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Association of AMP-activated protein kinase and uncoupling protein-2 gene expressions with hypertension in obese patients

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Background

The obesity is a multifactorial metabolic disease resulting from behavioral and genetic factors, which linked to hypertension.

Objective

The study was aimed to examine the association of uncoupling protein 2 (UCP2) and AMP-activated protein kinase (AMPK) gene expressions in obese and hypertension, to explore their potential roles in human obesity and hypertension. Moreover, their associations with lipid profiles were determined.

Materials and methods

Subcutaneous adipose tissues (SAT) were collected from 131 subjects, including 45 non-obese and non-hypertensive controls, 86 obese subjects (51 obese only and 35 with hypertension patients. Utilizing real-time reverse transcriptase polymerase chain reaction (RT-PCR), the expressions of the UCP2 and AMPK genes were determined.

Results and conclusion

The AMPK and UCP2 gene expressions were statistically significant when comparing the obese and hypertension groups to controls. Additionally, UCP2 and AMPK expression levels revealed high sensitivity and specificity using ROC curve analysis. Significant negative correlations were found between AMPK gene expression and cholesterol and LDL levels in hypertension and obese patients. While UCP2 expression showed a positive correlation with LDL and cholesterol in obese. These results indicated that UCP2 and AMPK gene expressions could play major roles as candidate genes for the development of obesity as well as hypertension. Correspondingly, their correlations with cholesterol and LDL implicated their roles in lipid metabolism. The correlations of the AMPK and UCP2 genes with hypertension and obesity may provide an alternative approach for managing obesity and its related-hypertension.

Keywords: obesity, hypertension, uncoupling protein 2 (UCP2), AMP-activated protein kinase (AMPK), gene expressions.

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Introduction

The abnormal deposition of fat in adipose tissue that may lead to health issues is referred to as obesity [1]. In general, an individual with a body mass index (BMI) of greater than 30 kg/m^2 is considered to be obese [2]. It is described as an imbalance in the management of energy balance resulting in an abnormal accumulation of body fat. Due to its role as a risk factor for numerous other chronic illnesses, such as cancer, type II diabetes, hypertension, chronic renal disorders, and cardiovascular disease, its rising prevalence represents a serious public health problem [3].

Since abdominal obesity affects the immunological and endocrine systems and increases the risk of insulin resistance, diabetes, hypertension, and cardiovascular disease, there is a tight relationship between obesity and hypertension [4]. Furthermore, regardless of race, ethnicity, or sex, obesity is known to be a significant risk factor for hypertension in children as well as adults, genetic predisposition has a significant role in the interaction between obesity and hypertension [5]. On the other hand, the precise genes that directly contribute to hypertension and obesity remain unidentified [6]. The pathophysiology of obesity-related hypertension is being studied to clarify the involvement of genetics, using a variety of molecular mechanisms (both genetic and epigenetic) [7].

The uncoupling proteins (UCPs) are mitochondrial proteins that disperse the inner membrane electrochemical potential as heat

(energy homeostasis) [8]. The gene UCP2 is thought to be extremely important for controlling intracellular adenosine triphosphate (ATP). On chromosome 11, the human UCP2 gene is located. These chromosomes contain sites for the UCP2 gene that are linked to obesity [9]. The significant function that UCP2 gene expression plays in some metabolic conditions originates from its function in modifying the rate of metabolism and raising BMI [3]. It results in an imbalance in the ratio of energy intake to energy expenditure, which can promote obesity [9]. Thus, it was postulated that UCP2 gene expression is crucial for obesity because of its pivotal involvement with weight gain [10].

The AMP-activated protein kinase (AMPK) is an important factor in controlling the metabolism of adipose tissue. The available data demonstrates that AMPK activation is linked to white brown adipose tissue thermogenesis, fatty acid oxidation, lipogenesis/adipogenesis, and browning of white adipose tissue [11; 12]. As a consequence of its critical role in physiology and pathology, AMPK, a key regulator of cellular metabolism, promotes phosphorylation of target substrates and is essential to maintaining and regulating of energy homeostasis, making it one of the most promising targets for both the prevention and the management of obesity [13; 14].

The present study was undertaken to examine the gene expressions of UCP2, and AMPK in order to assess the prospective roles of AMPK and UCP2 in patients with obesity and hypertension. Furthermore to elucidate the associations of the above-mentioned genes with anthropometric parameters and lipid profiles.

Materials and methods

The study design

The current study conducted on 131 individuals, dividing into three groups 1) Obese group: 51 obese patients without hypertension; 2) Hypertension group: 35 obese with hypertension; 3) Control group: 45 (age-sex matched) non-obese and non-hypertensive subjects. The world health organization (WHO) and the national institute of health both defined obesity as having a BMI of more than 30 kg/m^2 [15]. Hypertension patients were already diagnosed as having blood pressure ≥130/85 mm Hg or receiving drug therapy for hypertension. None of the cases had any other disease that may interfere with the study parameters or were deteriorated cases, including type 2 diabetes (T2D), cardiac, renal, liver, and thyroid diseases.

The current study was done in biochemistry department, national research centre (NRC), Egypt. Written informed consent was obtained from all participants, the study was approved by the medical research ethics committee of the national research centre (No. 19-162). The study was done according to the code of ethics of the world medical association (declaration of Helsinki) for studies involving humans.

Tissue samples collections from subcutaneous adipose tissue (SAT)

The SAT samples (~1–5 g) were obtained from obese patients admitted to kasr Al-ainy hospital, Cairo university, Egypt during bariatric surgery and from control subjects underwent an elective surgical procedure such as repair of hernias, gall bladder stone, and appendicitis. SAT were gathered at the site of the transverse lower abdominal incision.

Anthropometric measurements and biochemical estimation

The anthropometric measurements are including height and weight. The case report provided clinical information, including measurements of blood pressure. The BMI is measured via dividing body weight (kg) / height (m^2).

Fasting serum levels of total cholesterol, triglycerides, and the HDL were measured with an enzymatic colorimetric method (Stanbio Laboratory, Boerne, Texas, USA). The LDL was calculated using Friedewald's formula as: LDL-c (mg/dL) = TC - HDL-c - TG/5 [16].

Ribonucleic acid (RNA) extraction from adipose tissue

Following excision, the adipose tissue was promptly placed in liquid nitrogen and preserved at -80°C until required. RNeasy liquid tissue extraction minikit (Qiagen Inc., Germantown, Maryland, USA) was used to extract total RNA in accordance with the manufacturer's instructions. The absorbance at 260 and 280 nm was used to quantify RNA. Total RNA was kept at -80°C.

Real-time assessment of uncoupling protein 2 (UCP2) and AMP-activated protein kinase (AMPK) gene expression using reverse transcriptase polymerase chain reaction (RT-PCR)

Reverse transcription (RT) was used to convert RNA to cDNA using Qiagen's taqman RT reagents in accordance with the manufacturer's instructions. An applied biosystems 2700 real-time PCR was utilized for quantitative RT-PCR. The taqman universal PCR master mix was employed. Applied biosystems also provided taqman primers and probes for β actin (internal control), UCP2, and AMPK. A duplicate of each sample was run. The cDNA sample was added, but otherwise the same setup was employed for the negative control. Under control circumstances, no PCR product was found. To summarize, UCP2, AMPK, and β actin were separately amplified for 10 min at 95°C. After that, the annealing and extension stages were repeated in cycles consisting of 95°C for 15 sec and 60°C for 1 min. Real-time measurements of the rise in fluorescence were performed during the extension step. Every run of samples (on each plate) in the PCR system was conducted alongside a calibrator sample (controls). The fold changes in gene expression are displayed using the $2^{-\Delta\Delta Ct}$ method [17].

Statistical analysis

The quantitative data are expressed as mean ± SE. One-way ANOVA was used to compare the means of the studied groups. A correlation analysis was carried out to assess the relation between various variables and gene expressions. A significance value of $p < 0.05$ was applied to all statistical tests. SPSS Version 21 (SPSS Inc., Chicago, USA) was used for all analysis. Receiver operator characteristic (ROC) curves were used for obesity and hypertension diagnoses depending on the accuracy of UCP2 and AMPK genes.

Results and discussions

The clinical features of study participants, which involved 45 healthy controls, 51 obese and 35 obese with hypertension patients, are shown in Table 1. Hypertension group was significantly elevated compared with control and obese subjects according to age as the risk of high blood pressure increased in the elderly persons. The mean levels of BMI were significantly increased in obese and hypertension patients when compared to controls.

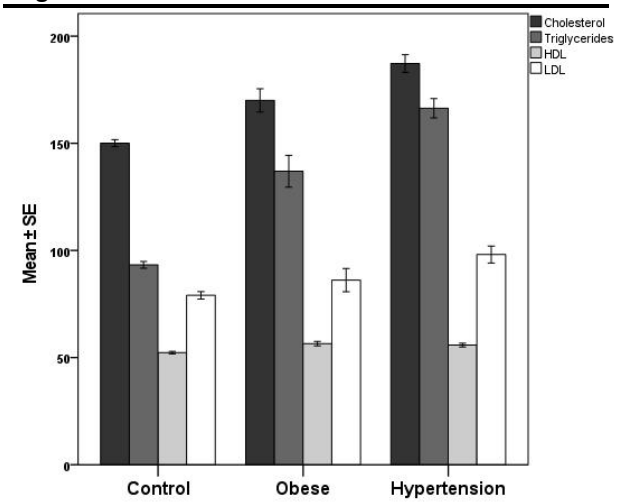
Regarding lipid profile of the studied groups, cholesterol and triglycerides were significantly raised in hypertension (187, 166 mg/dL; respectively) and obese groups (TC= 170 and TG= 137 mg/dL) compared to control group (TG= 93; TC= 150 mg/dL). While, LDL exhibited significantly higher levels in patients with hypertension (98 mg/dL) compared with obese (86 mg/dL) and controls (79 mg/dL). There was no remarkably association concerning HLD within groups (Figure 1).

Table 1 Demographic characteristic of the studied subjects

Variables	Control	Obese	Obese + Hypertension
Number	45	51	35
Sex (M:F)	15:30	11:40	14:21
Age (years)	34.1±0.9	33.7±1.4	42.7±1.8*†
BMI (Kg/m^2)	21.7±0.3	45±1*	42±1.6*

The variables are presented as mean ± SE or frequency. *p < 0.05 vs. control group; †p < 0.05, vs. obese group

Figure 1



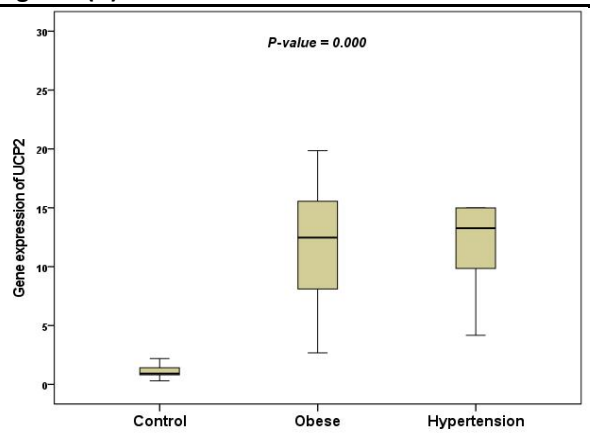
Lipid profile of study groups.

The UCP2 gene expression levels were higher in both hypertension (14.55) and obese (11.48) when compared to the controls (1.12). In addition, the levels of AMPK gene were more increase in hypertension and obese groups (3.8 and 2.85, respectively) than control group (1.14). UCP2 and AMPK gene expressions revealed higher significant associations between hypertension and obese groups when compared with the control group ($p< 0.001$) (Figure 2A & 2B).

The significant negative correlations ($p<0.05$) were found in a scatter plot between the

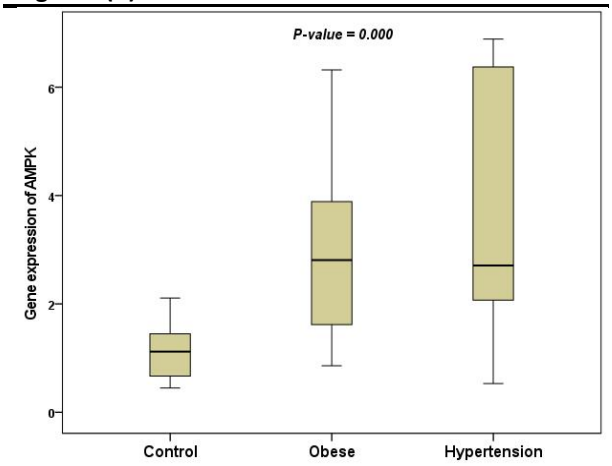
expression of the AMPK gene and LDL and cholesterol in the obese group (Figure 3A). Concerning hypertension group, there were significant negative correlations in cholesterol and LDL (Figure 3B). Whereas, the gene expression of UCP2 indicated positive relations with cholesterol and LDL levels for hypertension patients (Figure 4).

Figure 2(A)



Uncoupling protein 2 (UCP2) gene expressions of control, obese and hypertension groups.

Figure 2(B)

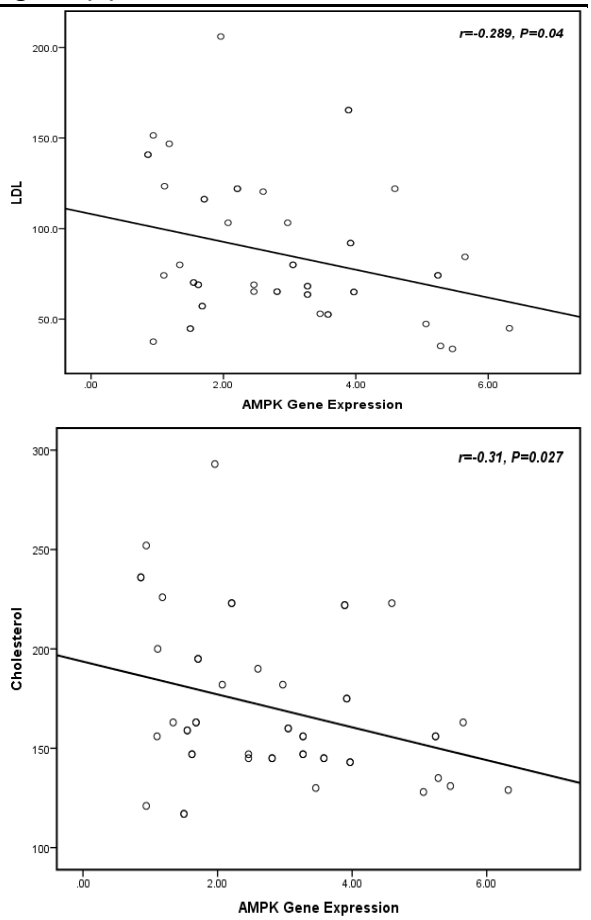


AMP-activated protein kinase (AMPK) gene expression of control, obese and hypertension groups.

The ROC curve analysis was used to evaluate the sensitivity and specificity of AMPK and UCP2 expressions in obese sera (Figure 5A). UCP2 expression levels exhibited high specificity and sensitivity, with 97.8% and 100% respectively, at a cut off value 2.44 while, the expression levels of AMPK displayed 86.3% sensitivity and 71.1% specificity, with a cut off value 1.34 (Table 2). Regarding to hypertension, the specificity and sensitivity of AMPK expression were 88.9 & 88.6 % respectively and UCP2 were 100 % (Table 3 and Figure 5B).

The obesity is considered as excessive accumulation of fat that presents a health risk. The interplay between obesity and hypertension including many factors as oxidative stress, hemodynamic alterations, insulin resistance, hyperinsulinemia, and renal injury, as well as mitochondrial factors, genetics, and epigenetics [5]. Numerous candidate genes are linked to obesity like the genes encoding uncoupling proteins (UCP) and AMP-activated protein kinase (AMPK) genes [14; 18].

Figure 3(A)



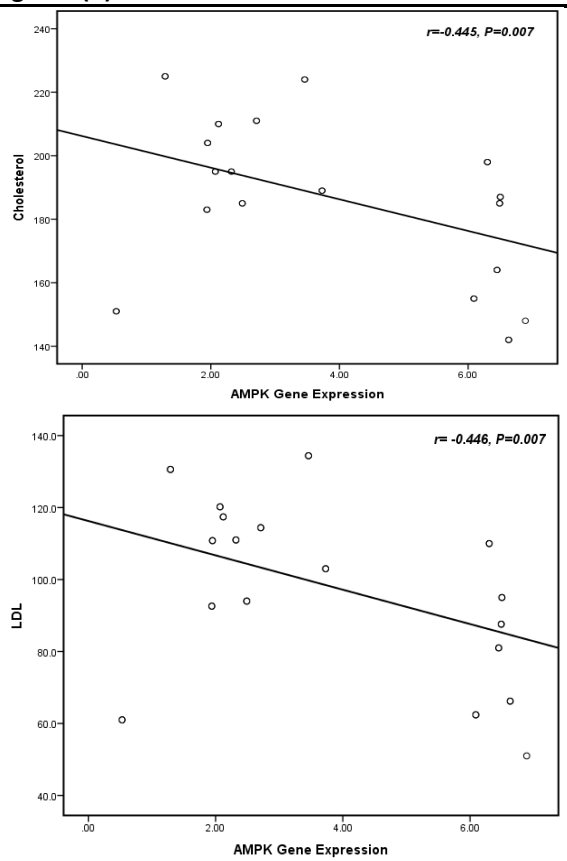
Correlations of AMP-activated protein kinase (AMPK) gene with lipid profile in obese group.

We are aware of no prior research evaluating UCP2 and AMP gene expressions in human adipose tissue of obese with its related-hypertension complication. Furthermore, associations of these gene expressions from both the hypertensive and obese groups with lipid profile were elucidated.

The UCP2 is defined as mitochondrial membrane transporter implicated in energy balance regulation which is expressed in white adipose tissue [9]. Though lean body mass is an essential organ for energy usage and

protection opposed to fat accumulation, the UCP2 function and expression in lean body mass have still unclear, however some reports display the relationship of UCP2 gene expression and fat mass in adipose tissues [3; 9; 19; 20]. Particularly, a prior research has suggested that UCP2 gene polymorphisms could elevate the possibility of metabolic syndrome along with central obesity in Asian population [21].

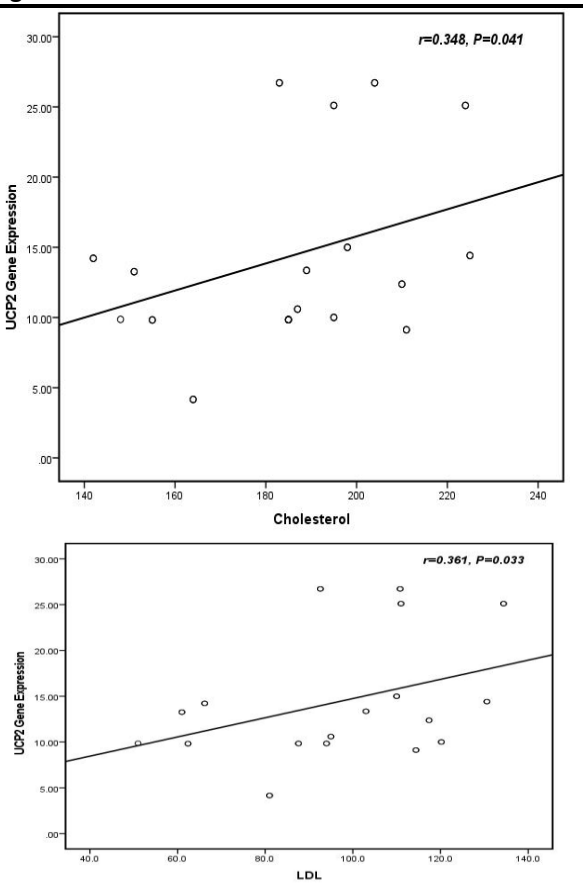
Figure 3(B)



Correlations of AMP-activated protein kinase gene with lipid profile in hypertension group.

To explore the risk of UCP2 gene in obesity and hypertension, a significant increase was observed in UCP2 gene expression among obese and hypertension compared to controls (p<0.001). In the same line, a recent study indicated an increased UCP2 level in obese women, subsequently reduction in energy expenditure should be associated with additional fat mass and overweight; therefore, higher abundance of UCP2 expression interrelated with the development of obesity in women. Collectively, UCP2 might be a diagnostic tool for obesity as well as a molecular target for curing obesity and its complications like hypertension [22; 23]. This could be explained as UCP2 is located in the center of energy metabolism; acting a vital role in the identification, diagnosis, and management of obesity. Where, UCP2 avoids adipose tissue accumulation via heat generation which controls energy consumption and thermogenesis [24; 25]. The UCP2 exhibit an apparent association with the grade of different diseases such as obesity and its comorbidities (hypertension, diabetes, etc.) [26].

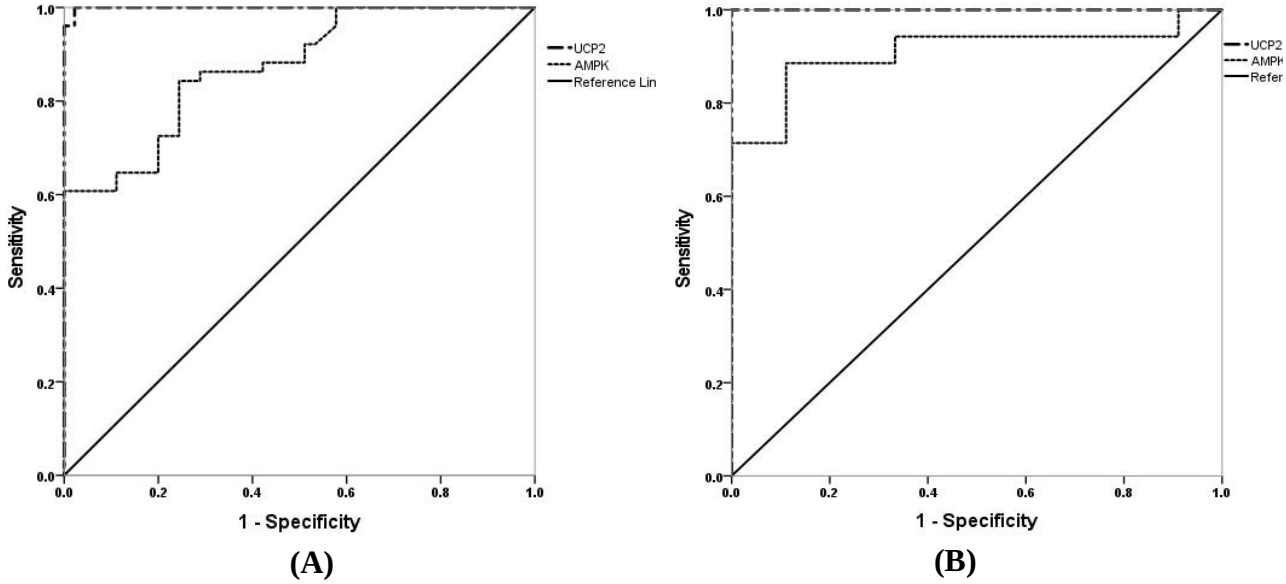
Figure 4



Correlations of uncoupling protein 2 gene with lipid profile in hypertension group.

On the other hand, because genetic variations could affect the results, a human investigation assessed inadequate evidence for the relationship between polymorphisms of UCP2 and BMI [27]. No correlation between UCP2 and variations in BMI was found in the Chinese populace [28]. There was confusion about whether a higher UCP2 expression was negatively correlated with the degree of obesity; Cortes-Oliveira C, et al. noticed that elevated UCP2 expression contributing decline weight in animals following a hypo-caloric diet intervention [29]. Further research showed that obese participants' lower UCP2 gene expression relative to non- obese individuals resulted in a reduction in energy expenditure and an increase in body fat storage [21; 30-32].

Figure 5



Receiver operating characteristic (ROC) curve analysis of AMP-activated protein kinase (AMPK) and uncoupling protein 2 (UCP2) in obese (A) and hypertension (B) groups.

Table 2 Results of ROC analysis for UCP2 and AMPK in obese patients

Genes	AUC	S.E	Sig.	Cutoff	Sensitivity %	Specificity %	Accuracy %	95% CI
UCP2	0.999	0.001	0.000	2.44	100%	97.8%	98.9%	0.997 - 1
AMPK	0.873	0.034	0.000	1.34	86.3%	71.1%	78.7%	0.806 - 0.94

AUC: area under the curve, SE: standard error, 95% CI: 95% confidence interval.

Table 3 Receiver operating characteristic curve analyses in hypertension group for uncoupling protein 2 (UCP2) and AMP-activated protein kinase (AMPK) genes

Genes	AUC	S.E	Sig.	Cutoff	Sensitivity %	Specificity %	Accuracy %	95% CI
UCP2	1	0.000	0.000	3.44	100%	100%	100%	1 - 1
AMPK	0.91	0.039	0.000	1.9	88.6%	88.9%	88.75%	0.834 - 0.986

AUC: Area under the curve; SE: Standard error; 95% CI: 95% Confidence interval

There is information on UCP2 gene expression in human adipose tissue from obese individuals, while there is less information from hypertension patients. According to Muhammad et al.'s findings, variations in the UCP2 gene were linked to alterations in blood pressure [33]. As a result, the current study may provide an overview of UCP2's function in human adipose tissue of hypertensive patients. Contradictory to our finding, some recent reports found that UCP2 gene expression is down-regulated in patients with obesity when compared with normal weight subjects. Hence, an inhibition in UCP2 expression may result in a decline in active thermogenesis and the deposition of fat in adipose tissue [32; 34; 35]. Likewise, according to Margaryan et al., UCP2 mRNA expression was lower in all subjects with metabolic diseases, such as hypertension, than in the controls [19] Prior research conducted in China and North America found that UCP2 expression raised the risk of obesity and was linked to abdominal obesity or elevated body mass index in comparison to controls [36; 37]. Nevertheless, a meta-analysis research revealed that European and Asian population lacked this link [38]. These results imply that the ethnic group has an impact on the ability of UCP2 to increase obesity predisposition [39].

The UCP2 gene may be involved in the control of lipid metabolism, as evidenced by its correlation with the lipid parameters. Potentially via its role as a transporter of free fatty acids, UCP2 could be involved in lipids management. In the present study, a positive correlation was observed in the hypertension group between TC and LDL and UCP2 gene expression. This insight could provide us with innovative therapeutic approaches for

overcoming diseases associated with obesity like hypertension. Accordingly, it has been shown that the UCP2 gene is linked to higher lipid profiles and greater likelihood of dyslipidemia [40; 41]. On the other hand, lack of association with lipid levels has also been reported [42; 43].

The AMPK acts as a nutrient and energy sensor that is currently known to be crucial in fat metabolism, particularly in controlling the energy expenditure of adipose tissue which has emerged as one of the most attractive targets in the avoidance and cure of obesity due to its essential role in pathology as well as physiology [14; 44]. The present study demonstrated a significant raise in the AMPK gene expression level of obese and hypertensive patients compared to controls, which is consistent with the AMPK's association with a broad range of pathological conditions, including obesity and metabolic syndrome disorders like hypertension [45; 46]. Moreover, Martinez-Agustin et al. [44] found an elevated level of total AMPK in subcutaneous adipose tissue of morbidly obese subjects in Spanish populace. Meanwhile, on humans and rodents exhibited that AMPK accumulation in adipose tissue is linked to the metabolic syndrome and its associated diseases [47]. In contrast, Gauthier et al. have proposed a direct correlation between lowered AMPK activity and obese patients who were insulin-resistant in American population [48]. Furthermore, there is an intense association between AMPK activity and BMI in Brazilians, indicating a relationship between AMPK gene expressions and a lower BMI. All of these findings illustrate a strong relationship between AMPK activity and the progression of obesity in various ethnicities [49].

Concerning the role of AMPK in lipid metabolism, there were negative correlations between AMPK gene expressions and TC and LDL in both obese and hypertension groups. The role of AMPK in lipolysis is controversial. While research implies that AMPK has an anti-lipolytic impact [50], another hypothesize that AMPK actually enhances lipolysis [51]. This variation might be attributed to the AMPK's tissue-specific action under certain circumstances. On this basis, an increase in lipolysis could indirectly activate AMPK [14].

Mao et al [52] examine the possible mechanism underlying the interaction between AMPK and UCP2 using gain and loss of function studies. The phosphorylation level of AMPK varied in response to UCP2 overexpression or silencing, while AMPK expression remained mostly unchanged. Some prior research indicated a relationship between UCP2 and AMPK in hypertension. Nonetheless, the exact mechanism is remaining unclear [53].

The current study has some limitations that need to be noted. Our sample size was relatively small due to challenges in approving and obtaining adipose tissue biopsies from our patients. Furthermore, we could only access subcutaneous fat that had been separated from the abdominal area. Consequently, we focused our analyses on this biological sample. Owing to the small amount of samples obtained, we could only examine the gene expression of the adipose tissue and could not verify our conclusions at the protein level.

Conclusion

These results suggested that UCP2 and AMPK gene expressions might be useful genetic biomarkers for obesity and hypertension in Egyptian patients. These data highlight the different metabolic role of these genes in the development of the obesity and its comorbidities. Nevertheless, further studies are required to illustrate the function of AMPK and UCP2 as prospective therapeutic targets for metabolic disorders like hypertension and obesity.

Conflicts of interest

The authors declare there are no conflicts of interest.

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Authors' contributions

Concepts and Design: Mie Afify, Mohamed D.E. Abdelmaksoud, and W. I. Hamimy; Definition of intellectual content: El sayed Mahdy, Hatem El mezyen, and W. I. Hamimy;

Literature search, Clinical studies, and Data acquisition: Mariem M. Kelany, Weaam Gouda, Lamiaa Mageed, and Amgad K. Hassan; Data analysis and Statistical analysis: Weaam Gouda and Lamiaa Mageed; Manuscript preparation: Mariem M. Kelany, Weaam Gouda, and Lamiaa Mageed; Manuscript editing and review: Mie Afify, Mohamed D.E. Abdelmaksoud, El sayed Mahdy, and Hatem El mezyen; Guarantor: Mie Afify.

Ethical considerations

This study was performed in line with the principles of the declaration of Helsinki. The approval was granted by the medical ethics committee of the national research centre (approval number: 19-162).

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