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# 25-Hydroxyvitamin D Serum Levels Linked to Single Nucleotide Polymorphisms (SNPs) (rs2228570, rs2282679, rs10741657) in Sports Performance in Elite Athletes

Fernández-Lázaro Diego[1], Celorrio San Miguel Ana María[1], Seco-Calvo Jesús[2,3], Roche Enrique[4,5], Fernández-Lázaro César I[1,6]

University of Valladolid[1] (Spain), University of León[2] (Spain), University of the Basque Country [3] (Spain), Miguel Hernández University[4] (Spain), Instituto de Salud Carlos III[5] (Spain), University of Navarra[6] (Spain).

## Introduction & Objective

- The extreme physical and energetic demands of CrossFit® training require high nutritional requirements with nutrients, vitamins, and minerals (1). Vitamin D seems to acquire a more relevant role, in CrossFit® athletes, by protecting bone, immune, and muscle health (2). Additionally, optimal serum levels of 25-hydroxyvitamin D (25-OH/D) are positively related to strength power, and endurance performance (3). Single nucleotide polymorphisms (SNPs) have been reported to influence nutrients, including vitamin D status (4), and appear to modify individual responses related to athlete health and athletic performance (5). We conducted a pilot study to evaluate the impact and presence of a possible connection between 3 SNPs as CYP2R1 (rs2282679), GC (rs2228570), and VDR (rs10741657), serum 25-OH/D concentrations and the relationship with the degree of sports performance in highly trained CrossFit® athletes.

## Material & Methods

- A multicenter epidemiological, observational, longitudinal, pilot study was conducted in 2 CrossFit® Box and we report it here according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (6). The cohort consisted of $37.5 \pm 11.3$-year-old of fifty highly trained male CrossFit® (77.6 ± 10.9 kg; 171.5 ± 5.6 cm; 35.3 ± 11.7 months experience). Genomic DNA was isolated from 20 mL of the blood sample using FlexiGene DNA Kit (Qiagen, Hilden, Germany). 3 SNPs (rs10741657 to CYP2R1, rs2282679 to GC and rs2228570 to VDR) were genotyped with KASPar assays (KBiosciences, Herts, United Kingdom and LGC Genomics, United Kingdom) according to manufacturer's instructions. We assayed for 25-OH/D concentration using a commercially available Enzyme-linked immunosorbent assay (ELISA) kit (Eagle Biosciences, Nashua, NH, USA). The study was approved by the Clinical Research Ethics Committee of the University Hospital Valladolid (Spain) PI-19-1350.

## Results

### Table 1. Characteristics-Related with CrossFit® athletes (n=50).

| | | |
|---|---|---|
| Gender, n (%) | Male | 50 (100) |
| Nationality, n (%) | Spanish | 38 (76) |
| | Other | 12 (24) |
| Body mass (kg), mean (SD) | | 77.6 (10.9) |
| Fat Mass (kg), mean (SD) | | 9.7 (2.9) |
| Fat Mass (%), mean (SD) | | 12.5 (2.3) |
| Free Fat Mass (kg), mean (SD) | | 67.9 (4.1) |
| Free Fat Mass (%), mean (SD) | | 65.2 (2.6) |
| Height (cm), mean (SD) | | 171.5 (5.6) |
| VO2 max (ml/kg/min), mean (SD) | | 43.5 (4.4) |
| Crossfit® experience (months), mean (SD) | | 35.3 (11.7) |
| Fran1 WODs (seconds), mean (SD) | | 231 (15) |

*Abbreviations* = SD: standard deviation; WODs: Workouts of the day; VO2max: maximum amount of oxygen; n: sample size; %: percentage; kg: kilograms; cm: centimeters; ml: milliliters; min: minutes; [1] three rounds of thrusters and pull-ups for 21, 15, and 9 repetitions.

### Table 2. Comparisons between SNPs Vs. 25-OH/D

| Gen (SNPs) | Alleles | 25-OH/D (ng/mL), mean (SD) | p-value |
|---|---|---|---|
| CYP2R1 (rs10741657) | AA | 38.2 (11.2) | 0.076 |
| | GA | 26.9 (7.5) | |
| | TT | 21.5 (4.7) | |
| GC (rs2282679) | TT | 42.6 (3.2) | <0.05 |
| | GT* | 25.4 (5.7) | |
| | GG* | 21.6 (5.1) | |
| VDR (rs228570) | AA | 35.9 (8.3) | <0.05 |
| | GA | 24.4 (5.6) | |
| | GG$ | 18.9 (4.9) | |

*Notes:* Values are expressed as mean (SD) for quantitative variables. Statistically significant values at p-value level <0.05. The multiple comparisons test is based on the Bonferroni test. *: Significant differences with respect to TT. $: Significant differences with respect to AA. *Abbreviations* = SNPs: Single nucleotide polymorphisms; 25-hydroxy vitamin D, 25-hydroxy vitamin D.

### Table 3. Correlations between 25-OH/D & SNPs

| Gen (SNPs) | Alleles | Full Cohort (n = 50) | |
|---|---|---|---|
| | | r | p-value |
| CYP2R1 (rs10741657) | AA | **0.17** | **0.034** |
| | GA | 0.089 | 0.424 |
| | TT | **-0.34** | **0.016** |
| GC (rs2282679) | TT | **0.29** | **0.041** |
| | GT | 0.07 | 0.526 |
| | GG | **-0.33** | **0.012** |
| VDR (rs228570) | AA | **0.15** | **0.030** |
| | GA | 0.06 | 0.172 |
| | GG$ | **-0.43** | **<0.001** |

*Notes:* Bold type equals statistically significant values at p-value level <0.05. Correlations (r) are based on Spearman's rank correlation coefficient. SNPs, Single nucleotide polymorphisms

### Figure 1. Correlation of Sports Performance by WODs CrossFit® Total and 25-OH/D
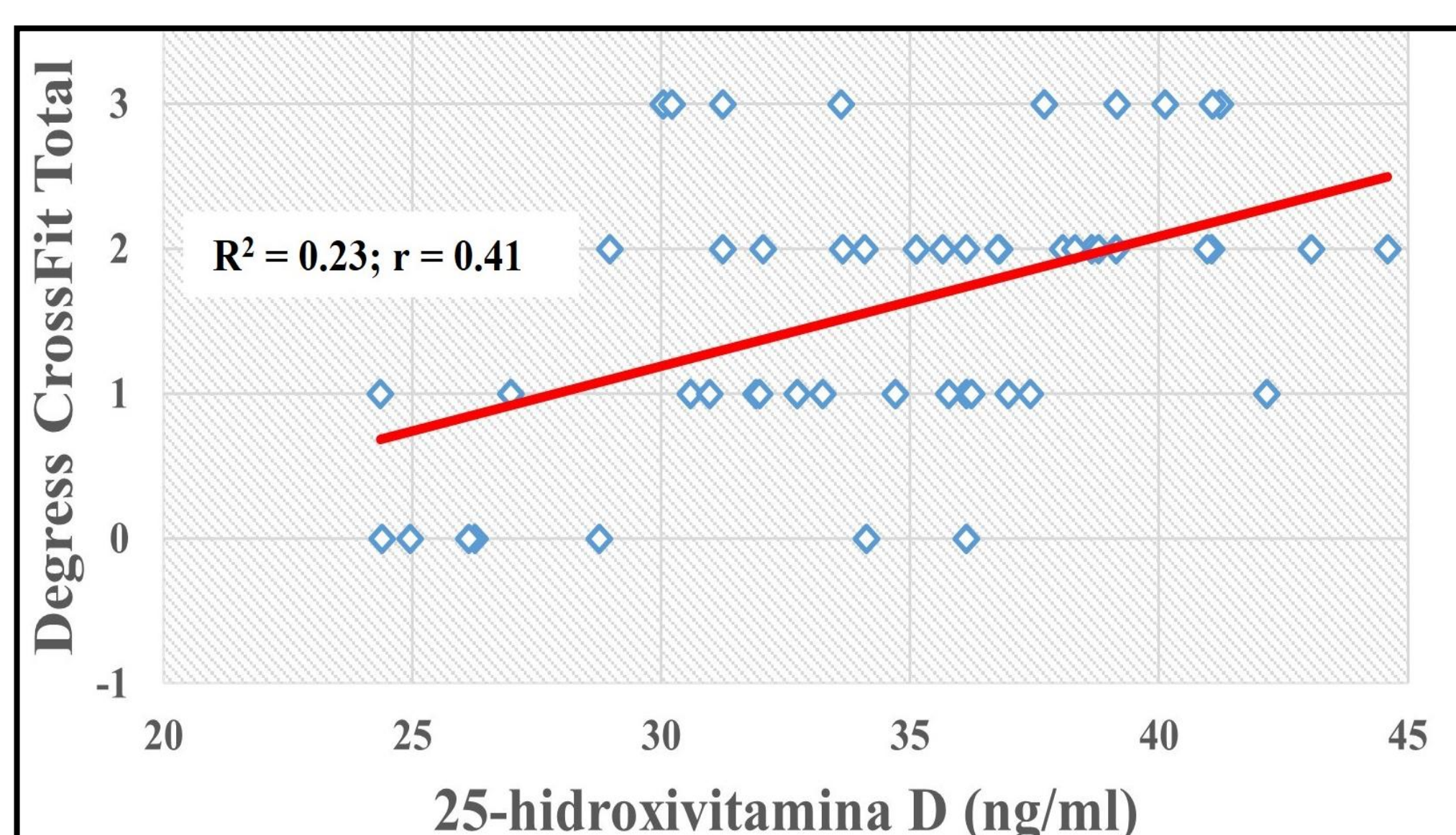


$R^2 = 0.23; r = 0.41$

### Table 4. Participants study characteristics and SNPs associated with 25-OH/D. Odds Ratio and 95% confidence intervals

| Variable | Full Cohort (n = 50) | |
|---|---|---|
| | OR (IC 95%) Crude | OR (IC 95%) Multivariate1 |
| Body mass index (BMI), (kg/m2) | 1.00 (ref..) | -- |
| VO2 max (ml/kg/min) | 1.62 (0.81-3.27) | 1.77 (0.54-3.65) |
| Age (years) | 0.91 (0.71-1.18) | 0.92 (0.62-1.46) |
| Free Fat Mass (kg) | 0.97 (0.84-1.191) | 1.14 (0.86-1.52) |
| CYP2R1 (rs10741657) | 1.00 (ref.) | -- |
| AA | 1.44 (0.74-2.87) | **2.01 (0.77-5.48)** |
| GA | 0.93 (0.782-1.05) | 1.02 (0.83-1.27) |
| GG | 0.83 (0.74-0.93) | 0.76 (0.65-0.89) |
| GC (rs2282679) | 1.00 (ref.) | -- |
| TT | 3.69 (2.28-5.99) | **3.67 (2.11-6.41)** |
| GT | 0.83 (0.68-1.02) | 0.76 (0.49-1.21) |
| GG | 0.67 (0.53-0.85) | **0.66 (0.51-0.89)** |
| VDR (rs228570) | 1.00 (ref.) | -- |
| AA | 2.93 (1.58-5.47) | **2.88 (1.43-5.92)** |
| GA | 1.01 (0.42-2.64) | 1.24 (0.29-6.11) |
| GG | 0.53 (0.23-1.42) | **0.31 (0.12-1.27)** |

*Abbreviations:* CI, confidence interval; BMI, body mass index; OR, Odds Ratio; ref, reference. Notes: **Bold letter** equals statistically significant values at p-value level <0.05. 1Multivariate model: adjusted for all variables in the table.

## Conclusions

Allelic variations in the CYP2R1 (rs10741657), GC (rs2282679), and VDR (rs2228570) SNPs affect the vitamin D status in CrossFit® athletes. Vitamin D levels have a moderate positive correlation (r = 0.41) with CrossFit® Total scores in athletes.

## References

## Acknowledgments