

Uncoupling protein-2 (UCP2) gene expression in subcutaneous and omental adipose tissue of Asian Indians

Relationship to adiponectin and parameters of metabolic syndrome

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Abbreviations: UCP2, uncoupling protein-2; SAT, subcutaneous adipose tissue; OAT, omental adipose tissues; RT-PCR, real time reverse transcriptase polymerase chain reaction; T2DM, type 2 diabetes; BMI, body mass index; ATP, adenosine triphosphate; ADP, adenosine diphosphate; TZD, thiazolidinediones; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high density lipoprotein; CVD, cardiovascular disease

Objective: UCP2 is a mitochondrial membrane transporter expressed in white adipose tissue and involved in regulation of energy balance. In this present study, we examined the depot specific comparison of UCP2 gene expression in different metabolic states, in order to explore the potential role of UCP2 in human obesity and diabetes. We also determined UCP2's association with adiponectin and insulin resistance with different parameters of the metabolic syndrome.

Methods: Subcutaneous adipose tissue (SAT) and omental adipose tissues (OAT) were obtained from 69 subjects, including 23 non-obese controls, 26 obese and 20 obese T2DM patients. Metabolic syndrome and other clinical features were studied. Adiponectin and UCP2 gene expression was quantitated by Real Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Results: UCP2 gene expression was significantly reduced in obese and diabetic patients compared with controls. Interestingly, we found that UCP2 gene expression was reduced more in omental fat compared with subcutaneous fat and this effect was observed only in males but not in females. Partial correlation analysis showed significant association with the obesity parameters waist circumference, insulin and HOMA-IR, the lipid parameter triglyceride and the adipokine adiponectin.

Conclusion: Reduced UCP2 gene expression in obese and diabetic patients and its association with obesity parameters and HOMA-IR confirms its role as a candidate gene in the study of obesity and diabetes in our population. Also, its association with triglycerides implicates its role in lipid metabolism. An association between adiponectin and UCP2 gene expression may provide us with an innovative therapeutic strategy to prevent obesity related diseases, like diabetes and CVD.

Introduction

India is currently experiencing an increasing epidemic of obesity and type 2 diabetes (T2DM). Asian Indians suffer from an increased susceptibility to T2DM, insulin resistance and cardiovascular disease in comparison to Western populations due to an increased prevalence of central obesity and high body fat (%), even at low body mass indexes (BMI).^{1,2} The presence of certain genes is responsible for the predisposition of Indians to T2DM. Further studies are needed to unravel the genetics of diabetes in the Indian population.

Uncoupling proteins (UCPs) are attractive candidate genes for obesity and T2DM as they have been mapped to the human chromosome 11q13 and mouse chromosome 7 in regions that have been linked to obesity and hyperinsulinemia.³⁻⁶ UCP belongs to the family of mitochondrial transporter proteins that uncouples the transport of protons across the inner mitochondrial membrane from the electron transport chain that results in the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP), hence generating heat rather than energy.^{4,5}

UCP1, the first member identified, is expressed primarily in brown adipose tissue and is responsible for non-shivering

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thermogenesis in infants and small mammals, but its role in the adult human is uncertain. In contrast, UCP2 is expressed widely, such as in white adipose tissue, skeletal muscle, pancreatic islets and the central nervous system. UCP3 exhibits a more limited tissue-specific expression, confined to skeletal muscle, cardiac muscle and fat tissue. The UCP literature currently indicates that the physiological role of the UCP1 homologs in mammals extend into fatty acid oxidation, the glucose disposal rate, insulin secretion, reactive oxygen species (ROS) production, apoptosis and aging.⁷⁻⁹

Several UCP2 gene polymorphisms were linked to an increased BMI in Pima Indians and other populations,¹⁰⁻¹² although this has not been confirmed by other studies.^{7,13} The association of UCP2 gene polymorphisms with insulin resistance and T2DM has also been reported in several studies.^{4,14-16}

Reduced UCP gene expression has been found in adipose tissue of obese subjects and in the first-degree relatives of T2DM patients.^{5,7,17,18} A few studies also reported that thiazolidinediones (TZD) stimulated UCP2 gene expression in human adipocytes.^{13,19} Thus, these reports indicate that this protein could be playing an important role in the regulation of energy expenditure and are likely to contribute to obesity and T2DM.

UCP2 levels in adipose tissue may also influence systemic metabolism via modification of the release of adipokines by adipose tissue.²⁰ The adipokine adiponectin is exclusively expressed in white adipose tissue and is located on chromosome 3q27, which has been mapped to a susceptibility locus for T2DM and metabolic syndrome.^{21,22} Novel anti-inflammatory, anti-atherogenic and insulin sensitizing properties of adiponectin make this cytokine an important candidate gene for T2DM. Low plasma adiponectin levels in insulin resistant states suggest that adiponectin might have several therapeutic advantages.²³ Villarroya and coworkers reported that circulating adiponectin levels and adiponectin gene expression in adipose tissue are reduced in UCP2-null mice, though this relation in human studies is not yet understood.²⁰

Animal and human studies do not yet afford a clear view of the role of UCP2 in the human adipose tissue of obese and diabetic patients. Though UCP expression studies in skeletal muscle of T2DM has been reported,^{5,8} to date, no study is available on UCP expression in human adipose tissue of diabetic patients. The present study was undertaken to test the hypothesis that UCP2 mRNA levels are decreased in obese and diabetic patients and also to elucidate the depot specific comparison of UCP2 gene expression and its association with insulin resistance, different parameters of metabolic syndrome and the adipokine, adiponectin.

Results

Clinical characteristics of the population. The clinical characteristics of study subjects, which included 23 non-obese controls, 26 obese and 20 T2DM patients, are shown in Table 1. Mean levels of BMI, waist size, systolic blood pressure, plasma glucose, insulin and HOMA-IR were significantly increased in obese subjects. Serum high density lipoprotein (HDL) cholesterol and adiponectin levels were significantly reduced in obese subjects

Table 1. Characteristics of study subjects (mean ± SE)

	Control	Obese	T2DM
Number	23	26	20
Sex (M:F)	8:15	9:17	8:12
BMI (kg/m ²)	20.56 ± 0.54	32.9 ± 1.8***	39.37 ± 2.29***,†
Waist (cm)	78.57 ± 1.13	103.58 ± 4.6***	117.6 ± 5.2***,†
SBP (mmHg)	114.78 ± 1.9	120.77 ± 1.6*	127.5 ± 1.76***,†
DBP (mmHg)	77.83 ± 1.5	78.46 ± 1.3	85.5 ± 1.7***,††
Glucose (mg/dl)	4.72 ± 0.09	5.13 ± 0.07***	9.8 ± 0.42***,†††
Total cholesterol (mg/dl)	3.87 ± 0.24	4.71 ± 0.21	6.16 ± 0.35***,†††
Triglyceride (mg/dl)	1.14 ± 0.06	1.31 ± 0.1	2.09 ± 0.14***,†††
HDL cholesterol (mg/dl)	1.24 ± 0.04	1.08 ± 0.04*	0.83 ± 0.03***,†††
Insulin (μU/ml)	11.66 ± 3.03	24.69 ± 5.12	33.62 ± 16.9
HOMA-IR	2.46 ± 0.67	5.75 ± 1.26*	8.17 ± 2.22*
Serum adiponectin (mg/l)	10.16 ± 1.06	6.2 ± 0.92**	7.55 ± 0.74*

***p < 0.001; **p < 0.01, *p < 0.05 vs control group; †††p < 0.001, ††p < 0.01, †p < 0.05, †p < 0.02 vs. obese group.

compared with control subjects. In the case of diabetic patients, mean levels of BMI, waist size, systolic and diastolic blood pressure, plasma glucose and triglycerides were significantly increased, while HDL cholesterol was significantly reduced compared with control and obese subjects. Diabetic patients exhibited insulin resistance condition as expected and serum adiponectin levels were significantly reduced compared with control subjects. Gender dimorphism was observed in serum adiponectin levels where female controls exhibited significantly higher levels compared with male controls (male controls, 11.81 ± 0.89 and female controls, 6.85 ± 1.8).

Depot-specific UCP2 gene expression. In non-obese controls, obese and diabetic patients, both in males and females, UCP2 gene expression of OAT was significantly higher compared with SAT (Fig. 1) *p < 0.05, **p < 0.01.

Metabolic condition based effect on UCP2 and adiponectin gene expression. Metabolic condition based analysis reported that UCP2 gene and adiponectin gene expression was significantly reduced in obese and diabetic patients compared with controls (**p < 0.001) and this reduction was almost 60–70%, irrespective of depots and gender (Fig. 2A and B).

When adiponectin gene expression was normalized to BMI, it exhibited a significantly reduced expression in obese and diabetic patients compared with controls (0.046 ± 0.001, 0.031 ± 0.001, 0.026 ± 0.001; ***p < 0.001 control, obese and diabetic patients values respectively). UCP2 gene expression was also independent of changes in BMI (0.054 ± 0.002, 0.037 ± 0.003, 0.034 ± 0.002; ***p < 0.001 control, obese and diabetic patients values respectively).

Gender-based effect on UCP2 gene expression. Using the 2^{-ΔΔCt} method, the data are presented as fold changes in gene expression normalized to an endogenous reference gene, relative to control (calibrator).

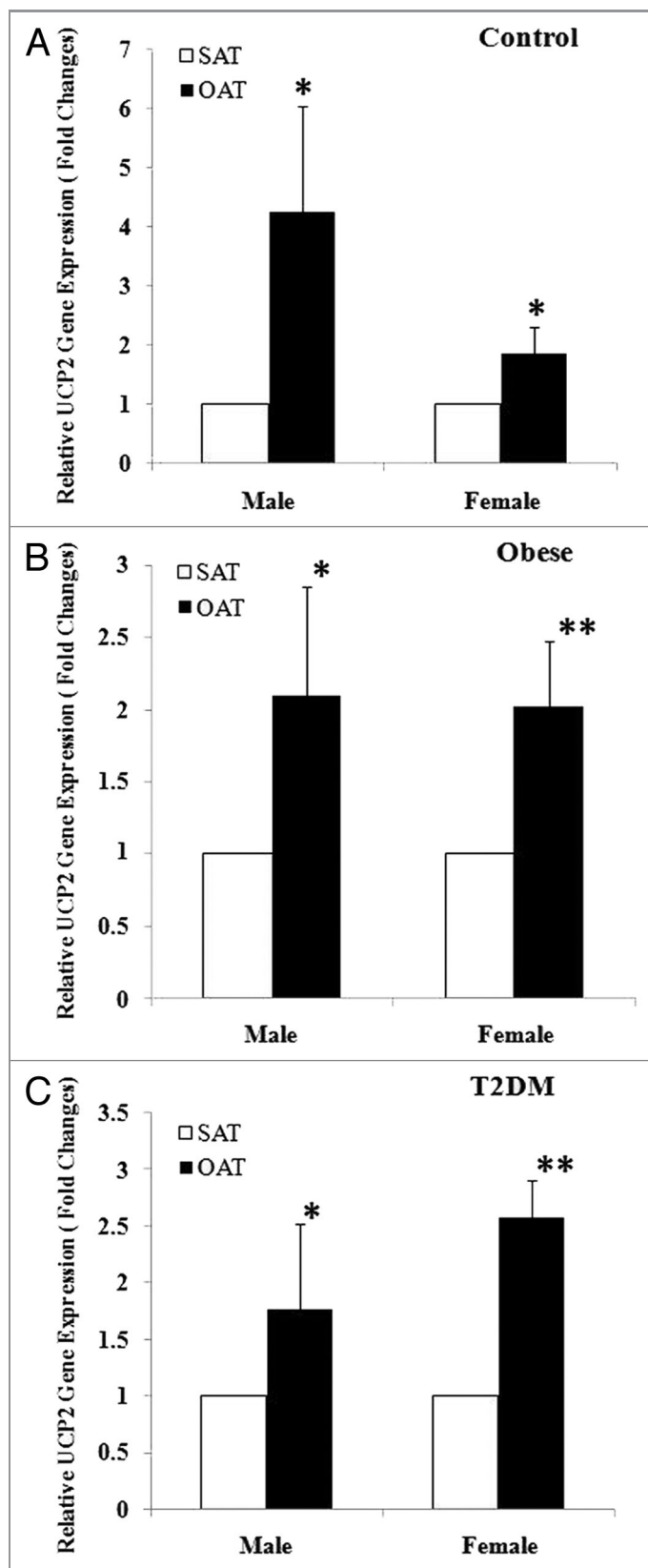


Figure 1. UCP2 gene expression from SAT (empty bar) and OAT (solid bar) of non-obese controls (A), obese (B) and T2DM (C) from both genders (male and female). UCP2 gene expression was measured with real-time PCR and normalized to β actin using the $2^{-\Delta\Delta Ct}$ method of relative quantification. Data are presented as mean \pm SE (results presented relative to SAT and mean value for SAT was set at 1). * $p < 0.05$, ** $p < 0.01$.

patients, where UCP2 gene expression was reduced more in omental compared with subcutaneous fat, and this effect is more prominent in males but not in females (* $p < 0.05$).

Relation between UCP2 gene expression and the metabolic syndrome parameter and adiponectin. With regard to association studies of UCP2 gene expression with different parameters of metabolic syndrome, gene expression from SAT but not OAT exhibits a significant negative association with the obesity parameters waist circumference, insulin, HOMA-IR and also with the lipid parameter of triglyceride and positive association with serum adiponectin and SAT and OAT adiponectin gene expression after adjustment for age (Table 3).

Discussion

The Asian Indian population seems to be genetically more prone to insulin resistance and T2DM due to their unique features of increased central obesity at a low BMI.^{1,2} Central obesity is associated with increased local adipose tissue inflammation. In the last decade, new endocrine functions have been discovered for adipose tissue, indicating that the adipocyte is an important regulator of systemic metabolism via the production of multiple proteins (adipokines).²¹ UCP2 is also widely expressed in human tissues, including white adipose tissue. Initially, several studies had reported the role of UCP2 in the regulation of whole energy homeostasis.²⁴ Recently, the UCP2 locus has been linked to obesity, hyperinsulinemia and resting energy expenditure, suggesting that variation in UCP2 expression could influence the development of obesity and its associated metabolic disorders, such as T2DM, hypertension and atherosclerosis.¹⁴⁻¹⁶ Hence, UCP2, a member of mitochondrial carrier proteins, has been hypothesized as an attractive candidate gene for obesity and T2DM.^{3,16,24}

Most of the literature has focused on polymorphism studies of the promoter region of the human UCP2 gene^{14-16,25} whereas regulation of UCP2 gene expression has been studied more extensively only in rodents.^{26,27} Interestingly, one rodent study reported that UCP2 controls adiponectin gene expression in adipose tissue of mice.²⁰ Association between these proteins was not studied in human adipose tissue. Since both adiponectin and UCP2 are important players in energy metabolism and adipose tissue function, studies exploring the association between these proteins are of great importance from a therapeutic point of view in obesity related disease.

Limited data are available on UCP2 gene expression in human adipose tissue of obese subjects,^{7,8,10,13,17} but no data are available from diabetic patients. Thus, these studies do not yet offer a clear view of the role of UCP2 in human adipose tissue of diabetic patients.

Gender-based effect in obesity exhibits no difference in UCP2 gene expression from SAT and OAT of male and female subjects (Table 2A). Table 2B presents the gender-based effect in diabetic

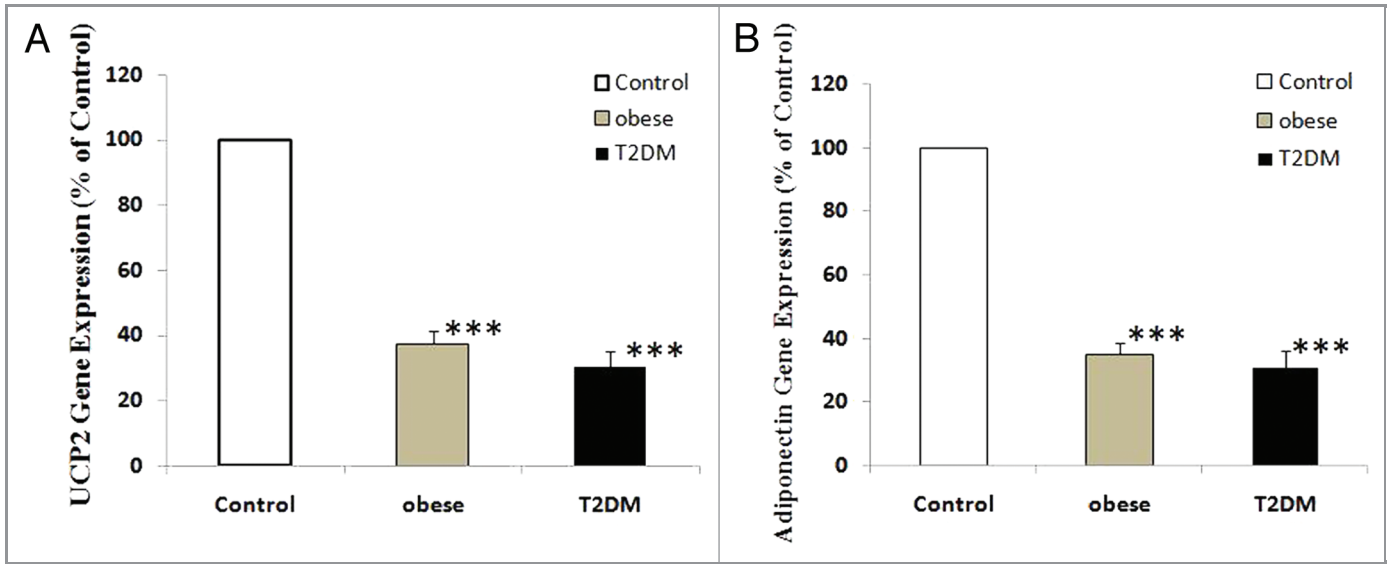


Figure 2. (A) Percentage (%) reduction in UCP2 gene expression of obese (gray bar) and diabetic patients (solid bar) compared with controls (empty bar). Data are reported as mean \pm SE, and results are presented as percent of control group (control value = 100%) [Using the $2^{-\Delta\Delta Ct}$ method, the data are presented as the fold changes in gene expression normalized to an endogenous reference gene and relative to control (calibrator)]. *** $p < 0.001$. (B) Percentage (%) reduction in adiponectin gene expression of obese (gray bar) and diabetic patients (solid bar) compared with controls (empty bar). Data are reported as mean \pm SE, and results are presented as percent of control group (control value = 100%) [Using the $2^{-\Delta\Delta Ct}$ method, the data are presented as the fold changes in gene expression normalized to an endogenous reference gene and relative to control (calibrator)]. *** $p < 0.001$.

To our knowledge, this is the first study wherein UCP2 gene expression was studied in diabetic patients in a depot-specific manner (including SAT and OAT). In the present study, UCP2 gene expression was evaluated in human adipose tissue of obese subjects. Furthermore, association of UCP2 gene expression from both the depots with insulin resistance, with different parameters of metabolic syndrome, and the adipokine adiponectin was also investigated to gain insight into adipocyte biology.

The main finding of the study is a reduced UCP2 gene expression in obese Indian subjects compared with non-obese controls, irrespective of depots and gender. These findings are consistent with other reports.^{7,13,17,18} This lower expression may result in a decreased production of UCP2, decreased energy expenditure and hence, increased accumulation of body fat.

Table 2. Gender-based effect in obese or diabetic subjects (mean \pm SE)

A. Obese subjects	SAT OB M	OAT OB M
UCP2 gene expression	(n = 6) 0.54 \pm 0.11	(n = 6) 0.25 \pm 0.1*
	SAT OB F	OAT OB F
UCP2 gene expression	(n = 14) 0.35 \pm 0.06	(n = 14) 0.37 \pm 0.08
B. Diabetic subjects	SAT DM M	OAT DM M
UCP2 gene expression	(n = 6) 0.38 \pm 0.1	(n = 6) 0.01 \pm 0.02 [†]
	SAT DM F	OAT DM F
UCP2 gene expression	(n = 9) 0.43 \pm 0.11	(n = 9) 0.29 \pm 0.06

Using the $2^{-\Delta\Delta Ct}$ method, the data are presented as the fold changes in gene expression normalized to an endogenous reference gene and relative to control (calibrator). SAT, subcutaneous adipose tissue; OB, obese; M, male; F, female; OAT, omental adipose tissue; DM, diabetic; * $p < 0.02$ (paired t-test); [†] $p < 0.05$ (paired t-test).

Consistent with previous reports, adiponectin gene expression was significantly reduced in obese and diabetic patients.^{28,29}

Interestingly, UCP2 gene expression was also reduced in Indian diabetic patients and this decrease was independent of changes in BMI, thus implicating this gene in the pathophysiology of both obesity and T2DM.

Oberkofler et al.¹⁷ found that UCP2 mRNA abundance was higher in the intra-peritoneal region than in the extra peritoneal adipose tissue. The reason could be that the lipolysis activity and triglyceride turnover is higher in omental fat compared with subcutaneous depot. The present study also reported that UCP2

Table 3. Association of UCP2 expression in SAT and OAT with different metabolic syndrome parameter

Parameter	UCP2 expression (SAT)	UCP2 expression (OAT)
BMI	NS	NS
Waist	-0.25*	NS
Insulin	-0.429*	NS
HOMA-IR	-0.425*	NS
Triglyceride	-0.287*	NS
HDL cholesterol	NS	NS
Serum adiponectin level	0.324*	NS
Adiponectin gene expression (SAT)	0.497**	NS
Adiponectin gene expression (OAT)	0.389*	NS

* $p < 0.05$, ** $p < 0.01$. Partial correlation analysis with adjustment for age.

gene expression was 2- to 4-fold higher in OAT compared with SAT of the Indian population.

The degree of obesity and fat distribution differed across genders. In gender-based analysis, in our population, we found that the reduction in UCP2 gene expression was more in OAT compared with SAT of male diabetic patients but not of females. In particular, one previous study has also demonstrated that UCP2 gene polymorphisms may increase the risk of central obesity and metabolic syndrome with greater effects on Asian men.¹⁴

In agreement with the results of previous studies,^{7,13} this present study found that UCP2 gene expression of SAT, but not OAT, was significantly associated with the obesity parameters waist circumference, insulin and HOMA-IR. Though UCP2 gene expression was higher overall in OAT of control, obese and T2DM patients, a reduction in UCP2 gene expression was found in omental compared with subcutaneous fat in obese and diabetic patients, compared with controls. The lack of physiological regulation of UCP2 gene expression in OAT with the parameters of metabolic syndrome could be due to defective omental fat compared with subcutaneous.¹⁷

The association of the UCP2 gene with the lipid parameter triglyceride suggests its role in the regulation of lipid metabolism. Possibly through its function as a free fatty acid transporter, UCP2 may participate in the regulation of lipids as fuel substrates. The most important finding of the association study is the positive correlation between adiponectin and UCP2 gene expression in the Indian population. This new insight may provide us with innovative therapeutic strategies to prevent obesity related diseases like diabetes and CVD.

In conclusion, reduced UCP2 gene expression in obese and diabetic patients in our population, and its association with obesity parameters and HOMA-IR, confirms its role as a candidate gene in the study of obesity and diabetes. Association of UCP2 gene expression with triglyceride implicates its role in lipid metabolism. The UCP2 gene also remains an interesting target for pharmacological upregulation in the treatment of obesity and diabetes with its association with the adipokine, adiponectin.

Materials and Methods

Subjects. The study population consisted of a total of 69 subjects, consisting of 23 non-obese controls, 26 obese and 20 T2DM patients. Diabetes was defined based on history (for patients taking oral hypoglycemic drugs) or according to WHO criteria³⁰ of fasting glucose ≥ 7.0 mmol/l or 2 h glucose ≥ 11.1 mmol/l for subjects without a clinical history of diabetes. Most of the diabetic patients were receiving anti-diabetic agent sulphonylurea or metformin or a combination of both. Diabetic patients were not receiving treatment for hypertension or any other illness at the time of study. None of the diabetic patients had significant renal, hepatic or cardiovascular disease. Obesity was defined if the BMI was ≥ 25 kg/m² according to the cut-off suggested for Asian Indians.³¹ Control subjects were classified as having normal glucose tolerance (fasting plasma glucose < 6.1 mmol/l and 2 h

glucose < 7.8 mmol/l). They were non-hypertensive and non-obese and were confirmed to have no known disease, including cardiac and thyroid disease. None of the controls have a family history of T2DM. All the subjects gave their informed consent after the procedure was explained to them. The ethics committee of Sir Harkisondas Nurrotumdas Hospital and Medical Research Society approved the project.

The study subjects were surgical patients from Sir H.N. Hospital and Research Centre and St. George's Hospital, Mumbai, who underwent abdominal surgery for various ailments such as gall bladder stone (cholelithiasis), kidney stones (renal calculi), appendicitis, ovarian cyst, umbilical/inguinal hernia and laparoscopic gastric bypass. All subjects fasted overnight before tissue removal. SAT and OAT samples (~1–5 g) were collected during the surgical procedure and were immediately sent to the laboratory. SAT was collected at the site of transverse lower abdominal incision and OAT was collected from greater omentum.

Anthropometric measurements and biochemical estimation. The subjects had an age range of 35–65 y. Anthropometric measurement including height, weight and waist circumference (abdominal circumference at the level of iliac crest) and clinical details including blood pressure measurement were obtained from the case report. BMI was calculated from the ratio of body weight in kg to height in square meters and expressed as kg/m² units. Fasting blood samples were obtained after surgery for the determination of serum adiponectin, insulin and plasma glucose levels. Fasting plasma glucose was measured by the glucose peroxidase method (Randox). ELISA Kits for human serum adiponectin and insulin were obtained from Linco Res Inc.

Insulin resistance measured as Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) using following formula:³²

$$\text{Insulin resistance} = \frac{\text{Fasting insulin (uU/ml)} \times \text{Fasting glucose (mmol/l)}}{22.5}$$

Isolation of RNA from adipose tissue. Adipose tissue was immediately put into liquid nitrogen after resection and then stored at -80°C until used. Total RNA was extracted using RNeasy Liquid Tissue Extraction Minikit (Qiagen Inc.) according to manufacturer instructions. RNA was quantified by measuring absorbance at 260 nm and 280 nm. RNA integrity was verified by carrying out formamide agarose gel electrophoresis. Total RNA was stored at -80°C .

Real time reverse transcriptase polymerase chain reaction (RT-PCR) measurement of UCP2 and adiponectin gene expression. RNA was converted into cDNA by reverse transcription (RT) using random hexamers and Multiscribe TM Reverse Transcriptase at 25°C for 10 min, 37°C for 120 min and 85°C for 5 min according to manufacturer's instructions (High Capacity cDNA Reverse transcription kit, Applied Biosystems).

Quantitative real-time PCR was performed using an Applied Biosystems 7500 Real-Time PCR system as described by the manufacturer (Applied Biosystems). Taqman universal PCR master mix was used. Taqman primers and probes for human UCP2, adiponectin and β actin as an internal control were also supplied by Applied Biosystems (Assay-by-Design). All samples

were run in duplicate. For the negative control, the same set-up was used with the exception of the addition of the cDNA sample. PCR product was not detected in the control conditions. In brief, UCP2 mRNA and β actin were amplified in separate wells at 95°C for 10 min and thereafter repeating cycles comprised of 95°C for 15 sec and 60°C for 1 min for annealing and extension steps. During the extension step, an increase in fluorescence was measured in real-time. A calibrator sample (controls) was run together with samples in every run (on each plate) in the PCR instrument.

Data were obtained as ct values according to the manufacturer's guidelines, where ct is defined as the cycle number at which fluorescence is statistically significant above background. Fold changes of gene expression were calculated by the $2^{-\Delta\Delta ct}$ method, where Δct is the difference in ct of the gene of UCP2 and ct of β actin; and $\Delta\Delta ct$ is the difference in Δct of unknown sample and Δct of the calibrator or control samples.³³ Using the $2^{-\Delta\Delta ct}$ method, the data are presented as the fold changes in gene expression normalized to an endogenous reference gene and relative to control (calibrator).

Statistical analysis. UCP2 gene expression was measured with the real-time PCR and normalized to β actin using the $2^{-\Delta\Delta ct}$ method of relative quantification. Quantitative data are expressed

as mean \pm SE from determinations. Group means were compared using a one-way ANOVA with post hoc tests (for multiple group comparisons) or unpaired t-test (for comparison between two groups). Student's paired t-test was used for comparing data from SAT and OAT depots in each individual subject. Partial correlation analysis was performed with adjustment for age for determining the relationship between adiponectin gene expression and different parameters of syndrome and UCP2 gene expression. The level of significance for all statistical tests was set at $p < 0.05$. All analysis was performed using SPSS (Version 16).

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

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