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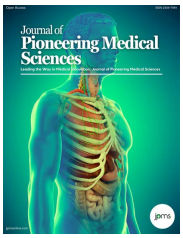

Serum Irisin Levels and Cardiovascular Risk in Obese Patients before and after Bariatric Surgery

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Abstract **Objectives:** Irisin has emerged as a potential cardiovascular biomarker with protective effects. This study aimed to evaluate serum irisin levels and FNDC5 gene expression in obese individuals undergoing bariatric surgery and to assess irisin's role as a cardiovascular biomarker in obesity and post-surgery. **Method:** Forty severely obese participants and 40 healthy, normal-weight controls were evaluated at baseline, 6 months and 12 months after surgery. Serum irisin was measured using ELISA and FNDC5 gene expression was assessed via Real-Time PCR. Various biochemical, anthropometric and clinical parameters were also measured. **Results:** Serum irisin levels decreased significantly from 10.45±2.49 ng/mL at baseline to 7.66±1.49 ng/mL at 12 months post-surgery ($p = 0.000$). FNDC5 gene expression also significantly declined ($p = 0.000$). Irisin levels were positively correlated with BMI and FNDC5 expression and inversely correlated with cholesterol, LDL, triglycerides, CRP, MDA and hs-Tn. Higher irisin levels were associated with lower cholesterol, LDL, CRP and MDA levels. At 12 months, irisin was negatively correlated with cholesterol, ApoB/ApoA ratio and hs-Tn. **Conclusion:** Irisin may serve as a cardiovascular biomarker in obese individuals both before and after bariatric surgery. Elevated irisin levels are associated with obesity but decrease following surgery, correlating with inflammatory markers and cardiovascular risk factors. These results suggest that higher irisin levels could be linked to a lower cardiovascular risk in obese patients.

Key Words Irisin, FNDC5, Real-Time PCR, BMI

INTRODUCTION

Obesity is an important global health issue, increasing the susceptibility to cardiovascular diseases (CVD), various cancers, type 2 diabetes mellitus (T2DM) and other related diseases [1]. This trend is exacerbated by unhealthy diets, sedentary habits and socioeconomic changes in the Eastern Mediterranean region [2]. In the Middle East, about 8% of cardiovascular diseases are directly attributable to obesity, with 4% of coronary heart disease, 11% of heart failure and 9% of atrial fibrillation is linked to obesity [3].

Individuals with obesity have an increased susceptibility to cardiovascular morbidity and mortality. Increased body fat, especially in the visceral or abdominal regions, is associated with cardiovascular risk and disease through diverse physiological mechanisms, including metabolic, endocrine function, hormone regulation, immune response, tissue mechanisms, vascular dynamics and functional capacity [4].

Despite knowledge of obesity-related cardiovascular risk factors, there are gaps in understanding effective biomarkers for early detection and intervention. Current research emphasizes the need for new potential biomarkers that could predict future cardiovascular events in obese individuals, allowing for accurate risk assessment and targeted prevention strategies.

One promising biomarker is irisin, a myokine discovered in 2012, originating in skeletal muscle tissue [5]. Irisin regulation is closely linked to physical activity, facilitated by the peroxisome proliferator-activated gamma co-activator 1-alpha (PGC-1α) receptor and its downstream effector, fibronectin type III domain-containing protein-5 (FNDC5).

FNDC5 cleavage releases irisin into circulation, influencing adipocyte physiology and promoting a 'browning' process that enhances thermogenic capacity, potentially mitigates cardiovascular risk [6].

Since its initial identification as the precursor to irisin, FNDC5 has garnered significant interest due to its identical structure in both mice and humans. This protein is prevalent across various body tissues, particularly in those requiring high energy. Moreover, FNDC5 has demonstrated protective roles against disturbances in cardiovascular and metabolic functions [7].

Indeed, research by Liu and colleagues showed that a lack of FNDC5 worsened conditions like obesity-associated high blood fat levels, lipid accumulation in the liver and disruptions in fatty acid oxidation and cellular autophagy processes. Conversely, increasing FNDC5 levels appeared to mitigate these issues [8].

Furthermore, FNDC5 is abundantly present in heart muscle tissue, where enhancing its expression or the infusion of irisin significantly reduced oxidative stress, apoptosis of cardiomyocyte and heart dysfunction induced by doxorubicin treatment [9]. These findings highlight the therapeutic potential of FNDC5 in cardio protection.

Additionally, early life Introduction of the FNDC5 gene has been suggested to delay the onset of cardiac dysfunction associated with aging. FNDC5 contributes to improving age-related heart problems by activating AMPKα, suggesting its potential as a therapeutic target to maintain cardiovascular health in older population [10] .

However, the exact mechanisms behind its effects remain unclear and limited research has investigated irisin's dynamics in obese individuals undergoing bariatric surgery-a population experiencing significant metabolic and cardiovascular changes post-operatively.

This study aims to elucidate serum irisin dynamics and its gene expression patterns in obese individuals before and after weight loss surgery. By correlating these dynamics with established cardiovascular disease markers, the study seeks to enhance understanding of irisin's potential as a cardiovascular biomarker in the context of obesity and bariatric surgery interventions.

METHODS

Study Design

This study enrolled 40 participants with severe obesity (SO) scheduled for bariatric surgery between 2022 and 2023 at Zagazig University Hospitals, with follow-up assessments at six and twelve months post-surgery. 40 healthy, normal weight individuals (BMI $\leq$25 kg/m²) were recruited as control group.

Ethics

The study obtained endorsement from the Zagazig University Ethics Committee (approval ZU-IRB 91/24-Jan-24). Prior to inclusion in the study, all participants provided informed consent.

Inclusion Criteria of the Obesity Group

Participants qualified for obesity surgery if their body mass index (BMI) met or exceeded 40 kg/m², or if it was 35 kg/m² or higher alongside at least one associated condition such as hypertension, dyslipidaemia, T2DM, or syndrome of obstructive sleep apnea. Participants had an average age of 50.59 years (SD±1.69) and stable weight (fluctuations <±2 kg) for at least three months prior to the operation. All had a documented history of SO for a minimum of five years and were not on medications for obesity or inflammation during the study.

Exclusion Criteria

Individuals with prior neoplastic, hepatic, or renal conditions, ongoing systemic diseases, or diagnosed with inflammatory/infectious diseases were excluded.

Pre-surgery assessments encompassed evaluations of medical history, physical condition, nutritional status and metabolic, cardiopulmonary and psychological assessments. Most patients (n = 35) underwent Roux-en-Y gastric bypass (RYGB) surgery, while five had gastric banding. Patients were evaluated at baseline, six months and twelve months post-surgery for various clinical and biological parameters.

Ethics

The study obtained endorsement from the Ethics Committee. Prior to inclusion in the study, all participants provided informed consent.

Sampling

Blood samples were collected after an eight-hour fast at baseline, six and twelve months post-surgery using vacuum tubes containing EDTA, sodium citrate and a separator for serum. Samples were centrifuged (2500 g, ten minutes) and serum/plasma was aliquoted and stored (-80°C) for analysis. Peripheral blood mononuclear cells (PBMC) were extracted using mononuclear cell preparation tubes and centrifuged (force 2000 g, thirty minutes). Isolated PBMCs were rinsed with a solution of 50% heat-inactivated fetal bovine serum and 1x phosphate-buffered saline, then preserved in fetal bovine serum containing dimethyl sulfoxide in liquid nitrogen for future use.

Laboratory Measurements

Colorimetric enzymatic techniques (Roche Diagnostics, Mannheim, Germany), assessed total cholesterol, triacylglycerides (TG), high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) levels. Blood glucose was determined using the hexokinase-glucose method, while HbA1c and high-sensitivity CRP were measured using high-resolution ion exchange liquid chromatography (Bio-Rad, Hercules, CA, USA). The homeostatic model assessment of insulin resistance (HOMA-IR) was computed.

A radioimmunoassay method (RIA) (Linco Research in St. Louis, Missouri) was used for analysis of adiponectin,

Table 1: The general demographic and biochemical data of studied groups

Parameters	Healthy control (a)	Baseline (b)	6 months post-surgery (c)	12 months post-surgery (d)	p-value
Age	49.47±2.34	50.59±1.69	50.59±1.69	50.59±1.69	-
Sex (M/F)	26/14	26/14	26/14	26/14	-
BMI (kg/m^2)	24.9±0.5	48.30±2.40	37.87±3.58	32.10±3.95	<0.05 $^{a\text{-}b,\ a\text{-}c,\ a\text{-}d,\ b\text{-}c,\ b\text{-}d}$
Glucose (mg/dL)	80.15±2.50	98.97±3.31	81.22±2.64	80.18±2.60	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
HOMA-IR	1.82±0.50	3.63±0.79	1.95±0.54	1.71±0.90	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
Cholesterol (mg/dL)	160.70±5.10	175.57±5.05	150.27±3.69	158.70±5.06	<0.05 $^{a\text{-}b,\ a\text{-}c,\ b\text{-}c,\ b\text{-}d}$
LDL (mg/dL)	94.55±4.09	104.13±2.98	91.34±4.39	94.35±4.18	<0.05 $^{a\text{-}b,\ a\text{-}c,\ b\text{-}c,\ b\text{-}d}$
HDL(mg/dL)	55.33±1.29	46.14±0.81	49.80±0.93	55.13±1.27	<0.05 $^{a\text{-}b,\ a\text{-}c,\ b\text{-}c,\ b\text{-}d}$
TG (mg/dL)	89.15±6.39	138.36±8.77	99.11±5.25	88.95±6.38	<0.05 $^{a\text{-}b,\ a\text{-}c,\ b\text{-}c,\ b\text{-}d}$
HBA1c (%)	5.47±0.08	6.51±0.25	5.49±0.22	5.37±0.08	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
Irisin (ng/mL)	7.36±1.52	10.45±2.49	9.16±1.42	7.66±1.49	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
Adiponectin (µg/mL)	9.91±2.61	8.00±2.04	9.78±2.80	9.90±2.51	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
FNDC5 expression	0.55±0.22	2.1±0.48	0.83±0.12	0.5±0.11	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
CRP (mg/L)	5.8±1.7	9.01±2.9	6.4±1.6	5.7±1.3	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
Apo B/Apo A ratio	0.52±0.15	0.72±0.20	0.61±0.15	0.52±0.15	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
MDA (µmol/L)	1.88±0.66	2.14±0.78	1.94±0.65	1.88±0.66	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$
Hs-Tn (ng/L)	4.9±1.6	10.2±1.6	5.9±1.7	4.9±1.6	<0.05 $^{a\text{-}b,\ b\text{-}c,\ b\text{-}d}$

Significant difference from baseline, $^{a\text{-}b}$: Healthy control-baseline, $^{a\text{-}c}$: Healthy control - 6 months post-surgery, $^{a\text{-}d}$: Healthy control - 12 months post-surgery, $^{b\text{-}c}$: Baseline - 6 months post-surgery, $^{b\text{-}d}$: Baseline - 12 months post-surgery, $^{c\text{-}d}$: 6 months post-surgery- 12 months post-surgery

while irisin was measured using an ELISA kit provided by Phoenix Europe GmbH in Karlsruhe, Germany. Plasma levels of apolipoprotein AI (ApoA1) and apolipoprotein B (ApoB) were determined utilizing immune-turbidimetric assays kit provided by Thermo-Electron.

Serum malondialdehyde (MDA) was assayed using a spectrophotometer commercial kit (Merck chemicals Laboratories, Ltd, Crumlin, UK).

High Sensitivity Cardiac Troponin I (hs-cTnI) Test Kit (Quantum Dots Fluorescence Immunochromatography) (Vazyme, China) was used for High Sensitivity Cardiac Troponin I assay.

RNA Extraction

Total RNA was extracted from PBMCs employing the TRIzol method (Qiagen) and converted into complementary DNA (cDNA) using "High-Capacity cDNA Reverse Transcription Kits" from Thermo-Fisher-Scientific.

Quantitative Real-Time Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed on the QuantStudio™ 7 Flex Real-Time PCR System [Applied Biosystems Inc., Thermo Fisher Scientific] using the PowerUp™ SYBR™ Green Master Mix from (Thermo Fisher Scientific). FNDC5 gene expression was analyzed with specific primers and expression levels were computed relative to a control condition using the 2^(-ΔΔCT) method, normalized against endogenous control gene GAPDH (Sino Biologicals).

Statistical Analysis

The minimal estimated sample size at 80% power and 95% confidence interval was 36 cases, calculated using OpenEpi. Results were presented as mean values±standard deviations (SD). One-way analysis of variance (ANOVA) was used to evaluate the impact of surgical intervention on measured parameters at baseline, six months and twelve months post-surgery. Post hoc analysis using the Tukey test identified variations among different groups. The Student independent sample T-test assessed differences in measured parameters between low and high irisin groups at baseline. Pearson correlation analysis identified associations between serum irisin levels, gene expression and metabolic changes post-bariatric surgery. Statistical evaluations were two-sided, with significance set at $p \leq 0.05$, using SPSS Statistics software (IBM, version 25; IBM Corp., Armonk, NY, USA).

RESULTS

The General Demographic and Biochemical Data of Studied Groups

Body Mass Index: The average body mass index (BMI) in obese patients at baseline (48.30±2.40) was significantly higher than when compared with control group (24.9±0.5) (p = 0.0001)(Table 1).

The average BMI showed a significant reduction at 6 months (37.87±3.58) and 12 months (32.10±3.95) following bariatric surgery, compared to the initial BMI of the obese group at baseline (48.30±2.40), (p = 0.0001) as presented in Table 1 and Figure 1.

Metabolic and biochemical parameters: All evaluated metabolic parameters were significantly lower in control group when compared to obese patients at baseline except for HDL, adiponectin, expression which was significantly higher in control group (Table 1).

All evaluated metabolic parameters showed significant reductions at both 6 and 12 months post-surgery, except for HDL, adiponectin, which exhibited significant increases at both 6 and 12 months following the operation (Table 1).

Irisin level and gene expression: Irisin concentration was significantly higher in individuals with obesity at baseline (10.45±2.49) than their concentration in healthy controls (7.36±1.52) (p<0.05). A significant decrease in irisin

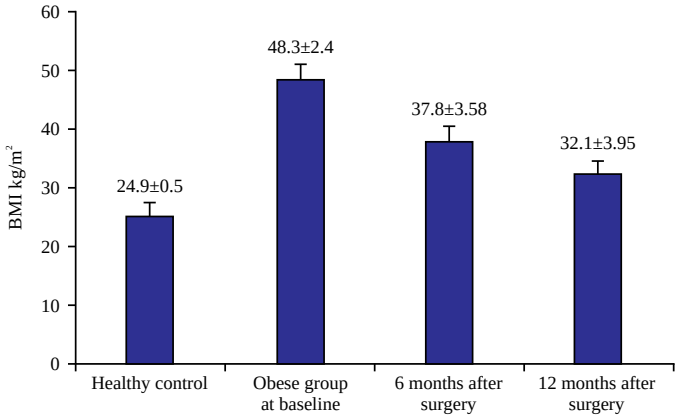

Figure 1: BMI of healthy control group, obese group at baseline and after surgery. The average body mass index (BMI) showed a significant reduction at 6 months (37.87±3.58) and 12 months (32.10±3.95) following bariatric surgery, compared to the initial BMI of the obese group at baseline (48.30±2.40), (p = 0.000 by one way ANOVA test)

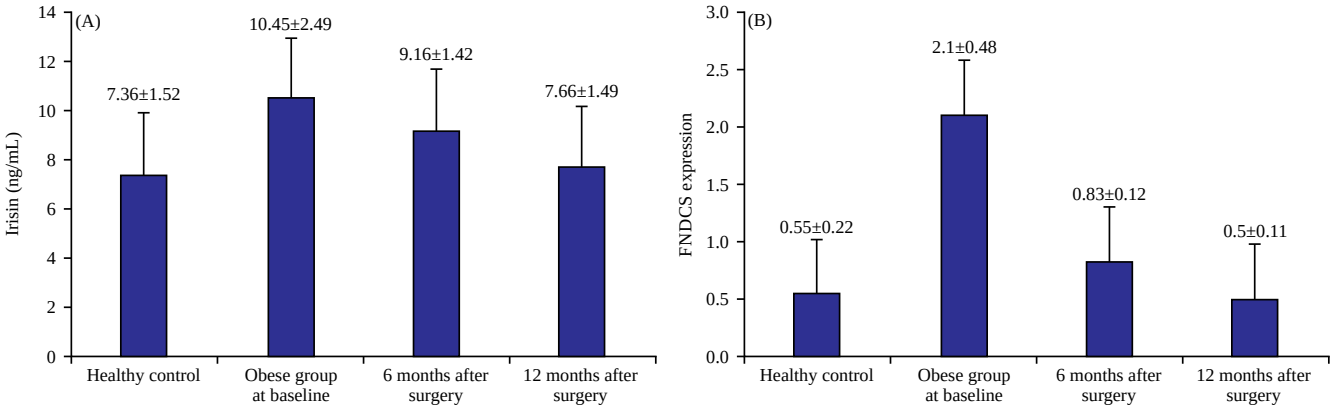

Figure 2: Serum Irisin level and FNDC5 expression in healthy control group and obese group at various study intervals before and after surgery. (A): The serum irisin level was notably elevated in obese patients at baseline (10.45±2.49) than its level in patients after 6 months (9.16±1.42ng/mL) and after 12 months (7.66±1.49) of bariatric surgery (p = 0.000 by one way ANOVA test). (B): The fold change expression of FNDC5 gene was significantly higher in obese patients at baseline (2.1±0.48) than its level in patients after 6 months (0.83±0.12) and after 12 months (0.5±0.11) of bariatric surgery (P=0.000, by one way ANOVA test).

concentrations were observed at 6 months (9.16±1.42) and 12 months (7.66±1.49) post-surgery within the group of obesity (p<0.05 for both) Table 1, Figure 2.

Gene expression analysis revealed a significantly higher fold change expression of FNDC5 gene in obese group at baseline when compared to control group (p<0.05). Within the obesity group, fold change expression of FNDC5 gene was significantly decreased at six and twelve months following the operation (p<0.05) Table 1, Figure 2.

CRP level (inflammatory parameter): The mean CRP level was significantly higher at obesity group at the baseline when compared to healthy control group (p<0.05). Within the obesity group, a significant decrease in the CRP level was observed at 6 months and 12 months following the operation (p<0.05).

Malondialdehyde (MDA) Oxidative parameter: The mean MDA level was significantly higher at obesity group at the baseline when compared to healthy control group (p<0.05). Within the obesity group, a significant decrease in the MDA level was observed at 6 months and 12 months following the operation (p<0.05).

High Sensitivity Cardiac Troponin (hs-Tn) Cardiovascular biomarker: High Sensitivity Cardiac Troponin (hs-Tn) was assayed to provide valuable insights into subclinical myocardial injury and cardiovascular risk. Obese individuals have significantly slightly elevated baseline level of hs-Tn as compared to healthy control individuals (p<0.05). Within the obesity group, a significant decrease in the hs-Tn level was observed at 6 months and 12 months following the operation (p<0.05).

Table 2: Comparison of demographic and biochemical characteristics between the low and the high irisin patient groups

Parameters	Low irisin group N = 19	High irisin group N = 21	p-value
Age	51.13±1.7	50.11±1.5	0.057
BMI	48.73±2.0	47.90±2.7	0.11
Glucose	99.3±3.68	98.67±3.0	0.89
HOMA-IR	3.69±0.87	3.76±0.58	0.75
Cholesterol	176.37±5.21	172.03±2.21	0.005*
LDL	104.04±2.8	101.15±1.4	0.000*
HDL	46.13±0.84	46.14±0.75	0.981
TG	137.37±8.7	141.24±8.6	0.233
HBA1c	6.50±0.28	6.54±0.13	0.671
irisin	9.2±1.4	13.70±1.3	0.000*
Adiponectin	8.1±1.8	7.6±2.5	0.554
FNDC5	1.9±0.75	2.3±0.32	0.034*
CRP	10.84±2.5	7.41±1.6	0.000*
ApoB/APoA ratio	0.76±0.20	0.68±0.19	0.21
MDA	2.53±0.55	1.75±0.55	0.000*
Hs -Tn	10.4±1.14	10.0±1.13	0.28

*Significant difference between the low and the high irisin group at baseline by independent sample t test

Table 3: Correlation analysis of irisin level and FNDC5 expression with studied parameters at baseline

	Serum Irisin		FNDCP expression	
Parameters	Pearson	p-value	Pearson	p-value
Age	-0.102	0.529	-0.025	0.880
BMI	0.652	0.000*	0.295	0.060
Glucose	-0.167	0.302	-0.129	0.426
HOMA-IR	0.037	0. 819	0.208	0.198
Cholesterol	-0.61	0.000**	-0.164	0.311
LDL	-0.706	0.000**	-0.419	0.007**
HDL	-0.166	0.306	0.161	0.341
TG	-0.333	0.036*	-0.261	0.104
HBA1c	-0.078	0.631	-0.123	0.451
Adiponectin	-0.185	0.253	-0.336	0.034*
FNDC5	0.358	0.023*	1	------
irisin	1	------	0.358	0.023*
CRP	-0.331	0.037	-0.110	0.501
ApoB/APoA ratio	-0.296	0.064	-0.083	0.612
MDA	−0.350	0.026*	-0.072	0.521
Hs-Tn	−0.595	0.001**	-0.171	0.558

*Significant at the p≤0.05 level, **Significant at the p≤0.01 level

Comparing Demographic and Biochemical Characteristics Between the Low and the High Serum Irisin Patient Groups

Obese participants at baseline were categorized into 2 groups based on their irisin levels: the low irisin group (6.1-10.1 ng/mL) consisting of 19 individuals and the high irisin group (10.2-16.3 ng/mL) comprising 21 individuals (Table 2).

Individuals with elevated serum irisin levels exhibited significantly lower levels of cholesterol (p = 0.005), LDL (p = 0.000), CRP (p = 0.000) and MDA (p = 0.000) in comparison to those with lower serum irisin levels, as indicated in (Table 2). No statistical variations was observed among the two groups concerning age, BMI, glucose, HOMA-IR, HDL, TG, HbA1c, adiponectin, MDA, hs-Tn and the ApoB/ApoA ratio.

Table 4: Correlation analysis of irisin level and FNDC5 expression with studied parameters at 6 months post-surgery

	6 months			
	Serum Irisin		FNDCP expression	
Parameters	Pearson	p-value	Pearson	p-value
Age	-0.144	0.375	-0.174	0.283
BMI	0.569	0.000**	0.175	0.280
Glucose	-0.379	0.016*	-0.368	0.020*
HOMA-IR	0.205	0.204	0.263	0.101
Cholesterol	-0.334	0.035*	-0.474	0.002**
LDL	-0.191	0.239	-0.095	0.559
HDL	0.223	0.166	0.192	0.236
TG	0.046	0.778	-0.406	0.009**
HBA1c	-0.023	0.886	-0.530	0.000**
Adiponectin	-0.076	0.642	-0.268	0.094
FNDC5	0.41	0.008**	1	-
irisin	1	-	0.412**	0.008**
CRP	-0.027	0.869	-0.170	0.295
ApoB/APoA ratio	-0.204	0.208	-0.021	0.899
MDA	-0.330	0.037*	-0.266	0.097
hs-Tn	-0.415	0.007**	-0.094	0.563

*Significant at the p≤0.05 level, **Significant at the p≤0.01level

Table 5: Correlation analysis of irisin level and FNDC5 expression with the studied parameters at 12 months post-surgery

	12 months			
	Serum Irisin		FNDCP expression	
Parameters	Pearson	p-value	Pearson	p-value
Age	-0.006	0.969	-0.041	0.799
BMI	0.385	0.014*	0.278	0.082
Glucose	-0.129	0.427	-0.058	0.720
HOMA-IR	0.097	0.552	0.045	0.783
Cholesterol	-0.333	0.036*	-0.019	0.908
LDL	-0.298	0.061	-0.086	0.596
HDL	0.102	0.533	0.095	0.560
TG	0.015	0.926	0.005	0.973
HBA1c	-0.036	0.826	-0.067	0.681
Adiponectin	-0.160	0.324	-0.247	0.125
FNDC5	0.425	0.006**	1	-
irisin	1	-	0.425	0.006**
CRP	-0.052	0.750	-0.009	0.954
ApoB/APoA ratio	-0.438	0.005**	0.077	0.637
MDA	-0.351	0.026*	-0.023	0.887
Hs-Tn	-0.395	0.011*	-0.088	0.589

*Significane at the p≤0.05 level, **Significane at the p≤0.01level

Correlation Analysis of Irisin Level and FNDC5 Expression with Studied Parameters at Baseline Obese Patients

Serum irisin exhibited significant positive association with significant positive association with body mass index (r = 0.652, p = 0.000) and with FNDC5 expression (r = 0.358, p = 0.023). However, they demonstrated negative associations with Cholesterol (r = -0.61, p = 0.000), LDL (r = -0.706, p = 0.000), TG (r = -0.333, p = 0.036) and CRP (r = -0.331, p = 0.037), MDA (r = −0.350, p = 0.026) and hs- Tn (r = −0.595, p = 0.001) (Table 3).

FNDCP expression were exhibited a negative association with LDL (r = -0.706, p = 0.007), adiponectin (r = -0.336,

p = 0.034) and positive association with irisin (r = 0.358, p = 0.023) as demonstrated in Table 3.

Correlation Analysis of Irisin Level and FNDC5 Expression with Studied Parameters in Obesity Group at 6 Months Post-surgery

Six months post-surgery, irisin in the serum exhibited positive association with positive association with (BMI) (r = 0.569, p = 0.000) and FNDC5 expression (r = 0.41, p = 0.008). But were exhibited negative association with glucose (r = -0.379, p = 0.016), Cholesterol (r = -0.334, p = 0.035), MDA (r = -0.330, p = 0.037) and hs- Tn (r = -0.415, p = 0.007) (Table 4).

FNDCP expression were negatively associated with glucose (r = -0.368, p = 0.020), cholesterol (r = -0.474, p = 0.002), TG (r = - 0.406, p = 0.009) and HBA1C (-0.530, p = 0.000) and were positively associated with irisin (r = 0.412, p = 0.008) (Table 4).

Correlation Analysis of Irisin Level and FNDC5 Expression with Studied Parameters in Obesity Group at Twelve Months after the Surgery

At twelve months after the operation, serum irisin exhibited positive association with body mass index (BMI) (r = 0.385, p = 0.014) and FNDC5 expression (r = 0.425, p = 0.006), but exhibited negative association with, Cholesterol (r = -0.333, p = 0.036), ApoB/APoA ratio (r = -0.438, p = 0.005), MDA (r = -0.351, p = 0.026) and hs-Tn (r = -0.395, p = 0.011) (Table 5). FNDCP expression and irisin were significantly positively associated (r = 0.425, p = 0.006) (Table 5).

DISCUSSION

Obesity, influenced by genetic and environmental factors, increases the risk of dyslipidemia, CVDs and insulin resistance. As a protein generated from the proteolysis cleavage of FNDC5, Irisin, may be linked to these disorders [11]. This study explores irisin levels and gene expression in individuals afflicted with morbid obesity pre- and post-bariatric surgery, examining correlations with CVD indicators and clinical parameters.

Initially, obese individuals had elevated serum irisin when compared with control group, which decreased at 6 and 12 months of weight loss following surgery, positively correlating with BMI. This aligns with findings from Gouda *et al.* [12], Kazeminasab *et al.* [13] and Bensmaine *et al.* [14], who reported elevated irisin in obese individuals compared to controls. Varela-Rodriguez *et al.* [15] and Perez-Sotelo *et al.* [16] suggested adipose tissue as the primary irisin source, secreting higher levels in obese individuals to counter metabolic dysregulation. Additionally, Gao *et al.* [17] found that irisin upregulates FNDC5 expression, enhancing irisin secretion in a feedback loop.

Contrarily, Moreno-Navarrete *et al.* [18] observed decreased irisin and FNDC5 expression in obese individuals, likely due to variability in participant profiles, including those with metabolic syndrome who exhibited decreased irisin concentrations due to compromised beiging of adipocytes [19-20].

Post-surgery, irisin and FNDC5 expression significantly decreased. This trend suggests a potential normalization of metabolic function as obesity-related stress diminishes. Consistent with findings by Huh *et al.* [21] who noted that both muscle expression of FNDC5 and irisin levels in the circulation exhibited a significant decrease six months following bariatric surgery. Furthermore, Carmona-Maurici *et al.* [22], noted an initial drop at 6 months post-surgery with a return to baseline at 12 months, possibly reflecting an adjustment to new energy intake and body weight.

Jamal *et al.* [23] reported a noteworthy increased FNDC5 expression at 6 months post-surgery, followed by a decline at 12 months, suggesting a role in the inflammatory response during recovery. Their findings were reinforced by the observed decrease in CRP levels following bariatric surgery, underscoring the favourable influence of weight loss surgery on inflammation.

Our results showed positive associations between serum irisin levels and FNDC5 expression, consistent with Panagiotou *et al.* [24], Palacios-Gonzalez *et al.* [25], Jang *et al.* [26] and Eslampour *et al.* [27], who linked elevated irisin to increased obesity risk and adiposity measures. Post-surgery, irisin levels correlated with BMI and FNDC5 expression, supporting its role as an adipomyokine influenced by body weight changes, specifically in relation to body fat mass.

Obesity increased cardiovascular risk, while bariatric surgery reduced atherogenic lipoproteins, lipids and cardiovascular risk [28]. In our study, after the surgical operation, LDL, triglycerides, cholesterol, apolipoproteins and CRP levels decreased, while HDL and adiponectin increased. The significant reduction in BMI and improvement in metabolic parameters post-surgery provide evidence on the efficacy of bariatric surgery in mitigating obesity-related metabolic dysfunctions.

Malondialdehyde (MDA) is a biomarker of oxidative stress that is often elevated in obese individuals, indicating a link between oxidative stress and obesity-related conditions such as insulin resistance, type 2 diabetes and cardiovascular diseases. Studies have shown a strong correlation between elevated MDA levels and body mass index (BMI), making MDA a reliable indicator of oxidative stress in obesity [29]. In our study, obese patients had significantly higher MDA levels at baseline compared to healthy controls ($p< 0.05$), but these levels decreased significantly at 6 and 12 months post-operation ($p<0.05$).

High-sensitivity troponin (hs-Tn) is a vital biomarker used for diagnosing and predicting outcomes in cardiovascular diseases, particularly myocardial infarction. Elevated hs-Tn levels can signify subclinical myocardial injury and are linked to a heightened risk of adverse cardiovascular events [30]. Our findings revealed that obese patients had mildly elevated

hs-Tn levels at baseline compared to the control group. This aligns with observations that obesity is associated with increased hs-Tn levels due to heightened myocardial stress and inflammation from excess adipose tissue [31].

Interestingly, interventions that target weight loss in obese individuals appear to have a beneficial impact on hs-Tn levels. In our study, a significant reduction in hs-Tn levels was observed at both 6 months and 12 months post-operation in the obesity group. This is consistent with research suggesting that weight loss in obese patients can lead to a marked decrease in hs-Tn levels, emphasizing the importance of managing obesity to mitigate cardiovascular risk [32].

Irisin is suggested to maintain lipid metabolism and energy expenditure by promoting adipocyte browning and lipid oxidation while inhibiting adipogenesis and cholesterol synthesis [33-36]. Also, Studies by Askin *et al.* [37] and Carmona-Maurici *et al.* [38] linked irisin to reduced endothelial dysfunction and cardiovascular disease progression.

Pre-surgery, serum irisin negatively correlated with cholesterol, LDL, TG, CRP, MDA and hs-Tn while FNDC5 expression negatively correlated with LDL and adiponectin. Bots *et al.* [39] and Woollard *et al.* [40] found inverse relationships between irisin and markers of vascular inflammation and atherosclerosis. Lu *et al.* [41] and Zhang *et al.* [42] demonstrated irisin's role in reducing atherosclerotic plaques, inflammation and apoptosis in aortic tissue. Interestingly, the rise in serum irisin levels in obese patients corresponded with a reduction in serum hs-Tn levels. Consistent with our findings, other studies have also reported an inverse relationship between irisin and hs-Tn levels in patients who have experienced a myocardial infarction [43, 44]. This suggests that increased irisin levels might have a protective effect on the heart by lowering markers of myocardial injury.

Combining the collective findings from these studies, we could speculate that decreased irisin levels may indicate a more unfavourable pathological condition during the initial stages of atherosclerosis and other cardiovascular diseases. Liu *et al.* [45] found high irisin levels linked to lower cardiovascular risk factors in overweight/obese Chinese individuals, suggesting irisin as a CVD marker.

At 12 months post-surgery, irisin negatively correlated with cholesterol, the ApoB/ApoA ratio and hs-Tn levels, supporting its use as a CVD predictor in obese individuals. Elevated irisin may indicate reduced cardiovascular complications in obesity, making it a potential biomarker for CVD susceptibility.

Also, the association of irisin with inflammatory and oxidative markers suggests that irisin may play a role in attenuating systemic inflammation and oxidative stress, thereby contributing to improved cardiovascular outcomes.

The results suppose that monitoring irisin serum levels may offer valuable insights into CVD risk among obese individuals and who have undergone bariatric surgery.

CONCLUSIONS

Our study found that irisin levels are higher in obese patients and decrease following weight loss from surgery. Irisin also showed correlations with markers of inflammation, oxidative stress and cardiovascular risk, suggesting it might serve as a molecular link between obesity and cardiovascular disease development. Nevertheless, further studies are needed to validate these findings and determine their applicability to different populations.

Limitations

- The study's observational design, limited sample size and short follow-up duration are limitations. Larger studies with longer follow-ups are needed to validate these findings. Exploring different weight loss surgery procedures' effects on irisin/FNDC5 expression may provide further insights.
- Additionally, exploring the effects of different weight loss surgery procedures on irisin/FNDC5 expression and their outcomes could provide further insights into the therapeutic potential of targeting the irisin/FNDC5 axis in obesity management.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Ethical Statement

The study obtained endorsement from the Zagazig University Ethics Committee (approval ZU-IRB 91/24-Jan-24). Prior to inclusion in the study, all participants provided informed consent.

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