Journal of Clinical & Translational Endocrinology 34 (2023) 100324

Contents lists available at ScienceDirect

Journal of Clinical & Translational Endocrinology

journal homepage: www.elsevier.com/locate/jcte

Prospective effects of cholecalciferol supplementation on irisin levels in sedentary postmenopausal women: A pilot study

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ARTICLE INFO

Keywords:
Vitamin D
Post-menopausal women
Irisin

ABSTRACT

Introduction: In postmenopausal women, vitamin D deficiency has been associated with disability, low muscle mass and fractures. Irisin is an important myokine that may contribute to the maintenance of muscle and bone density. Vitamin D is associated with the growth and function of muscle tissue through interactions between the vitamin D receptor and PGC-1α and activation of p38/MAPK (mitogen-activated protein kinase) in muscle, a mechanism similar to irisin action. The aim of this pilot study was to evaluate the effects of cholecalciferol supplementation on serum irisin levels in sedentary postmenopausal women with hypovitaminosis D (25(OH)D < 20 ng/mL).

Material and methods: 80 sedentary postmenopausal women with hypovitaminosis D and low sun exposure were supplemented with cholecalciferol (30,000 IU/month) for 12 months. Calcium, parathyroid hormone, alkaline phosphatase (AP) and irisin levels were measured before and after supplementation.

Results: 25(OH) vitamin D increased in all participants. Serum levels of irisin increased (from 0.52 ± 0.27 to 0.80 ± 0.53; $p < 0.003$), accompanied by a decrease in AP (from 80 ± 24 to 66 ± 23; $p < 0.001$).

Conclusions: Restoration of vitamin D status increased serum irisin levels in sedentary postmenopausal women. Whether increased serum irisin levels may have an impact on clinical outcomes deserves further evaluation.

Introduction

Hypovitaminosis D is highly prevalent worldwide [1–2] and has been associated with disability such as poor physical performance, low muscle strength, low muscle mass, bone demineralization, falls and fractures in post-menopausal women [3]. Vitamin D, alone or in combination with other factors, increases muscle strength and protects against muscle wasting [4].

Irisin, the cleaved form of fibronectin type III domain-containing protein 5 (FNDC5), is an exercise-induced myokine that has been shown to have multiple beneficial effects on health, mainly inducing skeletal muscle hypertrophy [5]. The expression of FNDC5, the precursor of irisin, is induced in muscle by exercise through a PGC1 alpha-dependent pathway [6] and irisin is regulated by the activation of p38/MAPK [7].

It has been shown that menopausal women have lower levels of irisin [8]. Guo M et al. showed that the expression levels of irisin and its precursor FNDC5 are reduced at the mRNA and protein levels in muscle during ageing [9]. After intraperitoneal administration of recombinant irisin protein, the authors observed an improvement in grip strength, muscle weights and fiber size, demonstrating the role of irisin in maintaining muscle physiology during ageing.

Recent studies show that hypovitaminosis D is directly associated with loss of muscle mass [10]. Vitamin D plays a role in the number and diameter of type II muscle cells and contributes to the growth and function of muscle tissue through interactions between the vitamin D receptor (VDR) and PGC-1α and activation of p38/MAPK (mitogen-activated protein kinase) in muscle [11–12]. This mechanism is similar for the expression of FNDC5, the precursor of irisin, induced in muscle by exercise. An experimental study reported that rats with hypovitaminosis D had low serum levels of irisin [13].

Physical exercise has been associated with increased irisin levels and improved skeletal muscle proteome analysis, and irisin administration mimics some molecular effects of exercise in the quadriceps muscle

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https://doi.org/10.1016/j.jcte.2023.100324
Received 24 May 2023; Received in revised form 27 July 2023; Accepted 8 September 2023
Available online 15 September 2023

[14]. Therefore, vitamin D administration might be an alternative treatment if physical capacity or mobility during ageing precluded exercise. To our knowledge, there is no study so far investigating the effect on serum irisin levels resulting from vitamin D supplementation in postmenopausal women with hypovitaminosis D.

Material and methods

After calculating the sample size, admitting a type I error of 20% and power of 80%, we aimed to include 80 post-menopausal women with hypovitaminosis D, defined as 25(OH) vitamin D < 20 ng/mL. This is a non-randomized longitudinal study with a 12-month follow-up.

Inclusion criteria were women aged ≥60 years, with hypovitaminosis D, low sun exposure, who received and used daily sunscreen for 12 months, sedentary, with estimated glomerular filtration rate >60 mL/min/1.73 m^2. We did not include women with sun exposure, moderate or high physical activity, osteoporosis or severe bone disease, and those in use regular use of non-steroidal anti-inflammatory drugs. All women included agreed to participate in the study by signing an informed consent form approved by Ethics Committee. Blood sample (5 mL) were taken from each woman at baseline (before cholecalciferol supplementation (T0)) and after supplementation with cholecalciferol 30,000 IU/month for 12 months (T12). At both times, the following tests were performed: fasting blood glucose, serum creatinine, alkaline phosphatase (AP), serum calcium, C-reactive protein (CRP), parathyroid hormone (PTH) and irisin. The enzymatic method was used to analyze blood glucose levels. AP levels were determined by the colorimetric method using the Roche Cobas Integra autoanalyzer (F Hoffmann-La Roche Ltd, Basel, Switzerland). Creatinine was measured by Jaffe's colorimetric-kinetic method. The estimated glomerular filtration rate of creatinine (eGFR Creat) was analyzed using the formula developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) group. Serum levels of irisin (catalogue number RAG018R – BioVendor, Brno, Czech Republic) were determined by immunoenzymatic assay (ELISA). Vitamin D (Architect 25-OH Vitamin D – catalogue number 3L52 – Abbott, Wiesbaden, Germany) and CRP (C-Reactive Protein – catalogue number 8G65-21 – Abbott, Wiesbaden, Germany) concentrations were determined by the immunochemiluminescence method and PTH by the immunoradiometric assay (N-TACT® PTH SP – catalogue number code 26,100 – RIA – DiaSorin, Rome, Italy).

Anthropometric data included abdominal circumference and body mass index (BMI).

Statistical analysis

The normality of the data was checked using the Shapiro-Wilk test. Continuous numerical variables were expressed as mean and standard deviation or median and percentiles (25–75%) according to their parametric or nonparametric distribution, respectively. Comparison between T0 and T12 were performed using the paired T-test or the Wilcoxon test, as appropriate. Correlations between independent variables were analyzed using the Pearson or Spearman test, as appropriate.

A 5% significance level ($p < 0.05$) was set for statistical tests. Analyses were performed using IBM SPSS STATISTICS for Windows software (IBM Corp., Armank, N.Y., U.S.A.) version 25.

Results

We included 80 women, with a mean age of 67 ± 5 years, and an average BMI indicating overweight/obesity. Supplementation with cholecalciferol was capable to increase levels of 25(OH) vitamin D and all women achieved a minimum of 25 ng/mL (Fig. 1). During the 12-month follow-up, there was no change in BMI, renal function, CRP, PTH and serum calcium. There was an increase in irisin (Fig. 2), and a decrease in glucose and AP.

We found no correlation between irisin and 25(OH) vitamin D (r = 0.17, p = 0.13) and any correlation between the differences (delta) of 25 (OH) vitamin D and irisin (r = 0.13, p = 0.51) (Fig. 3).

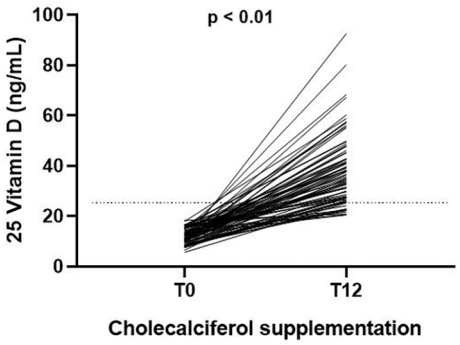

Fig. 1. Serum levels of 25 Vitam in D before (T0) and after (T12) cholecalciferol supplementation in postmenopausal women.

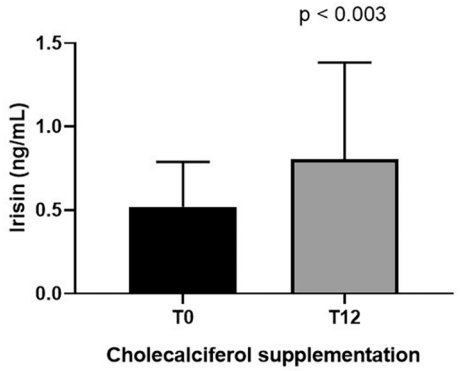

Fig. 2. Serum levels of irisin before (T0) and after (T12) cholecalciferol supplementation in postmenopausal women.

Discussion

In this pilot prospective study, we observed an increase in irisin levels after supplementation with cholecalciferol in sedentary postmenopausal women with hypovitaminosis D.

We found a decrease in glucose levels. A shred of recent evidence has shown that vitamin D deficiency impairs insulin secretion in response to glucose stimulation [15], and cholecalciferol administration has been reported to improve insulin resistance and glycemia [16].

Cholecalciferol plays an important role in calcium metabolism and bone health, and its deficiency has been associated with bone loss mineralization [17]. AP decreases after cholecalciferol administration, a

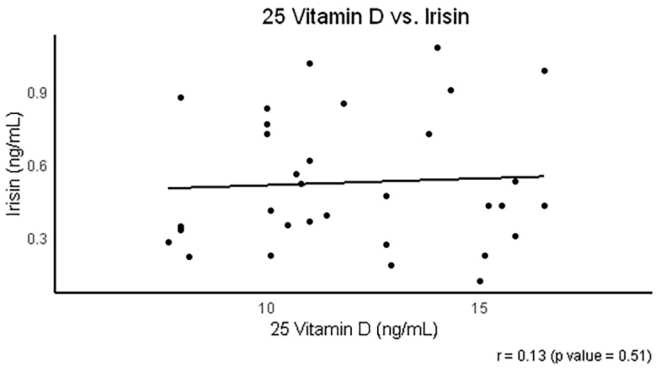

Fig. 3. Correlation between the differences of 25-D vitamin and irisin after cholecalciferol supplementation in postmenopausal women.

finding whose clinical significance should be confirmed in other studies. The study design and the lack of bone densitometry precluded further conclusions on this bone mineralization.

Serum irisin levels increased after cholecalciferol supplementation, but we did not observe a significant positive correlation between these variables. It is possible that the effect of cholecalciferol on irisin levels may not be dose dependent or other mechanisms not explored in this study may be involved. Although the mechanism of vitamin D on irisin synthesis is not fully understood, it has been reported that vitamin D activates p38/MAPK. This is the same mechanism that stimulates irisin synthesis [7,18]. Irisin is an important factor in muscle mass and bone metabolism, which may contribute to the maintenance of muscle and bone density in older individuals [19–20]. It has also been shown that FNDC5 and irisin synthesis are increased during myogenic differentiation of human myocytes in vitro, supporting the idea of irisin's the myogenic potential [21]. Some studies have shown that menopausal women have lower concentrations of irisin, which may favour the decrease of muscle resistance, affecting the performance of activities of daily living [22–23] and increasing osteoporosis [24].

Our study is subject to some limitations such as the small sample size, the lack of a control group, and the inclusion of postmenopausal women with vitamin D deficiency, which limit the generalizability of the results. However, the strength of our study is the prospective design, with a long-term follow-up. Whether increased serum irisin levels may influence muscle mass gain and clinical outcomes in postmenopausal women deserves further investigation.

CRediT authorship contribution statement

Luiz Phellipe Dell Aquila: Conceptualization, Investigation, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Armando Morales Junior:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Patricia Moreira:** Methodology, Data curation. **Maysa Seabra Cendoroglo:** Methodology, Data curation, Supervision, Resources. **Rosilene Motta Elias:** Validation, Methodology, Data curation, Writing – review & editing. **Maria Aparecida Dalboni:** Conceptualization, Methodology, Data curation, Formal analysis, Project administration, Funding acquisition, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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