

CARSWELL, A.T., JACKSON, S., SWINTON, P., O'LEARY, T.J., TANG, J.C.Y., OLIVER, S.J., SALE, C., IZARD, R.M., WALSH, N.P., FRASER, W.D and GREEVES, J.P. 2022. Vitamin D metabolites are associated with physical performance in young healthy adults. *Medicine and science in sports and exercise* [online], 54(11), pages 1982-1989. Available from: <https://doi.org/10.1249/MSS.0000000000002987>

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

CARSWELL, A.T., JACKSON, S., SWINTON, P., O'LEARY, T.J., TANG, J.C.Y., OLIVER, S.J., SALE, C., IZARD, R.M., WALSH, N.P., FRASER, W.D and GREEVES, J.P.

2022

This is a non-final version of an article published in final form in CARSWELL, A.T., JACKSON, S., SWINTON, P., O'LEARY, T.J., TANG, J.C.Y., OLIVER, S.J., SALE, C., IZARD, R.M., WALSH, N.P., FRASER, W.D and GREEVES, J.P. 2022. Vitamin D metabolites are associated with physical performance in young healthy adults. Medicine and Science in Sports and Exercise, 54(11), pages 1982-1989. Available from: <https://doi.org/10.1249/MSS.0000000000002987>

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

3 Alexander T. Carswell¹, Sarah Jackson², Paul Swinton³, Thomas J. O’Leary^{2,4}, Jonathan C. Y.
4 Tang¹, Samuel J. Oliver⁵, Craig Sale⁶, Rachel M. Izard⁷, Neil P. Walsh⁸, William D. Fraser^{1,9},
5 Julie P. Greeves^{1,2,4}

¹Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, Norfolk, United Kingdom.

²Army Health and Performance Research, Army HQ, Andover, Hampshire, United Kingdom.

³School of Health Sciences, Robert Gordon University, Aberdeen, United Kingdom.

⁴Division of Surgery and Interventional Science, University College London, London, United Kingdom.

⁵College of Human Sciences, Bangor University, Bangor, Gwynedd, United Kingdom.

⁶Musculoskeletal Physiology Research Group, SHAPE Research Centre, Nottingham Trent University, Nottingham, United Kingdom.

⁷Defence Science and Technology, Porton Down, Ministry of Defence, Salisbury, Wiltshire, United Kingdom.

⁸Faculty of Science, Liverpool John Moores University, Liverpool, United Kingdom.

⁹Departments of Endocrinology and Clinical Biochemistry, Norfolk and Norwich University Hospital, Norfolk, United Kingdom.

6 **Corresponding author:** Julie P. Greeves OBE PhD, Army Health and Performance

7 Research, Army HQ, Andover, Hampshire, United Kingdom, SP11 8HT. Tel: +441264

8 886785. Email: julie.greeves143@mod.gov.uk

9 **ABSTRACT**

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

12 **Methods.** In 1205 men and 322 women (94.8% white Caucasian, 22.0 ± 2.8 years)
13 commencing military training, we measured: serum vitamin D metabolites (25-
14 hydroxyvitamin D (25(OH)D) and 24,25-dihydroxyvitamin D (24,25(OH)₂D) by high-
15 performance liquid chromatography tandem mass spectrophotometry, and 1,25-
16 dihydroxyvitamin D (1,25(OH)₂D) by immunoassay); VDR SNPs (rs2228570, rs4516035,
17 and rs7139166 by polymerase chain reaction genotyping); and endurance performance by 2.4
18 km run, muscle strength by maximal dynamic lift, and muscle power by maximal vertical
19 jump.

20 **Results.** Serum 25(OH)D was negatively associated with 2.4 km run time and positively
21 associated with muscle power ($\beta = -12.0$ and 90.1), 1,25(OH)₂D was positively associated
22 with run time and negatively associated with strength and muscle power ($\beta = 5.6$, -1.06 , and
23 -38.4), and 24,25(OH)₂D was negatively associated with run time ($\beta = -8.9$; $P < 0.01$), after
24 controlling for age, sex, smoking, alcohol, physical activity, time outdoors, season, and BMI.
25 Vitamin D metabolites (25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D) together explained
26 variances of 5.0% in run time, 0.7% in strength, and 0.9% in muscle power ($\Delta F P < 0.001$).
27 All performance measures were superior with low 1,25(OH)₂D:24,25(OH)₂D ratio ($P < 0.05$).
28 VDR SNPs were not associated with physical performance ($\Delta F P \geq 0.306$).

29 **Conclusion.** Vitamin D metabolites accounted for a small portion of variance in physical
30 performance. Associations between vitamin D metabolites and run time were the most
31 consistent. VDR SNPs explained no variance in performance. Greater conversion of
32 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),

33 was favourable for performance, indicating 24,25(OH)₂D may have a role in optimising
34 physical performance.

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

37

38 INTRODUCTION

39 Serum 25-hydroxyvitamin D (25(OH)D) concentration is the recommended, and widely used,
40 indicator of an individual's vitamin D status (25(OH)D \geq 50 nmol·L⁻¹ is deemed sufficiency
41 (1)) due to its abundance and longer half-life relative to other circulating vitamin D
42 metabolites (1, 2). 1,25-dihydroxyvitamin D (1,25(OH)₂D)—synthesized from 25(OH)D by
43 1 α -hydroxylase—is the most biologically active vitamin D metabolite in humans.
44 1,25(OH)₂D circulates in pmol·L⁻¹ concentrations, with its actions mediated by vitamin D
45 receptors (VDRs) (3). Despite their proximity in the metabolic pathway, there is no direct
46 correlation between serum 25(OH)D and 1,25(OH)₂D due to the tight regulation of
47 hydroxylation enzymes (4). A dynamic relationship exists between 25(OH)D and
48 1,25(OH)₂D when expressed as a relative ratio with 24,25-dihydroxyvitamin D
49 (24,25(OH)₂D) (4). 24,25(OH)₂D is synthesized from 25(OH)D by 24-hydroxylase and like
50 25(OH)D, circulates in nmol·L⁻¹ concentrations. Although 24,25(OH)₂D has been labeled as a
51 purely catabolic metabolite of vitamin D, potential biological roles and the possible existence
52 of a 24,25(OH)₂D specific receptor have emerged (5-8). Individuals with low 25(OH)D,
53 normal 1,25(OH)₂D, but increased 1,25(OH)₂D:24,25(OH)₂D ratio have higher parathyroid
54 hormone (PTH) concentrations than those at the opposite end of the spectrum with high
55 25(OH)D, normal 1,25(OH)₂D, and decreased 1,25(OH)₂D:24,25(OH)₂D ratio (4). High
56 24,25(OH)₂D relative to 1,25(OH)₂D (*i.e.*, low 1,25(OH)₂D:24,25(OH)₂D) may reduce the
57 bioactivity of 25(OH)D and 1,25(OH)₂D, downregulating PTH secretion, whilst maintaining

58 1,25(OH)₂D within strict boundaries. Low 24,25(OH)₂D relative to 1,25(OH)₂D (*i.e.*, high
59 1,25(OH)₂D:24,25(OH)₂D) may upregulate PTH secretion and enhance the effects of vitamin
60 D (4). On the other hand, if 24,25(OH)₂D is itself biologically active, low
61 1,25(OH)₂D:24,25(OH)₂D may be beneficial. How this recently identified inverse
62 exponential relationship between serum 25(OH)D and 1,25(OH)₂D:24,25(OH)₂D relates to
63 physiological outcomes, such as physical performance, remains unexplored.

64

65 Beyond regulating calcium and phosphate homeostasis and augmenting bone mineralisation
66 (1), extra-skeletal functions of vitamin D and its metabolites have emerged following the
67 discovery of the VDR in almost all human tissues (3, 9). 1,25(OH)₂D stimulates skeletal
68 muscle protein synthesis by VDR-mediated signaling (9), and may improve cardiac, skeletal
69 muscle, and endothelial function (10-14). Avoiding low serum 25(OH)D and achieving
70 vitamin D sufficiency (1) may, therefore, be important for muscle strength and endurance
71 type exercise (15, 16). Cross-sectional studies investigating the influence of vitamin D on
72 physical performance in young healthy adults have reported both positive and no associations
73 between circulating 25(OH)D and physical performance (17-20), when controlling for
74 variables that influence performance (*e.g.*, sex, body composition, smoking, physical activity,
75 and season (21-23)). In contrast, improving vitamin D status by increasing serum 25(OH)D
76 with oral vitamin D₃ supplementation or increased sunlight exposure has not enhanced
77 physical performance in randomized controlled trials (24, 25). This inconsistency between
78 observational and interventional studies may be due to a focus on serum 25(OH)D as a
79 measure of vitamin D status, and not examining the relative concentrations of vitamin D
80 metabolites. Rather than simply increasing serum 25(OH)D, shifting
81 1,25(OH)₂D:24,25(OH)₂D from high to low may be necessary for a beneficial effect on
82 performance to emerge.

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

90

91 The purpose of the study was to examine the relationship: i) between vitamin D metabolites
92 (serum 25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D) and physical performance; and ii) between
93 VDR polymorphisms (rs2228570, rs4516035, and rs7139166) and physical performance. We
94 hypothesized a three-dimensional model of vitamin D metabolites incorporating serum
95 25(OH)D and 1,25(OH)₂D:24,25(OH)₂D would have a dynamic relationship with physical
96 performance; and SNPs in the VDR would be associated with variance in performance.

97

98 **METHODS**

99 **Participants**

100 1527 British Army recruits (1205 men and 322 women, 94.8% white Caucasian; Table 1)
101 voluntarily participated in the study, after providing informed written consent and passing a
102 physician-screened military medical assessment. All experimental procedures were
103 completed during week one of initial Army training. Participants were recruited during week
104 one of initial Army training between April 2013 and May 2017 from three military training
105 populations: male infantry recruits at Infantry Training Centre, Catterick; standard entry
106 female recruits at Army Training Centre, Pirbright; and male and female officer cadets at
107 Royal Military Academy, Sandhurst—thereby providing a representative sample of all

108 individuals commencing Army training in the UK. A subset of these data have been
109 published (25). The present study includes unpublished vitamin D metabolite and SNP data,
110 and is from a larger sample, more representative of all individuals commencing Army
111 training. The study received ethical approval from the UK Ministry of Defence Research
112 Ethics Committee (protocol number 165/Gen/10) and was conducted in accordance with the
113 Declaration of Helsinki (2013).

114

115 **Study design**

116 A cross-sectional, observational study design was used to determine whether serum vitamin
117 D metabolites and VDR SNPs were associated with physical performance in young healthy
118 adults. All assessments were performed during week one of initial Army training and are
119 listed here. Venous blood samples were obtained for analysis of: serum 25(OH)D, and its
120 metabolites 1,25(OH)₂D and 24,25(OH)₂D; and VDR SNPs in whole blood (rs2228570,
121 rs4516035, and rs7139166). Physical performance was assessed by a maximal effort 2.4 km
122 run, and tests of maximal dynamic lift strength and vertical jump peak power output. Body
123 mass and height (Seca, Hamburg, Germany) were measured in light clothing and without
124 shoes. Participants self-reported their alcohol intake; smoking habits; physical activity levels;
125 and typical time spent outdoors, using questionnaires.

126

127 **Experimental procedures**

128 *Blood collection and handling*

129 Whole blood samples were obtained by venipuncture from a prominent vein in the
130 antecubital fossa into one serum vacutainer and one EDTA vacutainer (Becton Dickinson,
131 Oxford, UK). Whole blood in the EDTA vacutainer was immediately frozen at -80°C for
132 later analysis. Whole blood in the serum vacutainer was left to clot in a vacutainer rack at

133 room temperature for 1 h before being centrifuged at 1500 g for 10 min at 4°C, with serum
134 aliquots immediately frozen at -80°C for later analysis.

135

136 *Biochemical analysis*

137 Total serum 25(OH)D (25(OH)D₂ and 25(OH)D₃) and total 24,25(OH)₂D (24,25(OH)₂D₂ and
138 24,25(OH)₂D₃) were measured with high-performance liquid chromatography tandem mass
139 spectrophotometry using a Micromass Quattro Ultima Pt electrospray ionisation mass
140 spectrometer, as described previously (27). Serum 1,25(OH)₂D was measured by
141 chemiluminescent immunoassay using a DiaSorin LIAISON® XL analyser (Stillwater,
142 Minnesota, USA). The measurement ranges of the assays were 0–200 nmol·L⁻¹ for 25(OH)D₂
143 and 25(OH)D₃, 0–25 nmol·L⁻¹ for 24,25(OH)₂D₂ and 24,25(OH)₂D₃, and 12–480 pmol·L⁻¹ for
144 1,25(OH)₂D. The mean coefficient of variation (CV) for intra-assay imprecision across the
145 measuring range of the assays was 4.9% for 25(OH)D₂, 8.3% for 25(OH)D₃, 7.7% for
146 24,25(OH)₂D₂, 9.0% for 24,25(OH)₂D₃, and 7.4% for 1,25(OH)₂D. The cumulative inter-
147 assay CVs were ≤7.4% for 25(OH)D₂, ≤9.6% for 25(OH)D₃, ≤10.6% for 24,25(OH)₂D₂,
148 ≤8.9% for 24,25(OH)₂D₃, and ≤9.3% for 1,25(OH)₂D. All biochemical analyses were
149 undertaken by the Good Clinical Laboratory Practice and Vitamin D External Quality
150 Assessment Scheme certified Bioanalytical Facility at the University of East Anglia.

151

152 *Single-nucleotide polymorphisms*

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

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

159

160 *Endurance performance*

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

170

171 *Muscle strength*

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

182 *Muscle power*

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

194

195 **Statistical analysis**

196 Hierarchical multiple linear regression was used to examine the association between vitamin
197 D metabolites and physical performance. Age, sex, smoking, alcohol intake, physical activity,
198 time spent outdoors, season, and body mass index (BMI) were included in regression models
199 as covariates (21-23, 31). The association between vitamin D metabolites and physical
200 performance was analyzed in two steps. Serum 1,25(OH)₂D and 24,25(OH)₂D were included
201 in step one, and 25(OH)D was added in step two, so the relationship between serum
202 1,25(OH)₂D and 24,25(OH)₂D, and physical performance could be examined, with and
203 without 25(OH)D. Given the large inter-individual differences in metabolites, these variables
204 were standardized by scaling them relative to their standard deviation to improve the
205 interpretation of beta coefficients. Cohen's f^2 effect sizes were calculated using a standard
206 formula (32). No signs of strong heteroscedasticity or deviations from a normal distribution

207 were detected for model residuals. Sensitivity analyses (data not shown) conducted with
208 vitamin D metabolites log transformed or categorized into tertiles, as described previously
209 (33), resulted in no substantive changes to the null-hypotheses tests or effect sizes. Variance
210 inflation factor for all multiple regression models was <4.2 , indicating no presence of
211 multicollinearity (34). The association between vitamin D metabolites and physical
212 performance was also explored by clustering participants into groups based on two
213 dimensions of serum 25(OH)D and 1,25(OH)₂D:24,25(OH)₂D ratio. Clustering was
214 performed using a k-means technique and the Bayesian information criterion to select the
215 number of clusters, with the n in each cluster determined by the algorithm (35). Pairwise
216 comparisons of the mean differences in physical performance across clusters were made
217 using the t-distribution. Multiple linear regression models were also used to investigate the
218 association between VDR SNPs and physical performance. Separate models were fitted for
219 each of the SNPs whilst controlling for the same variables used to assess the association
220 between vitamin D metabolites and physical performance. Associations were evaluated by
221 conducting F-tests for nested linear models. Pairwise comparisons of the mean differences
222 between vitamin D metabolites across seasons were made using the t-distribution. All
223 statistical tests were conducted within a general linear model framework and using R 3.6.2 (R
224 Foundation for Statistical Computing, Vienna, Austria). Statistical significance was accepted
225 at $P < 0.05$.

226

227 **RESULTS**

228 **Vitamin D metabolites and season**

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

231 were deficient. During winter, 30.7% were vitamin D sufficient, 38.6% insufficient, and
232 30.7% deficient.

233

234 **Vitamin D metabolite predictors of physical performance**

235 *Endurance performance*

236 Serum 1,25(OH)₂D was positively associated, and 24,25(OH)₂D was negatively associated
237 with 2.4 km run time after controlling for age, sex, smoking, alcohol intake, physical activity,
238 time spent outdoors, season, and BMI ($P < 0.01$ and $P < 0.001$, respectively; Table 3). These
239 relationships remained following the addition of serum 25(OH)D as a predictor ($P < 0.001$
240 and $P < 0.01$, respectively), with 25(OH)D negatively associated with 2.4 km run time ($P <$
241 0.001). Vitamin D metabolites (25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D) together
242 explained 5.0% of the variance in 2.4 km run time (significant $\Delta F P < 0.001$).

243

244 *Muscle strength*

245 Serum 1,25(OH)₂D was negatively associated, and 24,25(OH)₂D was positively associated
246 with maximal dynamic lift strength (muscle strength) after controlling for age, sex, smoking,
247 alcohol intake, physical activity, time spent outdoors, season, and BMI ($P < 0.01$ and $P <$
248 0.001 , respectively; Table 3). Following the addition of serum 25(OH)D as a predictor,
249 1,25(OH)₂D remained negatively associated with muscle strength ($P < 0.001$), but neither
250 24,25(OH)₂D nor 25(OH)D were associated with muscle strength ($P = 0.126$ and $P = 0.093$,
251 respectively). Vitamin D metabolites (25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D) together
252 explained 0.7% of the variance in muscle strength (significant $\Delta F P < 0.001$).

253

254

255

256 *Muscle power*

257 Serum 1,25(OH)₂D was negatively associated, and 24,25(OH)₂D was positively associated
258 with vertical jump peak power output (muscle power) after controlling for age, sex, smoking,
259 alcohol intake, physical activity, time spent outdoors, season, and BMI ($P < 0.05$ and $P <$
260 0.001 , respectively; Table 3). Following the addition of serum 25(OH)D as a predictor,
261 1,25(OH)₂D remained negatively associated ($P < 0.01$), 24,25(OH)₂D was not associated (P
262 $= 0.791$), and 25(OH)D was positively associated with muscle power ($P < 0.001$). Vitamin D
263 metabolites (25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D) together explained 0.9% of the
264 variance in muscle power (significant $\Delta F P < 0.001$).

265

266 **Relative concentrations of vitamin D metabolites and physical performance**

267 *Endurance performance*

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

270

271 *Muscle strength*

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

274

275 *Muscle power*

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

278

279

280

281 **Vitamin D receptor polymorphisms and physical performance**

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

287

288 **DISCUSSION**

289 Serum 25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D were associated with 2.4 km run time;
290 1,25(OH)₂D was associated with muscle strength; and 25(OH)D and 1,25(OH)₂D were
291 associated with muscle power, in young healthy adult men and women. Other factors
292 contributing to physical performance (age, sex, smoking, alcohol intake, physical activity,
293 time spent outdoors, season, and BMI) were controlled for as covariates using hierarchical
294 multiple linear regression. Vitamin D metabolites (25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D)
295 together explained variances of 5.0% in 2.4 km run time, 0.7% in muscle strength, and 0.9%
296 in muscle power. In terms of practical significance, the magnitude of the association between
297 vitamin D metabolites and physical performance can be considered small (Cohen's f^2 effect
298 sizes < 0.15). Nevertheless, in real-world terms, for every 1 SD increase in 25(OH)D (+28.0
299 nmol·L⁻¹), 2.4 km run time was 12 s faster and vertical jump peak power output 90 W higher;
300 for every 1 SD increase in 24,25(OH)₂D (+3.3 nmol·L⁻¹), 2.4 km run time was 9 s faster; and
301 for every 1 SD increase in 1,25(OH)₂D (+36.5 pmol·L⁻¹), 2.4 km run time was 6 s slower,
302 maximal dynamic lift strength 1 kg lower, and vertical jump peak power output 38 W lower.
303
304 As hypothesized, serum 25(OH)D and 1,25(OH)₂D:24,25(OH)₂D had a dynamic relationship
305 with physical performance: 2.4 km run times were faster, and muscle strength and muscle

306 power were greater in men and women with proportionally greater conversion of 25(OH)D to
307 24,25(OH)₂D relative to 1,25(OH)₂D (*i.e.*, low 1,25(OH)₂D:24,25(OH)₂D ratio). Examining
308 this relationship between vitamin D metabolites provides a unique insight into how the
309 vitamin D metabolic pathway is related to physical performance and suggests that
310 24,25(OH)₂D may have role in optimising physical performance. Contrary to our hypothesis,
311 VDR SNPs (rs2228570, rs4516035, and rs7139166) were not associated with physical
312 performance.

313

314 **Vitamin D metabolite predictors of physical performance**

315 The negative association between serum 24,25(OH)₂D and 2.4 km run time, and positive
316 associations between 24,25(OH)₂D and muscle strength and muscle power, were weaker or
317 absent when 25(OH)D was added as a predictor because of the tight correlation between
318 these metabolites (4). Serum 25(OH)D was itself negatively associated with 2.4 km run time
319 and positively associated with muscle power. In contrast, 1,25(OH)₂D was positively
320 associated with 2.4 km run time and negatively associated with muscle strength and muscle
321 power, even when 25(OH)D was included as a predictor. However, serum 1,25(OH)₂D alone
322 does not reflect vitamin D reserves or status because it is tightly regulated by the
323 hydroxylation enzymes expressed by CYP27B1 and CYP24A1, with 1,25(OH)₂D production
324 upregulated by PTH and downregulated by fibroblast growth factor 23 (FGF23) (36). No
325 metabolites were associated with muscle strength when controlling for age and sex in the
326 only published study to examine 25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D relationships with
327 muscle function (37). This non-significant finding may be explained by the small sample size
328 (116 adults, 20–74 years) (37), relative to the present study.

329

330

331 **Relative concentrations of vitamin D metabolites and physical performance**

332 An inverse exponential relationship exists between serum 25(OH)D and
333 1,25(OH)₂D:24,25(OH)₂D ratio (4). As the availability of 25(OH)D as a precursor
334 diminishes, the conversion of 25(OH)D to 24,25(OH)₂D is reduced, resulting in a
335 proportional increase in 1,25(OH)₂D (38). Superior physical performance in adults with low
336 1,25(OH)₂D:24,25(OH)₂D ratio suggests 24,25(OH)₂D is not a purely catabolic metabolite.
337 Given 1,25(OH)₂D is the most biologically active vitamin D metabolite, greater circulating
338 concentrations of 1,25(OH)₂D relative to 24,25(OH)₂D (*i.e.*, high 1,25(OH)₂D:24,25(OH)₂D)
339 might be expected to be favourable for physical performance (16). Our novel finding that
340 proportionally greater conversion of 25(OH)D to 24,25(OH)₂D relative to 1,25(OH)₂D (*i.e.*,
341 low 1,25(OH)₂D:24,25(OH)₂D ratio) was better for endurance performance, muscle strength,
342 and muscle power, suggests 24,25(OH)₂D might influence physical performance. Rather than
343 being a catabolic waste product, emerging evidence indicates 24,25(OH)₂D has a role in
344 osteoblastic differentiation and bone development (39), promotion of fracture healing (7, 8,
345 40), and protection against cartilage damage (6). The existence of 24,25(OH)₂D receptors,
346 and their possible function relevant to physical performance needs to be examined.

347

348 Vitamin D metabolites may improve physical performance by increasing the delivery of
349 oxygenated blood to muscle through improved endothelial function (13, 14) and maintenance
350 of normotension (41). Vitamin D metabolites can also increase mitochondrial oxidative
351 function, potentially attenuating the development of skeletal muscle fatigue (12). By
352 potentially increasing aerobic capacity, these mechanisms could account for why the
353 associations between vitamin D metabolites and 2.4 km run time were the most consistent.
354 Whether similar relationships exist between vitamin D metabolites and performance in
355 endurance events of longer duration is an interesting area for future study. Vitamin D

356 metabolites may exert beneficial downstream effects on physical performance by modulating
357 muscle remodelling, since the VDR/retinoid X receptor signaling pathway is upregulated
358 during the early stages of hypertrophy (42). Vitamin D can enhance skeletal muscle repair
359 following damaging exercise and help to protect against infection by supporting aspects of
360 innate and acquired immunity (16). By doing so, vitamin D may help to minimise the number
361 of training sessions missed by athletes and military personnel, and thus potentially lead to
362 improved physical performance.

363

364 **Vitamin D receptor polymorphisms and physical performance**

365 This is the largest study to examine the relationship between VDR SNPs (rs2228570,
366 rs4516035, and rs7139166) and physical performance in young adults, with no associations
367 observed. Previously, the rs2228570 allele related to increased VDR function was associated
368 with weaker muscle strength in older adults (men and women, mean 62 years (43); men, 58–
369 93 years (44)). Increased VDR function may increase CYP24A1 expression, leading to the
370 degradation and decreased availability of 1,25(OH)₂D (9). Associations between the
371 rs2228570 polymorphism and quadriceps strength were no longer statistically significant
372 after controlling for fat-free mass in women (mean 42 years) (45) and men (58–93 years)
373 (44), suggesting this polymorphism may influence muscle mass rather than strength *per se*. In
374 contrast to the present study, strength differed between groups of children (mean 10 years)
375 with different rs4516035 alleles, but no differences emerged for rs2228570 alleles (46). The
376 range of muscle or other performance assessments used, and differences in participants’
377 fitness and age (skeletal muscle VDR expression decreases with age (47)) have contributed to
378 these equivocal findings.

379

380

381 **Perspectives**

382 This study provides a unique insight into the dynamic relationship between vitamin D
383 metabolites and demonstrates that the relative circulating concentrations of 1,25(OH)₂D and
384 24,25(OH)₂D are related to physical performance. Randomized controlled trials have shown
385 vitamin D₃ supplementation does not improve physical performance (24), however, in these
386 studies vitamin D status has been assessed using 25(OH)D in isolation and the relative
387 concentrations of 1,25(OH)₂D and 24,25(OH)₂D have not been considered. Shifting
388 1,25(OH)₂D:24,25(OH)₂D ratio from high to low may be necessary for a beneficial effect on
389 performance to occur. Whether oral vitamin D₃ supplementation can correct high
390 1,25(OH)₂D:24,25(OH)₂D ratio and achieve high 25(OH)D and low
391 1,25(OH)₂D:24,25(OH)₂D ratio—and thereby enhance physical performance—remains to be
392 determined. How much and how often oral vitamin D₃ is needed to achieve a steady state of
393 vitamin D metabolites needs to be examined. Avoiding high serum 24,25(OH)₂D has been
394 recommended because 24,25(OH)₂D may act to block the activity of the VDR (48). This
395 recommendation, however, assumes 24,25(OH)₂D is purely a catabolic waste product—a
396 hypothesis that warrants further evaluation given that relatively high 24,25(OH)₂D was
397 associated with superior performance.

398

399 Avoiding a relative increase in serum 1,25(OH)₂D may be beneficial for performance,
400 therefore, supplementation with alfacalcidol (1-hydroxyvitamin D₃) or calcitriol
401 (1,25(OH)₂D) are unlikely to be effective for enhancing physical performance—especially
402 because serum 1,25(OH)₂D is maintained within a tight range, despite fluctuations in
403 25(OH)D and 24,25(OH)₂D (4, 36). Supplementation that increases 1,25(OH)₂D beyond its
404 normal plateau, thereby increasing the 1,25(OH)₂D:24,25(OH)₂D ratio, could be detrimental.

405

406 Genome wide studies have identified CYP24A1 as one of the major genetic determinants of
407 variability in vitamin D metabolism (49). Increased CYP24A1 activity increases
408 24,25(OH)₂D and decreases 1,25(OH)₂D and PTH (36). Whether supplementation, dietary or
409 lifestyle interventions can be used in individuals with genetically lower CYP24A1 activity (as
410 indicated by increased 1,25(OH)₂D:24,25(OH)₂D ratio), to manage their vitamin D
411 metabolism and enhance physical performance requires future study.

412

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

421

422 **Conclusions**

423 Serum 25(OH)D, 1,25(OH)₂D, and 24,25(OH)₂D were associated with 2.4 km run time;
424 1,25(OH)₂D was associated with muscle strength; and 25(OH)D and 1,25(OH)₂D were
425 associated with muscle power, after controlling for covariates. Vitamin D metabolites
426 accounted for a small portion of variance in physical performance. Polymorphisms in the
427 VDR were not associated with physical performance. Faster 2.4 km run times and greater
428 muscle strength and muscle power in adults with proportionally greater conversion of
429 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)
430 indicates 24,25(OH)₂D may have a role in optimising physical performance.

431 **ACKNOWLEDGMENTS**

432 The authors thank Dr Daniel Kashi, Xin Hui Aw Yong, Mark Ward, Claire Potter, and Dr
433 Laurel Wentz for their assistance with data collection. This work was funded by the Ministry
434 of Defence (Army), UK.

435

436 **CONFLICT OF INTEREST**

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

440 **REFERENCES**

- 441 1. Institute of Medicine. *Dietary reference intakes for calcium and vitamin D*. Washington
442 (DC): The National Academies Press; 2011. pp. 345-455.
- 443 2. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention
444 of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol*
445 *Metab*. 2011;96(7):1911-30.
- 446 3. Haussler MR, Whitfield GK, Kaneko I, et al. Molecular mechanisms of vitamin D action.
447 *Calcif Tissue Int*. 2013;92(2):77-98.
- 448 4. Tang JCY, Jackson S, Walsh NP, Greeves J, Fraser WD, Bioanalytical Facility team. The
449 dynamic relationships between the active and catabolic vitamin D metabolites, their ratios,
450 and associations with PTH. *Sci Rep*. 2019;9(1):6974.
- 451 5. St-Arnaud R, Glorieux FH. 24,25-Dihydroxyvitamin D-active metabolite or inactive
452 catabolite? *Endocrinology*. 1998;139(8):3371-4.
- 453 6. Boyan BD, Hyzy SL, Pan Q, et al. 24R,25-dihydroxyvitamin D3 protects against articular
454 cartilage damage following anterior cruciate ligament transection in male rats. *PLoS One*.
455 2016;11(8):e0161782.
- 456 7. Gal-Moscovici A, Gal M, Popovtzer MM. Treatment of osteoporotic ovariectomized rats
457 with 24,25(OH)2D3. *Eur J Clin Invest*. 2005;35(6):375-9.
- 458 8. St-Arnaud R. CYP24A1-deficient mice as a tool to uncover a biological activity for
459 vitamin D metabolites hydroxylated at position 24. *J Steroid Biochem Mol Biol*. 2010;121(1-
460 2):254-6.
- 461 9. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin
462 D in skeletal muscle: form, function, and metabolism. *Endocr Rev*. 2013;34(1):33-83.
- 463 10. Allison RJ, Close GL, Farooq A, et al. Severely vitamin D-deficient athletes present
464 smaller hearts than sufficient athletes. *Eur J Prev Cardiol*. 2015;22(4):535-42.
- 465 11. Ryan ZC, Craig TA, Folmes CD, et al. 1alpha,25-dihydroxyvitamin D3 regulates
466 mitochondrial oxygen consumption and dynamics in human skeletal muscle cells. *J Biol*
467 *Chem*. 2016;291(3):1514-28.
- 468 12. Sinha A, Hollingsworth KG, Ball S, Cheetham T. Improving the vitamin D status of
469 vitamin D deficient adults is associated with improved mitochondrial oxidative function in
470 skeletal muscle. *J Clin Endocrinol Metab*. 2013;98(3):E509-E13.
- 471 13. Tarcin O, Yavuz DG, Ozben B, et al. Effect of vitamin D deficiency and replacement on
472 endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab*. 2009;94(10):4023-
473 30.
- 474 14. Wolf ST, Jablonski NG, Ferguson SB, Alexander LM, Kenney WL. Four weeks of
475 vitamin D supplementation improves nitric oxide-mediated microvascular function in
476 college-aged African Americans. *Am J Physiol Heart Circ Physiol*. 2020;319(4):H906-H14.

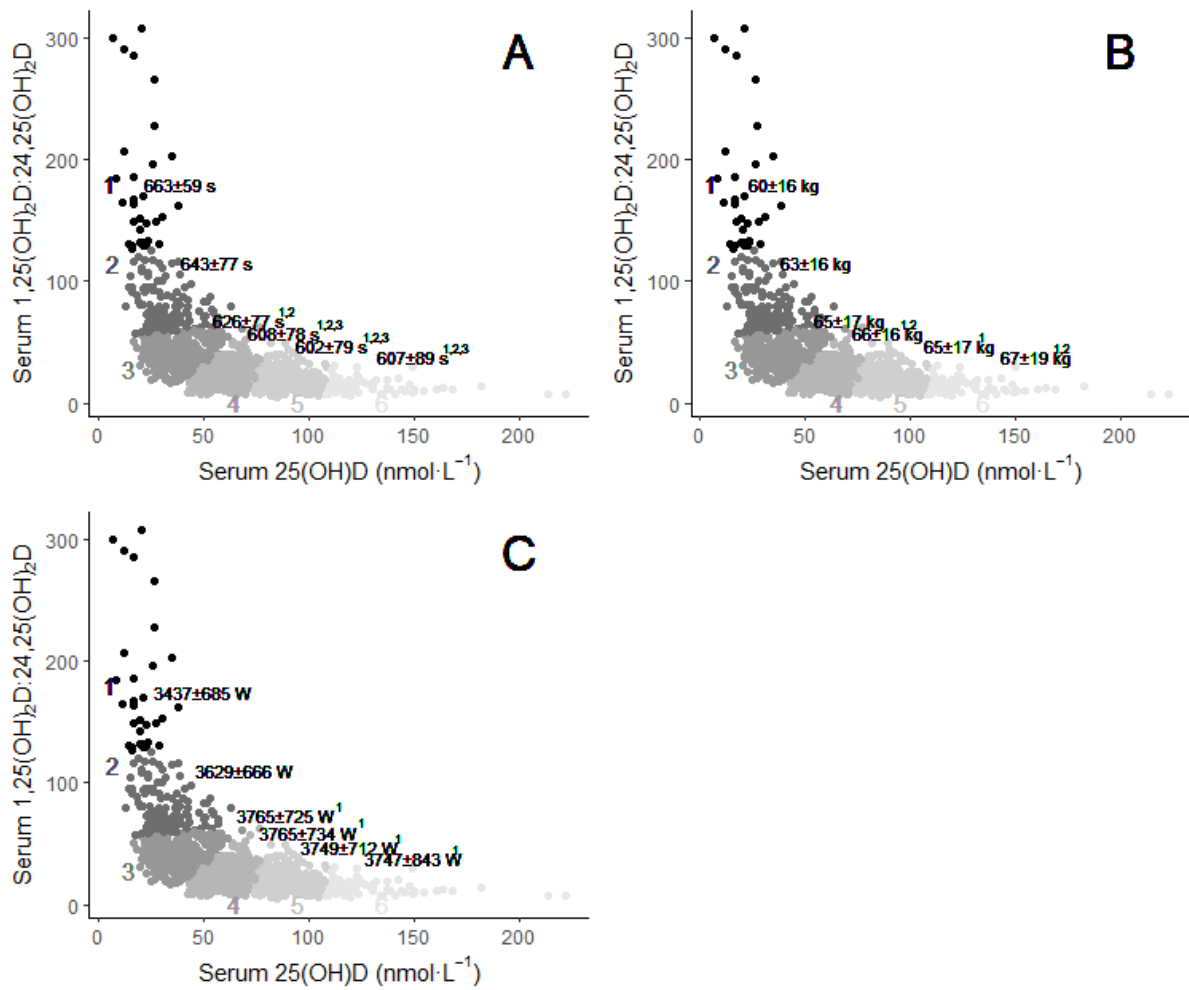
- 477 15. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine joint
478 position statement. Nutrition and athletic performance. *Med Sci Sports Exerc.*
479 2016;48(3):543-68.
- 480 16. Owens DJ, Allison R, Close GL. Vitamin D and the athlete: current perspectives and new
481 challenges. *Sports Med.* 2018;48(Suppl 1):3-16.
- 482 17. Grimaldi AS, Parker BA, Capizzi JA, et al. 25(OH) vitamin D is associated with greater
483 muscle strength in healthy men and women. *Med Sci Sports Exerc.* 2013;45(1):157-62.
- 484 18. Hamilton B, Whiteley R, Farooq A, Chalabi H. Vitamin D concentration in 342
485 professional football players and association with lower limb isokinetic function. *J Sci Med*
486 *Sport.* 2014;17(1):139-43.
- 487 19. Ardestani A, Parker B, Mathur S, et al. Relation of vitamin D level to maximal oxygen
488 uptake in adults. *Am J Cardiol.* 2011;107(8):1246-9.
- 489 20. Fitzgerald JS, Peterson BJ, Warpeha JM, Johnson SC, Ingraham SJ. Association between
490 vitamin D status and maximal-intensity exercise performance in junior and collegiate hockey
491 players. *J Strength Cond Res.* 2015;29(9):2513-21.
- 492 21. Cannell JJ, Hollis BW, Sorenson MB, Taft TN, Anderson JJ. Athletic performance and
493 vitamin D. *Med Sci Sports Exerc.* 2009;41(5):1102-10.
- 494 22. Mattila VM, Tallroth K, Marttinen M, Pihlajamaki H. Physical fitness and performance.
495 Body composition by DEXA and its association with physical fitness in 140 conscripts. *Med*
496 *Sci Sports Exerc.* 2007;39(12):2242-7.
- 497 23. Song EY, Lim CL, Lim MK. A comparison of maximum oxygen consumption, aerobic
498 performance, and endurance in young and active male smokers and nonsmokers. *Mil Med.*
499 1998;163(11):770-4.
- 500 24. Farrokhyar F, Sivakumar G, Savage K, et al. Effects of vitamin D supplementation on
501 serum 25-hydroxyvitamin D concentrations and physical performance in athletes: a
502 systematic review and meta-analysis of randomized controlled trials. *Sports Med.*
503 2017;47(11):2323-39.
- 504 25. Carswell AT, Oliver SJ, Wentz LM, et al. Influence of vitamin D supplementation by
505 sunlight or oral D3 on exercise performance. *Med Sci Sports Exerc.* 2018;50(12):2555-64.
- 506 26. Krasniqi E, Boshnjaku A, Wagner KH, Wessner B. Association between polymorphisms
507 in vitamin D pathway-related genes, vitamin D status, muscle mass and function: a
508 systematic review. *Nutrients.* 2021;13(9):3109.
- 509 27. Tang JCY, Nicholls H, Piec I, et al. Reference intervals for serum 24,25-
510 dihydroxyvitamin D and the ratio with 25-hydroxyvitamin D established using a newly
511 developed LC-MS/MS method. *J Nutr Biochem.* 2017;46:21-9.
- 512 28. Friedl KE, Knapik JJ, Hakkinen K, et al. Perspectives on aerobic and strength influences
513 on military physical readiness: report of an International Military Physiology Roundtable. *J*
514 *Strength Cond Res.* 2015;29 Suppl 11:S10-23.

- 515 29. Fortes MB, Diment BC, Greeves JP, Casey A, Izard R, Walsh NP. Effects of a daily
516 mixed nutritional supplement on physical performance, body composition, and circulating
517 anabolic hormones during 8 weeks of arduous military training. *Appl Physiol Nutr Metab*.
518 2011;36(6):967-75.
- 519 30. Sayers SP, Harackiewicz DV, Harman EA, Frykman PN, Rosenstein MT. Cross-
520 validation of three jump power equations. *Med Sci Sports Exerc*. 1999;31(4):572-7.
- 521 31. Wicherts IS, van Schoor NM, Boeke AJ, et al. Vitamin D status predicts physical
522 performance and its decline in older persons. *J Clin Endocrinol Metab*. 2007;92(6):2058-65.
- 523 32. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. Hillsdale (NJ):
524 Lawrence Erlbaum; 1988. 19-74 p.
- 525 33. Visser M, Deeg DJ, Lips P, Longitudinal Aging Study A. Low vitamin D and high
526 parathyroid hormone levels as determinants of loss of muscle strength and muscle mass
527 (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab*.
528 2003;88(12):5766-72.
- 529 34. Kutner MH, Nachtsheim CJ, Neter J, Li W. *Applied linear statistical models*. 5th ed. New
530 York (NY): McGraw-Hill; 2005.
- 531 35. Fraley C, Raftery AE. How many clusters? Which clustering method? Answers via
532 model-based cluster analysis. *Comput J*. 1998;41(8):578-88.
- 533 36. Henry HL. Regulation of vitamin D metabolism. *Best Pract Res Clin Endocrinol Metab*.
534 2011;25(4):531-41.
- 535 37. Hassan-Smith ZK, Jenkinson C, Smith DJ, et al. 25-hydroxyvitamin D3 and 1,25-
536 dihydroxyvitamin D3 exert distinct effects on human skeletal muscle function and gene
537 expression. *PLoS One*. 2017;12(2):e0170665.
- 538 38. Fraser WD, Tang JCY, Dutton JJ, Schoenmakers I. Vitamin D measurement, the debates
539 continue, new analytes have emerged, developments have variable outcomes. *Calcif Tissue*
540 *Int*. 2020;106(1):3-13.
- 541 39. Curtis KM, Aenlle KK, Roos BA, Howard GA. 24R,25-dihydroxyvitamin D3 promotes
542 the osteoblastic differentiation of human mesenchymal stem cells. *Mol Endocrinol*.
543 2014;28(5):644-58.
- 544 40. Martineau C, Naja RP, Hussein A, et al. Optimal bone fracture repair requires 24R,25-
545 dihydroxyvitamin D3 and its effector molecule FAM57B2. *J Clin Invest*. 2018;128(8):3546-
546 57.
- 547 41. Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the
548 renin-angiotensin system in humans. *Hypertension*. 2010;55(5):1283-8.
- 549 42. Chaillou T, Lee JD, England JH, Esser KA, McCarthy JJ. Time course of gene expression
550 during mouse skeletal muscle hypertrophy. *J Appl Physiol*. 2013;115(7):1065-74.

- 551 43. Hopkinson NS, Li KW, Kehoe A, et al. Vitamin D receptor genotypes influence
552 quadriceps strength in chronic obstructive pulmonary disease. *Am J Clin Nutr.*
553 2008;87(2):385-90.
- 554 44. Roth SM, Zmuda JM, Cauley JA, Shea PR, Ferrell RE. Vitamin D receptor genotype is
555 associated with fat-free mass and sarcopenia in elderly men. *J Gerontol A Biol Sci Med Sci.*
556 2004;59(1):10-5.
- 557 45. Windelinckx A, De Mars G, Beunen G, et al. Polymorphisms in the vitamin D receptor
558 gene are associated with muscle strength in men and women. *Osteoporos Int.*
559 2007;18(9):1235-42.
- 560 46. Bozsodi A, Boja S, Szilagyi A, Somhegyi A, Varga PP, Lazary A. Muscle strength is
561 associated with vitamin D receptor gene variants. *J Orthop Res.* 2016;34(11):2031-7.
- 562 47. Bischoff-Ferrari HA, Borchers M, Gudat F, Durmuller U, Stahelin HB, Dick W. Vitamin
563 D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res.*
564 2004;19(2):265-9.
- 565 48. Owens DJ, Tang JC, Bradley WJ, et al. Efficacy of high-dose vitamin D supplements for
566 elite athletes. *Med Sci Sports Exerc.* 2017;49(2):349-56.
- 567 49. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D
568 insufficiency: a genome-wide association study. *Lancet.* 2010;376(9736):180-8.
- 569

570 **FIGURE CAPTIONS**

571 **Figure 1.** Dynamic relationships between vitamin D metabolites and physical performance.
 572 Participants (1 per filled circle) are categorized into one of six clusters, with each cluster's
 573 mean \pm SD physical performance shown. Panel A, 2.4 km run time; Panel B, maximal
 574 dynamic lift strength; and Panel C, vertical jump peak power output. $^1P < 0.05$ vs cluster 1;
 575 $^2P < 0.05$ vs cluster 2; $^3P < 0.05$ vs cluster 3.



576

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 percent.

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

	Spring <i>n</i> = 358	Summer <i>n</i> = 472	Fall <i>n</i> = 394	Winter <i>n</i> = 303	All Seasons <i>n</i> = 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)₂D (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)₂D (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)₂D:24,25(OH)₂D	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)₂D	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)₂D, 24,25(OH)₂D, 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)₂D and 24,25(OH)₂D 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; f², Cohen's f² effect size, f² ≥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	Beta	R ²	ΔR ²	Sig. ΔF	f ²
		CC vs alternate	CC vs alternate				
		allele 1	allele 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; f², Cohen's f² effect size, f² ≥0.02, ≥0.15 and ≥0.35 represent small, medium and large effect sizes, respectively (32).