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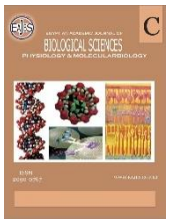

Relationship of Serum MicroRNA-26a and GSK-3β Levels with Metabolic Syndrome in Women with Obesity

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ABSTRACT

Background: Identification of the pathogenesis of metabolic derangement in obesity is important. MicroRNA-26a has a regulatory function in glucose and lipid metabolism. Glycogen synthase kinase 3β (GSK-3β) represents a target gene of MicroRNA-26a.**Objectives:** Our aim was to measure serum MicroRNA-26a and GSK-3β in women with obesity and detect their correlation to clinical and metabolic variables in this group of patients.**Methods:** In total, 70 obese females and 25 healthy non-obese females were involved in this cross-sectional study. Blood pressure, BMI, waist circumference, and thicknesses of triceps, subscapular, and suprailiac skinfolds were measured. The HbA1c, fasting blood glucose (FBG), lipid profile, ALT, AST were measured in serum. MicroRNA-26a and GSK-3β expression were evaluated via real-time PCR.**Results:** In obese women, the MicroRNA-26a expression was significantly decreased, and the GSK-3β expression level was significantly raised compared to healthy control ($P < 0.001$; for both). MicroRNA-26a had significant negative correlation to age (r = –0.260; $P = 0.030$), ALT (r = –0.300; $P = 0.012$), FBG (r = –0.353; $P = 0.003$), HbA1c (r = –0.588; $P < 0.001$), triglycerides (TG) (r = –0.347; $P = 0.003$) and GSK-3β (r = –0.627; $P <0 .001$). Nonetheless, GSK-3β was significantly positively correlated to age ($P < 0.001$) and BMI ($P = 0.022$), in addition to ALT (P<0.001), HbA1c (P<0.001), TG (P<0.001). Multivariate analysis reported that age (95% CI: 1.058–1.299; $P = 0.002$) and MicroRNA-26a (95% CI: 0.000–0.004; $P \leq 0.001$) were the significant variables correlated to metabolic syndrome (MetS).**Conclusion:** Serum MicroRNA-26a is significantly decreased, and serum GSK-3β is significantly escalated in obese patients. MicroRNA-26a and GSK-3β are significantly interplayed with MetS in obese patients.

Abbreviations:

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ER, Endoplasmic Reticulum; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MetS, metabolic syndrome; RT-PCR, real-time polymerase chain reaction; SBP, Systolic blood pressure.

INTRODUCTION

Obesity is a global health concern and the most crucial risk factor for impaired glucose metabolism, dyslipidemia, fatty liver and cardiovascular disorders (GBD 2015 Obesity Collaborators, 2017). Nonetheless, not all obese patients have metabolic derangement.

Metabolic syndrome (MetS) is a globally increasing metabolic disorder and appears to correspond to the escalating prevalence of obesity. MetS includes a cluster of risk factors that are related to insulin resistance (IR). These risk factors comprise obesity as well as hyperlipidemia, hyperinsulinemia, hypertension, and hyperglycemia. Interestingly, MetS is recognized for increasing the risk of developing atherosclerotic vascular disease and type 2 diabetes mellitus (T2DM) (Hayden, 2023)**.**

MetS specific mechanism in obese people is unclear. The significance of body fat distributions (Kwon *et al.*, 2017), genetic and epigenetic factors (Fathi , 2018), inflammation, and MicroRNA expression (Jiménez-Lucena *et al.*, 2018) have been shown in the pathogenesis of metabolic disturbance in people with obesity.

MicroRNAs are endogenous minute noncoding RNAs regulating gene expression and have critical roles in diverse physiological and pathological processes. Recent studies uncovered the MicroRNAs involvement in immune regulation, cardiac remodeling, malignancy, and genetic disorders (Seeley *et al.*, 2018; Gabisonia *et al.*, 2019; Suzuki, 2023). Moreover, the MicroRNAs have been reported to have a function in MetS pathogenesis and its correlated complications, suggesting them as a new class of endocrine factors (Włodarski *et al.*, 2020). However, further evaluation is required to determine which MicroRNAs are dysregulated and the target pathways they affect.

Prior research has indicated that MicroRNA-26a had anti-cancer characteristics by controlling the development of tumors, blood vessel creation, energy consumption, cell invasion, and metastasis to different regions of the body by focusing on different messenger RNAs (Fu *et al.*, 2015). New data regarding its role in obesity and metabolic processes has been found by recent studies (Fu *et al.*, 2015; Song *et al.*, 2018). In the liver, serum, and islets of obese mice, MicroRNA-26a was found to be downregulated (Fu *et al.*, 2015; Song *et al.*, 2018). On the other hand, it has been demonstrated that mice with upregulated MicroRNA-26a exhibit protection against diet-triggered obesity and diabetes through improved β-cell function (Song *et al.*, 2018) and enhanced glucose homeostasis and insulin sensitivity (Fu *et al.* ,2015).

Glycogen synthase kinase (GSK) constitutes a multifunctional protein kinase, which is essential in regulating different cellular processes like cell proliferation, cell survival, gene expression, and cellular structure (Jope and Johnson, 2004). Initially identified for its function in glycogen synthase regulation in response to insulin, GSK has also been associated with energy metabolism regulation and metabolic disorder development (Wang *et al.*,2022). Despite these established functions, the precise molecular mechanisms and pathways by which GSK3 influences metabolic activity necessitate further exploration. Studies have shown that MicroRNA-26a suppresses GSK3β expression in neuronal tissues (Lucci *et al.*, 2020) , cardiac muscles (Tang *et al.*,2020) , and the kidney (Murata *et al.*, 2023). In our current research, we determined the serum gene expression of MicroRNA-26a and GSK-3β in obese women and investigated their correlation to the existence of MetS in this patient group and their relation to each other.

MATERIALS AND METHODS

1- Study Design:

This cross-sectional study was carried out at Kasr Al-Ainy Hospital, a tertiary medical facility in Cairo, and authorized by the ethical committee of Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University. All participants signed informed

consent. Our protocols complied with the ethical guidelines established in the 1975 Declaration of Helsinki.

2- Subjects and Methods:

This study included 70 obese females and 25 healthy non-obese females as controls. The eligible participants were chosen from Cairo University's outpatient clinics for internal medicine in Kasr Al-Ainy Hospital, precisely the diabetes, endocrinology, and nutrition clinics between January and June of 2024. Individuals with autoimmune disorders, cancer, liver cirrhosis, chronic renal illness, pregnancy, and secondary causes of obesity were excluded. Every participant underwent a thorough medical evaluation that comprised measures of blood pressure, height, weight, body mass index (BMI), waist circumference, and thicknesses of the triceps, subscapular, and suprailiac skinfolds. Serum glucose, glycated hemoglobin (HbA1c), lipid profiles, and aspartate and alanine aminotransferases (AST and ALT) tests were also conducted on the subjects. To evaluate MicroRNA-26a and GSK-3β gene expression in the serum of healthy persons and obese individuals, real-time polymerase chain reaction (RT-PCR) was performed. Obese females are subdivided into 2 groups obese without MetS (n=35) and Obese with MetS (n=35). MetS was diagnosed using the IDF criteria (Alberti *et al.* 2006).

3-Biochemical Assay:

3.1. GSK-3β Gene Expression In Serum Via RT-PCR:

Following the protocols, we extracted the total RNA from the serum utilizing a Qiagen kit (Qiagen, USA). A NanoDrop®1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA) was used to evaluate the quality of the RNA. After that, complementary DNA (cDNA) was created employing SYBR Premix Ex TaqTM II (Perfect Real Time, TaKaRa, Japan) and RT-qPCR via High-Capacity cDNA Reverse Transcription Kits (Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania) per the protocols. The PCR reaction was conducted at the following conditions: 95 °C for 5 min, 40 cycles for 15 s at 95 °C, and 60 °C for 60 s. Using the computation of the 2-ΔΔCt control cells, the RQ of each target gene was evaluated quantitatively by normalizing against the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPGH) for GSK-3β.

The Applied Biosystem software was utilized to compute the relative quantitation. The cycle threshold (Ct) values have been calculated graphically for various target genes, including GAPDH. Prior to being expressed as ΔCt values, the Ct values of all target genes in the same sample were first modified to GAPDH. After that, $2^{-\Delta\Delta Ct}$ values were found to reflect distinct target gene quantities and were calculated as ΔCt (treated samples) – ΔCt (control samples). The final numbers that were displayed were given as ratios to control cells. Table 1, presents the primer sequences for the investigated genes.

3.2. MicroRNA-26a Gene Expression by RT-PCR:

Mirvana kit (Thermo Fisher, Massachusetts, USA) was used to extract microRNA from serum samples. The TaqMan® Micro RNA Assays are real-time PCR appliances of Applied Biosystems that were used to detect and precisely quantify mature MicroRNA. The TaqMan® Micro RNA Reverse Transcription Kit, USA, was used to synthesize single-stranded cDNA from MicroRNA samples. U6 snRNA was utilized as an endogenous control for normalization. The relative expression of the MicroRNA-26a gene was assessed in both groups using the comparative CT method (ΔΔCT). Table 1, shows the primer sequence for the investigated gene.

Table 1. Primers sequence of studied genes

Gene symbol	Primer sequence from 5′- 3′
GSK3-β	F: 5'-GGAACTCCAACAAGGGAGCA-3' R: 5'-TTCGGGGTCGGAAGACCTT A-3'
GAPDH	F: 5'-GCACCGTCAAGGCTGAGAAC-3' R: 5'-TGGTGAAGACGCCAGTGGA-3'
MicroRNA-26a	F: 5'-GGC CTC GTT CAA GTA ATC CA-3' R: 5'-GCC TTT AGC AGA AAG GAG GTT-3'
U6	F: 5'-GCTTCGGCAGCACATATACTAAAAT3' R: 5'-CGCTTCACGAATTTGCGTGTCAT-3'.

F: Forward primer, R: Reverse primer

2.4. Statistical Analysis:

Employing the SPSS version 28 (IBM Corp., Armonk, NY, USA), the data were coded and entered. Summary of the data involved the use of median and interquartile range for quantitative data, and frequency (count) along with relative frequency (percentage) for categorical data. The non-parametric Mann-Whitney and the Chi-square ($\chi 2$) tests were utilized for comparing quantitative variables and categorical data, respectively. An exact test was conducted when the expected frequency was < 5. To analyze associations between quantitative variables, the Spearman correlation coefficient was deployed. Additionally, logistic regression was carried out to identify the MetS independent predictors. $P < 0.05$ indicated statistically significant.

RESULTS

1- Clinical and Laboratory Features of Obese Women Compared to Healthy Non-Obese Women:

A total of 70 women with obesity and 25 healthy non-obese women were registered in this study. Obese patients had a mean BMI (42.33 ± 8.02) kg/m^2, 18.6 % had hypertension, and 42.9% had diabetes. In obese patients, the mean HbA1c (5.83 ± 1.07 vs. 5.39 ± 0.18; *P = 0.048*) and the low-density lipoprotein cholesterol (LDL-C) (122.00 ± 38.58 vs. 92.08 ± 9.89; *P < 0.001*) were significantly elevated compared to the control group. However, there was no significant difference between the 2 groups regarding triglycerides (TG) or high density lipoprotein cholesterol (HDL-C). In both groups, MicroRNA-26a was significantly lowered (0.38 ± 0.20 vs. 1.03 ± 0.08; *P < 0.*001), and GSK-3β was significantly raised (4.82 ± 2.37 vs. 1.01 ± 0.01; *P < 0.001)*. Table 2, manifested the clinical features and laboratory parameters of both groups.

2- Comparison of the Clinical and Laboratory Variables Between Obese Females with and Without MetS:

Females with MetS were older and had elevated BMI (*P < 0.001*; *P = 0.017*) than obese females without MetS. Furthermore, HbA1c, TG, ALT, and AST were significantly higher in females with MetS in comparison to females without (P<0.001; P<0.001; P<0.001;P=0.033). Nonetheless, waist circumference and thicknesses of triceps, subscapular, and suprailiac skinfolds in the group with MetS were not significantly different compared to those without MetS. In obese females with MetS, MicroRNA-26a was significantly lowered (0.38 ± 0.20 vs. 1.03 ± 0.08; *P < 0.001*), and GSK-3β was significantly raised (4.82 ± 2.37 vs. 1.01 ± 0.01; *P < 0.001*) compared to those without MetS (Table 3).

3- Associations of Serum MicroRNA-26a and GSK-3β levels with Various Parameters in Obese Females:

In obese women, MicroRNA-26a level manifested a significant negative correlation with age, TG, plasma glucose, and ALT. In addition, MicroRNA-26a expression elucidated a negative corrlation with serum GSK-3β (r = –0.627; *P < 0.001*, Fig.1) in obese females. Nevertheless, a positive correlation with systolic blood pressure (SBP) was observed (r = 0.385; *P = 0.001*, Table 4).

Furthermore, relations between various variables and GSK-3β were

investigated (Table 4). Serum GSK-3β was positively correlated to age (*P < 0.001*) and BMI (*P = 0.022*), in addition to FBG *(P< 0.001),* HbA1c *(P<0.001),* triglycerides *(P< 0.001),* and ALT (P<0.001). However, both serum MicroRNA-26a and GSK-3β levels had no significant correlation with waist circumference or any skinfold thickness in women with obesity (Table 4).

Table 2: Comparison of clinical and metabolic characteristics of women with obesity and healthy non-obese women.

Variable	Women with obesity (n=70)	Healthy non-obese (n=25)	*P value
Age (mean/years)	32.43±8.46	29.72±8.52	0.163
Body mass index(kg/m2)	42.33±8.02	23.47±2.03	< 0.001
Waist circumference (cm)	116.11±14.13	79.96±10.22	< 0.001
Triceps skinfold thicknesses(mm)	36.53±6.59	19.84±5.12	< 0.001
Subscapular skinfold thicknesses(mm)	43.00±6.17	20.40±4.17	< 0.001
Suprailiac skinfold thicknesses(mm)	40.00±6.81	18.84±7.69	< 0.001
Systolic blood pressure (mmHg)	120.74±17.30	119.85 ±14.98	0.914
Alanine transaminase (U/L)	20.81± 11.12	19.55±6.6	0.566
Aspartate transaminase (U/L)	23.14±7.75	22.15±6.35	0.560
Fasting blood glucose (mg/dl)	103.67±29.67	92.28±5.95	0.006
[1]HbA1c (%)	5.83±1.07	5.39±0.18	0.048
[2]LDL-C (mg/dL)	122.00±38.58	92.08±9.89	< 0.001
[3]HDL-C (mg/dL)	43.61±8.78	41.00±4.69	0.259
Triglycerides (mg/dL)	118.60±61.42	93.56±14.67	0.055
MicroRNA-26a	0.38±0.20	1.03±0.08	< 0.001
GSK-3β	4.82±2.37	1.01±0.01	< 0.001

*P value <0.05 is considered significant.[1]HbA1c: glycated hemoglobin; [2]LDL-C: low density lipoprotein cholesterol; [3]HDL-C: high density lipoprotein cholesterol; [4]GSK-3β: Glycogen synthase kinase-3 beta.

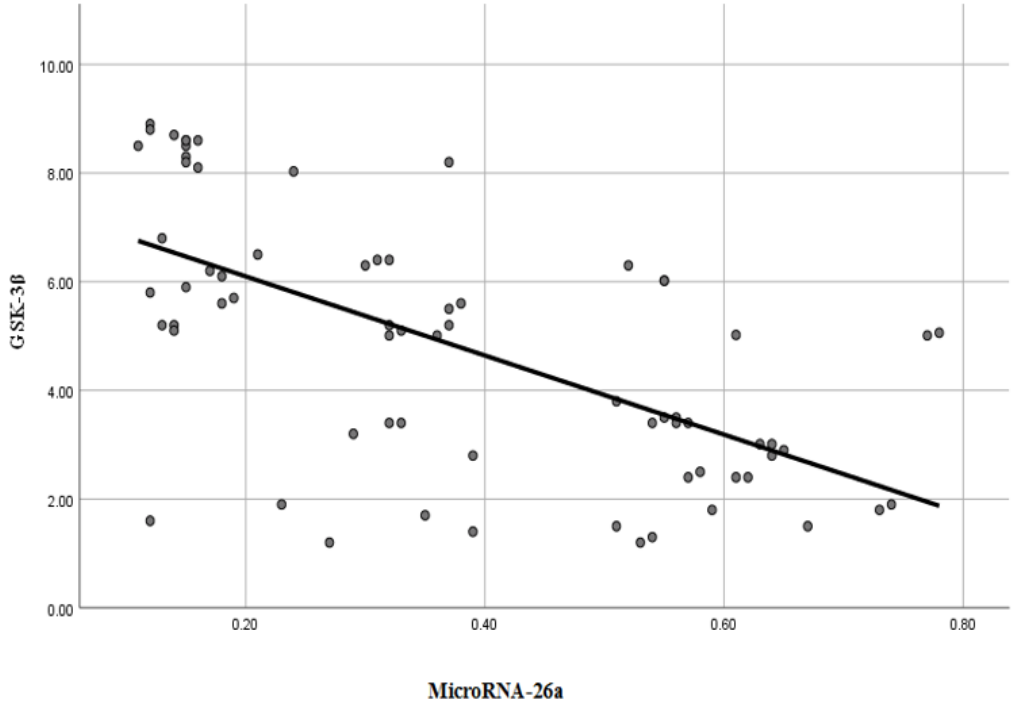

Fig.1. Correlation analysis of serum MicroRNA-26a and GSK-3β in obese women. There was a significant negative correlation between serum MicroRNA-26a and GSK-3β (*P < 0.001*).

Table 3: Comparison of clinical and metabolic characteristics of women with obesity and without and with metabolic syndrome.

	Obese without metabolic Syndrome (n=35)			**Obese with metabolic syndrome (n=35)**			**P value*
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	
Age (years)	29.00	21.00	33.00	37.00	32.00	40.00	< 0.001
BMI (kg/m2)	38.20	35.15	45.00	42.30	38.96	46.43	0.017
Waist circumference (cm)	112.00	101.00	130.00	115.00	107.00	126.00	0.219
Triceps skinfold thicknesses(mm)	35.00	34.00	41.00	35.00	30.00	40.00	0.479
Subscapular skinfold thicknesses(mm)	45.00	40.00	45.00	45.00	40.00	50.00	0.265
Suprailiac skinfold thicknesses(mm)	40.00	33.00	45.00	40.00	35.00	42.00	0.230
Systolic blood pressure (mmHg)	120.00	110.00	130.00	110.00	110.00	130.00	0.246
FBG (mg/dl)	92.00	90.00	96.00	106.00	96.00	113.00	< 0.001
[1]HbA1c (%)	5.20	4.90	5.50	6.10	5.80	6.70	< 0.001
[2]LDL-C (mg/dL)	110.00	100.00	140.00	115.00	99.00	177.00	0.282
[3]HDL-C (mg/dL)	45.00	35.00	55.00	43.00	37.00	48.00	0.309
Triglycerides (mg/dL)	94.00	70.00	101.00	120.00	99.00	170.00	< 0.001
[4]ALT (U/L)	14.00	10.00	20.00	26.00	16.00	30.00	< 0.001
[5]AST (U/L)	19.00	16.00	28.00	24.00	19.00	31.00	0.033
MicroRNA-26a	0.56	0.37	0.63	0.17	0.14	0.32	< 0.001
[6]GSK-3β	2.80	1.80	3.40	6.30	5.60	8.30	< 0.001

*P value <0.05 is considered significant.[1]HbA1c: glycated hemoglobin; [2]LDL-C: low density lipoprotein cholesterol; [3]HDL-C: high density lipoprotein cholesterol; [4]ALT: alanine aminotransferase; [5]AST: aspartate aminotransferase; [6]GSK-3β: Glycogen synthase kinase-3 beta.

Table 4: Correlations of serum MicroRNA-26a and GSK-3β with different variables in women with obesity.

variable	MicroRNA-26a		GSK-3β	
	r	*P	r	*P
Age (years)	-0.260	0.030	0.460	< 0.001
Body mass index (kg/m2)	-0.196	0.103	0.273	0.022
Waist circumference (cm)	-0.012	0.919	0.156	0.196
Triceps skinfold thicknesses(mm)	0.067	0.583	-0.056	0.646
Subscapular skinfold thicknesses(mm)	-0.136	0.262	0.086	0.480
Suprailiac skinfold thicknesses(mm)	0.230	0.055	-0.042	0.732
Systolic blood pressure (mmHg)	0.385	0.001	-0.211-	0.079
Alanine aminotransferase (U/L)	-0.300	0.012	0.425	< 0.001
Aspartate aminotransferase (U/L)	-0.228	0.058	0.251	0.036
Fasting blood glucose (mg/dL)	-0.353	0.003	0.465	< 0.001
[1]HbA1c (%)	-0.588	< 0.001	0.620	< 0.001
[2]LDL-C (mg/dL)	-0.045	0.709	0.111	0.359
[3]HDL-C (mg/dL)	0.158	0.192	-0.093	0.445
Triglycerides(mg/dL)	-0.347	0.003	0.474	< 0.001
MicroRNA-26a	-	-	-0.627	< 0.001

*P value <0.05 is considered significant. [1]HbA1c: glycated hemoglobin; [2]LDL-C: low density lipoprotein cholesterol; [3]HDL-C: high density lipoprotein cholesterol.

4- Regression Analysis of Factors Linked to MetS:

Logistic regression was conducted to evaluate independent factors linked to MetS. The findings demonstrated that the MetS risk was 1.17-fold higher in females with advanced age (95% confidence interval [CI]: 10.7–19.0%; *P = 0.002*). Conversely, a significant reduction was recognized in the MetS risk in women with higher MicroRNA-26a (OR: 0.000030, 95% CI: 0.000–0.004, *P < 0.001*).

DISCUSSION

The MetS is often linked to obesity as it poses a risk due to several factors such as IR, genetic predisposition, dysfunction of adipose tissue, inflammation, and abnormal distribution of body fat (Han and lean, 2016). However, not all obese individuals develop MetS, and additional investigation is needed to understand the factors associated with it. The primary outcome of the present research revealed a noteworthy correlation between MetS and serum levels of MicroRNA-26a and GSK-3β in obese women.

MicroRNA-26a has been recognized as a critical factor in the regulation of adipose tissue formation, acting as a significant controller of adipocyte progenitor cells' differentiation and adipose tissue mass (Acharya *et al.* 2019). Our study uncovered a reduction in the serum levels of MicroRNA-26a in obese women compared to non-obese healthy females. Furthermore, obese women with MetS exhibited even lower levels of MicroRNA-26a compared to those without MetS. Previous research has shown a decrease in the MicroRNA-26a expression in the liver, serum, and islets of obese mice. In addition, the MicroRNA-26a overexpression has been demonstrated to protect mice from diet-provoked obesity (Fu *et al.,* 2015; Song *et al.,* 2018).

In a previous experimental study, it was found that dysregulation of MicroRNA-26a is linked to obesity-associated MetS. Additionally, a slight decrease in MicroRNA-26a expression could participate in IR development (Fu *et al.*, 2015). Our current study revealed a significant negative correlation between MicroRNA-26a alongside blood glucose levels and TG in obese women. This correlation can be elucidated by the fact that MicroRNA-26a directly targets essential regulators that contribute to gluconeogenesis, fatty acid synthesis, and insulin signaling (Fu *et al.*, 2015). However, in contrast to our outcomes, Shvangiradze et al. have reported a positive correlation between MicroRNA-26a and LDL-C levels and waist circumference in patients with obesity and coronary artery disease (CAD) (Teona *et al.,* 2019). Importantly, our study did not detect a significant correlation between MicroRNA-26a and body fat distribution parameters or LDL-C levels. This discrepancy could be attributed to differences in the populations studied, as our research did not include patients with CAD who typically have elevated LDL-C levels.

Intriguingly, MicroRNA-26a has been found to have a significant impact on various pathways associated with energy dissipation. It also influences the shift of mitochondrial structure towards that observed in brown adipocytes. Moreover, MicroRNA-26a promotes uncoupled respiration by greatly enhancing the uncoupling protein 1 expression, which is a key protein in brown fat (Karbiener *et al.*, 2014).

These outcomes may establish an explanation for the lack of significant correlation between MicroRNA-26a and waist circumference as well as skinfold thicknesses in the present study.

Our findings demonstrate a significant correlation between GSK-3β and FBG as well as HbA1c in obese women, aligning with the increased expression and activity of GSK-3 in T2DM patients (Patel *et al.,* 2008; MacAulay *et al.,* 2007). Additionally, the specific overexpression of GSK3β has been shown to impair glucose tolerance and insulin action, whereas the GSK-3β knockout or inhibition has the opposite effect, highlighting the crucial function of GSK-3β in the insulin signaling

pathway (Henriksen , 2010).

In their study, Wang *et al.* (2018) discovered that inhibiting GSK3 had a positive impact on reducing inflammation in visceral adipose tissue (VAT) caused by obesity. This was evident through a decrease in pro-inflammatory M1 macrophages and an elevation in anti-inflammatory M2 macrophages in the VAT. Additionally, GSK3 inhibition led to a reduction in circulatory inflammatory monocytes. These anti-inflammatory impacts were attributed, at least partially, to the inhibition of macrophage apoptosis inhibitor production in macrophages through the inactivation of STAT3, which in turn reduced free fatty acid levels (Wang *et al.*, 2018). This finding provides some insight into the positive association between GSK-3β and TG levels observed in our study.

In cardiac muscles and sensory neurons, MicroRNA-26a has been discovered to have a negative impact on the expression of GSK-3β (Tang *et al,*. 2020; Wang *et al.*, 2018). Nevertheless, the role of MicroRNA-26a in MetS among individuals with obesity remains largely unknown. Our research revealed a negative correlation between GSK-3β and MicroRNA-26a in obese women. Similarly, Fu et al. (2015) have reported that MicroRNA-26a has a direct inhibitory influence on the GSK-3β expression in both mice and humans. This inhibition results in the phosphorylation of insulin receptor substrate proteins on Ser residues, ultimately dampening insulin signaling. These results indicate that MicroRNA-26a has a significant function in promoting insulin signaling (Fu *et al.*, 2015).

The present study demonstrated that an elevated level of MicroRNA-26a was linked to a decreased risk of MetS, independent of other factors. This phenomenon can be attributed to the direct targeting of various crucial regulators involved in gluconeogenesis, fatty acid synthesis, and insulin signaling by MicroRNA-26a. Furthermore, the interaction between hepatic metabolism as well as insulin signaling further strengthens and amplifies the effects of MicroRNA-26a. These findings imply that even a minor alteration in the expression of MicroRNA-26a can potentially cause significant physiological consequences (Zeng *et al.*, 2021). In line with our study, Fu and colleagues have found that a slight reduction in MicroRNA-26a levels (approximately 2-fold) may result in IR and diabetes onset. Conversely, in mice, a small rise in MicroRNA-26a expression was shown to be effective in preventing complications associated with obesity (Fu *et al.*, 2015).

There is a strong association between non-alcoholic fatty liver disease (NAFLD) and MetS (Radu *et al.*, 2023). The current study demonstrated higher levels of aminotransferases in obese women and MetS compared to obese women without MetS. Additionally, a significant negative relation was observed between MicroRNA-26a and aminotransferases in obese women. MicroRNA-26a is triggered by Endoplasmic Reticulum (ER) stress in liver cells, playing a crucial role in managing ER stress (Xu *et al.*, 2021). Previous research has indicated that overexpression of MicroRNA-26a in human hepatocytes effectively reduces ER stress as well as lipid accumulation. In contrast, a deficiency in MicroRNA-26a has the opposite effect (Ali *et al.*, 2018). Mechanistically, MicroRNA-26a targets the initiation factor 2α, a key player in ER stress response that regulates cellular translation, lipid accumulation, inflammation, and hepatic steatosis (Ali *et al.*, 2018).The current study uncovered that GSK-3β might participate in NAFLD development in obese patients, and GSK-3β showed a significant correlation with ALT in obese women. Accumulating evidence suggests that GSK-3β activation participates in the activation of the mitochondrial cell death pathway and occurs through the ER stress response (Cao *et al.* ,2014; Ibrahim *et al.*, 2011). Additionally, inhibiting GSK-3β has been found to have a protective effect against hepatic lipid accumulation commonly seen in NAFLD and against the key factors contributing to the more complicated condition of MetS, including IR and obesity (Emma *et al.*, 2020).

This study has some limitations. First, in obese patients, although there is a significant interplay between MicroRNA-26a and GSK-3β, which suggests a molecular pathway, the study did not include functional analyses. Second, our study was cross-sectional. To fully understand the dynamic changes in MicroRNA-26a and GSK-3β in patients with obesity and MetS, further prospective studies are required. Third, the measurement of MicroRNA-26a and GSK-3β was conducted in serum, and no gene study in tissues was conducted. Eventually, the participants were recruited from a specific region, so the results may not apply to individuals from other geographic areas. It is necessary to conduct further studies involving diverse ethnicities to validate our findings.

In conclusion, our study highlights the disturbances in MicroRNA-26a and GSK-3β levels in obese patients. The reduction in MicroRNA-26a and the elevation in GSK-3β in individuals with obesity could potentially contribute to the metabolic abnormalities linked to obesity. This research suggests that MicroRNA-26a and GSK-3β could be promising targets for treating MetS associated with obesity.

Declarations:

Ethical Approval: This study was approved by the Research Ethics Committee (REC) of Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University (Approval number: N-260-2023).

Conflict of interests: The authors declare no conflicts of interest.

Authors Contributions: All authors contributed equally, and have read and agreed to the published version of the manuscript.

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Availability of Data and Materials: Upon a reasonable request, the corresponding author provides access to all data.

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