

EFFECT OF VITAMIN D₃ ADMINISTRATION ON IRISIN LEVELS IN VITAMIN D DEFICIENT RAT MODEL

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ABSTRACT

Background: Irisin is a novel myokine, adipokine and neurokine which increases energy expenditure and improves glucose tolerance. Both myokine irisin and vitamin D (VD) are players in the musculoskeletal system; However, irisin-vitamin D relationship is still unclear.

Objective: Investigating the effect of chronic administration of cholecalciferol (vitamin D₃) on serum irisin level in relation to some metabolic parameters in vitamin D deficient rat model.

Material and Methods: Thirty six healthy weaned (21 days) male albino rats (80.70±10.1 gram) were used. Rats were divided randomly into two groups: Group I : Control group (n=12) fed on normal balanced diet for 6 weeks then given 1ml of pure canola oil as a vehicle every other day for 2 weeks via gavage and Group II: vitamin D-deficient (VDD) group (n=24) fed on vitamin D-deficient diet (20% lactose, 2% Ca, and 1.25% P) for 6 weeks, then subdivided randomly into two equal subgroups: Group IIa (VDD vehicle -treated group) which was given 1ml of pure canola oil as a vehicle every other day for 2 weeks via gavage, and Group IIb (VDD D₃- treated group): which was given 1 ml of 75 µg/ml of vitamin D₃ in 1ml of pure canola oil every other day for 2 weeks. For all groups, BMI, food intake, serum irisin, 25-hydroxy vitamin D 25-OHVD, calcium, phosphorus, parathrmone hormone (PTH), glucose, insulin and calculated homeostasis model assessment of insulin resistance (HOMA-IR) were estimated.

Results: VD deficient diet for six weeks induced significantly decrease in serum 25-OHVD together with insignificant changes in PTH, Ca, P levels, BMI and food intake. Regarding serum irisin levels, they were low in VDD rats in comparison to normal rats.

However, after 2 weeks of treatment with VD₃, serum irisin levels were significantly higher in VDD D₃-treated group than in VDD vehicle- treated rats. These elevated levels of irisin were significantly positive correlated to serum 25-OHVD in VDD vehicle- treated and VD deficient D₃- treated groups. Moreover, serum insulin levels and HOMA-IR were significantly higher in VDD rats than that of normal rats and, they were negatively correlated with serum 25-OHVD and irisin in both VDD vehicle- treated and VDD D₃-treated groups.

Conclusion: Hypovitaminosis D was significantly associated with reduced irisin levels and elevated insulin resistance. Moreover, irisin levels significantly elevated after restoration of VD. Both serum irisin and 25-OHVD were negatively correlated with insulin resistance. Hypovitaminosis D-induced metabolic deterioration could be resulted from decreased irisin levels.

Keywords: Irisin, vitamin D₃ supplementation, vitamin D deficiency, food intake.

INTRODUCTION

Irisin is a novel myokine secreted by skeletal muscle and adipose tissue in response to exercise. It has been shown that irisin promotes browning of white

adipose cells, increases energy expenditure, and improves glucose tolerance (Bostrom et al., 2012 and Polyzos et al., 2013). Besides metabolic role, irisin mediates positive effects on bone health. It was found that irisin enhances

osteoblast differentiation *in vitro* (Colaïanni et al., 2014). Also, serum irisin levels were lower in women with osteoporotic fractures compared to normal (Palermo et al., 2015 and Engin-Ustün et al., 2016).

Interestingly, similar to irisin, vitamin D appears to share some of its beneficial effects as it improves the parameters of glucose metabolism, increases insulin production, and plays a role in weight reduction (Holick, 2007 and Cavalier et al., 2011). Patients with rickets and osteomalacia displayed proximal myopathy, suggesting a direct link between hypovitaminosis D and muscle function (Girgis et al., 2013).

Accordingly, vitamin D and irisin interplay remains unclear. Controversy human studies investigated this relationship; Cavalier et al. (2014) failed to find any relation between vitamin D and irisin level however, irisin levels increased after vitamin D correction in Vitamin D deficient subjects (Al-Daghri et al., 2016). Thus, the present study aimed to investigate the association of irisin and vitamin D levels in normal and vitamin D deficiency state, and to examine the impact of vitamin D correction on circulating irisin in vitamin D deficient rats and their metabolic relationships.

MATERIAL AND METHODS

Animals: Thirty six weaned male albino rats of a local strain, aged 21 days, weighting (80.70 ± 10.1 g.) were obtained from the animal house in Faculty of Veterinary medicine -Zagazig University. Animals were kept in nine steel wire cages (40 Cm x 30 Cm x 18 Cm, 4 rats /cage) in the animal house in Faculty of Medicine of Zagazig University under hygienic conditions with an ambient

temperature range of $22 \pm 2^\circ\text{C}$ and a normal dark/ light cycle. All animals received care in compliance with the animal care guidelines and ethical regulations in accordance with the guide for the care and use of laboratory animals according to **Institute of Laboratory Animal Resources (1996)**. The study protocol was approved by the Institutional Review Board (IRB) and ethics committee of Faculty of Medicine Zagazig University. Animals were fed standard chow and had free access to water. Rats were accommodated to animal house conditions for one week before the experiments going on. From day one of the experiment, rats were divided randomly into two groups:

Group I : Control group (n=12) fed on normal balanced diet (carbohydrate 59.2%, fat 7.1%, protein 18.1%, fiber 4.8%, Ash 2.2%, moisture <10% of total Kcal , calcium 5.1g/kg, and vitamin D₃ 1000 IU/kg - AIN-93G, Bio-Serve, USA) for 6 weeks, then given 1ml of pure canola oil as a vehicle every other day for 2 weeks via gavage .

Group II: Vitamin D-deficient (VDD) group (n=24) fed on vitamin D-deficient diet containing (20% lactose, 2% Ca, and 1.25% P / total Kcal; carbohydrate 67.5%, fat 12.7% and protein 19.8% - TD.87095 Brown C.C, USA) (Stavenuiter et al., 2015) for 6 weeks.

After 6 weeks, VDD rats were subdivided randomly into two equal subgroups: **Group IIa (VDD vehicle - treated group)** which was given 1ml of pure canola oil as a vehicle every other day for 2 weeks via gavage.

Group IIb (VDD D₃- treated group): which was given 75 µg (0.66ml) of vitamin D₃ (Vi-De 3, Novartis Egypt) (Brouwer et al., 1998) in 1ml of pure canola oil every other day for 2 weeks.

All groups continued feeding its own type of diet until the end of experiments.

Food intake: was measured daily, Preweighed food was provided in standard stainless steel hoppers. After 24 h, rats were briefly removed from their cages and weighed, and the amount of food remaining, including any on the bottom of the cages or any that had spilled onto plastic sheets placed under each cage, was recorded. Intake was calculated as the weight (in grams) of food provided less that recovered (Vento et al., 2008).

Body mass index (BMI): At the end of treatment period, BMI was calculated by dividing body weight of rat in grams on the square of the nose to anus length in cm (Novelli et al., 2007).

Blood sampling: Animals were fasted overnight, anesthetized with diethyl ether, and sacrificed by capitulation. Blood was immediately collected in centrifuge tubes, and was allowed to clot for 2 hours at room temperature before centrifugation for 20 minutes at approximately 500 rpm. The separated serum was stored at -20° C until used for analysis of:

Serum irisin levels according to Bostrom et al. (2012) using rat irisin ELISA rat catalogue no MBS9356609 (Mybiosource USA).

25 hydroxy vitamin D (25-OHVD) levels according to Shuai et al. (2008)

using rat ELISA kits, catalogue no MBS288530 (Mybiosource USA).

Calcium (Ca) levels according to Gindler et al. (1972) using A calcium colorimetric assay kit (Randox UK, catalogue no CA590).

Serum phosphorus (P) levels according to Goldenberg et al. (1966) by colorimetric method using kits supplied by Bio-diagnostic Co. (Cairo, Egypt).

Serum parathormone (PTH) according to Kruger et al. (1995) catalogue no MBS702121 (Mybiosource USA).

Serum glucose level according to Tietz (1995) using glucose enzymatic-liquizyme rat kits (Biotechnology, Egypt).

Insulin level according to Temple et al. (1992) using KAP1251-INS-EASIA rat Kits (BioSource Europe S.A., Belgium).

Calculation of homeostatic model assessment of insulin resistance index (HOMA-IR): It based on serum insulin level (?IU/ml) and serum glucose level (mg/dl) according to the formula described by Matthews et al. (1985) as $HOMA-IR = \text{fasting serum glucose (mg/dl)} \times \text{fasting serum insulin (?IU/ml)} / 405$.

Statistical Analysis: The measured parameters were presented as the mean ± SD, and Pearson correlation coefficient was used to test their association. ANOVA with a post hoc test; Fisher's Least Significant Difference (LSD) was used to analyze the differences among studied groups. P values < 0.05 were considered to be significant. For the statistical analyses, SPSS version 19 (SPSS Inc. Chicago, IL, USA) was used.

RESULTS

Regarding the mean value of BMI, food intake and serum glucose insignificant changes ($P>0.05$) were detected among studied groups. Moreover, in group IIa (VDD vehicle- treated), there were significant ($P<0.001$) increases in the mean values of serum insulin (15.13 ± 1.47), and calculated HOMA-IR (2.93 ± 0.55) in comparison to that of control group (7.35 ± 0.98 and 1.48 ± 0.27 respectively), and that of group IIb (VDD D3- treated) (7.84 ± 0.84 and 1.59 ± 0.31 respectively), while there were no significant differences ($P>0.05$) between control and group IIb regarding both serum insulin and HOMAIR (**Table1**).

Concerning serum PTH, Ca and P, there were insignificant differences ($P>0.05$) among studied groups. Moreover, in group IIa (VDD vehicle- treated), there were significant ($P<0.001$) decreases in the mean values of both serum 25-OHVD (14.52 ± 2.69) and serum irisin (0.73 ± 0.15)

in comparison to that of control group (37.5 ± 5.69 and 1.47 ± 0.01) and that of group IIb (VDD D3- treated) (34.50 ± 4.41 and 1.55 ± 0.08 respectively) . There were no significant differences ($P>0.05$) in the mean values of serum 25-OHVD and serum irisin between control and group IIb (**Table 2**).

In VDD vehicle- treated) and (VDD D3-treated, there were significant positive correlations between irisin and 25-OHVD ($r=0.59$, $P< 0.05$; $r= 0.67$, $P <0.05$ respectively), however, there were significant negative correlations between irisin and both insulin level ($r=-0.79$, $P< 0.01$; $r= -0.64$, $P <0.05$ respectively) and HOMA-IR ($r=-0.72$, $P< 0.01$; $r= -0.88$, $P <0.001$ respectively). Additionally, in the same groups, 25-OH vitamin D showed significant negative correlations with insulin level ($r=-0.62$, $P< 0.05$; $r= -0.93$, $P <0.001$ respectively), and HOMA-IR ($r=-0.68$, $P< 0.05$; $r= -0.88$, $P <0.001$ respectively) (**Fig.1-10**).

Table (1): Statistical analysis of BMI, Food intake, serum glucose (mg/dL), serum Insulin (mIU/mL) and calculated HOMA-IR in the three studied groups.

Parameters	Control	VDD vehicle-treated		VDD D3- treated	
	Mean±SD	Mean±SD	P value	Mean±SD	P value
BMI (g/Cm2)	0.56±0.05	0.57±0.05	P=0.877 ^a	0.54±0.05	P=0.398 ^a P=0.319 ^b
Food intake	53.08±7.23	54.24±6.78	P=0.692 ^a	55.17±7.26	P=0.477 ^a P=0.751 ^b
Glucose (mg/dL)	78.06±9.10	81.78±7.87	P=0.29 ^a	80.67±8.39	P=0.75 ^a P=0.456 ^b
Insulin (mIU/mL)	7.35±0.98	15.13±1.47	P<0.001 ^a	7.84±0.84	P=0.293 ^a P=0.000 ^b
HOMA-IR	1.48±0.27	2.93±0.55	P<0.001 ^a	1.59±0.31	P=0.53 ^a P=0.000 ^b

a = p-value of significance versus control, b = p-value of significance versus VDD vehicle-treated group.

Table (2): Statistical analysis of serum PTH (Pg/ml), Ca (mg/dL), P (mg/dL), 25-OHVD (ng/ml) and Irisin (?g/ml) in the three studied groups.

Parameters	Groups	Control	VDD vehicle-treated	VDD D3-treated		
		Mean±SD	Mean±SD	P value	Mean±SD	P value
PTH(pg/ml)		16.68±2.62	16.99±2.46	P=0.759 ^a	17.74±2.46	P=0.308 ^a P=0.473 ^b
Serum Ca ⁺⁺ (mg/dL)		10.39±0.71	10.40±0.61	P=0.943 ^a	10.22±0.54	P=0.533 ^a P=0.487 ^b
P (mg/dL)		5.16±0.72	5.51±0.55	P=0.218 ^a	5.41±0.76	P=0.375 ^a P=0.724 ^b
25-OHVD (ng/ml)		37.5±5.69	14.52±2.69	P<0.001 ^a	34.50±4.41	P=0.108 ^a P<0.001 ^b
Irisin (?g/ml)		1.47 ± 0.01	0.73±0.15	P<0.001 ^a	1.55±0.08	P=0.099 ^a P<0.001 ^b

a = p-value of significance versus control, b = p-value of significance versus VDD vehicle-treated group.

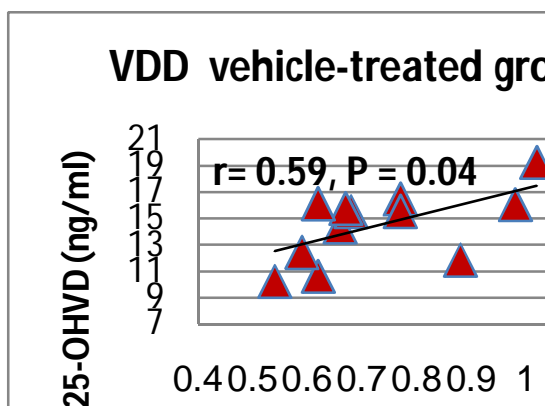


Figure (1): Pearson's correlation between irislin (ug/ml) and 25-OHVD (ng/ml) in VDD vehicle-treated group.

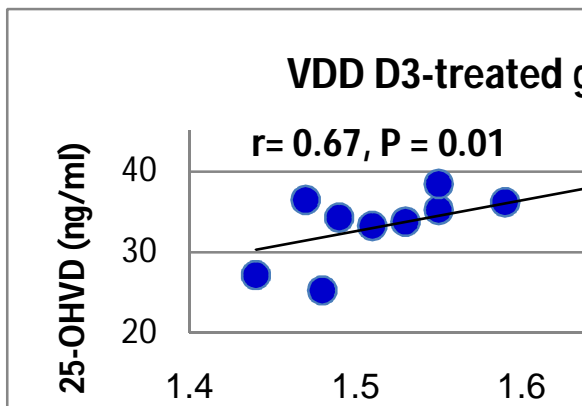


Figure (2): Pearson's correlation between irislin (ug/ml) and 25-OHVD (ng/ml) in VDD D₃-treated group.

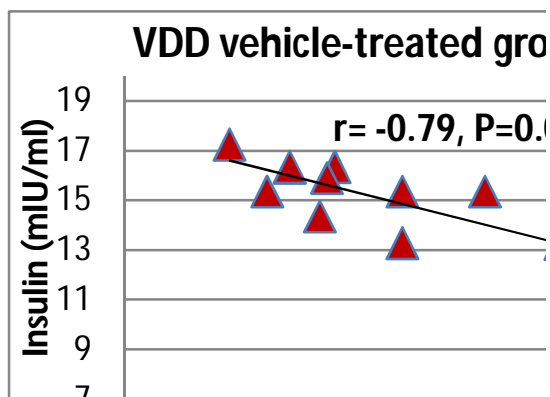


Figure (3): Pearson's correlation between irislin (ug/ml) and insulin (mIU/mL) in VDD vehicle-treated group.

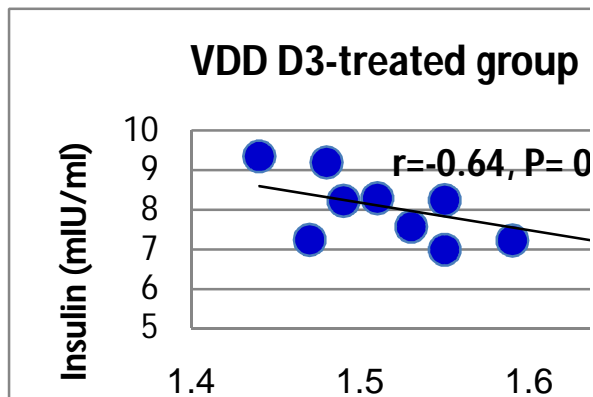


Figure (4): Pearson's correlation between irislin (ug/ml) and insulin (mIU/mL) in VDD D₃-treated group.

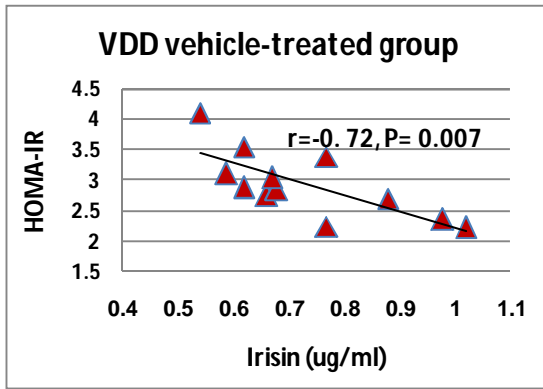


Figure (5): Pearson's correlation between irisin (ug/ml) and HOMA-IR in VDD vehicle-treated group.

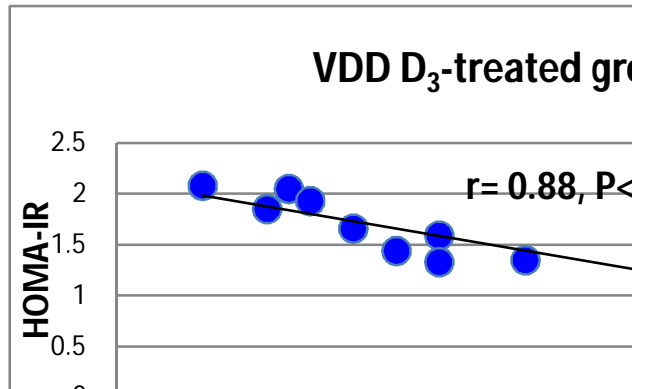


Figure (6): Pearson's correlation between irisin (ug/ml) and HOMA-IR in VDD D₃.treated group.

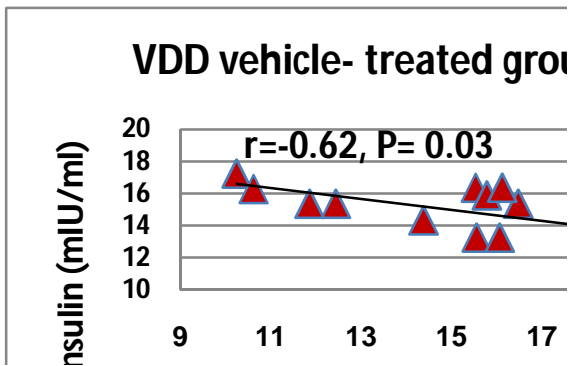


Figure (7): Pearson's correlation between 25-OHVD (ng/ml) and insulin (mIU/mL) in VDD vehicle-treated group.

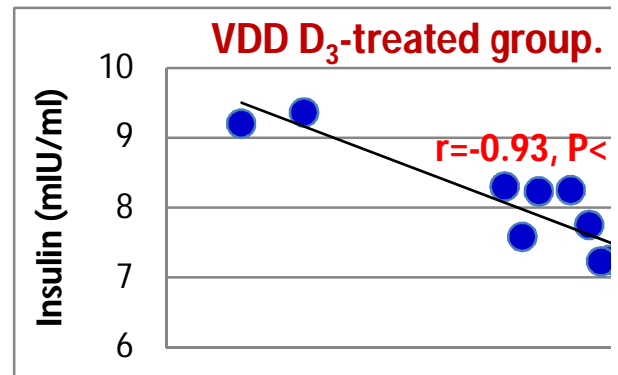


Figure (8): Pearson's correlation between 25-OHVD (ng/ml) and HOMA-IR in VDD D₃-treated group.

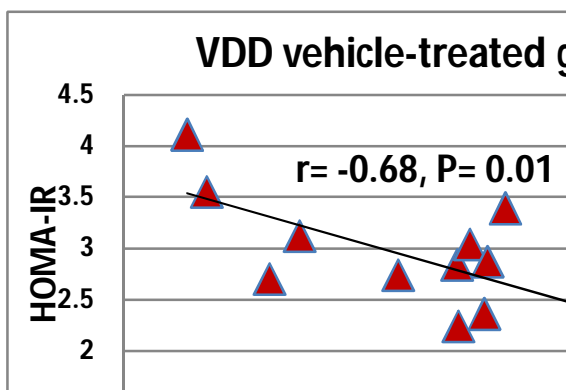


Figure (9): Pearson's correlation between 25-OHVD (ng/ml) and HOMA-IR in VDD vehicle-treated group.

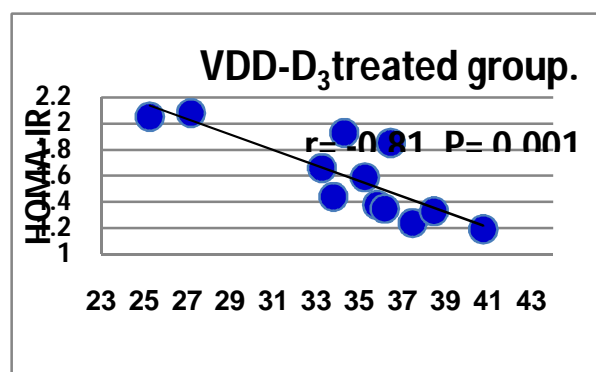


Figure (10): Pearson's correlation between 25-OHVD (ng/ml) and HOMA-IR in VDD D₃-treated group.

DISCUSSION

Vitamin D deficiency is a worldwide problem (Mithal et al., 2009). Low serum 25-hydroxyvitamin D levels have been associated with higher mortality rates and several diseases ranging from cardiovascular diseases and diabetes (Akin et al., 2012) to autoimmune diseases and liver diseases (Skaaby, 2015).

In this experimental study, six weeks of feeding a vitamin D deficient diet (but contained Ca 2%, P 1.25%, and 20% lactose) induced vitamin D deficiency in rats (serum 25-OHVD levels were < 20 ng/ml) together with insignificant changes in serum levels of PTH, Ca, and P levels (Stavenuiter et al., 2015).

Lactose is commonly used to counteract the otherwise total absence of VDR-dependent intestinal Ca and P absorption as it can increase the passive vitamin D-independent Ca absorption in the intestine (Bouillon et al., 2008). Together with use of Ca content (2%) higher than that in the standard rat chow (1%) were directed to both normalize serum Ca and prevent the development of hyperparathyroidism during vitamin D deficiency (Stavenuiter et al., 2015).

In the current study, there was a significant decrease in irisin levels in VD deficient rats in comparison to normal ones. Moreover, positive correlation was seen between irisin and VD in this group. After VD administration for 2 weeks, these levels of irisin increased significantly, and positively correlated to serum D in VDD D₃- treated group. Our findings were supported by Al-Daghri et al., (2016) who performed a year-long

intervention study on male and female subjects fed on vitamin D-rich foods and exposed to sunlight, besides performing normal physical activity. They found that levels of irisin in male subjects were significantly increased than control however irisin levels in females remain unchanged.

However, these results were different than those of Cavalier et al. (2014) who revealed that a single large dose of vitamin D (100,000 IU) did not impact irisin levels in young healthy subjects. Also, they concluded that the effects of vitamin D on muscle strength may not be interrelated to an irisin pathway. This controversy is due to some factors: Firstly they used single high dose of vitamin D, secondly, sample size used in their study was small, thirdly, their study was human (species difference), and finally, they selected subjects who were already with normal vitamin D. So, it might be possible that a single shot of vitamin D has no further effect on circulating irisin.

In the present work, hypovitaminosis D and low irisin levels were associated with insulin resistance as there was an inverse correlation between 25-OHVD and irisin levels with insulin levels and (HOMA-IR) in VD deficient rats. Many studies have demonstrated close relationships between vitamin D status with insulin resistance (Cheng et al., 2013; Heaney, 2013; Pilz et al., 2013 and Tepper et al., 2016).

Thus, after vitamin D treatment, a significant improvement in insulin sensitivity (HOMA-IR decreased) was seen in VDD D₃- treated rats rather than those untreated, and insulin resistance significantly negative correlated with 25(OH) Vitamin D and irisin levels.

It can be hypothesized that deficiency of VD leads to hyperinsulinemia and insulin resistance through decreased levels of irisin, as irisin could predict the onset of insulin resistance (**Crujeiras et al., 2014**). Moreover, **Song et al. (2014)** and **Zhang et al. (2014)** reported that functional mechanism of irisin were through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling indicating the relevance between irisin and insulin signaling.

This negative relationship between irisin and insulin resistance was consistent with the report of **Al-Daghri et al. (2014)** who found that irisin was negatively correlated with HOMA-IR in healthy women and **Moreno-Navarrete et al. (2013)** who showed a negative correlation between irisin levels and insulin resistance in men with obesity. Moreover, **Yan et al. (2014)** showed that fasting insulin, HbA1c and albumin/globulin ratio were negatively associated with serum irisin indicating that irisin may play potential role in insulin resistance and metabolic syndrome as well. Additionally, **Yang et al. (2015)** revealed that inhibited insulin action could be recovered by irisin addition.

Therefore, it was considered that irisin improves insulin resistance by marked up-regulation of uncoupling protein 1 (UCP1) and several mitochondrial genes, increase in oxygen consumption, improvement of glucose tolerance and reduction of insulinemia, demonstrating a greatly improved metabolic profile most likely via elevated energy expenditure (**Bostrom et al., 2012** and **Sanchis-Gomar et al., 2014**).

On the other hand, a positive correlation between HOMA-IR and irisin was reported (**Bostanc? et al., 2015**; **Chen et al., 2015** and **Fukushima et al., 2016**).

The reasons for this discrepancy might be partly due to the differences in the enrolled populations between studies or species difference

Other mechanisms by which vitamin D improved insulin sensitivity was reported by **Belenchia et al. (2013)** and **Poolsup et al. (2016)**. Vitamin D could reduce inflammation that indirectly improves insulin resistance and pancreatic β -cell function (**Sung et al., 2012** and **Pilz et al., 2013**). Additionally, the mechanism of action of vitamin D may also, mediated via the regulation of plasma ionized calcium levels, which influence insulin synthesis and secretion (**Pittas et al., 2007**; **Afzal et al., 2013**; and **Schottker et al., 2013**), and thus have a direct beneficial effect on pancreatic β -cell functions (**Palomer et al., 2008** and **Mitri et al., 2011**). Recently, **Sun et al. (2016)** showed that vitamin D supplementation for 1 year effectively improves fasting glucose level and insulin resistance in healthy Japanese adults.

In a trial to explain how vitamin D enhances irisin secretion, the expression of irisin precursor; fibronectin type III domain containing 5 (FNDC5) is induced in muscle by physical exercise via a peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1-alpha)- dependent pathway and there may be a coactivation between the vitamin D receptor and PGC1-alpha signaling pathways in muscle. Thus, by this interaction between vitamin D receptor and PGC-1 α , VD may increase expression of PGC-1 α

which upregulates the expression of FNDC5 mRNA promoting irisin secretion into blood (Bostrom et al., 2012 and Cavalier et al., 2014), However, Choi et al. (2013) found that vitamin D did not alter AMPK phosphorylation, the upstream of PGC1- α in rat. Recently, others also speculated that FNDC5 is not a direct PGC1- α target gene, but rather is upregulated in skeletal muscle in vivo via secondary mechanisms (Pekkala et al., 2013 and Norheim et al., 2014).

CONCLUSION

Hypovitaminosis D was significantly associated with reduced irisin levels and elevated insulin resistance. Additionally, irisin levels were significantly elevated after restoration of VD. Both serum irisin and 25(OH) vitamin D are negatively correlated with insulin resistance. Hypovitaminosis D-induced metabolic deterioration could be resulted from decreased irisin levels.

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تأثير إعطاء فيتامين د₃ على مستوى هورمون الأيريسين في مصل الدم لنموذج الجرذان المحدث لها تجريبيا نقص في فيتامين د

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خلفية البحث: الأيريسين هو هورمون يفرز من العضلات و الأنسجة الدهنية وله وظائف متعددة من ضمنها أنه يزيد من إستهلاك الطاقة ويحسن من إفراز الإنسولين . و كل من الأيريسين وفيتامين د لهما دور في وظائف الجهاز العضلي الهيكلي . ومع ذلك، فإن علاقة الأيريسين بفيتامين د لا تزال غير واضحة.

الهدف من البحث: التعرف على تأثير الإعطاء المزمن لفيتامين د على مستوى الأيريسين في مصل الدم للجرذان الطبيعية و الجرذان المحدث لها تجريبيا نقص في مستويات فيتامين د ، و أيضا علاقة كل منهما ببعض معلمات الأيض .

طرق البحث: تم إستخدام ٣٦ من ذكور الجرذان البيضاء السليمة عمرها حوالي ٢١ يوم، ووزنها حوالي (٨٠,٧٠ ± ١٠,١ جرام) ؛ و تم تقسيم الجرذان عشوائيا إلى مجموعتين: المجموعة الأولى: المجموعة الضابطة (العدد = ١٢)؛ ويقدم لها نظام غذائي طبيعي متوازن لمدة ٦ أسابيع، ثم تم إعطائها ١ مل من زيت الكانولا النقي ثلاث مرات أسبوعيا لمدة أسبوعين من خلال أنبوب تغذية والمجموعة الثانية: جرذان محدث لها تجريبيا نقص في مستوى فيتامين د بالدم و عددها ٢٤ جرذا و ذلك عن طريق تغذيتها بنظام غذائي لا يحتوي على فيتامين د ، بينما يحتوي على (٢٠٪ لاكتوز، ٢٪ كالسيوم، و ١,٢٥٪ فسفور) لمدة ٦ أسابيع؛ ثم تم تقسيمها عشوائيا إلى مجموعتين متساويتين فرعيتين: مجموعة (٢-أ): جرذان محدث لها نقص فيتامين د ومعالجة بالوسيط بزيت الكانولا النقي ثلاث مرات أسبوعيا لمدة أسبوعين من خلال أنبوب تغذية، و مجموعة (٢-ب): جرذان محدث لها نقص فيتامين د ومعالجة بإعطائها فيتامين د بجرعة ٧٥ ميكروغرام من فيتامين د ٣ ثلاث مرات أسبوعيا لمدة أسبوعين من خلال أنبوب تغذية. وبعد إنتهاء التجربة، تم قياس- لجميع الجرذان - مؤشر كتلة الجسم، و كمية تناول الطعام، و مستويات كل من الأيريسين ، ٢٥-هيدروكسي فيتامين د، و الكالسيوم، و الفوسفور، و هورمون الباراثورمون ، و جلوكوز الدم، و الإنسولين، و معادلة مقاومة الإنسولين .

النتائج: أسفرت النتائج عن أن الغذاء الذي لا يحتوي على فيتامين د لمدة ستة أسابيع أحدث انخفاضا ذا دلالة إحصائية في مستوى ٢٥-هيدروكسي فيتامين د بالدم ، بينما لم يتغير كلاً من مستويات هورمون الباراثورمون، و الكالسيوم، و مستويات الفوسفور، و مؤشر كتلة الجسم و كمية تناول الطعام. و وجد أن مستوى الأيريسين قد انخفض بمعدل ذو دلالة إحصائية في مجموعة الجرذان

المحدث لها نقص فيتامين د بمقارنتها بالجرذان الطبيعية . وكما وجد أن بعد أسبوعين من العلاج بفيتامين د ارتفع مستوى الإيريدين ارتفاعاً ذا دلالة إحصائية في مجموعة الجرذان المعالجة بفيتامين د عن مثيلاتها التي لم تعالج بالفيتامين . وهذا المستوى من الإيريدين له ارتباط إيجابي ذو دلالة إحصائية مع مستويات ٢٥ -هيدروكسي فيتامين د في كل من مجموعة الجرذان المحدث لها نقص فيتامين د ، وأيضاً المعالجة بفيتامين د . وعلاوة على ذلك، كانت مستويات الإنسولين ومعادلة مقاومة الأنسولين أعلى و ذات دلالة إحصائية لدى الجرذان المحدث لها نقص فيتامين د بالمقارنة بالجرذان الطبيعية و الجرذان المعالجة بفيتامين د . وكما أن مستوى الإنسولين و كذلك معادلة مقاومة الإنسولين لهما ارتباط سلبي بدلالة إحصائية مع كلا من مستوى الإيريدين و مستويات ٢٥ - هيدروكسي فيتامين د في كل من مجموعة الجرذان المحدث لها نقص فيتامين د وأيضاً المعالجة بفيتامين د .

الاستنتاج: يرتبط نقص فيتامين د بشكل ملحوظ مع انخفاض مستويات الإيريدين وارتفاع مقاومة الإنسولين، بينما تزيد مستويات الإيريدين بشكل كبير بعد تصحيح نسبة فيتامين د بالدم، وأيضاً وجد ارتباط سلبي بين كل من الإيريدين و ٢٥ -هيدروكسي فيتامين د مع مقاومة الإنسولين، وبالتالي فإن نقص فيتامين د يحدث خلافاً في الأيض من خلال انخفاض مستويات الإيريدين.