Egypt. J. Chem. **Vol. 65**, No. 12, pp. 175 – 180 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Circulating Irisin In Relation To Obesityand Anorexia Nervosa in Patients with Type 2 Diabetes

MoushiraZaki[a], HanaaReyadAbdallah[a], Hala T El-Bassyouni[b], Hend M. Tawfeek[c],Hanan Hanna [d], MagdiN.Ashour[e] and Eman R. Youness[e]*

a Biological Anthropology Department, Medical Researches and Clinical Studies Institute– National Research Centre Cairo, Egypt.

[b] Clinical Genetics Department, National Research Centre, Cairo, Egypt.

[c] Internal Medicine Faculty of Medicine, Al-Azhar University- Girls, Egypt.

[d] Chemical Pathology, Faculty of Physical Therapy –Delta University for Science and Technology- Gamsa – Egypt

[e] Medical Biochemistry, Departments, Medical Researches and Clinical Studies Institute – National Research Centre Cairo, Egypt

Abstract

Irisin is a myokine excreted via the activation of specific proteases (EC 3.4) from fibronectin type III domain-containing protein. Irisin stimulates proliferation of β-cell and biosynthesis and secretion of insulin. Augmented circulating irisin has been correlated with reduced IR and improved glucose tolerance.Nevertheless, the role of irisin in type 2 diabetes mellitus (T2DM) is indistinct. Studies have revealed that white adipose tissue (WAT) also secretes irisin and circulating irisin is increased in obese persons. Our objective was to estimate irisin concentrations in patients with type 2 diabetes mellitus (T2DM) along with obesity and anorexia nervosa (AN). Nevertheless, under conditions of severely altered body weight such as obesity and anorexia nervosa the regulation of irisin remain to be investigated.This observational parallel-group study was conducted in 120 diabetic women with AN (T2DM), 60 obese and 60 non-obesecompared with 60 healthy normal weight (NW) controls.Circulating irisin levels have been quantified in serum samples by ELISA. Anthropometric and body composition were performed.The obese T2DM showed significant higher levels of irisin levels compared to non-obese as well as to the NW controls. Irisin also associated positivelywith body weight, BMI, fat mass, obesity measures and blood pressure levels.In conclusion our study demonstrates the association between irisin levels and obesity in diabetic patients. Irisin is not primarily affected by the presence of anorexia nervosain obese T2DM.

Key word:Anorexia nervosa, body composition, irisin, obesity,T2DM

1. Introduction

Irisin, an innovative myokine, is processed proteolytically from the FNDC5 gene product preceding its release into the circulation and regulated by PPAR-c coactivator-1(PGC1)[1]. In long-term, irisin was found to be significantly diminished in new onset andtype 2 diabetes patients compared with nondiabetic ones[2]. Irisin stimulates the markers of the white adipose tissue browning process assisting tissue regeneration, cell proliferation, energy metabolism, reducing insulin resistance, and lowering blood glucose [3]. In patients with diabetes mellitus type 2 (T2DM), irisin is a probable upcoming therapeutic target as reports

*Corresponding author e-mail: hoctober2000@yahoo.com
Receive Date: 26 December 2021, Revise Date: 14 March 2022, Accept Date: 16 March 2022
DOI: 10.21608/EJCHEM.2022.112977.5143

have confirmed that irisin can alleviate pro-inflammatory conditions and induce browning of adipocytes[4]. It has been informed that raisedirisin levels in response to exercise mightdecrease weight in obese persons and insulin resistance in diabetic patients. Moreover, previous studies emphasized that the increased serum levels ofirisin reduces the risk of body mass index BMI augmentation and coronary atherosclerosis Additionally, the serum level of irisin positively correlates with body fat mass, higher waist circumference, and dyslipidemia [5][6].

The T2DM is a chronic metabolic disorder caused by imperfections in insulin activity,insulin secretion, or both. The chief mechanisms of insulin resistance (IR) in T2DM are lipotoxicity, endoplasmic reticulum stress,amyloid deposition in the pancreas, oxidative stress, and glucotoxicity[7]. Signals released by peripheral tissues, as the digestive tract or fat, are energetic contributors in regulation of body weight through acting at central level[8]. Because of the upsurge in the metabolic diseases prevalence associated with obesity, comprisingT2DM, numerous metabolic biomarkers have been recorded as potential controllers of glucose homeostasis[9]. Many studies revealed diminished irisin levels in T2DM patients irrespective of the diagnosis time and whether they are undertaking any medications and even lower levels in the occurrence of impediments of T2DM[10]. Similarly, other studies revealed significantly reduced levels of irisin in adults with T2DM irrespective of BMI, age, gender, and relating their findings with worsening in the PGC-1α expression in persons with T2DM[11]. Kurdiova et al. carried out an in vitro and in vivo studies, found divergent impacts in each of them; as from the in vitro study,in the in vivo study, irisin had a greater expression of FNDC5[12]. One study indicated the deficiency of relationship among T2DM and irisin[13]. body weight and food intake were regulated by a complex network of protein hormones & peptide inhibiting or stimulating food intake [14].
Based on literature data, the purpose of this workwas to compare the irisin concentrations in obese patients with type 2 diabetes, explore the correlation of circulating irisin and the body composition exploring the irisin role in anorexia nervosa.

2. Patients and Methods This study comprised 191 individuals, they included 60 obese patients with T2DM without anorexia nervosa (AN), 60 non- obese patients with T2DM and AN and 60 healthy control subjects. Eleven patients refused to participate in the study. All samples were collected after obtaining the patients' informed consent using a form approved by the Ethical Committee of the National Research Centre.
Insulin resistance (IR) was estimated based on calculation of the homeostasis model assessment (HOMA) index for each patient. This was done using the formula: (fasting plasma insulin in Iu/ml × fasting plasma glucose in mmol/l ÷ 22.5) [15].
Anthropometric parameters included body weight, height, mid upper arm circumference, waist and hip circumferences have been measured. Skin-fold thickness of biceps, triceps,subscapular, suprailiac and abdominal skin fold thickness were measured as well. All measurements were taken 3 times on the left side of the body and the mean of the 3 values was used. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Height was measured with the patients standing with their backs leaning against the stadiometer of the same scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters square (kg/m^2). Mid upper arm circumference (MUAC) was measured using a flexible tape at the midway between the olecranon and acromial process on the upper right arm with the elbow flexed 90 degrees. Waist circumference (WC) was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. Skin-fold thickness was measured to the nearest mm, except for low values (usually 5 mm or less) when it was taken to the nearest 0.5 mm. These readings were made at six sites on all subjects, at the biceps, triceps, subscapular and supra-iliac areas, using Holtain caliper (Ltd, Bryberian, Crymmych, Pembrokeshire). The subscapular skinfold was measured below the lower angle of the left scapula at a diagonal in the natural cleavage of the skin. Biceps skin fold thickness was measured at the level of the mid-point between the acromion (lateral edge of the acromion process) and the radius (proximal and lateral border of the radius bone) on the mid-line of the anterior surface of the arm, triceps skin fold thickness (vertical fold, midway between acromion, and olecranon processes on the posterior surface of the arm),and the position of the suprailiac skinfold was

the diagonal fold just above the iliac crest even with the anterior axillary line, abdominal skin fold was at 5 cm adjacent to the umbilicus to the right side. Subsequently, sum of skin folds was calculated. Anthropometric measurements were obtained according to standardized equipment and following the recommendations of the International Biological Program [16]. Systolic and diastolic blood pressures (SBP and DBP) were measured twice in the right arm in a sitting position after a10 min rest period; using a mercury sphygmomanometer the average of the two measurements was used for analysis. Blood pressure was measured according to a standardized operating procedure using a calibrated sphygmomanometer and brachial inflation cuff (HEM-7200 M3, Omron Healthcare, Kyoto, Japan). Fat mass was measured by Tanita Body Composition Analyzer (SC-330).

3. Venous blood samples were collected by direct venipuncture after an overnight fast (minimum 12 h). Fasting plasma glucose and serum lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides (TG) were measured by enzymatic colorimetric methodsusing a Hitachi auto analyzer 704 (Roche Diagnostics. Switzerland)[17] . Low density lipoprotein cholesterol (LDL-C) was calculated according to certain equation (LDL-C= Total cholesterol –Triglycerides/5+ HDL-C) [18](14). Serum insulin concentration was analyzed by chemiluminescent immunoassay (Immulite2000, Siemens, Germany[19]. Insulin resistance was determined by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) calculated as the product of the fasting plasma insulin level (IU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5[15]. Serum irisin concentrations were measured using commercialELISA kit.Fresh sera were used for the measurement of fasting blood glucose (FBG), which was assayed using the glucose oxidase method. The anorexia nervosa patients were diagnosed by experienced clinicians.Fasting glucose, glycated hemoglobin (HbA1c), insulin, total cholesterol, and triglycerides were quantified with commercialkits

4. Statistical analysis

Continuous variables were expressed as mean ± standard deviation. Nonparametric variables were log-transformed to improve normality for statistical testing, and the datawere expressed as median (min–max). A one-way ANCOVA with Bonferroni's post hoc comparisons wasused to compare the mean values among groups. Partial correlations with age and BMIas variables were used to explore the relationships between irisin and other variables. Comparisons between groups were performed using ANOVA or Kruskal-Wallis tests followed by Bonferroni or Dunn's posttests, as appropriate. The univariate associations between irisin, anthropometric,and biochemical parameters were examined by Pearson or Spearman correlation tests. A p value < 0.05 was considered significant.

5. Results

Figure 1 shows flow chart of the enrolled participants.

Table 1 shows the mean levels of irisin levels andHbA1c in different groups. Significant increase of serum irisin concentrations and HbA1c in the obese T2DM subjects compared with non- obese T2DM and controls was observed.

Table 2 shows univariate relationship between serum irisin level, anthropometric and biochemical parameters in obese T2DM. Significant positive association was observed between irisin levels, metabolic parameters, and obesity measures in patients withT2DM.

Figure 2 shows Boxplots of irisin concentrations in group categories of patients and controls. Irisin was increased in patients with obesity. Box-and-whiskers plot with median value, interquartile range, and upper & lower values is shown.

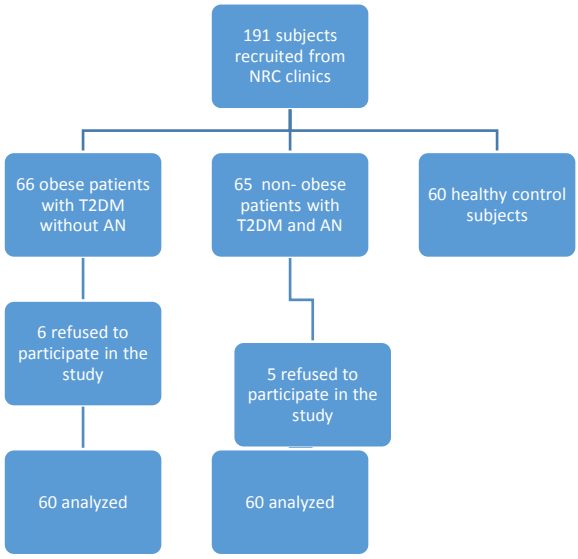

Fig. 1 Flowchart of enrolled participants

Table 1. Plasmairisinand HbA1c in the different groups

	Controls	Obese T2DM	Non-Obese T2DM	ANOVA *P* value
Irisin(ng/mL)	40.28 ± 4.34	88.86 ± 37.69*	41.20 ± 2.60	< 0.001
HbA1c	3.91± 1.01	7.90± 3.42#	5.60 ± 2.12	< 0.001

* p< 0.01 versus the Control group and non –obese T2DM
# p< 0.05 versus the Control group and non –obese T2DM

Table 2.Univariaterelationship between irisin level, anthropometric and biochemical parameters in obese T2DM

Fat %	r	0.414**
	p	0.0001
Mid upper arm circumference	r	0.316**
	p	0.000
Waist circ.	r	0.380**
	p	0.001
Sum of skinfolds	r	0.370**
	p	0.001
Systolic PB (mm Hg)	r	.216*
	p	.013
Systolic PB (mm Hg)	r	0.224*
	p	0.011
Abdominal skinfold	r	0.310**
	p	0.001
BMI	r	0.336**
	p	0.007
Fasting glucose	r	0.236**
	p	0.001
HBA1c	r	0.436**
	p	0.001
HOMA-IR	r	0.526**
	p	0.001

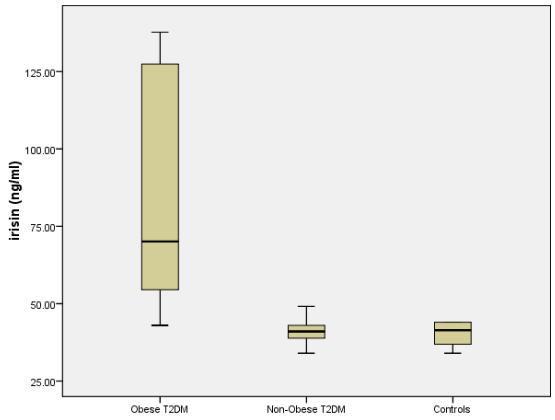

Figure 2: Boxplots of irisin concentrations by group categories: Irisin increases significantly with obesity

5. Discussion

Irisin is an innovative hormone that offers a probable solution for metabolicdisorders treatment due to its effect on the glycemic homeostasis and adipose tissue [20]. Concerning the effect of irisin, in both humans and animals, the results were paradoxical but fascinating. level of irisin seemed to be negatively interrelated with insulin levels,age, adiponectin and triglyceride concentrations, proposing that irisin might be implicated in metabolic regulation[21].Previous studies focused on insulin resistance [22][23] and assumed that itmight represent an independent risk factor affecting the adipokines circulating levels. In the presentwork, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was the chief factor that fluctuated more among the altered groups. A previous study postulated that insulin resistance might affect irisin levels and proposed to comprise the irisin/HOMA-IR ratio as a novel independent parameter. Remarkably, although irisin levels were not altered amongst the studied groups, the valuesof irisin/HOMA-IR were significantly augmentedwhile comparedto recreational & sedentary subjects, therefore approving a probable effect of insulin resistance on levels of irisin. Furthermore, the irisin level increased in obese patients (BMI >30) compared to anorexia nervosa subjects which indicated apositive correlation of body weight and irisin with BMI. Likewise, other work[24] conveyed a correlation of irisin and BMI. However, other investigators did not find an association between HOMA-IR and the circulating irisin levels. Irisin was recognized as a potential factor related with insulin resistance progression throughout the weight maintenance period next a dietary weight-lowering platformin obese patients[25]. Some evidence has confirmed the relationship between insulin resistance or diabetes and decreased serum irisin levels [26][27][28]. Presented suggestion about the muscle mass and adiposity effects on circulating irisin has been argumentative. Whereas a researcher reported that irisin was not associated to BMI in diabetic[29].A positive correlation between BMI andserum irisin has been consistently reported in three other studies [11][24][30].

In conclusion, our study highlighted the association of serum irisin with obese T2DM patients. However, it was not increased in theanorexia nervosa patients. Thus, the irisin could be used as a therapeutic approach for the treatment of metabolic disorders.

Conflict of interest

None

Funding

This research did not receive any fund

Acknowledgments

The authors thank all participants.

6. References

1. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*.481(7382):463-468 (2012).
2. Højlund K, Boström P. Irisin in obesity and type 2 diabetes. *J Diabetes Complications*. 27(4):303-304(2013).
3. de Oliveira M, De Sibio MT, Mathias LS, Rodrigues BM, Sakalem ME, Nogueira CR. Irisin modulates genes associated with severe coronavirus disease (COVID-19) outcome in human subcutaneous adipocytes cell culture. *Mol Cell Endocrinol*. 515:110917(2020).
4. Brinkmann C, Weh-Gray O, Bloch W, Brixius K, Predel H-G, Kreutz T. Effects of a combined endurance/strength training program on circulating irisin levels in overweight/obese men and women with type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. Nov 11.doi: 10.1055/a-1284-5428. Online ahead of print(2020).
5. Kaneda H, Nakajima T, Haruyama A, et al. Association of serum concentrations of irisin and the adipokines adiponectin and leptin with epicardial fat in cardiovascular surgery patients. *PLoS One*.13(8):e0201499(2018).
6. Dianatinasab A, Koroni R, Bahramian M, et al. The effects of aerobic, resistance, and combined exercises on the plasma irisin levels, HOMA-IR, and lipid profiles in women with metabolic syndrome: A randomized controlled trial. *J Exerc Sci Fit*. 18(3):168-176(2020).
7. Harding HP, Ron D. Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes*. 51(suppl 3):S455-S461(2002).
8. López M, Tovar S, Vazquez MJ, Williams LM, Diéguez C. Peripheral tissue–brain interactions in

the regulation of food intake. *Proc Nutr Soc*. 66(1):131-155(2007).
9. Zhang T, Zhang H, Li Y, et al. Long-term impact of temporal sequence from childhood obesity to hyperinsulinemia on adult metabolic syndrome and diabetes: the Bogalusa heart study. *Sci Rep*. 7(1):1-7(2017).
10. Zhang M, Chen P, Chen S, et al. The association of new inflammatory markers with type 2 diabetes mellitus and macrovascular complications: a preliminary study. *Eur Rev Med Pharmacol Sci*. 18(11):1567-1572(2014).
11. Liu J-J, Wong MDS, Toy WC, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. *J Diabetes Complications*. 27(4):365-369(2013).
12. Kurdiova T, Balaz M, Vician M, 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. *J Physiol*. 592(5):1091-1107(2014).
13. Sanchis-Gomar F, Alis R, Pareja-Galeano H, et al. Circulating irisin levels are not correlated with BMI, age, and other biological parameters in obese and diabetic patients. *Endocrine*. 46(3):674-677(2014).
14. Suzuki K, Jayasena CN, Bloom SR. Obesity and appetite control. *Exp Diabetes Res*. 824305 (2012).
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 28(7):412-419(1985).
16. Hiernaux J, Tanner JM, Jarman S. Growth and physical studies. *Hum Biol A Guid to F methods London IBP*. (1969).
17. Hirschler V, Oestreicher K, Maccallini G, Aranda C. Relationship between obesity and metabolic syndrome among Argentinean elementary school children. *Clin Biochem*. 43(4):435-441(2010).
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 18(6):499-502(1972).
19. Chu C-H, Lee J-K, Keng H-M, et al. Hyperthyroidism is associated with higher plasma endothelin-1 concentrations. *Exp Biol Med*. 2006;231(6):1040-1043.
20. Leustean L, Preda C, Teodoriu L, Mihalache L, Arhire L, Ungureanu M-C. Role of Irisin in Endocrine and Metabolic Disorders—Possible New Therapeutic Agent? *Appl Sci*. 11(12):5579(2021).
21. Huh JY, Mougios V, Skraparlis A, Kabasakalis A, Mantzoros CS. Irisin in response to acute and chronic whole-body vibration exercise in humans. *Metabolism*. 63(7):918-921(2014).
22. King-Morris KR, Deger SM, Hung AM, et al. Measurement and correlation of indices of insulin resistance in patients on peritoneal dialysis. *Perit Dial Int*. 36(4):433-441(2016).
23. Sperling M, Grzelak T, Pelczyńska M, et al. Concentrations of omentin and vaspin versus insulin resistance in obese individuals. *Biomed Pharmacother*. 83:542-547(2016).
24. Huh JY, Panagiotou G, Mougios V, 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*. 61(12):1725-1738(2012).
25. Crujeiras AB, Zulet MA, Lopez-Legarrea P, et al. Association between circulating irisin levels and the promotion of insulin resistance during the weight maintenance period after a dietary weight-lowering program in obese patients. *Metabolism*. 63(4):520-531(2014).
26. Crujeiras AB, Pardo M, Casanueva FF. Irisin:'fat'or artefact. *Clin Endocrinol (Oxf)*. 82(4):467-474(2015).
27. Lopez-Legarrea P, De La Iglesia R, Crujeiras AB, et al. Higher baseline irisin concentrations are associated with greater reductions in glycemia and insulinemia after weight loss in obese subjects. *Nutr Diabetes*. 4(2):e110-e110(2014).
28. Gamas L, Matafome P, Seiça R. Irisin and myonectin regulation in the insulin resistant muscle: implications to adipose tissue: muscle crosstalk. *J Diabetes Res*. Article ID 359159 | https://doi.org/10.1155/2015/359159(2015).
29. Timmons JA, Baar K, Davidsen PK, Atherton PJ. Is irisin a human exercise gene? *Nature*. 488(7413):E9-E10(2012).
30. Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity–correlation with body mass index. *Peptides*. 39:125-130(2013).