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Original Article

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The mRNA expression levels of uncoupling proteins 1 and 2 in mononuclear cells from patients with metabolic disorders: obesity and type 2 diabetes mellitus

Poziom ekspresji mRNA genów *UCP1* i *UCP2* w komórkach jednojądrzastych pacjentów z zespołem metabolicznym: otyłością i cukrzycą typu 2

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Introduction: Type 2 diabetes mellitus (T2DM) and obesity are metabolic disorders whose major hallmark is insulin resistance. Impaired mitochondrial activity, such as reduced ratio of energy production to respiration, has been implicated in the development of insulin resistance. Uncoupling proteins (UCPs) are proton carriers, expressed in the mitochondrial inner membrane, that uncouple oxygen consumption by the respiratory chain from ATP synthesis.

Aim: The aim of the study was to determine transcriptional levels of *UCP1* and *UCP2* in peripheral blood mononuclear cells (PBMCs) from patients with metabolic disorders: T2DM, obesity and from healthy individuals.

Material/Methods: The mRNA levels of *UCP1*, *UCP2* were determined by Real-Time PCR method using Applied Biosystems assays.

Results: The *UCP1* mRNA expression level was not detectable in the majority of studied samples, while very low expression was found in PBMCs from 3 obese persons. *UCP2* mRNA expression level was detectable in all samples. The median mRNA expression of *UCP2* was lower in all patients with metabolic disorders as compared to the controls (0.20±0.14 vs. 0.010±0.009, p=0.05). When compared separately, the differences of medians *UCP2* mRNA expression level between the obese individuals and the controls as well as between the T2DM patients and the controls did not reach statistical significance.

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Conclusions: Decreased *UCP2* gene expression in mononuclear cells from obese and diabetic patients might contribute to the immunological abnormalities in these metabolic disorders and suggests its role as a candidate gene in future studies of obesity and diabetes.

Keywords: **mRNA expression levels • mononuclear cells • type 2 diabetes mellitus**

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Abbreviations:

ATP – adenosine triphosphate, **BMI** – body mass index, **β2M –** β2microglobulin, **EDTA** – ethylenediaminetetraacetic acid, **mRNA –** messenger ribonucleic acid, **PBMCs** – peripheral blood mononuclear cells, **ROS** – reactive oxygen species, **RT-PCR –** real-time polymerase chain reaction, **T2DM –** type 2 diabetes mellitus, **UCPs –** uncoupling proteins, **3`UTR** – 3' untranslated region.

Introduction

Type 2 diabetes mellitus (T2DM) and obesity are metabolic disorders whose susceptibility is controlled by the interplay of genetic and environmental factors. It is established that a lifestyle of excessive consumption and physical inactivity raise the prevalence of these disorders [9]. Numerous studies suggested a link between T2DM and obesity involving insulin resistance, specific profile of pro-inflammatory cytokines, disturbed fatty acid metabolism, and mitochondrial dysfunction [5,11]. Mitochondria are engaged in several biological processes which regulate metabolic and energy homeostasis, and its defects might accelerate the progression of insulin resistance and dysregulated lipid metabolism [5,15,27].

The activation of oxidative stress pathway is engaged in early stage of T2DM development such as insulin resistance [20]. The reactive oxygen species (ROS) are produced in diabetes by mitochondrial and non-mitochondrial origins and include: NADPH oxidase, xanthine oxidase, uncoupled eNOS lipoxygenase, cytochrome P450 enzymes, and many others [13]. Mitochondrial ROS formation can be reduced by uncoupling proteins (UCPs) [21]. The UCPs are a family of mitochondrial transporters located in the inner mitochondrial membrane. Mammals express five UCPs proteins which are differently distributed in tissues [16]. All of them are anion carriers proteins which transport protons (H^+) to the mitochondrial matrix and in turn dissipate the proton motive force as heat and uncouple the oxidation substrate from the production of ATP. In this study we focus our attention on UCP1 and UCP2 since these proteins play a pathogenetic role in diabetes mellitus as was reviewed by Liu et al. [18].

UCP1 is mainly expressed in brown adipose tissue (BAT) and it is responsible for non-shivering thermogenesis. Recent studies showed protein or/and mRNA expression also in human white adipose tissue, skeletal muscle, longitudinal smooth muscle layers, retinal cells, and islet cells; however, its physiological functions in these tissues and organs are not well established [18]. Furthermore, *UCP1* mRNA expression was also detected in mitochondria isolated from whole thymus and thymocytes in mammals (rats and mouse) [4]. UCP1 activity facilitates a more rapid flux of electrons through the inner mitochondrial membrane which allows for the reduction of membrane potential and, as a consequence, a reduction of ROS production [3]. Therefore, the main role of UCP1 in other than adipose tissues seems to be protection against oxidative stress [2,8].

Unlike UCP1, UCP2 does not have thermogenic functions, but controls mitochondrial ROS emission [10,12] as well as glucose and lipid metabolism [7]. UCP2 is widely distributed in several tissues including the spleen, kidney, pancreas, central nervous system and immune system. UCP2 is a negative modulator of insulin secretion [28,30,32]. Moreover, this protein was reported to control the production of mitochondrial ROS and regulate immune system activation by modulating MAPK pathway [10].

Both T2DM and obesity are associated with chronic low-grade inflammation which affects insulin signaling and

pancreatic β-cell function [11,31]. Elevated levels of circulating pro-inflammatory cytokines have been detected in patients with both T2DM and obesity [22,23], and the main source of these molecules are leukocytes [6,14]. The possibility of a link between inflammation, leukocytes, and UCPs is largely unexplored. Thus, the purpose of our study was to determine transcriptional levels of *UCP1* and *UCP2* in peripheral blood mononuclear cells (PBMCs) isolated from patients with metabolic disorders: T2DM, obesity as well as in PBMCs from healthy individuals.

Materials and Methods

Patients

Peripheral blood samples were obtained from 20 patients with T2DM (mean age 54.5 ± 4.8), 19 patients with obesity (49.8 ± 5.2) and 22 healthy volunteers (44 ± 6.3 years). Patients were diagnosed at the "Surb Astvatsamayr" Medical Center (Yerevan, Armenia) based on criteria established by an expert committee on the diagnosis and classification of diabetes mellitus: i) random venous plasma glucose ≥11.1mmol/L, ii) fasting plasma glucose ≥7mmol/L, iii) laboratory hemoglobin A1c >48mmol/mol (6.5%). All the patients with T2DM were treated with diet therapy and oral hypoglycemic agents. No patients received insulin therapy. Obesity was defined as a BMI ≥ 30 kg/m^2. Obese patients have no diagnosed T2DM. None of healthy subjects reported presence of diabetes or a family history of this disease, obesity or other metabolic diseases. The study was approved by the Ethics Committee of the Institute of Molecular Biology of the NAS RA (IRB IORG0003427).

Sampling

Blood samples were collected from controls and patients into tubes treated with EDTA as an anticoagulant. Immediately after sampling, PBMCs were isolated from blood by using Histopaque®-1077 density gradient centrifugation. Isolated PBMCs were washed with PBS. Further, PBMCs were stored in 100µl RNAlater at – 20°C until use.

RNA extraction and RT-PCR

Total RNA was extracted from frozen PBMC using the Qiagen RNeasy Mini Kit according to the manufacturer's protocol. The RNA concentration was determined spectrophotometrically. The quality of the RNA was checked spectrophotometrically and also by visualization of the 28S:18S ribosomal RNA ratio on a 2% agarose gel. Immediately after isolation, the 500 ng of total RNA was reverse transcribed with the iScript cDNA Synthesis Kit (BioRad).

The mRNA levels of human *UCP1*, *UCP2,* β*2microglobulin* (β*2M*) and *18S rRNA* were determined using Applied Biosystems assays (Hs00222453_m1, Hs01075227_m1, Pre-developed TaqMan Assay Reagents Human β2M, and Pre-developed TaqMan Assay Reagents Human *18S rRNA*, respectively). All of the samples were assayed in duplicate on the ViiA 7 Real Time PCR System (Applied Biosystems). Due to higher expression stability in investigated samples β*2M* gene, not *18S rRNA,* was chosen as a reference gene. The results were calculated according to the ΔCt method (Livak and Schmittgen 2001) applying the β*2M* gene as a reference.

Statistical analysis

Statistical analysis was performed using the statistical software *Statistica version 7*. Groups were compared using nonparametric the Mann-Whitney U test. The summary statistics are presented as the median and ± standard deviation. The level of statistical significance was set at $p \leq 0.05$.

Results

UCP1 and *UCP2* mRNA levels were determined by RT-PCR in PBMCs isolated from three investigated groups: patients with T2DM, obesity, and healthy individuals. All samples from the controls and T2DM patients did not produce detectable levels of *UCP1* mRNA; however, we found very low expression of *UCP1* in 3 out of 19 obese patients (median: $3x10^{-8}$).

mRNA expression level of *UCP2* ($2^{-\Delta Ct}$) was detectable and ranged between 0.003-0.056 with median value: 0.013. The results for particular groups are presented in Table 1.

In a group of healthy individuals the median *UCP2* expression level was 0.020 (range: 0.004-0.056), for obese individuals we observed the median *UCP2* expression level 0.013 (range: 0.004-0.037), while in diabetes patients 0.009 (range: 0.003-0.038). If we consider patients with metabolic disorders as one group, then the

Table 1. The median, range (Min, Max), interquadrille range (Q1, Q3) and standard deviation (St. dev.) for *UCP2* mRNA expression levels in group of patients with metabolic disorders (obese subjects and T2DM patients) and the controls expressed as $^{2\text{-delta}}$ CT (two results were omitted due to highly outstanding values)

UCP2 mRNA expression	N	Median	Min	Max	Q1	Q2	St. dev.
controls	21	0.020	0.004	0.056	0.008	0.026	0.014
metabolic disorders	38	0.010	0.003	0.038	0.007	0.019	0.009
obese subjects	19	0.013	0.004	0.037	0.006	0.019	0.009
T2DM	19	0.009	0.003	0.038	0.007	0.022	0.010

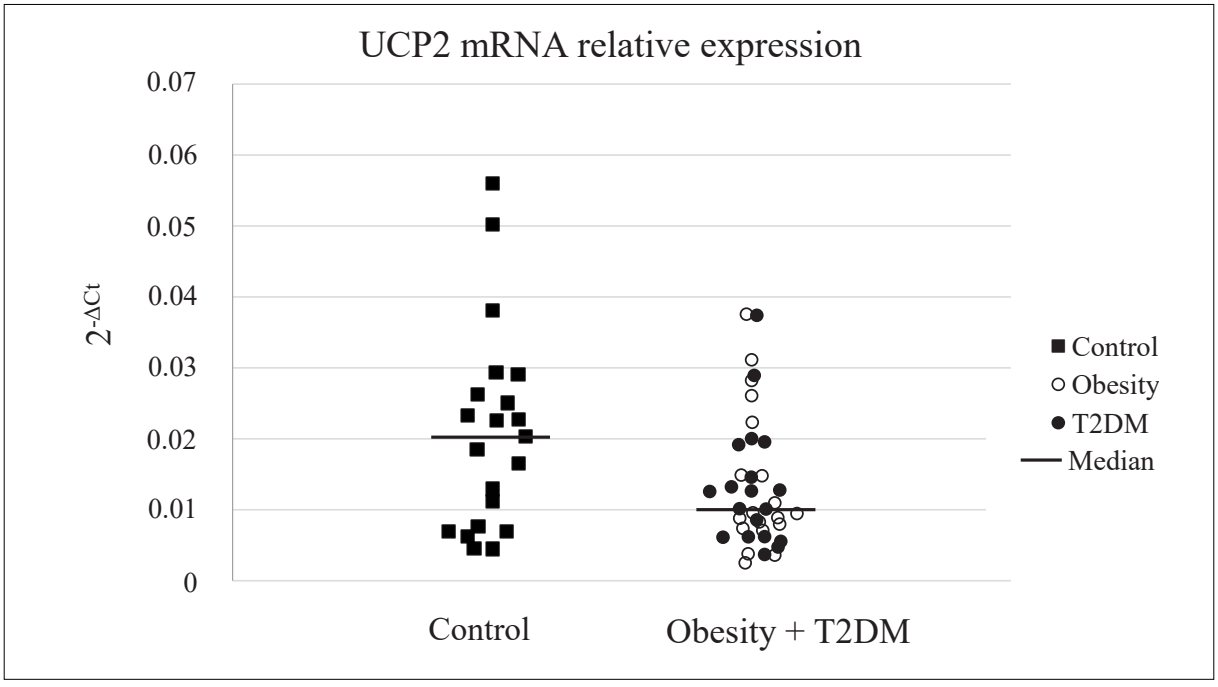

Fig. 1. mRNA level of *UCP2* in PBMCs isolated from healthy control subjects, patients with metabolic disorders: type 2 diabetes mellitus (T2DM) and obesity measured by quantitative RT-PCR. The expression of *UCP2* was normalized to that of *β2M* (expressed as $2^{-\Delta CT}$). Horizontal bars represent the median values for each group. The difference between *UCP2* mRNA expression level is statistically significant $p \leq 0.05$ (Mann-Whitney U test)

median *UCP2* expression level is significantly lower than in the case of control individuals (0.010 vs. 0.020, $p \leq 0.05$) (Figure 1). The differences between separately analyzed groups were not statistically significant.

Discussion

UCP1 was described as a marker of brown adipose tissue [17] and it was thought that this protein is exclusively expressed in BAT. Nowadays, there is evidence that UCP1 is expressed on the protein and/or mRNA levels in mouse and human beige/WAT cells, mammalian pancreatic islets, human skeletal muscle, bovine retina, human longitudinal smooth muscle layers (reviewed in [3]) as well as rat and mouse thymocytes [1]. Recently, Mori et al. reported UCP1 expression in human skin with immunohistochemistry staining, placing UCP1 in the granular layer of the epidermis, sweat glands, hair follicles, and sebaceous glands of various sites in the human body[24]. To the best of our knowledge, there was no data concerning *UCP1* mRNA expression in human leucocytes. Therefore, we investigated the *UCP1* mRNA expression in human PBMCs from 60 individuals using the real-time PCR technique. We were able to detect mRNAs of *UCP1* only in 3 samples. Interestingly, detectable, although very low (about 10^5 times lower than for *UCP2*), levels of *UCP1* mRNA expression were found among obese subjects. This surprising observation should be further confirmed in a larger group of obese individuals.

UCP2 is widely present in many tissues, among them skeletal muscles and immune cells [16,28,29]. Expression of this protein in mononuclear cells was mainly shown in mice [29]. To the best of our knowledge, this is the first study demonstrating expression of *UCP2* in human mononuclear cells from human peripheral blood. In our study we found lower mRNA *UCP2* expression level in PBMCs samples from metabolic disorder patients. However, when analyzed separately, T2DM patients vs. the controls and obese individuals vs. the controls, the differences in *UCP2* mRNA expression level did not reach statistical significance.

To date, the exact role of UCP2 is not clearly defined, especially in certain tissues. However, several clinical and experimental studies have strengthened the hypothesis that UCP2 negatively regulates insulin secretion and is a major link between obesity, β-cell dysfunction, and T2DM [32]. It was shown that UCP2 was significantly upregulated in obesity-induced model of diabetes in mice (ob/ob mouse) in islets, while UCP2-deficient ob/ob mice had restored first phase insulin secretion, increased serum insulin levels and decreased levels of glycemia. Besides, UCP2-deficient mice demonstrated higher ATP content and glucose-stimulate insulin secretion (GSIS) in islets [32]. Moreover, it was described that medical compounds exhibit curative effect on diabetes through downregulation of *UCP2* gene expression (reviewed in [18]). On the other hand, recent studies on knockdown mice have shown, that UCP2 knockdown caused elevation of ROS and/or oxidative stress, followed by the enhancement of GSIS (reviewed in [18]).

Most of the investigations describing the role of UCP2 are focused on its function in β-cells. The studies

about UCP2 function in other tissues are very limited. It has been shown that *UCP2* gene expression is decreased in white adipose tissues in obese and diabetes patients [19,26]. The authors speculate that low *UCP2* gene expression may result in a decreased production of UCP2 protein, decreased energy expenditure and, hence, increased accumulation of body fat [19]. Low mRNA expression was also found in the skeletal muscles of obese subjects. In this study, Nordfors et al. postulate that the decrease in UCP2 mRNA may be due to hereditary factors and/or a consequence of events associated with obesity, such as increased muscle strain or different exercise patterns [25].

In the present study, we also found decreased *UCP2* expression level in PBMCs from patients with metabolic disorders, albeit we were not able to strictly conclude that *UCP2* expression was lower in obese individuals, since the differences in expression levels between obese individuals and the controls did not reach statistical significance.

At present, it is difficult to confidently speculate whether this observed data on basal expression of *UCP2* gene is related to the pathogenesis of T2DM or obesity or whether it only reflects the constitutive status of the cells. This question should be addressed in future studies.

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