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

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Medicine and Science in Sports and Exercise

Published: 01/11/2022

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

03. May. 2024

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

2 **adults**

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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 (ΔF $P < 0.001$).

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 (ΔF $P \geq 0.306$).

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),

2

33 was favourable for performance, indicating 24,25(OH)$_2$D 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)$_2$D)—synthesized from 25(OH)D by

43 1α-hydroxylase—is the most biologically active vitamin D metabolite in humans.

44 1,25(OH)$_2$D 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)$_2$D due to the tight regulation of

47 hydroxylation enzymes (4). A dynamic relationship exists between 25(OH)D and

48 1,25(OH)$_2$D when expressed as a relative ratio with 24,25-dihydroxyvitamin D

49 (24,25(OH)$_2$D) (4). 24,25(OH)$_2$D 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)$_2$D 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)$_2$D specific receptor have emerged (5-8). Individuals with low 25(OH)D,

53 normal 1,25(OH)$_2$D, but increased 1,25(OH)$_2$D:24,25(OH)$_2$D 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)$_2$D, and decreased 1,25(OH)$_2$D:24,25(OH)$_2$D ratio (4). High

56 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

57 bioactivity of 25(OH)D and 1,25(OH)$_2$D, downregulating PTH secretion, whilst maintaining

58   1,25(OH)$_2$D within strict boundaries. Low 24,25(OH)$_2$D relative to 1,25(OH)$_2$D (*i.e.*, high

59   1,25(OH)$_2$D:24,25(OH)$_2$D) may upregulate PTH secretion and enhance the effects of vitamin

60   D (4). On the other hand, if 24,25(OH)$_2$D is itself biologically active, low

61   1,25(OH)$_2$D:24,25(OH)$_2$D may be beneficial. How this recently identified inverse

62   exponential relationship between serum 25(OH)D and 1,25(OH)$_2$D:24,25(OH)$_2$D 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)$_2$D 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 D$_3$ 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)$_2$D:24,25(OH)$_2$D 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)$_2$D and 24,25(OH)$_2$D; 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)D$_2$ and 25(OH)D$_3$) and total 24,25(OH)$_2$D (24,25(OH)$_2$D$_2$ and

138   24,25(OH)$_2$D$_3$) 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)$_2$D 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)D$_2$

143   and 25(OH)D$_3$, 0–25 nmol·L^{-1} for 24,25(OH)$_2$D$_2$ and 24,25(OH)$_2$D$_3$, and 12–480 pmol·L^{-1} for

144   1,25(OH)$_2$D. The mean coefficient of variation (CV) for intra-assay imprecision across the

145   measuring range of the assays was 4.9% for 25(OH)D$_2$, 8.3% for 25(OH)D$_3$, 7.7% for

146   24,25(OH)$_2$D$_2$, 9.0% for 24,25(OH)$_2$D$_3$, and 7.4% for 1,25(OH)$_2$D. The cumulative inter-

147   assay CVs were ≤7.4% for 25(OH)D$_2$, ≤9.6% for 25(OH)D$_3$, ≤10.6% for 24,25(OH)$_2$D$_2$,

148   ≤8.9% for 24,25(OH)$_2$D$_3$, and ≤9.3% for 1,25(OH)$_2$D. 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)$_2$D:24,25(OH)$_2$D 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 ΔF $P < 0.001$).

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 ΔF $P < 0.001$).

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 ΔF $P < 0.001$).

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

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

13

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

Relative concentrations of vitamin D metabolites and physical performance

An inverse exponential relationship exists between serum 25(OH)D and

1,25$(OH)_2$D:24,25$(OH)_2$D ratio (4). As the availability of 25(OH)D as a precursor

diminishes, the conversion of 25(OH)D to 24,25$(OH)_2$D is reduced, resulting in a

proportional increase in 1,25$(OH)_2$D (38). Superior physical performance in adults with low

1,25$(OH)_2$D:24,25$(OH)_2$D ratio suggests 24,25$(OH)_2$D is not a purely catabolic metabolite.

Given 1,25$(OH)_2$D is the most biologically active vitamin D metabolite, greater circulating

concentrations of 1,25$(OH)_2$D relative to 24,25$(OH)_2$D (*i.e.*, high 1,25$(OH)_2$D:24,25$(OH)_2$D)

might be expected to be favourable for physical performance (16). Our novel finding that

proportionally greater conversion of 25(OH)D to 24,25$(OH)_2$D relative to 1,25$(OH)_2$D (*i.e.*,

low 1,25$(OH)_2$D:24,25$(OH)_2$D ratio) was better for endurance performance, muscle strength,

and muscle power, suggests 24,25$(OH)_2$D might influence physical performance. Rather than

being a catabolic waste product, emerging evidence indicates 24,25$(OH)_2$D 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$(OH)_2$D 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

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

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.

17

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.

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.

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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 ± 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. [1]$P < 0.05$ *vs* cluster 1;

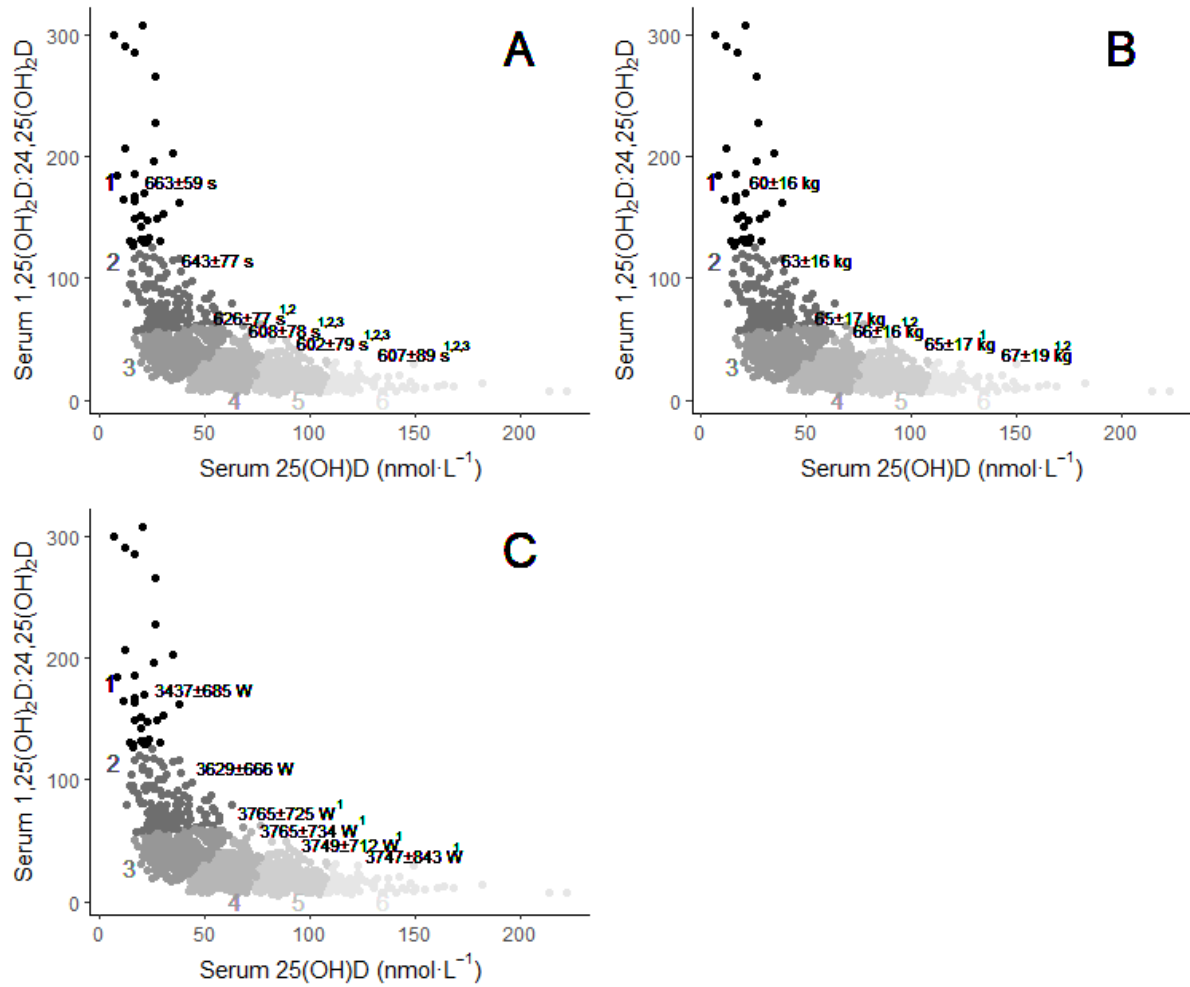575 [2]$P < 0.05$ *vs* cluster 2; [3]$P < 0.05$ *vs* cluster 3.

576

24

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	Summer	Fall	Winter	All Seasons
	$n = 358$	$n = 472$	$n = 394$	$n = 303$	$n = 1527$
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 $\geq$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, $f^2 \geq 0.02$, ≥ 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, $f^2 \geq 0.02$, ≥ 0.15 and ≥ 0.35 represent small, medium and large effect sizes, respectively (32).