 4.39)	0.96	0.03
Other unprocessed meat	1.93 (1.52, 2.34)	4.07 (3.25, 4.89)	2.14	<0.001
Processed meat	3.11 (2.63, 3.60)	3.41 (2.87, 3.95)	0.30	0.351
Fish and shellfish	2.17 (1.61, 2.72)	0.94 (0.68, 1.20)	-1.23	<0.001
Eggs	2.57 (2.02, 3.12)	2.73 (2.19, 3.28)	0.16	0.627
Nuts and seeds	3.97 (2.87, 5.07)	7.43 (5.74, 9.11)	3.46	<0.001
Soy and legumes	1.84 (1.35, 2.34)	3.88 (2.92, 4.84)	2.03	<0.001
Sub-total	*20.62 (18.39, 22.85)*	*29.83 (26.62, 33.04)*	*9.21*	*<0.001*

Food variables	Weekly intake frequency[a]		Mean change	*P* value[d]
	Before basic training[b]	During basic training[c]		
Grains				
White bread	3.81 (2.73, 4.89)	3.12 (1.89, 4.34)	-0.69	0.364
Wholegrain bread	2.78 (2.17, 3.39)	8.75 (7.19, 10.31)	5.97	<0.001
Rice	1.83 (1.49, 2.17)	4.84 (3.77, 5.91)	3.01	<0.001
Pasta and noodles	2.22 (1.67, 2.76)	2.42 (1.75, 3.08)	0.20	0.623
Other grains	0.23 (0.12, 0.34)	0.09 (0.03, 0.14)	-0.15	0.018
Porridge	0.98 (0.64, 1.32)	3.29 (2.57, 4.02)	2.31	<0.001
Muesli	1.29 (0.89, 1.69)	2.98 (2.37, 3.58)	1.69	<0.001
Other breakfast cereals	4.36 (3.31, 5.40)	3.99 (3.06, 4.92)	-0.36	0.493
Sub-total	*17.50 (15.21, 19.78)*	*29.47 (26.62, 32.32)*	*11.98*	*<0.001*
Dairy				
Milk	16.23 (13.29, 19.16)	19.09 (16.03, 22.15)	2.86	0.096
Cheese and yoghurt	5.09 (4.05, 6.13)	4.93 (4.02, 5.85)	-0.16	0.764
Milk alternatives	0.50 (0.19, 0.81)	0.05 (0.02, 0.08)	-0.45	0.005
Sub-total	*21.81 (18.35, 25.28)*	*24.07 (20.61, 27.53)*	*2.26*	*0.246*
Fats				
Butter or margarine	5.07 (3.85, 6.29)	11.07 (9.29, 12.86)	6.00	<0.001
Cream	0.62 (0.42, 0.82)	0.22 (0.08, 0.35)	-0.40	<0.001
Cooking oil	3.46 (2.60, 4.32)	3.48 (2.05, 4.91)	0.02	0.976
Sub-total	*9.15 (7.29, 11.01)*	*14.77 (12.53, 17.01)*	*5.62*	*<0.001*
Discretionary items				
Muffins and cakes	1.72 (1.00, 2.45)	2.83 (2.17, 3.48)	1.10	0.014
Sweet biscuits	1.88 (1.42, 2.34)	3.59 (3.05, 4.12)	1.70	<0.001
Crackers	2.26 (1.62, 2.90)	1.15 (0.77, 1.52)	-1.11	0.003
Muesli bars	1.28 (0.78, 1.78)	4.50 (3.79, 5.21)	3.22	<0.001
Potato crisps	1.56 (1.03, 2.09)	0.07 (0.03, 0.10)	-1.49	<0.001
Chocolate	2.15 (1.45, 2.84)	0.60 (0.35, 0.85)	-1.55	<0.001
Ice cream	1.02 (0.74, 1.29)	3.80 (3.24, 4.35)	2.78	<0.001
Meat pies	0.41 (0.27, 0.55)	0.20 (0.15, 0.25)	-0.21	0.006
Beef jerky	0.12 (0.05, 0.19)	1.58 (0.90, 2.26)	1.46	<0.001
Added sugar and syrups	6.91 (5.13, 8.68)	14.90 (12.05, 17.74)	7.99	<0.001
Sub-total	*19.30 (15.92, 22.67)*	*33.20 (29.40, 37.01)*	*13.90*	*<0.001*
Beverages				
Coffee	2.75 (1.61, 3.90)	3.83 (2.56, 5.10)	1.08	0.076
Tea	3.85 (2.29, 5.41)	1.39 (0.53, 2.25)	-2.46	0.005
Other beverages	6.99 (5.12, 8.86)	18.54 (15.80, 21.28)	11.55	<0.001
Fortified beverages	1.22 (0.47, 1.97)	0.12 (-0.01, 0.24)	-1.10	0.005
Sub-total	*14.81 (11.73, 17.89)*	*23.88 (20.51, 27.25)*	*9.07*	*<0.001*
Alcohol				
Alcohol	1.72 (1.31, 2.13)	0.41 (0.31, 0.52)	-1.31	<0.001
Sub-total	*1.72 (1.31, 2.13)*	*0.41 (0.31, 0.52)*	*-1.31*	*<0.001*
Total				
Total	162.80 (147.80, 177.79)	212.17 (196.22, 228.11)	49.37	<0.001

[a] Mean (95% confidence interval).

[b] Covers the 4-weeks before basic training commenced.

[c] Covers the final 4-weeks of basic training.

[d] *P* value determined using paired *t*-tests.

[e] Also known as sweet potato.

6.5 Discussion

Across the 16-week duration of basic training, FM and %BF significantly decreased and FFM significantly increased in female recruits in the New Zealand Army. During the first 8-weeks of basic training, all measures of physical fitness improved significantly. The frequency of food intake increased significantly during basic training, primarily due to increases in protein, grains, fats, discretionary items and beverages. Secondary findings were that a higher %BF at week 1 was significantly associated with impaired running and push-up performance and this association remained at week 8. There was no association between %BF and curl-ups.

While there was no change in BM, and therefore BMI in this study, FM decreased and FFM increased significantly. These findings are consistent with previous studies in female recruits following at least 8-weeks of basic training (Etzion-Daniel et al., 2008; Foulis et al., 2021; Margolis et al., 2012; Nindl et al., 2011) and highlight the caution required when interpreting BM and BMI data in this population. For 18-24-year-old females in a representative sample of the New Zealand population the mean BM was 75.1kg and the mean BMI was 27.5kg/m^2 (Ministry of Health, 2021). In a cohort of post-menarche, premenopausal New Zealand females (*n*=378) aged 16-45-years, the mean BMI was 27.1kg/m^2 and the mean %BF was 34.0% (Jayasinghe et al., 2019). These findings suggest that female recruits at the commencement of basic training are leaner than their civilian counterparts. Only one participant had a BMI ≥30 kg/m^2 at week 1, in line with the New Zealand Defence Force recruiting standard for a BMI of 18-30kg/m^2, with a BMI up to 33kg/m^2 eligible following additional assessment (New Zealand Defence Force, 2022).

In this study, %BF was reduced by 20.5% of initial %BF following 16-weeks of basic training. At an average of 1.3% per week this reduction in %BF is comparable to previous studies (1.1-1.8% per week) of female recruits undertaking basic training in the United States Marine Corps (Lieberman et al., 2008), United States Army (Foulis et al., 2021; Margolis et al., 2012; Proctor et al., 2018) and British Army (Blacker et al., 2009). Conversely, Richmond et al. (2012) reported no significant change in %BF during 14-weeks of basic training for female British Army recruits. The current finding of a positive association between initial %BF and change in %BF during basic training has been reported previously in a large cohort of female recruits in the United States Army (Foulis et al., 2021).

Participants with an initial %BF >30 had significantly lower aerobic fitness at both week 1 and week 8, compared to participants with a %BF ≤30; however, their run time improved on average, 9.3% compared to 6.7%, respectively. This supports the suggestion by Foulis et al. (2021), that females commencing basic training with a higher %BF are less physically fit and therefore experience greater training-induced fat loss. This is likely due to working at a relatively higher percentage of their maximal capacity, to achieve the same physical training and military task outputs as fitter recruits. In the total cohort, 98% increased their FFM, suggesting the 16-week programme was effective in stimulating an anabolic response in most female recruits. This may be due to the final 8-weeks of basic training placing greater emphasis on physical military tasks, which require explosive power, extensive lifting and prolonged load carriage. A further explanation for the improvements in body composition may be positive changes in dietary intake during basic training, where there is restricted access to energy-dense, nutrient-poor, ultra-processed food and alcohol.

Optimal dietary intake is required to maintain health and prevent injury in female recruits, whilst fuelling training, promoting recovery and enhancing training adaptations, such as gains in SMM (Thomas et al., 2016). There was no change in BM throughout basic training, suggesting energy balance was maintained, which has been observed previously in female recruits (Foulis et al., 2021; Margolis et al., 2012; Richmond et al., 2012). However, this doesn’t eliminate the risk of low energy availability, which may lead to impaired physiological functioning and performance (Mountjoy et al., 2018). Adequate energy and protein intake are required to facilitate skeletal muscle adaptations to physical training. Carbohydrate has a proven role as a key fuel substrate to support muscular work across a range of intensities and duration, with prolonged and/or intermittent activity (Thomas et al., 2016). The significant increases in frequency intake of protein, grains and starchy vegetables, coupled with the improvements in body composition and physical fitness suggests protein and carbohydrate requirements were likely to be met by most female recruits.

Positive findings regarding changes in habitual dietary intake during basic training include an increased frequency intake of unprocessed meat, with meat, fish and/or chicken served at each meal. In addition to supporting increased protein requirements, this suggests a corresponding increase in bioavailable iron intake, a critical nutrient for optimising physical performance (Beck et al., 2021). The mean frequency intake of vegetables and fruits was at least five and two times per day, respectively, both before and during basic training. If each eating occasion was an equivalent serving size, the recommendations for New Zealand adults have been achieved (Ministry of Health, 2020). While intake of 'potato and kūmara' were the only vegetables to increase significantly, intake frequency of non-starchy vegetables continued to average four times per day during basic training. A decrease in seasonal fruits that fall outside the standard training periods for basic training, such as stone fruit and berries contributed to the significant decrease in the intake of 'other fruits'. The significant increase in 'nuts and seeds', 'legumes', 'porridge', and 'muesli' all contribute positively towards increasing dietary fibre intake, essential for gut health, and in the case of 'nuts and seeds', useful sources of unsaturated fats also.

There was no significant change in dairy product intake observed in this cohort of female recruits. Milk and milk products are a key source of calcium, a critical nutrient for bone health and muscle function (Beck et al., 2021). The Recommended Dietary Intake (RDI) for Australian and New Zealand women aged 19-50-years and the MDRIs for calcium is 1000mg per day, and 1,300mg for women less than 19-years (Department of Health and Ageing and Ministry of Health, 2006; Headquarters

Departments of the Army et al., 2017). Calcium intake has been shown to be low in the diets of young New Zealand women (University of Otago and Ministry of Health, 2011). In this study, calcium requirements may have been achieved through the quantity of dairy consumed, with a mean frequency intake of three times per day, and/or the significant increase in 'grains' and 'non-alcoholic beverages', which contribute smaller amounts of calcium to the diet (University of Otago and Ministry of Health, 2011). The New Zealand Defence Force Catering Nutrition Standards consider the calcium requirements of recruits through the provision of a range of milk, including calcium-enriched plant-based milk at each meal, dairy products served daily as snack items, and milk-based dessert items, such as custard and ice-cream regularly available.

The significant increase in 'other beverages', which includes cocoa and chocolate-based beverages, cordial, and fruit and vegetable juice; and 'added sugar and syrups', which includes jam and honey is concerning. While the cordial option is typically artificially sweetened, there is undoubtedly an increase in free sugars consumed. While free sugars will contribute towards achieving the higher energy demands of female recruits, they negatively impact health. Free sugars in general are associated with dental caries and sugar-sweetened beverages may replace the intake of more nutrient dense wholefoods (World Health Organization, 2015). While the intake frequency of some discretionary items, such as potato crisps, chocolate and meat pies decreased significantly during basic training due to lack of availability; overall, there was an increase in high sugar discretionary items. This includes sweet biscuits and muesli bars, due to their inclusion as affordable, shelf-stable, packaged items in bag lunches, operational ration packs and recruit snack packs.

Food items available in the military dining hall during this study met the New Zealand Defence Force Catering Nutrition Standards; however, recruits' personal food choices ultimately determine dietary intake. In the context of quantity catering, with repetitive options over a 16-week period, the overall dietary behaviours appear positive with inclusion of a variety of nutritious foods. However, the absolute free sugar intake warrants further investigation.

While recruits require energy- and nutrient-dense foods during basic training to match their high energy expenditure, it is important that education is provided regarding nutrition requirements following basic training. Educating recruits to adjust their dietary intake, as their training volume reduces and access to ultra-processed food and alcohol increases, is required towards the end of basic training. The importance of this is highlighted by a report stating a reduction in %BF is not maintained during training immediately following basic training in the British Army (Scientific Advisory Committee on Nutrition, 2017).

The basic training setting is a strength of this study, due to the stable environmental and behavioural conditions over an extended duration. As 98% of female recruits, from intakes across 2.5-years volunteered for this study, the participants are representative of female recruits undertaking basic training in the New Zealand Army during the study period. The use of BIA for body composition assessment is a limitation due to its variable validity in comparison to a laboratory reference standard, such as dual energy x-ray absorptiometry (Sergi et al., 2017). However, the portability and speed of the InBody$_{230}$ analyser minimised the impact of data collection on real-time training. In addition, the InBody$_{230}$ analyser has demonstrated excellent reliability in repeat measurements (McLester et al., 2020; von Hurst et al., 2016) making it suitable for assessing changes in body composition in a research setting. Interpretation of dietary intake data from a non-quantitative FFQ is also limited and associations with body composition and physical fitness are difficult to identify. As recruits were not involved in food preparation or cooking during basic training, there may be underreporting of intake during this period. This particularly applies for items used in prepared meals, such as butter, oil, milk, cheese and vegetables. In addition, FFQs have several further limitations, including a reliance on memory of food intake over time, lack of information on meal combinations and being restricted to a defined list of foods (Bailey, 2021).

Future research should incorporate quantitative assessment of portions consumed to determine the intake of energy and nutrients, and further investigate associations with body composition and physical fitness. In addition, body composition and physical fitness should be assessed at weeks 1, 8 and 16 to explore changes in response to a shift in physical training from aerobic fitness to power, muscular endurance and muscular strength. While there were no significant differences in initial body composition or physical fitness between participants that finished basic training and those that did not in this study, 20% of female recruits were either discharged or back squadded, delaying their completion of basic training. This rate is high and warrants further investigation to minimise the personal and organisational impact of training delays and early discharge from military service.

In conclusion, our findings demonstrate the New Zealand Army 16-week basic training programme is effective in providing an anabolic environment. Adequate dietary intake supports training-induced adaptations in physical fitness and body composition in female recruits. Significant decreases in FM and %BF and increases in FFM were experienced alongside significant improvements in physical fitness measures in most female recruits, despite no change in BM. This highlights the limitations of using BMI data in this population of physically active females. It is recommended that further work is undertaken during the recruiting phase to encourage potential and selected recruits to enter basic training with a healthy body composition and strong physical fitness base.

6.6 References

Bailey, R. L. (2021). Overview of dietary assessment methods for measuring intakes of foods, beverages, and dietary supplements in research studies. *Current Opinion in Biotechnology*, *70*, 91-96. https://doi.org/10.1016/j.copbio.2021.02.007

Beck, K. L., Kruger, R., Conlon, C. A., Heath, A. L., Coad, J., Matthys, C., Jones, B., & Stonehouse, W. (2012). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women. *Journal of the Academy of Nutrition and Dietetics*, *112*(8), 1177-1187. https://doi.org/10.1016/j.jand.2012.05.012

Beck, K. L., von Hurst, P. R., O'Brien, W. J., & Badenhorst, C. E. (2021). Micronutrients and athletic performance: a review. *Food and Chemical Toxicology*, *158*, 112618. https://doi.org/10.1016/j.fct.2021.112618

Bell, N. S., Mangione, T. W., Hemenway, D., Amoroso, P. J., & Jones, B. H. (2000). High injury rates among female army trainees: a function of gender? *American Journal of Preventive Medicine*, *18*(3 Suppl), 141-146.

Blacker, S. D., Wilkinson, D. M., & Rayson, M. P. (2009). Gender differences in the physical demands of British Army recruit training. *Military Medicine*, *174*(8), 811-816. https://doi.org/10.7205/milmed-d-01-3708

Department of Health and Ageing and Ministry of Health. (2006). *Nutrient Reference Values for Australia and New Zealand: including Recommended Dietary Intakes*. Canberra: Commonwealth of Australia.

Edgar, D., Gill, N., & Driller, M. (2020). Physical characteristics of New Zealand Army, Navy and Airforce officer trainees' over a 6-week joint officer induction course. *The Journal of Sport and Exercise Science*, *4*, 63. https://doi.org/10.36905/jses.2020.02.01

Etzion-Daniel, Y., Constantini, N., Finestone, A. S., Shahar, D. R., Israeli, E., Yanovich, R., & Moran, D. S. (2008). Nutrition consumption of female combat recruits in army basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S677-684. https://doi.org/10.1249/MSS.0b013e3181894603

Foulis, S. A., Hughes, J. M., Walker, L. A., Guerriere, K. I., Taylor, K. M., Proctor, S. P., & Friedl, K. E. (2021). Body mass does not reflect the body composition changes in response to similar physical training in young women and men. *International Journal of Obesity (2005)*, *45*(3), 659-665. https://doi.org/10.1038/s41366-020-00730-0

Headquarters Departments of the Army, the Navy, & the Air Force Washington DC. (2017). *Army regulation 40-25, Nutrition and menu standards for human performance optimization*. 3 January 2017.

Herzman-Harari, S., Constantini, N., Mann, G., Lencovsky, Z., & Stark, A. H. (2013). Nutrition knowledge, attitudes, and behaviors of Israeli female combat recruits participating in a nutrition education program. *Military Medicine*, *178*(5), 517-522. https://doi.org/10.7205/milmed-d-12-00439

Jayasinghe, S. N., Breier, B. H., McNaughton, S. A., Russell, A. P., Della Gatta, P. A., Mason, S., Stonehouse, W., Walsh, D. C. I., & Kruger, R. (2019). Dietary patterns in New Zealand women: evaluating differences in body composition and metabolic biomarkers. *Nutrients*, *11*(7). https://doi.org/10.3390/nu11071643

Jones, B. H., Hauret, K. G., Dye, S. K., Hauschild, V. D., Rossi, S. P., Richardson, M. D., & Friedl, K. E. (2017). Impact of physical fitness and body composition on injury risk among active young adults: a study of army trainees. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S17-s22. https://doi.org/10.1016/j.jsams.2017.09.015

Knapik, J., Darakjy, S., Scott, S. J., Hauret, K. G., Canada, S., Marin, R., Rieger, W., & Jones, B. H. (2005). Evaluation of a standardized physical training program for basic combat training. *Journal of Strength and Conditioning Research*, *19*(2), 246-253. https://doi.org/10.1519/16324.1

Knapik, J. J., Hauret, K. G., Arnold, S., Canham-Chervak, M., Mansfield, A. J., Hoedebecke, E. L., & McMillian, D. (2003). Injury and fitness outcomes during implementation of physical readiness training. *International Journal of Sports Medicine*, *24*(5), 372-381. https://doi.org/10.1055/s-2003-40710

Knapik, J. J., Sharp, M. A., Canham-Chervak, M., Hauret, K., Patton, J. F., & Jones, B. H. (2001). Risk factors for training-related injuries among men and women in basic combat training. *Medicine and Science in Sports and Exercise*, *33*(6), 946-954.

Lieberman, H. R., Kellogg, M. D., & Bathalon, G. P. (2008). Female marine recruit training: mood, body composition, and biochemical changes. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S671-676. https://doi.org/10.1249/MSS.0b013e31818943b3

Margolis, L. M., Pasiakos, S. M., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Differential effects of military training on fat-free mass and plasma amino acid adaptations in men and women. *Nutrients*, *4*(12), 2035-2046. https://doi.org/10.3390/nu4122035

McLester, C. N., Nickerson, B. S., Kliszczewicz, B. M., & McLester, J. R. (2020). Reliability and agreement of various InBody body composition analyzers as compared to dual-energy x-ray absorptiometry in healthy men and women. *Journal of Clinical Densitometry*, *23*(3), 443-450. https://doi.org/10.1016/j.jocd.2018.10.008

Ministry of Health. (2017). *HISO 10001:2017 Ethnicity data protocols.* Wellington: Ministry of Health.

Ministry of Health. (2020). *Eating and activity guidelines for New Zealand adults: updated 2020.* Wellington: Ministry of Health.

Ministry of Health. (2021). *New Zealand Health Survey annual data explorer*. Retrieved 22 September 2022 from https://minhealthnz.shinyapps.io/nz-health-survey-2020-21-annual-data-explorer/_w_4f868c95/#!/

Mountjoy, M., Sundgot-Borgen, J. K., Burke, L. M., Ackerman, K. E., Blauwet, C., Constantini, N., Lebrun, C., Lundy, B., Melin, A. K., Meyer, N. L., Sherman, R. T., Tenforde, A. S., Klungland Torstveit, M., & Budgett, R. (2018). IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. *British Journal of Sports Medicine*, *52*(11), 687-697. https://doi.org/10.1136/bjsports-2018-099193

New Zealand Defence Force. (19 November 2020). *Defence Force Order 3: New Zealand Defence Force Human Resource Manual: Version 17/12 – AL 16*. Wellington, New Zealand.

New Zealand Defence Force. (2022). *Medical & Health Defence Careers*. Retrieved 22 September 2022 from https://www.defencecareers.mil.nz/defence-careers/how-do-i-join/medical-and-health/

Nindl, B. C., Jones, B. H., Van Arsdale, S. J., Kelly, K., & Kraemer, W. J. (2016). Operational physical performance and fitness in military women: physiological, musculoskeletal injury, and optimized physical training considerations for successfully integrating women into combat-centric military occupations. *Military Medicine*, *181*(1 Suppl), 50-62. https://doi.org/10.7205/milmed-d-15-00382

Nindl, B. C., McClung, J. P., Miller, J. K., Karl, J. P., Pierce, J. R., Scofield, D. E., Young, A. J., & Lieberman, H. R. (2011). Bioavailable IGF-I is associated with fat-free mass gains after physical training in women. *Medicine and Science in Sports and Exercise*, *43*(5), 793-799. https://doi.org/10.1249/MSS.0b013e31820065ea

Pierce, J. R., DeGroot, D. W., Grier, T. L., Hauret, K. G., Nindl, B. C., East, W. B., McGurk, M. S., & Jones, B. H. (2017). Body mass index predicts selected physical fitness attributes but is not associated with performance on military relevant tasks in U.S. Army soldiers. *Journal of Science and Medicine in Sport*, *20 Suppl 4*, S79-s84. https://doi.org/10.1016/j.jsams.2017.08.021

Proctor, S. P., Scarpaci, M. M., Maule, A. L., Heaton, K. J., Taylor, K., Haven, C. C., Rood, J., Ospina, M., & Calafat, A. M. (2018). Role of body composition and physical activity on permethrin urinary biomarker concentrations while wearing treated military uniforms. *Toxicology Letters*, *299*, 210-217. https://doi.org/10.1016/j.toxlet.2018.10.001

Richmond, V. L., Carter, J. M., Wilkinson, D. M., Homer, F. E., Rayson, M. P., Wright, A., & Bilzon, J. L. (2012). Comparison of the physical demands of single-sex training for male and female recruits in the British Army. *Military Medicine*, *177*(6), 709-715. https://doi.org/10.7205/milmed-d-11-00416

Scientific Advisory Committee on Nutrition. (2017). *SACN Statement on Military Dietary Reference Values for energy*. London, UK: Scientific Advisory Committee on Nutrition.

Sergi, G., De Rui, M., Stubbs, B., Veronese, N., & Manzato, E. (2017). Measurement of lean body mass using bioelectrical impedance analysis: a consideration of the pros and cons. *Aging Clinical and Experimental Research*, *29*(4), 591-597. https://doi.org/10.1007/s40520-016-0622-6

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine joint position statement. Nutrition and athletic performance. *Medicine and Science in Sports and Exercise*, *48*(3), 543-568. https://doi.org/10.1249/mss.0000000000000852

U.S. Department of Defense. (2022). *DoD Instruction 1308.3, DoD Physical fitness/body composition program*. Office of the Under Secretary of Defense for Personnel and Readiness.

University of Otago and Ministry of Health. (2011). *A focus on nutrition: key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.

Vaara, J. P., Groeller, H., Drain, J., Kyröläinen, H., Pihlainen, K., Ojanen, T., Connaboy, C., Santtila, M., Agostinelli, P., & Nindl, B. C. (2022). Physical training considerations for optimizing performance in essential military tasks. *Eur J Sport Sci*, *22*(1), 43-57. https://doi.org/10.1080/17461391.2021.1930193

von Hurst, P. R., Walsh, D. C. I., Conlon, C. A., Ingram, M., Kruger, R., & Stonehouse, W. (2016). Validity and reliability of bioelectrical impedance analysis to estimate body fat percentage against air displacement plethysmography and dual-energy x-ray absorptiometry [Article]. *Nutrition and Dietetics*, *73*(2), 197-204. https://doi.org/10.1111/1747-0080.12172

World Health Organization. (2015). *Guideline: sugars intake for adults and children*. Geneva: World Health Organization.

Yanovich, R., Evans, R., Israeli, E., Constantini, N., Sharvit, N., Merkel, D., Epstein, Y., & Moran, D. S. (2008). Differences in physical fitness of male and female recruits in gender-integrated army basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S654-659. https://doi.org/10.1249/MSS.0b013e3181893f30

Chapter 7 Discussion, conclusion and recommendations

7.1 Introduction

The aim of this research was to establish a situational awareness of iron and vitamin D status, dietary intake and body composition in female recruits in the New Zealand Army; determinants of iron and vitamin D status; and associations of iron status, dietary intake and body composition with physical fitness during 16-weeks of basic training. This discussion includes a summary of the research findings, methodological considerations, significance of the results, a conclusion and recommendations for both practice and future research.

7.2 Summary of findings

All iron status indicators, excluding haemoglobin (Hb), diminished during basic training in 76 participants undertaking 16-weeks of basic training in the New Zealand Army (**hypothesis 1 accepted**). There was a decrease in serum ferritin (SF) (effect size *r*=0.67, *P*<0.001) and transferrin saturation (TS) (*r*=0.28, *P*=0·014) and an increase in soluble transferrin receptor (sTfR) (*r*=0.63, *P*<0·001) and erythrocyte distribution width (RDW) (*r*=0.62, *P*<0·001). This decline in iron status was associated with impaired aerobic fitness, as measured by an increased time to complete a 2.4km run (**hypothesis 2 accepted**). Specifically, increased sTfR was positively associated (*P*=0.012) and decreased TS was negatively associated (*P*=0.005) with run time.

Both dietary and non-dietary determinants of suboptimal iron status were then explored at the commencement of basic training. In 101 participants, three dietary patterns (DPs) were identified: ‘vegetarian’, ‘meat and vegetables’ and ‘bread and beverages’ in the habitual dietary intake before commencing basic training. The ’vegetarian’ DP was significantly associated with SF, as a marker of iron stores, at the univariate level (*P*=0.023) (**hypothesis 3 accepted**). However, this association was lost when the ‘vegetarian’ DP was analysed in the context of non-dietary determinants (age, percent body fat (%BF), blood donation and ≥6-hours of exercise per week) in a multiple linear regression model. Serum ferritin was positively associated with %BF (*P*=0.009) and negatively associated with blood donation in the past year (*P*<0.011) compared to those that did not donate (**hypothesis 4 partially accepted: a history of iron deficiency and menstrual blood loss were not significant predictors**).

The vitamin D status of 87 recruits was then investigated. Serum 25-hydroxyvitamin D (25(OH)D) concentration decreased during basic training commenced in summer (*P*<0.001) and increased during basic training commenced in winter (*P*=0.033) (**hypothesis 5 accepted**). However, more than 60% of participants commencing basic training in winter still had suboptimal vitamin D status at the end of basic training. Age, body mass index (BMI), ethnicity, season, weekly exercise hours and SF were identified as potential determinants of 25(OH)D and entered in a hierarchical linear regression analysis. Commencing basic training in winter was negatively associated with 25(OH)D at baseline. Ethnicity was also a strong determinant of 25(OH)D. New Zealand European participants had the highest median 25(OH)D, followed by Māori, then Pacific at both the beginning and end time points (**hypothesis 6 accepted**).

Finally, the changes in physical fitness, body composition and dietary intake from the beginning to end of basic training were investigated. There were significant improvements in physical fitness during the first 8-weeks for 82 females, as measured by the timed 2.4km run, maximum continuous push-ups and maximum continuous curl-ups (all *P*<0.001). These improvements occurred alongside a significant decrease in fat mass (FM) and %BF, while fat free mass (FFM) significantly increased in 88 female recruits (all *P*<0.001) (**hypothesis 7 accepted**). The frequency of total food intake increased significantly during basic training (*P*<0.001) for 83 female recruits. This was primarily due to increases in protein, grains, fats, discretionary items and beverages (all *P*<0.001). When associations between body composition and physical fitness were explored, a higher %BF was associated with impaired aerobic fitness (**hypothesis 8 accepted**).

7.3 Methodological considerations

A series of analyses were designed to investigate the situational awareness of nutrition related health in female recruits in the New Zealand Army and associations with physical fitness. Methodological strengths and limitations need to be considered when evaluating the internal and external validity of these analyses. These considerations include the study population, study design, measurement of iron status and assessment of physical fitness, body composition and dietary intake.

7.3.1 Study population

Female recruits (*n*=108) from nine intakes of basic training over 2.5-years were provided the opportunity to participate in this research. The feasibility of recruitment determined the sample size. As 106 (98%) of the female recruits volunteered, the participants are considered representative of females undertaking basic training in the New Zealand Army during this period. Despite the high participation rate, the low absolute sample size limits the impact of the results. The limited sample size potentially restricted the statistical power to detect associations throughout this research.

Based on the concept of total response ethnicity (University of Otago and Ministry of Health, 2011), the ethnic group representation for the total participants (*n*=106) was 80.2% European, 29.2% Māori and 17.0% Pacific. The ethnic group representation in the latest New Zealand census data is 70.2% European, 16.5% Māori, 8.1% Pacific, 15.1% Asian, 1.5% Middle Eastern/Latin American/African (MELAA) and 1.2% Other (Stats NZ, 2018). The representation of Māori and Pacific in this research adds to the ethnic diversity of previous studies in female recruits that have primarily been conducted in the United States, United Kingdom and Israel. While the lack of Asian and MELAA participants represents the ethnicity of the female recruits during the study period, the participants are unlikely representative of young women living in New Zealand.

7.3.2 Study design

This research was primarily conducted with longitudinal prospective cohort analysis. The design was observational with quantitative data collected at the beginning and end of 16-weeks of basic training, except follow-up data on physical fitness, which was collected at week 8. While it is a limitation that physical fitness data were not conducted at the end of basic training with all other follow-up measures, this was done in coordination with the real-time programme during basic training. The overall longitudinal design facilitated analysis of the degree and direction of change over time for both individuals and the whole group. A particular challenge with this longitudinal design in female recruits was the loss to follow-up of 21 (20%) participants that self-withdrew or were removed from their original basic training due to back-squadding or discharge.

A cross-sectional observational design was used in the analysis of storage iron determinants in female recruits. Only data collected at the beginning of basic training were examined for associations. However, questions regarding previous lifestyle and medical history, such as blood donation in the previous 12-months and previous history of iron deficiency enabled the associations to span a period, rather than be limited to the moment in time the questions were asked.

The basic training setting is a considerable strength of this research due to the regulated environmental and behavioural factors, such as dietary intake; physical training type, intensity, duration and frequency; sleep; and social connection. All research was conducted at the same location, with recruits living in shared accommodation throughout the 16-weeks.

7.3.3 Measurement of iron status

It is recommended an individual's iron status is determined using multi-variable models to minimise misclassification due to the limitations of single tests (Looker et al., 1995; World Health Organization and Centers for Disease Control and Prevention, 2007). Iron status was determined using SF, Hb and C-reactive protein (CRP). To overcome the elevation in SF that occurs in response to an inflammatory process, participants with a CRP>10mg/L were excluded during analysis of iron status.

Iron deficiency non-anaemia was defined as SF<12µg/L and Hb≥120g/L and iron deficiency anaemia as SF<12µg/L and Hb<120g/L. While these cut-offs for SF and Hb were used in studies of female recruits in the United States Army, they required at least two indicators (SF, TS and/or RDW) to be abnormal (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2006). The rationale for using SF as a single indicator for iron deficiency non-anaemia in this research was based on the real-time clinical threshold applied to recruits for iron supplementation by medical staff during basic training. However, TS, RDW and sTfR were also measured to provide a deeper understanding of the changes in iron status during basic training and associations with physical fitness. Measuring sTfR provides a sensitive indicator of functional or tissue iron deficiency that is not affected by inflammation (Gibson, 2005).

7.3.4 Measurement of vitamin D status

Serum 25(OH)D is the metabolite routinely used to assess overall vitamin D status (Zittermann, 2003) as it reflects the amount of vitamin D from both diet and skin absorption. However, there is no consensus on 25(OH)D targets and a lack of standardised biochemical analysis for 25(OH)D (Dawson-Hughes et al., 2005). The Endocrine Society, a global community of physicians and scientists, has defined vitamin D status using 25(OH)D <50nmol/L as deficient, 50-74nmol/L as insufficient and ≥75nmol/L as sufficient. Their clinical practice guideline states that a 25(OH)D concentration ≥75nmol/L is required to achieve maximum musculoskeletal benefits from vitamin D (Holick et al., 2011). This is supported by studies associating lower stress fracture risk with 25(OH)D ≥75nmol/L in male military recruits (Davey et al., 2016; Ruohola et al., 2006) and 25(OH)D ≥100nmol/L in female recruits (Burgi et al., 2011). The analysis of vitamin D status in this research utilised the targets defined by the Endocrine Society and referred to all concentrations <75nmol/L as suboptimal. Finally, 25(OH)D was analysed using liquid chromatography-tandem-mass spectrometer (LC-MS/MS), the proposed gold standard for assays that enhances comparability between studies (Alexandridou et al., 2021).

7.3.5 Physical fitness assessment

The New Zealand Army base fitness test is comprised of three components, a 2.4km run, push-ups and curl-ups. It is routinely performed during weeks 1 and 8 of basic training as a standard requirement. Results from these tests were utilised to provide a situational awareness of physical fitness in female recruits and associations with iron status. During basic training in the New Zealand Army, the physical training focus during weeks 9 to 16 shifts from aerobic fitness to power, muscular endurance and muscular strength to support the field-based training components. During the final week of basic training the New Zealand Army’s Land Combat Fitness Test (LCFT) is conducted. The LCFT is a functional evaluation of physical performance that simulates the military-specific tasks of load carriage, manual handling of military stores, battlefield manoeuvre, casualty evacuation and battlefield endurance. It has been suggested that training for a combat fitness test is expected to stimulate a greater increase in FFM than aerobic fitness training (Foulis et al., 2021a). As the LCFT was introduced to basic training after this research was initiated it was not included in the study procedures. Although the LCFT is only conducted once during basic training, inclusion of the results in future research may provide further depth of analysis of the physical performance of female recruits, particularly with operational relevance.

7.3.6 Body composition assessment

Optimal body composition has been described as healthy %BF and high FFM (Foulis et al., 2021b) and BMI is reported to be an imprecise measure for body composition in military personnel (Foulis et al., 2021a). However, BMI remains the primary screening tool for body composition in military personnel internationally and there is currently no consensus on optimal %BF within the military.

Body composition was assessed via bioelectrical impedance analysis (BIA). A limitation of BIA is the variable validity in comparison to a laboratory reference standard, such as dual energy x-ray absorptiometry (Sergi et al., 2017). However, the portability and speed of BIA make it an ideal option for field-based research and minimised the impact of data collection on real-time training. In addition, the InBody$_{230}$ analyser has demonstrated excellent reliability in repeat measurements (McLester et al., 2020; von Hurst et al., 2016) making it suitable for assessing the change in body composition in a research setting. A strength of the methodology was the strict conduct followed for body composition measurements. This included ensuring participants had fasted overnight, performed no physical activity for at least 8-hours prior, consumed minimal water and urinated prior to testing, wore light clothing and removed all jewellery. The same BIA unit was also used for all measures.

7.3.7 Dietary assessment

The dietary assessment tool used in this research was a non-quantitative food frequency questionnaire (FFQ). The FFQ was designed to determine consumption patterns of foods and food groupings that contain iron or factors affecting iron bioavailability. A validation study in premenopausal women, using New Zealand food variables, demonstrated the FFQ was reproducible and reasonably valid for determining iron related food groups and DPs (Beck et al., 2012). In the analysis of storage iron determinants, the FFQ was used to collect data on dietary intake during the 4-weeks before basic training commenced. Dietary patterns were subsequently identified from the FFQ data using factor analysis as a strength of this research. Although one previous study in Israeli female recruits explored the association between mean dietary iron intake and iron status at the commencement of basic training, no studies have explored DPs in this population.

In the analysis of dietary intake changes during basic training, the FFQ was utilised as a standard FFQ to determine the intake frequency of food variables during the 4-weeks before basic training commenced and 4-weeks at the end of basic training. Interpretation of dietary intake from a non-quantitative FFQ is limited and associations with body composition and physical fitness are difficult to identify. Strategies to mitigate the methodological disadvantages of a FFQ, described in chapter two, included online administration of the FFQ to ensure complete data capture, clear written and verbal instructions on how to complete the FFQ and the presence of a registered dietitian throughout completion of the FFQ to answer any relevant questions. Participants (*n*=3) were excluded due to FFQ data with implausible intakes, for example, more than 50 serves of fruit and vegetables per day. This was most likely the result of inaccurate reporting.

In the empirical approach to DP analysis, two statistical techniques, factor analysis and cluster analysis, are commonly used to generate DPs from the dietary data collected (Newby & Tucker, 2004;

Sauvageot et al., 2017). In factor analysis, data are reduced into DPs based on correlations between food variables, while in cluster analysis, data are reduced into DPs based on individual differences in mean intake (Newby & Tucker, 2004). The advantage of factor analysis is each participant is allocated a score for all derived factors (dietary patterns), which is a continuous variable. This provides a greater understanding and the associations between DPs and health outcomes (Zhao et al., 2021). For this reason, factor analysis was the chosen methodology for including DPs in determining predictors of iron stores.

A common limitation of factor analysis studies are the necessary but subjective decisions made in determining DPs, such as the number of input variables, food groupings, model selection, rotation method, number of factors to retain and interpretation of the results (Newby & Tucker, 2004). Most studies collapse the original measured dietary data into a smaller number of input variables for factor analysis. This is commonly based on food groups, but also nutrient content, dietary use or nutrient profile (Newby & Tucker, 2004; Schulz et al., 2021). Selection of food variables with a specific consumption frequency has been utilised (Beck et al., 2012) and in the analysis of iron store determinants, food variables consumed on average ≥2.0 times per week were entered into the factor analysis to determine DPs. This was based on the consideration that consumption frequency of at least once every 4-days will likely affect iron status (Beck et al., 2012). However, it is acknowledged that differences in selection of input variables may have affected the DPs derived.

7.4 Discussion of key results

A major objective of this longitudinal research was to characterise iron status in females during basic training and investigate associations with physical fitness in the New Zealand Army. Establishing a situational awareness of iron status in female recruits and the effect on operationally relevant outcomes enables strategies that mitigate the risk of suboptimal iron status to be implemented.

Previous studies investigating iron status in females during basic training have been limited to the United States and 9-weeks duration (Karl et al., 2010; McClung et al., 2009a; McClung et al., 2009b; McClung et al., 2006). In this research, iron status indicators were measured with an interval of 15-weeks, reflecting haematological changes that are closer to the 120-day lifespan of erythrocytes.

Iron status indicators, SF, sTfR, TS and RDW all diminished during basic training. Reduced iron status, represented by an increase in sTfR and a decrease in TS, was associated with impaired aerobic fitness, while there was no significant association with push-ups or curl-ups. In this research, SF had the greatest change of any biomarker, with a 32% reduction from baseline. This finding supports the role of SF as an early indicator of suboptimal iron status. The association between aerobic fitness and indicators of tissue-iron deficiency, such as sTfR and TS, suggests these biomarkers can be used to determine iron deficiency non-anaemia with a functional impairment in aerobic capacity. Peeling et al. (2007) proposed that the SF cut-off value for stage one iron deficiency (iron depletion) be <35μg/L in athletic populations. While it is acknowledged this level of iron depletion is unlikely to have a substantial impact on aerobic fitness, early iron supplementation will likely prevent iron deficiency non-anaemia or iron deficiency anaemia manifesting. This is particularly relevant in female recruits with the expected decline in iron status during basic training.

A complementary approach to iron supplementation is to identify determinants of iron stores in females before commencing basic training in the New Zealand Army. This will enable targeted lifestyle education during the recruiting phase to assist females in maintaining or enhancing their iron status. Despite iron deficiency non-anaemia and iron deficiency anaemia reported previously in females commencing basic training (Dubnov et al., 2006; Israeli et al., 2008; Karl et al., 2010; McClung et al., 2009a; McClung et al., 2006), no studies have explored associations between iron stores, dietary and non-dietary determinants simultaneously in female recruits. The use of DPs to investigate associations between diet and iron status in this research was a novel approach in female recruits. Dietary patterns may present a clearer picture of the whole diet than the role of a single food or nutrient (Newby & Tucker, 2004). This takes into consideration the multiple components in food that affect iron bioavailability.

Percent body fat and blood donation in the past year were the strongest determinants of iron stores in females at the commencement of basic training in the New Zealand Army. A higher %BF appears to be protective of iron stores within this healthy, non-obese cohort. Inadequate intake of total energy compromising dietary iron intake and/or high physical activity levels that reduce iron absorption, through the stimulation of hepcidin (Newlin et al., 2012) may have contributed to the association between a lower %BF and diminished SF. Blood donation in the past year significantly decreased iron stores compared to those participants that did not donate blood. While the association between SF and DPs was lost in the context of non-dietary determinants, dietary advice regarding total energy requirements and iron bioavailability remains prudent. An estimated 200-250mg of iron is lost during the standard donation of 450-500mL of blood (Kiss et al., 2015; Reddy et al., 2020). Strategies to mitigate the risk of suboptimal iron status resulting from blood donation include pre-donation measurement of SF (Dijkstra et al., 2019), extending the inter-donation interval and post-donation iron supplementation (Kiss et al., 2015; Rigas et al., 2019). However, Merkel and Moran (2018) recommend that military recruits avoid blood donation during strenuous training periods, such as basic training.

Alongside iron, vitamin D has also been identified as a key micronutrient in supporting the health and performance of female military personnel and in particular, musculoskeletal health (Wardle et al., 2021). Another objective of this research was therefore to characterise vitamin D status in female recruits during basic training and investigate potential determinants at the commencement of basic training. Suboptimal vitamin D status has been reported in most female recruits at the end of basic training in previous studies conducted in the United States and United Kingdom (Andersen et al., 2010;

Carswell et al., 2018; Gaffney-Stomberg et al., 2019; Lutz et al., 2012). Establishing a situational awareness of the vitamin D status in female recruits in the New Zealand Army enables strategies that mitigate the risk of suboptimal vitamin D status to be implemented. Waiouru Military Camp is located at 39°S. Conducting vitamin D research in military populations at locations at least approaching 40° has been encouraged in previous research. This allows the effect of more extreme latitudes on vitamin D status to be measured.

Overall, 25(OH)D declined during basic training, with two-thirds of participants having suboptimal vitamin D status (25(OH)D <75nmol/L) at the end. Wintertime and being of Pacific or Māori ethnicity were the strongest determinants and negatively associated with 25(OH)D at the commencement of basic training. Further declines during basic training resulted in 100% of Pacific and 85% of Māori participants having suboptimal 25(OH)D at the end. Observational studies in recruits suggest an association between suboptimal vitamin D status and increased risk of injuries and illness. This may have a considerable impact on both individuals and the organisation regarding training delays and potential discharge. While controversy remains regarding 25(OH)D targets for healthy adults that infer maximum health benefits, early clinical screening and supplementation for military recruits with suboptimal vitamin D status appears prudent. This should be initiated before or at the commencement of basic training to account for the significant decline observed during basic training.

The final objective of this research was to characterise body composition, physical fitness and dietary intake during basic training and investigate associations between these three inter-related factors in female recruits. Improvements in each of these factors has the potential to substantially enhance functional outcomes in female recruits. The highly structured nature of basic training creates an environment capable of inducing positive changes in all three factors. Establishing a situational awareness of changes in body composition, physical fitness and dietary intake can inform potential changes in the delivery of physical training and nutrition services if required. Previous studies in female recruits have not explored the changes in, or associations between, these three factors simultaneously. In addition, few studies have incorporated measures of muscular endurance (push-ups and curl-ups) alongside aerobic fitness (running).

During basic training, FM and %BF decreased significantly and FFM increased significantly, while there was no change in body mass and BMI. The primary tool for assessing body composition in military personnel in the New Zealand Army is BMI; however, this study confirms previous findings in female recruits that suggest BMI may be an imprecise measure of body composition changes (Etzion-Daniel et al., 2008; Foulis et al., 2021b; Margolis et al., 2012; Nindl et al., 2011). Body mass index appears to mask body composition changes associated with functional outcomes. All measures of physical fitness improved during the first 8-weeks of basic training, as expected based on findings in previous studies (Bell et al., 2000; Blacker et al., 2009; Knapik et al., 2005; Knapik et al., 2003; Richmond et al., 2012; Yanovich et al., 2008). A higher %BF at week 1 was associated with impaired aerobic fitness, as measured by a 2.4km run, and muscular endurance, as measured by push-ups throughout the first 8-weeks. Dietary intake frequency significantly increased during basic training, primarily due to increases in protein, grains, fats, discretionary items and beverages. This suggests an increase in energy intake that is contrary to previous findings (Etzion-Daniel et al., 2008; Herzman-Harari et al., 2013; Margolis et al., 2012). There were no associations between the frequency intake of food categories and %BF.

Overall, the findings demonstrate that 16-weeks of basic training in the New Zealand Army is effective in providing an anabolic environment with adequate dietary intake to support training-induced adaptations in female recruits. Of concern, is the potential loss of positive changes experienced during basic training when soldiers are no longer living and training in a highly structured environment. Attention to lifestyle factors, such as dietary intake, physical training and sleep, as well as social connection are warranted following basic training. This is another critical link in supporting retention of females in the New Zealand Army.

7.5 Conclusion

Basic training in the New Zealand Army provides an anabolic environment in which dietary intake supports positive training-induced adaptations in physical fitness and body composition of female recruits. However, at the same time, factors within basic training are contributing towards diminishing nutritional status of female recruits that limits their health and performance potential. Iron status indicators (excluding Hb) were diminished during basic training and suboptimal iron status was associated with impaired aerobic fitness. In addition, vitamin D status was suboptimal for most female recruits at the end of basic training. At the commencement of basic training, a lower %BF and recent blood donation were identified as determinants of reduced iron stores, and wintertime and being of Pacific or Māori ethnicity were determinants of reduced vitamin D status. Education regarding dietary intake and lifestyle choices that support optimal nutritional status in females is recommended during the recruiting phase. In addition, clinical screening and early supplementation of iron and vitamin D, before or at the commencement of basic training, is recommended to counter the negative impact of basic training on female recruits. Collectively, these strategies appear critical for enabling success in female recruits during basic training in the New Zealand Army. This will in turn increase retention and improve the operational capability in the New Zealand Army and wider New Zealand Defence Force.

7.6 Recommendations

7.6.1 Future research

Based on the findings in this research, the following recommendations are suggested for future military nutrition research to maintain or enhance the health and performance of recruits in the New Zealand Defence Force and international militaries:

1. Investigate the iron status of female soldiers in the New Zealand Army during training conducted in the months following the completion of basic training.
2. Explore development of an iron status screening tool on commencement of basic training that includes both dietary and non-dietary determinants.
3. Establish a consensus amongst clinicians and researchers regarding cut-off values for iron status indicators that infer a negative effect on aerobic fitness, in the absence of iron deficiency anaemia.
4. Investigate the vitamin D status of ethnically diverse male recruits during basic training in the New Zealand Army.
5. Establish a consensus amongst clinicians and researchers regarding a serum 25(OH)D concentration that infers a negative effect on musculoskeletal health in military personnel.
6. Investigate physical fitness and body composition at weeks 1, 8 and 16 of basic training in recruits in the New Zealand Army and associations between these two factors.
7. Investigate the dietary intake of recruits in the New Zealand Army during basic training utilising quantitative dietary assessment tools.
8. Investigate the causes for back-squadding and discharge from basic training in female recruits in the New Zealand Army.

7.6.2 Translational impact

Based on the findings in this research, the New Zealand Army introduced clinical iron screening during week 1 of training for females joining as recruits and officer cadets. Iron supplementation is offered if suboptimal iron status is indicated (SF<30µg/L, Hb<120g/L or Hb≥120g/L). The following recommendations are suggested to maintain or enhance the health and performance of recruits in the New Zealand Army:

1. Provide clinical screening for iron status to females during the recruiting phase and prescribed treatment with iron supplementation if indicated. Cut-off values for iron status indicators should consider the expected declines in iron status during basic training. This will allow time for improvements compared to what is currently achieved during week 1 of training.
2. Provide clinical screening for vitamin D status to candidates during the recruiting phase and prescribed treatment with vitamin D supplementation if indicated. Cut-off values for vitamin D status indicators should be based on ensuring maximum musculoskeletal benefits and consider the expected changes in vitamin D status during basic training.
3. Provide lifestyle education during the recruiting phase to females regarding the maintenance of iron status that includes advice regarding blood donation and seeking medical advice if concerned about heavy menstrual bleeding.
4. Provide nutrition education during both the recruiting phase and basic training to candidates and recruits regarding optimal nutrition to fuel the demands of basic training. This education should incorporate total energy and foods to meet nutrient requirements.
5. Provide nutrition education towards the end of basic training to recruits regarding nutrition requirements following basic training. This education should advise recruits how to adjust their dietary intake appropriately as their training volume reduces and access to ultra-processed foods and alcohol increases.

7.7 References

Alexandridou, A., Schorr, P., Stokes, C. S., & Volmer, D. A. (2021). Analysis of vitamin D metabolic markers by mass spectrometry: recent progress regarding the “gold standard” method and integration into clinical practice. *Mass Spectrometry Reviews*. https://doi.org/10.1002/mas.21768

Andersen, N. E., Karl, J. P., Cable, S. J., Williams, K. W., Rood, J. C., Young, A. J., Lieberman, H. R., & McClung, J. P. (2010). Vitamin D status in female military personnel during combat training. *Journal of the International Society of Sports Nutrition*, *7*, 38. https://doi.org/10.1186/1550-2783-7-38

Beck, K. L., Kruger, R., Conlon, C. A., Heath, A. L., Coad, J., Matthys, C., Jones, B., & Stonehouse, W. (2012). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women. *Journal of the Academy of Nutrition and Dietetics*, *112*(8), 1177-1187. https://doi.org/10.1016/j.jand.2012.05.012

Bell, N. S., Mangione, T. W., Hemenway, D., Amoroso, P. J., & Jones, B. H. (2000). High injury rates among female army trainees: a function of gender? *American Journal of Preventive Medicine*, *18*(3 Suppl), 141-146.

Blacker, S. D., Wilkinson, D. M., & Rayson, M. P. (2009). Gender differences in the physical demands of British Army recruit training. *Military Medicine*, *174*(8), 811-816. https://doi.org/10.7205/milmed-d-01-3708

Burgi, A. A., Gorham, E. D., Garland, C. F., Mohr, S. B., Garland, F. C., Zeng, K., Thompson, K., & Lappe, J. M. (2011). High serum 25-hydroxyvitamin D is associated with a low incidence of stress fractures. *Journal of Bone and Mineral Research*, *26*(10), 2371-2377. https://doi.org/10.1002/jbmr.451

Carswell, A. T., Oliver, S. J., Wentz, L. M., Kashi, D. S., Roberts, R., Tang, J. C. Y., Izard, R. M., Jackson, S., Allan, D., Rhodes, L. E., Fraser, W. D., Greeves, J. P., & Walsh, N. P. (2018). Influence of vitamin D supplementation by sunlight or oral D3 on exercise performance. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/mss.0000000000001721

Davey, T., Lanham-New, S. A., Shaw, A. M., Hale, B., Cobley, R., Berry, J. L., Roch, M., Allsopp, A. J., & Fallowfield, J. L. (2016). Low serum 25-hydroxyvitamin D is associated with increased risk of stress fracture during Royal Marine recruit training. *Osteoporosis International*, *27*(1), 171-179. https://doi.org/10.1007/s00198-015-3228-5

Dawson-Hughes, B., Heaney, R. P., Holick, M. F., Lips, P., Meunier, P. J., & Vieth, R. (2005). Estimates of optimal vitamin D status. *Osteoporosis International*, *16*(7), 713-716. https://doi.org/10.1007/s00198-005-1867-7

Dijkstra, A., van den Hurk, K., Bilo, H. J. G., Slingerland, R. J., & Vos, M. J. (2019). Repeat whole blood donors with a ferritin level of 30 µg/L or less show functional iron depletion. *Transfusion*, *59*(1), 21-25. https://doi.org/10.1111/trf.14935

Dubnov, G., Foldes, A. J., Mann, G., Magazanik, A., Siderer, M., & Constantini, N. (2006). High prevalence of iron deficiency and anemia in female military recruits. *Military Medicine*, *171*(9), 866-869.

Etzion-Daniel, Y., Constantini, N., Finestone, A. S., Shahar, D. R., Israeli, E., Yanovich, R., & Moran, D. S. (2008). Nutrition consumption of female combat recruits in army basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S677-684. https://doi.org/10.1249/MSS.0b013e3181894603

Foulis, S. A., Hughes, J. M., Spiering, B. A., Walker, L. A., Guerriere, K. I., Taylor, K. M., Proctor, S. P., & Friedl, K. E. (2021a). US Army basic combat training alters the relationship between body mass index and per cent body fat. *BMJ Mil Health*. https://doi.org/10.1136/bmjmilitary-2021-001936

Foulis, S. A., Hughes, J. M., Walker, L. A., Guerriere, K. I., Taylor, K. M., Proctor, S. P., & Friedl, K. E. (2021b). Body mass does not reflect the body composition changes in response to similar physical training in young women and men. *International Journal of Obesity (2005)*, *45*(3), 659-665. https://doi.org/10.1038/s41366-020-00730-0

Gaffney-Stomberg, E., Nakayama, A. T., Guerriere, K. I., Lutz, L. J., Walker, L. A., Staab, J. S., Scott, J. M., Gasier, H. G., & McClung, J. P. (2019). Calcium and vitamin D supplementation and bone health in Marine recruits: effect of season. *Bone*, *123*, 224-233. https://doi.org/10.1016/j.bone.2019.03.021

Gibson, R. S. (2005). *Principles of nutritional assessment.* New York: Oxford University Press.

Herzman-Harari, S., Constantini, N., Mann, G., Lencovsky, Z., & Stark, A. H. (2013). Nutrition knowledge, attitudes, and behaviors of Israeli female combat recruits participating in a nutrition education program. *Military Medicine*, *178*(5), 517-522. https://doi.org/10.7205/milmed-d-12-00439

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., & Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism*, *96*(7), 1911-1930. https://doi.org/10.1210/jc.2011-0385

Israeli, E., Merkel, D., Constantini, N., Yanovich, R., Evans, R. K., Shahar, D., & Moran, D. S. (2008). Iron deficiency and the role of nutrition among female military recruits. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S685-690. https://doi.org/10.1249/MSS.0b013e31818946ae

Karl, J. P., Lieberman, H. R., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2010). 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. *American Journal of Clinical Nutrition*, *92*(1), 93-100. https://doi.org/10.3945/ajcn.2010.29185

Kiss, J. E., Brambilla, D., Glynn, S. A., Mast, A. E., Spencer, B. R., Stone, M., Kleinman, S. H., & Cable, R. G. (2015). Oral iron supplementation after blood donation: a randomized clinical trial. *JAMA*, *313*(6), 575-583. https://doi.org/10.1001/jama.2015.119

Knapik, J., Darakjy, S., Scott, S. J., Hauret, K. G., Canada, S., Marin, R., Rieger, W., & Jones, B. H. (2005). Evaluation of a standardized physical training program for basic combat training. *Journal of Strength and Conditioning Research*, *19*(2), 246-253. https://doi.org/10.1519/16324.1

Knapik, J. J., Hauret, K. G., Arnold, S., Canham-Chervak, M., Mansfield, A. J., Hoedebecke, E. L., & McMillian, D. (2003). Injury and fitness outcomes during implementation of physical readiness training. *International Journal of Sports Medicine*, *24*(5), 372-381. https://doi.org/10.1055/s-2003-40710

Looker, A. C., Gunter, E. W., & Johnson, C. L. (1995). Methods to assess iron status in various NHANES surveys. *Nutrition Reviews*, *53*(9), 246-254.

Lutz, L. J., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Vitamin D status, dietary intake, and bone turnover in female soldiers during military training: a longitudinal study. *Journal of the International Society of Sports Nutrition*, *9*(1), 38. https://doi.org/10.1186/1550-2783-9-38

Margolis, L. M., Pasiakos, S. M., Karl, J. P., Rood, J. C., Cable, S. J., Williams, K. W., Young, A. J., & McClung, J. P. (2012). Differential effects of military training on fat-free mass and plasma amino acid adaptations in men and women. *Nutrients*, *4*(12), 2035-2046. https://doi.org/10.3390/nu4122035

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Nindl, B. C., Young, A. J., & Lieberman, H. R. (2009a). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *American Journal of Clinical Nutrition*, *90*(1), 124-131. https://doi.org/10.3945/ajcn.2009.27774

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Young, A. J., & Lieberman, H. R. (2009b). Longitudinal decrements in iron status during military training in female soldiers. *British Journal of Nutrition*, *102*(4), 605-609. https://doi.org/10.1017/s0007114509220873

McClung, J. P., Marchitelli, L. J., Friedl, K. E., & Young, A. J. (2006). 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*, *25*(1), 64-69.

McLester, C. N., Nickerson, B. S., Kliszczewicz, B. M., & McLester, J. R. (2020). Reliability and agreement of various InBody body composition analyzers as compared to dual-energy x-ray absorptiometry in healthy men and women. *Journal of Clinical Densitometry*, *23*(3), 443-450. https://doi.org/10.1016/j.jocd.2018.10.008

Merkel, D., & Moran, D. S. (2018). Coping with iron deficiency and iron deficiency anemia in combat recruits. *Acta Haematologica*, *139*(3), 166-167. https://doi.org/10.1159/000487387

Newby, P. K., & Tucker, K. L. (2004). Empirically derived eating patterns using factor or cluster analysis: a review. *Nutrition Reviews*, *62*(5), 177-203. https://doi.org/10.1301/nr.2004.may.177-203

Newlin, M. K., Williams, S., McNamara, T., Tjalsma, H., Swinkels, D. W., & Haymes, E. M. (2012). The effects of acute exercise bouts on hepcidin in women. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(2), 79-88.

Nindl, B. C., McClung, J. P., Miller, J. K., Karl, J. P., Pierce, J. R., Scofield, D. E., Young, A. J., & Lieberman, H. R. (2011). Bioavailable IGF-I is associated with fat-free mass gains after physical training in women. *Medicine and Science in Sports and Exercise*, *43*(5), 793-799. https://doi.org/10.1249/MSS.0b013e31820065ea

Peeling, P., Blee, T., Goodman, C., Dawson, B., Claydon, G., Beilby, J., & Prins, A. (2007). Effect of iron injections on aerobic-exercise performance of iron-depleted female athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *17*(3), 221-231.

Reddy, K. V., Shastry, S., Raturi, M., & Baliga, B. P. (2020). Impact of regular whole-blood donation on body iron stores. *Transfusion Medicine and Hemotherapy. Offizielles Organ der Deutschen Gesellschaft für Transfusionsmedizin und Immunhämatologie*, *47*(1), 75-79. https://doi.org/10.1159/000499768

Richmond, V. L., Carter, J. M., Wilkinson, D. M., Homer, F. E., Rayson, M. P., Wright, A., & Bilzon, J. L. (2012). Comparison of the physical demands of single-sex training for male and female recruits in the British Army. *Military Medicine*, *177*(6), 709-715. https://doi.org/10.7205/milmed-d-11-00416

Rigas, A. S., Pedersen, O. B., Magnussen, K., Erikstrup, C., & Ullum, H. (2019). Iron deficiency among blood donors: experience from the Danish Blood Donor Study and from the Copenhagen ferritin monitoring scheme. *Transfusion Medicine*, *29 Suppl 1*, 23-27. https://doi.org/10.1111/tme.12477

Ruohola, J. P., Laaksi, I., Ylikomi, T., Haataja, R., Mattila, V. M., Sahi, T., Tuohimaa, P., & Pihlajamaki, H. (2006). Association between serum 25(OH)D concentrations and bone stress fractures in Finnish young men. *Journal of Bone and Mineral Research*, *21*(9), 1483-1488. https://doi.org/10.1359/jbmr.060607

Sauvageot, N., Schritz, A., Leite, S., Alkerwi, A., Stranges, S., Zannad, F., Streel, S., Hoge, A., Donneau, A. F., Albert, A., & Guillaume, M. (2017). Stability-based validation of dietary patterns obtained by cluster analysis. *Nutrition Journal*, *16*(1), 4. https://doi.org/10.1186/s12937-017-0226-9

Schulz, C. A., Oluwagbemigun, K., & Nöthlings, U. (2021). Advances in dietary pattern analysis in nutritional epidemiology. *European Journal of Nutrition*, *60*(8), 4115-4130. https://doi.org/10.1007/s00394-021-02545-9

Sergi, G., De Rui, M., Stubbs, B., Veronese, N., & Manzato, E. (2017). Measurement of lean body mass using bioelectrical impedance analysis: a consideration of the pros and cons. *Aging Clinical and Experimental Research*, *29*(4), 591-597. https://doi.org/10.1007/s40520-016-0622-6

Stats NZ. (2018). *2018 Census ethnic group summaries*. Retrieved 20 October 2022 from https://www.stats.govt.nz/tools/2018-census-ethnic-group-summaries/

von Hurst, P. R., Walsh, D. C. I., Conlon, C. A., Ingram, M., Kruger, R., & Stonehouse, W. (2016). Validity and reliability of bioelectrical impedance analysis to estimate body fat percentage against air displacement plethysmography and dual-energy x-ray absorptiometry [Article]. *Nutrition and Dietetics*, *73*(2), 197-204. https://doi.org/10.1111/1747-0080.12172

Wardle, S. L., O'Leary, T. J., McClung, J. P., Pasiakos, S. M., & Greeves, J. P. (2021). 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*, *24*(10), 995-1001. https://doi.org/10.1016/j.jsams.2021.08.011

World Health Organization and Centers for Disease Control and Prevention. (2007). *Assessing the iron status of populations: second edition; including literature reviews*. Geneva: World Health Organization.

Yanovich, R., Evans, R., Israeli, E., Constantini, N., Sharvit, N., Merkel, D., Epstein, Y., & Moran, D. S. (2008). Differences in physical fitness of male and female recruits in gender-integrated army basic training. *Medicine and Science in Sports and Exercise*, *40*(11 Suppl), S654-659. https://doi.org/10.1249/MSS.0b013e3181893f30

Zhao, J., Li, Z., Gao, Q., Zhao, H., Chen, S., Huang, L., Wang, W., & Wang, T. (2021). A review of statistical methods for dietary pattern analysis. *Nutrition Journal*, *20*(1), 37. https://doi.org/10.1186/s12937-021-00692-7

Zittermann, A. (2003). Vitamin D in preventive medicine: are we ignoring the evidence? *British Journal of Nutrition*, *89*(5), 552-572. https://doi.org/10.1079/bjn2003837

Chapter 8 Appendices

Appendix A Health and lifestyle questionnaire

General information:
The following questionnaire is designed to gain a better understanding of female recruits in the New Zealand Army. It is specifically looking at predictors of iron deficiency and factors related to performance. This questionnaire will help inform future policy designed to improve the performance and success of female recruits. The answers you provide are confidential and will not influence your time on basic training in any way. Please answer all questions honestly. Feel free to ask the research team any questions at any stage.

Questions	Response options
Date of birth	
Town/city of birth	
Which ethnic group(s) do you identify with? *Select all that apply.*	New Zealand European Māori Samoan Cook Island Māori Tongan Niuean Chinese Indian Other (please state ethnic group)
Do you have children?	Yes No *If yes, how old are your children?*
What were your living arrangements before basic training?	Living at home with parents Living on own Living with partner Flatting/house share Other (please specify)
How many people lived in your household?	
What is the highest level of education you have received?	NCEA Level 1/School Certificate/Equivalent NCEA Level 2/6th Form Certificate/Equivalent NCEA Level 3/Bursary/Equivalent Tertiary Certificate/Diploma Degree Postgraduate Qualification Other (please specify)
Do you smoke cigarettes?	Yes, everyday Yes, occasionally I used to Never *If yes, how many cigarettes do you smoke per day?*
Do you consider yourself to be:	Overweight Normal weight Underweight

How would you describe your eating pattern?	Eat a variety of all foods, including animal products Eat eggs, dairy, fish and chicken but avoid other meats Eat eggs and dairy products but avoid all meats and fish Eat eggs but avoid dairy products, all meats and fish Eat no animal products Other (please specify)
Do you follow any diet for cultural or religious reasons?	Yes No *If yes, what type of diet do you follow?*
Do you follow any diet for any health reasons (e.g., gluten free)?	Yes No *If yes, what type of diet do you follow?*
Have you dieted strictly in the last year?	Yes No *If yes, please explain.*
In the past 4-weeks, how many hours per week did you spend exercising at an intensity that raised your heart rate?	None Less than 1.0-hour 1.0-2.9-hours 3.0-5.9-hours 6.0-8.9-hours 9.0 or more hours
Did you change your usual exercise habits in preparation for basic training?	Yes No *If yes, please explain how.*
What activity or activities did you do in preparation for basic training? *Select all that apply.*	Jogging/running Pack walking Cycling Gym strength exercises (e.g., weights) Own body weight strength exercises (e.g., push-ups) Circuit training Swimming None Other (please specify)
If you participated in any activity or activities in preparation for AARC, how many hours per week did you spend on each activity?	Jogging/running Pack walking Cycling Gym strength exercises (e.g., weights) Own body weight strength exercises (e.g., push-ups) Circuit training Swimming Other (please specify)
Did you enjoy sport at school?	Yes No
How physically fit do you think you are?	Very unfit (e.g., can't walk 5 minutes without being out of breath) Unfit Neither fit nor unfit Fit Very fit (e.g., an international standard athlete)

Do you think you are physically fit enough to be a soldier?	Yes No I don't know
Do you have, or have you ever suffered from any acute or chronic illness which may affect your iron status? ***e.g., Meckel's diverticulum, ulcerative colitis, haemorrhoids.***	Yes No *If yes, please provide details on date, diagnosis, diagnosed by (e.g., doctor, nurse) and any further details.*
Have you ever suffered from low iron stores, iron deficiency or iron deficiency anaemia?	Yes No *If yes, please provide details on date, diagnosis, diagnosed by (e.g., doctor, nurse) and any further details.*
Have you ever been treated for iron deficiency or iron deficiency anaemia?	Yes No *If yes, please provide details on date, type of treatment (e.g., iron tablets), duration (e.g., 3-months) and any further details.*
Have you had a blood transfusion in the last year?	Yes No *If yes, do you know why you received the transfusion?*
Have you previously been pregnant?	Yes No *If yes, how many times?*
Are you currently pregnant or is there a chance you could be pregnant?	Yes No
Are you currently taking any medication (excluding dietary supplements)?	Yes No *If yes, please state what medication you are taking, how long you have been taking it and why?*
Are you currently using any contraception?	Oral contraception Implant contraception Contraception by injection (e.g., Depo-Provera) Intra-uterine device Other (please specify) *If yes, how long have you been using this contraceptive method?*
Have you taken any form of supplement in the last 6-months? (e.g., vitamin tablet, protein powder, iron supplements, sports supplements)	Yes No *If yes, please give details (e.g., brand name of the supplement, the type of supplement, the dose, the number taken and the frequency of intake).*
Do you have, or have you had any medical condition which has resulted in blood loss?	Yes No *If yes, please describe.*
Please describe any sources of blood loss such as coughing up blood, blood in stools or urine, blood loss through injury or blisters in the past year. *We will ask about your periods later.*	

Have you ever donated blood?	Yes No
When did you last donate blood?	DD/MM/YYYY
If you have donated blood, how many times have you donated blood in the past year?	
Do you get nose bleeds?	Yes No
If yes, how often do you get nose bleeds?	Times per month or year
If yes, how heavy are your nose bleeds?	Light Medium Heavy
Have you started your periods?	Yes No *If you want to explain further, please provide details here.*
How old were you when you had your first menstruation period?	Years Months
Have you had a period in the last 6-months?	Yes No
Would you describe your periods as regular (every month) or irregular?	Regular Irregular
How best describes your periods and the number of days in your cycle? *To count the number of days in your cycle count from the first day of your last period to the day you expect your period to start.*	I have had my first period but nothing since I have regular periods and my cycle is usually less than 21-days I have regular periods and my cycle is usually between 21 to 35-days I have regular periods and my cycle is usually between 36 to 90-days I have regular periods and my cycle is usually over 90-days My periods are so irregular it’s difficult to tell how many days my cycle is Other (please specify)
When did your last period start?	DD/MM/YYYY, or The week starting DD/MM/YYYY I don’t know
On average how many days does your period normally last for?	
How many ‘heavy’ days do you have during a period?	
How many ‘light’ days do you have during a period?	
Do you have any additional comments you would like to make?	

Appendix B Food frequency questionnaire

General information: This questionnaire focuses on your dietary habits, including intake of foods that contain iron and foods which affect iron absorption. This questionnaire has been developed specifically to look at the relationship between diet and iron status. When answering the questions, consider your intake of food over the past month. To help you do this, please think of an event in your life that happened one month ago and think about your eating patterns since that time.
To determine the frequency of food intake, participants selected one of the following nine options for each of the 143 food variables described below (under food categories): • I never eat this food • Less than once a month • 1-3 times a month • Once per week • 2-3 times per week • 4-6 times per week • Once per day • 2-3 times per day • 4 plus times per day
Unprocessed meat
Beef (e.g., roast, steak, chops, schnitzel, silverside, casseroles, stir fry, curry, hamburger meat, mince dishes)
Chicken, turkey, or duck (e.g., roast, fried, steamed, BBQ, casseroles, stir fry, curry, fried takeaway chicken)
Lamb, hogget, or mutton (e.g., roast, steak, chops, BBQ, casseroles, stir fry, curry)
Pork (e.g., roast, chops, steak, casseroles, stir fry, curry)
Veal
Liver, kidney, other offal (including pate)
Ham, bacon
Game meats (e.g., venison, mutton bird, rabbit)
Processed meat
Corned beef (canned)
Beef Jerky / Biltong
Sausages, frankfurters, saveloys
Luncheon sausage, salami, brawn, pastrami
Black pudding
Meat pies
Fish and seafood
Fresh and frozen fish (e.g., snapper, tarakihi, gurnard, flounder, hoki, salmon, whitebait, shark, eel)
Battered and crumbed fish (e.g., fish fingers, fish cakes)
Canned and bottled fish (e.g., tuna, salmon, herrings, sardines)
Mussels, pipi, paua, cockles, oysters
Scallops, crab sticks, crab, squid, crayfish, kina
Prawns, shrimps

Eggs
Eggs – boiled, fried, poached, scrambled, raw and egg-based dishes including quiche, soufflés, frittatas, omelettes
Nuts and seeds
Peanuts, mixed nuts, macadamias, pecan, hazelnuts, brazil nuts, walnuts, cashews, pistachios
Almonds
Pumpkin seeds, sunflower seeds, pinenuts
Sesame seeds, tahini
Peanut butter
Soy and legumes
Tofu, soybeans, tempeh
Beans in sauce (e.g., baked beans, chilli beans)
Beans (canned or dried) (e.g., black beans, butter beans, haricot beans, red kidney beans, white kidney beans, refried beans)
Lentils
Peas (e.g., chickpeas, hummus, falafels, split peas, cow peas)
Dahl (all varieties)
Milk, milk products, and alternatives
Cheese (e.g., Cheddar, Colby, Edam, Tasty, blue vein, camembert, parmesan, gouda, processed)
Cottage cheese, ricotta cheese
Cream, sour cream, cream cheese, cheese spreads, fromage frais (all varieties)
Milk (cow’s milk) as a drink (e.g., flavoured milk, milk shakes)
Milk (cow’s milk) (all varieties) added to drinks (e.g., in tea, coffee)
Milk (cow’s milk) (all varieties) added to food (e.g., cereals, dishes such as macaroni cheese, milk puddings such as rice pudding, custard, semolina, instant puddings, dairy food)
Soy Milk
Coconut Milk
Yoghurt
Ice cream
Fruits
Apples
Bananas, green bananas
Citrus fruits (e.g., oranges, tangelo, tangerine, mandarin, grapefruit, lemon)
Green kiwifruit
Zespri gold kiwifruit
Pears, Nashi pears
Stone fruit (e.g., apricots, nectarines, peaches, plums, lychees)
Avocados, olives
Feijoa, persimmon, tamarillos
Grapes
Mangos
Watermelon
Pawpaw (papaya), other melons (e.g., honey dew, rock melon)
Pineapple
Rhubarb
Fruit salad, canned

Strawberries, blackberries, cherries, blueberries, boysenberries, loganberries, cranberries, gooseberries, raspberries
Sultanas, raisins, currants, figs
Dried apricots, prunes, dates, mixed dried fruit
Vegetables
Potato (e.g., boiled, mashed, baked, roasted, fried, chips)
Kūmara (e.g., boiled, mashed, baked, roasted, fried, chips)
Green beans, broad beans, runner beans, asparagus
Broccoli (all varieties)
Red cabbage
Cabbage (all varieties), Brussels sprouts
Capsicum, peppers (all varieties)
Carrots
Cauliflower
Corn (all varieties)
Courgette, zucchini, cucumber, gherkins, or marrow (all varieties)
Beetroot
Radishes (all varieties)
Lettuce
Mushrooms
Onions (all varieties), leeks, celery
Tomatoes (all varieties)
Peas (green)
Spinach, silver beet, Swiss chard (all varieties)
Other green leafy vegetables (e.g., watercress, puha, witloof, chicory, kale, chard, collards, Chinese kale, Bok Choi)
Pumpkin, squash, yams
Parsnip
Taro leaves (palusami)
Breakfast cereals
Porridge, rolled oats, oat bran, oatmeal
Muesli (all varieties)
Weetbix (all varieties)
Cornflakes or rice bubbles
Bran based cereals (all varieties, e.g. All Bran, Sultana Bran)
Light and fruity cereals (e.g., Special K, Light and Tasty)
Chocolate based cereals (e.g., Milo cereal, Coco Pops)
Sweetened cereals (e.g., Nutri-Grain, Fruit Loops, Honey Puffs, Frosties)
Breakfast drinks (e.g., Up and Go)

Grains
White rice
Brown rice
Instant noodles
Pasta, noodles (white)
Pasta, noodles (whole wheat)
Couscous, polenta
Bulgur wheat (e.g., tabbouleh)
Wheat germ, wheat bran (flakes)
Bread, cakes, biscuits, and crackers
White bread and rolls (including specialty breads such as focaccia, panini, pita, naan, crumpets, pizza bases, tortillas, burrito, roti)
Brown bread and rolls (including multigrain, wholegrain, whole meal breads)
Breads fortified with iron (e.g., Mighty White Tip Top bread)
Fruit and currant bread / buns
White flour muffins (all varieties)
Whole meal muffins (all varieties)
Cakes (all varieties, excluding chocolate and fruit cake)
Chocolate cake
Fruit cake
Biscuits, plain sweet
Biscuits, chocolate, or chocolate covered
Crackers (e.g., crisp bread, water crackers, rice cakes, cream crackers, Cruskits, Meal Mates)
Iron fortified crackers (e.g., Vita wheat)
Miscellaneous foods and snacks
Marmite
Chocolate spread (e.g., Nutella)
Butter or margarine
Cooking oil (all varieties)
Soup, vegetable based, homemade or canned
Soup, meat based, homemade or canned
Sugar (all varieties) added to food / drinks
Jam, marmalade, honey, or syrups
Muesli or cereal bar (all varieties)
Potato crisps
Milk chocolate
Dark chocolate
White chocolate
Alcohol
Beer, cider (all varieties)
Red wine
White wine
Spirits (all varieties)
Ready to drink alcoholic beverages

Non-alcoholic beverages
Complan, Sustagen (all varieties)
Milo
Hot chocolate, drinking chocolate, Cocoa, Ovaltine, Nesquik
Coffee (all varieties)
Black tea
Herbal tea, fruit tea
Cordials (including syrups, powders) (e.g., blackcurrant, orange)
Fruit and vegetable juices (all varieties)
Sports drinks (e.g., Powerade)
Energy drinks (e.g., Red Bull, V)
Water (including tap water or bottled water)

Appendix C Supplementary table to chapter 6

This supplementary table describes the 142 food items (excluding water) from the food frequency questionnaire used in chapter 6. Changes in the weekly intake frequency of the food variables (e.g., green leafy vegetables, bananas and beef) were investigated from before basic training to during basic training. The table also displays how each variable was compressed into nine food categories (e.g., vegetables, fruits and protein). These categories were used to explore associations between the total weekly intake frequency and percent body fat at weeks 1 and 16 of basic training.

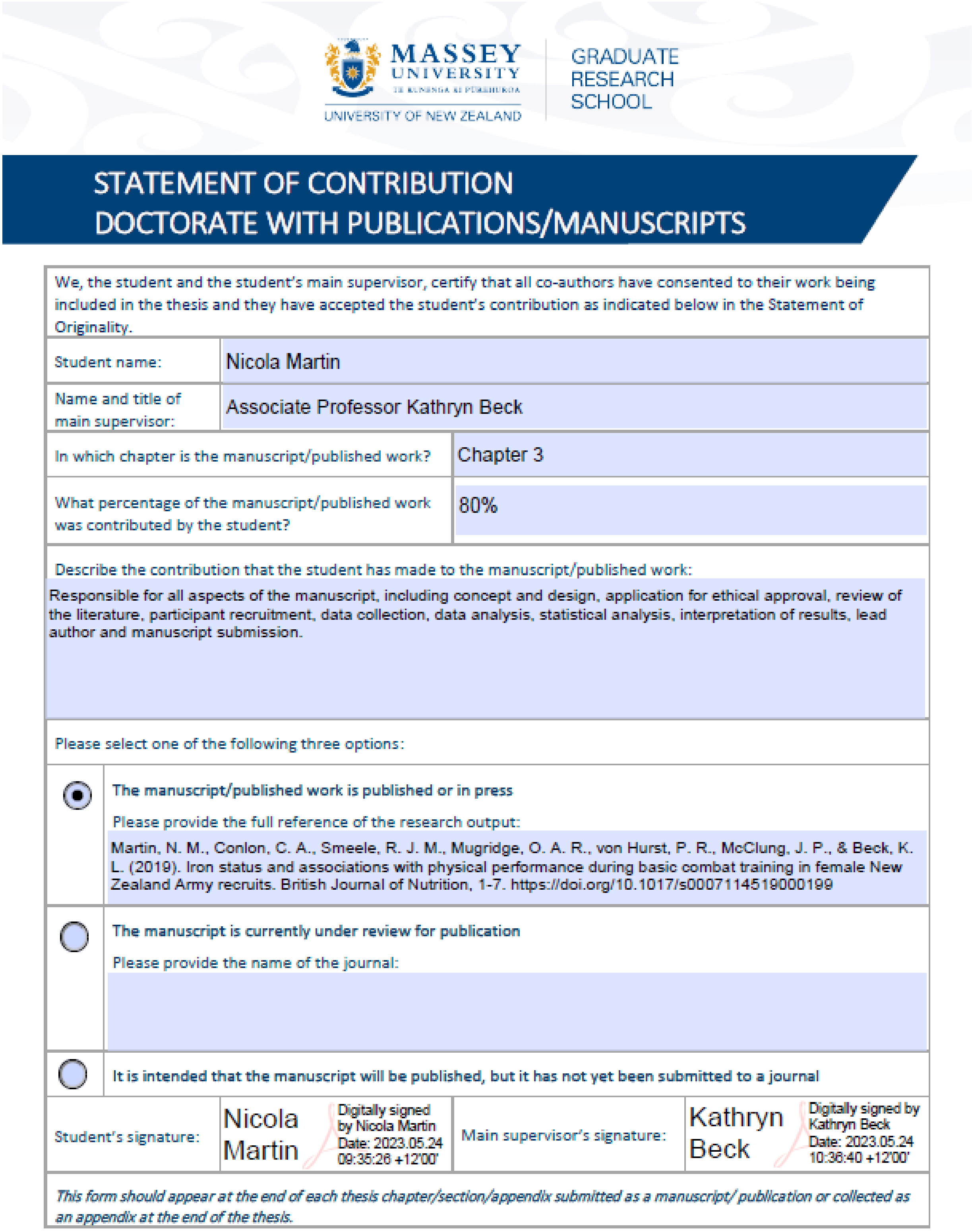
Food categories and food variables	
Vegetables	
Potato and kūmara[a]	All varieties of potato, kūmara and sweet potato
Green leafy vegetables	Lettuce, green cabbage, brussels sprouts, spinach, silver beet, Swiss chard, watercress, puha, witloof, chicory, kale, chard, collards, bok choy and taro leaves
Other vegetables	Fresh or frozen – beetroot, broccoli, red cabbage, capsicum, carrots, cauliflower, corn, courgette, zucchini, cucumber, gherkins, marrow, mushrooms, onions, leeks, celery, parsnip, green peas, pumpkin, squash, yams, radishes and tomatoes; homemade and canned soup
Fruits	
Apples	
Bananas	Yellow and green bananas
Citrus fruit	Oranges, tangelos, tangerines, mandarins, grapefruit and lemons
Other fruit	Fresh or frozen – green and gold kiwifruit, pears, nashi pears, stone fruit (e.g., apricots, nectarines, peaches, plums, lychees), feijoa, persimmon, tamarillos, grapes, mango, melons (e.g., watermelon, pawpaw, papaya, honeydew, rock melon), pineapple, rhubarb, berries (e.g., strawberries, blackberries, blueberries, boysenberries, loganberries, cranberries, gooseberries, raspberries), cherries, avocados and olives
Canned and dried fruit	Canned and dried fruit (e.g., sultanas, raisins, currants, figs, apricots, prunes, dates and cranberries)
Protein	
Beef	
Chicken, turkey and duck	
Other unprocessed meat	Lamb, hogget, mutton, pork, veal, liver, kidney, other offal and game meats
Processed meat	Ham, bacon, canned corned beef, sausages, frankfurters, saveloys, luncheon sausage, salami, brawn, pastrami and black pudding

Fish and shellfish	Fresh, frozen, battered, crumbed, canned or bottled fish; mussels, pipis, paua, cockles, oysters, scallops, crab sticks, crab, squid, crayfish, kina, prawns and shrimps
Eggs	
Nuts and seeds	Peanuts, peanut butter, mixed nuts, macadamias, pecans, hazelnuts, Brazil nuts, walnuts, cashews, pistachios, almonds, pumpkin seeds, sunflower seeds, pine nuts, sesame seeds and tahini
Soy and legumes	Tofu, soybeans, tempeh; canned beans in sauce (e.g., baked beans, chilli beans); dried or canned beans (e.g., black beans, butter beans, haricot beans, red kidney beans, white kidney beans, refried beans), peas (e.g., chickpeas, hummus, falafel, split peas, cow peas), lentils and dahl
Grains	
White bread	Sliced and specialty white bread, panini, pita, crumpets, pizza bases, tortillas, naan, roti and fruit bread
Wholegrain bread	Sliced and specialty multigrain, wholegrain and wholemeal bread
Rice	White and brown rice
Pasta and noodles	White and wholewheat pasta and instant noodles
Other grains	Couscous, polenta, bulgur wheat, wheat germ and wheat bran
Rolled oats	Porridge, oat bran and oatmeal
Muesli and granola	
Other breakfast cereals	Weetbix, bran-based cereals (e.g., All Bran, Sultana Bran), light and fruity cereals (e.g., Special K, Light and Tasty), cornflakes, rice bubbles, chocolate-based cereals (e.g., Milo cereal, Coco-Pops) and sweetened cereals (e.g., Nutri-grain, Fruit Loops, Honey Puffs, Frosties)
Dairy	
Cow's milk	As a drink, added to drinks (e.g., in coffee and tea) and added to food (e.g., breakfast cereal, cheese sauce, rice pudding, custard, semolina and instant pudding)
Cheese and yoghurt	Hard and soft cheese, including cottage and ricotta cheese
Cream	Cream, sour cream, cream cheese, cheese spreads and fromage frais
Milk alternatives	Soy and coconut milk
Fats	
Butter or margarine	
Cooking oil	
Discretionary items	
Muffins and cakes	Sweet and savoury muffins
Sweet biscuits	
Crackers	
Muesli bars	Muesli or cereal bars
Potato crisps	
Chocolate	Milk, dark or white chocolate
Ice cream	
Meat pies	
Beef jerky	Beef jerky/biltong
Added sugar and syrups	Sugar added to food and drinks, jam, marmalade, honey, syrups and chocolate spreads

Beverages	
Coffee	
Tea	Black, herbal and fruit tea
Other beverages	Sugar based or artificially sweetened – hot chocolate, drinking chocolate, Cocoa, Ovaltine, Nesquik, Milo, cordial, fruit and vegetable juice, sports drinks, energy drinks
Fortified beverages	Nutrition supplement beverages (e.g., Complan, Sustagen) and fortified breakfast beverages (e.g., Up & Go)
Alcohol	
Alcohol	Beer, cider, wine, spirits and ready to drink alcoholic beverages

[a] Also known as sweet potato.

Appendix D Statements of contribution



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE
RESEARCH
SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nicola Martin
Name and title of main supervisor:	Associate Professor Kathryn Beck
In which chapter is the manuscript/published work?	Chapter 3
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:

Responsible for all aspects of the manuscript, including concept and design, application for ethical approval, review of the literature, participant recruitment, data collection, data analysis, statistical analysis, interpretation of results, lead author and manuscript submission.

Please select one of the following three options:

● **The manuscript/published work is published or in press**

Please provide the full reference of the research output:

Martin, N. M., Conlon, C. A., Smeele, R. J. M., Mugridge, O. A. R., von Hurst, P. R., McClung, J. P., & Beck, K. L. (2019). Iron status and associations with physical performance during basic combat training in female New Zealand Army recruits. British Journal of Nutrition, 1-7. https://doi.org/10.1017/s0007114519000199

○ **The manuscript is currently under review for publication**

Please provide the name of the journal:

○ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Nicola Martin Digitally signed by Nicola Martin Date: 2023.05.24 09:35:26 +12'00'	Main supervisor's signature:	Kathryn Beck Digitally signed by Kathryn Beck Date: 2023.05.24 10:36:40 +12'00'

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE
RESEARCH
SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nicola Martin
Name and title of main supervisor:	Associate Professor Kathryn Beck
In which chapter is the manuscript/published work?	Chapter 4
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:

Responsible for all aspects of the manuscript, including concept and design, application for ethical approval, review of the literature, participant recruitment, data collection, data analysis, statistical analysis, interpretation of results, lead author and manuscript submission.

Please select one of the following three options:

● **The manuscript/published work is published or in press**

Please provide the full reference of the research output:

Martin, N. M., von Hurst, P. R., Conlon, C. A., Smeele, R. J. M., Mugridge, O. A. R., & Beck, K. L. (2023). Body fat percentage and blood donation are the strongest determinants of iron stores in premenopausal women joining the New Zealand Army. Military Medicine. https://doi.org/10.1093/milmed/usad023

○ **The manuscript is currently under review for publication**

Please provide the name of the journal:

○ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Nicola Martin Digitally signed by Nicola Martin Date: 2023.05.24 09:30:46 +12'00'	Main supervisor's signature:	Kathryn Beck Digitally signed by Kathryn Beck Date: 2023.05.24 10:52:20 +12'00'

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nicola Martin
Name and title of main supervisor:	Associate Professor Kathryn Beck
In which chapter is the manuscript/published work?	Chapter 5
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:

Responsible for all aspects of the manuscript, including concept and design, application for ethical approval, review of the literature, participant recruitment, data collection, data analysis, statistical analysis, interpretation of results, lead author and manuscript submission.

Please select one of the following three options:

○ **The manuscript/published work is published or in press**

Please provide the full reference of the research output:

● **The manuscript is currently under review for publication**

Please provide the name of the journal:

Accepted and awaiting publication in The Journal of Sport and Exercise Science.

○ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Nicola Martin Digitally signed by Nicola Martin Date: 2023.05.24 10:04:42 +12'00'	Main supervisor's signature:	Kathryn Beck Digitally signed by Kathryn Beck Date: 2023.05.24 10:52:56 +12'00'

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE
RESEARCH
SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nicola Martin
Name and title of main supervisor:	Associate Professor Kathryn Beck
In which chapter is the manuscript/published work?	Chapter 6
What percentage of the manuscript/published work was contributed by the student?	80%

Describe the contribution that the student has made to the manuscript/published work:

Responsible for all aspects of the manuscript, including concept and design, application for ethical approval, review of the literature, participant recruitment, data collection, data analysis, statistical analysis, interpretation of results, lead author and manuscript submission.

Please select one of the following three options:

○ **The manuscript/published work is published or in press**

Please provide the full reference of the research output:

○ **The manuscript is currently under review for publication**

Please provide the name of the journal:

● **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Nicola Martin Digitally signed by Nicola Martin Date: 2023.05.24 10:06:08 +12'00'	Main supervisor's signature:	Kathryn Beck Digitally signed by Kathryn Beck Date: 2023.05.24 10:54:06 +12'00'

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.