International Journal of Advanced Research in Medicine

E-ISSN: 2706-9575
P-ISSN: 2706-9567
IJARM 2023; 5(4): 16-21
Received: 11-06-2023
Accepted: 16-07-2023

Mahrous Mohammed Elmetwaly
Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt

Sahbaa Mahmoud AboELnasr
Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt

Ghada Abdelmoemen Soliman
Department of Clinical Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt

Ingy Abd Elwahab Ibrahim
Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt

Corresponding Author:
Mahrous Mohammed Elmetwaly
Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt

The relation between serum irisin levels with the anthropometric and metabolic parameters in obese and non-obese patients with type 2 diabetes mellitus

Mahrous Mohammed Elmetwaly, Sahbaa Mahmoud AboELnasr, Ghada Abdelmoemen Soliman and Ingy Abd Elwahab Ibrahim

DOI: https://doi.org/10.22271/27069567.2023.v5.i4a.518

Abstract
Background: The browning of white adipose tissue caused by irisin, which has a role in thermogenesis. The purpose of this research was to assess the irisin in individuals with type 2 diabetes mellitus (T2DM), comparing obese and non-obese patients, to evaluate its correlation with metabolic and anthropometric parameters.
Methods: This survey work setting was done on 200 patients with clinical criteria of type two diabetes mellitus. Patients were categorized in to 2 groups according to body mass index (BMI): group I: 94 patients with obese type 2 DM and Group II: 106 patients with non-obese type 2 DM. Detection and quantification of Irisin in human and mouse plasma, serum, urine, cell, and tissue lysates were performed using Irisn Elisa Kits.
Results: A significant positive correlation was between irisin levels and anthropometric measures. Conversely, a significant negative correlation was found between irisin levels and indicators of fat-free mass. There was an insignificant correlation between irisin and parameters as (Height, systolic BP, diastolic BP, total cholesterol, triglycerides, HDL and LDL).
Conclusion: The elevation in irisin levels in individuals with obesity may suggest a physiological role in enhancing glucose tolerance, a common impairment observed in obese individuals. However, this compensatory irisin secretion seems to transition towards a state of inadequate secretion as diabetes develops. Irisin has the ability to have beneficial effects on glucose and insulin levels.

Keywords: Serum irisin levels, anthropometric, obese and non-obese patients, type 2 DM

Introduction
Obesity is identified by an aggregation of adipose tissue, leading to various health complications such as metabolic syndrome, chronic illnesses, cardiovascular diseases, and type 2 diabetes mellitus (T2DM). This condition is linked with the development of a state of low-grade inflammation [1]. In the last thirty years, obesity has emerged as a global pandemic, posing a significant risk to human well-being [2].
The association between obesity and T2DM has been postulated to be mediated through insulin resistance [3].
The pathogenesis of subsequent improvement and insulin resistance of T2DM include changes of phenotypic and molecular in the liver, adipose tissue and skeletal muscle. It is worth mentioning that skeletal muscle plays a significant part in facilitating over 75% of the overall glucose absorption triggered by insulin in the body. This highlights the importance of tissue abnormalities in the regulation of glucose homeostasis among persons diagnosed with T2DM [4].
The endocrine functions of skeletal muscle have been recently shown via the secretion of bioactive metabolites referred to as myokines [5] in response to a variety of factors, including the contraction of muscles during activity and/or changes in diets [6].
Irisin has been characterized as a myokine that is stimulated by exercise, with a peptide structure consisting of 112 amino acids [7]. One of the primary physiological roles of irisin is its potential involvement in the control of thermogenesis [8], it causes browning of white adipose tissue [9]. Numerous global investigations have begun investigating the association between irisin and diabetes mellitus [10].

As a consequence, there is a correlation between enhanced glucose homeostasis and a decrease in insulin resistance, prompting several research to investigate the involvement of irisin in glucose control. However, conflicting findings have emerged, casting doubt on the existence of this hormone [11].
The purpose of this work was evaluation of circulating serum irisin levels in obese and non-obese patients with T2DM to elucidate its corelation with metabolic and anthropometric parameters.

Patients and Methods

This survey work setting was done on 200 patients, both genders, with clinical criteria of T2DM obese and non-obese patients.
The research was conducted between February 2020 and April 2022, with the consent of the Ethical Committee of Tanta University Hospitals in Tanta, Egypt. Prior to the commencement of the study, a comprehensive and well-informed written permission was acquired from either the patient or their respective families.
Exclusion criteria were macro-and microvascular complications of diabetes mellitus, thyroid dysfunction, alcoholic patients and pregnant female.
Patients were categorized in to 2 groups according to BMI, group I: 94 patients with obese type 2 DM and Group II: 106 patients with non - obese type 2 DM.
Patients underwent a comprehensive medical evaluation, which included various assessments and tests. These assessments encompassed a medical examination, where measurements such as waist size in centimetres were taken. Additionally, a detailed medical history was obtained. Other measurements included body mass index (BMI), muscle mass, and fat-free mass (FFM). The FFM Index was also calculated. Furthermore, blood pressure was measured, neuropathy was assessed, and a fundus examination was conducted using an ophthalmoscope. Electrocardiography (ECG) and echocardiography were performed to evaluate cardiac function. Laboratory assessments were conducted, which involved analysing various parameters. These parameters included a complete blood count (CBC), C-reactive protein (CRP) levels, kidney function tests (serum urea and creatinine), albumin creatinine ratio in urine, estimated glomerular filtration rate (GFR), liver function tests (SGPT, SGOT), a complete lipid profile (serum cholesterol, LDL, HDL), and serum triglyceride levels. Additionally, HbA1C, HOMA-IR, and serum irisin levels were measured using enzyme-linked immunosorbent assay (ELISA).
The BMI is calculated by dividing an individual's weight in kilograms by the square of their height in meters. Persons may be classified into three groups depending on their BMI. These categories include lean persons, who have a BMI ranging from 18.5 to 24.9 kg/m2, overweight adults, who have a BMI ranging from 25 to 29.9 kg/m2, and obese individuals, who have a BMI of 30 kg/m2 or more.
The assessment of FFM and muscle mass may be conducted by the use of Bioelectrical Impedance Analysis (BIA). The measurement of body composition was conducted using BIA using the Gatherum body fitness B-5010 equipment from Germany. This device recorded many parameters including body weight (BW), water content, percentage of FM and FFM. The term FFM encompasses all components of the body except for fat, such as water, organs, bone, and muscle mass.
The eGFR is calculated by the use of the Modification of Diet in Renal Disease (MDRD) formula. The serum creatinine, which is quantified in milligrams per deciliter (mg/dL). The estimated eGFR is quantified as 186 mL/min/1.73 m^2. The equation used for age estimation incorporates a constant value of -1.154, along with supplementary coefficients of -0.203 for females and 0.742 for males. The numerical value of 1.21 corresponds to those who self-identify as black.

Irisn Elisa Kits

Irisin detection and quantification enzyme immunoassay designed for human or mouse plasma, serum, urine, cell or tissue lysate samples. The detection sensitivity limit of the kit is 6.25 ng/mL Irisin. Each kit contains enough chemicals to run up to 96 experiments, including standard curve and unknown samples.

Statistical analysis

The data was subjected to analysis using the Statistical Program for Social Science version 20 (SPSS Inc., Chicago, IL, USA). Quantitative variables were described using statistical measures such as the mean and standard deviation. Quantitative measures, including numerical values and proportions, were used to depict qualitative characteristics. The student t-test was used to evaluate the statistical significance of differences in parametric quantitative variables between two groups. When the frequency of occurrences fell below five, the comparison of qualitative variables was conducted using either the chi-square (X2) test or Fisher's exact test. The Pearson correlation coefficients were computed in order to assess the relationship between two variables that have a normal distribution. A variable is considered to have statistical significance when its associated p-value is less than or equal to 0.05, indicating that the observed results are unlikely to have occurred by chance. This criterion is particularly pertinent in scenarios when the variable does not conform to a normal distribution.

Results

Patient characters showed no significant variation among analysis, while anthropometric measurements were significantly higher in group I. While FFM (kg) was significantly lower in group I. Table 1

Table 1: Baseline features and anthropometric measurements in both groups

Demographic data		Obese type 2 DM (n = 94)	Non-obese type 2 DM (n = 106)	P value
Gender	Male	43 (45.7%)	39 (36.8%)	0.199
	Female	51 (54.3%)	67 (63.2%)	
Age (years)		50.50 (37.0-60.0)	49.50 (38.0-63.0)	0.699
< 35		20 (21.3%)	17 (16.0%)	0.442
35 – 45		12 (12.8%)	22 (20.8%)	
45 – 55		23 (24.5%)	24 (22.6%)	

≥ 55	39 (41.5%)	43 (40.6%)	
Smoking	15 (16.0%)	12 (11.3%)	0.338
Anthropometric measurements			
Weight (Kg)	105.4±13.62	68.2±5.9	<0.001*
Height (m)	1.70 (1.65-1.75)	1.72(1.69-1.76)	0.307
BMI (Kg/m^2)	36.24 (32.66-39.24)	22.96 (22.01-23.72)	<0.001*
Waist circumference (cm)	99.2±13.66	69.4±7.08	<0.001*
Hip circumference (cm)	131.6±3.83	102.4±1.31	<0.001*
Waist–to–hip ratio	0.75 (0.66-0.85)	0.70 (0.61-0.74)	<0.001*
Fat mass (kg)	35.7±12.66	10.5±4.6	<0.001*
Fat mass (%)	40.4±7.81	20.4±9.21	<0.001*
Fat free mass (kg)	67.4±16.33	59.8±6.81	<0.001*
Fat–free mass (%)	64.5±15.92	85.9±10.91	<0.001*
Fat–free mass index (kg/m^2)	21.1±4.04	17.98±3.05	<0.001*

Data are demonstrated by number (%) or average ± SD. BMI: body mass index. DM: diabetes mellitus. Fat mass percent normal range: 20-30% for women, and 10-20% for men, fat-free mass normal range is 13.4 to 21.7 and 24.6 to 33.2 for men and women, fat-free mass index normal range is 1.8 to 5.2 kg/m2 for men and 3.9 to 8.2 kg/m^2 for women. SBP was significantly higher in obese group while duration of diabetes, type of drug and DBP was insignificantly different between both groups. Table 2

Table 2: Duration of diabetes, type of drug, systolic and diastolic BP in the studied groups

	Obese type 2 DM (n = 94)	**Non-obese type 2 DM (n = 106)**	**P value**
Duration of diabetes	3 (2-4)	4 (3-3)	0.412
Type of drug			
Oral hypoglycemic	67 (71%)	81 (76%)	0.975
Metformin	21 (22.3%)	34 (32%)	
SGLT2	17 (18%)	10 (9%)	
DDPL	14 (14.8%)	18 (16.9%)	
Sulfonyl	15 (15.9%)	19 (17.9%)	
Insulin	27 (28.7%)	25 (23.5%)	
Systolic BP (mm Hg)	148.40±23.66	129.34±12.79	<0.001*
Diastolic BP (mm Hg)	78.36±12.23	76.14±11.07	0.803

Data are demonstrated by Mean ± SD or median IQR, BP: blood pressure, SD: Standard deviation, DM: diabetes mellitus.

The obese group had substantially higher levels of lipid profile, fasting blood glucose, fasting insulin, HbA1c, HOMA-IR, and serum irisin. The HDL levels were significantly reduced in obese group. There was no significant difference observed in the renal function tests, namely the albumin-creatinine ratio, between 2 groups. Table 3.

Table 3: Laboratory investigation in the studied groups

	Obese type 2 DM (n = 94)	**Non-obese type 2 DM (n = 106)**	**P value**
Fasting blood glucose (mg/dL)	165.2±12.18	131.5±10.76	<0.001*
HbA1c (%)	9.4±1.79	7.8±1.22	<0.001*
Fasting insulin (IU/mL)	24.3±8.11	14.8±8.84	<0.001*
HOMA–IR	9.2 (5.82-12.17)	3.7 (2.4-7.5)	<0.001*
Albumin\Creatinine ratio	4.085 (3.52 - 4.62)	4.05 (3.67- 4.66)	0.431
Serum irisin (ng/mL)	11 (9.15-12.47)	4.70 (4.14-5.84)	<0.001*
Lipid profile			
Total cholesterol (mg/dL)	220.41±13.92	182.96±11.93	<0.001*
Triglycerides (mg/dL)	180.97±14.88	170.40±10.67	<0.001*
HDL cholesterol (mg/dL)	46.99±5.57	50.12±5.92	<0.001*
LDL cholesterol (mg/dL)	124.25±17.96	96.20±14.93	<0.001*
Renal function			
Blood urea (mg/dL)	35.16±8.49	36.23±8.02	0.362
Serum creatinine (mg/dL)	0.97 (0.89 – 1.08)	0.96 (0.88 – 1.07)	0.469
EGFR (ml/min/1.73 m^2)	78.75±12.15	82.03±12.42	0.061

Data are demonstrated by Average ± SD or median IQR, HDL: high density lipoprotein. LDL: low density lipoprotein. EGFR: estimated glomerular filtration rate.

In the obese group, there was a substantial positive association between serum irisin and a variety of indicators, and there was a significant negative correlation between serum irisin and laboratory examination. Both of these correlations were statistically significant. There was a link between serum irisin and a variety of measures, although it was not significant. Table 4.

Table 4: Association between Serum irisin and different parameters in obese group (n = 94)

	Serum irisin	
	r	p
Weight (Kg)	0.25	0.015*
Height (cm)	0.042	0.684
BMI (Kg/m^2)	0.27	0.009*
Waist circumference (cm)	0.321	0.002*
Hip circumference (cm)	0.379	<0.001*
Waist–to–hip ratio	0.250	0.015*
Fat mass (kg)	0.291	0.004*
Fat mass (%)	0.279	0.007*
Fat free mass (kg)	-0.218	0.035*
FFM (%)	-0.339	< 0.001*
Fat–free mass index (kg/m^2)	-0.263	0.011*
SBP (mm Hg)	0.032	0.761
DBP (mm Hg)	-0.10	0.823
Total cholesterol (mg/dL)	0.008	0.937
Triglycerides (mg/dL)	-0.036	0.733
HDL cholesterol (mg/dL)	-0.097	0.352
LDL cholesterol (mg/dL)	0.021	0.837
Fasting blood glucose (mg/dL)	-0.308	0.003*
Fasting insulin (IU/mL)	-0.311	0.002*
HbA1c (%)	-0.336	< 0.001*
HOMA–IR	-0.242	0.019*
Blood urea (mg/dL)	-0.002	0.982
Serum creatinine (mg/dL)	-0.145	0.164
EGFR (ml/min/1.73 m^2)	0.116	0.267

r: Pearson coefficient, HDL: high density lipoprotein. LDL: low density lipoprotein. EGFR: estimated glomerular filtration rate. BMI: body mass index.

In the non-obese cohort, a significant positive association was observed between serum irisin levels and various parameters, as well as a significant negative correlation between numerous parameters and serum irisin levels. A negligible connection was observed between several parameters and serum irisin levels. Table 5.

Table 5: Correlation between Serum irisin and different parameters in non–obese group (n = 106)

	Serum irisin	
	r	p
Weight (Kg)	0.252	0.009*
Height (cm)	0.042	0.666
BMI (Kg/m^2)	0.226	0.020*
Waist circumference (cm)	0.283	0.003*
Hip circumference (cm)	0.447	<0.001*
Waist–to–hip ratio	0.227	0.019*
Fat mass (kg)	0.382	0.001*
Fat mass (%)	0.229	0.018*
Fat free mass (kg)	-0.227	0.019*
FFM (%)	-0.222	0.022*
Fat–free mass index (kg/m^2)	-0.227	0.019*
SBP (mm Hg)	0.175	0.073
DBP (mm Hg)	0.186	0.057
Total cholesterol (mg/dL)	0.153	0.118
Triglycerides (mg/dL)	0.061	0.532
HDL cholesterol (mg/dL)	-0.059	0.549
LDL cholesterol (mg/dL)	0.006	0.952
Fasting blood glucose (mg/dL)	-0.240	0.013*
Fasting insulin (IU/mL)	-0.201	0.039*
HbA1c (%)	-0.203	0.037*
HOMA–IR	-0.310	0.001*
Blood urea (mg/dL)	0.024	0.808
Serum creatinine (mg/dL)	0.371	0.088
EGFR (ml/min/1.73 m^2)	-0.031	0.754

r: Pearson coefficient. HDL: high density lipoprotein. LDL: low density lipoprotein. EGFR: estimated glomerular filtration rate. BMI: body mass index.

Discussion

The prevalence of obesity has shown a significant increase on a global scale [12]. Simultaneously, the incidence of comorbid disorders associated with obesity has increased, such as metabolic syndrome, cardiovascular disease (CVD), DM, insulin resistance, hypertension, chronic kidney disease, heart failure, cancer, and dementia [13].

In our work, there was a positive significant association between different serum irisin and anthropometric parameters.

Similar to our findings, Binay *et al.* [14] established the connection between anthropometric measurements and metabolic markers in obese children, including irisin and oxytocin levels. In this research, 30 healthy controls and 90 obese children were included. They found that children who were obese had greater levels of irisin than the controls (p=0.018).

In their study, Shoukry *et al.* [15] study reported that the serum irisin of obese nondiabetic controls were considerably greater than those of lean nondiabetic controls. They assessed the levels irisin in people with obesity and T2D, and they clarified any potential connections between these conditions' serum irisin levels and metabolic and anthropometric parameters. Our findings are in agreement with Shoukry *et al.* [15] they found a favorable association between serum irisin and BMI (r = 0.985, P 0.001), W/H ratio (r = 0.880, P 0.001), and irisin levels in those with type 2 diabetes (r = 0.218, P = 0.007).

Our results are confirmed by Gumpina *et al.* [16] studied the correlation of serum irisin with clinical, biochemical and anthropometric parameters in patients with T2DM. They demonstrated that irisin levels strongly correlated with weight, BMI, waist circumference and W/H ratio.

In agreement with our results, Alzoughool *et al.* [17]. The aim of this research is to evaluate the levels of serum irisin in persons diagnosed with T2DMand explore any possible

correlations between serum irisin, anthropometric obesity indices, and biochemical markers. The researchers performed a prospective observational study with a cohort of 90 individuals who had been diagnosed with T2DM. The results suggest that there exists a significant positive relationship between serum irisin levels and weight (r = 0.338, $p<0.05$), BMI (r = 0.332, $p<0.05$), WC (r = 0.409, $p<0.01$), and (r = 0.432, $p<0.01$). The study results indicate that irisin is synthesized by both adipose tissue and muscle tissue, with subcutaneous adipose tissue exhibiting a greater synthesis of irisin compared to visceral adipose tissue. [18].

Our findings are consistent with Zhang *et al.* [19]. The scientists reported a positive relationship between plasma irisin levels and both BMI and insulin resistance.

Elevated irisin concentrations were also correlated with heightened BMI, W\H ration, body fat percentage, and overall fat mass [14].

The current investigation revealed a statistically significant positive correlation between blood irisin levels and both fat mass and fat mass percentage. Moreover, a significant inverse association was seen between serum irisin levels and certain other metrics.

Our results are in agreement with Shoukry *et al.* [15] Previous study has shown a positive correlation between blood irisin concentrations and adipose tissue mass in individuals without diabetes as well as those diagnosed with T2DM).The correlation coefficient between the first variable and the second variable is r = 0.959, which indicates a strong positive relationship. The significance threshold for this correlation is P < 0.001, suggesting that the observed connection is statistically significant. The correlation coefficient for the second variable is r = 0.202, and it is statistically significant at a significance level of P = 0.013

However, in contrast to our results Gumpina *et al.* [16] reported that serum irisin strongly linked with triglycerides.

Our findings disagree with, Ebert *et al.* [20] who reported that irisin, but not other adipokines, was inversely correlated with dyslipidemia and fat mass.

The findings of our investigation revealed a significant inverse relationship between several metabolic markers and serum irisin levels, including fasting blood glucose, fasting insulin, HbA1c (%), and HOMA–IR.

Serum irisin levels strongly correlated with fasting plasma glucose & insulin and HOMA IR in Gumpina *et al.* [16] and that agreed with our findings.

Our findings are consistent with Zhang *et al.* [19] they reported that both diabetes patients with obesity and those with normal weight had a negative connection between HbA1c, HOMA-IR, and plasma irisin

In the same line with our findings, Ebert *et al*. The current study investigated the correlation between metabolic indices and the rs726344 variant in FNDC5 gene with serum irisin concentrations. The study aimed to examine the fluctuations in serum irisin levels during an OGTT within a specific subgroup of the population (n = 136). A negative connection was observed between levels of irisin and concentrations of fasting glucose.

Also, Choi *et al.* [21]. A cross-sectional research was undertaken to assess the correlation between blood irisin levels and a range of metabolic indicators. It was observed that elevated levels of irisin were correlated with a decreased frequency of newly diagnosed T2D Furthermore, the findings from the multiple regression analysis revealed a significant association between serum irisin levels and 2-hour plasma glucose, with the latter being included as the independent variable (p= 0.004). The findings of this work indicate that irisin may have a significant impact on the development of T2D and glucose intolerance.

In contrast to our results, Binay *et al.* [14]. The study indicated a correlation between increased levels of irisin and elevated levels of glucose, insulin, and HOMA-IR. Different sample size and age of participants may be appropriate explanation for this difference.

In our research, the obese group had substantially higher levels of fasting blood glucose, fasting insulin, HbA1c, HOMA–IR, and serum irisin.

Our results are in harmony with Zhang *et al.* [19]. The research findings indicated that individuals with obesity and diabetes had substantially elevated levels of fasting blood glucose, fasting insulin, HbA1c, HOMA–IR, and serum irisin, as stated by the researchers.

However, Shoukry *et al.* [15]. Both serum irisin and fasting insulin levels were shown to be positively correlated with one another (r = 0.989, P 0.001 and r = 0.207, P = 0.011, respectively). Conversely, it might be postulated that in patients with obesity, a sustained elevation in irisin levels may contribute to both increased insulin secretion and reduced insulin sensitivity over an extended period of time. The precise mechanism responsible for the elevation of irisin levels in individuals with obesity has yet to be fully elucidated

Limitations: single-centre research, where the number of participants was limited, the sample size was average, and the individuals' levels of physical activity were not determined.

Conclusions

The elevation in irisin levels in individuals with obesity may suggest a physiological role in enhancing glucose tolerance, a common impairment observed in obese individuals. However, this compensatory irisin secretion seems to transition towards a state of inadequate secretion as diabetes develops. Irisin has the ability to have beneficial effects on glucose levels and insulin.

Financial support and sponsorship: Nil

Conflict of Interest: Nil

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How to Cite This Article
Elmetwaly MM, AboELnasr SM, Soliman GA, Ibrahim IAE. The relation between serum irisin levels with the anthropometric and metabolic parameters in obese and non-obese patients with type 2 diabetes mellitus. International Journal of Advanced Research in Medicine. 2023;5(4):16-21.