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Vitamin D metabolites are associated with musculoskeletal injury in young adults: a prospective cohort study

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Vitamin D metabolites are associated with musculoskeletal injury in young adults: a prospective cohort study

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ABSTRACT

The relationship between vitamin D metabolites and lower body (pelvis and lower limb) overuse injury is unclear. In a prospective cohort study, we investigated the association between vitamin D metabolites and incidence of lower body overuse musculoskeletal and bone stress injury in young adults undergoing initial military training during all seasons. In 1637 men and 530 women (age, 22.6 ± 7.5 years; BMI, 24.0 ± 2.6 $kg \cdot m^{-2}$; 94.3% white ethnicity), we measured serum 25-hydroxyvitamin D (25(OH)D) and 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) by high-performance liquid chromatography tandem mass spectrometry, and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) by immunoassay during week 1 of training. We examined whether the relationship between 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$ ratio was associated with overuse injury. During 12 weeks training, 21.0% sustained ≥1 overuse musculoskeletal injury, and 5.6% sustained ≥1 bone stress injury. After controlling for sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course (Officer, standard, or Infantry), lower body overuse musculoskeletal injury incidence was higher for participants within the second lowest *versus* highest quartile of $24,25(OH)_2D$ (OR: 1.62 [95%CI 1.13–2.32; $P = 0.009$]) and lowest *versus* highest cluster of 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$ (OR: 6.30 [95%CI 1.89–21.2; $P = 0.003$]). Lower body bone stress injury incidence was higher for participants within the lowest *versus* highest quartile of $24,25(OH)_2D$ (OR: 4.02 [95%CI 1.82–8.87; $P < 0.001$]) and lowest *versus* highest cluster of 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$ (OR: 22.08 [95%CI 3.26–149.4; $P = 0.001$]), after controlling for the same covariates. Greater conversion of 25(OH)D to $24,25(OH)_2D$, relative to $1,25(OH)_2D$ (*i.e.*, low $1,25(OH)_2D$:$24,25(OH)_2D$), and higher serum $24,25(OH)_2D$ were associated with a lower incidence of lower body overuse musculoskeletal and bone stress injury. Serum $24,25(OH)_2D$ may have a role in preventing overuse injury in young adults undertaking arduous physical training.

Key words: Vitamin D, injury, nutrition, exercise, musculoskeletal.

INTRODUCTION

Vitamin D, mainly obtained from sunlight (by exposure of dermal 7-dehydrocholesterol to ultraviolet B radiation) and to a lesser extent from dietary sources (*e.g.*, oily fish or vitamin D supplements), is hydroxylated by 25-hydroxylase to 25-hydroxyvitamin D (25(OH)D). Serum 25(OH)D is the most abundant vitamin D metabolite,[1] and is used to determine an individual's vitamin D status.[2] The most widely recognised function of vitamin D is to maintain calcium and phosphate homeostasis,[1,2] and thereby promote bone mineralisation essential for the development and maintenance of skeletal health.[3] Vitamin D increases the absorption of intestinal calcium, which is maximised at the threshold for vitamin D sufficiency (serum 25(OH)D ≥50 nmol·L^{-1}).[3] Vitamin D deficiency and insufficiency (25(OH)D <30 and 30–49.9 nmol·L^{-1}) are associated with rickets, low bone mineral density, osteomalacia, and the development of osteoporosis.[3,4] A meta-analysis of randomised controlled trials concluded vitamin D and calcium supplementation reduces fracture risk in older men and women (mean age 66 years).[5] Emerging evidence suggests a role for vitamin D in promoting skeletal muscle repair and remodelling,[6,7] and tendon healing.[8] In young adults, and physically active individuals in particular, the relationship between vitamin D status and overuse injury to bone, muscle, or connective tissues is unclear.[9,10] Athletes and military personnel who sustained a musculoskeletal injury,[11,12] or bone stress injury,[13,14] had lower serum 25(OH)D concentrations than their uninjured peers. However, serum 25(OH)D was not associated with markers of bone health or musculoskeletal injury in other observational studies.[15-19] Serum 25(OH)D may not be the best biomarker of vitamin D status for skeletal health because other measures of vitamin D status were more strongly associated with bone density and fracture risk in other studies.[16,20,21]

The clinical relevance and optimal concentration of 25(OH)D is the subject of ongoing debate.[16,20-23] 25(OH)D is relatively metabolically inactive[2] and is hydroxylated by 1α-

hydroxylase to form biologically active 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). $1,25(OH)_2D$ circulates in picomolar concentrations, with its actions mediated by binding with vitamin D receptors, which are found in most human tissues.[24] 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) is generated from the hydroxylation of 25(OH)D by 24-hydroxylase, and like 25(OH)D, circulates in nanomolar concentrations. $24,25(OH)_2D$ is generally regarded as a vitamin D catabolite, however, possible biological roles in bone development, fracture healing, and protection against cartilage damage have emerged.[25-30] No direct correlation exists between serum 25(OH)D and $1,25(OH)_2D$, but a dynamic relationship between 25(OH)D and $1,25(OH)_2D$ occurs when $1,25(OH)_2D$ is expressed as a ratio with $24,25(OH)_2D$.[31] Serum 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$ has been shown to be associated with physical performance,[32] but existing evidence does not support a beneficial effect of vitamin D supplementation on physical performance or muscle function.[6,33] Whether this inverse exponential relationship between serum 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$ relates to other physiological processes and meaningful outcomes, such as overuse injury, warrants further investigation.

The relationship between vitamin D metabolites (serum 25(OH)D, $1,25(OH)_2D$, and $24,25(OH)_2D$) and overuse injury in young healthy adults is unclear. We used a prospective cohort study to examine the association between vitamin D metabolites and the incidence of lower body (pelvis and lower limb) overuse musculoskeletal and bone stress injury in men and women undertaking military training across all seasons. We hypothesised that a dynamic relationship between vitamin D metabolites incorporating serum 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$ ratio would be associated with lower body overuse musculoskeletal and bone stress injury.

MATERIALS AND METHODS

British Army recruits volunteered to participate in this prospective cohort study between April 2013 and March 2017 and provided informed written consent. All participants were free from injury at the beginning of the study, having passed a physician-screened medical assessment during the first week of training. Initial military training follows a syllabus of basic military skills including physical training, regimental drill, weapon handling, map reading, and fieldcraft. Recruits followed a progressive, structured physical training programme, consisting of endurance and circuit training, agility-based gymnasium work, assault course practice, and marching whilst carrying load. The physical demands of initial military training[34,35] and incidence of lower body overuse injury[36] have been reported previously. The UK Ministry of Defence Research Ethics Committee approved the study (MODREC 165/Gen/10), and all procedures were conducted in accordance with the Declaration of Helsinki (2013). Participants also took part in a wider programme of concurrent studies examining injury, illness, and physical performance in Army recruits undergoing initial military training,[32,37-41] that determined the study size.

Participants

Medical records were available for 2167 of the 2277 British Army recruits who volunteered; therefore, 2167 recruits were eligible and participated in the study (1637 men and 530 women; Table 1). Participants were recruited during the first week of their initial military training from three Army training sites: 403 men and 106 women at Royal Military Academy, Sandhurst, UK (latitude, 51°N; Army Officer training); 424 women at Army Training Centre, Pirbright, UK (latitude, 51°N; Army standard training); and 1234 men at Infantry Training Centre, Catterick, UK (latitude, 54°N; Army Infantry training). By recruiting participants from these three sites, a representative sample of all individuals commencing Army training in the UK were studied. Participants commenced initial military

training during all seasons (20.8% in spring, 26.4% in summer, 31.5% in fall, and 21.3% in winter).

Experimental procedures

During the first week of initial military training, a venous blood sample was obtained for the measurement of vitamin D metabolites (serum 25(OH)D, 1,25$(OH)_2$D, and 24,25$(OH)_2$D), and physical fitness, body mass, and height were measured. Physical fitness was assessed by a maximal effort 2.4 km run. Body mass and height (Seca, Hamburg, Germany) were measured in light clothing and without shoes. Participants self-reported their ethnicity, smoking habits, and bone injury history (bone fracture and bone stress injury), using questionnaires. After 12 weeks of military training, participants' medical records were accessed to obtain a record of clinician-diagnosed lower body overuse musculoskeletal and bone stress injuries.

Blood collection and handling

Whole blood samples were obtained by venipuncture from a prominent vein in the antecubital fossa into serum vacutainers (Becton Dickinson, Oxford, UK). Whole blood was left to clot for 1 h at room temperature before being centrifuged at 1500 *g* for 10 min at 4°C, with serum aliquots immediately frozen at −80°C for later analysis.

Biochemical analysis

Total serum 25(OH)D (25$(OH)D_2$ and 25$(OH)D_3$) and total 24,25$(OH)_2$D (24,25$(OH)_2D_2$ and 24,25$(OH)_2D_3$) were measured with high-performance liquid chromatography tandem mass spectrophotometry using a Micromass Quattro Ultima Pt electrospray ionisation mass spectrometer, as described previously.[42] Serum 1,25$(OH)_2$D was measured by chemiluminescent immunoassay using a DiaSorin LIAISON® XL analyser (Stillwater, Minnesota, USA). The measurement ranges of the assays were 0.1–200 $nmol \cdot L^{-1}$ for

$25(OH)D_2$ and $25(OH)D_3$, 0.8–25 $nmol \cdot L^{-1}$ for $24,25(OH)_2D_2$, 0.1–25 $nmol \cdot L^{-1}$ for $24,25(OH)_2D_3$, and 12–480 $pmol \cdot L^{-1}$ for $1,25(OH)_2D$. Results above the assay upper limit were repeated on dilution to produce a value within the working range of the assay. Values below the lower limit of quantification were not included in the calculation of total 25(OH)D or total $24,25(OH)_2D$. The mean coefficient of variation (CV) for intra-assay imprecision across the measuring range of the assays was 4.9% for $25(OH)D_2$, 8.3% for $25(OH)D_3$, 7.7% for $24,25(OH)_2D_2$, 9.0% for $24,25(OH)_2D_3$, and 7.4% for $1,25(OH)_2D$. The cumulative inter-assay CVs were ≤7.4% for $25(OH)D_2$, ≤9.6% for $25(OH)D_3$, ≤10.6% for $24,25(OH)_2D_2$, ≤8.9% for $24,25(OH)_2D_3$, and ≤9.3% for $1,25(OH)_2D$. Our 25(OH)D and $24,25(OH)_2D$ assays showed <6% accuracy bias against Centers for Disease Control and Prevention's reference method on the Vitamin D External Quality Assessment Scheme (DEQAS), and <9% bias against the method-specific mean for $1,25(OH)_2D$. We met the certification performance standards set by DEQAS throughout the time the analyses were performed. All biochemical analyses were undertaken by the Good Clinical Laboratory Practice and DEQAS certified Bioanalytical Facility at the University of East Anglia.

Lower body overuse musculoskeletal and bone stress injury

Injuries reported to the medical centre by participants during military training were diagnosed by clinicians and recorded in their medical records. Defence General Practitioners and physiotherapists diagnosed injuries by taking detailed histories, conducting physical examinations, and referring recruits for imaging and further investigations, where necessary (*e.g.*, magnetic resonance imaging, X-ray). For all lower body overuse musculoskeletal and bone stress injuries sustained during the first 12 weeks of military training, the diagnosis and number of lost training days (lost in full or on reduced duties), and date of diagnosis (standard and Infantry training) were retrieved from medical records by a physician independent of their clinical care. The number of lost training days was counted until a

participant either returned to full training in their original platoon or were transferred to another platoon to complete missed training, for physical rehabilitation, or for discharge from the Army. The outcome of training for each participant was categorised as either a pass, transfer to another platoon for physical rehabilitation, medical discharge from the Army, or discharge from the Army for non-medical reasons. Lower body bone stress injuries were recorded separately from lower body overuse musculoskeletal injuries. Overuse musculoskeletal injuries were defined as pain, inflammation, or a functional disorder that involved joints, muscles, tendons, ligaments, and associated connective tissues, with the mechanism of injury a result of use over time, rather than a traumatic event[36] (*e.g.*, plantar fasciitis, Achilles tendinopathy, patellar tendinitis, iliotibial band syndrome). Bone stress injuries encompassed the spectrum of overuse injuries caused by microdamage accumulation in bone, frequently referred to as stress reactions and stress fractures[43] (*e.g.*, medial tibial stress syndrome, femoral, tibial, calcaneal, and metatarsal stress fractures).

Statistical analysis

Potential relationships between vitamin D metabolites and overuse injury were explored by first quantifying associations with injuries sustained at any point during initial military training using logistic regression, and second by quantifying injury rates using Cox proportional hazards regression. Concentrations of vitamin D metabolites were categorised using the following approaches: 1) vitamin D status for 25(OH)D, using commonly accepted thresholds (deficient <30; insufficient 30–49.9; sufficient ≥50 nmol·L^{-1});[3] and in the absence of equivalent definitions for other metabolites, 2) quartiles for 1,25$(OH)_2$D and 24,25$(OH)_2$D; and 3) clustering participants into groups based on two dimensions, simultaneously, of serum 25(OH)D and 1,25$(OH)_2$D:24,25$(OH)_2$D ratio. For all analyses, the reference category was the category containing the highest concentration of metabolites, in keeping with the rationale that higher concentrations would be beneficial for musculoskeletal health. Clustering was

performed using a standard k-means technique and the Bayesian information criterion (BIC) to select the number of clusters.[44] Briefly, clusters were considered representative of a Gaussian mixture model, with the BIC calculated using penalised maximum likelihood to identify the number of clusters that best predicted the observed data whilst penalising for adding more clusters. Once the number of clusters was selected, the Hartigan and Wong algorithm was used to assign individual data points by minimising the within-group sum of squares based on Euclidean distances.[44] This clustering technique has been used previously to analyse associations between vitamin D metabolites and physical performance.[32]

Adjusted regression models controlled for covariates previously shown to be associated with injury risk during military training (sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course (Officer, standard, or Infantry)).[45-47] Tests of differences between Army training courses (Officer, standard, or Infantry, stratified by sex), seasons, and lower body injury incidence status (overuse musculoskeletal injury, bone stress injury, or no overuse injury) were conducted with Kruskal-Wallis tests for continuous variables and Chi-squared tests for categorical variables. Where significant differences were observed, *post hoc* analysis for pairwise comparisons were undertaken. All statistical tests were conducted using the R environment, version 4.2.1 (R Core Team), with Cox proportional models conducted with the 'survival' package.[48] Statistical significance was accepted at $P < 0.05$.

RESULTS

Demographic, anthropometric, physical fitness, lifestyle behaviour, and bone fracture history characteristics were different between participants commencing different Army training courses ($P < 0.05$, Table 1). 74.1% of participants passed initial military training, 8.4% were transferred from their original platoon to another for physical rehabilitation, 5.3% were medically discharged from the Army, and 12.2% were discharged from the Army for non-medical reasons.

Vitamin D metabolites and season

There was some seasonal variation in vitamin D metabolites ($P < 0.001$, Table 2). Across all seasons, 12.0% of participants were vitamin D deficient, 23.7% were insufficient, and 64.3% were sufficient. During winter, 31.0% of participants were vitamin D deficient, 41.0% were insufficient, and 28.0% were sufficient.

Lower body overuse musculoskeletal and bone stress injury incidence

Overuse musculoskeletal injury

During 12 weeks training, 21.0% of participants sustained ≥1 lower body overuse musculoskeletal injury. The incidence of lower body overuse musculoskeletal injury was higher in men undertaking Officer training, compared with men undertaking Infantry training; higher in women undertaking Officer training compared with men undertaking Infantry training; and higher in women undertaking standard training compared with men undertaking Infantry training ($P < 0.05$, Table 3).

Bone stress injury

During 12 weeks training, 5.6% of participants sustained ≥1 lower body bone stress injury. The incidence of lower body bone stress injury was higher in women undertaking standard

training, compared with men undertaking Officer training, and higher in men undertaking Infantry training compared with men undertaking Officer training ($P < 0.05$, Table 3).

Lost training days

Overuse musculoskeletal injury

Each lower body overuse musculoskeletal injury resulted in a median (interquartile range (IQR)) 6 (3–12) lost training days. The number of lost training days due to lower body overuse musculoskeletal injury was higher in men undertaking Infantry training, compared with women undertaking standard training ($P < 0.05$, Table 3).

Bone stress injury

Each lower body bone stress injury resulted in a median (IQR) 20 (10–25) lost training days. The number of lost training days due to lower body bone stress injury was higher in men undertaking Infantry training, compared with women undertaking standard training ($P < 0.05$, Table 3).

Vitamin D metabolites and overuse injury

The prevalence of vitamin D insufficiency was higher in participants who sustained a lower body overuse musculoskeletal injury than those who were not injured ($P < 0.05$, Table 4). Serum $1,25(OH)_2D$ and $1,25(OH)_2D$:$24,25(OH)_2D$ concentrations were higher in participants who sustained a lower body bone stress injury, compared with participants who sustained a lower body overuse musculoskeletal injury and those who were not injured ($P < 0.05$, Table 4). All covariates (except smoking and bone stress injury history) were different between participants categorised by whether they sustained a lower body overuse musculoskeletal injury, bone stress injury, or were not injured ($P < 0.05$, Table 4).

Vitamin D metabolite relationships with overuse injury incidence and risk

Overuse musculoskeletal injury

The incidence of lower body overuse musculoskeletal injury was higher for participants within quartile 2 *versus* the highest quartile of $24,25(OH)_2D$; and quartile 3 *versus* the highest quartile of $24,25(OH)_2D$, after controlling for sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course ($P < 0.01$). The incidence of lower body overuse musculoskeletal injury was also higher for participants within cluster 1 *versus* the highest cluster of 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$; cluster 3 *versus* the highest cluster; and cluster 4 *versus* the highest cluster, after controlling for the same covariates ($P < 0.05$, Table 5).

The risk of lower body overuse musculoskeletal injury at any given time during military training was higher for participants within quartile 1 *versus* the highest quartile of $24,25(OH)_2D$; quartile 2 *versus* the highest quartile of $24,25(OH)_2D$; and quartile 3 *versus* the highest quartile of $24,25(OH)_2D$, after controlling for sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course ($P < 0.05$). The risk of lower body overuse musculoskeletal injury at any given time during military training was also higher for participants within cluster 1 *versus* the highest cluster of 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$, after controlling for the same covariates ($P < 0.05$, Table 6 and supplemental Figure 1).

Bone stress injury

The incidence of lower body bone stress injury was higher for participants within quartile 1 *versus* the highest quartile of $24,25(OH)_2D$; and quartile 2 *versus* the highest quartile of $24,25(OH)_2D$, after controlling for sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course ($P < 0.05$). The incidence of lower body bone stress injury was also higher for participants within cluster 1 *versus* the highest cluster of 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$; and cluster 3 *versus* the highest cluster, after controlling for the same covariates ($P < 0.05$, Table 5).

The risk of lower body bone stress injury at any given time during military training was higher for participants within quartile 1 *versus* the highest quartile of 24,25$(OH)_2$D; and quartile 2 *versus* the highest quartile of 24,25$(OH)_2$D, after controlling for sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course ($P < 0.05$, Table 6 and supplemental Figure 2).

Relative concentrations of vitamin D metabolites and proportion who sustained an overuse injury

The dynamic relationship between vitamin D metabolites is illustrated in Figure 1, with clusters 1 to 6 (lowest to highest) of 25(OH)D and 1,25$(OH)_2$D:24,25$(OH)_2$D indicated.

Overuse musculoskeletal injury

The proportion of participants who sustained a lower body overuse musculoskeletal injury was higher for participants within cluster 1 *versus* cluster 2, and higher for participants within cluster 3 *versus* clusters 2 and 5 ($P < 0.05$). The number of lost training days was higher for participants within cluster 1 *versus* clusters 2 and 5, and higher for participants within cluster 3 *versus* clusters 2 and 5 ($P < 0.05$, Figure 1A).

Bone stress injury

The proportion of participants who sustained a lower body bone stress injury was higher for participants within cluster 1 *versus* clusters 2, 3, 4, 5, and 6 ($P < 0.05$). The number of lost training days was higher for participants within cluster 1 *versus* clusters 2 and 5, and higher for participants within cluster 3 *versus* clusters 2 and 5 ($P < 0.05$, Figure 1B).

DISCUSSION

Greater conversion of 25(OH)D to 24,25$(OH)_2$D, relative to 1,25$(OH)_2$D (*i.e.*, low 1,25$(OH)_2$D:24,25$(OH)_2$D), and higher serum 24,25$(OH)_2$D, measured at the time of commencing arduous physical training, were associated with a lower incidence of lower body overuse musculoskeletal and bone stress injury in young adults. We controlled for factors associated with injury risk (sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course) as covariates in our regression models. As hypothesised, we demonstrated that a dynamic relationship between vitamin D metabolites incorporating serum 25(OH)D and 1,25$(OH)_2$D:24,25$(OH)_2$D ratio was related to clinician-diagnosed lower body overuse musculoskeletal and bone stress injury. By examining this relationship between vitamin D metabolites, we provide a unique insight into how the vitamin D metabolic pathway is related to overuse injury. To the best of our knowledge, this study is the first to investigate serum 1,25$(OH)_2$D and 24,25$(OH)_2$D relationships with injury in young, physically active adults. These novel data demonstrate that serum 24,25$(OH)_2$D may have a role in protecting against overuse injury in young men and women. Serum 1,25$(OH)_2$D:24,25$(OH)_2$D ratio $\leq$32, or 24,25$(OH)_2$D $\geq$7.7 nmol·L^{-1} when analysed in isolation, may help to protect against lower body overuse musculoskeletal and bone stress injury (32 = maximum 1,25$(OH)_2$D:24,25$(OH)_2$D ratio in the highest cluster; 7.7 nmol·L^{-1} = minimum 24,25$(OH)_2$D concentration in the highest quartile; Table 5).

Higher 25(OH)D has previously been associated with a lower risk of bone stress injury (stress fracture) in military personnel, with control for some covariates.[14,49,50] Others have shown a higher incidence of musculoskeletal injury[11] and self-reported bone fracture (caused by trauma or overuse)[12] in athletes with lower 25(OH)D, but these studies may have overestimated the possible influence of vitamin D because they did not control for any covariates. Our study includes control for multiple covariates, and examines 25(OH)D

together with two other major vitamin D metabolites, rather than analysing 25(OH)D in isolation. Using this approach, we reveal a more nuanced association, with low $1,25(OH)_2D$:$24,25(OH)_2D$ resulting in a lower incidence of overuse musculoskeletal and bone stress injury. Serum $1,25(OH)_2D$:$24,25(OH)_2D$ <35 has been estimated as a threshold for vitamin D sufficiency,[31] which is similar to our threshold of ≤32 for protection against overuse injury. The relationships we report are consistent for three methods of analysis (Tables 5 and 6, and Figure 1). The reason why overuse musculoskeletal injury incidence, for example, was greater in clusters 1, 3, and 4 compared with the highest cluster, but was not greater in cluster 2 compared with the highest cluster remains unclear. This might be explained by interindividual differences during military training, or genetic differences that may influence vitamin D and musculoskeletal pathways.

Serum 25(OH)D and $1,25(OH)_2D$ were not related to overuse injury when examined in isolation from $24,25(OH)_2D$. Serum $1,25(OH)_2D$ does not reflect vitamin D reserves or status because its availability is tightly regulated by parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23).[51] The relatively short half-life and low serum concentration of $1,25(OH)_2D$ mean it is not a suitable marker of vitamin D status. Increased $24,25(OH)_2D$:25(OH)D (independent of vitamin D binding protein)[23] and $24,25(OH)_2D$ were associated with a reduced risk of hip fracture in elderly adults, but 25(OH)D was not associated with risk of hip fracture.[21] Increased $24,25(OH)_2D$:25(OH)D, but not 25(OH)D, was associated with a reduced risk of fracture and slower decline in bone mineral density in elderly adults.[20] We did not measure the bioavailability of vitamin D metabolites in this study, but our findings support the possibility that serum 25(OH)D alone is not the best clinical marker of vitamin D status for predicting injury risk, and that other vitamin D metabolites and their bioavailability need to be considered.

An inverse exponential relationship exists between serum 25(OH)D and $1,25(OH)_2D$:$24,25(OH)_2D$ ratio.[31] When the availability of 25(OH)D decreases, the conversion of 25(OH)D to $24,25(OH)_2D$ and $1,25(OH)_2D$ is reduced, but $24,25(OH)_2D$ production appears to be reduced more, which increases $1,25(OH)_2D$:$24,25(OH)_2D$.[52] Vitamin D deficiency will result in a decrease in serum calcium and release of PTH,[2] which may then lead to an increase in FGF23.[53] Possible effects of elevated FGF23 may help to explain why higher $1,25(OH)_2D$:$24,25(OH)_2D$ was associated with an increased incidence of overuse injury. Examining whether PTH and FGF23 are associated with overuse injury is a logical area for future study. Our novel finding that proportionally greater conversion of 25(OH)D to $24,25(OH)_2D$ relative to $1,25(OH)_2D$ (*i.e.*, low $1,25(OH)_2D$:$24,25(OH)_2D$) was associated with a lower incidence of overuse injury, suggests $24,25(OH)_2D$ is not a purely catabolic metabolite. The existence of $24,25(OH)_2D$ receptors,[25,27,54] and their possible function in the musculoskeletal system needs to be examined.

Mechanisms

Vitamin D supports musculoskeletal health, and may reduce the risk of bone stress injury by its classical effects on the maintenance of calcium and phosphate homeostasis, intracellular calcium, and promoting the mineralisation of osteoid.[1] Bone remodelling can become imbalanced during arduous physical training, resulting in microdamage, weakening of bone, and bone stress injuries.[22] Overproduction of $1,25(OH)_2D$ in sarcoidosis and other granulomatous diseases can cause increased osteoclastic bone resorption and hypercalcaemia.[55] Increasing $24,25(OH)_2D$ (thereby lowering the $1,25(OH)_2D$:$24,25(OH)_2D$ ratio) may protect against these effects and therefore warrants investigation. Rather than being a catabolic waste product, $24,25(OH)_2D$ may be important for normal bone development and integrity. It enhances bone formation and fracture healing in animal models;[26-28,56-58] however, studies are needed to examine if these effects occur in humans.

Beyond bone, 24,25$(OH)_2$D may be involved in cartilage development[29] and prevent articular cartilage damage,[30] supporting its possible role in preventing overuse musculoskeletal injuries.

High 25(OH)D and low 1,25$(OH)_2$D:24,25$(OH)_2$D were associated with greater muscle strength and muscle power, and better endurance performance in the same population of military recruits.[32] Strength training can reduce the risk of overuse musculoskeletal injury.[59] Delaying the onset of fatigue and avoiding its negative effect on lower body biomechanics for longer during training, competition, or military operations could lower the risk of musculoskeletal injury.[60] It may be that vitamin D helps to protect against overuse injury by enhancing muscle strength and performance in endurance type exercise, but a causal effect of vitamin D on physical performance has not been clearly demonstrated.

Perspectives

Key strengths of this study include analysing records of clinician-diagnosed lower body overuse injuries, in a large sample of men and women, and the measurement of lost training days that indicate the severity and burden of injury. This study has limitations. The concentration of vitamin D metabolites (which undergo a seasonal variation) were measured during the first week of training, not at the time that injuries were reported. The availability of vitamin D metabolites at the time of injury, and whether they were associated with injury risk at that time, is, therefore, not known. Nevertheless, our prospective measure of vitamin D metabolites could be valuable for predicting an individual's risk of overuse injury and allow time for preventative action to be taken. This study only reports associations. Further validation is required to examine cause and effect. It may be that higher concentrations of vitamin D metabolites are reflective of a more physically active, outdoor lifestyle before commencing military training, which could reduce the likelihood of sustaining an overuse

injury. We controlled for the effect of physical fitness on injury incidence by including 2.4 km run time as a covariate.[45,46] Most participants in the present study were white; whether vitamin D metabolites are similarly associated with overuse injury in other ethnic groups is not known and requires further study.

Eight weeks vitamin D and calcium supplementation (800 IU·day^{-1} and 2000 mg·day^{-1}) reduced the incidence of stress fracture by 20% in female military recruits,[61] but neither serum 25(OH)D nor any other vitamin D metabolites were measured. Beyond this single study, no randomised controlled trials have assessed the influence of supplementation with vitamin D or its metabolites on injury risk in young adults.[22] Randomised, placebo-controlled trials in otherwise healthy adults at risk of vitamin D deficiency and overuse injury are warranted (*e.g.*, in athletes or military personnel undertaking arduous physical training). Vitamin D and calcium supplementation (1000 IU·day^{-1} and 2000 mg·day^{-1}) was beneficial for bone health during initial military training.[62,63] A negative association between calcium intake and serum 25(OH)D[64] indicates calcium consumption potentially influences vitamin D metabolite ratios. The influence of calcium intake on vitamin D metabolites and overuse injury warrants further investigation.

Whether oral vitamin D supplementation can achieve high 25(OH)D and low $1,25(OH)_2D$:$24,25(OH)_2D$ ratio—and reduce the incidence of overuse injury—remains to be determined. High doses of vitamin D have increased $24,25(OH)_2D$ concentrations and lowered $1,25(OH)_2D$:$24,25(OH)_2D$ ratios.[65] Optimal concentrations of vitamin D metabolites and their ratios—that may result in beneficial effects—need to be determined. The high incidence of lower body overuse injuries in athletes and military personnel, and long rehabilitation times required for bone stress injuries in particular (typically >80 days),[36]

mean a safe, low-burden, and low-cost intervention (*e.g.*, an oral vitamin D supplement) that lowers the risk of overuse injury would be attractive if found to be efficacious.

Conclusions

Greater conversion of 25(OH)D to 24,25$(OH)_2$D, relative to 1,25$(OH)_2$D (*i.e.*, low 1,25$(OH)_2$D:24,25$(OH)_2$D), and higher serum 24,25$(OH)_2$D, were associated with a lower incidence of lower body overuse musculoskeletal and bone stress injury, after controlling for covariates. Serum 24,25$(OH)_2$D may have a role in helping to protect against overuse injury in young adults undergoing arduous physical training.

Disclosure summary

The authors have no conflicts of interest regarding the study.

Author roles

ATC: data collection and interpretation, drafted the manuscript. TJO: data collection and interpretation. PS: formal data analysis. SJ: study design, data collection and interpretation. JCYT: data collection and interpretation, biochemical analysis. SJO: study design, data collection. RMI: study design. NPW: study design, data collection. WDF: study design, data interpretation, biochemical analysis. JPG: study concept and design, data interpretation. All authors critically revised the manuscript and approved the final version.

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Data availability

The data that support the findings of the study are not publicly available but are available from the corresponding author upon reasonable request.

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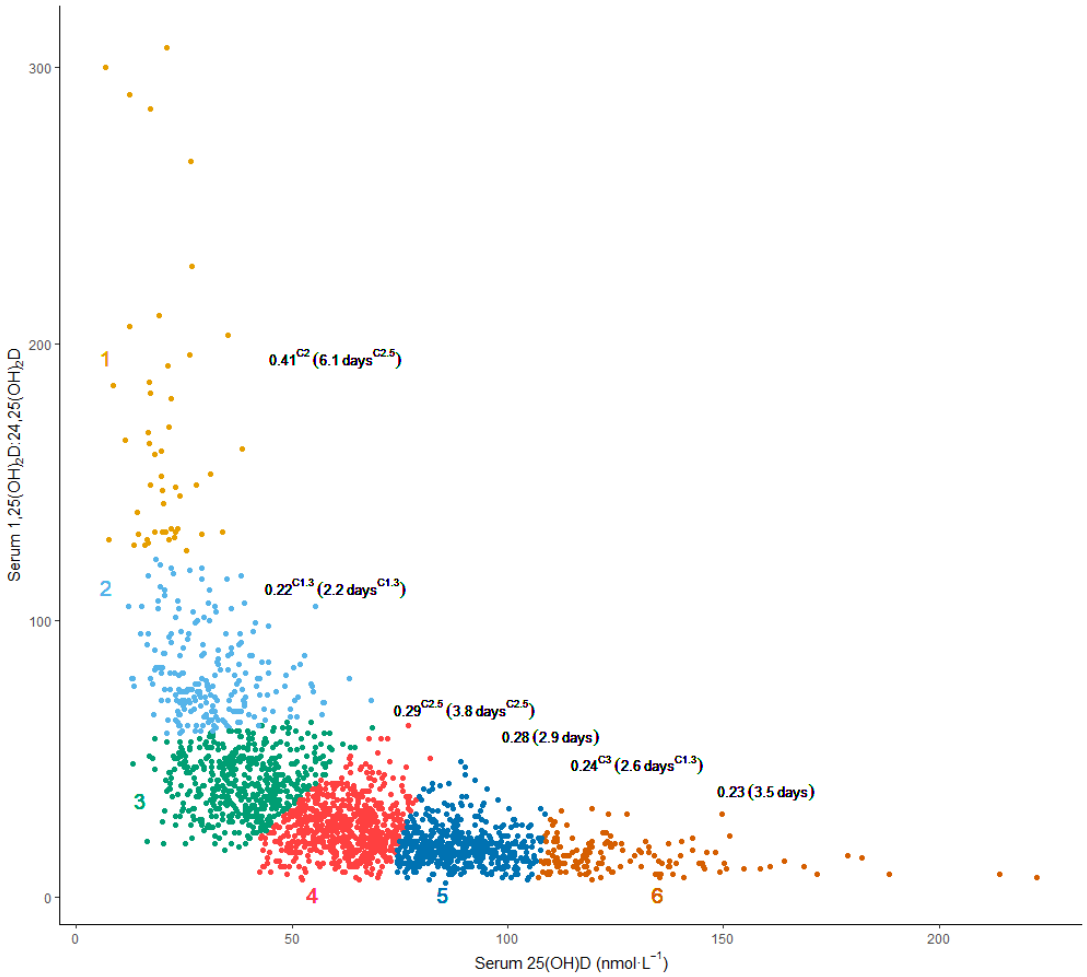
FIGURE LEGENDS

FIGURE 1: Dynamic relationship between vitamin D metabolites and overuse injury. Participants (1 per filled circle) are categorised into one of six clusters (with each cluster a different colour). Clusters are annotated with the proportion of participants injured during initial military training, and the number of lost training days across all participants (injured or not). A: lower body overuse musculoskeletal injury; B: lower body bone stress injury. Cx,y, $P < 0.05$ *vs* cluster x and y. Comparisons of binary coding of lower body overuse musculoskeletal and bone stress injury were conducted using logistic regression with full data set and reference cluster rotated to assess all pairwise comparisons. Comparisons of the number of lost training days were conducted using tobit regression with full data set and reference cluster rotated to assess all pairwise comparisons.

FIGURE 1

A



B

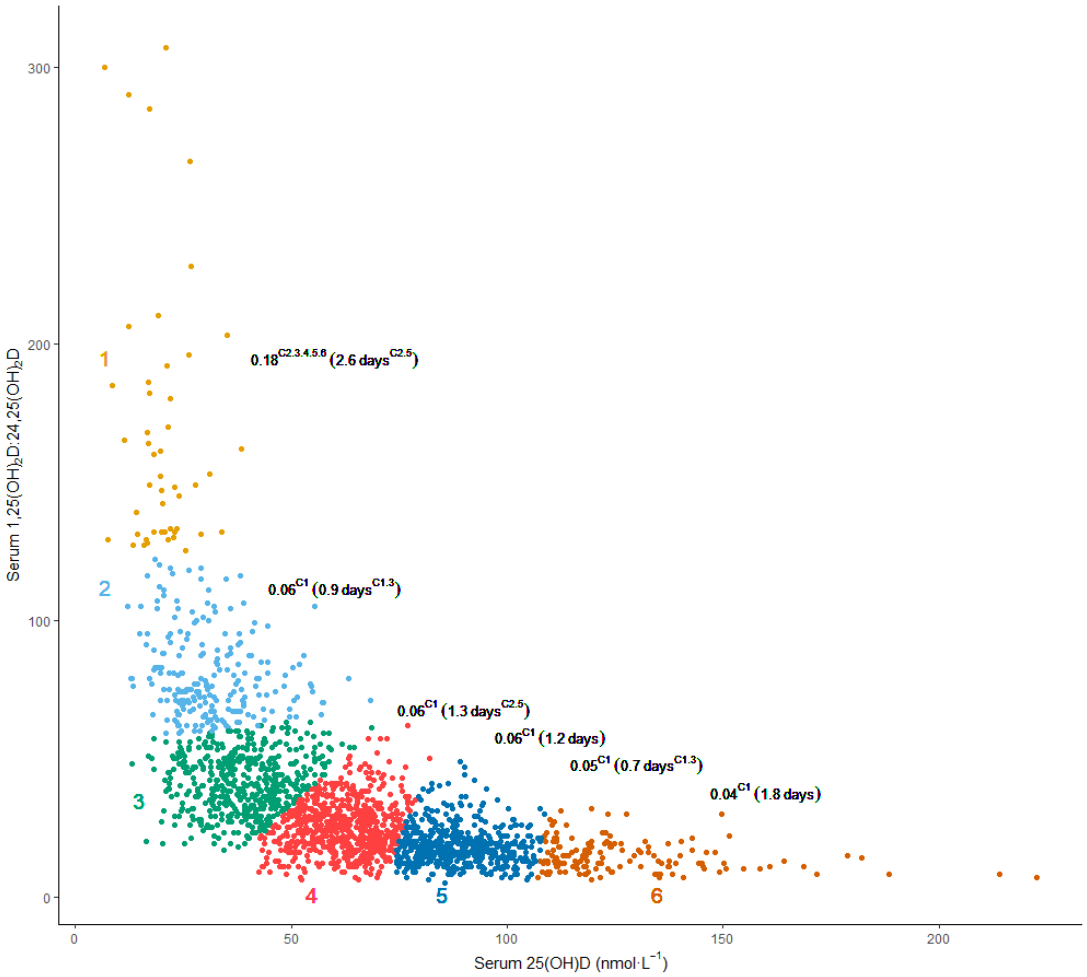


TABLE 1: Demographic, anthropometric, physical fitness, lifestyle behaviour, and bone injury history characteristics.

	All participants	Army Officer training		Army standard training	Army Infantry training	*P* value
Sex	75.5% Men	Men	Women	Women	Men	
n	2167	403	106	424	1234	
Ethnicity (%)						
White	94.3	91.1[c,d]	91.2	95.0[a]	95.3[a]	**0.001**
Other	5.7	8.9	8.8	5.0	4.7	
Age (years)	22.6 ± 7.5	23.1 ± 1.7[c,d]	23.5 ± 1.7[c,d]	22.2 ± 3.3[a,b,d]	22.4 ± 9.5[a,b,c]	**<0.001**
Body mass (kg)	73.5 ± 10.6	79.5 ± 8.6[b,c,d]	65.1 ± 6.7[a,d]	64.8 ± 8.2[a,d]	75.3 ± 9.9[a,b,c]	**<0.001**
Height (m)	1.75 ± 0.08	1.80 ± 0.07[b,c,d]	1.67 ± 0.06[a,c,d]	1.65 ± 0.06[a,b,d]	1.77 ± 0.06[a,b,c]	**<0.001**
BMI (kg·m^{-2})	24.0 ± 2.6	24.7 ± 2.3[b,c,d]	23.5 ± 2.0[a]	23.7 ± 2.4[a]	24.0 ± 2.7[a]	**<0.001**
2.4 km run time (s)	617 ± 79	546 ± 38[b,c,d]	659 ± 54[a,c,d]	717 ± 70[a,b,d]	610 ± 60[a,b,c]	**<0.001**
Smoker (%)	30.6	19.8[b,d]	3.9[a,c,d]	23.6[b,d]	38.7[a,b,c]	**<0.001**
Previous bone fracture (%)	42.8	48.1[c]	35.9	28.8[a,d]	46.7[c]	**<0.001**
Previous bone stress injury (%)	4.1	4.8	6.9	3.6	3.8	0.106

BMI: body mass index. Data are *n*, percent, or mean ± SD. Missing data: ethnicity, *n* = 60; age, *n* = 44; body mass, *n* = 60; height, *n* = 58; BMI, *n* = 62; 2.4 km run time, *n* = 361; smoking, *n* = 54; bone fracture history, *n* = 68; bone stress injury history, *n* = 75. [a], $P < 0.05$ *vs* Officer men; [b], $P < 0.05$ *vs* Officer women; [c], $P < 0.05$ *vs* standard women; [d], $P < 0.05$ *vs* Infantry men.

TABLE 2: Seasonal variation in vitamin D status and serum vitamin D metabolites.

	All Seasons	Spring	Summer	Fall	Winter	*P* value
Vitamin D status (%)						
Deficient	12.0	9.6[b,d]	2.1[a,c,d]	9.0[b,d]	31.0[a,b,c]	**<0.001**
Insufficient	23.7	25.0[b,d]	10.3[a,c,d]	22.3[b,d]	41.0[a,b,c]	**<0.001**
Sufficient	64.3	65.4[b,d]	87.6[a,c,d]	68.7[b,d]	28.0[a,b,c]	**<0.001**
25(OH)D (nmol·L^{-1})	63.3 ± 28.8	62.3 ± 27.3[b,d]	78.8 ± 26.0[a,c,d]	65.0 ± 27.1[b,d]	42.9 ± 23.0[a,b,c]	**<0.001**
1,25(OH)$_2$D (pmol·L^{-1})	137.6 ± 37.4	143.6 ± 36.9[c,d]	142.7 ± 35.9[c,d]	134.2 ± 38.5[a,b]	130.0 ± 36.5[a,b]	**<0.001**
24,25(OH)$_2$D (nmol·L^{-1})	5.6 ± 3.4	5.1 ± 3.1[b,c,d]	6.7 ± 2.9[a,c,d]	6.3 ± 3.7[a,b,d]	3.7 ± 3.0[a,b,c]	**<0.001**
1,25(OH)$_2$D:24,25(OH)$_2$D	35.7 ± 30.1	40.1 ± 31.2[b,c,d]	27.2 ± 20.3[a,d]	28.9 ± 22.3[a,d]	52.3 ± 40.2[a,b,c]	**<0.001**
25(OH)D:24,25(OH)$_2$D	12.8 ± 4.3	13.7 ± 4.3[b,c]	12.7 ± 3.6[a,c]	11.5 ± 3.6[a,b,d]	13.6 ± 5.4[c]	**<0.001**

25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 24,25(OH)$_2$D, 24,25-dihydroxyvitamin D. Vitamin D deficient 25(OH)D <30; insufficient, 30-49.9; sufficient ≥50 nmol·L^{-1}. Data are percent or mean ± SD. Missing data: 25(OH)D, n = 41; 1,25(OH)$_2$D, n = 39; 24,25(OH)$_2$D, n = 39; 1,25(OH)$_2$D:24,25(OH)$_2$D, n = 56; 25(OH)D:24,25(OH)$_2$D, n = 42. [a], $P < 0.05$ *vs* spring; [b], $P < 0.05$ *vs* summer; [c], $P < 0.05$ *vs* fall; [d], $P < 0.05$ *vs* winter.

TABLE 3: Lower body overuse musculoskeletal and bone stress injury incidence and lost training days during initial military training.

	All participants	**Army Officer training**		**Army standard training**	**Army Infantry training**	***P* value**
Sex	75.5% Men	Men	Women	Women	Men	
Lower body overuse musculoskeletal injury						
Incidence (%)	21.0	25.8[d]	31.1[d]	26.4[d]	16.7[a,b,c]	**<0.001**
Lost training days/injury	6 (3–12)	3 (5–10)	5 (3–9)	5 (2–10)[d]	7 (4–14)[c]	**0.019**
Lower body bone stress injury						
Incidence (%)	5.6	3.0[c,d]	3.8	7.8[a]	5.9[a]	**0.019**
Lost training days/injury	20 (10–25)	20 (12–25)	20 (9–29)	10 (6–21)[d]	20 (14–28)[c]	**0.034**

Data are percent or median (IQR). [a], $P < 0.05$ *vs* Officer men; [b], $P < 0.05$ *vs* Officer women; [c], $P < 0.05$ *vs* standard women; [d], $P < 0.05$ *vs* Infantry men.

TABLE 4: Vitamin D metabolites, covariates, and lower body overuse musculoskeletal and bone stress injury.

	All participants	No lower body overuse musculoskeletal or bone stress injury	Lower body overuse musculoskeletal injury	Lower body bone stress injury	*P* value
***n* (%)**	2167 (100%)	1589 (73.3%)	456 (21.0%)	122 (5.6%)	
Vitamin D status (%)					
Deficient	12.0	12.6	9.6	13.9	0.178
Insufficient	23.7	22.7[b]	28.1[a]	20.5	**0.040**
Sufficient	64.3	64.7	62.3	65.6	0.612
Serum vitamin D metabolites					
25(OH)D (nmol·L^{-1})	63.3 ± 28.8	63.9 ± 29.1	62.1 ± 27.9	61.3 ± 28.6	0.353
1,25(OH)$_2$D (pmol·L^{-1})	137.6 ± 37.4	136.5 ± 36.8[c]	138.2 ± 38.9[c]	150.2 ± 38.4[a,b]	**<0.001**
24,25(OH)$_2$D (nmol·L^{-1})	5.6 ± 3.4	5.7 ± 3.5	5.5 ± 3.2	5.2 ± 3.2	0.318
1,25(OH)$_2$D:24,25(OH)$_2$D	35.7 ± 30.1	35.2 ± 28.8[c]	34.9 ± 27.6[c]	45.6 ± 47.9[a,b]	**0.010**
25(OH)D:24,25(OH)$_2$D	12.8 ± 4.3	12.8 ± 4.2	12.5 ± 4.1	13.6 ± 5.5	0.207
Sex (% men)	75.5	78.1[b,c]	68.2[a]	69.7[a]	**<0.001**
BMI (kg·m^{-2})	24.0 ± 2.6	24.1 ± 2.6[b]	23.7 ± 2.4[a]	24.0 ± 2.7	**0.032**
2.4 km run time (s)	617 ± 79	612 ± 74[b,c]	630 ± 89[a]	640 ± 97[a]	**0.002**
Smoker (%)	30.6	29.2	34.9	32.8	0.054
Previous bone fracture (%)	42.8	41.1[c]	45.5	54.7[a]	**0.029**
Previous bone stress injury (%)	4.1	3.5	5.5	6.3	0.192
Army training course (%)					
Officer	23.5	22.4[b,c]	30.0[a,c]	13.1[a,b]	**<0.001**
Standard	19.6	17.6[b,c]	24.6[a]	27.1[a]	**<0.001**
Infantry	56.9	60.0[b]	45.4[a,c]	59.8[b]	**<0.001**

25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 24,25(OH)$_2$D, 24,25-dihydroxyvitamin D; BMI, body mass index. Vitamin D deficient 25(OH)D <30; insufficient, 30-49.9; sufficient ≥50 nmol·L^{-1}. Data are *n*, percent, or mean ± SD. [a], $P < 0.05$ *vs* no lower body overuse musculoskeletal or bone stress injury; [b], $P < 0.05$ *vs* lower body overuse musculoskeletal injury; [c], $P < 0.05$ *vs* lower body bone stress injury.

TABLE 5: Vitamin D metabolite relationships with lower body overuse musculoskeletal and bone stress injury incidence.

	Lower body overuse musculoskeletal injury	Lower body bone stress injury
Vitamin D status		
25(OH)D (nmol·L^{-1})	$R^2 = 0.16$	$R^2 = 0.19$
Deficient: 6.9 – 29.9	0.69 [0.42 – 1.12]	2.27 [0.93 – 5.54]
	P = 0.136	*P* = 0.072
Insufficient: 30.0 – 49.9	1.14 [0.84 – 1.56]	1.56 [0.84 – 2.92]
	P = 0.399	*P* = 0.158
Sufficient: 50 – 222.5	*Reference*	*Reference*
Serum vitamin D metabolites		
1,25(OH)$_2$D (pmol·L^{-1})	$R^2 = 0.16$	$R^2 = 0.19$
Quartile 1: 32.3 – 112.0	0.98 [0.68 – 1.40]	0.50 [0.23 – 1.09]
	P = 0.895	*P* = 0.082
Quartile 2: 113.0 – 135.0	0.94 [0.66 – 1.35]	1.01 [0.53 – 1.92]
	P = 0.753	*P* = 0.970
Quartile 3: 136.0 – 160.0	0.89 [0.64 – 1.24]	1.02 [0.57 – 1.83]
	P = 0.489	*P* = 0.948
Quartile 4: 161.0 – 380.0	*Reference*	*Reference*
24,25(OH)$_2$D (nmol·L^{-1})	$R^2 = 0.16$	$R^2 = 0.20$
Quartile 1: 0.4 – 3.1	1.49 [0.99 – 2.24]	**4.02 [1.82 – 8.87]**
	P = 0.058	***P* < 0.001**
Quartile 2: 3.2 – 5.1	**1.62 [1.13 – 2.32]**	**2.39 [1.16 – 4.92]**
	***P* = 0.009**	***P* = 0.018**
Quartile 3: 5.2 – 7.6	**1.59 [1.13 – 2.24]**	1.25 [0.62 – 2.52]
	***P* = 0.008**	*P* = 0.536
Quartile 4: 7.7 – 29.6	*Reference*	*Reference*
25(OH)D and 1,25(OH)$_2$D:24,25(OH)$_2$D	$R^2 = 0.17$	$R^2 = 0.21$
Cluster 1: 6.9 – 38.5 nmol·L^{-1} and 125 – 307	**6.30 [1.89 – 21.2]**	**22.08 [3.26 – 149.4]**
	***P* = 0.003**	***P* = 0.001**
Cluster 2: 12.0 – 68.3 nmol·L^{-1} and 59 – 122	1.01 [0.46 – 2.23]	2.30 [0.43 – 12.20]
	P = 0.984	*P* = 0.327
Cluster 3: 13.1 – 68.6 nmol·L^{-1} and 17 – 63	**2.35 [1.21 – 4.55]**	**5.00 [1.20 – 20.81]**
	***P* = 0.011**	***P* = 0.027**
Cluster 4: 42.4 – 82.0 nmol·L^{-1} and 6 – 62	**2.07 [1.10 – 3.88]**	3.10 [0.79 – 12.13]
	***P* = 0.024**	*P* = 0.104
Cluster 5: 74.3 – 108.6 nmol·L^{-1} and 5 – 49	1.72 [0.92 – 3.21]	1.55 [0.41 – 5.88]
	P = 0.089	*P* = 0.521
Cluster 6: 107.2 – 222.5 nmol·L^{-1} and 6 – 32	*Reference*	*Reference*

Logistic regression with control for covariates: sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course. R^2 for all variables in the model. 25(OH)D, 25-

hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; $24,25(OH)_2D$, 24,25-dihydroxyvitamin D. Vitamin D deficient 25(OH)D <30; insufficient, 30-49.9; sufficient ≥50 $nmol \cdot L^{-1}$. Vitamin D metabolites are minimum – maximum. Data are odds ratio [95% confidence interval].

TABLE 6: Vitamin D metabolite relationships with lower body overuse musculoskeletal and bone stress injury risk.

	Lower body overuse musculoskeletal injury	Lower body bone stress injury
Vitamin D status		
25(OH)D (nmol·L^{-1})	$R^2 = 0.43$	$R^2 = 0.77$
Deficient: 6.9 – 29.9	0.80 [0.49 – 1.30]	1.82 [0.47 – 7.12]
	P = 0.366	*P* = 0.388
Insufficient: 30.0 – 49.9	1.15 [0.86 – 1.52]	2.04 [0.86 – 4.89]
	P = 0.714	*P* = 0.106
Sufficient: 50 – 222.5	*Reference*	*Reference*
Serum vitamin D metabolites		
1,25(OH)$_2$D (pmol·L^{-1})	$R^2 = 0.44$	$R^2 = 0.79$
Quartile 1: 32.3 – 112.0	1.21 [0.84 – 1.77]	0.60 [0.21 – 1.74]
	P = 0.304	*P* = 0.347
Quartile 2: 113.0 – 135.0	0.98 [0.69 – 1.40]	1.14 [0.50 – 2.60]
	P = 0.910	*P* = 0.763
Quartile 3: 136.0 – 160.0	0.85 [0.61 – 1.19]	0.63 [0.27 – 1.46]
	P = 0.341	*P* = 0.278
Quartile 4: 161.0 – 380.0	*Reference*	*Reference*
24,25(OH)$_2$D (nmol·L^{-1})	$R^2 = 0.46$	$R^2 = 0.83$
Quartile 1: 0.4 – 3.1	**1.57 [1.01 – 2.45]**	**5.15 [1.67 – 18.26]**
	***P* = 0.045**	***P* = 0.005**
Quartile 2: 3.2 – 5.1	**1.63 [1.10 – 2.42]**	**3.60 [1.24 – 10.40]**
	***P* = 0.015**	***P* = 0.018**
Quartile 3: 5.2 – 7.6	**2.03 [1.41 – 2.92]**	1.75 [0.64 – 4.75]
	***P* < 0.001**	*P* = 0.273
Quartile 4: 7.7 – 29.6	*Reference*	*Reference*
25(OH)D and 1,25(OH)$_2$D:24,25(OH)$_2$D	$R^2 = 0.45$	$R^2 = 0.82$
Cluster 1: 6.9 – 38.5 nmol·L^{-1} and 125 – 307	**3.89 [1.30 – 11.42]**	1.31 [0.76 – 26.40]
	***P* = 0.015**	*P* = 0.077
Cluster 2: 12.0 – 68.3 nmol·L^{-1} and 59 – 122	1.00 [0.44 – 2.67]	1.83 [0.20 – 17.01]
	P = 0.998	*P* = 0.594
Cluster 3: 13.1 – 68.6 nmol·L^{-1} and 17 – 63	1.70 [0.84 – 3.44]	5.04 [0.84 – 22.65]
	P = 0.141	*P* = 0.076
Cluster 4: 42.4 – 82.0 nmol·L^{-1} and 6 – 62	1.77 [0.91 – 3.45]	2.43 [0.46 – 12.90]
	P = 0.091	*P* = 0.296
Cluster 5: 74.3 – 108.6 nmol·L^{-1} and 5 – 49	1.40 [0.73 – 2.67]	1.10 [0.23 – 5.31]
	P = 0.311	*P* = 0.906
Cluster 6: 107.2 – 222.5 nmol·L^{-1} and 6 – 32	*Reference*	*Reference*

Cox proportional hazards regression with control for covariates: sex, BMI, 2.4 km run time, smoking, bone injury history, and Army training course. R^2 for all variables in the model.

25(OH)D, 25-hydroxyvitamin D; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 24,25$(OH)_2$D, 24,25-dihydroxyvitamin D. Vitamin D deficient 25(OH)D <30; insufficient, 30-49.9; sufficient ≥50 nmol·L^{-1}. Vitamin D metabolites are minimum – maximum. Data are hazard ratio [95% confidence interval].