Vitamin D metabolites are associated with physical performance in young healthy adults

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ABSTRACT

Purpose. To determine vitamin D metabolites and vitamin D receptor (VDR) single-nucleotide polymorphisms (SNPs) relationships with physical performance.

Methods. In 1205 men and 322 women (94.8% white Caucasian, 22.0 ± 2.8 years) commencing military training, we measured: serum vitamin D metabolites (25-hydroxyvitamin D (25(OH)D) and 24,25-dihydroxyvitamin D (24,25(OH)$_2$D) by high-performance liquid chromatography tandem mass spectrophotometry, and 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) by immunoassay); VDR SNPs (rs2228570, rs4516035, and rs7139166 by polymerase chain reaction genotyping); and endurance performance by 2.4 km run, muscle strength by maximal dynamic lift, and muscle power by maximal vertical jump.

Results. Serum 25(OH)D was negatively associated with 2.4 km run time and positively associated with muscle power ($\beta = -12.0$ and 90.1), 1,25(OH)$_2$D was positively associated with run time and negatively associated with strength and muscle power ($\beta = 5.6$, –1.06, and –38.4), and 24,25(OH)$_2$D was negatively associated with run time ($\beta = -8.9$; $P < 0.01$), after controlling for age, sex, smoking, alcohol, physical activity, time outdoors, season, and BMI. Vitamin D metabolites (25(OH)D, 1,25(OH)$_2$D, and 24,25(OH)$_2$D) together explained variances of 5.0% in run time, 0.7% in strength, and 0.9% in muscle power (ΔF $P < 0.001$). All performance measures were superior with low 1,25(OH)$_2$D:24,25(OH)$_2$D ratio ($P < 0.05$). VDR SNPs were not associated with physical performance (ΔF $P \geq 0.306$).

Conclusion. Vitamin D metabolites accounted for a small portion of variance in physical performance. Associations between vitamin D metabolites and run time were the most consistent. VDR SNPs explained no variance in performance. 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 ratio), was favourable for performance, indicating 24,25(OH)$_2$D may have a role in optimising physical performance.

Key words: Vitamin D, exercise, endurance, muscle strength, muscle power, polymorphisms.

INTRODUCTION

Serum 25-hydroxyvitamin D (25(OH)D) concentration is the recommended, and widely used, indicator of an individual's vitamin D status (25(OH)D $\geq$50 nmol·L^{-1} is deemed sufficiency (1)) due to its abundance and longer half-life relative to other circulating vitamin D metabolites (1, 2). 1,25-dihydroxyvitamin D (1,25(OH)$_2$D)—synthesized from 25(OH)D by 1α-hydroxylase—is the most biologically active vitamin D metabolite in humans. 1,25(OH)$_2$D circulates in pmol·L^{-1} concentrations, with its actions mediated by vitamin D receptors (VDRs) (3). Despite their proximity in the metabolic pathway, there is no direct correlation between serum 25(OH)D and 1,25(OH)$_2$D due to the tight regulation of hydroxylation enzymes (4). A dynamic relationship exists between 25(OH)D and 1,25(OH)$_2$D when expressed as a relative ratio with 24,25-dihydroxyvitamin D (24,25(OH)$_2$D) (4). 24,25(OH)$_2$D is synthesized from 25(OH)D by 24-hydroxylase and like 25(OH)D, circulates in nmol·L^{-1} concentrations. Although 24,25(OH)$_2$D has been labeled as a purely catabolic metabolite of vitamin D, potential biological roles and the possible existence of a 24,25(OH)$_2$D specific receptor have emerged (5-8). Individuals with low 25(OH)D, normal 1,25(OH)$_2$D, but increased 1,25(OH)$_2$D:24,25(OH)$_2$D ratio have higher parathyroid hormone (PTH) concentrations than those at the opposite end of the spectrum with high 25(OH)D, normal 1,25(OH)$_2$D, and decreased 1,25(OH)$_2$D:24,25(OH)$_2$D ratio (4). High 24,25(OH)$_2$D relative to 1,25(OH)$_2$D (*i.e.*, low 1,25(OH)$_2$D:24,25(OH)$_2$D) may reduce the bioactivity of 25(OH)D and 1,25(OH)$_2$D, downregulating PTH secretion, whilst maintaining

$1{,}25(OH)_2D$ within strict boundaries. Low $24{,}25(OH)_2D$ relative to $1{,}25(OH)_2D$ (*i.e.*, high $1{,}25(OH)_2D{:}24{,}25(OH)_2D$) may upregulate PTH secretion and enhance the effects of vitamin D (4). On the other hand, if $24{,}25(OH)_2D$ is itself biologically active, low $1{,}25(OH)_2D{:}24{,}25(OH)_2D$ may be beneficial. How this recently identified inverse exponential relationship between serum 25(OH)D and $1{,}25(OH)_2D{:}24{,}25(OH)_2D$ relates to physiological outcomes, such as physical performance, remains unexplored.

Beyond regulating calcium and phosphate homeostasis and augmenting bone mineralisation (1), extra-skeletal functions of vitamin D and its metabolites have emerged following the discovery of the VDR in almost all human tissues (3, 9). $1{,}25(OH)_2D$ stimulates skeletal muscle protein synthesis by VDR-mediated signaling (9), and may improve cardiac, skeletal muscle, and endothelial function (10-14). Avoiding low serum 25(OH)D and achieving vitamin D sufficiency (1) may, therefore, be important for muscle strength and endurance type exercise (15, 16). Cross-sectional studies investigating the influence of vitamin D on physical performance in young healthy adults have reported both positive and no associations between circulating 25(OH)D and physical performance (17-20), when controlling for variables that influence performance (*e.g.*, sex, body composition, smoking, physical activity, and season (21-23)). In contrast, improving vitamin D status by increasing serum 25(OH)D with oral vitamin D_3 supplementation or increased sunlight exposure has not enhanced physical performance in randomized controlled trials (24, 25). This inconsistency between observational and interventional studies may be due to a focus on serum 25(OH)D as a measure of vitamin D status, and not examining the relative concentrations of vitamin D metabolites. Rather than simply increasing serum 25(OH)D, shifting $1{,}25(OH)_2D{:}24{,}25(OH)_2D$ from high to low may be necessary for a beneficial effect on performance to emerge.

Several single-nucleotide polymorphisms (SNPs) within vitamin D pathway-related genes are associated with circulating 25(OH)D and may be responsible for some inter-individual variability in the vitamin D endocrine system (26). SNPs in the gene that encodes for the VDR have been studied in relation to muscle strength and function in mostly elderly and sedentary adults with equivocal results (26). The relationship between rs2228570, rs4516035, and rs7139166 VDR polymorphisms and physical performance remains to be determined in young, physically active adults.

The purpose of the study was to examine the relationship: i) between vitamin D metabolites (serum 25(OH)D, 1,25(OH)$_2$D, and 24,25(OH)$_2$D) and physical performance; and ii) between VDR polymorphisms (rs2228570, rs4516035, and rs7139166) and physical performance. We hypothesized a three-dimensional model of vitamin D metabolites incorporating serum 25(OH)D and 1,25(OH)$_2$D:24,25(OH)$_2$D would have a dynamic relationship with physical performance; and SNPs in the VDR would be associated with variance in performance.

METHODS

Participants

1527 British Army recruits (1205 men and 322 women, 94.8% white Caucasian; Table 1) voluntarily participated in the study, after providing informed written consent and passing a physician-screened military medical assessment. All experimental procedures were completed during week one of initial Army training. Participants were recruited during week one of initial Army training between April 2013 and May 2017 from three military training populations: male infantry recruits at Infantry Training Centre, Catterick; standard entry female recruits at Army Training Centre, Pirbright; and male and female officer cadets at Royal Military Academy, Sandhurst—thereby providing a representative sample of all individuals commencing Army training in the UK. A subset of these data have been published (25). The present study includes unpublished vitamin D metabolite and SNP data, and is from a larger sample, more representative of all individuals commencing Army training. The study received ethical approval from the UK Ministry of Defence Research Ethics Committee (protocol number 165/Gen/10) and was conducted in accordance with the Declaration of Helsinki (2013).

Study design

A cross-sectional, observational study design was used to determine whether serum vitamin D metabolites and VDR SNPs were associated with physical performance in young healthy adults. All assessments were performed during week one of initial Army training and are listed here. Venous blood samples were obtained for analysis of: serum 25(OH)D, and its metabolites $1,25(OH)_2D$ and $24,25(OH)_2D$; and VDR SNPs in whole blood (rs2228570, rs4516035, and rs7139166). Physical performance was assessed by a maximal effort 2.4 km run, and tests of maximal dynamic lift strength and vertical jump peak power output. Body mass and height (Seca, Hamburg, Germany) were measured in light clothing and without shoes. Participants self-reported their alcohol intake; smoking habits; physical activity levels; and typical time spent outdoors, using questionnaires.

Experimental procedures

Blood collection and handling

Whole blood samples were obtained by venipuncture from a prominent vein in the antecubital fossa into one serum vacutainer and one EDTA vacutainer (Becton Dickinson, Oxford, UK). Whole blood in the EDTA vacutainer was immediately frozen at −80°C for later analysis. Whole blood in the serum vacutainer was left to clot in a vacutainer rack at room temperature for 1 h 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$(OH)_2D$ (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 (27). Serum 1,25$(OH)_2D$ was measured by chemiluminescent immunoassay using a DiaSorin LIAISON® XL analyser (Stillwater, Minnesota, USA). The measurement ranges of the assays were 0–200 nmol·L^{-1} for 25(OH)D_2 and 25(OH)D_3, 0–25 nmol·L^{-1} for 24,25$(OH)_2D_2$ and 24,25$(OH)_2D_3$, and 12–480 pmol·L^{-1} for 1,25$(OH)_2D$. 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$(OH)_2D$. All biochemical analyses were undertaken by the Good Clinical Laboratory Practice and Vitamin D External Quality Assessment Scheme certified Bioanalytical Facility at the University of East Anglia.

Single-nucleotide polymorphisms

Whole blood samples in EDTA vacutainers were defrosted and resuspended for 15 min on a rotating wheel. Genomic DNA was isolated from whole blood using the ReliaPrep™ Blood gDNA Miniprep System (Promega, Southampton, UK) according to the manufacturer's instructions. Using samples of DNA, Kompetitive Allele Specific PCR (KASP™, LGC

Genomics, Teddington, Middlesex, UK) genotyping was used for SNP genotyping of rs2228570, rs4516035, and rs7139166 in the VDR gene.

Endurance performance

Endurance performance was assessed as the time to complete a maximal effort 2.4 km run, recorded to the nearest second. After an 800 m warm up, the 2.4 km run was performed on a standardized running course at each training site. The time to complete a 2.4 km run is indicative of maximal aerobic capacity (28) and is assessed during selection, training, and throughout a military career. All participants were accustomed to performing this test from selection before commencing military training. Faster 2.4 km run times indicated better endurance performance. Therefore, negative associations with run time indicated improved endurance performance, and positive associations with run time indicated worsened endurance performance.

Muscle strength

Maximal dynamic lift strength was determined as the maximal weight lifted using an incremental lift machine that simulates a power clean weightlifting movement, as described previously (29). The device consisted of a vertically moving carriage with handgrips positioned 0.30 m above the ground. Participants lifted the weight (20 kg starting mass) to a height where the handgrips were 1.45 m from the ground, the height of a British Army four tonne truck. With each successful lift, the weight was increased by 5 kg. The test was terminated when participants failed to lift the weight to 1.45 m on their second attempt. Differences in body height may have affected the participants ability to lift weight to the same absolute height, however, this measure of maximal dynamic lift strength was chosen because it correlates with and predicts success in military and functional tasks (28).

Muscle power

Vertical jump peak power output was assessed by countermovement vertical jump using a jump mat (Takei Scientific Instruments, Tokyo, Japan) and validated equation (30): peak power (W) = (51.9 x maximal vertical jump height (cm)) + (48.9 x body mass (kg)) – 2007, as described previously (29). We analyzed this estimate of muscle power rather than jump height because lower body power is important for the performance of military specific tasks (28). A belt was fitted around the waist of each participant and secured to a rubber mat. Participants were instructed to jump as high as possible three times, with their hands placed on their hips. A fourth jump was performed if jump height increased across the three attempts, indicative of a learning effect. Maximal vertical jump height was recorded as the highest score achieved. Test-retest reliability of $r \geq 0.90$ has been reported for these performance tests (29).

Statistical analysis

Hierarchical multiple linear regression was used to examine the association between vitamin D metabolites and physical performance. Age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and body mass index (BMI) were included in regression models as covariates (21-23, 31). The association between vitamin D metabolites and physical performance was analyzed in two steps. Serum 1,25(OH)$_2$D and 24,25(OH)$_2$D were included in step one, and 25(OH)D was added in step two, so the relationship between serum 1,25(OH)$_2$D and 24,25(OH)$_2$D, and physical performance could be examined, with and without 25(OH)D. Given the large inter-individual differences in metabolites, these variables were standardized by scaling them relative to their standard deviation to improve the interpretation of beta coefficients. Cohen's f^2 effect sizes were calculated using a standard formula (32). No signs of strong heteroscedasticity or deviations from a normal distribution were detected for model residuals. Sensitivity analyses (data not shown) conducted with vitamin D metabolites log transformed or categorized into tertiles, as described previously (33), resulted in no substantive changes to the null-hypotheses tests or effect sizes. Variance inflation factor for all multiple regression models was <4.2, indicating no presence of multicollinearity (34). The association between vitamin D metabolites and physical performance was also explored by clustering participants into groups based on two dimensions of serum 25(OH)D and 1,25$(OH)_2$D:24,25$(OH)_2$D ratio. Clustering was performed using a k-means technique and the Bayesian information criterion to select the number of clusters, with the *n* in each cluster determined by the algorithm (35). Pairwise comparisons of the mean differences in physical performance across clusters were made using the t-distribution. Multiple linear regression models were also used to investigate the association between VDR SNPs and physical performance. Separate models were fitted for each of the SNPs whilst controlling for the same variables used to assess the association between vitamin D metabolites and physical performance. Associations were evaluated by conducting F-tests for nested linear models. Pairwise comparisons of the mean differences between vitamin D metabolites across seasons were made using the t-distribution. All statistical tests were conducted within a general linear model framework and using R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was accepted at $P < 0.05$.

RESULTS

Vitamin D metabolites and season

There was some seasonal variation in vitamin D metabolites ($P < 0.001$, Table 2). Across all seasons, 66.7% of participants were vitamin D sufficient, 21.4% were insufficient, and 11.9%

were deficient. During winter, 30.7% were vitamin D sufficient, 38.6% insufficient, and 30.7% deficient.

Vitamin D metabolite predictors of physical performance

Endurance performance

Serum 1,25(OH)$_2$D was positively associated, and 24,25(OH)$_2$D was negatively associated with 2.4 km run time after controlling for age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and BMI ($P < 0.01$ and $P < 0.001$, respectively; Table 3). These relationships remained following the addition of serum 25(OH)D as a predictor ($P < 0.001$ and $P < 0.01$, respectively), with 25(OH)D negatively associated with 2.4 km run time ($P < 0.001$). Vitamin D metabolites (25(OH)D, 1,25(OH)$_2$D, and 24,25(OH)$_2$D) together explained 5.0% of the variance in 2.4 km run time (significant ΔF $P < 0.001$).

Muscle strength

Serum 1,25(OH)$_2$D was negatively associated, and 24,25(OH)$_2$D was positively associated with maximal dynamic lift strength (muscle strength) after controlling for age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and BMI ($P < 0.01$ and $P < 0.001$, respectively; Table 3). Following the addition of serum 25(OH)D as a predictor, 1,25(OH)$_2$D remained negatively associated with muscle strength ($P < 0.001$), but neither 24,25(OH)$_2$D nor 25(OH)D were associated with muscle strength ($P = 0.126$ and $P = 0.093$, respectively). Vitamin D metabolites (25(OH)D, 1,25(OH)$_2$D, and 24,25(OH)$_2$D) together explained 0.7% of the variance in muscle strength (significant ΔF $P < 0.001$).

Muscle power

Serum 1,25$(OH)_2$D was negatively associated, and 24,25$(OH)_2$D was positively associated with vertical jump peak power output (muscle power) after controlling for age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and BMI ($P < 0.05$ and $P < 0.001$, respectively; Table 3). Following the addition of serum 25(OH)D as a predictor, 1,25$(OH)_2$D remained negatively associated ($P < 0.01$), 24,25$(OH)_2$D was not associated ($P = 0.791$), and 25(OH)D was positively associated with muscle power ($P < 0.001$). Vitamin D metabolites (25(OH)D, 1,25$(OH)_2$D, and 24,25$(OH)_2$D) together explained 0.9% of the variance in muscle power (significant ΔF $P < 0.001$).

Relative concentrations of vitamin D metabolites and physical performance

Endurance performance

Run times were faster in participants within clusters 3 to 6 *vs* cluster 1 and 2, and participants within clusters 4 to 6 *vs* cluster 3 ($P < 0.05$, Fig. 1A).

Muscle strength

Maximal dynamic lift strength was higher in participants within clusters 4 to 6 *vs* cluster 1, and participants within clusters 4 and 6 *vs* cluster 2 ($P < 0.05$, Fig. 1B).

Muscle power

Vertical jump peak power output was higher in participants within clusters 3 to 6 *vs* cluster 1 ($P < 0.05$, Fig. 1C).

Vitamin D receptor polymorphisms and physical performance

Vitamin D receptor SNPs did not explain any of the variance in 2.4 km run time, muscle strength, or muscle power after controlling for age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and BMI (significant ΔF $P \geq 0.306$, Table 4). There were no between genotype differences in 2.4 km run time, muscle strength, or muscle power when no confounding factors were controlled for ($P \geq 0.086$).

DISCUSSION

Serum 25(OH)D, 1,25$(OH)_2$D, and 24,25$(OH)_2$D were associated with 2.4 km run time; 1,25$(OH)_2$D was associated with muscle strength; and 25(OH)D and 1,25$(OH)_2$D were associated with muscle power, in young healthy adult men and women. Other factors contributing to physical performance (age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and BMI) were controlled for as covariates using hierarchical multiple linear regression. Vitamin D metabolites (25(OH)D, 1,25$(OH)_2$D, and 24,25$(OH)_2$D) together explained variances of 5.0% in 2.4 km run time, 0.7% in muscle strength, and 0.9% in muscle power. In terms of practical significance, the magnitude of the association between vitamin D metabolites and physical performance can be considered small (Cohen's f^2 effect sizes <0.15). Nevertheless, in real-world terms, for every 1 SD increase in 25(OH)D (+28.0 nmol·L^{-1}), 2.4 km run time was 12 s faster and vertical jump peak power output 90 W higher; for every 1 SD increase in 24,25$(OH)_2$D (+3.3 nmol·L^{-1}), 2.4 km run time was 9 s faster; and for every 1 SD increase in 1,25$(OH)_2$D (+36.5 pmol·L^{-1}), 2.4 km run time was 6 s slower, maximal dynamic lift strength 1 kg lower, and vertical jump peak power output 38 W lower.

As hypothesized, serum 25(OH)D and 1,25$(OH)_2$D:24,25$(OH)_2$D had a dynamic relationship with physical performance: 2.4 km run times were faster, and muscle strength and muscle power were greater in men and women with proportionally 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 ratio). Examining this relationship between vitamin D metabolites provides a unique insight into how the vitamin D metabolic pathway is related to physical performance and suggests that 24,25(OH)$_2$D may have role in optimising physical performance. Contrary to our hypothesis, VDR SNPs (rs2228570, rs4516035, and rs7139166) were not associated with physical performance.

Vitamin D metabolite predictors of physical performance

The negative association between serum 24,25(OH)$_2$D and 2.4 km run time, and positive associations between 24,25(OH)$_2$D and muscle strength and muscle power, were weaker or absent when 25(OH)D was added as a predictor because of the tight correlation between these metabolites (4). Serum 25(OH)D was itself negatively associated with 2.4 km run time and positively associated with muscle power. In contrast, 1,25(OH)$_2$D was positively associated with 2.4 km run time and negatively associated with muscle strength and muscle power, even when 25(OH)D was included as a predictor. However, serum 1,25(OH)$_2$D alone does not reflect vitamin D reserves or status because it is tightly regulated by the hydroxylation enzymes expressed by CYP27B1 and CYP24A1, with 1,25(OH)$_2$D production upregulated by PTH and downregulated by fibroblast growth factor 23 (FGF23) (36). No metabolites were associated with muscle strength when controlling for age and sex in the only published study to examine 25(OH)D, 1,25(OH)$_2$D, and 24,25(OH)$_2$D relationships with muscle function (37). This non-significant finding may be explained by the small sample size (116 adults, 20–74 years) (37), relative to the present study.

Relative concentrations of vitamin D metabolites and physical performance

An inverse exponential relationship exists between serum 25(OH)D and $1,25(OH)_2D:24,25(OH)_2D$ ratio (4). As the availability of 25(OH)D as a precursor diminishes, the conversion of 25(OH)D to $24,25(OH)_2D$ is reduced, resulting in a proportional increase in $1,25(OH)_2D$ (38). Superior physical performance in adults with low $1,25(OH)_2D:24,25(OH)_2D$ ratio suggests $24,25(OH)_2D$ is not a purely catabolic metabolite. Given $1,25(OH)_2D$ is the most biologically active vitamin D metabolite, greater circulating concentrations of $1,25(OH)_2D$ relative to $24,25(OH)_2D$ (*i.e.*, high $1,25(OH)_2D:24,25(OH)_2D$) might be expected to be favourable for physical performance (16). 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 $1,25(OH)_2D:24,25(OH)_2D$ ratio) was better for endurance performance, muscle strength, and muscle power, suggests $24,25(OH)_2D$ might influence physical performance. Rather than being a catabolic waste product, emerging evidence indicates $24,25(OH)_2D$ has a role in osteoblastic differentiation and bone development (39), promotion of fracture healing (7, 8, 40), and protection against cartilage damage (6). The existence of $24,25(OH)_2D$ receptors, and their possible function relevant to physical performance needs to be examined.

Vitamin D metabolites may improve physical performance by increasing the delivery of oxygenated blood to muscle through improved endothelial function (13, 14) and maintenance of normotension (41). Vitamin D metabolites can also increase mitochondrial oxidative function, potentially attenuating the development of skeletal muscle fatigue (12). By potentially increasing aerobic capacity, these mechanisms could account for why the associations between vitamin D metabolites and 2.4 km run time were the most consistent. Whether similar relationships exist between vitamin D metabolites and performance in endurance events of longer duration is an interesting area for future study. Vitamin D metabolites may exert beneficial downstream effects on physical performance by modulating muscle remodelling, since the VDR/retinoid X receptor signaling pathway is upregulated during the early stages of hypertrophy (42). Vitamin D can enhance skeletal muscle repair following damaging exercise and help to protect against infection by supporting aspects of innate and acquired immunity (16). By doing so, vitamin D may help to minimise the number of training sessions missed by athletes and military personnel, and thus potentially lead to improved physical performance.

Vitamin D receptor polymorphisms and physical performance

This is the largest study to examine the relationship between VDR SNPs (rs2228570, rs4516035, and rs7139166) and physical performance in young adults, with no associations observed. Previously, the rs2228570 allele related to increased VDR function was associated with weaker muscle strength in older adults (men and women, mean 62 years (43); men, 58–93 years (44)). Increased VDR function may increase CYP24A1 expression, leading to the degradation and decreased availability of 1,25(OH)$_2$D (9). Associations between the rs2228570 polymorphism and quadriceps strength were no longer statistically significant after controlling for fat-free mass in women (mean 42 years) (45) and men (58–93 years) (44), suggesting this polymorphism may influence muscle mass rather than strength *per se*. In contrast to the present study, strength differed between groups of children (mean 10 years) with different rs4516035 alleles, but no differences emerged for rs2228570 alleles (46). The range of muscle or other performance assessments used, and differences in participants' fitness and age (skeletal muscle VDR expression decreases with age (47)) have contributed to these equivocal findings.

Perspectives

This study provides a unique insight into the dynamic relationship between vitamin D metabolites and demonstrates that the relative circulating concentrations of $1,25(OH)_2D$ and $24,25(OH)_2D$ are related to physical performance. Randomized controlled trials have shown vitamin D_3 supplementation does not improve physical performance (24), however, in these studies vitamin D status has been assessed using 25(OH)D in isolation and the relative concentrations of $1,25(OH)_2D$ and $24,25(OH)_2D$ have not been considered. Shifting $1,25(OH)_2D$:$24,25(OH)_2D$ ratio from high to low may be necessary for a beneficial effect on performance to occur. Whether oral vitamin D_3 supplementation can correct high $1,25(OH)_2D$:$24,25(OH)_2D$ ratio and achieve high 25(OH)D and low $1,25(OH)_2D$:$24,25(OH)_2D$ ratio—and thereby enhance physical performance—remains to be determined. How much and how often oral vitamin D_3 is needed to achieve a steady state of vitamin D metabolites needs to be examined. Avoiding high serum $24,25(OH)_2D$ has been recommended because $24,25(OH)_2D$ may act to block the activity of the VDR (48). This recommendation, however, assumes $24,25(OH)_2D$ is purely a catabolic waste product—a hypothesis that warrants further evaluation given that relatively high $24,25(OH)_2D$ was associated with superior performance.

Avoiding a relative increase in serum $1,25(OH)_2D$ may be beneficial for performance, therefore, supplementation with alfacalcidol (1-hydroxyvitamin D_3) or calcitriol ($1,25(OH)_2D$) are unlikely to be effective for enhancing physical performance—especially because serum $1,25(OH)_2D$ is maintained within a tight range, despite fluctuations in 25(OH)D and $24,25(OH)_2D$ (4, 36). Supplementation that increases $1,25(OH)_2D$ beyond its normal plateau, thereby increasing the $1,25(OH)_2D$:$24,25(OH)_2D$ ratio, could be detrimental.

Genome wide studies have identified CYP24A1 as one of the major genetic determinants of variability in vitamin D metabolism (49). Increased CYP24A1 activity increases 24,25(OH)$_2$D and decreases 1,25(OH)$_2$D and PTH (36). Whether supplementation, dietary or lifestyle interventions can be used in individuals with genetically lower CYP24A1 activity (as indicated by increased 1,25(OH)$_2$D:24,25(OH)$_2$D ratio), to manage their vitamin D metabolism and enhance physical performance requires future study.

The present study is limited by its cross-sectional design. Associations between vitamin D metabolites and physical performance could be explained by reverse causation, *i.e.*, fitter, more physically active individuals spend more time outdoors exposed to sunlight and, in-turn, had higher serum concentrations of vitamin D metabolites. However, we included participants' self-reported physical activity levels and typical time spent outdoors as covariates in our regression models. Almost all of the participants in the present study were white Caucasian. Whether the vitamin D metabolites and SNPs we examined are associated with physical performance in other ethnic groups is unknown and warrants further study.

Conclusions

Serum 25(OH)D, 1,25(OH)$_2$D, and 24,25(OH)$_2$D were associated with 2.4 km run time; 1,25(OH)$_2$D was associated with muscle strength; and 25(OH)D and 1,25(OH)$_2$D were associated with muscle power, after controlling for covariates. Vitamin D metabolites accounted for a small portion of variance in physical performance. Polymorphisms in the VDR were not associated with physical performance. Faster 2.4 km run times and greater muscle strength and muscle power in adults with proportionally 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 ratio) indicates 24,25(OH)$_2$D may have a role in optimising physical performance.

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CONFLICT OF INTEREST

The authors have nothing to disclose. The results of the present study do not constitute endorsement by the ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE CAPTIONS

Figure 1. Dynamic relationships between vitamin D metabolites and physical performance. Participants (1 per filled circle) are categorized into one of six clusters, with each cluster's mean ± SD physical performance shown. Panel A, 2.4 km run time; Panel B, maximal dynamic lift strength; and Panel C, vertical jump peak power output. $^{1}P < 0.05$ *vs* cluster 1; $^{2}P < 0.05$ *vs* cluster 2; $^{3}P < 0.05$ *vs* cluster 3.

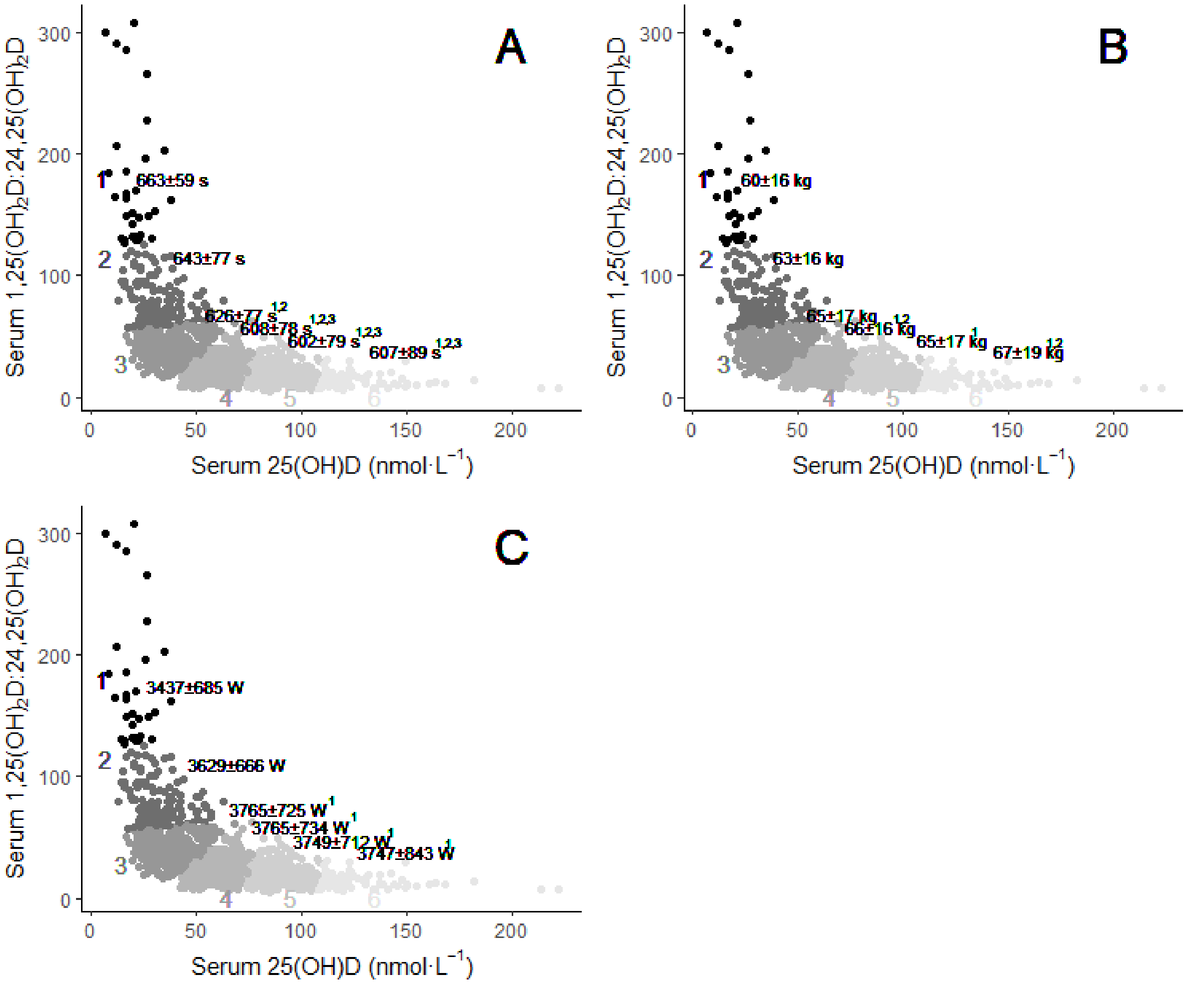

Table 1. Demographic, anthropometric, lifestyle behavior, and physical performance characteristics.

Demographics				
Age (years)	22.0 ± 2.8			
Sex (% men)	78.9			
Ethnicity				
White Caucasian (%)	94.8			
Other (%)	5.2			
Anthropometrics				
Body mass (kg)	74.1 ± 10.4			
Height (m)	1.75 ± 0.08			
BMI (kg·m^{-2})	24.1 ± 2.5			
Lifestyle behaviors				
Smoker (%)	36.6			
Alcohol user (%)	89.5			
Physical activity (h·week^{-1})	9.6 ± 11.2			
Time spent outdoors (h·week^{-1})	**<1**	**1–3.5**	**3.5–6**	**>6**
April-September (%)	2.3	28.7	33.1	35.9
October-March (%)	6.7	39.0	26.7	27.6
Physical performance				
2.4 km run time (s)	617 ± 79			
Maximal dynamic lift strength (kg)	65 ± 17			
Vertical jump peak power output (W)	3702 ± 748			

BMI, body mass index. Data are mean ± SD or precent.

Table 2. Seasonal variation in vitamin D status and serum vitamin D metabolites.

	Spring ***n* = 358**	**Summer** ***n* = 472**	**Fall** ***n* = 394**	**Winter** ***n* = 303**	**All Seasons** ***n* = 1527**
Vitamin D status					
Sufficient (%)	66.8	85.6	71.8	30.7	66.7
Insufficient (%)	22.9	11.9	18.0	38.6	21.4
Deficient (%)	10.3	2.5	10.2	30.7	11.9
25(OH)D (nmol·L^{-1})	62.3 ± 26.8 [a]	76.8 ± 25.8	65.2 ± 26.0 [a]	43.5 ± 22.7 [a,b,c]	63.8 ± 28.0
1,25(OH)$_2$D (pmol·L^{-1})	141.8 ± 34.8	142.9 ± 36.5	132.1 ± 37.1 [a,c]	131.1 ± 35.8 [a,c]	137.5 ± 36.5
24,25(OH)$_2$D (nmol·L^{-1})	5.1 ± 3.0 [a,b]	6.6 ± 3.0	6.5 ± 3.2	3.8 ± 3.2 [a,b,c]	5.6 ± 3.3
1,25(OH)$_2$D:24,25(OH)$_2$D	40.2 ± 32.1 [d]	28.3 ± 21.6 [c,d]	26.9 ± 20.6 [c,d]	52.8 ± 41.3	35.6 ± 30.5
25(OH)D:24,25(OH)$_2$D	13.8 ± 4.3	12.7 ± 3.8 [c,d]	11.1 ± 3.3 [a,c,d]	13.7 ± 5.4	12.8 ± 4.3

Vitamin D sufficient, serum 25(OH)D ≥50 nmol·L^{-1}; insufficient, serum 25(OH)D 30–<50 nmol·L^{-1}; and deficient, serum 25(OH)D <30 nmol·L^{-1}. Data are mean ± SD or percent. a, lower than summer; b, lower than fall; c, lower than spring; d, lower than winter, $P < 0.001$.

Table 3. Serum 1,25$(OH)_2$D, 24,25$(OH)_2$D, and 25(OH)D predictors of 2.4 km run time (endurance), muscle strength (maximal dynamic lift), and muscle power (vertical jump peak power output).

	Serum vitamin D metabolites	**Beta**	**R^2**	**ΔR^2**	**Sig. ΔF**	***f^2***
2.4 km run time	1,25$(OH)_2$D	4.1**	0.488	0.044	<0.001	0.09
	24,25$(OH)_2$D	-18.2***				
	1,25$(OH)_2$D	5.6***	0.494	0.050	<0.001	0.10
	24,25$(OH)_2$D	-8.9**				
	25(OH)D	-12.0***				
Muscle strength	1,25$(OH)_2$D	-0.95**	0.668	0.007	<0.001	0.02
	24,25$(OH)_2$D	1.41***				
	1,25$(OH)_2$D	-1.06***	0.668	0.007	<0.001	0.02
	24,25$(OH)_2$D	0.75				
	25(OH)D	0.86				
Muscle power	1,25$(OH)_2$D	-27.8*	0.672	0.006	<0.001	0.02
	24,25$(OH)_2$D	63.7***				
	1,25$(OH)_2$D	-38.4**	0.675	0.009	<0.001	0.03
	24,25$(OH)_2$D	-5.5				
	25(OH)D	90.1***				

After controlling for covariates (age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and BMI) serum 1,25$(OH)_2$D and 24,25$(OH)_2$D were entered in step one, and 25(OH)D was entered in step two as predictors of physical performance. Beta, standardized beta coefficient; Sig. ΔF, significant F change *P* value; f^2, Cohen's f^2 effect size, f^2 ≥0.02, ≥0.15 and ≥0.35 represent small, medium and large effect sizes, respectively (32). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 4. VDR SNP (rs2228570, rs4516035, and rs7139166) predictors of 2.4 km run time (endurance), muscle strength (maximal dynamic lift), and muscle power (vertical jump peak power output).

	VDR SNP	**Beta** CC *vs* alternate allele 1	**Beta** CC *vs* alternate allele 2	**R^2**	**ΔR^2**	**Sig. ΔF**	**f^2**
2.4 km run time	rs2228570	0.48	-4.0	0.429	0.000	0.610	<0.001
	rs4516035	0.59	-0.23	0.429	0.000	0.716	<0.001
	rs7139166	0.71	0.56	0.429	0.000	0.978	<0.001
Muscle strength	rs2228570	0.39	0.52	0.650	0.000	0.743	<0.001
	rs4516035	0.57	0.86	0.650	0.000	0.575	<0.001
	rs7139166	-0.29	-0.88	0.650	0.000	0.599	<0.001
Muscle power	rs2228570	19.4	19.0	0.666	0.000	0.610	<0.001
	rs4516035	39.2	51.1	0.666	0.000	0.306	0.002
	rs7139166	-12.2	-50.8	0.666	0.000	0.312	0.002

After controlling for covariates (age, sex, smoking, alcohol intake, physical activity, time spent outdoors, season, and BMI), VDR SNP genotypes were entered as predictors of physical performance. rs2228570: 40%, 44%, and 16% of participants had CC, CT (alternate allele 1), and TT (alternate allele 2) genotypes; rs4516035: 18%, 46%, and 36% of participants had CC, TC (alternate allele 1), and TT (alternate allele 2) genotypes; rs7139166: 36%, 46%, and 18% of participants had CC, GC (alternate allele 1), and GG (alternate allele 2) genotypes. VDR, vitamin D receptor; SNP, single-nucleotide polymorphism; Beta, standardized beta coefficient; Sig. ΔF, significant F change *P* value; f^2, Cohen's f^2 effect size, f^2 ≥0.02, ≥0.15 and ≥0.35 represent small, medium and large effect sizes, respectively (32).