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Different gene expression profiles in subcutaneous & visceral adipose tissues from Mexican patients with obesity

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***Background & objectives*: Obesity is a health problem that requires substantial efforts to understand the physiopathology of its various types and to determine therapeutic strategies for its treatment. The objective of this study was to characterize differences in the global gene expression profiles of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) between control patients (normal weight) and patients with obesity (IMC≥30) using microarrays.**

***Methods*: Employing RNA isolated from SAT and VAT samples obtained from eight control and eight class I, II and III patients with obesity, the gene expression profiles were compared between SAT and VAT using microarrays and the findings were validated via real-time quantitative polymerase chain reaction.**

***Results*: A total of 327 and 488 genes were found to be differentially expressed in SAT and VAT, respectively ($P \leq 0.05$). Upregulation of *PPAP2C, CYP4A11* and *CYP17A1* genes was seen in the VAT of obese individuals.**

***Interpretation & conclusions*: SAT and VAT exhibited significant differences in terms of the expression of specific genes. These genes might be related to obesity. These findings may be used to improve the clinical diagnosis of obesity and could be a tool leading to the proposal of new therapeutic strategies for the treatment of obesity.**

Key words Adipose tissue - gene expression - obesity - subcutaneous adipose tissue - visceral adipose tissue

[#]Equal contribution

The World Health Organization (WHO) has referred to obesity as a 'global epidemic' due to its increasing prevalence[1]. According to the 2012 National Health and Nutrition Survey[2] of the Mexican population, the prevalence of being overweight or obese was 71.2 per cent in adults over the age of 20 yr and 34.4 per cent in children. The economic impact of treating this disease is of considerable concern, due to the increase in associated co-morbidities and mortality[2]. Adipose tissue is now recognized as an important tissue not only for energy storage but also due to its important endocrine functions, including the secretion of many factors, such as adipocytokines and adipokines[3]. Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) are the two main types of adipose tissue in adult humans. Numerous clinical studies[4,5] have demonstrated that important differences exist with respect to endocrine production in different types of adipose tissue. The observation that the metabolism and secretion of lipids vary at different sites between SAT and VAT suggests site-specific functions[4,5]. It has been demonstrated that VAT exhibits a more pro-inflammatory and metabolically harmful profile than SAT[6]. VAT differs both morphologically and functionally from SAT, including via the variation in the function of insulin in the regulation of lipolysis.

Passaro *et al*[7] evaluated gene expression through microarray analysis in gluteal and abdominal SAT in eight healthy controls identifying expression differences in several *HOX* genes between gluteal and abdominal depots. Gerhard *et al*[8] determined the gene expression profiles of SAT and VAT in six patients with extreme obesity using microarrays. The authors identified differential expression in many genes related to DNA replication and repair, cell morphology and development[8]. Lee *et al*[9] identified differential gene expression profile in subcutaneous abdominal fat biopsies from 19 obese individuals through microarray analysis. The upregulated and downregulated genes were classified as being associated with the following terms: inflammation and immune response, signalling, transcription regulation, cell cycle control, cell adhesion, transport carrier, structural protein/cytoskeleton organization, energy metabolism, response to stress, cell growth and apoptosis pathways[9]. Linder *et al*[10] examined differential gene expression between seven obese males and 11 obese females through representational difference analysis of cDNA from SAT and VAT. The results revealed a sex-specific pattern of gene expression in pathways related to the immune response, lipid metabolism, protein biosynthesis, signal transduction, cell structure, carbohydrate and amino acid metabolism, mitochondrial genes and other genes with unknown functions[10].

Because there is no specific evidence that genes or groups of genes show alterations and are involved in the development of this disease, and further considering the lack of any sufficiently advanced molecular biological studies on the specific functions and physiological roles of SAT and VAT or their differences in human obesity, this study was designed to examine the transcriptomes of SAT and VAT samples from obese patients and normal weight individuals with the aim of contributing to the current understanding of pathophysiology of obesity.

Material & Methods

The study protocol was approved by the Ethics Committee of the Military Central Hospital, Mexico City, Mexico (No. DINV-78798). The study was explained to all individuals before obtaining their written informed consent. Adult individuals of both sexes were classified as obese or lean according to their body mass index (BMI) following the WHO criteria[1] (BMI of $\geq$30 or <24.9 kg/m^2, respectively) and were recruited during the period from September 2015 to February 2017. The samples were obtained during inguinal hernia repair and laparoscopic cholecystectomy, performed in the department of Surgery at the Military Central Hospital, Mexico City, Mexico. The following inclusion criteria were applied: voluntary patients with age of 18-50 yr and absence of chronic diseases, including cancer, HIV infection and thyroid disorders. The exclusion criteria included pregnancy or treatment with drugs that may alter metabolism and compromise body weight. Briefly, after the administration of general anaesthesia, incisions (1/4″ to 1/2″) were made (one near the navel and the others lower on the abdomen). A laparoscope was inserted through one of the openings, and SAT samples were obtained. A mesh was positioned at the hernia and fastened in place with sutures. Finally, the instrument was removed, and the holes were closed with surgical tape. In the laparoscopic gallbladder removal procedure, briefly, several small incisions (<1″ long) were made (rather than one large longitudinal incision at the inferior aspect of the umbilicus), and the laparoscope was inserted and subsequently advanced through the subcutaneous fat (taking a sample) to the anterior rectus sheath. The dissection and extraction of the gallbladder were realized, and the incisions were closed.

Two different groups of patients with available electronic health records were included in these experiments: (*i*) a group of 16 patients (8 non-diabetic normal weight individuals and 8 patients with obesity) were used for the microarray experiments, and (*ii*) a confirmation group of 72 patients (35 non-diabetic normal weight individuals and 37 obese individuals, including the 16 patients in the microarray experiment) were used for validation of genes identified in the microarray analysis via real-time polymerase chain reaction (qPCR).

The range of BMI among the 35 individuals classified as non-diabetic normal weight was 18.5-24.9 kg/m^2. The range of the BMI of the 37 obese individuals was 30-34.9 kg/m^2, 12 of whom exhibited class II obesity (BMI=35-39.9 kg/m^2), while six exhibited class III obesity (BMI≥40 kg/m^2). The anthropometric and biochemical characteristics of the 16 individuals included in the microarray experiment group are summarized in Table I.

SAT and VAT samples were obtained from each patient. Isolation of total RNA was performed using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). RNA quality and integrity were assessed using QIAxcel and QIAxpert equipment (Qiagen).

For the microarray analysis, the synthesis of cDNA and subsequent fluorescent labelling of cRNA were performed with eight replicates in both the control (n=8) and obesity (n=8) groups, according to the manufacturer's protocol (Two-Color Microarray-Based Gene Expression Analysis/Low Input Quick Amp Labeling; Agilent Technologies, CA, USA)[11]. The arrays were scanned using a NimbleGen microarray scanner (Roche, Switzerland), and signal intensities in TIFF images were calculated using Feature Extraction software (FE, version 12.0; Agilent). All of the arrays were scanned and quantified using Imagen Feature Extraction software (*www.agilent.com/en/product/mirna-microarray-platform/mirna-microarray-software/feature-extraction-software-228496*), and the associated biological pathways were determined with GeneSpring GX 13.0 software (Agilent). The data were deposited in the Gene Expression Omnibus (GEO accession no. GSE84599). Differentially expressed genes were selected using the filtering criteria of a change in expression of at least 2.0 fold and $P \leq 0.05$. The Benjamini-Hochberg[12] algorithm was employed to compute false discovery rates.

Table I. Anthropometric and biochemical parameters of patients from the microarray analysis group

Parameters	Normal weight (n=8) (6 females, 2 males)	Obese (n=8) [Grade I (n=6), Grade II (n=2)] All females
Age (yr)	35.63±9.30	41.13±8.59
Weight (kg)	63.19±5.90	78.31±8.22***
BMI (kg/m^2)	23.52±1.34	33.03±3.15***
Glucose (mg/dl)	118.88±21.34	126.57±39.29
Cholesterol (mg/dl)	166.75±22.29	169.943±22.29
Triglycerides (mg/dl)	157.25±37.67	160.71±21.53
HDL (mg/dl)	35.39±6.06	35.88±2.13
LDL (mg/dl)	124.37±22.38	128.78±20.80
Creatinine (mg/dl)	0.81±0.14	0.80±0.06
Urea (mg/dl)	26.63±9.55	21.50±3.21
Uric acid (mg/dl)	6.04±1.11	6.98±0.66

***P=0.001, compared to controls. HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index

For microarray confirmation, reverse transcription for qPCR was performed according to the manufacturer's protocol using a One-Step qPCR assay (One-Step qRT-PCR KAPA SYBR FAST® Kit, Kapa Biosystems, Merck, Darmstadt, Germany) and Rotor-Gene Q equipment (Qiagen) with primers specific to genes of interest (synthesized by Integrated DNA Technologies, Iowa, USA) (Table II). Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ relative quantification method[13].

Statistical analysis: All statistical analyses were performed using commercially available GraphPad Prism version 6.0 (La Jolla, CA, USA) software and XLSTAT for Excel 2018 (Addinsoft, NY, USA). The data are expressed as the mean±SD. The Kolmogorov-Smirnov normality test was performed based on the null hypothesis that the distribution was normal. Differences between groups were tested using the unpaired Student's *t* test and ANOVA with Bonferroni *post hoc* analysis, and a correlation analysis was performed using the Pearson test.

Results

Gene expression levels in the SAT and VAT samples were analyzed using microarrays, as shown in Fig. 1. Two independent microarray analyses were performed employing Agilent arrays; the first was used to examine gene expression in SAT samples from 16 volunteers,

Table II. Primers used for real-time quantitative polymerase chain reaction

Gene name	Gene symbol	Accession number	Primer sequence	Product length (bp)
Adiponectin	*ADPN*	NC_000003.12	F 5'- CATCTCCTCCTCACTTCCATTC - 3' R 5' - GGCAGAGCTAATAGCAGTAGAACAG - 3'	158
Mevalonate diphosphate decarboxylase	*MVD*	NC_000016.10	F 5'- CGCCCATCTCTTACCTCAATG - 3' R 5' - ACACAGCAGCCACAAACTCAG - 3'	165
Peroxisome proliferator-activated receptor gamma	*PPARG*	NC_000003.12	F 5'- GCTGTCATTATTCTCAGTGGAGAC - 3' R 5' - GTCTGAGGTCTGTCATTTTCTGG - 3'	166
Cytochrome P450 family 17, subfamily A, member 1	*CYP17A1*	NC_000010.11	F 5'- CGATCAGAAGCTGGAGAAGATC - 3' R 5' - CCCCATTCTTGTAGGAGGTATT G - 3'	161
Cytochrome P450 family 4, subfamily A, member 11	*CYP4A11*	NC_000001.11	F 5'- TCCCATGGTTCCTACAGATTC - 3' R 5' - TCCAGCATCACTCGTACAGAG - 3'	167
Cytochrome P450 family 1, subfamily B, member 1	*CYP1B1*	NC_000002.12	F 5'- CTAGGCAAAGGTCCCAGTTC - 3' R 5' - CACCGACAGGAGTAGCAGG - 3'	158
Patatin-like phospholipase domain-containing 3	*PNPLA3*	NC_000022.11	F 5' - TCATCTCCGGCAAAATAGGC - 3' R 5' - TGAAGGAAGGAGGGATAAGGC - 3'	153
Acyl-CoA synthetase long-chain family member 3	*ACSL3*	NC_000002.12	F 5'- CAGCTGTAACATTTGCCACC - 3' R 5' - GGTAGATGGTTTTGAAGACACG - 3'	147
Succinate-CoA ligase alpha subunit	*SUCLG1*	NC_000002.12	F 5' - GTACGAGTCAAGCACAAACTGC - 3' R 5' - GATCTGGACACAATGCCAATC - 3'	149
Isocitrate dehydrogenase [NADP(+)] 2, mitochondrial	*IDH2*	NC_000015.10	F 5'- GTGGAGATGGATGGTGATGAG - 3' R 5' - CCAGTGCAGAGTCAATGGTG - 3'	157
Glycerol-3-phosphate acyltransferase, mitochondrial	*GPAM*	NC_000010.11	F 5'- AGAAATGGTTGCCACTGTCTC - 3' R 5' - TGAACTGGTAGAAACAGAAGCG - 3'	165
Phospholipid phosphatase 2	*PLPP2*	NC_000019.10	F 5'- ATTTTACTGCGGGGATGACTC - 3' R 5' - AAGTCCGAGCGAGAATAGAGC - 3'	159
Beta-2-microglobulin	*B2M*	NC_000015.10	F 5' - CAACTTCAATGTCGGATGGATG - 3' R 5' - TCGCGCTACTCTTCTCTTTCTGG - 3'	152
Actin, beta	*ACTB*	NC_000007.14	F 5' - CTGGCACCCAGCACAATG - 3' R 5' - GGGCCGGACTCGTCATAC - 3'	152
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	NC_000012.12	F 5' - GAGCCAAAAGGGTCATCATCTC - 3' R 5' - CCTTCCACGATACCAAAGTTGTC - 3'	152

divided into two groups: a control group (n=8) and a group with obesity (n=8); the second was employed to assess gene expression in VAT samples from the same groups. Microarray analysis demonstrated significant changes in the tissue transcriptomes, as shown in Tables III and IV. The analysis revealed decreased expression of 327 genes and increased expression of 488 genes in subcutaneous compared with those in visceral adipose tissue ($P \leq 0.05$). A 'volcano plot' showing the differential expression of all transcripts tested between VAT and SAT is provided in Fig. 2.

In the RT-PCR confirmation of gene expression, the expression levels of the adiponectin (*ADPN*) and peroxisome proliferator-activated receptor gamma (*PPARG*) genes were not affected by obesity in either tissue (SAT and VAT). Furthermore, the gene expression of mevalonate diphosphate decarboxylase (*MVD*) was decreased by obesity in SAT, while the levels of phosphatidic acid phosphatase type 2C (*PPAP2C*), cytochrome P450 4A11 (*CYP4A11*) and cytochrome P450 17A1 (*CYP17A1*) were significantly increased in VAT of obese patients. The gene expression level of *ADPN* was slightly increased in SAT in comparison with VAT in the obesity group. The isozyme levels of the long-chain fatty acid-coenzyme A ligase gene (*ACSL3*), succinyl-CoA ligase (*SUCGL*), isocitrate dehydrogenase 2 (*IDH2*), mitochondrial glycerol-3-phosphate acyltransferase (*GPAM*), patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) and cytochrome P450 1B1 (*CYP1B1*)

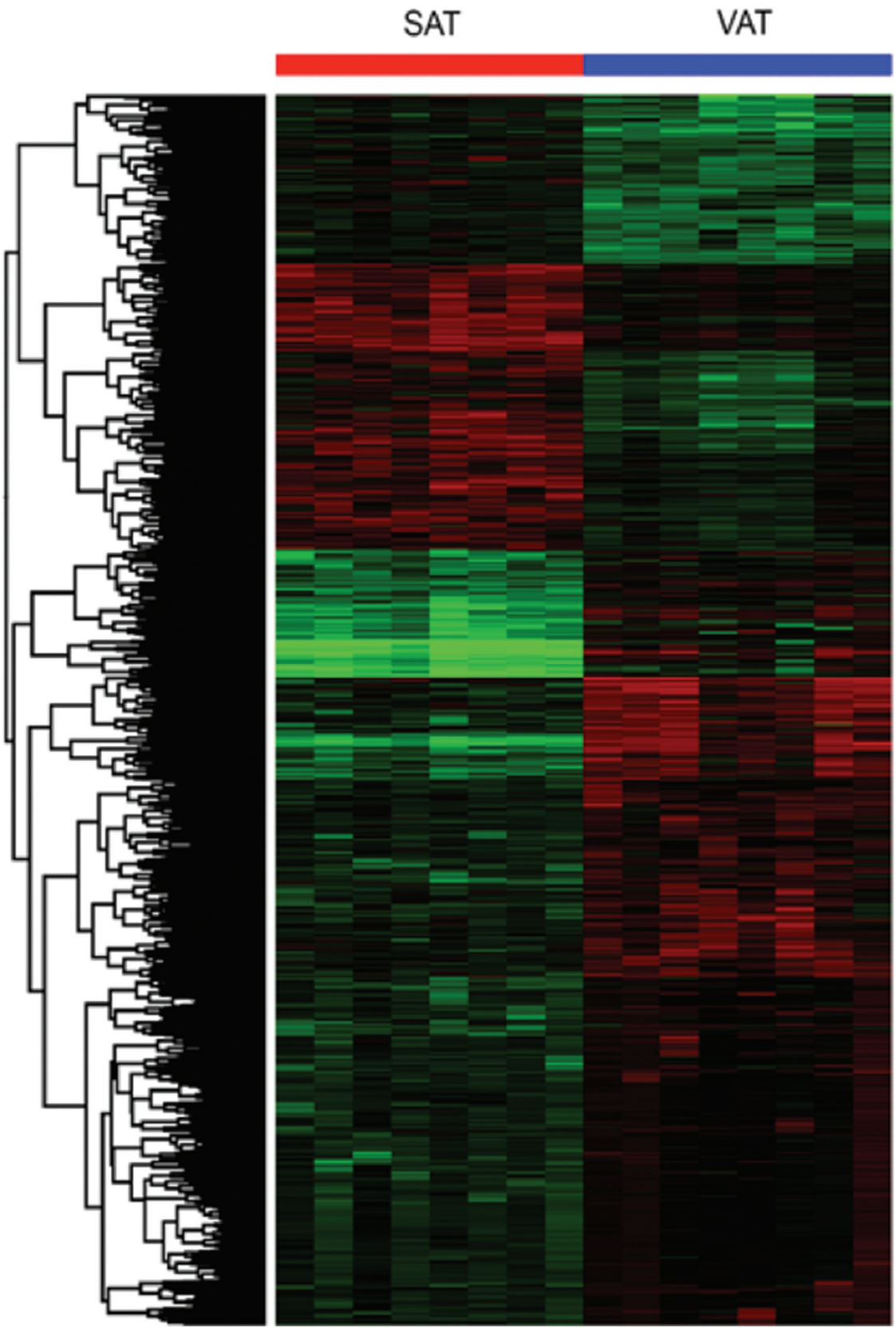

Fig. 1. Hierarchical cluster analysis of subcutaneous adipose tissue and visceral adipose tissue microarrays.

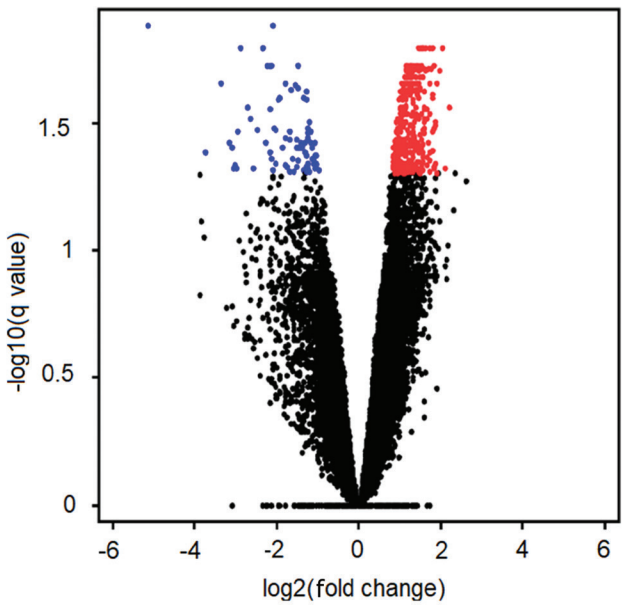

Fig. 2. Volcano plot of subcutaneous adipose tissue and visceral adipose tissue microarrays.

were significantly downregulated, whereas *PPAP2C*, *CYP4A11* and *CYP17A1* gene expression did not differ in the VAT of obese individuals (Figs 3 and 4).

Discussion

Several genes involved in signalling pathways exhibited altered expression in samples of both types of adipose tissue from the obese patients. One of these genes was *ADPN*. Several insulin-resistant states, such as obesity and type 2 diabetes, as well as cardiovascular diseases have been found to be associated with low levels of plasma *ADPN* in human obesity[14]. Our results were in agreement with previous reports demonstrating reduced *ADPN* expression in adipose tissue[15,16]. Another gene exhibiting altered expression in both SAT and VAT from obese individuals was *PPARG*. The *PPARG* signalling in metabolism regulation is considered a master regulator of adipogenesis[17]. Our results confirmed that downregulation of the *PPARG* gene induced downregulation of the *ADPN* gene and, thus, proliferation of adipocytes, lipoatrophy and alterations of insulin metabolism in the SAT and VAT of obese individuals in comparison with normal weight individuals[18].

Our study also showed that *MVD* gene expression was significantly downregulated in the SAT of obese individuals. A recent study in a high-fat diet-induced obese mouse model demonstrated that *MVD* gene expression was downregulated in the mouse liver after the administration of a *Polygala tenuifolia* plant extract, which is used as anti-obesity therapy[19]. *ACSL3* gene expression was significantly downregulated in the VAT of the obese individuals compared with that of the controls. Considering the evidence of the important role of *ACSL3* in hepatic lipogenesis and its regulation, the *ACSL3* gene and protein can be assumed to be essential for triglyceride metabolism in VAT, regulated by the *PPAR* gene, in addition to preventing the development of lipotoxicity in peripheral tissues and contributing to the modulation of steroidogenic genes in adipose tissue[20,21]. *SUCGL* and *IDH2* gene expression was also downregulated in the VAT of obese individuals. SUCGL deficiency in humans has only been related to mitochondrial hepatoencephalomyopathy, and expression of *IDH2* has been related to the development of acute myeloid leukaemia and glioma of the central nervous system[22,23]. Considering all of the physiological functions of these proteins, one can assume that these also play an important role in promoting metabolic changes in obesity in addition to their functions in VAT, as these are well known to induce alterations in the oxidative stress response, apoptotic processes and hypoxia levels in hypertrophic fat cells[24,25]. Thus, further study is warranted.

Our study also showed that the *GPAM* gene was expressed at a lower level in the VAT from obese individuals. These results were consistent with those of a previous study[26] showing that the total activity of the enzyme decreased as a function of adipocyte size in omental and subcutaneous fat. It is well known that adipocyte hypertrophy is prevalent in the VAT of obese individuals. Inhibition of *GPAM* has been proposed as a potential treatment for insulin resistance and type 2 diabetes[27]. The *PNPLA3* gene was found to be downregulated in the VAT of obese patients, supporting previous findings that *PNPLA3* expression was decreased in obese individuals[28]. In addition, differential expression of the *PNPLA3* gene has been observed in the SAT of obese patients on the initiation of weight loss with a low-calorie diet and after maintaining a stable body weight, where increased expression of this gene corresponds to body weight maintenance[29]. The reductions in the expression levels of these genes might have contributed to the reduction of *de novo* lipogenesis in the obese individuals, thereby contributing to alterations in glucose metabolism. No studies examining the expression of *PPAP2C* in human adipose tissue have been published; however, previous studies have demonstrated that this enzyme presents a wide variety of important biological functions and that

Table III. List of genes showing altered expression in the microarray analysis of subcutaneous adipose tissue samples from obese patients compared with controls

Wiki pathway	Microarray			
	Upregulated	Downregulated	Symbol	Name
Adipocyte-secreted products	1	1	*ADPN*	Adiponectin
			RETN†	Resistin
Transcription factors	2	5	*PPARG*	Peroxisome proliferator-activated receptor gamma
			PPARA†	Peroxisome proliferator-activated receptor alpha
			SREBP2†	Transcription factor, regulation of lipid, fatty acid and steroid metabolism
			IRS2	Insulin receptor substrate 2
			CEBPB	CCAAT/enhancer-binding protein beta
			MEF2A	Myocyte enhancer factor 2
			MEF2D	Myocyte-specific enhancer factor 2
Cholesterol synthesis	0	1	*MVD*	Mevalonate diphosphate decarboxylase
Fatty acid beta oxidation	0	2	*ACSL1*	Acyl-CoA synthetase long-chain family, member 1
			ACSL3	Acyl-CoA synthetase long-chain family, member 3
Glycolysis and gluconeogenesis	1	3	*GAPDHS*	Glyceraldehyde-3-phosphate dehydrogenase
			PGK1	Phosphoglycerate kinase 1
			HK1†	Hexokinase-1
			LDHC	Lactate dehydrogenase C
Inflammatory response	2	0	*CD40 LG*†	CD40 ligand
			VTN†	Vitronectin
Insulin signalling	1	6	*IRS*	Glucose homeostasis, intracellular insulin signalling cascade
			RAPGEF1†	Rap guanine nucleotide exchange factor 1
			MAP3K2	Mitogen-activated protein kinase kinase kinase 2
			MAP3K6	Mitogen-activated protein kinase kinase kinase 6
			MAP3K9	Mitogen-activated protein kinase kinase kinase 9
			MAP3K14	Mitogen-activated protein kinase kinase kinase 14
			AKT1	Serine/threonine protein kinase
Leptin signalling	1	1	*BAX*†	Bcl-2-like protein
			AKT1	Serine/threonine protein
Peroxisome lipid metabolism	0	1	*ABCD1*	ATP-binding cassette subfamily D, member 1
Apoptosis	1	3	*BAX*†	Bcl-2-like protein
			CDKN2A	Cyclin-dependent kinase inhibitor 2A
			CASP 4	Caspase 4
			CASP11	Caspase 11
Eicosanoid synthesis	1	1	*ALOX5*†	Arachidonate 5-lipoxygenase
			PNPLA3	Patatin-like phospholipase domain-containing protein 3
Cytochromes P450	2	0	*CYP7A1*†	Cytochrome P450 7A1
			CYP19A1†	Cytochrome P450 19A1

Differentially expressed genes were selected using the filtering criteria of a change of at least 2.0 fold and $P \leq 0.05$. †Upregulated genes

Table IV. List of genes showing altered expression in the microarray analysis of visceral adipose tissue samples from obese patients compared with those from controls

Wiki pathway	Microarray			
	Upregulated	Downregulated	Symbol	Name
Product secretion of adipocytes	0	2	*ADPN*	Adiponectin
			RETN	Resistin
Transcription factors	0	2	*PPARG*	Peroxisome proliferator-activated receptor gamma
			PPARGC1A	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
Cholesterol synthesis	0	1	*MVD*	Mevalonate diphosphate decarboxylase
Fatty acid beta oxidation	0	3	*ACSL3*	Acyl-CoA synthetase long-chain family member 3
			CHKB	Choline kinase beta
			HADHA	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA
Glycolysis and gluconeogenesis	1	0	*SLC2A5*†	Solute carrier family 2, member 5
Inflammatory response	2	0	*CD40LG*†	CD40 ligand
			CD86†	Cluster of differentiation 86
Insulin signalling	4	6	*RAPGEF1*†	Rap guanine nucleotide exchange factor 1
			EGR1†	Early growth response protein 1
			FOS†	Finkel-Biskis-Jinkins murine osteogenic sarcoma virus
			SUCGL	Succinyl-CoA ligase
			PTEN	Phosphatase and tensin homolog
			MAP3K2	Mitogen-activated protein kinase kinase kinase 2
			MAP3K4†	Mitogen-activated protein kinase kinase kinase 4
			MAP3K12	Mitogen-activated protein kinase kinase kinase 12
			MAP3K13	Mitogen-activated protein kinase kinase kinase 13
			MAP3K14	Mitogen-activated protein kinase kinase kinase 14
Leptin signalling	2	1	*BAX*†	Bcl-2-like protein
			SRC†	Proto-oncogene tyrosine protein kinase
			PDE3B	Phosphodiesterase
Peroxisome lipid metabolism	0	4	*ABCD1*	ATP-binding cassette subfamily D, member 1
			IDH1	Isocitrate dehydrogenase 1
			ACOX2	Acyl-CoA oxidase
			SLC27A2	Solute carrier family 27
Apoptosis	3	1	*BAX*†	Bcl-2-like protein
			PRF1†	Pore-forming protein
			CASP2†	Caspase 2
			CDKN2A	Cyclin-dependent kinase inhibitor 2A
Triglyceride synthesis	1	1	*GPAM*	Glycerol-3-phosphate acyltransferase, mitochondrial
			PPAP2C†	Phosphatidic acid phosphatase type 2C
Eicosanoid synthesis	0	1	*PNPLA3*	Patatin-like phospholipase domain-containing protein 3
Carbohydrate metabolism	3	0	*GALT*†	Galactose-1-phosphate uridylyltransferase
			G6PC3†	Glucose-6-phosphatase
			SLC2A5†	Solute carrier family 2, member 5

Contd...

Wiki pathway	Microarray			
	Upregulated	Downregulated	Symbol	Name
Nuclear receptors	0	1	*PPARG*	Peroxisome proliferator-activated receptor gamma
Oxidative stress	2	0	*FOS*†	Finkel-Biskis-Jinkins murine osteogenic sarcoma virus
			NOX4†	NADPH oxidase 4
Cytochromes P450	2	1	*CYP1B1*	Cytochrome P450 1B1
			CYP4A11†	Cytochrome P450 4A11
			CYP17A1†	Cytochrome P450 17A1

Differentially expressed genes were selected using the filtering criteria of a change of at least 2.0 fold and $P \leq 0.05$. †Upregulated genes

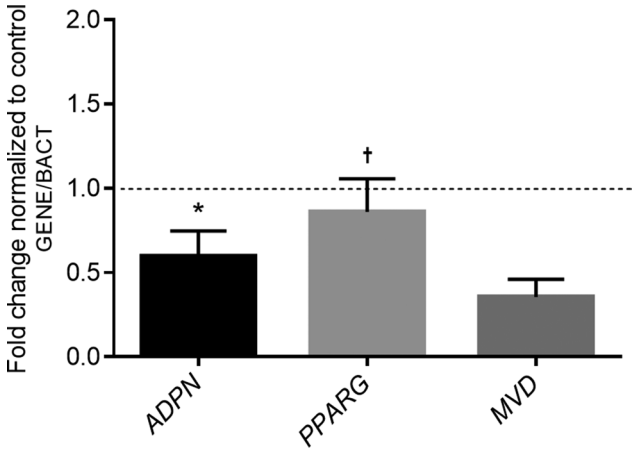

Fig. 3. Relative expression levels of selected studied genes in subcutaneous adipose tissue from obese individuals (n=37) and normal weight individuals (n=35). The data are expressed as fold changes relative to the control group or normal weight individuals (dashed line), taken as 100 per cent or 1.0. Differences between groups were assessed using ANOVA with Bonferroni *post hoc* analysis, $^{*}P<0.05$ vs. *MVD* gene expression and $^{\dagger}P<0.05$ vs. *ADPN* and *MVD* gene expressions.

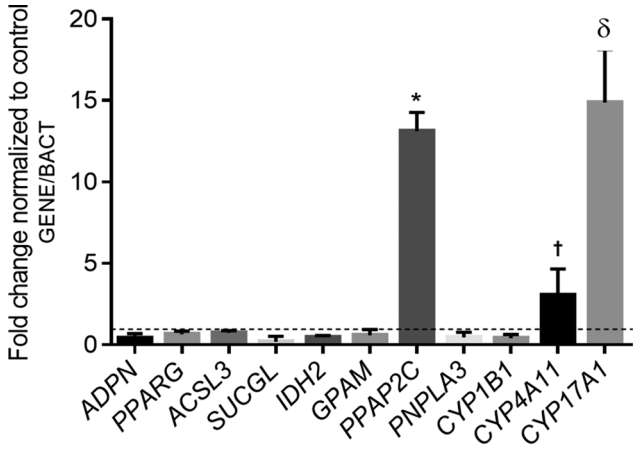

Fig. 4. Relative expression levels of selected studied genes in visceral adipose tissue from patients with obesity (n=37) and normal weight individuals (n=35). The data are expressed as fold changes relative to the control group or normal weight individuals (dashed line), taken as 100 per cent or 1.0, respectively. Differences between groups were assessed using ANOVA with Bonferroni *post hoc* analysis.$^{*}P<0.05$ vs. *ADPN, PPARG, ACSL3, IDH2, GPAM, PNPLA3* and *CYP1B1* gene expressions, $^{\dagger}P<0.05$ vs. *ADPN, PPARG, ACSL3, SUCGL, IDH2, GPAM, PPAP2C, PNPLA3* and *CYP1B1* gene expressions and $^{\delta}P<0.05$ vs. *ADPN, PPARG, ACSL3, SUCGL, IDH2, GPAM, PPAP2C, PNPLA3, CYP1B1* and *CYP4A11* gene expressions.

it potentially influences physiological processes such as cell proliferation, differentiation and survival, cell migration and apoptosis[30]. In addition, significantly increased expression of some CYP450 enzymes was detected in the VAT from obese individuals. An increase in the level of *CYP4A11* was observed in the obese individuals in this study. This result was consistent with those of other experimental studies[31,32]. Our results also demonstrated that the *CYP17A1* gene was upregulated in the VAT of obese individuals, in agreement with a study by Tabur *et al*[33]. However, a significant decrease was seen in *CYP1B1* gene expression in comparison with those of other CYPs. The downregulation of this CYP can likely be explained by its response to the endogenous activity of nuclear receptors; *PPARG* gene expression was also found to be reduced in VAT in the present study.

In the present study SAT and VAT depots exhibited distinct expression profiles. Differential gene expression of *MVD, ACSL3, SUCGL, IDH2, PPAP2C, CYP4A11* and *CYP1B1* in the SAT and VAT of obese individuals was shown. These novel findings could facilitate the identification of candidate genes and aid in the clinical diagnosis of obesity.

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***Conflicts of Interest*:** None.

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