

Uncoupling Protein 2 Expression Modulates Obesity in Chronic Kidney Disease Patients

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Abstract

Background: Obesity is a multifactorial metabolic disease resulting from behavioral and genetic factors. Obesity is linked to diabetes mellitus and hypertension, which are considered as major risk factors for chronic kidney disease (CKD); moreover, it has a direct effect on developing CKD and end stage renal disease (ESRD). Here was aimed to examine the association between uncoupling protein 2 (UCP2) gene expression and obesity in CKD patients.

Methods: UCP2 gene expression was analyzed by real time polymerase chain reaction (RT-PCR) in 93 participants divided into three groups. The groups included 31 non-obese CKD patients, 31 obese CKD patients, and 31 healthy, age-matched, unrelated volunteers as a control group.

Results: UCP2 gene expression was significantly relevant when comparing the non-obese CKD and obese CKD groups to the control group ($p < 0.001$). No significant association was found when the groups were compared by gender; Chi-square (X^2) was 2.38 and $p = 0.304$. A significant negative correlation was found between UCP2 gene expression and BMI in CKD ($p < 0.05$).

Conclusions: These results indicate that UCP2 gene expression plays a significant role as a risk factor for obesity in CKD patients.

Keywords: Chronic Kidney Disease (CKD), Obesity, Uncoupling Protein 2 (UCP2) Gene Expression, Real-Time Polymerase Chain Reaction (RT-PCR).

Introduction

Chronic kidney disease (CKD) is characterized by kidney function or structure abnormalities for more than three months with health implications. Chronic kidney disease criteria are based on the presence of kidney damage, such as albuminuria or decreased glomerular filtration rate (GFR) (1).

Kidney disease is increasing and considered a public health threat globally with an estimated more than 750 million patients (2). Epidemiologic data is mostly related to the disease's late stages; more than 2 million patients are treated with renal replacement therapy (RRT), which includes renal transplantation, peritoneal dialysis, and hemodialysis, yet this number may describe

only 10% of patients who need continuous treatment to survive (3).

The worldwide prevalence of obesity and overweightness has doubled since 1980 to an extent that nearly one third of the world's population is now classified as overweight or obese (4). Obesity and overweightness are defined by the World Health Organization (WHO) as excessive or abnormal accumulation of fatty tissue that represents a significant health risk.

The body mass index (BMI) is a metric used to classify degrees of adiposity, calculated by dividing the body weight in kilograms by the height squared in meters. According to the National Institute of Health (NIH), obesity is

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defined as BMI equal to or greater than 30 (5).

Obesity adversely affects nearly all the body's physiological functions and comprises a major health threat. It increases the risk for developing many disease conditions, such as cardiovascular diseases, diabetes mellitus (6,7), various cancers (8), an array of musculo-skeletal disorders (9), and poor mental health (10), all of which have negative effects on the quality of daily life, productivity, and healthcare costs.

Among its various complications, obesity has been shown to directly affect kidney function with or without other etiological factors (11) and is considered a major risk factor for chronic kidney disease development. Moreover, it has been linked to acceleration of existing kidney disease (12).

In addition, obesity and overweightness in patients with end-stage kidney disease represent a significant risk factor for its development after adjusting for other probable parameters including smoking, age, myocardial infarction (MI) history, hypertension, lipid profile, and diabetes (13). Thus, new therapeutic approaches are needed to prevent or decelerate obesity's harmful effects on the kidney.

Uncoupling protein 2 (UCP2) is located in the inner mitochondrial membrane. UCP2 is a transporter involved in dispersing the proton gradient, which can decrease reactive oxygen species (ROS) production and thus enhance cell survival (14). It is widely produced in various cell types, including renal tubular cells (15,16), and has a major role in controlling the mitochondrial production of ROS (17,18). Ultimately, UCP2 protects tissue cells from injury caused by oxidative stress.

Moreover, UCP2 is involved in other pathological and physiological processes, including glucose metabolism, neurodegeneration, inflammatory response, fatty acid oxidation, and apoptosis. It is also involved in energy balance regulation (19). On the basis of the central role of UCP2 gene expression in the development of obesity and its consequences, it was hypothesized that UCP2 gene expression is a risk factor for obesity in CKD patients.

Materials and Methods

The study was conducted in the Medical Biochemistry and Internal Medicine Departments, Faculty of Medicine, Zagazig University. Approval for the study was obtained from the Institutional Review Board (IRB).

This case-control study included 93 adult subjects divided into three groups; group I included 31 CKD patients with BMIs > 30, group II included 31 normal-weight CKD patients, and group III included 31 healthy subjects as controls who were age- and sex-matched with the patients. Individuals on hormonal therapy and/or with histories of congenital renal disorders were excluded.

Blood Sampling

Whole blood samples were collected in sterile EDTA-containing tubes and stored at -20 °C until analysis. Total RNA was extracted from all samples using a Simply P kit (Qiagen, Germany).

Reverse Transcription

RNA was reverse transcribed using a Maxime RT Premix kit with these conditions: 75 °C for 5 min, 60 °C for 60 min, and 90 °C for 5 min.

Real-Time RT-PCR

Real-time PCR was conducted using a real-time cyclor. All reactions were performed in duplicate. Samples were amplified in 20 µL reactions containing 1 µL of template DNA, 1 µL of each primer (1 µM), and 10 µL of 2X PCR Master mix solution (Qiagen, Germany). Relative gene expression changes were calculated using the $2^{-\Delta\Delta CT}$ method, as described by Livak and Schmittgen (12).

Statistical Analysis

Data were analyzed using SPSS version 23, and expressed as mean±SD, median, frequency, and percentage. Student's t test, ANOVA F-test, and chi-squared (X^2) test were used when appropriate. $p < 0.05$ was considered statistically significant. The analysis was based on the accuracy of the identified UCP2 gene to diagnose the presence of obesity in CKD patients

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as determined using receiver operator characteristic (ROC) curves as area under the curve (AUC) value, sensitivity, and specificity.

Results

Group I included 16 males and 15 females with a mean age of 55.2 ± 6.3 . Their mean BMI was 33.99 ± 1.4 Kg/m². Group II included 18 males and 13 females, with a mean age of 52.8 ± 6.3 years. Their mean BMI was 23.1 ± 1.04 Kg/m² (Tables 1 and 2) No significant association was found when the groups were compared according to gender (X² was 2.38 and $p = 0.304$). (Table 1). The UCP2 gene expression means were 0.2 in group I, 0.63 in group II, and 0.95 in the

control group. UCP2 gene expression showed a significant association between groups I and II when compared with the control group ($p < 0.001$) (Table 3).

A scatter plot identified a significant negative correlation between UCP2 gene expression and BMI for group II ($p = 0.001$) (Fig. 1).

The sensitivity and specificity of UCP2 expression in group II sera, a good predictive test for obesity, were assessed using ROC curve analysis. UCP2 expression levels showed high sensitivity and specificity, at 96.8 and 91.2% respectively, with a cut off value $\leq 0.375\%$ (Fig. 2).

Table 1. Socio-demographic characteristics of control, non-obese CKD, and obese CKD groups.

Control group (N= 31)	Non obese CKD Group (N= 31)	Obese CKD group (N= 31)	Test of sig.	p	
Age per year Mean \pm SD Median (min-max)	52.7 ± 2.5 53(47-57)	52.8 ± 6.3 53(43-66)	55.2 ± 6.3 55(42-65)	F=2.1	0.13
Sex no. (%) Male Female	10(32.3) 21(67.7)	13(41.9) 18(58.1)	16(51.6) 15(48.4)	χ^2 = 2.38	0.304

Table 2. Anthropometric measures of control, non-obese CKD, and obese CKD groups.

	Control group (N= 31)	Non obese CKD group (N= 31)	Obese CKD group (N= 31)	F test	p value
Weight/kg Mean \pm SD Median (Range)	75.29 ± 5.1 74 (65-85)	75.1 ± 3.7 74 (68-83)	100.5 ± 8.4 97 (88-115)	178.9	<0.001
Height/cm Mean \pm SD Median (Range)	181 ± 6.5 181 (169-191)	180.4 ± 6.1 180 (169-191)	171.8 ± 5 170 (165-182)	23.9	<0.001
BMI Mean \pm SD Median (Range)	22.9 ± 0.98 23.2 (21.9-24.4)	23.1 ± 1.04 23.6 (20.9-24.4)	33.99 ± 1.4 34 (30.2-36.3)	919.9	<0.001

Table 3. UCP2 gene expression in control, non-obese CKD, and obese CKFD groups.

	Control group (N= 31)	Non obese CKD group (N= 31)	Obese CKD group (N= 31)	F test	p value
UCP2 gene expression Mean \pm SD Median (range)	0.997 ± 0.25 0.95 (0.42:1.53)	0.618 ± 0.144 0.63 (0.35:0.91)	0.196 ± 0.093 0.2 (0.02:0.43)	158.6	<0.001

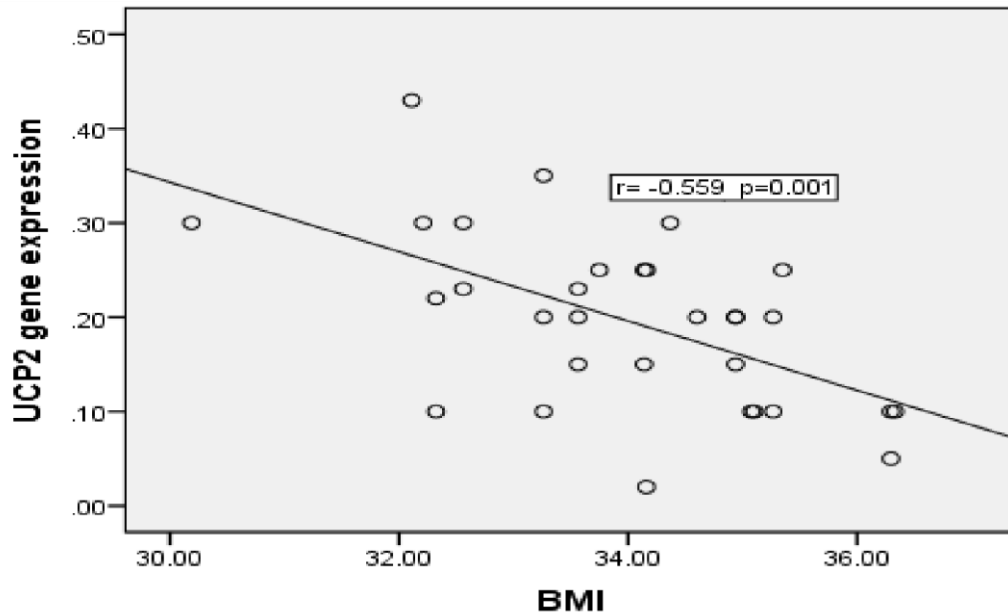


Fig. 1. Scatter plot showing the correlation between UCP2 gene expression and BMI for group I.

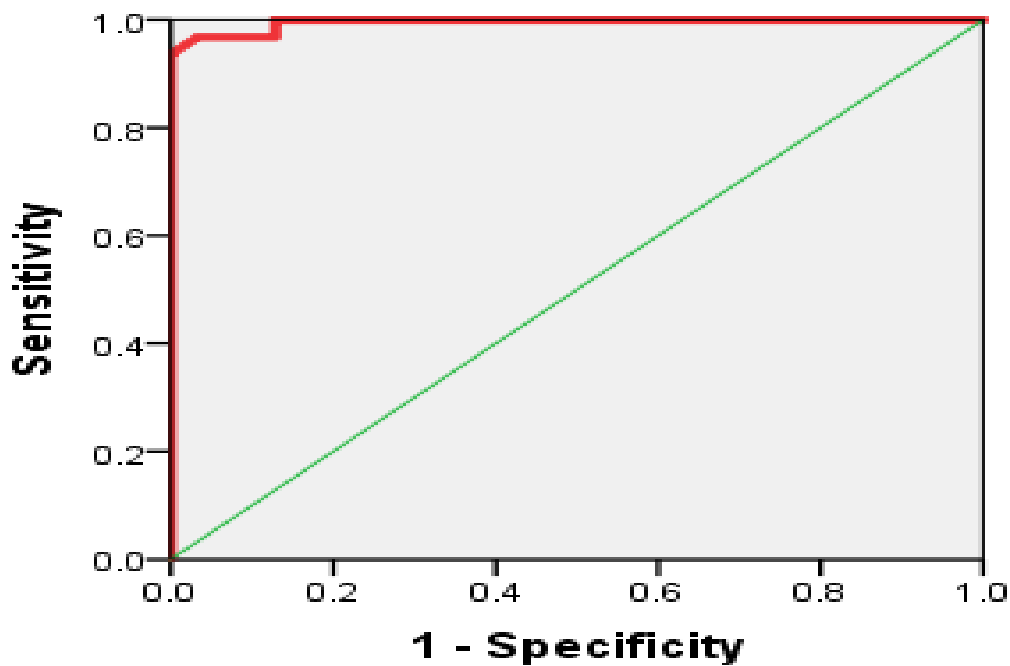


Fig. 2. Receiver operating characteristic (ROC) curve analysis.

Discussion

Obesity prevalence has risen globally over the past thirty years (20). The WHO defines obesity as excessive accumulation of fatty tissue, which represents a significant risk to health (4). Despite its simple definition, obesity is actually a syndrome that results from positive energy balance chronicity. Excessive carbohydrates are

converted to fat, which is stored in adipose tissue that increases in size, leading to increasing body fat and weight gain (21). As a mitochondrial transporter protein regulating glucose oxidation, lipid metabolism, and energy homeostasis, UCP2 plays a pivotal role in the development and management of obesity.

Damage to the mitochondria or its dysfunction are linked to various kidney diseases, including acute renal injury and CKD (22).

A significant mitochondrial function is to supply energy required by the kidney to clear wastes from blood and to regulate water and electrolyte balance. The energy production in cells occurs via the electron transport chain (ETC), which is located on the inner mitochondrial membrane and participates in the process of oxidative phosphorylation (23).

UCPs, also found on the inner mitochondrial membrane, comprise a superfamily of anion carrier proteins that uncouple oxidation from energy production with energy released as heat (23). Therefore, we explored the possible role of UCP2 gene expression in Egyptians as risk factor for obesity in CKD patients.

There was a significant association of UCP2 gene expression in the non-obese and obese CKD groups when compared with the control group. However, no significant association was seen when the groups were compared according to gender.

Moreover, our results showed a significant negative correlation between UCP2 gene expression and BMI for the obese CKD group (group II).

Our results agreed with those of Kovesdy et al., as they reported that obesity affects kidney function; also, Ikizler et al. and Stenvinkel et al. found that adipose tissue-specific events may contribute to pathological changes that aggravate CKD complications and negatively influence outcomes (24, 25, 26).

These findings could be attributed to pro-inflammatory adipokines and hormones

released from adipose tissue, such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, and leptin, which have been incriminated in the development of vascular and renal injury observed in obese individuals (27).

In line with our data, Oberkofler et al. and Sujata et al. showed that UCP2 gene expression was less in obese subjects than in controls (28, 29). Our results also agreed with Nordfors et al., who reported that UCP2 gene expression was 28% less in obese subjects than in controls ($p=0.001$) (30).

Moreover, Margaryan et al. and Brandao et al. reported that UCP2 mRNA expression was less in obese than in normal weight subjects. The reduction in UCP2 expression might induce less active thermogenesis and adipose tissue fat accumulation (31, 32). Oliveira et al. findings also supported ours, as they found that UCP2 gene expression was down regulated in obese patients (33).

On the other hand, Millet et al. found no major alteration of UCP2 gene regulation in obese subjects (34). In another study, Simoneau et al. suggested that UCP2 expression is not related to basal energy expenditure in humans (35).

This could be attributed to different study parameters as Millet et al. examined the effect during fasting, and in case of Simoneau et al., there was decrease in the lean body mass. Our results suggest that UCP2 gene expression could be used as a genetic obesity biomarker in Egyptian CKD patients.

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References

1. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest.* 2006;116:288-296.
2. GBD 2015 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of

Disease Study 2015. *Lancet.* 2016;388(10053):1603-1658.

3. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney Int.* 2011;80(12):1258-70.

4. Obesity and overweight. World Health Organization. 2015.

5. Glasgow RE, Terborg JR, Hollis JF, Severson HH, Boles SM. Take heart: results from the

- initial phase of a work-site wellness program. *Am J Public Health*. 1995;85(2):209-216.
6. Singh GM, Danaei G, Farzadfar F, Stevens GA, Woodward M, Wormser D, et al. The age-specific quantitative effects of metabolic risk factors on cardiovascular diseases and diabetes: a pooled analysis. *PLoS One*. 2013;8(7):e65174.
 7. Czernichow S, Kengne AP, Stamatakis E, Hamer M, Batty D, Stamatakis E. Body mass index, waist circumference and waist-hip ratio: which is the better discriminator of cardiovascular disease mortality risk? evidence from an individual-participant meta-analysis of 82 864 participants from nine cohort studies. *Obes Rev*. 2011;12(9):680-687.
 8. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K, et al. Body fatness and cancer—viewpoint of the IARC working group. *The New England Journal of Medicine*. 2016;375(8):794-798.
 9. Anandacoomarasamy A, Caterson I, Sambrook P, Fransen M, March L. The impact of obesity on the musculoskeletal system. *international journal of obesity*. 2008;32(2):211-222.
 10. Anstey KJ, Cherbuin N, Budge M, Young J. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. *Obesity Reviews*. 2011;12(5):e426-e437.
 11. Mathew AV, Okada S, and Sharma K. Obesity related kidney disease. *Current Diabetes Reviews*. 2011;7(1):41–49.
 12. Othman M, Kawar B, El Nahas AM. Influence of obesity on progression of non-diabetic chronic kidney disease: A retrospective cohort study. *Nephron Clinical Practice*. 2009;113:c16-c23.
 13. Hsu CY, McCulloch CE, Iribarren C, Darbinian J, Go AS. Body mass index and risk for end-stage renal disease. *Ann Intern Med*. 2006;144(1):21-8.
 14. Dutra MRH, Feliciano RDS, Jacinto KR, Gouveia TLF, Brigidio E, Serra AJ. Protective role of UCP2 in oxidative stress and apoptosis during the silent phase of an experimental model of epilepsy induced by pilocarpine. *Oxidative Medicine and Cellular Longevity*. 2018;2018:6736721.
 15. Gimeno RE, Dembski M, Weng X, Deng N, Shyjan AW, Gimeno CJ, et al. Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes*. 1997;46(5):900-906.
 16. 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(9):1857-1871.
 17. Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, et al. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat Genet*. 2000;26(4):435-9.
 18. Mattiasson G, and Sullivan PG, (2006). The emerging functions of UCP2 in health, disease, and therapeutics. *Antioxid Redox Signal*. 2006;8(1-2):1-38.
 19. Surniyantoro HNE, 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(2):E64-E72.
 20. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384(9945):766-81.
 21. Ladabaum U, Mannalithara A, Myer PA, Singh G. Obesity, abdominal obesity, physical activity, and caloric intake in US adults: 1988 to 2010. *Am J Med*. 2014;127(8):717-727.e712.
 22. Bhargava P, Schnellmann RG. Mitochondrial energetics in the kidney. *Nat Rev Nephrol*. 2017;13(10):629-646.
 23. Rousset S, Alves-Guerra MC, Mozo J, Miroux B, Cassard-Doulier AM, Bouillaud F, et al. The biology of mitochondrial uncoupling proteins. *Diabetes*. 2004;53(suppl 1):S130-5.
 24. Kovesdy CP, Furth S, Zoccali C. Obesity and kidney disease: hidden consequences of the

epidemic. *Iranian Journal of Kidney Diseases*. 2017;11:99-108.

25. Ikizler TA. Resolved: being fat is good for dialysis patients: the Godzilla effect: pro. *J Am Soc Nephrol*. 2008;19(6):1059-62.

26. Stenvinkel P, Lindholm B. Resolved: being fat is good for dialysis patients: the Godzilla effect: con. *J Am Soc Nephrol* 2008. 2008;19(6):1062-4.

27. Tomaszewski M, Charchar FJ, Maric C, J McClure, L Crawford, W Grzeszczak, et al. Glomerular hyperfiltration: a new marker of metabolic risk. *Kidney Int*. 2007;71(8):816-21.

28. Oberkofler H, Dallinger G, Liu YM, Hell E, Krempler F, Patsch W. Uncoupling protein gene: quantification of expression levels in adipose tissues of obese and non-obese humans. *J Lipid Res*. 1997;38(10):2125-33.

29. Sujata RM, Lele RD, Saranath D, Seth A, Parikh V. Uncoupling protein-2 (UCP2) gene expression in subcutaneous and omental adipose tissue of Asian Indians. *Adipocyte*. 2012;1(2):101-107.

30. Nordfors L, Hoffstedt J, Nyberg B, Thörne A, Arner P, Schalling M, et al. Reduced gene expression of UCP2 but not UCP3 in skeletal muscle of human obese subjects. *Diabetologia*. 1998;41:935-939.

31. Margaryan S, Witkowicz A, Partyka A, Yepiskoposyan 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. *Postępy Higieny i Medycyny Doświadczalnej*. 2017;71:895-900.

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

33. Oliveira M, Rheinheimer J, Moehlecke M, Rodrigues M, Assmannab TS, Leitão CB. UCP2, IL18, and miR-133a-3p are dysregulated in subcutaneous adipose tissue of patients with obesity. *Molecular and cellular endocrinology*. 2020;509.

34. Millet L, Vidal H, Andreelli F, Larrouy D, Riou JP, Ricquier D, Laville M, Langin D. Increased uncoupling protein-2 and -3 mRNA expression during fasting in obese and lean humans. *J Clin Invest*. 1997 Dec 1;100(11):2665-70.

35. Simoneau JA, Kelley DE, Neverova M, Warden CH. Overexpression of muscle uncoupling protein 2 content in human obesity associates with reduced skeletal muscle lipid utilization. *The FASEB journal*. 1998;12(15):1739-1745.