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Original article

Evaluation of circulating irisin levels in healthy young individuals after a single 100,000 IU vitamin D dose

Évaluation d'une dose unique de 100 000 UI sur les taux circulants d'irisine chez de jeunes adultes

Étienne Cavalier^{a,*}, Valentine Mismetti^b, Jean-Claude Souberbielle^c

^a Department of Clinical Chemistry, University of Liege, CHU Sart-Tilman, Liège, Belgium

^b Faculty of Medicine, University of Saint-Étienne, Saint-Étienne, France

^c Laboratoire d'Explorations fonctionnelles, Hôpital Necker-Enfants-Malades, Paris, France

Abstract

Irisin is a newly discovered hormone produced by the muscle. This hormone is involved in glucose metabolism, muscle strength and energy expenditure. As vitamin D is also known as a key player in that field, we aimed to see if a single dose of 100,000 IU of vitamin D would modify circulating levels of irisin in 29 young adults. Irisin was determined with the Phoenix Elisa assay at day 0, day 3, day 7, day 15 and day 28 after the loading dose. If we observed a significant increase in 25(OH)D levels (from 19.8 ± 8.4 ng/mL to 33.0 ± 8.5 ng/mL), irisin levels remained constant throughout the study (median 104.5 ng/mL, with values ranging from 69.9 to 390.9 ng/mL). Interestingly, one subject presented irisin values that were three times higher than all the others.

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Keywords: Irisin; Vitamin D; Muscle; Energy

Résumé

L'irisine est une hormone produite par le muscle jouant un rôle dans la force musculaire, le métabolisme glucidique et la dépense énergétique. Comme la vitamine D est également associée aux mêmes effets, nous avons voulu évaluer l'impact d'une prise de 100 000 UI de vitamine D sur les taux circulants d'irisine de 29 sujets adultes. Nous avons dosé l'irisine avec le kit Elisa de Phoenix avant la prise et après 3, 7, 14 et 28 jours. Si les taux de 25(OH)-vitamine D se sont élevés de façon significative (de $19,8 \pm 8,4$ ng/mL à $33,0 \pm 8,5$ ng/mL), nous n'avons pas constaté de variation du taux d'irisine (taux médian de 104,5 ng/mL, s'étalant de 69,9 à 390,9 ng/mL). Par contre, un sujet présentait de façon assez intéressante, des valeurs trois fois plus élevées que tous les autres.

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Mots clés : Irisine ; Vitamine D ; Muscle ; Énergie

1. Introduction

New findings have emerged to explain the role played by physical exercise on different organs. Recently, a newly discovered hormone, named irisin, was found to be produced by the muscle after exercise and has been shown to act on white adipose

cells in culture and in vivo to stimulate the expression of uncoupling protein 1 (UCP1) expression and browning of these cells in mice [1]. Interestingly, irisin can cause an increase in energy expenditure in mice with no changes in movement or food intake, potentially resulting in improvements in obesity and glucose homeostasis. Other fields of research have also suggested that vitamin D (VTD) – alone or in combination with other factors – plays a role in weight reduction, improves the parameters of glucose metabolism and increases muscular strength [2–4]. As the expression of FNDC5, the precursor of irisin, is induced in muscle by physical exercise via a PGC1 alpha-dependent pathway

* Corresponding author at: Service de Chimie Médicale, CHU de Liège, Domaine Universitaire du Sart-Tilman, 4000 Liège, Belgium.

E-mail address: Etienne.cavalier@chu.ulg.ac.be (É. Cavalier).

and that there may be, in muscle, an interplay between the vitamin D receptor and PGC1 alpha signaling pathways [5], a link between vitamin D and irisin may be investigated, even if Choi et al. have demonstrated in rats that vitamin D did not alter AMPK phosphorylation, the upstream of PGC1 alpha [6]. In this study, we aimed to see if a single supplementation of a dose of 100,000 IU of VTD in healthy individuals could have an impact of irisin circulating levels.

2. Patients, materials and methods

This study is approved by the local Ethics Committee of the University Hospital of Liège. Twenty-nine young laboratory healthy volunteers (19 females, 29 ± 8.2 -years-old, BMI $23.2 \pm 4.0 \text{ kg/m}^2$) were included. A single oral dose of 100,000 IU vitamin D3 (4 vials of D-Cure each containing 25,000 IU cholecalciferol, SMB Laboratories, Belgium) was administered to each subject at baseline. Blood was collected from fasting participants at baseline, 3, 7, 15 and 28 days, aliquoted and stored at -80°C until determination. All the analytical tests were performed in the 2 months after sampling. Irisin was determined with an Elisa method (Phoenix Pharmaceuticals, Burlingame CA), 25-hydroxyvitamin-D (25(OH)D) with an automated immunoanalyzer (DiaSorin Liaison, Stillwater, MN) and 1.25(OH)₂ vitamin D with another automated method (IDS iSYS, Boldon, UK) according to the manufacturer's instructions in our ISO 15189 Laboratory. We used Wilcoxon paired test to compare the results of the subjects before and after supplementation, with a *P*-value being considered as significant if it was < 0.05 .

3. Results

At baseline, median 25(OH)D values were $19.8 \pm 8.4 \text{ ng/mL}$, rose to $29.7 \pm 9.6 \text{ ng/mL}$ at day 3 (+50%, $P < 0.05$), reached $33.0 \pm 8.9 \text{ ng/mL}$ at day 7 (+11%, $P = 0.085$) and significantly decreased from day 7 to 28 (−7%, $P < 0.05$). Before repletion, 51.7% of the participants ($n = 15$) presented a serum 25OHD concentration $\leq 20 \text{ ng/mL}$ and 27.6% ($n = 8$) $\geq 30 \text{ ng/mL}$. At days 3, 7 and 28, these values changed to 3.4% ($n = 1$) and 48.3% ($n = 14$), 3.4% ($n = 1$) and 65.5% ($n = 19$) and 3.4% ($n = 1$) and 51.7% ($n = 15$), respectively. Subjects with 25OHD values $\leq 20 \text{ ng/mL}$ at baseline achieved significant increase of +70% ($P < 0.05$) at day 28 while those with values $> 20 \text{ ng/mL}$ presented an increase of +25% ($P < 0.05$). 1.25(OH)₂D significantly increased from $64.1 \pm 21.0 \text{ pg/mL}$ at baseline to $82.6 \pm 26.2 \text{ pg/mL}$ at day 3 ($P < 0.05$). Subjects with 25OHD values $\leq 20 \text{ ng/mL}$ at baseline presented a significant 1.25OHD increase at day 28 (+21%, $P < 0.05$) whereas those with 25OHD values $> 20 \text{ ng/mL}$ did not. At baseline, the median irisin values were 104.5 ng/mL , with values ranging from 69.9 to 390.9 ng/mL . There was no correlation between irisin levels and BMI. We did not observe any significant change in irisin values after 3, 7, 15 or 28 days. Interestingly, one subject presented values of approximately three times higher than the mean of the population throughout all the study (Fig. 1).

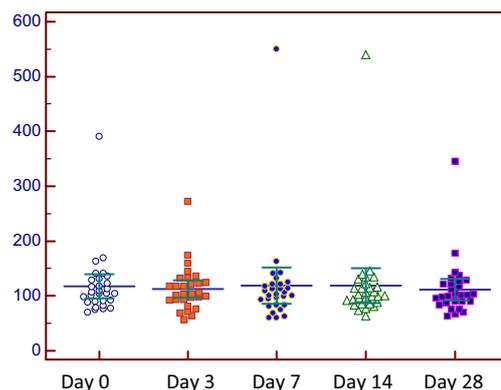


Fig. 1. Evolution of irisin levels (ng/mL) in 29 healthy adults after a single oral dose of 100,000 IU of vitamin D.

4. Discussion

Irisin is a newly discovered hormone that increases muscle strength and energy expenditure. Vitamin D has also been shown to play a similar role in some [7] but not all [8] studies. In this study, we failed to show that a single large (100,000 IU) dose of vitamin D increased the circulating levels of irisin in young healthy individuals. We have however to acknowledge that our population globally did not present vitamin D deficiency (30% of them were even above 30 ng/mL) and, accordingly, it is possible that a further supplementation on the irisin production (cleavage & release into the circulation) might have no further effect.

A correlation between irisin levels and BMI has been found in severe obese patients and in patients with anorexia nervosa [9]. We did not find such a correlation, but our population was composed of young healthy subjects presenting BMI values ranging from 18 to 30 kg/m^2 explaining probably this lack of correlation.

The results that we observed here were in the same range as those observed by Huh et al. ($113.1 \pm 20.6 \text{ ng/mL}$) compared to those that Stengel et al. obtained in normal weight individuals [10]. This is quite intriguing, because we used in this study the same kit as Stengel whereas Huh et al. used the Aviscera assay. To overcome a stability problem, we designed a study in which we stored 19 EDTA freshly collected samples with and without aprotinin for 1 month at -80°C . Our results show that storage at -80°C did not alter irisin concentration. Also, we did not find any difference between the samples kept with or without aprotinin. The discrepancies observed between different kits for irisin measurement have recently been highlighted by Sanchis-Gomar et al. [11]. These authors clearly questioned the validation of the antibodies used in the kits, the source and nature of the antigen and even wondered if irisin really existed. . .

In conclusion, our results have shown that a single 100,000 IU dose did not impact irisin levels in non-obese subjects and that the effects of vitamin D on muscle strength may not be linked to an irisin pathway. Interestingly, one subject presented irisin values that were three times higher than the others. These high values were later confirmed on another set of samples. This young lady did not present any particular characteristics even if she acknowledged having facilities in losing weight and if her father, aged 51, presented the same weight as when he was 17,

even after stopping smoking. A familial and genetic study may be of interest to see if there was a link between these observations, even if circulating irisin levels seem maternally inherited [12]. Finally, irisin may be a promising hormone in the future for therapeutical or diagnostic approaches, but some issues still remain on its determination.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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