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# Determinants and longitudinal changes of serum 25-hydroxyvitamin D during basic military training in female New Zealand Army recruits

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ARTICLEINFO	A B S T R A C T
Received: 14.08.2022 Accepted: 03.02.2023 Online: 27.10.2023	Suboptimal vitamin D status is reported to negatively impact military readiness. This longitudinal study aimed to describe vitamin D status during basic military training (BMT) and identify determinants of serum 25-hydroxyvitamin D (25(OH)D) in female recruits in the New Zealand Army. Serum 25(OH)D was measured during week one (baseline) and
Keywords: Vitamin D Military Predictors	week 16 (end) of BMT ( $n = 87$ ) 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 baseline using a hierarchical linear regression model. From baseline to end, mean $\pm$ SD 25(OH)D was 102.5 $\pm$ 33.6 vs $67.4 \pm 22.6$ nmol/L ( $p < 0.001$ ) for BMT commenced in summer and $67.4 \pm 21.5$ vs 73.8 $\pm$ 18.9 nmol/L ( $p = 0.033$ ) for BMT commenced in winter. Regardless of the season basic training commenced, less than one-third of participants overall had sufficient ( $\geq$ 75 nmol/L) vitamin D status at the end. Age, BMI, ethnicity, and season explained 48.2% of the variance in 25(OH)D at baseline. Wintertime and being of Pacific or Māori ethnicity were the strongest negative determinants of 25(OH)D at baseline. These results suggest seasonal 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 BMT.

# 1. 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 military training (BMT) 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)<sub>2</sub>D) stimulates intestinal absorption of dietary calcium and phosphorus. This maintains homeostasis of serum concentrations, essential for bone mineralisation (DeLuca, 2004)

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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 BMT. 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 BMT (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 baseline and the end of BMT 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., 2019; 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 BMT and to investigate potential determinants at baseline.

## 2. Methods

#### 2.1. Participants

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. New Zealand Army recruits undertake 16-weeks of BMT at Waiouru Military Camp, latitude 39°S and 792 m above sea level. All procedures involving human subjects were approved by the Massey University Human Ethics Committee: Southern A (Application – 13/85). Investigators also adhered to the Defence Force Order 3 (New Zealand Defence Force, 19 November 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.

## 2.2. Study procedures

Data was collected over nine intakes of BMT, categorised by the season BMT started. Summer intakes (n = 5) started in February or early March (end of summer) and ended in May (late autumn/fall) 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 week one (baseline) and week 16 (end) of BMT. Body composition, demographic and lifestyle data were collected at baseline.

## 2.3. Basic military training

Basic military 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.

### 2.4. Blood sampling and analysis

A fasted venepuncture blood sample of 18ml was collected between 0600 and 0730 hours at baseline and the end. 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  $25(OH)D \ge 150 \text{ nmol/L to } 12\% \text{ at} < 25 \text{ nmol/L}.$  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 (< 50 nmol/L), insufficient (50 - 74nmol/L) or sufficient (≥ 75 nmol/L) (Holick et al., 2011). All concentrations < 75 nmol/L were referred to as suboptimal.

#### 2.5. Ethnicity

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).

#### 2.6. Body composition

Body composition measures were determined at baseline. 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 body fat percentage were measured via bioelectrical impedance analysis using the InBody<sub>230</sub> (Biospace Co. Ltd., Seoul, South Korea). Body mass index (BMI) was calculated as mass (kg)/height (m<sup>2</sup>).

#### 2.7. Lifestyle

A questionnaire was administered at baseline 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 '< 3.0-hours', '3.0-5.9-hours', '6.0-8.9hours' and ' $\geq$  9.0-hours.'

#### 2.8. Statistical approach

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. 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 BMT and participants that did not. Comparison of mean values of 25(OH)D between ethnicity groups at baseline and the end 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 baseline. These determinants included age, BMI, body fat percentage, SF and CRP, entered as continuous variables; and season and smoking status, entered as binary variables. 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's  $\geq$  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 DurbinWatson statistic between 1.5 and 2.5 for autocorrelation of residuals, a Variance Inflating Factor < 5 for assessment of multi-collinearity and a satisfactory normal P-P plot of regression standardised residual.

#### 3. Results

Of the 108 female recruits invited to take part in this study, 106 volunteered to participate. Eighteen participants did not finish BMT: 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 BMT. Following these exclusions, 87 participants (age =  $19.2 \pm 2.2$  years; height =  $165.1 \pm 5.4$  cm; body mass =  $65.5 \pm 9.3$  kg; body mass index =  $24.0 \pm 2.8$  kg/m<sup>2</sup>; body fat percentage =  $26.8 \pm 5.4\%$ ) were included in the analysis. One participant consumed a vitamin D supplement before BMT commenced; however, their vitamin D status was deficient at baseline. Therefore, their supplement use did not impact further analysis and they were included in the results presented. Table 1 describes the characteristics of study participants at baseline.

Table 1: Characteristics of participants at baseline of basic military training (n = 87).

Characteristic	n	%			
Ethnicity					
NZ 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 <sup>a</sup>					
< 3.0	13	14.9			
3.0 - 5.9	38	43.7			
6.0 - 8.9	23	26.4			
$\geq$ 9.0	13	14.9			

*Note:* <sup>a</sup>Exercise at an intensity that elevated the heart rate. Abbreviations: NZ, New Zealand; NCEA, National Certificate of Educational Achievement.

Overall, the mean  $\pm$  SD 25(OH)D declined during BMT from 92.0  $\pm$  34.4 nmol/L at baseline to 69.3  $\pm$  21.7 nmol/L at the end, p < 0.001. There was no significant difference in 25(OH)D at baseline between those participants that finished BMT and the 18 participants that did not. From baseline to the end, mean  $\pm$  SD 25(OH)D was 105.2  $\pm$  33.7 to 76.3  $\pm$  17.4 nmol/L (p < 0.001) for

New Zealand Europeans,  $78.2 \pm 28.2$  to  $65.5 \pm 24.5$  nmol/L (p = 0.002) for Māori, and  $60.8 \pm 13.5$  to  $43.7 \pm 9.7$  nmol/L (p = 0.460) for Pacific. Figure 1 and Table 2 display ethnic differences in serum 25(OH)D and vitamin D status, respectively.

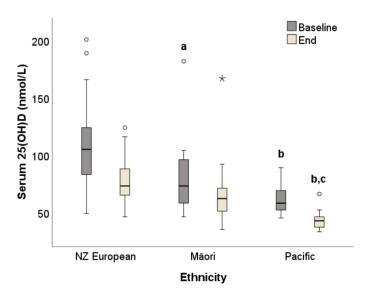


Figure 1: Boxplot of serum 25-hydroxyvitamin D at baseline and end of basic military training by ethnicity. Abbreviations: 25(OH)D, serum 25-hydroxyvitamin D; NZ, New Zealand. Boxes represent the middle 50<sup>th</sup> percentile. Vertical lines represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Lines inside the box represent the median values. •Values 1.5 times the interquartile range. \*Values 3 times the interquartile range. <sup>a</sup>p = 0.001 between Māori and NZ European, <sup>b</sup>p < 0.001 between Pacific and NZ European and <sup>c</sup>p < 0.001 between Pacific and Māori.

Table 2: Vitamin D status at baseline and the end of basic military training by ethnicity and season of intake (n = 87).

	Sufficient <sup>a</sup>		<b>Insufficient</b> <sup>a</sup>		Deficient <sup>a</sup>	
	n	%	n	%	n	%
Baseline						
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
End						
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
Summer						
Baseline	47	77.0	14	23.0	0	0
End	18	29.5	30	49.2	13	21.3
Winter						
Baseline	7	26.9	13	50.0	6	23.1
End	10	38.5	14	53.8	2	7.7

*Note:* <sup>a</sup>Serum 25(OH)D  $\geq$  75 nmol/L (sufficient), 50 – 74 nmol/L (insufficient), and < 50 nmol/L (deficient). NZ, New Zealand.

The hierarchical linear regression model for predicting serum 25(OH)D at baseline is illustrated in Table 3. Age, ethnicity, and season were significant determinants of 25(OH)D at baseline, 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 48.2% of the variance in 25(OH)D.

## 4. 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 BMT, 25(OH)D declined significantly for participants that commenced BMT in summer and increased significantly for those that commenced BMT in winter. Despite the increase observed for the winter intakes, two-thirds of all participants had suboptimal vitamin D status at the end of BMT, regardless of the season that they commenced training. For Pacific and Māori, 100% and 85% of participants had suboptimal vitamin D status following 16-weeks of BMT, respectively. Wintertime and being of Pacific or Māori ethnicity were the strongest determinants and inversely associated with 25(OH)D at baseline, when age and body composition were controlled for. This model explained 48.2% of the variance in 25(OH)D at baseline.

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 BMT 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 BMT in the United States Marines without vitamin D supplementation. For participants that commenced BMT in winter, a significant mean 25(OH)D increase (p < 0.05) was also reported during BMT. 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

Predictor		$B^{\mathrm{a}}$	SE B	95% CI	β <sup>b</sup>	R <sup>2</sup> Change
Step 1						
Age		-4.428	1.650	[-7.708, -1.148]	-0.280	$0.078^{*}$
Step 2						
Age		-4.388	1.637	[-7.644, -1.132]	-0.277	
BMI		-1.931	1.272	[-4.461, 0.598]	-0.157	0.025
Step 3						
Age		-3.059	1.560	[-6.162, 0.044]	-0.193	
BMI		-1.172	1.175	[-3.509, 1.166]	-0.095	
Ethnicity	NZ European			Reference		
	Māori	-22.303	7.582	[-37.385, -7.220]	-0.298	
	Pacific	-41.124	10.571	[-62.152, -20.096]	-0.383	$0.171^{**}$
Step 4						
Age		-2.376	1.331	[-5.024, 0.273]	-0.150	
BMI		-0.159	1.014	[-2.178, 1.859]	-0.013	
Ethnicity	NZ European			Reference		
	Māori	-21.714	6.445	[-34.538, -8.890]	-0.290	
	Pacific	-46.275	9.030	[-64.242, -28.307]	-0.431	
Season	Summer					
	Winter	-34.927	6.127	[-47.119, -22.736]	-0.467	$0.208^{**}$

Table 3: Hierarchical linear regression model for determinants of serum 25-hydroxyvitamin D at baseline of basic military training (n = 87).

*Note:* F(5, 81) = 15.054, p < 0.001,  $R^2 = 0.482$ , adjusted  $R^2 = 0.450$ . <sup>a</sup>Unstandardised coefficients. <sup>b</sup>Standardised coefficients. <sup>\*</sup>p < 0.01. <sup>\*\*</sup>p < 0.001. Age and BMI were entered as continuous variables. Ethnicity and season were entered as categorical variables. Abbreviations: BMI, body mass index; NZ, New Zealand.

aged 15 years and over was 62.4 nmol/L, 57.2 nmol/L and 46.0 nmol/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 BMT that commenced in summer and winter, the prevalence of suboptimal vitamin D at the end was 71% and 62%, respectively. These results support the findings of two studies in female recruits following completion of BMT in the United States Army at a training location of 34°N (Andersen et al., 2010; Lutz et al., 2012). For participants that commenced BMT at the end of summer, 75% had suboptimal vitamin D status at the end of training (Andersen et al., 2010). For participants that commenced BMT at the end of training (Andersen et al., 2010). For participants that commenced BMT 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, JSES | https://doi.org/10.36905/jses.2023.02.06

most female recruits have suboptimal vitamin D status at the end of BMT, if not also throughout BMT.

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 body fat percentage at baseline. 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, there was a positive association between baseline 25(OH)D and body fat percentage 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 BMT during winter lost more body fat than those undertaking training during summer.

The incidence of medically diagnosed stress fractures during BMT has been reported up to 5% for men and 18% for women (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  $\geq$  75 nmol/L has been linked with lower stress fracture rates in male military 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 ( $\geq$  100 nmol/L) had a lower stress fracture risk than those in the lowest quintile (< 50 nmol/L), OR = 0.51,  $p \leq 0.01$ , 95% CI [0.34, 0.76], (Burgi et al., 2011).

In this study, two-thirds of participants overall had suboptimal vitamin D status at the end of BMT. In the five participants that incurred medically diagnosed stress fractures and did not finish BMT, they all had suboptimal 25(OH)D at baseline (range 44-68 nmol/L). It is therefore worth considering screening and supplementing recruits with 25(OH)D < 75 nmol/L at baseline 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 cutoffs, 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 BMT, 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 BMT 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 BMT. In addition, there was no examination of dietary intake as a potential determinant of 25(OH)D. Although diet is a relatively modest contributed 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 JSES | https://doi.org/10.36905/jses.2023.02.06

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 Maori 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 BMT. 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 BMT in summer and increased significantly for those that commenced BMT in winter. Regardless of the season BMT commenced, two-thirds of participants overall had suboptimal vitamin D status at the end of 16-weeks of 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 military recruits focus on preventing suboptimal vitamin D status. This can be achieved through education, screening, and supplementation, before or at the commencement of BMT.

#### **Conflict of Interest**

The authors declare no conflict of interests.

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