Association of Vitamin D Supplementation in Cardiorespiratory Fitness and Muscle Strength in Adult Twins: A Randomized Controlled Trial

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Abstract

Background: Although vitamin D is related to cardiorespiratory fitness and muscle strength, there is no evidence in the literature about the genetic influence of the response to the increase in body vitamin D. Therefore, we evaluate the effect of longitudinal supplementation of vitamin D on parameters of physical fitness in monozygotic twins.

Methods: In total, 74 participants were included, with a mean age of 25 years, divided into two groups, one group received supplementation with cholecalciferol for 60 days and the other group did not. Cardiorespiratory fitness and muscle strength were measured before and after supplementation, through maximal treadmill tests and dynamometry, respectively. Wilcoxon tests were used to compare intra-group results and the Mann-Whitney test to examine intergroup differences.

Results: There was an increase in the serum concentration of vitamin D in participants who ingested the supplementation. Cardiorespiratory fitness improved after supplementation through increases in the values of maximum oxygen consumption of 28% (P <0.001). Muscle strength in right hand grip significantly increased in participants who received the supplement to 33.0 (P = 0.007).

Conclusion: 60 days of cholecalciferol supplementation improved cardiorespiratory fitness and upper limb muscle strength.

Trial registration: RBR-3qy2f2. Registered 28 March 2016, http://www.ensaiosclinicos.gov.br/rg/RBR-3qy2f2/2

Key Points
- Our work is innovative for using twin siblings in a clinical trial, decreasing the genetic differences between the control and intervention groups. Each pair of twins of the same sex was divided and one identical sibling was allocated to the control group and the other to the intervention group. Twins of different sexes were not included, only female sibling pairs or male sibling pairs.
- Supplementation with 2000 IU cholecalciferol for only 60 days generated benefits in cardiorespiratory fitness and muscle strength, by significantly increasing the Maximum Oxygen Consumption and the right hand grip.

Introduction

Vitamin D is involved in several non-skeletal functions, playing an important role in immunity and in cardiopulmonary and muscle functions [1]. Vitamin D receptors are present in several types of cells, including skeletal muscle, heart muscle, and vascular smooth muscle [2]. Thus, vitamin D insufficiency has been shown to be related to muscle weakness, arterial thickening, myocardial hypertrophy, and hypertension [3, 4].
Aerobic capacity measured through Maximum Oxygen Consumption (VO_{2}\text{max}), has been widely used in studies as a good indicator of cardiovascular condition [5, 6]. Low levels of cardiorespiratory fitness have been associated with health and quality of life problems, such as lower longevity, lower disposition, higher body fat content, worse lipid profile, and disturbances in glucose metabolism [7]. Thus, better cardiorespiratory fitness can reduce the risk of death from cardiovascular and metabolic diseases [8, 9].

There is significant evidence that vitamin D is an influential factor for VO_{2}\text{max} [10, 11, 12]. However, the majority of studies on this theme are observational, raising the question as to whether the serum concentration of 25-hydroxyvitamin D (25 (OH) D) is a cause of increased VO_{2}\text{max} or simply a result with little significance [13]. This increases the importance of clinical trials that help elucidate the relationship between vitamin D and VO_{2}\text{max} [14].

It is also known that vitamin D affects muscle strength, muscle size, and neuromuscular performance [15, 16] and the reduction in muscle strength and power also impairs the quality of life of individuals, as these factors influence the functional capacity to perform basic activities of daily life, generating limitations for their execution [17].

Both VO_{2}\text{max} and muscle strength are influenced by genetic factors [18] and the only human study model that enables control of the genetic factor is the study of monozygotic twins (MZ). MZ twins develop from a single egg cell, giving rise to two individuals who share the same genetic load and, because they have the same genotype, the differences between them occur mainly due to the external influences of the environment where they are inserted; the greater the differences in these influences, the greater the probabilities of different phenotypic observations. Thus, the realization of a study with twins enables analysis of the influence on the phenotype through the isolation of the interference of the genetic factor [25], in the case of the current study, the divergent external influence will be vitamin D supplementation. Thus, clinical trials with MZ allow control of one of these factors, making them extremely relevant [19].

Therefore, knowing the relationship of vitamin D with cardiorespiratory fitness and muscle strength, we aimed to establish whether low doses of this nutrient would bring improvements to the health of individuals. Therefore, the aim of this study is to evaluate the effect of cholecalciferol supplementation on cardiorespiratory fitness and muscle strength in healthy adult MZ twins.

**Methods**

**Study Population and Randomization**

In total, 74 men and women volunteer MZ twins participated in the study. To obtain a statistical power of 0.958, using the values of Mean and Standard Deviation, at least 25 individuals were required before supplementation and 25 individuals after supplementation. Zygosity in twins was determined using a validated zygosity questionnaire with 93.3% accuracy [20]. This was a randomized clinical trial by lot, performed by the researcher responsible, which took place between 2016 and 2018, where each pair of
twins was separated, with one twin allocated to the control group (CG, without intervention) and the other to the supplemented group (SG, with intervention) (Fig 1). All analyses were performed on the first day of inclusion in the research (T0) and after 60 days of supplementation (T60).

Eligibility criteria included twins of the same sex, from 18 to 40 years of age, with no diagnosis of any chronic non-communicable disease, non-obese, without dyslipidemia, and non-smokers. The exclusion criteria included individuals who could not perform the physical tests, who were users of illicit drugs, and those using energy or protein supplements, or supplements containing vitamin D, pregnant women, nursing mothers, and patients with metallic implants and pacemakers.

The twins included in the SG consumed 1 oily capsule of cholecalciferol per day, with a concentration of 2,000 IU, for a period of 60 days in the morning. This concentration does not exceed the maximum tolerable daily intake limit (UL), which is 4,000 IU/day [21], in addition to being considered a dose for maintaining adequate levels of vitamin D in individuals after treatment for deficiency of this nutrient. All participants in the SG received a follow-up form to daily record when they ingested the capsule. Sixty days after the start of supplementation, these forms were returned to the researchers, for monitoring compliance. The researchers responsible for conducting physical tests and blood collection did not know which group the individual was included in, the CG or SG.

During the intervention period, the twins in the CG and SG maintained their usual routine, without dietary, sports, or lifestyle changes. After 30 days of inclusion in the study, participants from the SG group were interviewed to confirm appropriate capsule intake and participants from both groups were interviewed to confirm maintenance of their routine.

At T0 and T60, all participants in the CG and SG groups performed collection of peripheral blood samples after a 12-hour fast, on a different day from the physical tests. The chemiluminescence technique (Access 2, Beckman Coulter, United States) was used to measure 25 (OH) D in serum. Values below 20 ng/mL were considered as deficiency [22].

At the beginning of the research, the primary endpoint was considered as the change in the serum concentration of 25 (OH) D after 60 days of supplementation with cholecalciferol. Our secondary endpoint was the variation in physical fitness after 60 days of cholecalciferol supplementation.

**Determination of Muscle strength:**

Isometric strength of the upper limbs was evaluated through dynamometry to determine muscle strength, using the handgrip and scapular strength test. The handgrip test followed the recommendations of the American Society of Hand Therapists, where the subject remained seated comfortably, positioned with the elbow at 90°, the forearm in a neutral position, and the wrist in a position varying from 0° to 30° of extension [23], using a hydraulic dynamometer (JAMAR®, precision of 0.2 kilogram-force - kgf). All subjects received verbal commands to perform the test, with standardized instructions. The test always started on the right side.
The recorded value of each individual was the highest value among the three measurements, performed alternately between the hands, with 5 seconds of maximum contraction in each handgrip and 60 a second rest between the measurements.

Scapular strength was measured using a 50 kgf Crow® scapular dynamometer. Each participant, standing, with their feet in an anatomical position, held the dynamometer at chest level, without touching the chest, with their elbows open and parallel to the ground. At the command of the evaluator, the participant performed a maximum scapular contraction lasting 3 to 5 seconds, with three repetitions, and an interval of 60 seconds between them. The highest value between attempts was considered as the result.

**Determination of Cardiorespiratory Fitness**

To determine maximum oxygen consumption, the cardiopulmonary stress test was performed (TECP) on a Cortex ergospirometer (Metalyzer® 3B), following a treadmill ramp protocol. Progressive and continuous speed and inclination increments were applied, according to the individual capacity estimated for each participant, based on the American College of Sports Medicine (ACSM) [24], and aiming to achieve absolute and relative VO₂max in a test period of 8 to 12 minutes [5]. An initial speed and incline pattern was adopted for sedentary and active individuals, 3km/h and 0% incline and 4km/h and 0% incline, respectively. The final speed and incline were determined according to the following prediction formulas, considering walking for sedentary and running for active individuals [24]:

1 - Walking \( \rightarrow \) \( \text{VO}_2 \) = Speed \( \times 1.675 + 0.3015 \). Speed. Incline + 3.50;

2 - Running \( \rightarrow \) \( \text{VO}_2 \) = Speed \( \times 3.35 + 0.15075 \). Speed . Incline + 3.50.

Oxygen consumption was continuously recorded during the TECP (\( \text{VO}_2 \)) as well as the respiratory exchange ratio (RER), breath by breath, through analysis of pulmonary gas exchange (metabolic analyzer Metalyzer® 3B). The equipment was calibrated prior to the development of the research and at the beginning of each TECP through self-calibration.

**Statistical Analysis**

The statistical software IBM SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all analyses. The Kolmogorov-Smirnov test was used to assess the distribution of variables, which presented non-parametric distribution. Categorical variables are shown as number (percentage) and compared by the Chi-square test. Continuous variables are shown as median (minimum and maximum). The differences between the medians of vitamin D, cardiorespiratory fitness, and muscle strength of the CG and SG groups were assessed by the Mann-Whitney test and the Wilcoxon test was used to evaluate the differences in the median intragroup values of the CG and SG between T0 and T60. All differences were considered significant when \( p \leq 0.05 \).
This study was approved by the Brazilian Registry of Clinical Trials under code RBR-3qy2f2 (05/09/2017) and the Research Ethics Committee of the Federal University of Rio Grande do Norte (UFRN), protocol number 1.385.218, CAAE 51186615.5.0000.5292.

Results

All 74 twins, 54 female and 20 male, with an average age of 25 years, completed the study, with 37 individuals in the CG and 37 individuals in the SG. The BMI (kg/m$^2$) of the CG was 25.2 and of the SG 24.9, with no differences between groups ($p = 0.798$) at T0 and T60. All SG individuals ingested 100% of the capsules provided. Family income, training frequency, and training duration were similar between the CG and SG (Table 1).

Vitamin D concentration in the SG twins increased 70% after 60 days of consuming 2000 IU cholecalciferol daily (T0 = 30.00 ng/mL and T60 = 50.87 ng/mL $p < 0.001$). In the CG there was no variation in this concentration (T0 = 30.02 ng/mL and T60 = 31.81 ng/mL $p = 0.950$). At T0 there were no differences between the CG and SG ($p = 0.189$). No subject presented potentially toxic concentrations of 25-hydroxy-vitamin D.

Cardiorespiratory fitness and muscle strength presented no differences between the CG and SG at T0. However, at T60, absolute VO$_2$max was different between the CG and SG ($p < 0.015$), since after supplementation, at T60, absolute VO$_2$max increased by 28% in the SG ($p < 0.001$). All subjects maintained their usual routine during the two months of participation in the study, including the level of physical exercise, which can be confirmed by the lack of variation in RER in both groups (Table 2).

Muscle strength of the left arm also increased in the SG after supplementation ($p = 0.007$) (Table 2). It should be noted that only 1 individual in each group used their left hand as predominant, the others used their right hand for writing and other tasks.

Discussion

In this clinical trial with healthy male and female MZ twins, we found that vitamin D supplementation for two months improved cardiorespiratory fitness and muscle strength by increasing the VO$_2$max and manual strength of the left arm.

To the best of our knowledge, this is the first study to explore the links between vitamin D supplementation and cardiorespiratory fitness in MZ twins. We chose to work with MZ twins to reduce the genotypic differences in phenotypic responses, since each MZ pair comes from a single zygote and they present genetically identical elements [25].
We observed alterations in oxygen consumption in the group that received supplementation, through improvement in absolute VO$_{2}$max, however, when considering body mass we did not find significant changes in relative VO$_{2}$ max, suggesting that regardless of genetics, absolute VO$_{2}$max can be modified by healthy eating habits and possibly by an increase in serum vitamin D.

This occurs because absolute VO$_{2}$max is less influenced by genetics when compared to relative VO$_{2}$max [26], so, as our study used monozygotic twins, we evidenced that genetics were decisive for responding to our external factor, in this case, supplementation. However, we did notice a slight increase in the 75th percentile of the supplemented group in relation to the relative VO$_{2}$ max, which could be a possible indication that this supplementation can alter the relative VO$_{2}$ max if used in larger doses or for a longer period of time. Thus, further research on the topic with MZ twins is needed to complement this study.

The hereditary and environmental contribution to biochemical, anthropometric, and functional markers as risk indicators for physical health, has been observed in studies on heritability [27, 28]. These studies aim to understand the relative influence of genotype and environment on phenotypic variation. Tarnoki [29] suggests that studies with twins may reveal genetic alterations, which are not clearly manifested, associated with biomarkers.

Cardiorespiratory fitness is recognized as a central determinant of survival in healthy individuals or those with comorbidities [30, 31]. Low fitness has been proposed as a new modifiable marker to improve risk stratification [30], since risk factors tend to group in sedentary people, regardless of age and sex [32].

In view of the increase in cardiovascular disorders, there is a need to investigate efforts to prevent and screen cardiovascular risk factors. In addition, good cardiorespiratory fitness is a predictor of life expectancy, both in patients with or without heart disease [33].

Pitz et al.[34] demonstrated the existence of a direct and indirect action of vitamin D on the function of cardiomyocytes and morphology of the heart, since low levels of vitamin D were associated with a higher prevalence of myocardial dysfunction. Gallagher and collaborators [35] showed that vitamin D can improve aerobic performance through indirect action on VO$_{2}$max. This corroborates with our results, since an increase in blood levels of 25 (OH) D was observed, increasing the maximum volume of oxygen captured and taken to the tissues through the cardiovascular system for energy production, in a unit of time.

It is possible that vitamin D contributed to this increase due to the greater expression of the vitamin D receptor (VDR) existing in several cells that make up the cardiovascular system, actively participating in cardiac function, through the regulation of muscle cell growth and the degree of myocardial contractility, among others [36, 37].

Vitamin D participates in cellular mechanisms that act on muscle growth and strength [35], being important for maintaining the mass, strength, and speed of contraction of skeletal muscle [38]. In this sense, vitamin D is considered fundamental for muscle quality and strength production [39], and its
deficiency has been related to decreases in these factors and in muscle mass [40], with impaired balance and an increased incidence of falls, which can cause irreversible functional limitations [41, 42, 43].

Greater muscle strength may also be associated with better health and cardiovascular protection [44], a lower incidence of cardiovascular disease, and lower risk of all-cause mortality [45, 46, 47].

Thus, vitamin D supplementation has been gaining prominence as a strategy to control physical fitness, including muscle strength [48, 49]. However, as the majority of studies were carried out in older adults or athletes [41, 50, 1], the possible benefits that may exist from vitamin D supplementation in healthy non-athlete adults are not yet known.

Zhu et al., [51] after supplementing 302 older women, aged between 70 and 90 years, who were deficient in vitamin D, with 1,000 IU/day for one year, concluded that the association of vitamin D with calcium for supplementation is used for improving muscle strength and mobility. Supplementation with 1,000 IU/day of vitamin D alone for nine months in 160 postmenopausal women with a mean age of 58 years showed a significant increase in muscle strength in the lower limbs through the sit and stand test [52].

Handgrip dynamometry is often used for functional and nutritional assessment of healthy and pathological individuals, not only to measure hand strength, but to assess total body strength [53, 54] and the nutritional status of pre and post-surgical patients [55, 56, 57]. Handgrip dynamometry is an inexpensive method, which is easy to use, and its results are well accepted in research and clinical evaluations [58, 59].

Our results indicate a possible benefit of the increase in the serum concentration of vitamin D in the long term, since after only two months of supplementation in low concentration, it was possible to observe a slight increase in manual strength of the non-dominant arm in SG, and only one individual used the left hand as the dominant hand. Dominance is an individual factor that significantly influences the measurement of manual dynamometry [60], as the dominant hand has higher handgrip strength values than the non-dominant hand [61, 62]. It is possible that in the long run, this increase is most likely to bring about more significant improvements in muscle strength.

The differences observed in both sexes between the strength of the dominant and non-dominant hand is usually around 10% [62]; the dominant hand being the one with the best performance and the most strength [63]. This fact may have contributed to the increase in strength only of the non-dominant hand, since this gain was probably not due to training with a load higher than that performed on a daily basis, but due to the supplementation which increased the serum vitamin D concentration and probably contributed to this strength improvement, since there were no changes in lifestyle or training during the intervention period. This occurs because the vitamin D receptor (VDR) is found in skeletal muscle cells, and vitamin D is important for maintaining the mass, strength, and speed of contraction of this muscle, due to its actions on the regulation of calcium transport, synthesis protein, and kinetics of contraction [64].
It is possible to conclude that vitamin D has beneficial effects on physical fitness, improving VO$_2$max and increasing manual muscle strength. However, better clarification of the molecular mechanisms of this role of vitamin D in improving skeletal and cardiorespiratory muscles is necessary.

**Study Limitations**

The main limitation of this study was the low sample size, since we selected a very restricted study population, MZ twins, to better assess the influence of the environmental factor, and the greater proportion of women in relation to men. Another point was the use of only one concentration of supplement and the evaluation carried out at only one time point after supplementation, two months, without further analysis and evaluation of these long-term findings. Therefore, for future research we suggest increasing the sample number and evaluating different concentrations of supplementation with different times of use.

**Conclusion**

Increasing the serum concentration of vitamin D to values above 50 generates health benefits, improving cardiorespiratory fitness and slightly increasing muscle strength of the non-dominant hand. Our findings contribute to the growing evidence regarding the non-skeletal benefits of vitamin D.

**Abbreviations**

**VO2max**: Maximum Oxygen Consumption

**25 (OH) D**: 25-hydroxyvitamin D

**MZ**: Monozygotic twins

**CG**: Control group

**SG**: Supplemented group

**T0**: first day of inclusion in the research

**T60**: after 60 days of supplementation

**UL**: Maximum tolerable daily intake limit

**IU**: International unity

**Kgf**: Kilogram-force

**TECP**: Cardiopulmonary stress test was performed
ACSM: American College of Sports Medicine

RER: Respiratory exchange ratio

UFRN: Federal University of Rio Grande do Norte

Declarations

Availability of data and materials

The additional datasets generated they are with corresponding author available on reasonable request.

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- Funding

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- Ethical Approval and Consent to participate

This work was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte and the Brazilian Registry of Clinical Trials. All participants signed a written informed consent. The study was performed in accordance with the standards of ethics outlined in the Declaration of Helsinki.

- Consent for publication

All authors made revisions to the manuscript, and have read and approved the final version.

- Competing interests

All authors declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work, and no other relationships or activities that could appear to have influenced the submitted work.

- Authors' contributions

Conception and design of the study: JFPM and ADL. Investigation and methodology of the study, drafting the work: JFPM, MVOB, ADL, and VNS. Acquisition and analysis of data and revision of the work: JFPM, MVOB, AAS, ECS, JRRC, WACS, MVBS, PMSD, VNS, and ADL.

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Tables

Table 1. Family income and physical activity levels in monozygotic twins.

|                          | CG                  | SG                  | p-value |
|--------------------------|---------------------|---------------------|---------|
|                          | (n = 37)            | (n = 37)            |         |
| Age, Y                   | 25.0 (21.0 – 37.0)  | 25.0 (21.0 – 37.0)  |         |
| Family income level n, (%) |                    |                     |         |
| < 1 Minimum wage         | 2.0 (5.4)           | 0.0 (0.0)           |         |
| > 3 < 5 Minimum wages    | 11.0 (30.0)         | 9.0 (24.3)          |         |
| > 5 < 7 Minimum wages    | 9.0 (24.3)          | 1.0 (2.7)           |         |
| > 7 < 10 Minimum wages   | 7.0 (18.9)          | 6.0 (16.2)          |         |
| > 10 < 20 Minimum wages  | 2.0 (5.4)           | 9.0 (24.3)          |         |
| > 20 Minimum wages       | 3.0 (8.1)           | 2.0 (5.4)           |         |
| Do not know              | 3.0 (8.1)           | 10.0 (27.1)         |         |
| Training duration per day| 3.0 (6.7)           | 7.0 (15.6)          |         |
| Less than 1 hour n, (%)  | 20.0 (54.0)         | 14.0 (38.0)         | 0.117   |
| More of 1 hour n, (%)    | 17.0 (46.0)         | 23.0 (62.0)         |         |
| Frequency of physical activity, days | 3.0 (1.0 – 6.0) | 5.0 (1.0 – 7.0) | 0.130   |

Categorical variables are shown as number (percentage) and compared by Chi-square test. Continuous variables are shown as median (minimum and maximum). CG, Control group; SG, Supplemented group. Brazilian minimum wage per month = US$ 265.73. p-value < 0.05 were considered significant.

Table 2. Cardiorespiratory fitness and muscle strength in monozygotic twins after supplementation with cholecalciferol.
| Variables                          | Control group (n = 37)                                                                 | Supplemented group (n = 37)                                                                 | p-value |
|-----------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|---------|
|                                   | T0                                      | T60                                      | T0                                      | T60                                      |         |
| **VO\textsubscript{2}max absolute, l/min** |                                    |                                           |                                           |                                           | 0.359   |
|                                   | 2.2 (1.8 - 2.6)                          | 2.1 (1.2 - 2.4)                          | 2.0 (1.6 - 2.6)                          | 2.6 (2.1 - 3.2)                          | < 0.001 |
| **VO\textsubscript{2}max relative, ml/kg\textsuperscript{-1}/ min\textsuperscript{-1**} | 34.5 (31.0 - 40.0)                      | 34.5 (31.0 - 40.8)                      | 33.5 (29.0 - 38.8)                      | 33.5 (29.3 - 47.0)                      | 0.414   |
|                                   | 1.1 (1.0 - 1.2)                          | 1.1 (1.1 - 1.2)                          | 1.1 (1.0 - 1.1)                          | 1.1 (1.0 - 1.1)                          | 0.098   |
| **Right Hand Grip, kgf**          | 32.5 (29.5 - 38.0)                       | 36.0 (31.0 - 37.0)                       | 32.0 (28.5 - 39.0)                       | 34.0 (29.0 - 40.0)                       | 0.631   |
|                                   | 29.0 (25.3 - 38.0)                       | 31.0 (27.0 - 37.3)                       | 28.0 (24.0 - 32.5)                       | 33.0 (29.0 - 40.5)                       | 0.606   |
| **Scapular Force, kgf**           | 23.0 (19.5 - 28.0)                       | 23.0 (18.5 - 28.3)                       | 24.0 (17.5 - 30.0)                       | 23.0 (18.8 - 28.3)                       | 0.952   |

Variables are shown as median (percentile 25 - percentile 75) and compared by Wilcoxon test. VO\textsubscript{2} max, Maximum oxygen consumption; RER max, Respiratory exchange ratio; CG, Control group; SG, Supplemented group; T0, first analysis; T60, analysis 60 days after the first. p-value < 0.05 were considered significant.

**Figures**

Image not available with this version

**Figure 1**

Flowchart illustrating the selection of the study population