Int J Clin Exp Med 2016;9(7):13086-13092
www.ijcem.com /ISSN:1940-5901/IJCEM0021873

Original Article

Impact of vitamin D correction on circulating irisin: a 12 month interventional study

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Received December 14, 2015; Accepted May 17, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Irisin, a novel myokine secreted by skeletal muscle, promotes browning of white fat and influences glucose and energy homeostasis. Irisin and vitamin D are also known for their roles in the musculoskeletal system. In this study, we examined the impact of vitamin D correction on circulating irisin in Saudi subjects. For this, vitamin D deficient subjects (≤50 nmol/l) were advised to receive adequate sunlight, eat vitamin D enriched foods and retain normal physical activity for correction/improvement in vitamin D status. Seventy-eight (30 male and 48 female) subjects, who responded to vitamin D intervention, were selected for further analyses. Anthropometry and routine biochemical assay were performed. Serum 25 (OH) D was analyzed using electrochemiluminiscence. Irisin levels were analyzed at baseline and after 12-month of intervention. Serum 25 (OH) D levels were significantly increased at post-intervention compared to baseline value (51.0±4.0 vs. 27.8±2.3 in males and 69.1±4.5 vs. 27.1±1.8 in females). With an increase in the levels of vitamin D adjusted for age and BMI, a corresponding increase in irisin level was observed in males (P=0.026) and to a lesser extent in females (P=0.093). Thus, vitamin D correction had a significant impact on irisin levels in male Saudi subjects but in females it failed to reach at significance. Further studies are needed in other ethnic population to better understand the influence of vitamin D on irisin regulation and secretion.

Keywords: Irisin, vitamin D, skeletal muscle, Saudi subjects

Introduction

Vitamin D is a fat-soluble vitamin with a well-known impact on calcium metabolism and bone health [1]. This sterol vitamin has other physiological actions such as modulation of cell growth immune function and inflammation [2-4]. Recently, vitamin D was reported to affect growth and function of muscle tissues as well [5]. In addition, patients with rickets and osteomalacia displayed proximal myopathy, suggesting a direct link between hypovitaminosis D and muscle function [6, 7].

Accumulating evidence indicates that skeletal muscle can function as an endocrine organ that secretes a number of "myokines", including the newly identified irisin [8]. The latter, secreted in response to activation by peroxisome proliferator-activated receptor (PPARγ) co-activator-1 (PGC-1α), is thought to play an important role in metabolism by stimulating adipose tissue browning [8, 9]. This change includes activation of uncoupling protein 1 (UCP-1) in brown adipocytes, leading to increased respiration and energy expenditure [8]. Brown adipose tissue is a positive predictor of femoral bone structure and correlates with muscle mass [10].

Besides metabolic improvement, irisin mediates the positive effects of skeletal muscle on bone health and, thus, represents a therapeutic target for bone diseases, including osteoporosis [8-10]. It was recently demonstrated that irisin enhances osteoblast differentiation *in vitro* [11]. Moreover, irisin levels were lower in women with osteoporotic fractures than those without [12]. The relevance of these findings opens a new window of opportunity in examin-

ing the effects of vitamins and hormones on musculoskeletal system including irisin secretion. In spite of our understanding of the roles of vitamin D and irisin in muscular strength and bone growth, the influence of long-term vitamin D correction on circulating irisin has not been studied. Therefore, in this study, we investigated the impact of a 12-month vitamin D intervention on circulating irisin in Saudi subjects.

Materials and methods

Subjects

Vitamin D-deficient subjects (serum 25 (OH) D below 50 nmol/l) were selected from the cohort for vitamin D studies of Prince Mutaib Chair for Biomarkers of Osteoporosis Research, King Saud University (KSU), Riyadh, KSA [13, 14]. A total of 78 (30 males; 48 females) adult (aged 30 to 65 years) non-diabetic vitamin D deficient Saudis who responded to 1 year vitamin D intervention were chosen. Patients with diabetes mellitus (type 1 and 2), morbid obesity, or complicated comorbidities, those taking vitamin D supplementation and sun screen protection were excluded from the study. Written informed consents were taken before inclusion. Approval was granted by the Ethics Committee of the College of Science, KSU, Riyadh, KSA. All subjects were asked to complete a questionnaire regarding demographic information, including past and present medical history, and to come back to their respective PHCCs in fasting condition (> 10 h) for anthropometry and blood withdrawal. They were also asked to come back 12 months later for the repeat of assessments.

Anthropometry and blood collection

Subjects visited their respective PHCCs in an overnight fasted state (> 10 hours) for anthropometry and blood withdrawal by the nurses and physician on the duty, respectively. Anthropometry included height (rounded off to the nearest 0.5 cm), weight (rounded off to the nearest 0.1 kg), waist and hip circumference (centimetres), and mean systolic and diastolic blood pressure (millimetres of Hg) (average of 2 readings). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Fasting blood samples were collected and transferred immediately to a non-heparinized tube for centrifugation. Collected serum was then transferred to pre-labelled plain tubes; stored in ice; and delivered to the Biomarkers Research Program (BRP) at KSU, Riyadh, KSA, for immediate storage at -20°C.

Sunlight exposure and vitamin D diet

After the collection of information and blood withdrawal, subjects were given verbal advice to expose themselves to sunlight for 5 to 30 min twice a week either before 10:00 AM and/or after 3:00 PM (minimum body parts exposed were face, neck, hands and arms). The time for sun exposure was based on a previous study done by Hannan and colleagues [15] in Riyadh, KSA, detailing the hours of daylight during which ultraviolet radiation levels are considered carcinogenic and should be avoided. They were also regularly encouraged every week through Short Message Service (SMS) to take increased amounts of vitamin D-rich foods, such salmon, tuna, cow liver, dairy products, and vitamin D-fortified foods. To ensure compliance, they were instructed to keep a diary in which they recorded sun exposure times and outdoor physical activity; such diaries were submitted to the investigators at the end of the study period as done previously [16].

Sample analyses

Fasting glucose, lipid profile, calcium, and phosphorous were measured using a chemical analyzer (Konelab 20XTi, Thermo Fischer Scientific, Vantaa, Finland). Serum 25-Hydroxyvitamin D was measured using COBAS e-411 automated analyzer (Roche Diagnostics, Indianapolis, IN, USA) in a DEQAS-certified laboratory (PMCO). For serum 25-hydroxyvitamin D assay, the inter- and intra-assay coefficients of variation (CV) were 8.0% and 5.6%, respectively, with a lower detection limit (LOD) of < 4 ng/ml).

All measurements were done in a DEQAS (Vitamin D External Quality Assessment Scheme) participating laboratory, the Biomarkers Research Program (BRP) of KSU, Riyadh, KSA. Irisin was measured using an enzyme-linked immunosorbent assay (ELISA) (Phoenix Pharmaceuticals Inc, Burlingame, CA, USA) as previously reported by our group [17, 18] with an intra-assay variability of < 10% and inter-assay variation of < 15%. All fasting samples fell within the detection range.

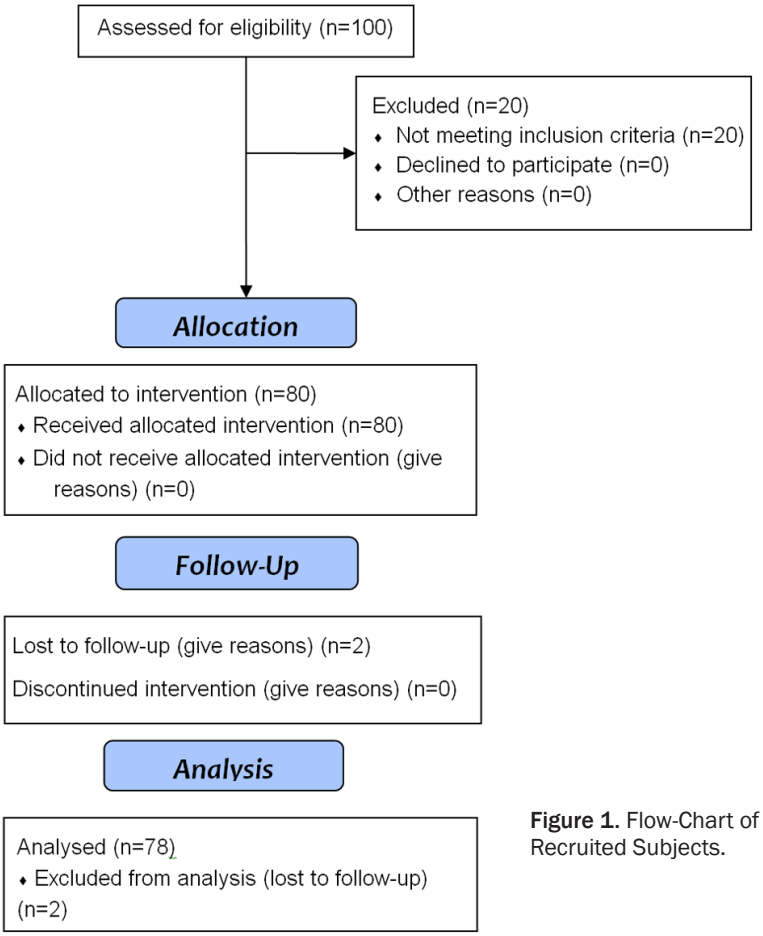

Figure 1. Flow-Chart of Recruited Subjects.

Data analysis

Data were analyzed using SPSS version 16.5 for Windows (Chicago, Illinois, USA). Normal continuous variables were presented as mean ± standard deviation. Paired Student t-test was done to determine intervention effects in anthropometric and metabolic parameters at baseline and at 12 months of intervention. General linear model (GLM) repeated measures was used to determine differences in irisin and vitamin D levels in males and females at baseline and after 12 months, with age and BMI as co-variates. *P*-value < 0.05 was considered significant.

Results

Figure 1 shows the flow chart of the subjects included in the present interventional study. **Table 1** highlights the general clinical characteristics of the subjects. We observed significantly higher vitamin D level at 12 months of intervention ($P < 0.001$). A corresponding significant increase in circulating irisin was also observed ($P = 0.007$) compared to baseline value. Significantly higher levels of calcium were also observed at 12 months than at baseline ($P=0.001$). However, no significant changes were observed in BMI, blood pressure and fasting blood glucose overtime. **Table 2** represents gender-based anthropometrics and biochemical characteristics at baseline and 12 months of intervention. In both males and females, significantly higher levels of serum calcium were observed at 12 months than at baseline ($P=0.016$ and 0.02 respectively). In males, the increase in vitamin D was accompanied by a significant increase in circulating irisin ($P=0.028$). The significant increase in irisin persisted even after adjusting for age and BMI ($P=0.026$). In female subjects, irisin levels increased post-intervention but the effect was not statistically significant before ($P=0.087$) and after ($P=0.093$) adjustments for age and BMI (**Table 2**).

Discussion

This is the first study on the Middle Eastern population that observed a positive relation between 25 (OH) D and irisin. In this intervention study, we observed a significant increase in the circulating irisin level in the overall group and strongly in men 12 months post-intervention. A similar trend was also observed in females but failed to reach statistical significance.

Vitamin D deficiency is increasingly recognized as a worldwide “epidemic”, including the Middle Eastern region and, specifically in KSA, where, paradoxically, the intensity of sunlight is proportional to the prevalence of vitamin D deficiency [19, 20]. Besides the well-known function of vitamin D in bone health and calcium metabolism, accumulating evidence suggests extra-skeletal functions of this sterol. Hypovitaminosis

Table 1. Clinical and Biochemical Characteristics of Subjects

Parameter	Baseline	12 Months	*P*-value
N	78		
M/F	30/48		
Age (years)	44.8 ± 1.26		
BMI (kg/m^2)	32.0 ± 0.81	32.0 ± 0.82	0.99
Glucose (mmol/l)	6.38 ± 0.26	6.2 ± 0.19	0.43
Triglycerides (mmol/l)#	1.84 ± 0.12	1.75 ± 0.1	0.67
Total Cholesterol (mmol/l)	4.93 ± 0.1	4.65 ± 0.12	0.042
HDL-Cholesterol (mmol/l)	0.78 ± 0.06	0.91 ± 0.05	0.088
Calcium (mmol/l)	2.23 ± 0.03	2.35 ± 0.03	0.001
Albumin (g/l)	38.2 ± 0.78	38.9 ± 0.85	0.49
Phosphate (mmol/l)	1.16 ± 0.07	1.1 ± 0.04	0.45
Vitamin D (nmol/l)#	27.3 ± 1.4	62.1 ± 3.3	< 0.001
Irisin (ng/ml)#	279.9 ± 17.97	384.5 ± 51.9	0.007

Note: Data presented as mean ± standard error; *p*-value significant at < 0.05. # denotes Non-Gaussian variables.

D has been associated with cardiovascular risk in adults and cardiometabolic risk in children [21, 22].

We found a significant increase in circulating irisin at 12 months post-vitamin D intervention. The elevation in circulating irisin was parallel to the increase in vitamin D level, which further strengthens the pleiotropic extra-skeletal functions of vitamin D.

As this is the first study of this kind and as no other study has been conducted to see the impact of long term vitamin D intervention on circulating irisin, published data are lacking to support our findings. Recently, Cavalier et al. studied the effect of a single dose of 100,000 IU of vitamin D and did not observe any change in circulating irisin level [23]. However this study differed significantly than ours. First, unlike single shot of a high dose of vitamin D, we performed a year-long intervention study by advising the subjects to receive appropriate sunlight exposure and to increase the intake of vitamin D-rich foods, besides normal physical activity. Second, sample size used in the study of Cavalier *et al*. was small and, importantly, Cavalier *et al*. selected subjects who were already vitamin D sufficient, so it might be possible that a single shot of vitamin D has no further effect on circulating irisin. In a different set of experiments, some other researchers also studied the effect of dietary pattern and food intake on irisin secretion [24, 25]. Park et al. [24] in a cross sectional study did not find any association of dietary quality with circulating irisin in humans. In an almost similar study, Schlogl et al. [25] observed even a decrease in serum irisin concentration with an increase in 24-hour ad libitum food intake. These observations, thus, failed to observe the influence of nutritional intake on circulating irisin.

When subjects were divided on the basis of gender, significantly higher levels of irisin were observed in males at the end of the baseline. But in females a similar increase was only a trend. Gender differences observed in irisin levels as a result of vitamin D correction may relate to sex-based differences in the content of brown adipose tissue (BAT) [26, 27]. As females have greater levels of BAT than males, this adipose tissue might inhibit further rises in irisin level. Furthermore, gender differences in irisin levels have been previously reported, with females having higher baseline irisin levels than males similar to the present study [28]. Moreover, sexual dimorphism in the actions of vitamin D might also influence irisin secretion. We recently described marked differences in the plasma proteome of men and women associated with normal physiological processes, regulating metabolism, including the actions of vitamin D [29]. These differences were phenotypically confirmed in vitamin D deficient males having an age and sex disadvantage over females [30]. Exactly how vitamin D enhances irisin secretion, specifically in males, remains to be further investigated. One theory may involve musculo-skeletal cross-talks between irisin, a muscle-derived biomarker, and vitamin D, a known biomarker for skeletal wellness [31]. Another possible mechanism may involve the direct involvements of both irisin and vitamin D in energy metabolism and regulation as well as adipocyte biology through uncoupling proteins (UCPs) [32, 33].

Recently, great interest in irisin research has developed due to its role in obesity, diabetes and disorders of the musculoskeletal system [8, 28, 32, 33]. Although the physiology and

Table 2. Clinical and Biochemical Characteristics of Male and Female Subjects

	MALES			FEMALES		
Parameter	Baseline	12 Months	*P*-value	Baseline	12 Months	*P*-value
N	30			48		
Age (years)	43.5 ± 1.5			45.6 ± 1.8		
BMI (kg/m^2)	31.0 ± 1.6	31.0 ± 1.6	0.93	32.7 ± 0.8	32.7 ± 0.9	0.96
Glucose (mmol/l)	6.7 ± 0.5	6.3 ± 0.3	0.42	6.2 ± 0.23	6.1 ± 0.1	0.88
Triglycerides (mmol/l)#	2.2 ± 0.2	1.9 ± 0.16	0.72	1.64 ± 0.1	1.63 ± 0.1	0.74
Total Cholesterol (mmol/l)	4.96 ± 0.2	4.67 ± 0.2	0.2	4.9 ± 0.1	4.6 ± 0.15	0.12
HDL-Cholesterol (mmol/l)	0.72 ± 0.1	0.76 ± 0.06	0.65	0.82 ± 0.08	1.0 ± 0.07	0.09
Calcium (mmol/l)	2.2 ± 0.06	2.4 ± 0.05	0.016	2.2 ± 0.04	2.3 ± 0.04	0.02
Albumin (g/l)	40.1 ± 1.5	39.1 ± 2.5	0.74	37.4 ± 0.9	38.9 ± 0.7	0.19
Phosphate (mmol/l)	1.09 ± 0.05	1.06 ± 0.08	0.7	1.2 ± 0.1	1.1 ± 0.05	0.51
Vitamin D (nmol/l)	25.7 ± 1.9	57.6 ± 5.3	< 0.001	24.6 ± 2.6	63.9 ± 6.0	< 0.001
Irisin (ng/ml)	245.1 ± 17.2	292.6 ± 25.6	0.044	247.19 ± 19.6	280.07 ± 24.5	0.315

Note: Data presented as mean ± standard error; *p*-value significant at < 0.05. # denotes Non-Gaussian variables.

action of this hormone have not been fully characterized, the results of this study suggest the improvement of irisin level with correction of vitamin D status.

We acknowledge some limitations in this study. Data on sun exposure and vitamin D-rich foods were based on administered questionnaires and advise. Other factors related to vitamin D status, such as skin colour and diet were not taken into consideration, although several studies already suggested that diet per se does not seem to affect irisin levels [24, 28]. Furthermore, although we encourage retaining habitual physical activity, it is possible that sun exposure without physical activity might have influenced the results. A systematic approach of administering a fixed dosage of vitamin D supplementation for a given time period with additional variables such as muscle features that are easily measured using bioelectric impedance may help strengthen the present findings. Strengths of the study include the longitudinal approach, the use of a homogenous cohort (Arab ethnic group) and the use of validated approaches in both irisin and vitamin D measurements.

In conclusion, vitamin D correction significantly improved the serum irisin level in male Saudi subjects and while a similar trend was observed in female but it failed to reach at significance level. Further studies are needed in other ethnic population to better understand the influence of vitamin D on irisin regulation and secretion.

Acknowledgements

The authors thank the Deanship of Scientific Research, Prolific Research Group Program (PRG-1436-15), Vice Rectorate for Graduate Studies and Scientific Research in King Saud University (KSU), Riyadh, Saudi Arabia for funding the study. We also thank Malak Nawaz Khan Kattak for the data analysis.

Disclosure of conflict of interest

None.

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