


Low serum 25-hydroxyvitamin D is associated with increased risk of stress fracture during Royal Marine recruit training

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Abstract

Summary The aim of this study was to investigate vitamin D status and stress fracture risk during Royal Marine military training. Poor vitamin D status was associated with an increased risk of stress fracture. Vitamin D supplementation may help to reduce stress fracture risk in male military recruits with low vitamin D status.

Introduction Stress fracture is a common overuse injury in military recruits, including Royal Marine (RM) training in the UK. RM training is recognised as one of the most arduous basic training programmes in the world. Associations have been reported between serum 25-hydroxyvitamin D (25(OH)D) and risk of stress fracture, but the threshold of 25(OH)D for this effect remains unclear. We aimed to

determine if serum 25(OH)D concentrations were associated with stress fracture risk during RM training.

Methods We prospectively followed 1082 RM recruits (males aged 16–32 years) through the 32-week RM training programme. Troops started training between September and July. Height, body weight and aerobic fitness were assessed at week 1. Venous blood samples were drawn at weeks 1, 15 and 32. Serum samples were analysed for 25(OH)D and parathyroid hormone (PTH).

Results Seventy-eight recruits (7.2 %) suffered a total of 92 stress fractures. Recruits with a baseline serum 25(OH)D concentration below 50 nmol L⁻¹ had a higher incidence of stress fracture than recruits with 25(OH)D concentration above this threshold ($\chi^2_{(1)}=3.564$, $p=0.042$; odds ratio 1.6 (95 % confidence interval (CI) 1.0–2.6)). Baseline serum 25(OH)D varied from 47.0±23.7 nmol L⁻¹ in February, to 97.3±24.6 nmol L⁻¹ in July (overall mean 69.2±29.2 nmol L⁻¹, $n=1016$). There were weak inverse correlations between serum 25(OH)D and PTH concentrations at week 15 ($r=-0.209$, $p<0.001$) and week 32 ($r=-0.214$, $p<0.001$), but not at baseline.

Conclusion Baseline serum 25(OH)D concentration below 50 nmol L⁻¹ was associated with an increased risk of stress fracture. Further studies into the effects of vitamin D supplementation on stress fracture risk are certainly warranted.

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Introduction

Stress fracture is an overuse injury of the bone often seen in military recruits and athletes. These fractures result from repetitive sub-maximal loading that, over time, exceeds the bone's intrinsic ability to repair [1], leading to an

accumulation of micro-damage in bone [2]. These injuries are costly to the military medical services, often requiring lengthy rehabilitation [3].

Vitamin D is an important factor for calcium absorption and bone health [4, 5]. Vitamin D status is positively associated with greater bone mineral density (BMD) and quantitative ultrasound (QUS) measures in adolescents and adults [6–8]. Supplementation with vitamin D reduces the risk of fracture in older adults [9]. Low BMD has been reported as a risk factor for stress fracture in a number of military studies [10–13].

Vitamin D status is therefore an attractive, potentially modifiable, risk factor for stress fracture in military training. High serum 25-hydroxyvitamin D (25(OH)D) concentration (as a marker of vitamin D status) has been linked with lower stress fracture rates [14–16]. In contrast, other studies have reported no difference in serum 25(OH)D concentration between stress fractured and non-fractured recruits [12, 17]. Supplementation with vitamin D and calcium reduced the incidence of stress fracture in female Navy recruits [18]. Vitamin D status is an important determinant of serum parathyroid hormone (PTH) concentration, where inadequate vitamin D is a potential cause of secondary hyperparathyroidism [19, 20]. The relationship between serum PTH and stress fracture risk is far from clear. A study in Finnish military recruits reported a higher serum PTH concentration, but not lower serum 25(OH)D, in stress fractured recruits compared with non-fractured recruits [12]. Longitudinal studies have reported mixed patterns of serum 25(OH)D and PTH during military training [21–23].

Royal Marine (RM) recruit training is an intensive 32-week programme undertaken at the Commando Training Centre Royal Marines (CTCRM) in the South West of England. Potential recruits (males only, aged 16–32 years) undergo a vigorous selection procedure challenging both physical and mental capabilities. Despite high physical entry standards, the prevalence of stress fracture in RM recruits has remained between 4 and 7 % over recent years [13, 24]. To date, no studies in the UK Armed Forces have investigated vitamin D status in relation to stress fracture risk. Thus, the aim of this study was to investigate serum 25(OH)D and PTH concentrations during RM recruit training and associations with stress fracture risk. We hypothesised that a poor vitamin D status would be associated with an increased risk of stress fracture during RM training.

Materials and methods

Study participants

The present study was part of *Surgeon General's Bone Health Project*, which aimed to identify risk factors for stress fracture

during UK initial military training. Recruits from 20 training troops ($n=2013$), who commenced RM training between Sept 2009 and July 2010, were invited to participate in the study. New training troops were normally formed every 2 weeks throughout the year, with 2-week periods of block leave being scheduled at Christmas and Easter (April) and 3-week block leave in the summer (August). Recruits were provided with a study brief during week 1 of training. Written informed consent was obtained from volunteer recruits ($n=1090$); $n=23$ recruits declined to participate in the study. A further eight recruits were discharged within the first 2 weeks as medically unfit for service due to pre-existing medical conditions, leaving 1082 participants. The study was approved by the UK Ministry of Defence Research Ethics Committee and was conducted in accordance with the ethical standards of the Declaration of Helsinki. All volunteers had passed the medical examination and met the minimum aerobic and strength physical fitness standards prior to commencing RM training; this was reconfirmed during week 1 of training.

Anthropometry and physical fitness

In week 2, each recruit underwent measurement of height (Invicta, Leicester, UK) and body weight in shorts and t-shirt to the nearest 0.1 kg (Seca, Hamburg, Germany). Body mass index (BMI) was calculated. Maximum oxygen uptake (VO_{2max}), as an index of aerobic fitness, was estimated from the Multi-Stage Fitness Test undertaken as part of the *Royal Marine Fitness Assessment* completed in week 1 of training.

Smoking

Self-reported smoking status was assessed from a questionnaire administered in week 2 of training. Recruits reported whether they were a current cigarette smoker, an ex-smoker, or had never smoked cigarettes.

Stress fracture diagnosis

Recruits reporting to the CTCRM Medical Centre with symptoms of a potential stress fracture underwent examination and X-ray or magnetic resonance imaging (MRI) scanning to confirm stress fracture diagnosis. Stress fracture diagnosis was based on a positive X-ray or MRI scan. Depending on the fracture site, a negative initial X-ray was followed up by a further X-ray or MRI to confirm diagnosis. The date of reporting to CTCRM Medical Centre was taken as the date of injury. All recruits with stress fractures were removed from RM training and underwent rest and rehabilitation in situ under medical supervision.

Serum analyses

A 7-mL non-fasted blood sample was drawn by medical personnel, using serum separation vacutainers at week 1, week 15, and week 32 of training. These samples were left to clot for 1 h, after which they were centrifuged (at 5000 rpm for 15 min within 1–2 h of sample draw). The serum was subsequently aspirated and initially stored in situ in plastic 1.5-mL Eppendorf tubes at -20°C , prior to transportation in a frozen state from CTCRM to the Institute of Naval Medicine, Gosport, UK, where samples were stored at -80°C prior to analysis. Start of training serum samples were analysed at the Specialist Assay laboratory, Clinical Biochemistry, Manchester Royal Infirmary for 25(OH)D by liquid chromatography tandem mass spectrophotometry (LC-MS/MS) using an ABSciex 5500 tandem mass spectrophotometer (AB Sciex UK Ltd., Warrington, UK) and the MassChrom[®] 25OHD3/D2 kit for LC-MS/MS (Chromsystems Instruments and Chemicals GmbH, Gräfelfing, Germany) following the manufacturers' instructions (laboratory intra- and inter-assay coefficient of variation (CV) 3.7 and 4.8 % respectively). The laboratory is accredited by Clinical Pathology Accreditation UK (CPA number 0865). Middle and end of training serum samples were analysed for 25(OH)D at University Hospital Birmingham, UK. 25(OH)D was analysed by liquid chromatography separation followed by tandem mass spectrometry (Waters 2795 Quattro Premier XE system, Waters Corporation, Milford, MA, USA). Both laboratories have been certified as proficient by the Vitamin D External Quality Assessment Scheme (DEQAS). Its objective is to ensure the analytical reliability of 25(OH)D and 1,25 dihydroxyvitamin D (1,25(OH)₂D) assays. Both laboratories have a % bias from the target value of less than 10 %. A total of 25 samples were analysed through DEQAS comparing the two labs to ensure comparability of the measurements. The mean values were 63.52 ± 4.96 nmol/L for the Birmingham and 64.91 ± 5.24 nmol/L for the Manchester labs. The correlation coefficient was $r=0.97$, $p<0.0001$, and there is no significant difference between the measured values for the 25 samples analysed between April 2013 and April 2014 ($p=0.796$).

All samples were analysed for parathyroid hormone (PTH) by an automated immunoassay performed on a Roche Modular system (Roche Diagnostics, Indianapolis, IN, USA) at University Hospital Birmingham, UK. The CV for PTH analysis is 4.8 % at this site.

Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS; Version 20, 2011). Data were checked for normality using the Kolmogorov–Smirnov test. Any uncertainties arising were further checked using graphical methods and standardised skewness and kurtosis

values to see where the violation of normality occurred and whether it was sufficiently large to cause problems with subsequent analysis. The only variable to violate normality was age. A Mann–Whitney U test was performed to compare differences in age between the groups. For all other variables, independent t tests were performed to evaluate differences between stress fractured recruits and non-fractured control recruits. Two-way mixed design (time of training by group) analysis of co-variance (ANCOVA) was undertaken to compare 25(OH)D and PTH concentrations between stress fractured recruits and non-fractured controls, after adjustment for body weight and aerobic fitness. The repeated measures factor assessed the differences in 25(OH)D and PTH across the 32 weeks of RM training (i.e. weeks 1, 15 and 32). Chi-square tests and point biserial correlations were administered to assess for associations between pairs of categorical variables, or between one categorical variable and one continuous variable. Seasons were defined as spring (21 Mar to 20 Jun), summer (21 Jun to 20 Sep), autumn (21 Sep to 20 Dec) and winter (21 Dec to 20 Mar).

A sub-set of stress fracture cases ($n=75$) were retrospectively matched for age, height, body weight and fitness to recruits ($n=75$) who did not stress fracture during training. Paired t tests were performed to evaluate differences between stress fractured recruits and non-fractured controls. Fisher's exact one-tailed test was used to explore differences in the incidence of stress fracture using a threshold of 25(OH)D of 50 nmol L^{-1} .

Results

25(OH)D, PTH and stress fracture

The 25(OH)D and PTH status of recruits who fractured and those who did not fracture are presented in Table 1. There were no differences in 25(OH)D or PTH at week 1 between stress fracture recruits and non-fracture recruits when

Table 1 Serum 25(OH)D and PTH in recruits with and without stress fracture

	25(OH)D (nmol L ⁻¹)		PTH (pmol L ⁻¹)	
	With fracture	Without fracture	With fracture	Without fracture
Week 1	64.2 ± 28.2 ($n=75$)	69.6 ± 29.3 ($n=940$)	3.37 ± 1.36 ($n=42$)	3.08 ± 1.07 ($n=457$)
Week 15	68.8 ± 27.7 ($n=52$)	69.8 ± 26.1 ($n=405$)	3.29 ± 1.01 ($n=53$)	3.25 ± 1.12 ($n=407$)
Week 32	$44.5 \pm 22.9^*$ ($n=17$)	56.8 ± 22.4 ($n=365$)	3.77 ± 1.45 ($n=17$)	3.38 ± 0.94 ($n=367$)

25(OH)D 25-hydroxyvitamin D, PTH parathyroid hormone

*Significantly lower than recruits without fracture ($p<0.05$)

controlled for body weight and fitness (ANCOVA analyses). Using a threshold of 50 nmol L^{-1} for 25(OH)D, recruits with a serum 25(OH)D concentration below 50 nmol L^{-1} at week 1 had a higher incidence of stress fracture during RM training than recruits with 25(OH)D concentration above this threshold at week 1 ($\chi^2_{(1)}=3.564$, $p=0.042$; Fisher's exact one-tailed test). Compared with a 25(OH)D of at least 50 nmol L^{-1} at the start of training, the odds ratio (OR) for stress fracture if 25(OH)D was less than 50 nmol L^{-1} was 1.6 (95 % confidence interval (CI) 1.0–2.6). Recruits who stress fractured within the first 10 weeks of training had a baseline serum 25(OH)D of $49.5 \pm 18.7 \text{ nmol L}^{-1}$ ($n=8$) compared with $66.4 \pm 29.2 \text{ nmol L}^{-1}$ ($n=70$) for those who fractured later in training ($p=0.043$). Serum 25(OH)D concentration was lower at week 32 in the fractured recruits than in the non-fractured recruits ($44.5 \pm 22.9 \text{ nmol L}^{-1}$ ($n=17$) compared with $56.8 \pm 22.4 \text{ nmol L}^{-1}$ ($n=365$); $p<0.05$). However, there were differences in the seasonal distribution of when fractured and non-fractured recruits completed training (week 32 blood sample taken). (Season for week 32: fractured—spring 6 %, summer 6 %, autumn 47 % and winter 41 %; non-fractured—spring 8 %, summer 22 %, autumn 40 % and winter 31 %.)

Vitamin D and PTH across training

Figure 1 illustrates the variation in baseline (week 1) serum 25(OH)D and PTH by month in which recruits started RM training. Baseline serum 25(OH)D varied from a low of $47.0 \pm 23.7 \text{ nmol L}^{-1}$ in recruits who started RM training in February,

to a high of $97.3 \pm 24.6 \text{ nmol L}^{-1}$ in recruits who started training in July (overall mean $69.2 \pm 29.2 \text{ nmol L}^{-1}$, $n=1016$). Conversely, baseline serum PTH was lowest in recruits who started training in September ($2.6 \pm 0.7 \text{ pmol L}^{-1}$) and highest in those who started training in March ($3.8 \pm 1.3 \text{ pmol L}^{-1}$).

When changes in 25(OH)D were investigated across training (2×3 mixed design ANCOVA), there was a decrease in 25(OH)D between weeks 15 and 32 ($F_{(1,350)}=3.925$, $p=0.048$). Neither body weight ($p=0.051$) nor aerobic fitness ($p=0.950$) was significant covariates. Similar analysis, controlling for body weight and fitness, showed no effect of time on PTH concentration. There were weak inverse correlations between serum 25(OH)D and serum PTH concentrations at week 15 ($r=-0.209$, $p<0.001$) and week 32 of training ($r=-0.214$, $p<0.001$), but not at baseline.

Stress fracture

Seventy-eight recruits (7.2 %) suffered a total of 92 stress fractures. The number of stress fractures reported per week of training is presented in Fig. 2. Five recruits suffered two fractures, three recruits suffered three fractures, and one recruit suffered four stress fractures during RM training. One stress fractured recruit was Afro-Caribbean, two were mixed race Afro-Caribbean/Caucasian, and the remainder were Caucasian. The physical characteristics of fractured and non-fractured recruits are presented in Table 2. There was no association between the month recruits started training and the occurrence of stress fracture. Similarly, there was no

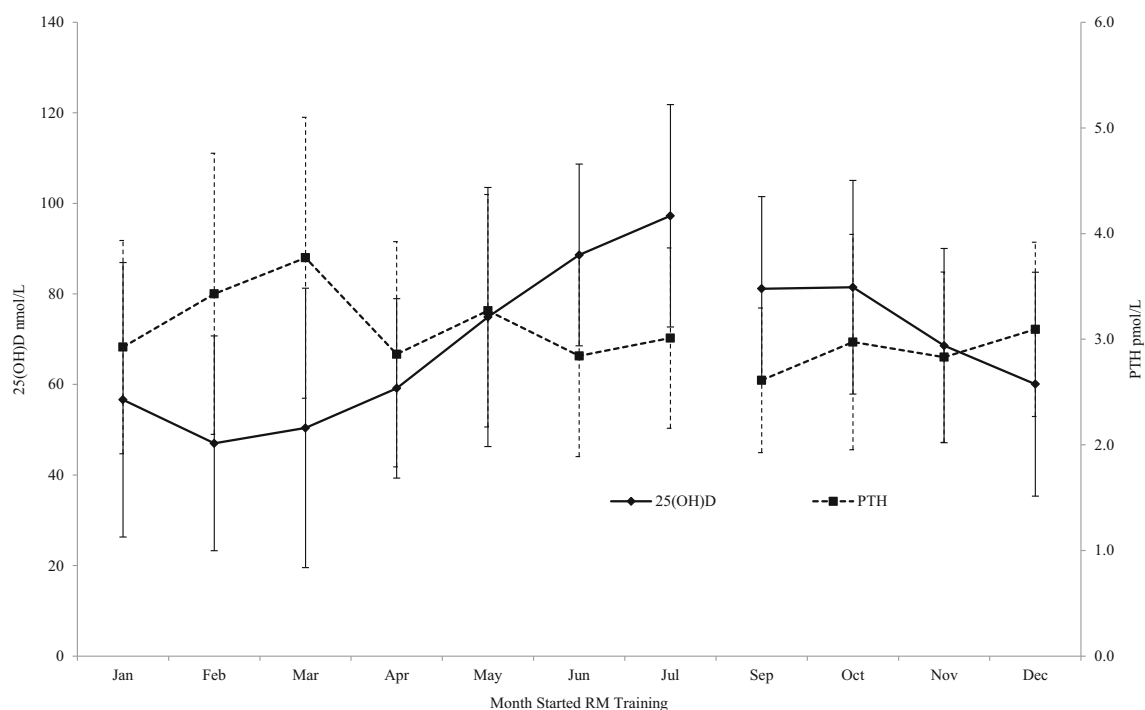


Fig. 1 Serum 25(OH)D and PTH concentrations for Royal Marine recruits at the start of training (week 1; baseline) across the RM training year (Mean and SD). Note: No troops started training in August due to summer block leave

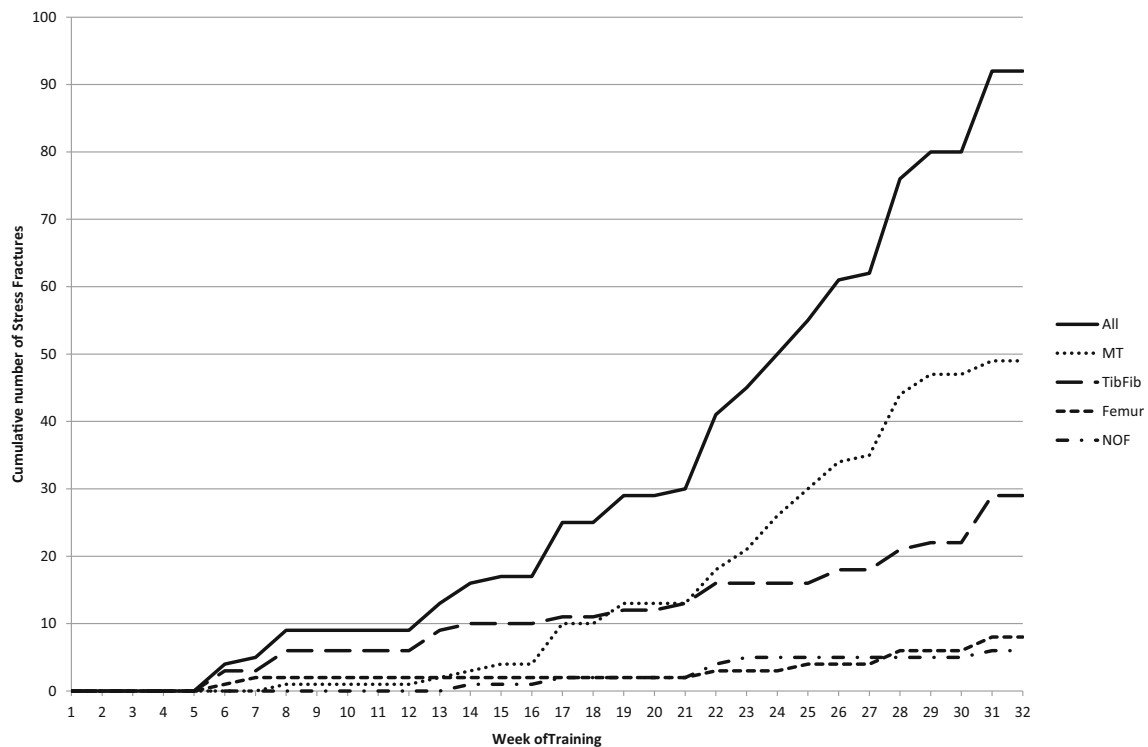


Fig. 2 Cumulative stress fractures during Royal Marine training. Note: *MT* metatarsal, *Tib/Fib* tibia or fibula, *NOF* neck of femur

discernible pattern in the occurrence of stress fracture in relation to the season a recruit joined CTCRM.

Training outcome

From the 1082 recruits participating in the study, 347 (32 %) completed training with their original troop (i.e. in 32 weeks), 255 (24 %) completed training with a later troop (due to illness, injury or professional reasons), and 480 (44 %) did not complete training. Reasons for a recruit not completing

training included withdrawing themselves from training, failing to pass physical or professional standards, or due to an injury which could not be fully rehabilitated such that a recruit would be unable to continue with a RM career.

Case-control matched pairs

Seventy-five of the stress fracture cases were retrospectively matched for age, body weight, height and aerobic fitness at baseline with recruits who did not stress fracture during RM

Table 2 Royal Marine recruit participant characteristics at week 1 of RM training (baseline)

	With stress fracture (<i>n</i> =78) Mean ± SD	Without stress fracture (<i>n</i> =954) Mean ± SD	<i>p</i>
Age (years) ^a	20 (6)	20 (4)	0.891 ^b
Height (m)	1.77±0.06	1.77±0.06	0.483
Body weight (kg)	72.6±7.7	74.9±7.6	0.013
BMI (kg m ⁻²)	23.3±2.0	23.8±2.0	0.014
VO _{2max} (mL kg ⁻¹ min ⁻¹) ^c	52.2±3.0	52.9±3.2	0.093
History of cigarette smoking (current and ex-smokers), <i>n</i> (%)	31 (39.7 %)	321 (33.8 %)	0.287 ^d
Current smoker, <i>n</i> (%)	6 (7.7 %)	115 (11.5 %)	0.254 ^d
Ex-smoker, <i>n</i> (%)	25 (32.1 %)	203 (20.2 %)	0.026 ^d

SD standard deviation, *BMI* body mass index, *VO_{2max}* maximum oxygen uptake

^aMedian (IQR)

^bMann–Whitney *U* Test

^cFor VO_{2max} *n*=71 stress fracture, *n*=901 non-fracture

^dChi-square test

training. There were no differences in age, body weight, height or aerobic fitness between the two recruit groups (Table 3). The stress fracture group had a lower 25(OH)D concentration compared with the non-stress fracture group (64.19 ± 28.18 vs. 78.58 ± 35.90 nmol L⁻¹; $t_{(74)}=2.970$, $p=0.004$). In this sub-sample, those recruits with a serum 25(OH)D concentration below 50 nmol L⁻¹ at week 1 had a higher incidence of stress fracture during RM training than recruits with 25(OH)D concentration above this threshold at week 1 ($\chi^2_{(1)}=5.365$, $p=0.016$; Fisher's exact one-tailed test). The OR for stress fracture if 25(OH)D was less than 50 nmol L⁻¹ was 2.3 (95 % CI 1.1–4.8) times the risk of fracture when 25(OH)D was at least 50 nmol L⁻¹ at the start of training. Using a threshold of 75 nmol L⁻¹, the OR was 1.9 (1.0–3.7).

Discussion

To the authors' knowledge, this is the largest reported cohort study prospectively investigating a threshold for serum 25(OH)D concentration in relation to risk of stress fracture in a military population, and the first in the UK. The results are consistent with our hypothesis that recruits with a poor vitamin D status at the start of RM training (serum 25(OH)D concentration below 50 nmol L⁻¹) were at increased risk of developing a stress fracture during the 32 weeks of training (OR=1.6).

Threshold of 25(OH)D for stress fracture risk

This increased risk of stress fracture with low serum 25(OH)D was consistent with findings from previous studies [14–16]. However, the threshold of 50 nmol L⁻¹ for reduced risk of fracture is lower than reported for other military populations [15, 16] but is consistent with the Institute of Medicine's definition of vitamin D sufficiency.[4] In Finnish male military

recruits ($n=22$ stress fractures), the risk of stress fracture was 3.6 times greater if serum 25(OH)D concentrations were below the median (75.8 nmol L⁻¹) at the start of training (July), compared with the risk if concentrations exceeded this population-specific median [16]. A threshold of 75 nmol L⁻¹ in the case–control analysis in the present study resulted in an almost doubling of risk of fracture (OR 1.9). A case–control study in female US Navy recruits reported that stress fracture risk was lowest in those recruits with a serum 25(OH)D concentration in the highest quintile (mean 125 nmol L⁻¹) around the time of fracture [15]. These authors suggest a target serum 25(OH)D concentration of 100 nmol L⁻¹ in female Navy recruits to reduce stress fracture risk.

In contrast, a recently reported study in US Military Academy cadets reported no differences in anthropometric measures, serum 25(OH)D, nor PTH concentrations at the start of training (July) between cadets who stress fractured during the 4-year programme and those who did not fracture (in both male and female cadets) [17]. However, the authors did not report the incidence of fracture in relation to a threshold of serum 25(OH)D. Consistent with the above study and others [12, 25], the present study found no difference in mean baseline serum 25(OH)D concentration between recruits who stress fractured during training and those who did not. These findings together would suggest that mean serum 25(OH)D status does not routinely differ at baseline between recruits susceptible to stress fracture and those that do not fracture during military training. However, stress fracture risk increases when serum 25(OH)D is below a threshold concentration. The higher OR in the case–control sub-analysis (at a threshold of 50 nmol L⁻¹, OR for matched analysis was 2.3 compared with 1.6 for the entire cohort) is likely due to the influence of body weight and aerobic fitness on the risk of stress fracture—factors which were matched in this sub-analysis.

Differences in thresholds for 25(OH)D associated with reduced fracture risk may be due to differences in the military

Table 3 Physical characteristics of 75 matched pairs of stress fractured and non-fractured recruits (matched for age, height, weight and aerobic fitness at baseline)

	With stress fracture ($n=75$) Mean \pm SD	Without stress fracture ($n=75$) Mean \pm SD	p
Age (years) ^a	21 (6)	21 (6)	0.959
Height (m)	1.77 \pm 0.06	1.77 \pm 0.06	0.689
Body weight (kg)	72.7 \pm 7.6	74.2 \pm 6.7	0.198
BMI (kg m ⁻²)	23.3 \pm 1.9	23.6 \pm 1.8	0.202
VO _{2max} (mL kg ⁻¹ min ⁻¹)	52.3 \pm 3.1	52.6 \pm 3.0	0.593
25(OH)D (nmol L ⁻¹)	64.2 \pm 28.2	78.6 \pm 35.9	0.004 ^b
PTH	3.21 \pm 1.40	3.37 \pm 1.40	0.630

SD standard deviation, BMI body mass index, 25(OH)D 25-hydroxyvitamin D, PTH parathyroid hormone, VO_{2max} maximum oxygen uptake

^a Median (IQR)

^b t test ($t_{(74)}=2.970$, $p=0.004$)

training programme undertaken, ultraviolet radiation B (UVB) exposure, and gender. Males have a lower risk of stress fracture compared with females [17, 26–28], such that the threshold of 25(OH)D below which stress fracture risk increases may well differ. To date, this hypothesis has not been investigated. In the only supplementation trial reported in the literature, supplementation of female Navy recruits with 2000 mg calcium and 800 IU vitamin D per day resulted in a 20 % lower incidence of stress fracture compared with a control group [18]. Vitamin D status was not assessed in this study, such that it was not possible to identify the proportion of the study population with poor vitamin D status at the start of the intervention, nor a threshold for serum 25(OH)D which was associated with reduced fracture risk.

An interesting finding from the present study was the lower serum 25(OH)D concentration at the end of training in the fractured recruits compared with that in the non-fractured recruits. A possible explanation for this could be the seasonal distribution of when fractured and non-fractured recruits completed training (week 32 blood sample taken). (Season for week 32: fractured—spring 6 %, summer 6 %, autumn 47 % and winter 41 %; non-fractured—spring 8 %, summer 22 %, autumn 40 % and winter 31 %).

Seasonal variation in 25(OH)D

A major difference to previous studies is that the present study involved recruits who started training over a 12-month period rather than at a single time point. As expected, there was variation in mean serum 25(OH)D concentration according to the month recruits started training. The mean circulating serum 25(OH)D concentrations in RM recruits starting training in the summer and winter seasons were consistent with those reported previously for young adults in Northern Europe [29]. In the UK, vitamin D deficiency is currently defined as a serum 25(OH)D concentration of $<25 \text{ nmol L}^{-1}$. [30] Applying this cutoff to data reported from the present study, RM recruits had a lower prevalence of 25(OH)D deficiency compared with males aged 19–24 years in the general UK population (i.e. 4 vs. 24 %) [31]. However, this threshold for deficiency does not inform optimal 25(OH)D levels, or the concentration below which the risk of stress fracture may be increased in individuals engaged in sport or military physical training.

Some recruits reported having taken an overseas holiday in the weeks immediately before starting RM training, thus increasing UVB exposure, and this may partly explain the variation in the start of training 25(OH)D status—especially in the months when 25(OH)D status is usually poor in the UK [31]. During the 8-month training programme, recruits experienced the usual seasonal variations in vitamin D status observed in the UK population, due to the inability to endogenously synthesise vitamin D from the action of the sunlight in

the winter months at the UK latitude of 50° – 60° N (October to March) [30].

25(OH)D and PTH across training

Consistent with findings from the present study, a decrease in serum 25(OH)D has previously been reported during shorter cycles of basic military training in male and female recruits [21–23]. The mechanism behind a decrease in vitamin D status during training is far from clear. Recruits undertake at least 50 % of their training outside, but exposure of the skin to sunlight may be limited due to the clothing and protective equipment worn. There was no effect of time on PTH concentration in the present study in contrast to previous studies in female recruits [21, 23]. PTH concentrations decreased after 2 months of military training in male Israeli recruits but returned to near baseline after 4 months [22]. The reasons for this difference remain unclear, and the findings suggest a complex relationship between PTH, 25(OH)D, other factors involved in bone metabolism and the response of bone to the physical challenge of military training.

Vitamin D deficiency is associated with secondary hyperparathyroidism [29]. However, there have been mixed findings in identifying a threshold in serum 25(OH)D concentration below which PTH concentration begins to rise [19, 32–34]. Data from the present study were similarly mixed with regards to the inter-relationship between serum PTH and 25(OH)D. There was no relationship between PTH and serum 25(OH)D evident at the start of training, but inverse correlations were identified at week 15 and week 32. Higher PTH, without lower 25(OH)D concentration, has previously been associated with increased risk of stress fracture in male military recruits [12]. This study did not investigate a threshold below which stress fracture risk increased, probably due to the low number of fracture cases ($n=15$) [12].

Other risk factors for stress fracture

Low body weight is frequently reported as a risk factor for stress fracture during military training [10, 14, 28]. This was supported in the present study, despite a minimum body weight of 65 kg for entry into RM recruit training leading to censored body weight data. There was a tendency for a lower baseline aerobic fitness between recruits who stress fractured during training and those who did not fracture ($p=0.93$), consistent with many studies in military recruits [12, 16, 26, 27, 35]. However, other studies have reported no difference in fitness [17, 36]. The high fitness standard on entry to RM training offers a possible explanation for the lack of difference between the stress fractured recruits and non-fractured recruits in the present study.

Limitations

A limitation of the study was that a blood sample could not be taken at the time of fracture due to time and logistical constraints. Burgi et al. reported the lowest incidence of stress fractures of the tibia or fibula in female US Navy recruits in the highest quintile for 25(OH)D concentration determined from a blood sample drawn near the time of fracture [15]. In the present study, there was a greater reduction in 25(OH)D during the second half of training in recruits who fractured compared with those who did not fracture, supporting the hypothesis that 25(OH)D status across training may influence stress fracture risk. RM training is exclusively open to male recruits. Findings from the present study may not be applicable to females, who are more susceptible to stress fracture [17, 26–28].

A further limitation was the necessity to use two different labs for our 25(OH)D measurements. The change in laboratories with respect to the study was somewhat out of our control but rather related to MoD policy of laboratory use and concomitant costs. However, as detailed in the “Materials and methods” section, both laboratories are participants of DEQAS and there is excellent agreement between the laboratories for their respective analyses of 25(OH)D status, with both Manchester and Birmingham having a % bias from the target value of <10 %. We are very confident that our findings were true results and not due to differences in methodology measurements.

In conclusion, serum 25(OH)D concentration, as a marker of vitamin D status, below 50 nmol L⁻¹ at the start of training was associated with an increased risk of stress fracture during RM recruit training. Vitamin D represents an easily modifiable risk factor for stress fracture. Further studies into the effects of vitamin D supplementation on stress fracture risk are firmly warranted.

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Conflicts of interest None.

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