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

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Abstract: Vitamin D substantially influences sports performance and post-exercise recovery because it offers anti-inflammatory, antioxidant, and cellular protective properties. However, deficient levels of 25-hydroxyvitamin D 25(OH)D (25(OH)D) (<30 ng/mL) could expose the health of individuals , lead to musculoskeletal disorders, and decrease athletic performance. Therefore, it would be appropriate to know the interactions between genes and vitamin D. We evaluated 25(OH)D a possible connection between the presence of certain SNPs in CYP2R1 (rs10741657) GC (rs2282679), and muscle VDR (rs2228570) genes, with serum 25(OH)D concentrations and the degree of WOD performance in highly trained CrossFit® practitioners. Knowing these relationships could be instrumental for personalized vitamin D supplementation and training strategies. Using a standardized commercial enzyme-linked immunosorbent assay procedure, the concentrations of 25(OH)D were determined and the genotyping procedures for each SNPs were done by specific assays with the KASpar® test. 25(OH)DA performance level in grades was established based on the CrossFit® Total score (sum in kilograms of 1 Repetition Max Squat, Press, and Deadlift). Significant differences ($p<0.05$) in 25(OH)D concentration were found between each of the SNPs of CYP2R1 and GC with 25(OH)D . We discovered statistically significant weak positive correlations ($p<0.05$) between 25(OH)D and AA-alleles of the CYP2R1 and VDR genes, and TT-alleles of the GC gen. Additionally, AA (rs10741657 and rs2228570) and TT (rs2282679) have a probability between 2 and 4 of having major concentrations of 25(OH)D. 25(OH)D25(OH)D Conversely, GG alleles present a probability of suboptimum values of 25(OH)D of 69%, 34%, and 24% for VDR, GC, and CYP2R1 respectively. , showing a strong moderate positive correlation ($r=0.41$) between the degrees of sports performance and 25(OH)D25(OH)D plasma levels. The different polymorphisms of our 3-candidate gen CYP2R1 (rs10741657), GC (rs2282679), and VDR (rs2228570) disturb 25(OH)D concentration and play a critical role sports performance in elite CrossFit® practitioners . These results could highlight that the evaluation of genetic factors is key to designing a vitamin D supplementation strategy to improve sports performance.

Keywords: sports performance; 25-OH vitamin D; SNPs; genetics; elite athletes

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1. Introduction

CrossFit® is a gimmicky exercise very high-intensity interval exercise (HIIT) (1), performed through the so-called "Workouts of the Day" (WODs) (1), that allow training with standardized routines (2). The extreme physical and energetic demands of CrossFit® training necessitate high nutritional requirements. CrossFit® athletes seem to take their bodies to the physical limit and achieve maximum sports performance requires supplementation with nutrients, vitamins, and minerals (3–5). Consequently, nutritional practices must be implemented allowing to cover the specific requirements of CrossFit® practitioners. (3).

In this way, vitamin D is of particular importance to athletes (6) because of its multimodal role in the nervous, immune, muscular, and skeletal systems (7). Especially optimal levels, vitamin D seems to acquire a more relevant role, in CrossFit® athletes, by protecting bone, immune, and muscle health (6). Athletes who are deficient in vitamin D and perform high-intensity and duration training are at high risk of musculoskeletal injuries, immunosuppression, or arthritis (8,9). In addition, optimal levels of serum 25-hydroxyvitamin D (25(OH)D) correlate positively with sports performance, including strength and power, running, endurance and aerobic abilities(10). These physical capacities and physiological demands correspond to CrossFit® (11).

Genetic determinants that may influence circulating 25-(OH)D should be considered (12). Recently Fernández-Lázaro et al. has described that single nucleotide polymorphisms (SNP) influence nutrients, including the behavior of vitamin D (12), and could specifically condition each athlete's healthy state and sports performance. (13). Therefore, certain SNPs could modulate (increase or decrease) the concentration of bioactive nutrients in plasma(14). Regarding vitamin D, SNPs in CYP2R1 gene have an impact on vitamin D metabolism. CYP2R1 codes for the hepatic 25-hydroxylase of the cytochrome P450 family (15), responsible for the first hydroxylation to the active form of vitamin D. In addition, SNPs in the GC gene coding for vitamin D-binding protein (VDBP) have an influence in vitamin D transport. VDBP is a protein belonging to the albumin family, is the main carrier of vitamin D in blood (16,17). Finally, SNPs in the vitamin D-receptor (VDR) gene influence vitamin D biological activity in many tissues, being muscle of particular interest in sport performance 25(OH)D(16–20).

In view of the foregoing information, we conducted a pilot study to assess a possible connection between the presence of certain SNPs in CYP2R1 (rs10741657), GC (rs2282679), and muscle VDR (rs2228570) genes, with serum 25(OH)D concentrations and the degree of WOD performance in highly trained CrossFit® practitioners. Knowing these relationships could be instrumental for personalized vitamin D supplementation and training strategies. 25(OH)D25(OH)D2. Material and Methods

2.1. Study design

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 (21). Study´s participants involved were highly trained CrossFit® athletes (n = 126) training CrossFit® Box in Spain (Figure 1). The study was approved by the Clinical Research Ethics Committee (CREC) of Valladolid Clinical Hospital (PI-19-1350) (Spain). Following the Declaration of Helsinki and the 2013 Fortaleza Revision (22), the informed consent document was drafted that all CrossFit® practitioners read, accepted, and signed.

2.2. Inclusion Criteria

Healthy adults of legal age made up the study sample of highly trained CrossFit® athletes in 2 CrossFit® Box in Salamanca (Spain) and Soria (Spain). Our athletes met the following criteria: i) ≥ 20 months of experience training CrossFit®; ii) ≥ 2 participations in CrossFit® competitions in the last season; iii) completed "Fran" WODs < 250 seconds; iv) pass a pre-study medical exam to rule out pre-existing illnesses or injuries; v) Do not use products or drugs from "The List of Prohibited Substances and Methods of 2023" established by the World Anti-Doping Agency (WADA) , including vitamin D supplementation;; vi) Know and sign the informed consent where the potential benefit/risk of our pilot study was exhaustively explained.2.3. Data collection

Table 1. shows the sociodemographic data, anthropometric measurements, sports performance parameters, and dietary evaluation of CrossFit® practitioners. **Table 1.** Characteristics of the study participants.

Characteristics	**CrossFit® Athletes**	
Sample size (n)	50	
Age (years)	35.7 ± 11.3	
Gener n (%)	Male	50 (100)
	Female	0 (0)
Nationality n (%)	Spanish	38 (76)
	Other	12 (24)
Body mass (kg)	77.6 ± 10.9	
Fat Mass (kg)	9.7 ± 2.9	
Fat Mass (%)	12.5 ± 2.3	
Free Fat Mass (kg)	67.9 ± 4.1	
Free Fat Mass (%)	65.2 ± 2.6	
Height (cm)	171.5 ± 5.6	
VO2 max (ml/kg/min)	43.5 ± 4.4	
Crossfit® experience (months)	35.3 ± 11.7	
Fran[1] WODs (seconds)	231±15	

Data are expressed as mean ± standard deviation (SD). *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.

2.4. Sociodemographic and anthropometric

Gender, age, nationality (Spanish or other), body mass, fat mass, free fat mass, and height were included as sociodemographic and anthropometric characteristics. Bioelectrical impedance (BC-730; Tanita, Japan) was used to assess body mass, fat mass, and free fat mass (23). The height was measured with a tape measure from the base of the floor to the measurement marked on the wall. Read and record height to the nearest millimeter.

2.5. Physical Performance

Fran and CrossFit® Total WODs, and maximum amount of oxygen (VO_2max) were assessed as physical performance variables. Fran and CrossFit® Total tests were evaluated following the CrossFit® training guide by Glasman (1,2). VO_2max was determined by a modified Bruce treadmill protocol (24).

2.6. Dietary Assessment

The nutritional evaluation was carried out following our planned studies in elite athletes (25,26).

2.6.1. Quantification of plasma 25(OH)D concentration level

Quantification of plasma 25(OH)D concentration level of DNA was carried out following the methodology of our previous studies (12). According to Larson-Mayer et al. (8), which establishes 5 levels and reference ranges of based on 25(OH)D level for athletes: i) < 20 ng/mL deficiency; ii) < 30-32 ng/mL insufficient; iii) > 30-32 ng/mL sufficient; iv) 40-100 ng/mL optimum; v) > 150 ng/mL + hypercalcemia toxic.

2.6.2. Single Nucleotide Polymorphism (SNPs) determination by DNA Isolation and Genotyping

We evaluated 3 SNPs of the genes rs10741657 to CYP2R1, rs2282679 to GC and rs2228570 to VDR. The 3 candidate genes, CYP2R1, GC and VDR with all their biallelic varieties have an influence on the bioactive concentration of 25(OH)D (16,18). Isolation and genotyping of DNA was carried out following the methodology of our previous studies (12).

2.6.3. CrossFti® Total Level

CrossFit® Total degree was evaluated following the CrossFit® training guide level 1 by Glassman (1,2), which establishes level: i) < 270 kg beginner; ii) 271-360 kg intermediate; iii) 361-450 kg advance; iv) ≥ 451 kg elite. Mandatory requirement to be able to compete locally and amateur in CrossFit® ≥ 360 kg is the cut-off point in CrossFit® Total (27).

2.6.4. CrossFit Training

The training routine consisted of non-consecutive days of the week (Monday, Wednesday, Friday, and Saturday). The 80-minute CrossFit® training consisted of 4 parts: specific warm-up of the working muscle groups, a technique part based on strength and skills, the main part of WODs, and cool down through muscle stretching. All training is planned and led by a certified CrossFit® trainer with a Grade I or II certificate.

2.6.5. Statistical analysis

Statistical analyses were performed using StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC. We calculated means and standard deviations (continuous variables) and frequencies and percentages (categorical variables) in the descriptive statistical analyzes. . A general univariate linear test of fixed factors was performed comparing each SNP of the 3 genes and 25(OH)D. Subsequently, a Bonferroni post-hoc test correction was applied to determine the differences between the polymorphisms. Spearman's rank correlation coefficient was used to obtain correlations between polymorphisms and 25(OH)D.

Analyzes were performed to determine odds ratios (OR) and 95% confidence intervals (CI) to quantify the association between the different variables and each SNP evaluated and 25(OH)D. We considered a two-sided p-value less than 0.05 to be considered statistically significant. Regression models were used and the Pearson correlation coefficient (r) was calculated according to the coefficient of determination (R2), for the different sports levels of CrossFit and 25(OH)D. . P values less than 0.05 were considered statistically significant. .

3. Results

3.1. CrossFit® athlete´s characteristics and dietary assessment

The sociodemographic and anthropometric characteristics, physical performance and nutritional characteristics are shown in Table 1. Table 2 recorded the energy and micronutrient consumption in CrossFit® athletes.

Table 2. Macronutrients, energy and micronutrient consumption in CrossFit® athletes.

CrossFit® Athletes	**n = 50**

Energy (kcal/kg)	40.3±4.8
Proteins (g)	141.3±37.9
Fats (g)	134.3±43.2
Carbohydrates (g)	341.2±97.6
Ca (mg)	1026.3±224.1
Mg (mg)	544.3±97.2
P (mg)	2120.6±67.1
Fe (mg)	23.1±3.6
Zn (mg)	13.4±1.1
Vitamin A (μg)	1862.3±1177.1
Vitamin E (mg)	16.0±1.8
Vitamin B_1 (mg)	2.9±0.4
Vitamin B_2 (mg)	2.6±0.3
Vitamin B (mg)	40.9±6.1
Vitamin B_6 (mg)	4.3±0.5
Vitamin B_9 (mg)	637.2±172.1
Vitamin B_{12} (μg)	9.6±2.7
Vitamin C (μg)	351.1±140.2

Data are expressed as mean ± standard deviation. *Abbreviations* = kg: kilograms; g: grams; mg: milligrams; μg: micrograms; Kcal: kilocalories.

3.2.25(. OH)D plasma level

25(OH)D plasma level of highly trained male CrossFit® was 34.7 ± 5.2 ng/mL and 68.0 % had 25(OH)D level sufficiency described in Table 3.

Table 3. Distribution of CrossFit® athletes according to 25-hydroxy vitamin D ranges for sports population.

Age (years)	**Sample (n)**	**25-OH/D (ng/mL) mean (SD)**	**[1]Deficiency n (%) < 20 ng/mL**	**[1]Insufficiency n (%) < 30-32 ng/mL**	**[1]Sufficiency (%) > 30-32 ng/mL**	**[1]Optimum (%) 40 -100 ng/mL**	**[1]Toxic (%) > 150 ng/mL + hypercalcemia**
< 35	19	36.2 (4.3)	-	2 (10.5)	14 (73.7)	3 (15.8)	-
> 35	31	33.1 (6.8)	-	6 (19.3)	20 (64.5)	5 (16.2)	-
35.7 (11.3)	50	34.7 (5.2)	-	8 (16.0)	34 (68.0)	8 (16.0)	-

Values are expressed as mean (standard deviation) for quantitative variables and as frequency (percentage) for categorical variables. *Abbreviations* = SD: standard deviation; ng: nanograms; mL: milliliters; %: percentage. [1]Characterization of 25-hydroxy vitamin D levels and ranges in athletes´ populations by Larson-Mayer et al. (25).

3.3. CrossFit® Total degrees

The description of the SNPs, 25-OH/ and CrossFit® Total degrees show in Table 4. Seventeen athletes were classified as competitors according to Competition RuleBook CrossFit® Games 2023 (27).

Table 4. CYP2R1, GC and VDR genes polymorphism, 25-hydroxy vitamin D plasm level and sports performance levels in the CrossFit® Total.

Gen	**SNPs**	**Allele**	**n (%)**	**Degrees of CrossFit® Total (Level n)[1]**				**CrossFit® Athletes**
				Beginner (Level 0) < 270 kg	**Intermediate (Level 1) 271-360 kg**	**Advanced (Level 2) 361-450**	**Elite (Level 3) ≥ 451**	**Competitors[2] (+360 kg / +1000 lb)**

CYP2R1	**rs10741657**	**AA**	17 (34.0)	0	6	8	3	11
		GA	25 (50.0)	2	18	4	1	5
		GG	8 (16.0)	4	3	1	0	1
		AA/GA/GG	50 (100.0)	6	27	13	4	17
GC	**rs2282679**	TT	19 (38.0)	1	8	7	3	10
		GT	22 (44.0)	3	15	4	0	4
		GG	9 (18.0)	2	4	2	1	3
		TT/GT/GG	50 (100.0)	6	27	13	4	17
VDR	**rs2228570**	AA	21 (42.0)	0	11	6	4	10
		GA	18 (36.0)	2	10	6	0	6
		GG	11 (22.0)	4	6	1	0	1
		AA/GA/GG	50 (100.0)	6	27	13	4	17

Values are expressed as frequency (percentage) for categorical variables. *Abbreviations* = SNPs: Single nucleotide polymorphisms; 25(OH)D: 25-hydroxy vitamin D; %: percentage; kg: kilograms; lb: pounds. [1]Glassman G. CrossFit training guide level 1; [2]Competition RuleBook CrossFit® Games 2023.

3.4. Comparisons between 25-hydroxy vitamin D and single nucleotide polymorphisms of the CYP2R1, GC and VDR genes.

Table 5 shows the existence of significant differences ($p < 0.05$) in the plasma concentration of 25(OH)D between the 3 biallelic combinations of the SNPs r2228570 (VDR) and rs2282679 (GC). Furthermore, statistically significant differences ($p < 0.05$) were observed in the concentration of 25(OH)D between athletes carrying the GG genotype with respect to the homozygous bialleles TT [rs2282679 (GC)] and AA [r2228570 (VDR)]. Also, the heterozygous biallele GA compared to AA for the SNP r2228570 (VDR) showed significant differences ($p < 0.05$) in 25OH/D plasm level.

Table 5. Comparisons between 25-hydroxy vitamin D and single nucleotide polymorphisms of the CYP2R1, GC and VDR genes.

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)	
		GG	21.5 (4.7)	
GC	**rs2282679**	TT	42.6 (3.2)	<0.05
		GT*	25.4 (5.7)	
		GG*	21.6 (5.1)	
VDR	**rs2228570**	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. *Abbreviations* = SNPs: Single nucleotide polymorphisms; 25(OH)D: 25-hydroxy vitamin D; ng: nanograms; mL: milliters. The multiple comparisons test is based on the Bonferroni test. *: Significant differences with respect to TT. [$]: Significant differences with respect to AA.

3.5. Correlations between 25-hydroxy vitamin D and single nucleotide polymorphisms of the CYP2R1, GC and VDR genes.

For homozygous AA (rs1074165; rs2228570) and TT (rs2282679) bialleles showed remarkably weak positive correlations ($p < 0.05$). Moreover, mild negative correlations ($p < 0.05$) were obtained for all homozygous GG of rs1074165 ($r = -0.34$; $p = 0.016$), rs2282679 ($r = -0.33$; $p = 0.012$) and rs2228570 ($r = -0.43$ ($p < 0.001$) (Table 6).

Table 6. Correlations between 25-hydroxy vitamin D and single nucleotide polymorphisms of the CYP2R1, GC and VDR genes. .

Gen (SNPs)	Full Cohort (n=50)	
	r	***p*-valor**
CYP2R1 (rs10741657)		
AA	**0.17**	**0.034**
GA	0.089	0.424
GG	**-0.34**	**0.016**
GC (rs2282679)		
TT	**0.29**	**0.041**
GT	0.07	0.526
GG	**-0.33**	**0.012**
VDR (rs2228570)		
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. *Abbreviations* = SNPs: Single nucleotide polymorphisms.

3.6. Single nucleotide polymorphisms of the CYP2R1, GC and VDR genes associated with 25-hydroxy vitamin D plasma level.

Table 7 shows the set of results of the multivariate logistic regression analysis to generate a diagnostic/predictor model of the plasma concentration of 25(OH)D that consisted of 3 variables that were the 3 SNPs of our study: CYP2R1, GC and VDR. Athletes who include an AA homozygous bialleic genotype (rs10741657 [OR 2.01, 95% CI 0.77-5.48]); (rs2228570[OR 2.88, 95% CI 1.43-5.92]) and TT (rs2282679[OR 3.67 95% CI 2.11-6.41]) could be more prone to have higher levels of 25(OH)D than other genotypes of these SNPs. On the contrary, our results have shown that athletes carrying the homozygous biallele GG (CYP2R1, GC and VDR) were associated with a lower concentration of 25(OH)D. Being more relevant in VDR rs2228570 (OR 0.31, 95% CI 0.12-1.27).

Table 7. Participant study characteristics and single nucleotide polymorphisms of the CYP2R1, GC and VDR genes associated with 25-hydroxy vitamin D concentration. Odds Ratio (OR) and 95% confidence intervals (95% CI).

Variable	Full Cohort (n=50)	
	OR (IC 95%) Crude	**OR (IC 95%) Multivariate[1]**
Body mass index (BMI), (kg/m^2)	1.00 (ref..)	--
VO_2 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 (rs2228570)	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)**

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

3.7. Correlation of sports level degree in CrossFit® Total and 25-hydroxy vitamin D (25(OH)D) plasma level

Figure 2 shows the correlation of sports level degree in CrossFit® Total and 25(OH)D plasma level. Figure 2 shows an R2 = 0.23 indicating that at least 23% of the changes in the CrossFit® Total score are reliable for the 25(OH)D level. In addition, a positive correlation (r = 0.41) is shown between the sports level degree in CrossFit® Total and 25(OH)D concentration.

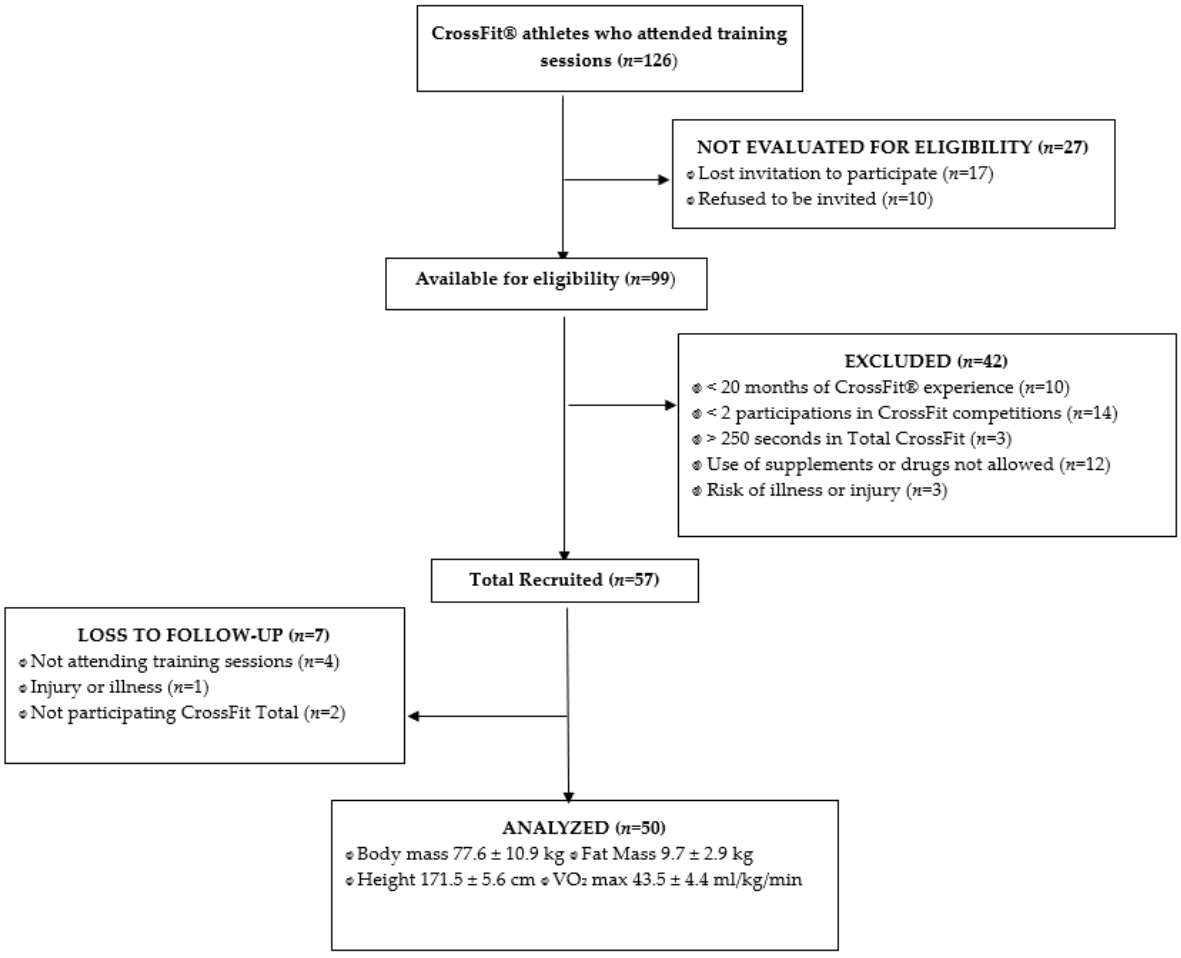

Figure 1. STORBE Flow Diagram for recruitment.

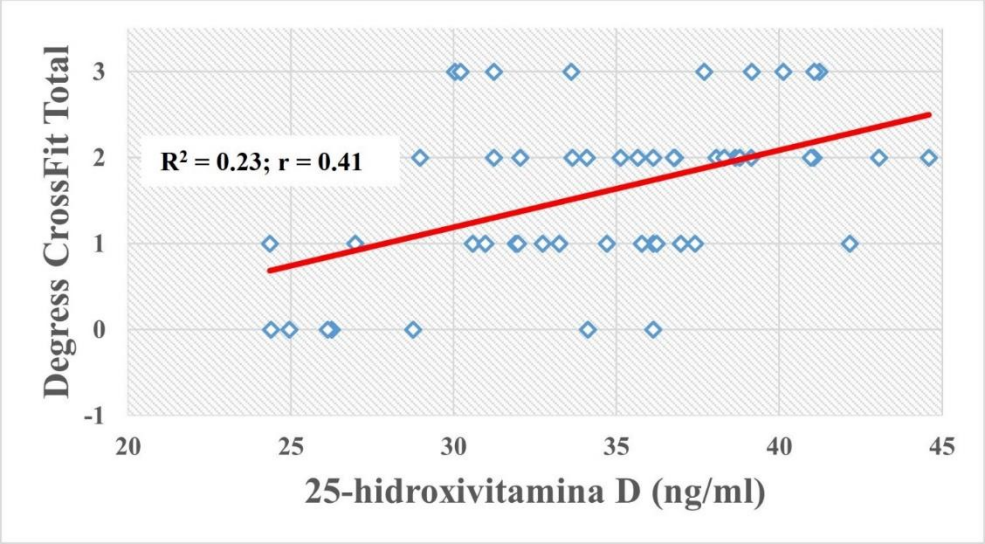

Figure 2. Correlation of sports level degree in CrossFit® Total and 25-hydroxy vitamin D (25(OH)D) plasm levels.

4. Discussion

Vitamin D substantially influences sports performance and post-exercise recovery because it offers anti-inflammatory, antioxidant, and cellular protective properties (28), on muscle cells (9). The pathways described that reveal the effects of vitamin D in restoring and sustaining the optimal healthy condition of skeletal muscle are genomic and/or non-genomic (29), in the same way as other nuclear steroids (30), through the vitamin D receptor (VDR) based in myocytes (31). Henceforth, adequate expression of VDR is essential, because vitamin D alone could not control or modulate the mass and/or functionality of skeletal muscle. (32). The loss or decrease in VDR expression is related to muscle pathologies and aging (33). However, increases in VDR expression are related to regeneration after muscle damage (34). Thus, the increased expression of VDR and the higher vitamin D plasma levels would favor this interaction (Vitamin D-VRD) (31). In humans, rs2228570 is the only VDR polymorphism that has distinct structural consequences for the VDR protein (35). Also, 25(OH)D ≥ 30 ng/ml induces muscular positive regulation of VDR (31,32) and exogenous vitamin D induces upregulation of VDR in the systemic extracellular matrix in primary muscle cells (34,36).

We have identified that VDR genotype variants (rs2228570) have significant differences ($p < 0.05$) in blood levels of 25(OH)D. Our results showed a negative correlation ($r = -0.43$; $p < 0.001$) were obtained between the total concentration of 25(OH)D and the homozygous GG biallele. Furthermore, CrossFit® athletes carrying AA (rs2228570) were 3 times more likely (OR 2.88, 95% CI 1.43-5.92) to have higher levels of vitamin D. VDR with the homozygous FokI AA genotype results in increased VDR protein activity compared to GA or GG genotypes (37). Thus, VDR gen´s polymorphisms may condition VDR expression and protein stability (38). In this sense, the A allele of VDR rs2228570 was associated with VDR mRNA copy number (18,39). Also, VDR expression is elevated acutely (1 to 3 hours) after resistance exercise (31). Although low levels of VDR expression in skeletal muscle don´t rule out direct actions on its physiological effects (31). Our findings suggest that the A allele is an element that safeguards achieving optimal levels of 25(OH)D (12) and full VDR protein acquisition with optimal physiological functioning (35), which enables improved sports performance. In this sense, we identified 12 advanced athletes and 4 elite athletes' carriers with the A allele of VDR (rs2228570), which means that 16 athletes were defined as level competitors in CrossFit® games, based on their sports performance.

The steps prior to the binding of 1-25OH/D and VDR require the hydroxylation of vitamin D by CYP2R1 (15) and 1-25 OH/D transport to the target VDR, by DBP (16,17).

Both SNPs, GC, and CYP2R1, substantially affect vitamin D status (13). GG genotype of CYP2R1 (rs10741657) was significantly more likely to have inadequate 25(OH)D levels (16) and GG and GT bialleles (GC rs2282679) was associated with lower 25(OH)D concentrations (17). Consistent with these studies (16,17), in our CrossFit® athletes the A allele of CYP2R1 rs10741657 and the T allele of GC rs2282679 were associated with 2-3 times more likely to present higher levels of 1-25OH/D, as in older adult patients (12). In fact, we found that 16 and 14 "competitive" sports-grade athletes carried the A allele (rs10741657) and the T allele (rs2282679) respectively.

On the other hand, non-genomic VDR pathways should be considered due to the direct action of vitamin D that optimizes the contractile process of skeletal muscle through greater mobilization of calcium towards the sarcoplasmic reticulum (40,41). Plasma levels in physiological ranges (> 30 ng/mL of 25(OH)D), as in the athletes in our study, will potentially improve skeletal muscle functionality (42) indirectly, which is key to athletic performance in CrossFit®.

Optimal 25(OH)D plasma levels of acts by promoting the improvement of neuromuscular function in older people (8,41). In this way, increases in skeletal muscle strength and physical capacity linked to levels in the adequate physiological range of 25(OHD). (12,43). These results are consistent with those reported in our CrossFit® athletes in the strength performance test The influence of the blood concentration of 25(OH)D in the skeletal muscle of our athletes will be responsible for 23 % of the effects on the sports level establishing a moderate positive correlation (r = 0.41) between the CrossFit® Total grade and 25(OH)D plasma levels. In our study, allelic variations in the SNPs GG (rs10741657), GC (rs2282679) and VDR (rs2228570) affect the plasma concentration of vitamin D in CrossFit® athletes, which may condition their level of sports performance. Especially the effects induced by the 1,25OH/D - VDR complex, through genomic and non-genomic pathways, leading to progresses in muscle health, muscle functionality, strength, muscle recovery, and potentially physical work. (41,44). In fact, rs2228570 (FokI) is the only polymorphism that perturbs the length and functionality of the VDR protein. Furthermore, deficient (>10 ng/mL) or toxic (>150 mg) levels of 25(OH)D inactivate the biologically active form of VDR (45). Our athletes maintained an adequate plasma concentration of 25(OH)D (34.7 ± 5.2 ng/mL), which would not structurally affect the VDR, maintaining its activity mediated by genomic and non-genomic pathways on skeletal muscle.

Regarding the possible practical applications of our findings, through SNPs, could allow athletes, coaches and/or sports nutritionists to recognize persons at potential risk of hypovitaminosis D and adjust possible nutritional actions by improving intake or supplementing. With the purpose of improving health, functionality and muscle performance in athletes, these tips are important for precision personalized nutrition and/or supplementation.

5. Conclusion

In this research proved those allelic variations in the CYP2R1 (rs10741657), GC (rs2282679), and VDR (rs2228570) SNPs disturb the (OH)D behavior in CrossFit® athletes. Thus, genetic polymorphisms of the genes (CYP2R1, GC and VDR) could be the key elements in the modulation of plasma 25(OH)D concentration. will depend and its availability to modulate the expression of genes involved skeletal muscle performance and/or health, such as VDR. Finally, we stated that the concentration of 25(OH)D has a moderate positive correlation (r = 0.41) with sports level degree in CrossFit® Total.

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