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

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Background
Obesity is a multifactorial metabolic disease resulting from behavioral and genetic factors, which are linked to hypertension.
Objective
The study aimed to examine the association of uncoupling protein 2 (UCP2) and AMP-activated protein kinase (AMPK) gene expressions in obesity and hypertension, to explore their potential roles in human obesity and hypertension. Moreover, their associations with lipid profiles were determined.
Patients and methods
Subcutaneous adipose tissues were collected from 131 participants, including 45 nonobese and nonhypertensive controls, 86 obese participants (51 obese only and 35 patients with hypertension also). Utilizing real-time reverse transcriptase PCR, expressions of the UCP2 and AMPK genes were determined.
Results and conclusion
AMPK and UCP2 gene expressions were statistically significant when comparing obese and hypertension groups to controls. In addition, UCP2 and AMPK expression levels revealed high sensitivity and specificity using receiver operating characteristic curve analysis. Significant negative correlations were found between AMPK gene expression and cholesterol and low-density lipoprotein (LDL) levels in hypertension and obese patients. However, 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 and hypertension. Correspondingly, their correlations with cholesterol and LDL implicated their roles in lipid metabolism. Correlations of the AMPK and UCP2 genes with hypertension and obesity may provide an alternative approach for managing obesity and its associated hypertension.

Keywords:
AMP-activated protein kinase, gene expressions, hypertension, obesity, uncoupling protein 2

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Introduction

The abnormal deposition of fat in the adipose tissue that may lead to health issues is referred to as obesity [1]. In general, an individual with a BMI 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].

As 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]. However, 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].

Uncoupling proteins (UCPs) are mitochondrial proteins that dissipate the inner membrane

electrochemical potential as heat (energy homeostasis) [8]. The gene UCP2 is thought to be extremely important for controlling intracellular 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 the 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 energy homeostasis, making it one of the most promising targets for both the prevention and management of obesity [13,14].

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

Patients and methods

The study design

The current study was conducted on 131 individuals, divided into three groups: the obese group included 51 obese patients without hypertension, the hypertension group included 35 obese patients with hypertension, and the control group: 45 (age–sex-matched) nonobese and nonhypertensive patients. The 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 more than or equal to 130/85 mmHg 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, cardiac, renal, liver, and thyroid diseases.

The current study was done in the 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 sample collections from subcutaneous adipose tissue

The subcutaneous adipose tissue (SAT) samples (~1–5 g) were obtained from obese patients admitted to Kasr Al-Ainy Hospital, Cairo University, Egypt during bariatric surgery and from controls, who underwent an elective surgical procedure such as repair of hernias, gall bladder stone, and appendicitis. SAT samples were gathered at the site of the transverse lower abdominal incision.

Anthropometric measurements and biochemical estimation

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

Fasting serum levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein were measured using the enzymatic colorimetric method (Stanbio Laboratory, Boerne, Texas, USA). The low-density lipoprotein (LDL) was calculated using Friedewald's formula as LDL-cholesterol (mg/dl)=TC–high-density lipoprotein-cholesterol–TG/5 [16].

RNA extraction from adipose tissue

Following excision, the adipose tissue was promptly placed in liquid nitrogen and preserved at −80°C until required. RNeasy lipid tissue mini extraction kit (Qiagen Inc., Germantown, Maryland, USA) was used to extract total RNA following 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 and AMP-activated protein kinase gene expression using reverse transcriptase PCR

Reverse transcription (RT) was used to convert RNA to cDNA using Qiagen's TaqMan RT reagents following the manufacturer's instructions. Applied Biosystems 2700 real-time PCR was used for quantitative RT-PCR. The TaqMan universal PCR master mix was used. 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 used 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 s 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 analysis of variance was used to compare the means of the studied groups. A correlation analysis was carried out to assess the relationship between various variables and gene expressions. A significance value of *P* value less than 0.05 was applied to all statistical tests. SPSS Inc., Chicago, USA (version 21) was used for all analyses. Receiver operating characteristic curves were used for obesity and hypertension diagnoses depending on the accuracy of UCP2 and AMPK genes.

Results and discussions

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

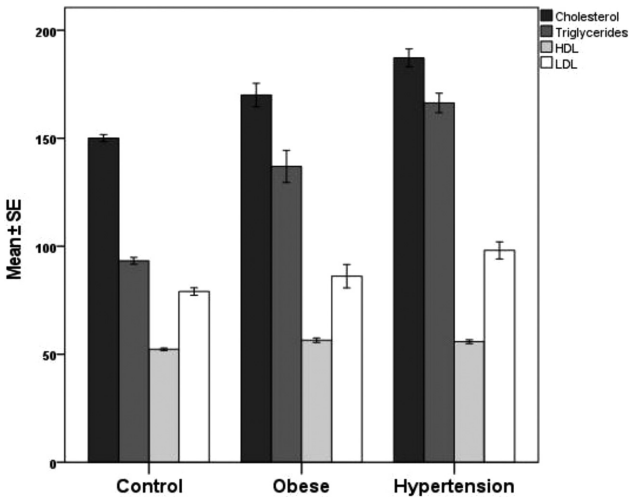
Regarding the lipid profile of the studied groups, cholesterol and TGs were significantly raised in hypertension (187, 166 mg/dl, respectively) and obese groups (TC=170 and TG=137 mg/dl) compared with the control group (TG=93; TC=150 mg/dl). However, LDL exhibited significantly higher levels in patients with hypertension (98 mg/dl) compared with obese

Table 1 Demographic characteristics of the studied participants

Variables	Control	Obese	Obese+hypertension
Number	45	51	35
Sex (male : female)	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*

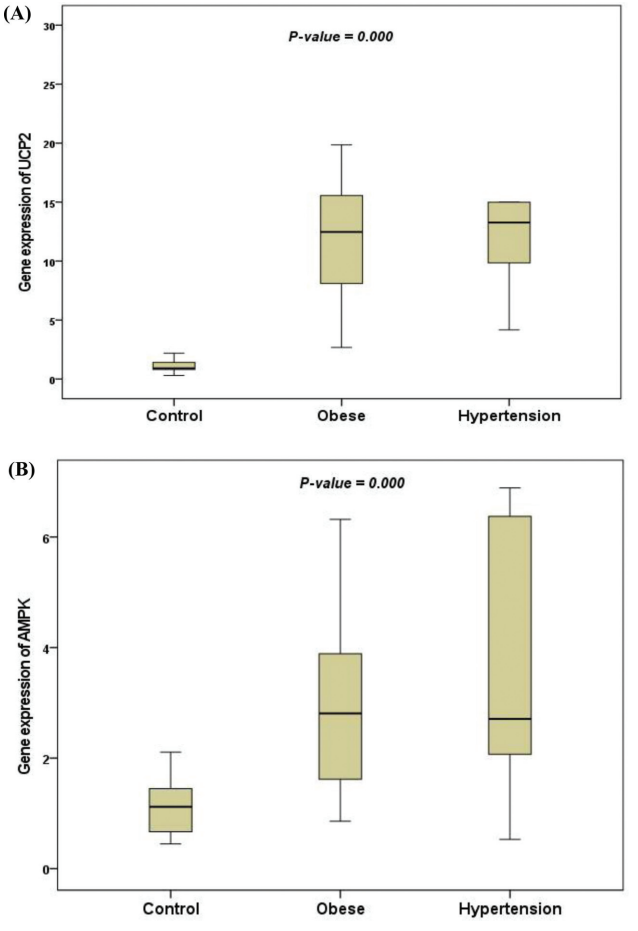
The variables are presented as mean±SE or frequency. **P* value less than 0.05 versus control group. †*P* value less than 0.05 versus obese group.

Figure 1



Lipid profile of study groups.

Figure 2



(a) Uncoupling protein 2 (UCP2) gene expressions of control, obese, and hypertension groups and (b) AMP-activated protein kinase (AMPK) gene expression of control, obese, and hypertension groups.

(86 mg/dl) and controls (79 mg/dl). There was no significant association concerning HDL within groups (Fig. 1).

UCP2 gene expression levels were higher in both hypertension (14.55) and obese (11.48) patients when compared with the controls (1.12). In addition, the levels of the AMPK gene were increased in hypertension and obese groups (3.8 and 2.85, respectively) than the control group (1.14). UCP2 and AMPK gene expressions revealed higher significant associations between hypertension and obese groups when compared with the control group ($P<0.001$) (Fig. 2a and b).

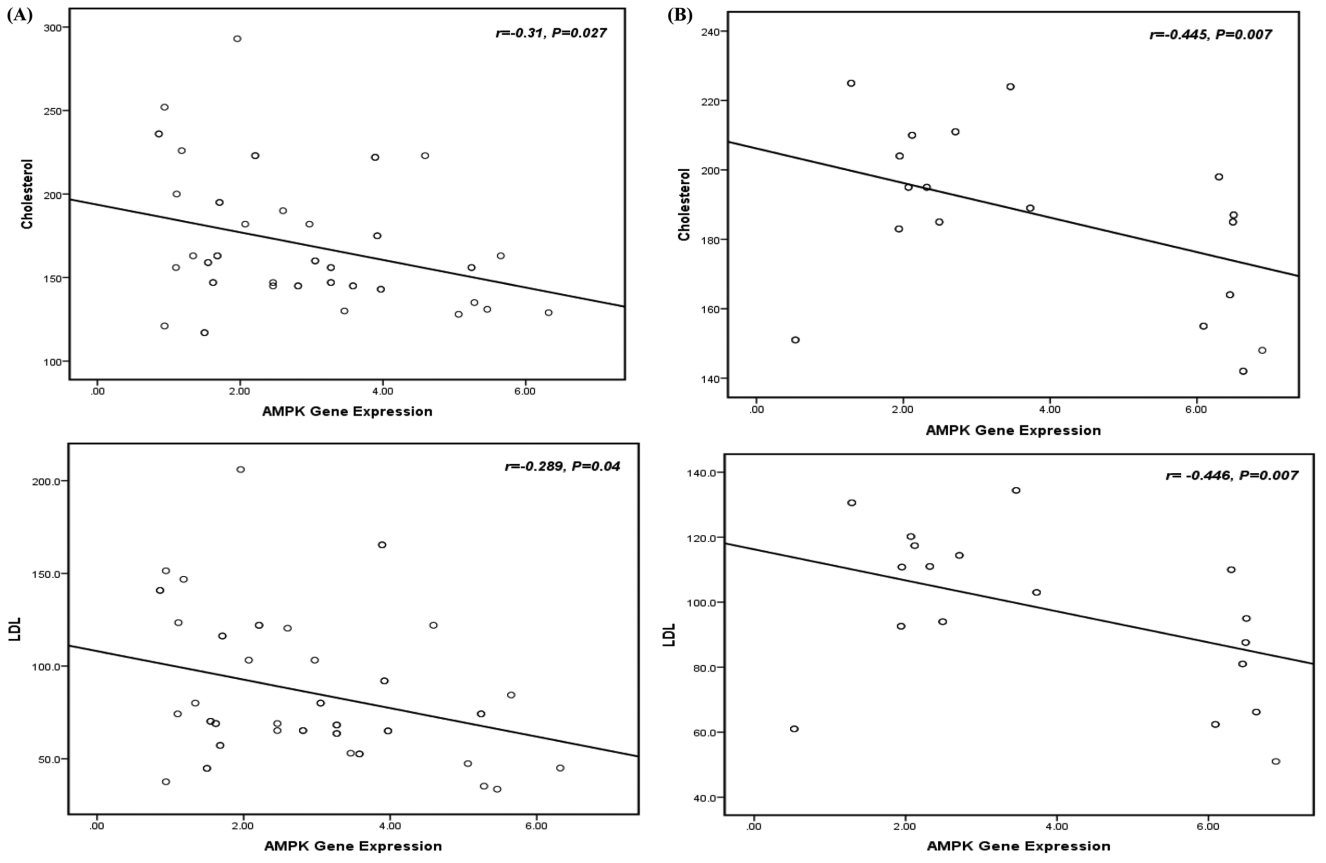
Significant negative correlations ($P<0.05$) were found in a scatterplot between the expression of the AMPK gene and LDL and cholesterol in the obese group (Fig. 3a). Concerning the hypertension group, there were significant negative correlations in cholesterol and LDL (Fig. 3b); however, the gene expression of UCP2 indicated positive relations with cholesterol and LDL levels for hypertension patients (Fig. 4).

The receiver operating characteristic curve analysis was used to evaluate the sensitivity and specificity of AMPK and UCP2 expressions in obese sera (Fig. 5a). UCP2 expression levels exhibited high specificity and sensitivity, with 97.8 and 100%, respectively, at a cutoff value of 2.44 while the expression levels of AMPK displayed 86.3% sensitivity and 71.1% specificity, with a cutoff value of 1.34 (Table 2). Regarding hypertension, the specificity and sensitivity of AMPK expression were 88.9 and 88.6%, respectively, and UCP2 levels were 100% (Table 3 and Fig. 5b).

Obesity is considered an excessive accumulation of fat that presents a health risk. The interplay between obesity and hypertension includes many factors such 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 UCP and AMPK genes [14,18].

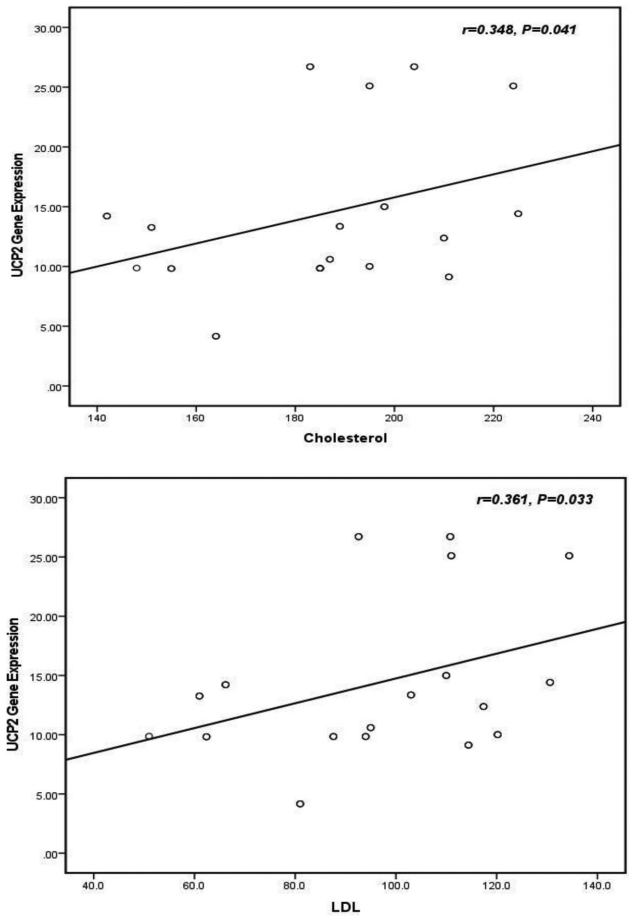
We are aware of no prior research evaluating UCP2 and AMP gene expressions in human adipose tissue of obese with its associated hypertension complication. Furthermore, associations of these gene expressions from both hypertensive and obese groups with lipid profiles were elucidated.

Figure 3



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

Figure 4



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

The UCP2 is defined as a mitochondrial membrane transporter implicated in energy balance regulation, which is expressed in the white adipose tissue [9]. Though lean body mass is an essential organ for energy use and protection compared with fat accumulation, the UCP2 function and expression in lean body mass remain unclear. However, some reports indicate a relationship between UCP2 gene expression and fat mass in adipose tissues [3,9,19,20]. Particularly, prior research has suggested that UCP2 gene polymorphisms could elevate the possibility of metabolic syndrome along with central obesity in the Asian population [21].

To explore the risk of the UCP2 gene in obesity and hypertension, a significant increase was observed in UCP2 gene expression among obese and hypertension compared with controls ($P<0.001$). Along the same line, a recent study indicated an increased UCP2 level in obese women, subsequently a reduction in energy expenditure should be associated with additional fat mass and overweight; therefore, a higher abundance of UCP2 expression is 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. However, UCP2 avoids adipose tissue accumulation through heat generation which controls energy consumption and thermogenesis [24,25]. The UCP2 exhibits an apparent association with the grade of different diseases such as obesity and its comorbidities (hypertension, diabetes, etc.) [26].

However, 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 *et al.* [29] noticed that elevated UCP2 expression contributed decline in weight in animals following a hypocaloric diet intervention. Further research showed that obese participants' lower UCP2 gene expression relative to nonobese individuals resulted in a reduction in energy expenditure and an increase in body fat storage [21,30–32].

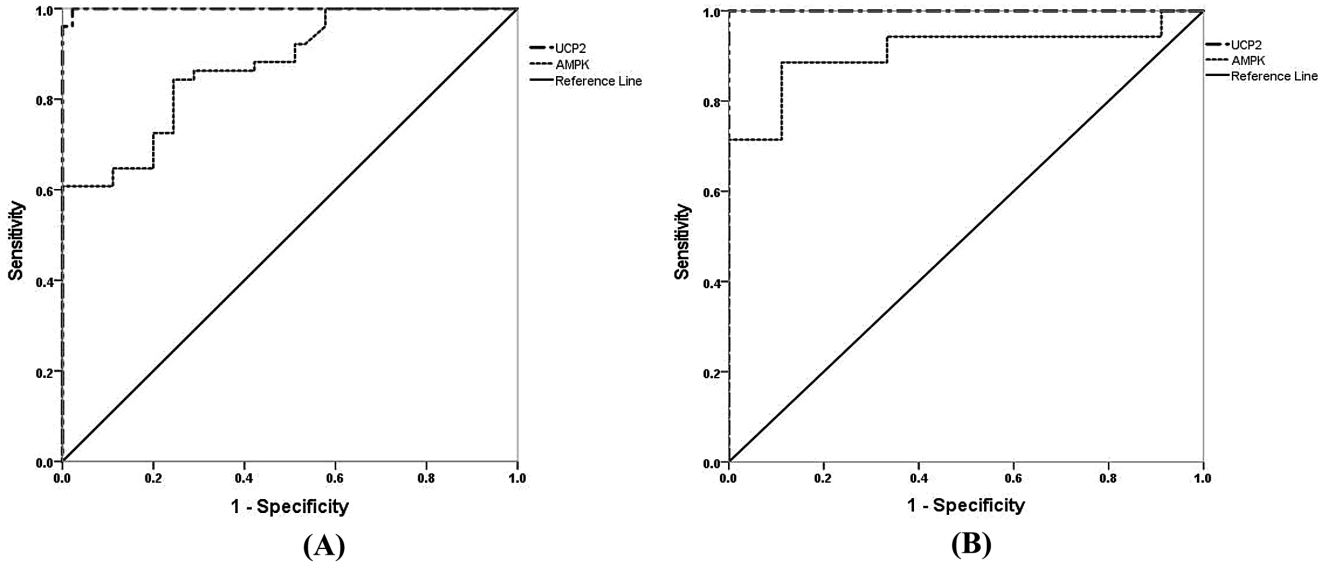
There is information on UCP2 gene expression in human adipose tissue from obese individuals, while there is less information on hypertension patients. According to the Muhammad *et al.*'s [33] findings, variations in the UCP2 gene were linked to alterations in blood pressure. As a result, the current study may provide an overview of UCP2's function in the human adipose tissue of hypertensive patients. Contradictory to our finding, some recent reports found that UCP2 gene expression is downregulated in patients with obesity when compared with normal-weight

Table 2 Results of receiver operating characteristic analysis for uncoupling protein 2 and AMP-activated protein kinase in obese patients

Genes	AUC	SE	Significance	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

95% CI, 95% confidence interval; AMPK, AMP-activated protein kinase; AUC, area under the curve; UCP2, uncoupling protein 2.

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 3 Receiver operating characteristic curve analyses in the hypertension group for uncoupling protein 2 and AMP-activated protein kinase genes

Genes	AUC	SE	Significance	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

95% CI, 95% confidence interval; AMPK, AMP-activated protein kinase; AUC, area under the curve; UCP2, uncoupling protein 2.

participants. 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.* [19], UCP2 mRNA expression was lower in all participants with metabolic diseases, such as hypertension, than in controls. Earlier 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 showed that the 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 lipid parameters. Potentially through its role as a transporter of free fatty acids, UCP2 could be involved in lipid management. In this 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 a greater likelihood of dyslipidemia [40,41]. However, a 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 rise in the AMPK gene expression level of obese and hypertensive patients compared with 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, Martínez-Agustin *et al.* [44] found an elevated level of total AMPK in the SAT of morbidly obese patients in the Spanish populace. Meanwhile, humans and rodents exhibited that AMPK accumulation in adipose tissue is linked to metabolic syndrome and its associated diseases [47].

In contrast, Gauthier *et al.* [48] have proposed a direct correlation between lowered AMPK activity and obese patients who were insulin-resistant in the American population. 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 suggests that AMPK has an anti-lipolytic impact [50], others hypothesize that AMPK 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 remains 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 roles of these genes in the development of obesity and its comorbidities. Nevertheless, further studies are required to illustrate the function of AMPK and UCP2 as prospective therapeutic targets for metabolic disorders such as hypertension and obesity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world-a growing challenge. N Engl J Med 2007; 356:213–215.

2 World Health Organization. Media Centre. Obesity and Overweight, 2015; Fact Sheet No. 311. Available at: http://www.who.int/mediacentre/factsheets/fs311/en/

3 Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, *et al.* Body-mass index and mortality among 1.46 million white adults. N Engl J Med 2010; 363:2211–2219.

4 Fahed G, Aoun L, Bou Zerdan M, Allam S, Bou Zerdan M, Bouferraa Y, *et al.* Metabolic syndrome: updates on pathophysiology and management in 2021. Int J Mol Sci 2022; 23:786.

5 El Meouchy P, Wahoud M, Allam S, Chedid R, Karam W, Karam S. Hypertension related to obesity: pathogenesis, characteristics and factors for control. Int J Mol Sci 2022; 23:12305.

6 Xi Y, Gao W, Zheng K, Lv J, Yu C, Wang S, *et al.* The roles of genetic and early-life environmental factors in the association between overweight or obesity and hypertension: a population-based twin study. Front Endocrinol 2021; 12:743962.

7 Shams E, Kamalumpundi V, Peterson J, Gismondi RA, Oigman W, de Gusmão Correia ML. Highlights of mechanisms and treatment of obesity-related hypertension. J Hum Hypertens 2022; 36:785–793.

8 Dalgaard LT, Andersen G, Larsen LH, Sørensen TI, Andersen T, Drivsholm T, *et al.* Mutational analysis of the UCP2 core promoter and relationships of variants with obesity. Obes Res; 2003; 11:1420–1422.

9 Surniyantoro HN E, Sadewa AH, Hastuti P. Uncoupling protein 2 (UCP2) as genetic risk factor for obesity in Indonesia is different in gender stratification. Kobe J Med Sci 2018; 64:E64–E72.

10 Rubattu S, Cotugno M, Bianchi F, Sironi L, Gelosa P, Stanzione R, *et al.* A differential expression of uncoupling protein 2 associates with renal damage in stroke –resistant spontaneously hypertensive rat/stroke –prone spontaneously hypertensive rat-derived stroke congenic lines. J Hypertens. 2017; 35:1857–1871.

11 Day EA, Ford RJ, Steinberg GR. AMPK as a therapeutic target for treating metabolic diseases. Trends Endocrinol Metab 2017; 28:545–560.

12 López M. EJE PRIZE 2017: hypothalamic AMPK: a golden target against obesity? Eur J Endocrinol 2017; 176:R235–R246.

13 Jeon SM. Regulation and function of AMPK in physiology and diseases. Exp Mol Med 2016; 48:e245.

14 Wang Q, Sun J, Liu M, Zhou Y, Zhang L, Li Y. The new role of AMP-activated protein kinase in regulating fat metabolism and energy expenditure in adipose tissue. Biomolecules 2021; 11:1757.

15 World Health Organization. WHO factsheet on obesity and overweight: world health organization fact sheet, 2020. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-andoverweight [Accessed April 12, 2021].

16 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499–502.

17 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 2001; 25: 402–408.

18 Hassan NE, El-Masry SA, Zarouk W, El Banna RA, Mosaad RM, Al-Tohamy M, *et al.* Obesity phenotype in relation to gene polymorphism among samples of Egyptian children and their mothers. Genes Dis 2018; 5:150–157.

19 Margaryan S, Witkowicz A, Partyka A, Yepiskoposyan L, Manukyan G, Karabon L. The mRNA expression levels of uncoupling proteins 1 and 2 in mononuclear cells from patients with metabolic disorders: obesity and type 2 diabetes mellitus. Adv Hyg Exp Med 2017; 71:895–900.

20 Seyedrafie A, Sepideh A. Brown fat tissue: therapeutic potential for insulin resistance, new hopes for tomorrow. New Insights Obes. Gene Beyond 2020; 4:022–023.

21 Mahadik SR, Lele RD, Saranath D, Seth A, Parikh V. Uncoupling protein 2 (UCP2) gene expression in subcutaneous and omental adipose tissue of Asian Indians: relationship to adiponectin and parameters of metabolic syndrome. Adipocyte 2012; 1:101–107.

22 Pishva H, Eshraghian MR, Hedayati M. UCP2, SHBG, Leptin, and T3 levels are associated with resting energy expenditure in obese women. Endocr Metab Immune Disord Drug Targets 2020; 20:234–241.

23 Kim YJ. A mixture of the aqueous extract of Garcinia cambogia, soy peptide and L: –carnitine reduces the accumulation of visceral fat mass in rats rendered obese by a high fat diet. Genes Nutr 2008; 2:353–358.

24 Su J, Liu J, Yan XY, Zhang Y, Zhang JJ, Zhang LC, *et al.* Cytoprotective effect of the UCP2-SIRT3 signaling pathway by decreasing mitochondrial oxidative stress on cerebral ischemia-reperfusion injury. Int J Mol Sci 2017; 18:1599.

25 Cheng WC, Tsui YC, Ragusa S, Koelzer VH, Mina M, Franco F, *et al.* Uncoupling protein 2 reprograms the tumor microenvironment to support the anti-tumor immune cycle. Nat Immunol 2019; 20:206–217.

26 Rask-Andersen M, Olszewski PK, Levine AS, Schiöth HB. Molecular mechanisms underlying anorexia nervosa: focus on human gene association studies and systems controlling food intake. Brain Res. Rev. 2010; 62:147–164.

27 Schonfeld-Warden NA, Warden CH. Physiological effects of variants in human uncoupling proteins: UCP2 influences body-mass index. Biochem Soc Trans 2001; 29:777–784.

28 Liu X, Zhang B, Liu X, Shen Y, Li J, Zhao N, *et al.* A 45-bp insertion/deletion polymorphism in uncoupling protein 2 is not associated with obesity in a Chinese population. Biochem Genet 2012; 50:784–796.

29 Cortes-Oliveira C, Nicoletti CF, de Souza Pinhel MA, de Oliveira BAP, Quinhoneiro DCG, Noronha NY. UCP2 expression is associated with weight loss after hypocaloric diet intervention. Eur J Clin Nutr 2017; 71: 402–406.

30 Pinkney JH, Boss O, Bray GA, Bulmer K, Coppack SW, Mohamed-Ali V. Physiological relationships of uncoupling protein-2 gene expression in human adipose tissue in vivo. J Clin Endocrinol Metab 2000; 85: 2312–2317.

31 Pedersen SB, Nyholm B, Kristensen K, Nielsen MF, Schmitz O, Richelsen B. Increased adiposity and reduced adipose tissue mRNA expression of uncoupling protein-2 in first-degree relatives of type 2 diabetic patients: evidence for insulin stimulation of UCP-2 and UCP-3 gene expression in adipose tissue. Diabetes Obes Metab 2005; 7:98–105.

32 Shawky S, Mahmoud O, Saad E, Sarhan W. The association between uncoupling proteins (UCP2) gene expression and obesity. Zagazig Univ Med J 2023; 29:155–159.

33 Muhammad H, Sulistyoningrum DC, Huriyati E, Lee YY, Muda WMW. Variation of uncoupling protein 2 (-866G/A), dietary fat intake and blood pressure: an Indonesian Nutrigenetic Cohort. Eur Heart J 2020; 41: ehaa946.2795.

34 Brandao CFC, Carvalho FGD, Nicoletti CF, Junqueira-Franco MVM, Couto-Lima CA, Souza AO, *et al.* UCP2 expression is negatively correlated with and body fat mass after combined physical training: a pilot study. Nutrire 2020; 45:13.

35 Oliveira M, Rheinheimer J, Moehlecke M, Rodriguesa M, Assmannab TS, Leitão CB. UCP2, IL18, and miR-133a-3p are dysregulated in subcutaneous adipose tissue of patients with obesity. Mol Cell Endocrinol 2020; 509:110805.

36 Xiu LL, Weng JP, Sui Y, Wang J, Yan JH, Huang ZM. Common variants in beta 3-adrenergic-receptor and uncoupling protein-2 genes are associated with type 2 diabetes and obesity. Zhonghua Yi Xue Za Zhi 2004; 84:375–379.

37 Yu X, Jacobs DRJr, Schreiner PJ, Gross MD, Steffes MW, Fornage M. The uncoupling protein 2 Ala55Val polymorphism is associated with diabetes mellitus: the CARDIA study. Clin Chem 2005; 51:1451–1456.

38 Qian L, Xu K, Xu X, Gu R, Liu X, Shan S, Yang T. UCP2-866G/A, Ala55Val and UCP3-55C/T polymorphisms in association with obesity susceptibility – a meta-analysis study. PLoS One 2013; 8:e58939.

39 da Fonseca ACP, Assis ISDS, Salum KCR, Palhinha L, Abreu GM, Zembrzuski VM, *et al.* Genetic variants in DBC1, SIRT1, UCP2 and ADRB2 as potential biomarkers for severe obesity and metabolic complications. Front Genet 2024; 15:1363417.

40 Reis AF, Dubois-Laforgue D, Bellannì e-Chantelot C, Timsit J, Velho G. A polymorphism in the promoter of UCP2 gene modulates lipid levels in patients with type 2 diabetes. Mol Genet Metab 2004; 82:339–344.

41 Cha MH, Kim IC, Kim KS, Kang BK, Choi SM, Yoon Y. Association of UCP2 and UCP3 gene polymorphisms with serum high-density lipoprotein cholesterol among Korean women. Metabolism 2007; 56:806–813.

42 D'Adamo M, Perego L, Cardellini M, Marini MA, Frontoni S, Andreozzi F, *et al.* The –866 A/A genotype in the promoter of the human uncoupling protein 2 gene is associated with insulin resistance and increased risk of type 2 diabetes. Diabetes 2004; 53:1905–1910.

43 Bulotta A, Ludovico O, Coco A, Di Paola R, Quattrone A, Carella M, *et al.* The common – 866G/A polymorphism in the promoter region of the UCP-2 gene is associated with reduced risk of type 2 diabetes in Caucasians from Italy. J Clin Endocrinol Metab 2005; 90:1176–1180.

44 Martínez-Agustin O, Hernández-Morante JJ, Martínez-Plata E, Sánchez de Medina F, Garaulet M. Differences in AMPK expression between subcutaneous and visceral adipose tissue in morbid obesity. Regul Pept 2010; 163:31–36.

45 Steinberg GR, Kemp BE. AMPK in health and disease. Physiol Rev 2009; 89:1025–1078.

46 Tian Z, Liang G, Cui K, Liang Y, Wang Q, Lv S, *et al.* Insight into the prospects for RNAi therapy of cancer. Front Pharmacol 2021; 12:644718.

47 Gauthier MS, Ruderman NB. Adipose tissue inflammation and insulin resistance. All obese humans are not created equal. Biochem J 2010; 430:e1–e4.

48 Gauthier M, O'Brien EL, Bigornia S, Mott M, Cacicedo JM, Xu XJ, *et al.* Decreased AMP-activated protein kinase activity is associated with increased inflammation in visceral adipose tissue and with whole-body insulin resistance in morbidly obese humans. Biochem Biophys Res Commun 2011; 404:382–387.

49 Ferrazâ Bannitz F, Welendorf CR, Coelho PO, Salgado W, Nonino CB, Rebeca A, *et al.* Bariatric surgery can acutely modulate ERâ stress and inflammation on subcutaneous adipose tissue in non-diabetic patients with obesity. Diabetol Metab Syndr 2021; 13:19.

50 Anthony NM, Gaidhu MP, Ceddia RB. Regulation of visceral and subcutaneous adipocyte lipolysis by acute AICAR induced AMPK activation. Obesity 2009; 17:1312–1317.

51 Koh HJ, Hirshman MF, He H, Li Y, Manabe Y, Balschi JA, *et al.* Adrenaline is a critical mediator of acute exercise-induced AMP-activated protein kinase activation in adipocytes. Biochem J 2007; 403:473–481.

52 Mao JY, Su LX, Li DK, Zhang HM, Wang XT, Liu DW. The effects of UCP2 on autophagy through the AMPK signaling pathway in septic cardiomyopathy and the underlying mechanism. Ann Transl Med 2021; 9:259.

53 Rubattu S, Bianchi F, Busceti CL, Cotugno M, Stanzione R, Marchitti S, *et al.* Differential modulation of AMPK/PPARα/UCP2 axis in relation to hypertension and aging in the brain, kidneys and heart of two closely related spontaneously hypertensive rat strains. Oncotarget 2015; 6:18800–18818.