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Expression of Energy Metabolism-related Genes in Adipose Tissue of Individuals with Different Grades of Obesity

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Abstract

Obesity is a metabolic condition caused by a chronic disruption in energy balance, leading to the accumulation of excess body fat with subsequent detrimental health consequences. The global epidemic of obesity emphasizes the necessity of understanding the molecular mechanisms of energy regulation. This study aimed to evaluate the expression levels of energy metabolism-related genes, including SREBP-1c, AMPK, UCP-2, and FASN, in the adipose tissue of individuals with different grades of obesity and determine their potential as obesity biomarkers. Subcutaneous adipose tissue samples were collected from 20 non-obese controls and 70 obese individuals, categorised as obese class I (BMI = 30–34.9 kg/m^2), obese class II (BMI = 35–39.9 kg/m^2), and obese class III (BMI ≥ 40 kg/m^2). mRNA was extracted from those samples, and the expression levels were measured using a real-time quantitative polymerase reaction. The study found that obese individuals have higher levels of FASN, SREBP-1c, UCP-2, and AMPK gene expression compared to non-obese individuals. These genes were expressed more in obese class II than in obese class I, but less in obese class III. Additionally, BMI and serum levels of total cholesterol, triglycerides, and LDL cholesterol were found to have a positive correlation with the expression of the studied genes, while HDL cholesterol showed a negative correlation. The investigated genes demonstrated excellent accuracy in discriminating between obese and non-obese individuals. The study's findings indicate that the genes examined may play a significant role in obesity and could be used as early predictors of obesity risk in humans. These results could be a step towards improving the clinical diagnosis of obesity and the development of new treatment strategies.

Keywords: Obesity; Gene Expression; Energy Metabolism; Subcutaneous adipose tissue.

1. Introduction

Obesity is one of the most common metabolic disorders, which represents a public health challenge worldwide (**1**). It is characterized by body fat accumulation caused by an imbalance between energy intake and energy expenditure **(2).** The prevalence of obesity has significantly increased globally over the past 20 years. It is expected that by 2030, 17% of individuals will be obese, with 177 million of those being extremely obese **(3).** Egypt is ranked 18th in the world in terms of obesity prevalence. According to Egypt's "100 million health" survey of 49.7 million adults conducted in 2019, 39.8% of Egyptians were obese (**4**). Obesity poses a serious threat to human health by significantly increasing the risk of hypertension, fatty liver disease, type 2 diabetes, cardiovascular diseases, and cancer. Additionally, obesity can reduce a person's life expectancy and have a negative impact on his quality of life (**4, 5**). The body mass index (BMI) is typically used to diagnose and categorise obesity. This index has a direct relationship with body fat mass and can be calculated using a person's weight and height (**6**). Obesity is defined as having a BMI of 30 kg/m^2 or higher, and it is further classified into three categories based on the degree of body fat excess **(7**). Disturbances in energy metabolism due to

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overeating and a lack of exercise are thought to be the main causes of obesity. However, environmental factors cannot entirely explain the rising rates of obesity incidence. The aetiology of obesity is regarded as more complex as it is influenced by a combination of genetic and environmental factors (**8).** Previous studies revealed that genes play a role in the development of obesity and weight control, with many genes being downregulated or activated to regulate energy metabolism (**8–10**). Obesity is significantly influenced by genetic factors, where the heritability of BMI is estimated to be 40%–70% (**11**). Understanding the genetic factors that contribute to energy metabolism is expected to improve the management of obesity. Among these genetic factors are the uncoupling proteins (UCPs), which play a significant role in thermoregulation and energy utilisation (**12**). There are five UCP isoforms identified in mammals (UCP-1 to UCP-5). Uncoupling proteins are a transporter family located in the mitochondrial inner membrane. Uncoupling protein-2 (UCP-2) is a crucial regulator of energy homeostasis that is abundant in white adipose tissue (**13**). It uncouples the transfer of protons generated by the electron transport chain across the inner membrane of mitochondria. This decoupling process reduces the amount of adenosine triphosphate (ATP) generated from fuel oxidation, resulting in heat instead of energy (**14**). Due to its location on the obesity-related regions of mouse chromosome 7 and human chromosome 11, UCP-2 is regarded as a promising candidate gene for obesity (**15**)**.** Several studies suggest that alterations in UCP-2 expression may lead to obesity by influencing energy metabolism (**13, 16**). Another key regulator of cellular and whole-body energy balance is adenosine monophosphate-activated protein kinase (AMPK)**.** AMPK is involved in the regulation of thermogenesis, fatty acid metabolism, and the development of adipose tissue. It induced metabolic changes such as phosphorylation of key enzymes and altered expression of genes involved in metabolic regulation (**17)**. Activated AMPK stimulates ATP-producing catabolic pathways, such as fatty acid oxidation, and inhibits ATP-consuming processes, such as lipogenesis (**18**). As a result, AMPK was of particular relevance in obesity research (**18–20**). The sterol regulatory element-binding proteins (SREBPs) are a membrane-bound transcription factor family that plays an essential role in energy metabolism. They control the expression of genes encoding enzymes that are involved in cholesterol and fatty acid biosynthesis (**21)**. In mammals, there are two types of SREBP: 1 (a, c) and 2 (**22**). SREBP-1 regulates fatty acid and glucose metabolism, whereas SREBP-2 regulates cholesterol production (**23**). SREBP-1c levels were reported to be significantly higher in obese individuals and obese animal models (**24, 25**). An increase in de novo lipogenesis (DNL) is an important contributor to increased fat mass, while a reduction in lipogenesis may be protective against the development of obesity. Fatty acid synthase (FASN) is a key regulator of lipid metabolism and is thought to play a role in the aetiology of human obesity. It is the central enzyme in DNL that catalyses the conversion of malonyl-CoA to palmitate (**26**). This enzyme is mostly found in adipose tissue and the liver. The FASN gene has been identified as a potential gene for body weight regulation (**27**). The inhibition of FASN was found to significantly reduce food intake and weight loss, suggesting that this enzyme may contribute to obesity through regulating energy balance (**28–30**). This study aimed to analyze the mRNA levels of energy metabolism-related genes, including AMPK, SREBP-1c, UCP-2, and FASN, in the adipose tissue of obese individuals with different BMIs and validate their potential as biomarkers for obesity. This could help in the development of strategies for preventing and managing obesity.

2. Subjects and methods

1.1. Study design

The study followed a case-control study design. All participants were recruited from the surgical department of the El Kasr el Aini hospital in Cairo, Egypt. The study was approved by the National Research Center's Ethical Committee (No. 19-162), and all subjects gave their informed consent to participate.

2.2. Study participants

The present study included 90 participants aged 30-45, divided into two BMI-based groups: obese (≥ 30 kg/m^2) and normal-weight control (18.5-24.9 kg/m^2). Seventy individuals who underwent bariatric surgery comprised the obese group, while the control group consisted of twenty healthy, normal-weight individuals who had undergone cholecystectomy or hernia repair procedures. The participants were matched in terms of gender and age. Obese participants were subdivided into three categories based on BMI. Those with a BMI of 30 to 34.9 kg/m^2 were classified as obese class I (mildly obese).

Those with a BMI of 35 to 39.9 kg/m^2 were classified as obese class II (moderately obese). Those with a BMI of 40 kg/m^2 or more were classified as obese class III (morbidly obese) (**31**). The BMI was calculated by measuring the height and weight of all participants. Individuals with thyroid dysfunction, liver or kidney diseases, and women taking contraceptive medication were excluded from the study.

2.3. Specimens collection

Five millilitres of blood were collected from each subject before surgery and after an overnight fast. The serum was then separated and immediately frozen at -80°C for subsequent biochemical analyses. During surgery, subcutaneous adipose tissue biopsies were taken and immediately stored at -80°C in RNA stabilization solution (RNAlater; Thermo Fisher Scientific, USA) for the subsequent gene expression analysis.

2.4. Biochemical analyses

Total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) levels were determined using an enzymatic colorimetric method, as described by Richmond (**32**) and Gordon *et al*. (**33**), respectively. Triglyceride (TG) levels were measured enzymatically using glycerol kinase and glycerol-phosphate oxidase **(34)**. The following equation was used to calculate low-density lipoprotein cholesterol (LDL-c) level: LDL-C (mg/dL) = total cholesterol - HDL-C - (triglycerides/5) (**35**). The liver enzyme activities (alanine transaminase- ALT and aspartate aminotransferase- AST) were measured using an enzymatic kinetic method according to Bergmeyer *et al.* (**36)**. Serum urea was assessed by an enzymatic colorimetric method (**37**), and serum creatinine was determined using the Jaffé method (**38**).

2.5. Extraction of RNA and quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from subcutaneous adipose tissue (100 mg) using the TRIzol reagent (Thermo Fisher Scientific, USA) and the phenol-chloroform extraction method described by Chomczynski and Sacchi (**39**). Following the manufacturer's instructions, the total purified RNA (1 µg) was reverse transcribed into cDNA by the TOPscript™ cDNA synthesis kit (Enzynomics, Daejeon, Korea). A quantitative real-time PCR was used to assess mRNA levels. The reaction mixture contained 10 µL of SYBR Green Master Mix, 2.0 µL of cDNA template, and 2.0 µL of each primer for each gene. The final volume of the reaction was completed at 25 µL by nuclease-free water. Sequences of primers are shown in Table 1. The following PCR amplification procedure was used: initial activation step at 95 °C for 10 minutes, then three-step cycling (denaturation at 94°C for 15 seconds; annealing at 55°C for 30 seconds ; extension at 70°C for 30 seconds) for 40 cycles, then 4 °C forever. The housekeeping gene, beta-actin, was used to normalize the expression of each gene. The threshold cycle (Ct value) was determined for each amplification curve, and the ΔCt value was calculated by subtracting the Ct value for the housekeeping gene from the Ct value for each sample and control. The fold change in gene expression was calculated using the $2^{\wedge}(-^{\Delta\Delta Ct})$ formula in comparison to endogenous controls.

Table (1): Sequences of primers used in qRT-PCR

Gene	5'→ 3' Forward
	5'→ 3' Reverse
FASN[30]	CGCGTGGCCGGCTACTCCTAC
	CGGCTGCCACACGCTCCTCT
SREBP-1c[40]	GCGGAGCCATGGATTGCAC
	CTCTTCCTTGATACCAGGCCC
AMPK[41]	CAAGCTTTTCAGGCATCCTC
	CAAATAGCTCTCCTCCTGAGACA
UCP-2[42]	GCCTCTACAATGGGCTGGTT
	GAGCATGGTAAGGGCACAGT
β-actin[43]	GTGGGCCGCTCTAGGCACCAA
	CTCTTTGATGTCACGCACGATTTC

2.6. Statistical Analysis

The mean and standard deviation (±SD) are used to express all data. The software SPSS version 21.0 was used for statistical analysis and graphics (Chicago, USA). A one-way ANOVA test was used to compare differences between independent variables. The Kruskall-Wallis test was used to identify genes differentially expressed between different groups. The correlations between variables were done using the Spearman's rank correlation coefficient test. Receiver operating characteristic (ROC) analysis was used to determine the potential of the studied genes as biomarkers for obesity. The level of significance was established at $p \leq 0.05$, whereas a p value of <0.01 was considered highly significant.

Table (2): Clinical and biochemical measurements of study participants

Variables \ Groups	Control (n= 20) Mean ± S.D.	Obese class I (n= 13) Mean ± S.D.	Obese class II (n= 16) Mean ± S.D.	Obese class III (n= 41) Mean ± S.D.	P value
Gender n (%) Males	7 (35.00%)	7 (53.80%)	2 (12.50%)	12 (29.30%)	0.115
Females	13 (65.00%)	6 (46.20%)	14 (87.50%)	29 (70.70%)	
Age (years)	33.65 ± 6.31	39.23 ± 7.54	33.50 ± 4.07	36.10 ± 8.43	0.167
Weight (Kg)	58.55 ± 4.74	96.69 ± 12.91	104.13 ± 8.07	133.06 ± 11.09	<0.001
Height (cm)	163.90 ± 6.58	170.23 ± 8.61	167.94 ± 5.57	165.88 ± 8.32	0.151
BMI(Kg/m^2)	21.88 ± 2.46	33.30 ± 2.59	36.86 ± 1.10	48.14 ± 5.44	<0.001
TC (mg/dL)	165.75 ± 20.36	178.34 ± 26.37	184.81 ± 27.28	222.77 ± 23.00	0.010
HDL-c (mg/dL)	56.23 ± 6.14	47.85 ± 3.72	43.75 ± 4.52	39.98 ± 6.83	<0.001
TG (mg/dL)	110.50 ± 25.64	147.02 ± 20.73	151.38 ± 22.15	169.23 ± 21.14	0.035
LDL-c (mg/dL)	95.26 ± 11.70	114.69 ± 19.38	127.66 ± 12.60	143.49 ± 15.59	<0.001
Urea (mg/dL)	19.00 ± 1.78	18.38 ± 1.61	18.56 ± 1.67	18.54 ± 1.48	0.353
Creatinine (mg/dL)	0.90 ± 0.13	0.85 ± 0.06	0.89 ± 0.06	0.86 ± 0.08	0.289
AST (U/L)	20.45 ± 3.59	20.08 ± 2.02	20.06 ± 2.79	19.61 ± 2.31	0.436
ALT (U/L)	20.70 ± 2.34	19.54 ± 1.98	21.00 ± 3.54	19.78 ± 2.83	0.103

P value<0.001 is considered statistically highly significant; p value< 0.01 and p value<0.05 are considered statistically significant.TC, total cholesterol; TG, triglyceride; BMI, body mass index; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine transaminase.

3. Results

The clinical and biochemical characteristics of the study subjects are described in Table 2. The BMI, weight, total cholesterol, LDL cholesterol, and triglyceride levels were significantly higher ($p<0.001$) in obese subjects from different classes compared to control subjects. Conversely, serum HDL cholesterol levels were significantly lower in obese individuals than in controls ($p = 0.005$). There was no significant difference ($p > 0.05$) in age or gender between the control and obese groups. Morbidly obese individuals (Obese class III) had the lowest HDL cholesterol level and the highest triglyceride, LDL, and total cholesterol levels. Figure 1 indicated that the mRNA expression levels of AMPK, SREBP-1c, UCP-2, and FASN were significantly higher ($p<0.001$) in obese subjects from various classes compared to control subjects. When comparing the expression levels of these genes among obese classes I, II, and III, no statistically significant differences were observed (data not shown). However, the morbidly obese (obese class III) exhibited lower expression levels for the investigated genes compared to class II and were similar to class.

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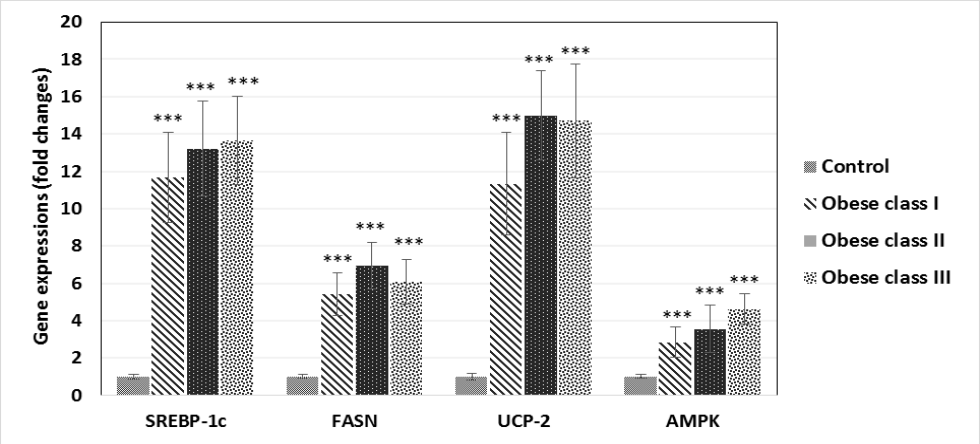

Figure (1): Gene expressions (fold changes) of the studied genes in obese individuals compared to healthy controls. ***P value<0.001for comparisons between groups.

Table (3): Association between the mRNA expression levels of studied genes in obese subjects

Genes	SREBP-1c	FASN	UCP-2	AMPK
SREBP-1c		0.278*	0.135	0.349**
FASN	0.278*		0.017	0.309**
UCP-2	0.135	0.017		-0.076
AMPK	0.349**	0.309**	-0.076	

* Significant correlation at p< 0.05. ** Significant correlation at p< 0.01.

Table 3 showed a significant positive correlation between the expression of SREBP-1c and the expressions of FASN and AMPK (r = 0.278, p = 0.020, and r = 0.349, p = 0.003, respectively). Additionally, a strong positive correlation was observed between FASN and AMPK mRNA expression levels (r = 0.309, p = 0.009). However, the UCP-2 gene did not show a statistically significant association with the FASN, AMPK, or SREBP-1c genes.

Table (4): Correlation between the studied gene expressions and the different measured parameters in obese class I

Genes / Parameters	SREBP-1c	FASN	UCP-2	AMPK
BMI (Kg/m^2)	0.761**	0.603**	0.833**	0.611**
TC (mg/dl)	0.678**	0.538**	0.627**	0.497*
HDL-c (mg/dl)	-0.306	-0.391*	-0.266	-0.129
TG (mg/dl)	0.771**	0.593**	0.783**	0.595**
LDL-c (mg/dl)	0.633**	0.489*	0.575**	0.469*

* Significant correlation at p< 0.05. ** Significant correlation at p< 0.01.

The expression levels of SREBP-1c, AMPK, UCP-2, and FASN were found to have a significant (p <0.001) positive correlation with BMI in mildly obese individuals, as shown in Table 4. Furthermore,

the mRNA levels of these genes were significantly ($p<0.001$) directly associated with total cholesterol, triglycerides, and LDL cholesterol. However, these genes showed a non-significant ($p > 0.05$) negative correlation with HDL-cholesterol, except for FASN, which demonstrated a significant negative correlation ($r = -0.391$, $p = 0.02$) in this class of obese patients (class I). Table 5 showed that in moderately obese individuals (obese class II), there was a significant ($p<0.001$) positive correlation between BMI, triglycerides, LDL, and total cholesterol with the expression levels of SREBP-1c, AMPK, UCP-2, and FASN, except for the AMPK gene, which has no significant correlation with LDL or total cholesterol. Furthermore, there was a significant ($p<0.01$) negative correlation between the studied genes and HDL-cholesterol.

Table (5): Correlation between the studied gene expressions and the different measured parameters in obese class II

Genes / Parameters	SREBP-1c	FASN	UCP-2	AMPK
BMI (Kg/m^2)	0.802**	0.671**	0.775**	0.632**
TC (mg/dl)	0.428*	0.563**	0.535**	0.319
HDL-c (mg/dl)	-0.485*	-0.670**	-0.688**	-0.449*
TG (mg/dl)	0.630**	0.848**	0.676**	0.629**
LDL-c (mg/dl)	0.328*	0.390*	0.363*	0.217

* Significant correlation at $p< 0.05$. ** Significant correlation at $p< 0.01$.

Table (6): Correlation between the studied gene expressions and different measured parameters in obese class III

Genes / Parameters	SREBP-1c	FASN	UCP-2	AMPK
BMI (Kg/m^2)	0.738**	0.705**	0.712**	0.605**
TC (mg/dl)	0.538**	0.310*	0.416**	0.113
HDL-c (mg/dl)	-0.275*	-0.170	-0.223	-0.147
TG(mg/dl)	0.526**	0.555**	0.609**	0.486*
LDL-c (mg/dl)	0.377*	0.185	0.233	0.001

* Significant correlation at $p< 0.05$. ** Significant correlation at $p< 0.01$.

In individuals with class III obesity (morbidly obese), the levels of SREBP-1c, FASN, and UCP-2 showed a significant ($p<0.01$) positive correlation with BMI, total cholesterol, and triglycerides. Additionally, a significant ($p<0.001$) direct association was observed between AMPK expression and both BMI and triglycerides. However, there was no statistically significant ($p > 0.05$) correlation found between LDL, HDL, or total cholesterol and AMPK expression levels. Only the SREBP-1c gene was significantly associated with HDL and LDL cholesterol ($r = -0.275$, $p = 0.032$, and $r = 0.377$, $p = 0.003$, respectively) in this group of obese participants (Table 6).

Table (7): ROC analysis of mRNA levels of studied genes in obese participants

	AUC	Cut-off value	Sensitivity %	Specificity %	P value	95% CI for AUC
SREBP-1c	1.000	3.105	100.0 %	100.0 %	<0.001	1.000 - 1.000
FASN	0.994	2.455	95.7 %	100.0 %	<0.001	0.983 - 1.000
UCP-2	0.999	3.085	98.6 %	100.0 %	<0.001	0.997 - 1.000
AMPK	0.900	2.115	72.9 %	100.0 %	<0.001	0.837 - 0.963

P value<0.001 is considered highly significant

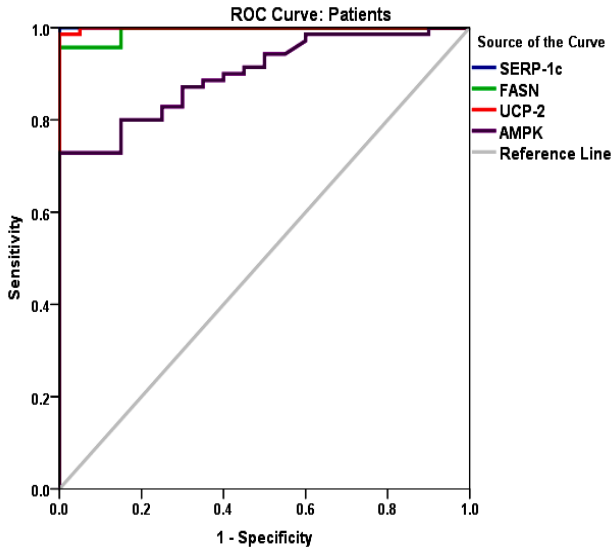

Figure (2): ROC curve of the studied genes in obese subjects

Table 7 indicated that the optimal cut-off values for the studied genes in determining obesity risk were as follows: 3.105 for SREBP-1c, 2.455 for FASN, 3.085 for UCP-2, and 2.160 for AMPK. Additionally, 7 and Figure 2 demonstrated that SREBP-1c exhibited the best performance in distinguishing between obese and normal-weight subjects (100% sensitivity and an area under the curve of 1.00), followed by UCP-2 and FASN, with sensitivities of 98.6% and 95.7, respectively, and areas under the curves of 0.999 and 0.994. On the other hand, AMPK displayed the lowest accuracy (72.9% sensitivity).

4. Discussion

Obesity prevalence has increased worldwide and reached epidemic proportions over the past few decades (**8**). The disruption of energy metabolism caused by overeating and physical inactivity is considered the major cause of obesity. However, genetic predisposition can also play a significant role in the imbalanced energy metabolism (**44**).The present study aimed to evaluate the expression of energy metabolism-related genes, such as SREBP-1c, FASN, AMPK, and UCP-2, in obese individuals. These genes play a critical role in energy regulation and fat metabolism, and their expression could enhance our understanding of the molecular mechanisms related to obesity **(10**). This study found that the expression of the investigated genes was higher in the adipose tissue of individuals with different grades of obesity compared to non-obese controls. Interestingly, the expression of these genes was higher in moderately obese individuals (obese class II) but not statistically significant compared to mildly obese individuals (obese class I). However, their expressions in morbidly obese individuals (obese class III) were more similar to those in obese class I. These findings were consistent with those of Auguet *et al*. (**45**) and Clemente-Postigo *et al.* (**46**), who found that morbidly obese patients have a lower expression of lipogenic and fatty acid oxidative genes. They suggested that as BMI increases, lipid storage capacity may decrease. This decrease could be related to adipose tissue performing a defense mechanism to prevent further expansion and fat accumulation in those classified as obese class III (morbidly obese). SREBP-1c is a transcription factor that upregulates important enzymes involved in lipid metabolism (**47**). The present study found that the mRNA level of SREBP-1c was significantly higher in the adipose tissue of obese individuals compared to non-obese individuals. Jannat *et al*. (**24**) revealed that SREBP-1c gene expression was significantly increased in the subcutaneous adipose tissue of obese

individuals, consistent with the present data. Our findings are also in agreement with those previously reported by Ebrahimi *et al*. (**48**). Furthermore, Crewe *et al.* (**49**) found an increase in SREBP-1c expression in obese mouse models. The present results suggest that SREBP-1c may play a significant role in the development of obesity, as increased levels of SREBP-1c will promote higher production of lipogenic enzymes like acetyl-CoA carboxylase and fatty acid synthase. This results in increased fatty acid synthesis and triglyceride production, leading to weight gain (**50**). On the other hand, Auguet and colleagues (**45**) demonstrated that SREBP-1c mRNA expression was significantly reduced in severely obese patients compared to controls, suggesting that adipose tissue may play a protective role against excessive fat accumulation in morbidly obese patients. A significant positive association was found between BMI, triglycerides, LDL, total cholesterol, and SREBP-1c mRNA levels in obese classes I, II, and III. These results suggested that as the expression of SREBP-1c mRNA increases, lipid levels and BMI increase, which may contribute to dysregulation of lipid metabolism and potentially increase the risk of cardiovascular disease and other obesity-related health issues (**48**). The current study also showed that the increased expression of SREBP-1c was accompanied by a corresponding increase in FASN expression. Furthermore, increased FASN expression was found to be positively associated with increased SREBP-1c expression in obese individuals. These results corroborated the regulatory relationship between SREBP-1c and FASN at the mRNA level (**21**). Supporting the present data, a previous study reported that the transcription factor SREBP-1c regulates FASN expression in adipose tissue and the liver (**51).** Jannat *et al.* (**24**) and Ebrahimi *et al*. (**48**) found that FASN expression levels were higher in obese adipose tissue than in non-obese individuals, which is consistent with the present data. Berndt *et al.* (**52**) also reported that FASN gene expression and protein levels were higher in obese adipose tissue than in normal-weight individuals and are positively correlated with BMI. Fatty acid synthase is a key enzyme in lipid metabolism and is thought to play a role in the etiology of human obesity. Elevated levels of FASN can lead to an increase in fatty acids, which can be stored as fat in the body, resulting in weight gain and obesity (**53**). FASN is also involved in the differentiation of pre-adipocytes into mature adipocytes, contributing to an increase in fat storage and obesity (**52**). The direct positive association observed in this study between FASN expression level and BMI, triglycerides, LDL, and total cholesterol in obese classes I, II, and III suggested that higher FASN expression may contribute to the dysregulation of lipid metabolism caused by obesity (**28**). The present results highlight the potential importance of targeting FASN as a potential therapeutic strategy for obesity. One key regulator of energy balance is the mitochondrial carrier protein, uncoupling protein-2. It disrupts the transfer of protons generated by the electron transport chain from ATP synthesis (**54**). Most studies have mainly focused on the polymorphisms of the UCP-2 gene in humans, while its expression is more emphasized in rodents (**55–57**). Thus, the present study assessed the expression of the UCP-2 gene in human adipose tissue and found that UCP-2 expression was higher in obese individuals compared to controls. Given that UCP-2 is involved in increasing energy expenditure in adipose tissue, this result may appear paradoxical since obese individuals have a high fat mass (**14**). The increased UCP-2 expression revealed in this study could be a protective mechanism against the higher beta-oxidation of fatty acids and the generation of reactive oxygen species linked to obesity (**58**).The present data were consistent with those of Souza et al. (**59**), who demonstrated that human blood cells exhibited increased levels of UCP-2 expression. In addition, Pheiffer and colleagues **(56)** observed a 4.6 and 3.0-fold increase in UCP-2 mRNA and protein levels, respectively, in severely obese rats. Additionally, we observed a significant positive correlation between UCP-2 mRNA levels and BMI in obese individuals from different classes. This finding may support the idea that UCP-2 could be a candidate gene for studying obesity (**15**). Another important finding of the current study was that mRNA levels of the AMPK gene were significantly overexpressed in subcutaneous fat samples of obese subjects than in lean controls. These findings corroborate those of Martnez-Agustin *et al.* (**60**), who found that individuals with morbid obesity have higher levels of AMPK gene expression in subcutaneous tissue. Kola et al. (**61**) also observed changes in AMPK activity in individuals with obesity and metabolic syndrome. Other research has shown that AMPK activity decreases in the adipose tissue of both obese individuals and animal obesity models (**62–64**). AMPK is a vital energy sensor that regulates cellular metabolism, particularly lipid metabolism. Its role in maintaining energy balance involves activating ATP-producing pathways and inhibiting energy-consuming pathways (**18**). In obese individuals, higher AMPK expression might be a result of the body's attempt to restore energy balance and regulate metabolism. This could result in

increased energy expenditure and potentially prevent further fat storage (**65**). Consequently, AMPK is considered a potential target for treating hyperlipidemia and obesity (**66, 67**). It has been established that AMPK decreases FASN expression by down-regulating SREBP-1c (**41**). However, the current study observed a direct and significant association between AMPK and both SREBP-1c and FASN expression levels. This divergent result may be due to variations in metabolic traits, ethnicity, or the size of the study population. The data obtained from ROC curve analysis demonstrated that the studied genes could effectively differentiate between obese individuals and control subjects. These results suggested that these genes could be used as early predictors of obesity risk.

5. Conclusion

The present study concludes that SREBP-1c, UCP-2, FASN, and AMPK may have a significant role in obesity and could potentially serve as early indicators to assess an individual's susceptibility to obesity. The findings of this study may also help in understanding the molecular mechanisms related to obesity, which could lead to the development of prevention and treatment strategies for obesity.

6. Acknowledgments

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7. Conflicts of interest

There are no conflicts of interest.

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