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The Effect of Vitamin D Supplementation on Serum and Muscle Irisin Levels, and FNDC5 Expression in Diabetic Rats

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Abstract

Background: Diabetes mellitus and metabolic disorders are a major burden on the healthcare system. Irisin is a novel myokine reported to have beneficial effects on glucose and lipid metabolism. Vitamin D deficiency has been implicated in the development of diabetes and hold a critical role in diabetes-related complications. In the present study, we examined the efficacy of vitamin D supplementation on serum irisin levels, skeletal muscle irisin levels, and the expression of the irisin precursor, FNDC5 (fibronectin-type III domain-containing 5) in type I diabetes mellitus rats.

Methods: Thirty-six adult male Sprague-Dawley rats (150 – 250 g) were randomly divided into four groups: group I: healthy control rats with no treatment (n=8), group II: healthy control rats receiving sesame oil as a placebo (n=8), group III: diabetic rats receiving sesame oil as placebo (n=10), group IV: diabetic rats treated with 4300 IU/kg/week vitamin D (n=10). Diabetes was induced by intraperitoneal (IP) injection of streptozotocin. At the end of the vitamin D intervention blood and triceps muscle samples were collected. RNA was extracted from muscle and real-time PCR was performed to examine FNDC5 gene expression.

Results: Our study showed that the administration of vitamin D (4300 IU/kg/week) in a streptozotocin-diabetic rat model resulted in increased serum vitamin D levels, FNDC5 gene expression and muscle irisin levels. However, the levels of serum irisin were not significantly changed by the administration of vitamin D.

Conclusions: In conclusion, we show that vitamin D supplementation enhances serum vitamin D levels, FDNC5 gene expression and muscle irisin levels in the streptozotocin-diabetic rat model. Our study highlights the potential therapeutic effect of vitamin D supplementation for diabetes mellitus.

Keywords: Diabetes, FNDC5, Irisin, Vitamin D.

Introduction

Diabetes mellitus (DM) is defined as a group of multifactorial endocrine diseases characterized by chronic hyperglycemia resulting from insulin resistance or defects in insulin secretion (1). Due to increasing prevalence worldwide, DM has become a major public health burden and has been considered a global epidemic (2). Chronic hyperglycemia is associated with long-term damage and failure of several organ systems affecting the eyes, nerves, kidneys, and heart. The pathological characteristics of DM includes the occurrence of both microvascular and

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macrovascular complications such as retinopathy, vascular disease, neuropathy and nephropathy (3).

Skeletal muscles act as an endocrine organ releasing signaling proteins called myokines which are involved in modulating many of the positive effects of exercise on metabolism (4). Myokines have been reported to have a critical role in preventing the development of DM and other metabolic disorders due to their ability to improve lipolysis and glucose uptake. One such myokine, irisin, has been shown to stimulate the browning of white adipose tissue (5). Exercise leads to the production of peroxisome proliferator-activated receptor-γ (PPAR- γ) coactivator-1α (PGC-1α), which stimulates the expression of protein fibronectin-type III domain-containing 5 (FNDC5) (6), which is the precursor to irisin. Cleavage of FNDC5 results in the production of irisin. Irisin induces the expression of uncoupling protein 1 (UCP1) in adipose tissue which converts white adipocytes to brown adipocytes (7). The transformation from white to brown fat increases energy expenditure and thermogenesis which can improve insulin resistance. Abnormalities in irisin have been reported to play a critical role in the pathogenesis of metabolic diseases like DM (8). Irisin levels have been reported to be decreased in DM. Additionally, irisin is associated with DM complications such as renal functions in chronic kidney disease, diabetic nephropathy patients, endothelial dysfunction and advance glycation end-products (AGEs) (9). Interventions which modulate irisin levels and return them to healthy levels may offer an effective therapeutic approach for treating DM. Vitamin D (cholecalciferol) is a fat-soluble vitamin with a well-known role in calcium metabolism and bone health. The secretion and synthesis of insulin has been reported to be impaired in hypovitaminosis D (10). Epidemiological studies suggest an association between vitamin D deficiency in early life and the later onset of T1DM (11) and in T2DM (12). Vitamin D has been reported to affect the growth and function of muscle tissues and be involved in improving glucose metabolism(13). Research has reported an interaction between the vitamin D receptor (VDR) and PGC-1α. Additionally, vitamin D has been shown to activate p38/MAPK (mitogen-activated protein kinase) in muscle. PGC-1α and irisin are regulated by the activation of p38/MAPK (14, 15).

The impact of vitamin D supplementation on FNDC5 gene expression and irisin concentration in diabetic rats has not been previously investigated. The present study examined potential mediators involved in the beneficial effects of vitamin D in diabetes. We hypothesize that the positive effects of vitamin D in diabetes is through FNDC5 and irisin.

Materials and methods

Experimental animals

Thirty-six adult male Sprague–Dawley rats (150 – 250 g) were bred and raised at the central animal house of the Pharmacology College at Tehran University of Medical Sciences (TUMS), Tehran, Iran. All experimental animals were acclimatized in standard cages under the appropriate conditions for animal procedures (temperature 22 ± 2 °C with a 12 hours- 12 hours' light - dark cycle and humidity 55-65%). The experimental animals were provided with access to standard commercial chow (Pars Dam Co, Tehran, Iran) and tap water. All animal procedures were based on standards for laboratory animal care approved by the Institutional Animal Care and Use Committee of TUMS. The ethical TUMS code was 28826/103/01/94.

Streptozotocin-induced diabetic rat model

The T1DM was induced in male Sprague-Dawley rats by administering a single dose of Streptozotocin (STZ) (50 mg/kg) (Sigma-Aldrich Co. St Louis, MO, USA) via intraperitoneal (IP) injection. All rats were fasted overnight receiving no food or water for at least 16 hours. The development of T1DM was confirmed by evaluating fasting blood sugar (FBS) levels 72 hours following the STZ injection using a glucometer (Bionime GM300, Swiss Design, Berneck, Switzerland). Blood samples were obtained from the tail veins and FBS over 250 mg/dL were considered diabetic. The rats with lower serum glucose levels were removed from subsequent experiments. As a control the healthy control group received a single IP injection of 1 ml sterile citrate buffer.

Study design

Adult male Sprague–Dawley rats were divided in four groups using a block randomization scheme as follows:

- Group 1 (n=8): Healthy Control (HC) (1 ml sodium citrate IP injection).
- Group 2 (n=8): NC + sesame oil (SO) group (1 ml sodium citrate and 0.5 ml sesame oil IP injection).
- Group 3 (n=10): Diabetes mellitus (DM) group + SO (0.5 ml sesame oil IP injection).
- Group 4 (n=10): DM + Vitamin D (4300 IU/rat/week vitamin D (Osveh Co., Iran) dissolved in 0.5 ml sesame oil IP injection).

The intervention period for this study was a total of 4 weeks. In the diabetic groups, two rats died during the study period therefore, analysis was performed for 32 rats. Twenty hours prior to the final day of the experiment, animals were fasted overnight with no food or water. At the end of the experiment all rats were anesthetized by an IP injection of ketamine (50 mg/kg) and xylazine (30 mg/kg). Blood samples were collected by cardiac puncture and immediately centrifuged at 3500 rpm for 20 min. Serum samples were stored at -70 °C until biochemical analysis. Body weight and food intake was recorded weekly throughout the experiment.

Muscle Sample preparation

Following the experiment, triceps muscles of all rats were collected to measure irisin protein levels and FNDC5 mRNA expression. Fifty mg of muscle tissue samples were incised and added to 10 ng of PBS (Phosphate-buffered saline) (pH=7.4). The sample was then homogenized in the homogenizer. Samples were centrifuged (at 2000-3000 RPM) for approximately 20 minutes. Supernatants were collected for muscle irisin levels and FNDC5 gene expression. The muscle samples were immediately stored at –80 °C.

Biochemical measurements

The FBS levels were measured by enzymatic and glucose oxidase methods (Pars Azmoon kit, Iran). Serum vitamin D levels were examined through commercial ELISA kit (Immunodiagnostic systems (IDS) CO, London, UK) and were expressed as ng/ml. Irisin concentrations were measured by ELIZA KIT (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions.

RNA extraction and real-time PCR gene expression quantification

Muscle samples were crushed, and cytoplasmic RNA was extracted using the RiboEx isolation kit (QIAGEN, Lilden, Germany) according to the manufacturer's instructions. The sequence primers depicted in Table 1. The quality and quantity of the extracted RNA was measured using the NanoDrop spectrophotometer (Thermo Fisher Scientific, San Jose, CA, USA). The cDNA was synthesized by QuantiTect Reverse Transcription Kit (Takara-Clontech, Tokyo, Japan). Quantitative Real-time PCR was performed using SYBR Premix Ex Taq II (Takara-Clontech, Tokyo, Japan). The gene expression changes were calculated by the $2^{\Delta Ct}$ method in comparison with the housekeeping Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (16).

Table 1. The sequences of primers used for real time PCR reactions.

Primer		Sequence (5′→3′)
FNDC5	Forward	5'-CATCATCAAGGACAACGAGC-3'
	Reverse	5'-CATATCTTGCTTCGGAGGAG-3'
GAPDH	Forward	5'-CATTCTTCCACCTTTGATGCTG-3'
	Reverse	5'-TGGTCCAGGGTTTCTTACTCC-3'

FNDC5: fibronectin type III domain containing-5 GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The normality of the data was determined using the Kolomogrov-Smirnov test. Differences among groups were analyzed by one-way analysis of covariance (ANCOVA) with Bonferroni post hoc test. The data was adjusted for

weight and food intake. A p value <0.05 was determined as statistically significant. All statistical analysis was performed using SPSS Statistics V. 17.01 software (SPSS Inc., Chicago, USA).

Results

Effects of vitamin D supplementation on body weight

As shown in Table 2, the induction of T1DM by means of STZ injection resulted in a decrease in body weight in the diabetic control group compared to the healthy control group (p value >0.05). The administration of vitamin D in diabetic rats did not lead to significant changes in body weight compared with the diabetic control group. The weight of the diabetic rats receiving vitamin D supplementation remained to be significantly lower than the healthy control group (p value <0.05).

Table 2. Fasting blood sugar (FBS), food intake, body weight and serum vitamin D in different experimental groups.

Variable	NC	NC+ SO	DM	DM+ Vit D	P value
FBS (mg/dl)	58.12±8.45	50.62±2.66	349.50±32.25[a,b]	320.62±52.49 [a,b]	<0.001
Initial Weight (g)	239.4±1.01	241.2±1.64	241.53±2.04	240.14±6.5	0.59
Final weight (g)	248.7±1.009	249.6±0.90	215.97±51.70 [a,b]	217±25.29 [a,b]	0.04
Food intake (g/day)	25.02±0.02	24.66±1.29	31.17±3.64[a,b]	29.86±1.23 [a,b]	<0.001
Serum Vit D (ng/ml)	28.98±3.44	26.67±4.30	27.60±6.5	40.74±2.16 [a,b,c]	<0.001

Results are expressed as mean ± SD, one-way ANOVA and post hoc Bonferroni test. DM: diabetes mellitus, FBS: Fasting Blood Sugar, HC: healthy control, SO: sesame oil. [a] p<0.05 compared with the control group, [b] p<0.05 compared with the control group with sesame oil. [c] p<0.05 compared with the diabetic control group,

Effects of vitamin D supplementation on FBS levels, food intake and serum vitamin D levels

The effect of vitamin D supplementation on FBS levels, food intake, and serum vitamin D levels is shown in Table 2. The induction of T1DM led to a significant increase in FBS compared to the healthy control group (p value <0.05). Our findings showed that vitamin D supplementation in the T1DM group did not alter FBS levels compared with T1DM control group. The food intake was not significantly different among the experimental groups. Following the induction of T1DM, there was a significant increase in the level of food intake among the DM groups. Supplementation with vitamin D did not result in any changes in the level of food intake in the T1DM group. Following the administration of vitamin D in the T1DM group, serum vitamin D levels were observed to significantly increase in the DM + Vit D group compared with healthy control and T1DM control groups not receiving vitamin D.

Table 3. Serum and muscle Irisin (total protein) concentration and FNDC5 gene expression in different experimental groups of Sprague–Dawley rats.

Variable	NC	NC+ SO	DM	DM+ Vit D	P value
Serum irisin (ng/ml)	1.62±0.16	1.63±0.17	1.46±0.22	1.71±0.39	0.28
Muscle irisin (total protein) (ng/mg)	3.89±1.47	4.82±2.29	1.33±0.45	4.73±1.80[c]	0.004
FNDC5 expression ($2^{-\Delta Ct}$)	78.21×10^{-5} $\pm46.32\times10^{-5}$	65.38×10^{-5} $\pm48.36\times10^{-5}$	46.85×10^{-5} $\pm55.61\times10^{-5}$	201.7×10^{-5} $\pm111.17\times10^{-5c}$	0.01

Results are expressed as mean ± SD, one-way ANOVA and post hoc Bonferroni test. DM: diabetes mellitus, HC: healthy control, SO: sesame oil. [a] p<0.05 compared with the control group, [b] p<0.05 compared with the control group with sesame oil. [c] p<0.05 compared with the diabetic control group.

Effects of vitamin D supplementation on serum irisin and muscle irisin levels

As shown in Table 3, serum irisin levels were not altered in the T1DM rats compared with the healthy control group. Additionally, supplementation with vitamin D had no detectable influence on serum irisin levels in the DM + Vit D group compared to the diabetic control group or healthy control group.

Muscle irisin levels were not altered following the induction of diabetes, no significant differences were observed compared with the healthy controls.

Administration of vitamin D following the induction of T1DM significantly increased muscle irisin levels in the DM + Vit D group. In addition, there were no significant differences between the muscle irisin levels in the DM + Vit D group and healthy control group.

Effects of vitamin D administration FNDC5 gene expression

As shown in Table 3, Real-Time PCR revealed that FNDC5 gene expression did not change following the induction of T1DM compared with the healthy control group. Administration of vitamin D following the induction of T1DM resulted in a significant increase in FNDC5 gene expression compared to the housekeeping gene, GAPDH, in the DM + Vit D group. No significant differences between FNDC5 gene expression and GAPDH expression were observed in the DM + Vit D group compared with the healthy control group.

Discussion

In the present study we have demonstrated that following the induction of T1DM in rats no significant changes in the irisin serum levels, muscle irisin protein levels, and FNDC5 gene expression occurred. Administration of vitamin D did not result in any changes in serum irisin levels among both the healthy control and T1DM rats. However, the muscle irisin levels and FNDC5 gene expression significantly increased in response to vitamin D supplementation among the T1DM rats.

Irisin holds a critical metabolic role in DM-relevant organs, such as the liver and pancreas, having a positive effect on glucose metabolism and insulin sensitivity (17). Research on obese mice has revealed that the overexpression of FNDC5 leads to enhanced energy expenditure and insulin sensitivity, and reduced hyperglycemia, hyperlipidemia, and hypertension (18). Treatment of muscle cells with irisin has been shown to enhance glucose and fatty acid uptake, similar to the metabolic effect of insulin. Additionally, irisin has been shown to increase *GLUT4* and *PPAR-alpha* gene expression, both of which are involved in glycogenolysis and gluconeogenesis, respectively (19). In obese patients, synthesis of FDNC5 and irisin is enhanced in order to maximize glucose uptake in the muscle and prevent hyperglycemia (20). However, following the development of diabetes, the expression of FNDC5 in muscle cells decreases by about 15% (19). Meta-analysis have confirmed that the levels of irisin are lower in patients with prediabetes or T2DM (21, 22). This observation may be a result of FNDC5 reduction and decreased irisin secretion in the skeletal muscle tissue of patients with obesity and diabetes (23). The inflammatory response has been implicated as a potential factor leading to reduced irisin levels (19). The biological phenomenon responsible for the elevated levels of irisin in obesity and low irisin secretion in diabetes has yet to be fully elucidated. However, chronic hyperglycemia and hyperlipidemia have been reported as potential triggers (23). Glucose is a regulator of irisin secretion from muscles in diabetes (19, 23). Previous research has shown irisin levels to remain unchanged following euglycemic–hyperinsulinemia clamp in diabetes, eliminating a potential role for insulin in modulating irisin secretion in diabetes (19, 23).

Recent evidence has shown that in pancreatic tissue, β-cells express the vitamin D receptor (VDR) and variations in the genes controlling vitamin D metabolism and expression of the VDR have been implicated in T1DM and T2DM pathogenesis (24). Vitamin D deficient mice have been shown to have impaired insulin secretion to glucose stimulation that was resolved following vitamin D_3 administration (24). Additionally, vitamin D has been reported to improve glycemic control (25). Deficiency in vitamin D have been linked to diabetic complications such as cardiovascular disease, neuropathy, dementia and bone loss (26). Poor glycemic control reduces the response of osteoblasts, osteocytes and osteoclasts to vitamin D_3 in TD2M (27). Therefore, poor glycemic control is linked to low bone mineral density (BMD) and high circulating levels of bone formation inhibitors (28). Elevated levels of irisin have been reported to improve bone metabolism in T1DM patients (29). In addition to the importance of mechanical stress on BMD, myokines have also been correlated with bone and fat tissue cross-talk (30). Research has indicated that treatment with irisin can improve bone health in both healthy and pathological states (31) by enhancing the activity

and differentiation (32) of osteoblasts through inhibiting sclerostin expression (31). Previous reports have shown a correlation between decreased levels of circulating irisin in women with osteoporotic fractures (33, 34). Similar to our findings, a previous interventional study in which a single dose of 100,000 IU of vitamin D was given to healthy subjects did not result in significant changes in serum irisin levels following 28 days of intervention (13). Two weeks of vitamin D supplementation at a dose of 75 μg / ml resulted in significant changes in serum irisin in albino vitamin D deficient rats (35). Following twelve months of vitamin D supplementation resulted in elevated serum irisin levels in vitamin D deficient subjects (36). Here we showed that although serum levels of irisin did not change following the administration of vitamin D, the total protein irisin levels in muscle and FNDC5 gene expression significantly increased following vitamin D supplementation.

The receptor for irisin has yet to be clearly identified, however the MAPK signaling pathway has been suggested as one of pathways in which irisin acts (4). Irisin induces osteoblast proliferation and differentiation via activation of the ERK and p38 MAPK signaling pathways (37). Activation of hepatic AMPK (AMP-activated protein kinase) exhibits an antidiabetic function through modifying glucose and lipid metabolism within the liver (38). Interestingly, irisin has also been reported to activate AMPK in skeletal muscle. Activation of Akt is directly linked with β-cell survival in an insulin-resistant state (39). Irisin activates the Akt signaling pathway in myocardial cells (39). The exact pathway of vitamin D on irisin secretion and FBNDC5 expression remains to be elucidated. Previous work has demonstrated that vitamin D supplementation and exercise elevates the levels of irisin in diabetic rats. These findings highlight the potential clinical applications for vitamin D supplementation, irisin administration, and exercise in the treatment of diabetes (40).

Limitations of the present student include many confounding variables that have the potential to alter the effect of vitamin D supplementation on irisin secretion. Such factors include the level of physical activity and lean body mass, both of which were not controlled for in this study. An additional limitation to our study was its short-term length. The long-term administration of vitamin D may have a different effect on the T1DM rats. Additionally, the role of the immune mediators, such as MAPK or ERK signaling molecules, were not examined. These signaling molecules have been previously implicated to influence the effect of vitamin D on irisin levels and function(14, 15). In efforts to better understand the interplay between irisin, vitamin D, and T1DM, these factors should be considered for future studies.

In conclusion, vitamin D supplementation in T1DM rats caused a significant increase in serum vitamin D levels, FNDC5 expression and total protein of irisin in muscle. However, the serum levels of irisin were not significantly altered in response to vitamin D. Further research should explore the therapeutic potential of vitamin D in diabetes and the effects of long-term vitamin D supplementation of diabetic rats.

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References

1. Blair M. Diabetes Mellitus Review. Urologic nursing. 2016;36(1):27-36.
2. Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, et al. Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes research and clinical practice. 2010;87(3):293-301.
3. Papatheodorou K, Papanas N, Banach M, Papazoglou D, Edmonds M. Complications of diabetes 2016. Journal of diabetes research. 2016;2016.
4. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481(7382):463.
5. Kamenov Z, Assyov Y, Angelova P, Gateva A, Tsakova A. Irisin and Testosterone in Men

with Metabolic Syndrome. Hormone and Metabolic Research. 2017;49(10):755-9.
6. Crujeiras AB, Pardo M, Arturo RR, Santiago NC, Zulet MA, Martínez JA, et al. Longitudinal variation of circulating irisin after an energy restriction-induced weight loss and following weight regain in obese men and women. American Journal of Human Biology. 2014;26(2):198-207.
7. Ates I, Arikan M, Erdogan K, Kaplan M, Yuksel M, Topcuoglu C, et al. Factors associated with increased irisin levels in the type 1 diabetes mellitus. Endocrine regulations. 2017;51(1):1-7.
8. Hee Park K, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, et al. Circulating irisin in relation to insulin resistance and the metabolic syndrome. The Journal of Clinical Endocrinology & Metabolism. 2013;98(12):4899-907.
9. Gouveia M, Vella J, Cafeo F, Affonso Fonseca F, Bacci M. Association between irisin and major chronic diseases: a review. Eur Rev Med Pharmacol Sci. 2016;20(19):4072-7.
10. Chiu KC, Chu A, Go VLW, Saad MF. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. The American journal of clinical nutrition. 2004;79(5):820-5.
11. Karvonen M, Jäntti V, Muntoni S, Stabilini M, Stabilini L, Muntoni S, et al. Comparison of the seasonal pattern in the clinical onset of IDDM in Finland and Sardinia. Diabetes care. 1998;21(7):1101-9.
12. Holick MF. Vitamin D: a millenium perspective. Journal of cellular biochemistry. 2003;88(2):296-307.
13. Cavalier E, Mismetti V, Souberbielle J-C, editors. Evaluation of circulating irisin levels in healthy young individuals after a single 100,000 IU vitamin D dose. Annales d'endocrinologie; 2014: Elsevier.
14. Sanchis-Gomar F, Perez-Quilis C. The p38–PGC-1α–irisin–betatrophin axis: Exploring new pathways in insulin resistance. Adipocyte. 2014;3(1):67-8.
15. Ronda AC, Buitrago C, Colicheo A, de Boland AR, Roldán E, Boland R. Activation of MAPKs by 1α, 25 (OH) 2-Vitamin D3 and 17β-estradiol in skeletal muscle cells leads to phosphorylation of Elk-1 and CREB transcription factors. The Journal of steroid biochemistry and molecular biology. 2007;103(3-5):462-6.
16. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C T method. Nature protocols. 2008;3(6):1101.
17.Leung PS. The potential of irisin as a therapeutic for diabetes. Future Science; 2017.
18. Xiong X-Q, Chen D, Sun H-J, Ding L, Wang J-J, Chen Q, et al. FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2015;1852(9):1867-75.
19. Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, et al. Physiology and role of irisin in glucose homeostasis. Nature Reviews Endocrinology. 2017;13(6):324.
20. Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism. 2012;61(12):1725-38.
21. Choi Y-K, Kim M-K, Bae KH, Seo H-A, Jeong J-Y, Lee W-K, et al. Serum irisin levels in new-onset type 2 diabetes. Diabetes research and clinical practice. 2013;100(1):96-101.
22. Liu J-J, Wong MD, Toy WC, Tan CS, Liu S, Ng XW, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. Journal of Diabetes and its Complications. 2013;27(4):365-9.
23. Kurdiova T, Balaz M, Vician M, Maderova D, Vlcek M, Valkovic L, et al. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. The Journal of physiology. 2014;592(5):1091-107.
24. Giri D, Pintus D, Burnside G, Ghatak A, Mehta F, Paul P, et al. Treating vitamin D deficiency in children with type I diabetes could improve their glycaemic control. BMC research notes. 2017;10(1):465.
25. Felício KM, de Souza ACCB, Neto JF, de Melo FTC, Carvalho CT, Arbage TP, et al. Glycemic variability and insulin needs in patients with type 1 diabetes mellitus supplemented with

vitamin D: a pilot study using continuous glucose monitoring system. Current diabetes reviews. 2018;14(4):395-403.
26. Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and diabetes. Endocrinology and Metabolism Clinics. 2010;39(2):419-46.
27. Inaba M, Nishizawa Y, Mita K, Kumeda Y, Emoto M, Kawagishi T, et al. Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1, 25-dihydroxyvitamin D3 in patients with type 2 diabetes. Osteoporosis International. 1999;9(6):525-31.
28. Faienza MF, Ventura A, Delvecchio M, Fusillo A, Piacente L, Aceto G, et al. High Sclerostin and Dickkopf-1 (DKK-1) serum levels in children and adolescents with type 1 diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism. 2016;102(4):1174-81.
29. Faienza MF, Brunetti G, Sanesi L, Colaianni G, Celi M, Piacente L, et al. High irisin levels are associated with better glycemic control and bone health in children with Type 1 diabetes. Diabetes research and clinical practice. 2018;141:10-7.
30.Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nature Reviews Endocrinology. 2012;8(8):457.
31. Colaianni G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, et al. The myokine irisin increases cortical bone mass. Proceedings of the National Academy of Sciences. 2015;112(39):12157-62.
32. Colaianni G, Cuscito C, Mongelli T, Oranger A, Mori G, Brunetti G, et al. Irisin enhances osteoblast differentiation in vitro. International journal of endocrinology. 2014;2014.
33. Anastasilakis A, Polyzos S, Makras P, Gkiomisi A, Bisbinas I, Katsarou A, et al. Circulating irisin is associated with osteoporotic fractures in postmenopausal women with low bone mass but is not affected by either teriparatide or denosumab treatment for 3 months. Osteoporosis International. 2014;25(5):1633-42.
34.Palermo A, Strollo R, Maddaloni E, Tuccinardi D, D'onofrio L, Briganti SI, et al. Irisin is associated with osteoporotic fractures independently of bone mineral density, body composition or daily physical activity. Clinical endocrinology. 2015;82(4):615-9.
35. Raafat NA, Abulmeaty MM. Effect of Vitamin D3 Administration on Irisin Levels in Vitamin D Deficient Rat Model. Al-Azhar Medical Journal. 2017 Jan;331(4146):1-4.
36. Al-Daghri NM, Rahman S, Sabico S, Amer OE, Wani K, Al-Attas OS, et al. Impact of vitamin D correction on circulating irisin: a 12 month interventional study. Int J Clin Exp Med. 2016;9(7):13086-92.
37. Qiao X, Nie Y, Ma Y, Chen Y, Cheng R, Yin W, et al. Irisin promotes osteoblast proliferation and differentiation via activating the MAP kinase signaling pathways. Scientific reports. 2016;6:18732.
38. Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. The Journal of clinical investigation. 2006;116(7):1776-83.
39. Jetton TL, Lausier J, LaRock K, Trotman WE, Larmie B, Habibovic A, et al. Mechanisms of compensatory β-cell growth in insulin-resistant rats: roles of Akt kinase. Diabetes. 2005;54(8):2294-304.
40. Kucukkaraca H, Sogut MU. Effect of exercise and vitamin d supplementation on the level of irisin hormone in diabetic rats. Clinical Nutrition. 2018;37:S97.