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ORIGINAL ARTICLE

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Changes in the expression of meteorin-like (METRNL), irisin (FNDC5), and uncoupling proteins (UCPs) after bariatric surgery

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Abtract

Objective: Bariatric surgery is currently the most effective treatment for severe obesity. This study aims to investigate the changes in expression levels of meteorin-like protein (METRNL), irisin (FNDC5), and uncoupling proteins (UCP) 1/2/3 following bariatric surgery to understand their involvement in enhancing metabolism after surgery.

Method: A total of 40 participants were enrolled in this interventional study, 20 with obesity BMI ≥ 35 kg/m^2 and 20 with BMI ≤ 25 kg/m^2. Bariatric surgery (laparoscopic sleeve gastrectomy and Roux-en-Y gastric bypass) was performed. The levels of various molecules of interest were analyzed before and after surgery.

Results: Gene expression analysis revealed significantly higher levels of *METRNL*, *UCP1*, and *UCP3* in individuals with obesity when compared with healthy individuals before surgery ($p < 0.05$). Gene expression levels of *METRNL* and *UCP2* showed a significant increase after bariatric surgery ($p < 0.05$). METRNL plasma level was significantly higher in individuals with obesity before surgery (mean [SEM], 55,222.6 [1,421.1] pg/mL, $p = 0.0319$), as well as at 6 and 12 months (57,537.3 [1,303.9] pg/mL, $p = 0.0005$; 59,334.9 [1,214.3] pg/mL, $p < 0.0001$) after surgery.

Conclusion: The changes in the levels of various molecules of interest support their possible involvement in the inflammatory and thermogenic responses following bariatric surgery.

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INTRODUCTION

Bariatric surgery is currently one of the most effective treatment options to combat severe obesity. It is associated with an improved quality of life, a reduction in obesity-related comorbidities and mortality, and a 45% to 95% rate of remission in type II diabetes mellitus (T2D) [1]. These procedures are recommended for people with BMI of 40 kg/m^2 or more, those with BMI of 35 kg/m^2 or more with associated comorbidities, or those with a lower BMI who have metabolic syndrome [2]. The safety of bariatric surgery is widely documented in literature [3]. In terms of weight loss and diabetes improvement or remission, short-term studies have indicated that sleeve gastrectomy (SG) is as successful as Roux-en-Y gastric bypass (RYGB) [4, 5]. It has also been demonstrated that SG, like RYGB, is effective in improving T2D, independent of weight loss [4, 5]. The positive impact of bariatric surgery in resolving obesity-related complications has drawn interest toward understanding the molecular mechanisms underlying its efficacy [6–10].

In a previous study done by our group, the effect of SG surgery on various molecules involved in thermogenesis were investigated in a diet-induced obesity rat model. A dysregulation in the expression levels of two adipomyokines, namely, meteorin-like protein (METRNL) and irisin, and its impact on the level of various uncoupling proteins (UCPs) were reported [11]. METRNL is an adipomyokine also known as subfatin and cometin that is secreted by white adipose tissue and skeletal muscle [12–16]. Rao et al. showed that the mRNA level of METRNL in beige and brown adipose tissue (BAT) increased with increasing peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) level. They also reported that exercise and cold exposure stimulated METRNL expression, increased its level in circulation, and induced genes associated with thermogenesis [15]. METRNL also was shown to promote interleukin-4 (IL-4) expression, which improves anti-inflammatory cytokines [15]. Furthermore, several studies have reported that it plays a role in improving insulin sensitivity [12, 14]. Irisin is another adipomyokine, upregulated by PGC-1α overexpression. It was shown to trigger a thermogenic response in white adipose tissue by activating UCP1 [17]. An interesting aspect of this protein is that the amino acid sequence is identical among mammalian species, suggesting that its function is highly conserved [17–19]. UCP1, 2, and 3 belong to an anion-carrier protein family located in the inner mitochondrial membrane. They have structural similarities but differential tissue expression [20–23]. These proteins possess tissue-specific functions such as thermogenesis and energy expenditure (UCP1), regulation of free fatty acid metabolism (UCP2 and UCP3), reduction in reactive oxygen species (ROS) formation (UCP1-3), and regulation of insulin secretion by pancreatic beta cells, glucose metabolism, and neuronal activity (UCP2) [20–22].

Based on our previous data from the animal model and considering that bariatric surgery improves metabolic homeostasis by increasing thermogenesis, the aim of this study was to measure the level of METRNL and irisin/fibronectin type III domain-containing protein 5 (FNDC5) in healthy individuals and in individuals with obesity (before and after bariatric surgery). Furthermore, UCP1, 2, and 3 levels were investigated to understand whether bariatric surgery could impact the expression of these molecules in association with the adipomyokines of interest.

Study Importance

What is already known?

- The beneficial effects of bariatric surgery at the physiological level are known.
- Previous animal studies performed by our group and others highlighted the important role of adipomyokines, meteorin-like protein (METRNL), and irisin (FNDC5), as well as the uncoupling proteins (UCPs). These molecules gained great interest as potential targets to combat obesity, type 2 diabetes mellitus, and insulin resistance.

What does this study add?

- For the first time, the gene expression level of *UCP2* in immune cells is reported with a significant increase following bariatric surgery, supporting its possible protective role in combating obesity.
- Furthermore, in this study, the molecules such as irisin, UCP1, and UCP3 that are typically not expressed in immune cells are seen to be expressed under the disease state.

How might these results change the direction of research or the focus of clinical practice?

- The study will direct the research toward other important roles that the adipomyokines and the uncoupling proteins may play, such as alleviating oxidative stress that could eventually improve insulin sensitivity.
- Bariatric surgery may improve obesity and insulin resistance through affecting the levels of METRNL and irisin, which in turn modulate the levels of UCPs that are known to play an important role in regulating mitochondrial energy homeostasis.

METHODS

Study design

This study involved a cohort of 40 participants 18 to 65 years of age, including 20 individuals with obesity (13 male, 7 female) with BMI ≥ 35.0 kg/m^2 and 20 healthy, normal-weight individuals considered as controls (14 male, 6 female) with BMI ≤ 25.0 kg/m^2 recruited in the

period between October 2019 to April 2021. The inclusion criteria for bariatric surgery was BMI ≥ 40 kg/m^2 or BMI ≥ 35 kg/m^2 with high-risk comorbidities such as severe T2D or cardiovascular risk factors. No individuals with age below 18 or above 65, BMI ≤ 35 kg/m^2, BMI between 35 and 40 kg/m^2 without any comorbidities or a severe medical condition, or unstable mental health concerns were recruited in this study. The study was approved by the Ministry of Health of Kuwait and the Institutional Ethics Committee of Kuwait University and Dasman Diabetes Institute in accordance with the Declaration of Helsinki (MOH/2017/630, P116-13MM-06, RA-HM 2019 026), and a written informed consent was signed by all participants. Laparoscopic SG (LSG) and RYGB were the two bariatric surgeries employed in the study; 17 patients (85%) had LSG and 3 patients (15%) had RYGB. Comorbidities involved obstructive sleep apnea (OSA) and diabetes (5%), OSA (10%), metabolic-associated fatty liver disease (15%), diabetes and hypertension (10%), hypertension (5%), and polycystic ovary syndrome (10%).

In patients who underwent LSG, more than 70% to 80% of their stomach volume was removed using a linear stapler transecting along the greater curvature from 5 cm proximal to the pylorus up to 1 cm away from the gastroesophageal junction. A 36-Fr. (French) bougie was used as a calibrator. Laparoscopic RYGB was performed by creating a small gastric portion with a volume of 30 to 50 mL. The jejunum was divided 50 cm distal to the ligament of Treitz and the proximal end of the jejunum was anastomosed to the distal part of the jejunum 150 cm below the site of transection (jejunojejunostomy). The 150-cm Roux limb of the proximal jejunum was brought up and anastomosed to the proximal gastric pouch. Physiological monitoring of all surgical patients was followed in accordance with the institutional protocol for bariatric surgery.

Blood sampling

Blood samples were collected after an 8-hour fast at baseline and 6 and 12 months after surgery using vacuum ethylenediaminetetraacetic acid (EDTA), sodium citrate, and serum separator tubes. The blood was centrifuged at 2500*g* for 10 minutes. The plasma and serum were separated, aliquoted, and stored at −80 °C until the assay was performed. Clinical biochemical tests were performed on all participants, including complete blood count, vitamin, liver, renal, lipid, bone, and thyroid profile test.

Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were separated from the collected whole blood by centrifugation at 2000*g* for 30 minutes with mononuclear cell preparation tubes (Becton, Dickinson and Company). The isolated PBMC were washed with 50% heat-inactivated fetal bovine serum/1x phosphate-buffered saline (HI-FBS/1x PBS) solution and stored in HI-FBS with dimethyl sulfoxide (DMSO) in liquid N_2 until further use.

RNA extraction and gene expression via quantitative real-time polymerase chain reaction

Total RNA was extracted from PBMC using the TRIzol method according to the manufacturer's protocol (Qiagen). The extracted RNA was quantified for quality and concentration using an Epoch Microplate Spectrophotometer (BioTek Instruments). RNA samples were reverse-transcribed to produce complementary DNA (cDNA) using High-Capacity cDNA Reverse Transcription Kits (Cat# 4374966, Thermo Fisher Scientific). Real-time polymerase chain reaction (RT-PCR) was performed on QuantStudio Flex 7 (Applied Biosystems, Inc., Thermo Fisher Scientific) using Power SYBR Green Master Mix (Cat# 4368702, Thermo Fisher Scientific) under optimized conditions following the manufacturer's protocol. Gene expression analysis was done for the following genes: *METRNL*, *FNDC5*, *UCP1/2/3*, and the primer sequences are included in Table 1. The data were analyzed by normalizing with the endogenous control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cat# RP300644, Sino Biologicals). Relative expression was calculated using the ΔΔCT method [24].

Measurements of plasma levels of METRNL, irisin, and C-reactive protein using enzyme-linked immunosorbent assay

Plasma levels of METRNL, irisin, and C-reactive protein (CRP) were detected using an enzyme-linked immunosorbent assay (ELISA). Plasma samples were thawed on ice and centrifuged for 5 minutes at 10,000*g* at 4 °C to remove any remaining cells or platelets. The plasma level of METRNL was measured using the human ELISA kit (Cat# LS-F13315, LifeSpan BioSciences, Inc.). The plasma level of irisin was measured using the irisin recombinant ELISA kit (Cat# EK-067-29,

TABLE 1 mRNA analyzed by quantitative RT-PCR and the nucleotide sequence of qRT-PCR primers

No.	mRNA	Accession No.	Amplicon	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
1	*UCP1*	NM_021833	161	ACAGCACCTAGTTTAGGAAG	CTGTACGCATTATAAGTCCC
2	*UCP2*	NM_003355	182	AGTTTTTCTCCATCTCCTGG	CTTTCTCCTTGGATCTGTAAC
3	*UCP3*	NM_003356	196	CATCATGAGGAATGCTATCG	TGGAGGTGAGTTCATATACC
4	*METRNL*	NM_001004431	123	AGTGGATGTACCCAACAG	CCAAATAAATATTGGCCCCC
5	*FNDC5*	NM_001171940	142	GGAGGATACGGAGTACATAG	CTCTTTCATGGTTACCTCATC

Abbreviations: FNDC5, fibronectin type III domain-containing protein 5; METRNL, meteorin-like protein; UCP1/2/3, uncoupling protein 1/2/3.

TABLE 2 Clinical and laboratory characteristics included in the study

Variable	Healthy controls (a)	Baseline (individuals with obesity), (b)	6 months (post-op) (c)	12 months (post-op) (d)	*p* value
Age (y)	36 ± 1.1	40 ± 3.1	40 ± 3.1	40 ± 3.1	NA
Sex (M/F)	13/7	13/7	13/7	13/7	NA
Weight (kg)	71.6 ± 2.4	111.7 ± 5.2	86.4 ± 3.3	79.2 ± 3.6	<0.05[a−b,a−c,b−c,b−d]
Height (cm)	169.4 ± 2.0	167.6 ± 2.2	168.7 ± 2.2	168.7 ± 2.2	NA
BMI (kg/m^2)	24.9 ± 0.5	39.7 ± 1.4	30.6 ± 1.0	27.9 ± 1.0	<0.05 [a−b,a−c,b−c. b−d]
Heart rate (beats/min)	75.3 ± 1.7	76.3 ± 1.9	82.8 ± 2.7	75.5 ± 1.9	ns
Systolic blood pressure (mm Hg)	119.5 ± 2.0	122.3 ± 2.1	111.9 ± 2.2	132.3 ± 2.9	<0.05 [a−d,b−c,b−d,c−d]
Diastolic blood pressure (mm Hg)	79.9 ± 1.8	77.4 ± 1.9	73.2 ± 2.1	79.3 ± 1.3	ns
blood glucose (mmol/L)	5.2 ± 0.2	6.3 ± 0.4	4.9 ± 0.2	5.2 ± 0.2	<0.05 [a−b,b−c,b−d]
Hemoglobin A_{1c} (%)	5.5 ± 0.1	6.1 ± 0.3	5.1 ± 0.1	5.4 ± 0.1	<0.05 [b−c,b−d]
TC (mmol/L)	5.6 ± 0.2	4.9 ± 0.2	4.8 ± 0.1	5.2 ± 0.2	<0.05 [a−c]
HDL (mmol/L)	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	<0.05 [a−c]
LDL (mmol/L)	3.5 ± 0.2	3.8 ± 0.0	3.2 ± 0.1	3.2 ± 0.1	<0.05 [b−c,b−d]
TG (mmol/L)	1.6 ± 0.3	1.4 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	ns
Insulin (mU/L)	3.8 ± 0.6	5.3 ± 1.2	3.4 ± 0.6	3.5 ± 0.5	ns
HOMA-IR	0.9 ± 0.2	1.5 ± 0.4	0.7 ± 0.2	0.8 ± 0.2	ns
Total protein (mg/dL)	74.6 ± 1.2	69.9 ± 0.9	72.8 ± 0.8	72.3 ± 0.8	<0.05 [a−b]
Albumin (mg/dL)	44.1 ± 0.7	39.8 ± 0.5	44.7 ± 0.5	43.6 ± 0.6	<0.05 [a−b,b−c,b−d]
ALT (IU/L)	32.2 ± 5.8	31.9 ± 4.0	17.1 ± 1.5	21.1 ± 5.8	ns
AST (IU/L)	28.9 ± 2.9	25.1 ± 2.0	15.4 ± 0.3	19.6 ± 1.1	<0.05 [a−c,a−d,b−c]
ALP (IU/L)	68.8 ± 3.0	81.7 ± 6.7	70.1 ± 3.8	84.9 ± 5.6	ns
GGT (IU/L)	23.1 ± 2.6	40.0 ± 13.5	17.9 ± 2.5	21.1 ± 4.6	ns
Total billirubin (μmol/L)	12.1 ± 0.9	12.1 ± 1.1	24.7 ± 8.5	12.1 ± 2.0	ns
FT4 (pmol/L)	11.1 ± 0.4	11.8 ± 0.4	18.6 ± 0.4	12.2 ± 0.3	<0.05 [a−c,b−c,c−d]
TSH (mIU/mL)	1.8 ± 0.1	2.4 ± 0.4	2.1 ± 0.3	1.9 ± 0.2	ns
CRP (mg/L)	6.4 ± 1.7	17.0 ± 2.3	8.3 ± 1.4	4.0 ± 0.7	<0.05 [a−b,b−c,b−d]
METRNL (pg/mL)	50,397.8 ± 897.9	55,221.6 ± 1,421.1	57,537.3 ± 1,303.9	59,334.9 ± 1,214.3	<0.05 [a−b,a−c,a−d]
Irisin (ng/mL)	21.0 ± 1.1	21.4 ± 1.3	17.6 ± 1.2	19.1 ± 1.3	ns

Note: Data are presented as mean ± SEM. Statistical assessment was two-sided and considered statistically significant at $p < 0.05$.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; F, female; FT4, free thyroxine; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein cholesterol; M, male; METRNL, meteorin-like protein; NA, not applicable; ns, not significant; TC, total cholesterol; TG, triglycerides; TSH, thyrotropin.
The statistical significance among groups is represented as:
[a−b]Healthy controls-baseline (individuals with obesity).
[a−c]Healthy controls-6 months (post-op).
[a−d]Healthy controls-12 months (post-op).
[b−c]Baseline (individuals with obesity)- 6 months (post-op).
[b−d]Baseline (individuals with obesity)- 12 months (post-op).
[c−d]6 months (post-op)- 12 months (post-op).

Phoenix Pharmaceuticals, Inc.). CRP level was measured in plasma using the human CRP ELISA kit (Cat# K 9710s, Immundiagnostik AG). Samples were diluted with sample diluent, and the assays were performed as per kit instructions. Concentrations for each analyte were calculated by plotting a five-parameter logistic curve. The intra-assay coefficient of variation for METRNL was 5.0% to 10.0%, whereas the interassay coefficient of variation was < 10%. The intra-assay coefficient for irisin ELISA was 1.0% to 7.0%, whereas the interassay coefficient was < 20%. The intra-assay coefficient for CRP ELISA was 5.0% to 10.0%, whereas the interassay coefficient was < 15%.

Statistical analysis

One-way ANOVA was used to compare the effect of surgery on various anthropometric and biochemical parameters as well as the

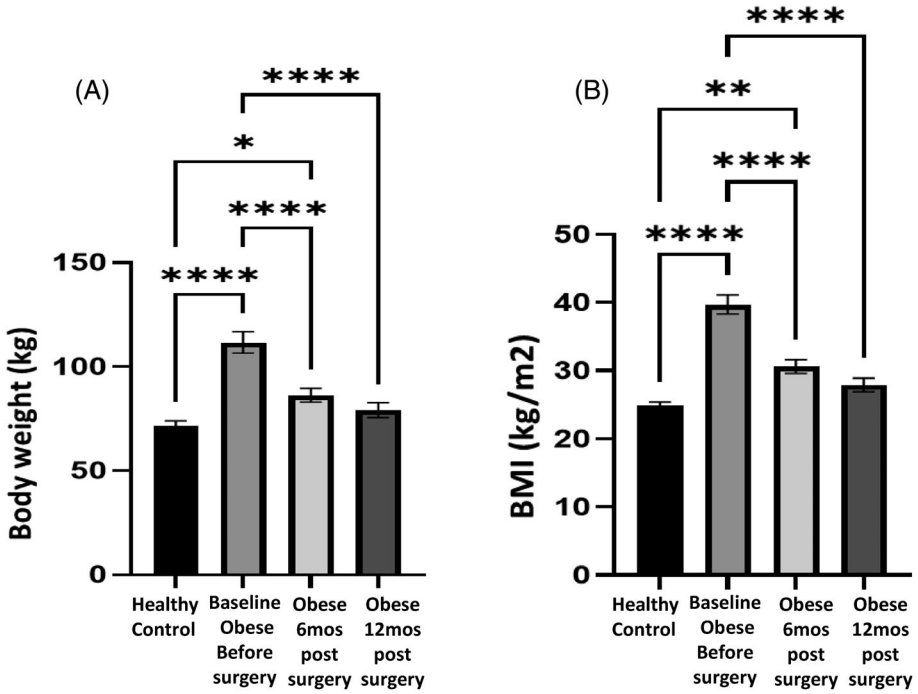

FIGURE 1 (A) Body weight and (B) BMI at baseline and after surgery, Statistical assessment was two-sided and considered statistically significant at *$p < 0.05$; **$p < 0.001$; ****$p < 0.0001$.

molecules of interest (METRNL, irisin, and UCP1, 2 and 3) at different time points (baseline before surgery and 6 and 12 months after surgery). Post hoc analysis was performed to assess differences between the various groups under study using Tukey test. All data were reported as mean ± standard error of mean (SEM). Spearman correlation analysis was performed to establish the link between the gene expression changes and the metabolic changes after bariatric surgery. Statistical assessment was two-sided and considered statistically significant at $p < 0.05$. Calculations were made using GraphPad Prism version 9.2. Multivariable linear regression analysis was performed to examine the predictive effect of each factor with METRNL, UCP1, UCP2, UCP3, and irisin as dependent variables in healthy groups and groups with obesity. The following variables were included: age, gender, total cholesterol, triglycerides (TG), fasting blood glucose, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and CRP.

RESULTS

Characteristics of the study population

The anthropometric and biochemical characteristics of the study population are presented in Table 2. The mean weight and BMI observed in individuals with obesity prior to bariatric surgery were significantly higher (mean [SEM], 111.7 [5.2] kg and 39.7 [1.4] kg/m^2, respectively) when compared with healthy individuals (71.6 [2.4] kg and 24.9 [0.5] kg/m^2, respectively). A significant decrease in mean weight and BMI were observed at 6 and 12 months after surgery within the group with obesity (Figure 1 A and B). The mean weight and BMI at 6 months were 86.4 (3.3) kg and 30.6 (1.0) kg/m^2, respectively, and at 12 months after bariatric surgery were 79.2 (3.6) kg and 27.9 (1.0) kg/m^2. The percentage of total weight loss at 6 months after surgery was 29.3% and at 12 months was 41.0%. Additionally, the percentage of excess weight loss was calculated, and it was 60.7% at 6 months and 77.9% at 12 months after surgery. All participants with comorbidities such as hypertension, polycystic ovary syndrome, OSA, metabolic-associated fatty liver disease, and/or diabetes reported either complete resolution or improvement in their condition. One-way ANOVA showed a significant difference in systolic blood pressure and fasting blood glucose in all groups. Post hoc analysis revealed significant differences in glycosylated hemoglobin (A_{1c}) and low-density lipoprotein cholesterol (LDL) levels among the obesity group before and after bariatric surgery (Table 2).

Gene expression of *METRNL*, *FNDC5* (irisin), and *UCP* in PBMC

Gene expression analysis revealed a significantly higher gene expression of *METRNL*, *UCP1*, and *UCP3* in individuals with obesity at baseline prior to surgery as compared with healthy controls ($P < 0.05$; Figure 2A, 2C, and 2F). Interestingly, *FNDC5* was not detected in PBMC of healthy controls; however, it was seen in PBMC of the

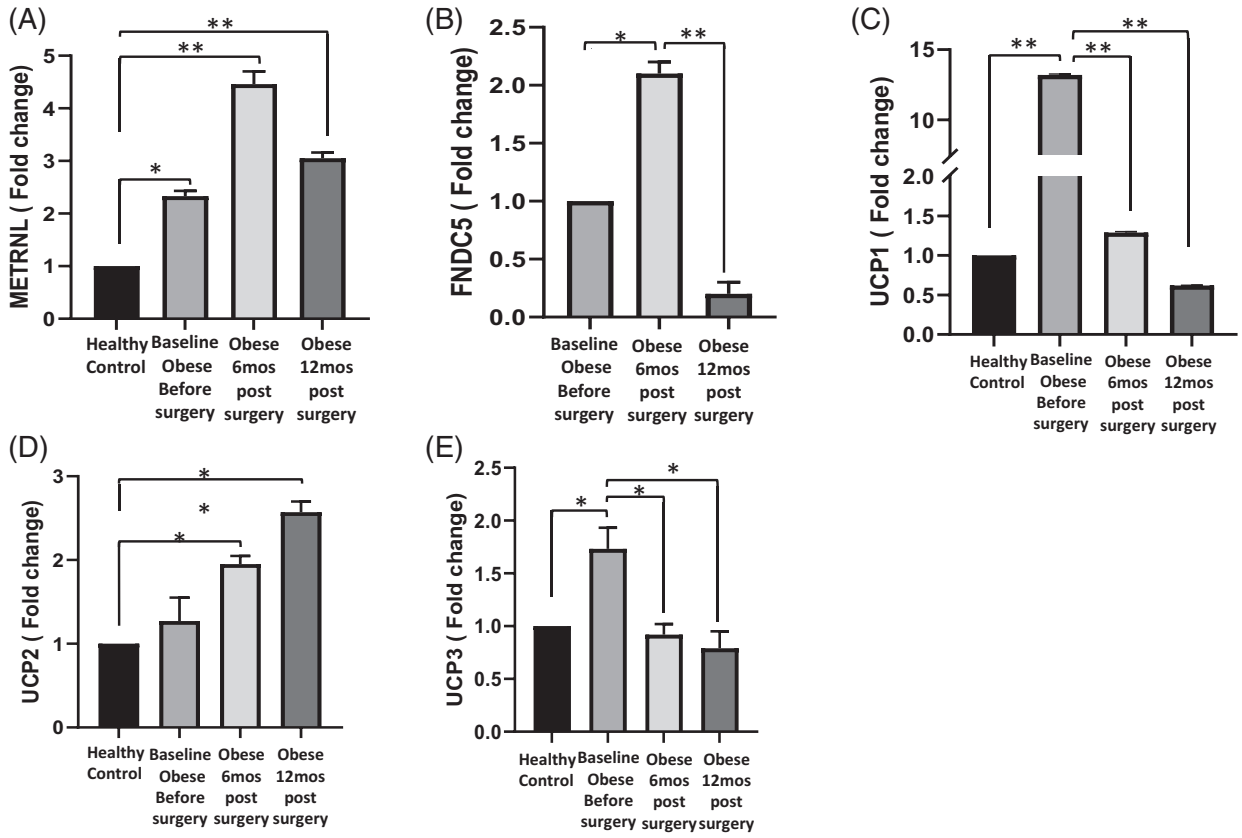

FIGURE 2 Gene expression analyses. Fold change differences at different time points after surgery of (A) *METRNL*, (B) irisin (*FNDC5*), (C) *UCP1*, (D) *UCP2*, and (E) *UCP3* compared with healthy controls. Statistical assessment was two-sided and considered statistically significant at $^{*}p < 0.05$; $^{**}p < 0.001$.

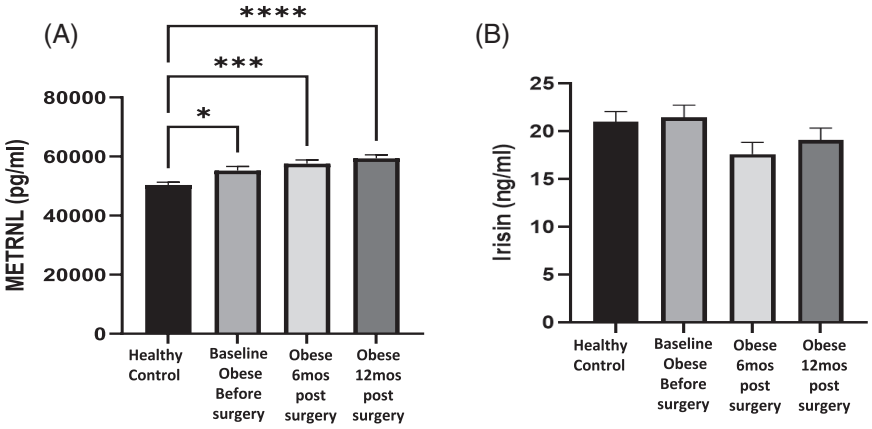

FIGURE 3 Changes in (A) METRNL and (B) irisin in plasma at different time points after surgery. Statistical assessment was two-sided and considered statistically significant at $^{*}p < 0.05$; $^{***}p < 0.0005$; $^{****}p < 0.0001$.

individuals with obesity at baseline. A significant increase in the gene expression levels of *METRNL*, *FNDC5*, and *UCP2* was observed in the group with obesity at 6 months after surgery ($P < 0.05$; Figure 2A, 2B, and 2D), followed by a reduction in the levels of *METRNL* and *FNDC5* at 12 months. Nevertheless, the *UCP2* gene expression level continued to show an increase at 12 months after surgery. The gene expression levels of *UCP1* and *UCP3* were significantly decreased at 6 and 12 months after surgery ($P < 0.001$).

METRNL and irisin levels in plasma

METRNL and irisin levels were measured in the circulation. The METRNL plasma level for individuals with obesity was significantly higher at baseline (mean [SEM], 55,222.6 [1,421.1] pg/mL, $P = 0.0319$), 6 months after surgery (57,537.3 [1,303.9] pg/mL, $P = 0.0005$), and 12 months after surgery (59,334.9 [1,214.3] pg/mL, $P < 0.0001$) compared with healthy individuals (50,397.8 [897.9] pg/

TABLE 3 Correlation analysis between changes in gene expression of METRNL, FNDC5, UCP1, UCP2, and UCP3 and various metabolic variables (baseline vs. postsurgery)

	METRNL		FNDC5		UCP1		UCP2		UCP3	
Variables	Spearman	*p* value	Spearman	*p* value	Spearman	*p* value	Spearman	*p* value	Spearman	*p* value
BMI	−0.533*	0.002	−0.149	0.458	−0.364*	0.037	−0.101	0.569	−0.131	0.492
Hemoglobin A_{1c}	0.027	0.885	−0.311	0.114	−0.511**	0.003	−0.075	0.683	0.064	0.742
TC	0.251	0.152	0.376	0.053	0.099	0.578	0.259	0.139	0.144	0.439
HDL	0.029	0.914	−0.309	0.385	0.069	0.799	0.050	0.854	0.143	0.626
LDL	0.102	0.708	0.498	0.143	−0.461	0.011	0.134	0.621	−0.095	0.748
TG	0.124	0.483	0.060	0.764	−0.180	0.308	0.098	0.582	−0.057	0.759
Glucose	0.034	0.851	−0.390*	0.049	−0.485**	0.006	−0.120	0.514	−0.103	0.602
Insulin	−0.096	0.600	0.008	0.968	−0.287	0.118	−0.222	0.222	−0.008	0.969
HOMA-IR	−0.188	0.319	−0.033	0.878	−0.371	0.048	−0.299	0.108	−0.015	0.943
Total rotein	0.158	0.396	0.387	0.045	0.509**	0.004	0.152	0.413	0.046	0.816
Albumin	0.038	0.838	0.133	0.516	0.416*	0.022	0.058	0.755	0.058	0.769
ALT	−0.078	0.675	−0.348	0.081	−0.318	0.087	−0.125	0.504	−0.132	0.502
AST	0.048	0.797	−0.534**	0.005	−0.404*	0.027	−0.099	0.596	−0.120	0.544
ALP	−0.273	0.151	−0.251	0.237	−0.334	0.077	−0.381*	0.042	−0.308	0.118
GGT	−0.220	0.234	−0.262	0.196	−0.409*	0.025	−0.269	0.143	−0.340	0.077
Total bilirubin	−0.078	0.677	0.227	0.265	−0.387	0.046	−0.114	0.543	−0.151	0.444
Free T4	−0.105	0.573	0.320	0.111	0.479**	0.007	−0.068	0.716	−0.008	0.966
TSH	0.208	0.262	−0.039	0.850	0.087	0.649	0.132	0.479	0.134	0.495

Note: Statistical assessment was two-sided and considered statistically significant at *$p < 0.05$; **$p < 0.001$.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FNDC5, fibronectin type III domain-containing protein 5; free T4, free thyroxine; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, Low-density lipoprotein cholesterol; METRNL, meteorin-like; TC, total cholesterol; TG, triglycerides; TSH, thyrotropin; UCP1/2/3, uncoupling proteins 1/2/3.

mL) (Figure 3A). However, no change was observed in the level of irisin (Figure 3B).

CRP level in plasma

To assess the impact of bariatric surgery on inflammation, CRP level was measured in circulation. The CRP plasma level was significantly higher at baseline in individuals with obesity (mean [SEM], 17.0 [2.3] mg/L) as compared with healthy controls (6.4 [1.7] mg/L, $P < 0.001$). A significant reduction in CRP plasma level was observed at 6 months (8.3 [1.4] mg/L, $P < 0.01$) and 12 months (4.0 [0.7] mg/L, $P < 0.0001$) following bariatric surgery (Table 2).

Correlation analysis between changes in gene expression and metabolic changes

Spearman correlation was performed to understand whether there is an association between the changes in the gene expression of the various molecules of interest with the metabolic changes observed after bariatric surgery (Table 3). A significant correlation was seen between the change in gene expression level of METRNL with change in BMI ($r = -0.533$; $P = 0.002$). The change in gene expression of FNDC5 showed a significant correlation with changes in glucose level ($r = -0.390$; $P = 0.049$) and AST ($r = -0.534$; $P = 0.05$). Interestingly, the change in gene expression level of UCP1 showed a significant correlation with changes in BMI ($r = -0.364$; $P = 0.037$) and glucose ($r = -0.485$; $P = 0.006$), as well as other parameters such as glycosylated hemoglobin (A_{1c}) ($r = -0.511$; $P = 0.003$), LDL ($r = -0.461$; $P = 0.011$), homeostatic model assessment for insulin resistance ($r = -0.371$; $P = 0.048$), total protein ($r = 0.509$; $P = 0.022$), albumin ($r = 0.416$; $P = 0.022$), AST ($r = -0.404$; $P = 0.027$), GGT ($r = -0.409$; $P = 0.025$), and free thyroxine (free T4) ($r = 0.479$; $P = 0.007$). The change in gene expression of UCP2 showed a correlation with ALP ($r = -0.381$; $P = 0.042$). No correlation was seen between the change in gene expression of UCP3 with changes in the metabolic parameters. Stepwise multivariate linear regression analysis was performed with METRNL, UCP1, UCP2, UCP3, and FNDC5 as dependent variables in the healthy group and the group with obesity, for which the results showed that in the group with obesity, total cholesterol was independently associated with METRNL ($\beta = 0.279$, $P = 0.006$), UCP2 ($\beta = 0.370$, $P = 0.003$), and UCP3 ($\beta = 0.288$, $P = 0.039$); ALT ($\beta = -0.832$, $P = 0.019$), GGT ($\beta = -0.192$, $P = 0.012$), and CRP ($\beta = 0.806$, $P = 0.029$) were independently associated with METRNL, UCP1, and UCP2, respectively. In the healthy group, none of the parameters showed significance with METRNL, UCP1, UCP2, UCP3, and FNDC5 as dependent variables.

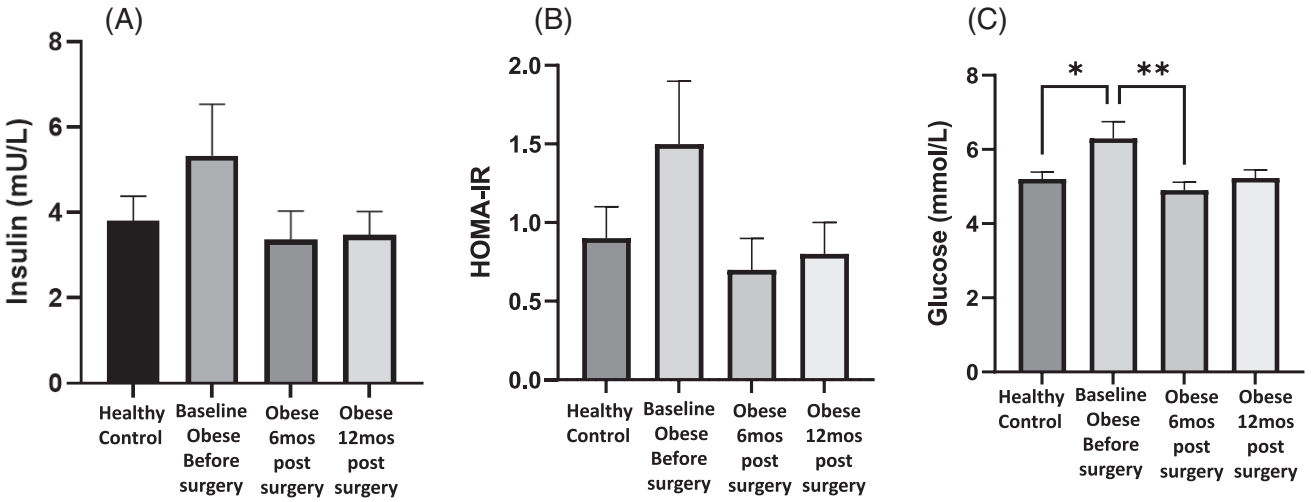

FIGURE 4 Changes in (A) insulin, (B) homeostatic model assessment for insulin resistance (HOMA-IR), and (C) glucose assessment at different time points after surgery. Statistical assessment was two-sided and considered statistically significant at $^{*}P < 0.05$; $^{**}P < 0.001$.

DISCUSSION

In this study, the effect of bariatric surgery was investigated in individuals with obesity. Our results showed a significant reduction in body weight and BMI among the individuals with obesity who underwent bariatric surgery as compared with their presurgery baseline levels. In addition, an improvement in the levels of insulin, glucose, and homeostatic model assessment for insulin resistance was observed after bariatric surgery (Figure 4A–C). The beneficial outcomes of bariatric surgery have been attributed to rapid metabolic improvements [25]. This study was based on our previous investigation on animal models in which the results suggested increased mitochondrial metabolism leading to increased thermogenesis [11]. Knowing that specific adipomyokines such as irisin and METRNL are associated with thermogenesis, in this study, the levels of these molecules were investigated. Furthermore, the mitochondrial markers UCP1, 2, and 3, which are known to play an important role in energy homeostasis, were also analyzed. In accordance with previously reported data, the plasma level of METRNL was significantly higher among the individuals with obesity at baseline as compared with healthy controls. A slight change in the level of irisin was seen among the same groups [19]. Further increases in plasma levels of METRNL were seen at 6 months and 12 months after surgery, whereas no significant change in irisin level was seen after surgery. However, in PBMC, *METRNL* and irisin (*FNDC5*) gene expression levels were significantly higher with obesity. This increase was even more pronounced at 6 months after surgery, which then decreased 1 year after surgery, supporting roles in the inflammatory response and thermogenesis.

PBMC are key cells that aid in the study of human body immunity. They are composed of several types of cells such as lymphocytes, monocytes, or macrophages. They provide selective responses to the immune system [26]. PBMC are an important tool that provide information on the pro- and anti-inflammatory responses in various conditions [27, 28]. METRNL and irisin are known to regulate inflammatory responses [29, 30]. The higher levels of these molecules in PBMC of individuals with obesity at baseline compared with healthy individuals could indicate the association of these molecules with obesity-induced chronic inflammation. The increased gene expression of these molecules at 6 months after surgery further supports their involvement in the inflammatory response during the healing period immediately after surgery, especially that the levels of these molecules decrease 12 months after surgery. In support of this conclusion, in the current study, a reduction in CRP level following bariatric surgery was observed, which reflects the beneficial effect of bariatric surgery on inflammation.

UCP are inner mitochondrial membrane proteins that regulate proton transport across the membrane [31]. UCP1 is exclusively found in BAT and is involved in thermogenesis and systemic energy homeostasis [32]. UCP2 is expressed in many tissues such as the spleen, lung, intestine, skin, brain, fibroblasts, immune cells, kidney, pancreatic islets, skeletal muscle, heart, and adipose tissue. It has been shown to play a role in inflammation, energy metabolism, and oxidative stress [33]. In this study, the gene expression level of *UCP2* in the immune cells (PBMC) was investigated. The results showed a significantly higher gene expression level of *UCP2* in obesity. This agrees with previous reports that showed UCP2 as a key regulator of obesity-related inflammation [34]. Obesity enhances the production of ROS, resulting in increased oxidative stress, which in turn leads to chronic low-grade inflammation. Because UCP2 plays a pivotal role in maintaining steady-state levels of ROS, the overproduction of ROS causes an increase in the expression of UCP2 [35, 36]. This explains the slight increase in *UCP2* expression with obesity. On the other hand, it is known that UCP2 plays a protective role in lowering oxidative stress, inflammation, and the consequent cellular damage [31, 34, 37]. Studies have also reported that bariatric surgery causes a reduction in oxidative stress through increased weight loss and a decrease in ROS production [38]. Therefore, the increased gene expression levels of *UCP2* after bariatric surgery observed in this study shed

light on the possible role of UCP2 in regulating oxidative stress after surgery. UCP3 is primarily expressed in the skeletal muscles and BAT and in lower levels in immune cells [39, 40]. It is involved in the regulation of oxidative stress and is associated with fatty acid metabolism within cells [23, 41]. Studies show that high-fat diets upregulate UCP3 [41, 42]. This supports the increase in *UCP3* gene expression with obesity in the current study. The *UCP3* gene expression level was normalized to that of healthy controls after bariatric surgery, indicating the beneficial role of bariatric surgery on fatty acid oxidation and oxidative stress [43]. For the first time, the gene expression level of *UCP1* was studied in PBMC. A highly significant increase in *UCP1* gene expression was seen with obesity, and this then decreased to normal levels at 6 and 12 months after surgery. Both *UCP1* and *UCP3* showed similar gene expression patterns, which is in agreement with previous reports showing that *UCP1* and *UCP3* have similar expression pattern in BAT [23, 44]. The functional significance of this similarity in immune cells is yet to be deciphered. Interestingly, the negative association observed between the change in *UCP1* gene expression level with multiple metabolic parameters further highlights the beneficial effect of bariatric surgery and the possible role that this molecule may play in regulating metabolic homeostasis at the cellular level. One of the limitations of the current study is the lack of detailed clinical and physiological profiles at various time points and the healthy controls who were included in the study.

CONCLUSION

The gene expression and plasma levels of adipomyokines METRNL and irisin (FNDC5) were significantly higher in individuals with obesity as compared with healthy subjects. These levels were further augmented following bariatric surgery, supporting the involvement of these molecules in the inflammatory and thermogenic responses after bariatric surgery. This study highlights the important role of adipomyokines in inflammation, because METRNL and FNDC5 were elevated with obesity in PBMC. Furthermore, the molecules such as FNDC5, UCP1, and UCP3 that are typically not expressed in immune cells were seen to be expressed under the disease state. The beneficial effects of bariatric surgery have been explored extensively for its physiological effects. This study further highlights the beneficial role of this surgery on the molecular level. For the first time, the gene expression of *UCP2* in immune cells was shown to have a significant increase following bariatric surgery, supporting its possible protective role in combating oxidative stress and inflammation. Further studies are required to understand the role that these molecules play in the immune cells under disease states and after bariatric surgery.O

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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