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## The endocrine profile of subcutaneous and visceral adipose tissue of obese patients

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**Key words:** obesity; metabolic syndrome; subcutaneous adipose tissue; visceral adipose tissue; isolated adipocytes; gene expression.

**SUMMARY**

The aim of the present study was to evaluate the expression profile of genes potentially related to metabolic complications of obesity in the whole adipose tissue and isolated adipocytes from subcutaneous (SAT) and visceral adipose tissue (VAT) from twelve non-diabetic obese women and twelve lean women. Real-time polymerase chain reaction was used for expression analysis of 41 genes of interest and two housekeeping genes. We found increased expression of specific proinflammatory and adipogenic genes and reduced expression of specific lipogenic and insulin signaling pathway genes in obese relative to lean women with no preferable localization in SAT or VAT depot. The gene expression significantly differed between adipocytes and adipose tissue but both contributed to the proinflammatory profile in obesity. We conclude that both SAT and VAT exhibit alterations in the expression of specific genes possibly contributing to proinflammatory and insulin resistance state and consequently to metabolic complications of obesity.

## INTRODUCTION

The global increase in the prevalence of obesity and its associated comorbidities commonly referred to as metabolic or insulin resistance syndrome has stimulated intensive research focused on the better understanding of energy metabolism regulation and the possibilities to prevent and/or treat obesity. Numerous large scale studies have demonstrated that the presence of metabolic syndrome markedly increases cardiovascular morbidity and mortality (Frayn and Coppack, 1992; Eckel et al., 2005 and Fujita et al., 2006).

During the last decade, a lot of new knowledge has been gained in this field, including the discovery of endocrine function of adipose tissue (Hotamisligil et al., 1993 and Zhang et al., 1994). It is now generally accepted that adipose tissue secretes a variety of factors that exert numerous paracrine and endocrine effects and play an important role in metabolic regulations (Fasshauer et al., 2001 and Eckel et al., 2005). The human white adipose tissue is now recognized as a central player in the subclinical inflammatory state that is characteristic for patients with obesity and insulin resistance or metabolic syndrome. Obesity enhances adipocyte size and significantly alters its endocrine characteristics towards more proinflammatory and insulin resistance-inducing factors production. Furthermore, obesity is accompanied by markedly increased infiltration of adipose tissue with immunocompetent cells that in turn produce increased amounts of proinflammatory factors such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), resistin and many others (Bastard et al., 2006 and Matsuzawa, 2006).

Numerous clinical studies have demonstrated that important differences exist with respect to endocrine production of different adipose tissue depots. In particular, visceral adipose tissue and epicardial adipose tissue appears to have more proinflammatory and metabolically harmful profile according to most but not all studies (Abate et al., 1995; Wajchenberg, 2000; Wagenknecht et al., 2003 and Kremen et al., 2006).

Although a lot of new knowledge has been gained with respect to the role of adipose tissue in human physiology the studies of the wide-ranging gene profile in the subcutaneous and visceral adipose depots of obese subjects have been limited and bring up some controversies. Most gene expression studies have been performed on whole adipose tissue and the expression of a very restricted number of genes has been quantified in human studies (Tan et al., 2005 and Kolak et al., 2007). Thus, the aim of the present study was to evaluate an expression profile of genes potentially related to metabolic complications of obesity in the samples of whole adipose tissue and isolated adipocytes from both subcutaneous (SAT) and visceral adipose tissue (VAT) depots from obese insulin resistant patients and normal-weight healthy subjects.

## **METHODS**

### *Study subjects*

Twelve non-diabetic obese women (mean age,  $44.9 \pm 2.7$  yr; mean body mass index,  $41.0 \pm 1.9$  kg/m<sup>2</sup>) and twelve normal-weight age-matched control women (mean age,  $48.9 \pm 2.6$  yr; mean body mass index,  $23.6 \pm 0.7$  kg/m<sup>2</sup>) were included in the study. None of the subjects suffered from diabetes (defined as fasting plasma glucose  $> 7.0$  mmol/l). Seven out of twelve obese subjects had impaired fasting glucose (between 5.6 to 6.9 mmol/l), the subjects with diabetes have been excluded from the study. All women included in the study were hospitalized at the General University Hospital, Prague. The samples of subcutaneous and visceral adipose tissue were obtained from the abdominal region during gastric banding surgery in obese patients and elective cholecystectomy in control women. Body weight of studied patients remained stable for at least 3 months prior the study. None of the patients had malignant tumor, thyroid disease, or acute infectious disease. Control subjects had no history of obesity and/or diabetes. Written informed consent was signed by all participants before

being enrolled in the study. The study was approved by the Human Ethical Review Committee, First Faculty of Medicine, and General University Hospital, Prague, Czech Republic, and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

#### *Anthropometric examination, blood sampling and adipose tissue sampling*

Anthropometric examination of all subjects was performed one day before the operation. All subjects were measured and weighted, and body mass index was calculated. Blood samples for biochemical and hormonal measurements were withdrawn between 07:00 h and 08:00 h after an overnight fasting at basal state (at the day of operation before the start of anesthesia). Serum was obtained by centrifugation and the samples were subsequently stored in aliquots at  $-70^{\circ}\text{C}$  until further analysis.

Samples of subcutaneous and visceral adipose tissue for mRNA expression analysis were obtained from abdominal region at the beginning of surgery. All samples were taken from approximately the same localization. The samples were obtained from the tissue that has not been previously traumatized mechanically or by cauterization to avoid of the influence of local tissue damage on studied parameters. The tissue samples were collected to RNA stabilization reagent (RNAlater, Qiagen, Hilden, Germany) and stored at  $-80^{\circ}\text{C}$  until further processing.

#### *Hormonal and biochemical assays*

Serum adiponectin was measured by commercial RIA kit (Linco Research, St. Charles MO) and C-reactive protein (CRP) was measured by high sensitive assay (high sensitive CRP - hsCRP) (Diagnostic Systems Laboratories Inc, Webster, TX). Sensitivity was 1.0 ng/ml for adiponectin and 1.6 ng/ml for hsCRP. Intra- and interassay variability for the kits were  $< 5\%$

and 9.3%, respectively. Serum leptin and resistin concentrations were measured by commercial ELISA kit (BioVendor, Brno, Czech Republic). Sensitivity was 0.2 ng/ml for resistin and 0.12 ng/ml for leptin, and the intra- and interassay variability were < 5% and 8.0%, respectively. Blood glucose was measured on an ABL 700 analyzer (Radiometer Medical A/S, Copenhagen, Denmark). Serum insulin concentrations were measured by commercial RIA kit (Cis Bio International, Gif-sur-Yvette Cedex, France). Sensitivity was 2.0  $\mu$ IU/ml and the intra- and interassay variability were 4.2% and 8.8%, respectively.

Biochemical parameters were measured in the Department of Biochemistry of General University Hospital by standard laboratory methods. Homeostasis model assessment (HOMA-IR) index was calculated as previously described (Matthews et al., 1985) using the following formula: fasting serum insulin (mIU/l) x fasting serum glucose (mmol/l)/22.5.

#### *Adipocyte isolation*

Immediately after removal ~ 1 g of adipose tissue was transferred to 5 ml of sterile 37°C KRBHA solution with glucose and bovine albumin (118.5 mM NaCl, 4.75 mM KCl, 2.70 mM CaCl<sub>2</sub>, 1.19 mM KH<sub>2</sub>PO<sub>4</sub>, 1.09 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 25 mM NaHCO<sub>3</sub>, 5 mM glucose and 3.33% bovine albumin). Within 10 min, samples were dissected to smaller sections and transferred to 5 ml sterile KRBHA medium with 0.3% collagenase type VIII (from *Clostridium histolyticum*, Sigma-Aldrich, Germany) and incubated in 37 °C shaking at 120 RPM for 35 min. After enzymatic digestion, the undigested tissue was separated by filtration through the nylon mesh of 250  $\mu$ m pore size (Spectrum Laboratories, Inc., CA, USA). Floating adipocyte layer was washed three times with 37 °C sterile KRBHA medium without collagenase. RNA was immediately extracted from isolated adipocytes on MagNA Pure instrument using MagNA Pure Compact RNA Isolation (Tissue) kit (Roche Diagnostics GmbH, Germany) (see below).



*Determination of mRNA expression*

Approximately 100 mg of tissue was collected to 1 ml of RNA stabilization reagent (RNAlater, Qiagen, Germany) and stored at -80 °C until further analysis. Samples of subcutaneous and visceral adipose tissue were homogenized on a MagNA Lyser Instrument using MagNA Lyser Green Beads (Roche Diagnostics GmbH, Germany). Total RNA was extracted from the homogenized sample on MagNA Pure instrument using MagNA Pure Compact RNA Isolation (Tissue) kit (Roche Diagnostics GmbH, Germany). The RNA concentration was determined from absorbance at 260 nm (BioPhotometer, Eppendorf AG, Germany). All samples had a 260/280 nm absorbance ratio  $1.78 \pm 0.06$ . The integrity of the RNA was checked by visualization of 18S and 28S ribosomal bands on 1 % agarose gel with an ethidium bromid. 1 µg of total RNA was used for reverse transcription to synthesize the first strand cDNA using the oligo (dT)<sub>18</sub> primers following the instructions of the RevertAid First Strand cDNA synthesis kit (Fermentas Life Science, Lithuania).

Measurement of gene expression was performed on an 7900HT Fast Real-Time PCR System instrument (Applied Biosystems, Foster City, CA, USA) using TaqMan® Custom Array. For reaction was used a mix of TaqMan® Universal PCR Master Mix, NO AmpErase® UNG (Applied Biosystems, Foster City, CA, USA), nuclease-free water (Fermentas Life Science, Lithuania) and approximately 12 µg of cDNA sample.

Controls with no cDNA template were performed with each assay and all samples were run at least in duplicates. The increase in fluorescence was measured in real time and data were obtained as threshold cycle ( $C_T$ ) values. To compensate for variations in input RNA amounts and efficiency of reverse transcription,  $\beta$ -2-microglobulin (B2M) and 18S rRNA were used as an endogenous references and results were normalized to the means of these

values. Relative expression of genes was calculated using the formula  $2^{-\Delta\Delta(\text{CT}_{\text{cytokine}} - \text{CT}_{\text{reference gene}})}$ .

### *Statistical analysis*

The statistical analysis was performed on SigmaStat software (SPSS Inc., Chicago, IL). The anthropometric, biochemical and hormonal results are expressed as means  $\pm$  SEM. Differences of gene expression and serum parameters between obese and non-obese were evaluated using unpaired *t* test or One Way ANOVA as appropriate. Statistical significance was assigned to  $P < 0.05$ .

Expression levels of the genes of interest are in arbitrary units and are normalized to two housekeeping genes 18S rRNA and  $\beta$ -2-microglobulin (B2M). The fold changes of the mRNA expression of genes of interest in obese (relatively to non-obese group) were calculated. The mean value of mRNA expression for each gene of interest in non-obese group was taken as 1.0.

## **RESULTS**

### *Anthropometric, biochemical and hormonal characteristics of the patients*

Anthropometric, biochemical and hormonal characteristics of the patients are summarized in Table 1. As expected, obese patients had markedly increased body mass index, C-reactive protein, fasting serum insulin and glucose levels and significantly increased homeostasis model assessment index of insulin resistance. Glycated hemoglobin, total cholesterol and fasting serum triacylglycerides did not differ between the groups. Serum leptin levels were markedly increased while serum adiponectin levels were significantly decreased in obese relative control group. Serum resistin did not significantly differ between the groups.

*Identification of differentially expressed genes between obese patients and normal-weight women*

From a total number of 41 genes studied, we identified 14 genes with significantly different expression between obese and control subjects. The complete summary of the fold changes in the gene expression in VAT and SAT and isolated adipocytes from both depots in obese relative to non-obese is shown in Table 2.

In the isolated adipocytes from SAT, we found markedly increased chemokine C-C motif ligand 3 (CCL3) and c-fos-induced growth factor expression and significantly reduced expression of fatty acid synthase (FASN), peroxisome proliferator-activated receptor alpha (PPAR- $\alpha$ ), facilitated glucose transporter, member 4 (GLUT4), sterol regulatory element binding transcription factor 1 (SREBF1) and vascular endothelial growth factor (VEGF) in obese patients compared with control subjects. Interleukin-10 (IL-10) and resistin were undetectable in adipocytes from both VAT and SAT in the both groups of subjects.

In the isolated adipocytes from VAT, we found significantly decreased gene expression of adiponectin, uncoupling protein 2 and VEGF and markedly increased expression of vascular endothelial growth factor C (VEGFC) in obese patients compared with control subjects. IL-10, TNF- $\alpha$  and resistin were undetectable in adipocytes from both VAT and SAT in both studied groups.

In the whole SAT, we found significantly increased expression of angiopoietin 2 and significantly decreased expression of FASN, GLUT4 and SREBF1 in obese relative to lean subjects.

In the whole VAT, we found marked increase in the expression of interleukin-6 (IL-6) and significantly decreased expression of immunocompetent cell marker CD14 and of insulin receptor substrate 1 (IRS1) in obese compared with non-obese subjects.

The expression of other studied genes was not significantly different in obese compared with lean subjects (Table 2).

*Depot-specific differences in the expression of genes in obese and lean subjects: comparison of subcutaneous and visceral adipose tissue and isolated adipocytes from both depots*

The overview of the depot-specific relative differences in the expression of the studied genes is summarized in Table 3. The expression of an immunocompetent cells marker CD45 was significantly higher in SAT than in SAT isolated adipocytes in obese, but not in non-obese subjects. The expression of CD14 was significantly higher in adipocytes from both SAT and VAT than in whole tissues of both study groups. The expression of IL-6 was markedly higher in visceral adipocytes than in the VAT in both obese (40-fold) and non-obese group (20-fold). The expression of CCL3 was 13.6-fold higher in adipocytes from SAT than in adipocytes from VAT and 22.8-fold higher in SAT adipocytes than in whole SAT in obese subjects. The expression of CCL3 was 4-fold higher in SAT relatively to VAT in obese subjects. Neither of these relative differences in CCL3 expression was seen in non-obese group. IL1- $\beta$  expression was 25-fold and 2.7-fold higher in VAT adipocytes than in VAT in the obese and non-obese group, respectively. Leptin and leptin receptor expression was significantly higher in SAT and VAT than in respective adipocytes in the obese group. In non-obese group, the same results were found, except for lack of difference in the expression of leptin between adipocytes from VAT and whole VAT. The expression of SERPINE1 was 24-fold higher in SAT adipocytes than in VAT adipocytes in the obese group only.

## **DISCUSSION**

We quantified expression of genes potentially involved in metabolic complications of obesity in SAT and VAT of morbidly obese and normal-weight subjects. We found that the

presence of obesity and insulin resistance is associated with changes of the specific proinflammatory, lipogenic, adipogenic and insulin signaling resistance pathway genes with no preferable localization in SAT or VAT depot.

Previous clinical studies have demonstrated that VAT exhibits metabolically harmful profile more closely related to metabolic complications of obesity than SAT (Wajchenberg, 2000; Hotamisligil, 2003; Wagenknecht et al., 2003 and Freedland, 2004). Here we show that both SAT and VAT of obese subjects exhibit markedly increased expression of specific proinflammatory genes (CCL3 in the SAT vs. IL-6 in the VAT) relative to lean subjects. The exact source of proinflammatory cytokines within the adipose tissue (adipocytes vs. stroma-vascular fraction) is still not completely clear (Savage et al., 2001; McTernan et al., 2002; Weisberg et al., 2003 and Kusminski et al., 2005). Thus, we measured the expression of two immunocompetent cells markers in adipose tissue to assess whether increased adipose tissue infiltration by immunocompetent cells can explain increased IL-6 in VAT of obese. However, the expression of CD14 in VAT was decreased rather than increased and the expression of CD45 was unchanged in obese relative to lean group, indicating that IL-6 is probably expressed by adipocytes rather than by immunocompetent cells in obese (Coppack, 2001). Alternatively, higher secretory activity rather than increased number of macrophages in obese subjects could explain their increased proinflammatory genes expression in. This hypothesis, however, does not explain much higher expression of CCL3 in subcutaneous adipocytes relative to whole SAT from obese. Such a finding rather suggests that some of proinflammatory factors are highly expressed by adipocytes. In contrast, the lack of the expression of resistin and TNF- $\alpha$  in isolated adipocytes from both depots (resistin) and from VAT (TNF- $\alpha$ ) suggests their unitary extraadipocyte production by macrophages at least in the respective depot (Weisberg et al., 2003 and Kusminski et al., 2007).

Overall, our present data do not confirm that VAT more actively contributes to proinflammatory and insulin resistant state of obese subjects relative to SAT as has been numerously demonstrated in some (Wajchenberg, 2000 and Wagenknecht et al., 2003), but not all (Abate et al., 1995 and Goodpaster and Kelley, 1998) previous studies. Furthermore, we do not confirm that increased infiltration of adipose tissue by macrophages is crucial for the development of proinflammatory state and insulin resistance. There might be an important crosstalk between adipocytes from both depots and other cell types and the production of many factors might be constitutive. We bear in mind that the observed upregulation of inflammation-related genes in adipocytes of obese subjects could be due to contamination of the isolated adipocytes by macrophages (Lee et al., 2005). This is, however, unlikely since all samples were subject to similar procedure.

Several genes involved in insulin signaling pathway presented altered expression in both adipose tissue depots from our obese patients, in conceptual agreement with the defective insulin-mediated glucose uptake by obese adipocytes reported earlier (Graham and Kahn, 2007, Kovacs et al., 2003). Here we show that both SAT and VAT of obese exhibit depot-specific alterations of expression of typical (e.i., GLUT4, IRS1) and less typical (e.i., PPAR- $\alpha$ ) (Loviscach et al., 2000) genes involved in insulin signaling pathway.

As expected, the altered expression of critical elements of insulin-stimulated glucose uptake (GLUT4) and of lipogenic pathway (FASN) might be related to altered expression of transcriptional factors, such as SREBF1 (Griffin and Sul, 2004 and Im et al., 2007). Here we show that SREBF1 expression in SAT is reduced simultaneously with the expression of both GLUT4 and FASN suggesting that this transcriptional factor could play an important role in the regulation of expression of critical elements of glucose uptake and lipogenic pathway in adipose tissue of obese. The reduction of FASN and SREBF1 in the SAT of obese might

contribute to the reduction of de novo lipogenesis in obese and thus contribute to the alterations in glucose metabolism and excess in other cell types and tissues.

The explanation of increases of some genes and decreases of the others when comparing isolated adipocytes and adipose tissue may lie in the predominant functional participation of the product of specific gene in processes localized to adipocyte itself or to the tissue in general. The expression of genes in adipose tissue may be influenced by the varying numbers of cell types present in the tissue, whereas the expression of genes in isolated adipocytes may be influenced not only by the local or systemic pathophysiological changes but possibly also by the isolation procedure. Bearing that in mind, we believe that comparing whole adipose tissue and isolated adipocytes is an important tool to specify the predominant location of gene expression within the fat for future studies.

We are aware, that our study has several limitations. The first one is a relatively low number of subjects included and the presence of impaired fasting glucose in some of the obese patients. These facts together with a huge variance of the data may partly explain some of the unexpected findings in obese vs. non obese subjects (e.g., unchanged expression of leptin, TNF- $\alpha$  in obese). Secondly, we are aware that the procedure for isolating adipocytes could trigger dramatic changes in expression of mRNA, especially the induction of genes encoding inflammatory mediators, chemokines and transcription factors including in the inflammatory response (Ruan et al., 2003 and Fain et al., 2004). Finally, it is noteworthy that the expression of the gene is not equal to protein production.

We conclude that both SAT and VAT depots of obese subjects exhibit alterations in the expression of specific proinflammatory, adipogenic, insulin signaling pathway and lipogenic genes possibly contributing to proinflammatory and insulin resistance state and consequently to metabolic complications of obesity. Adipocytes are at least partly responsible for the

expression of proinflammatory cytokines in adipose tissue. The expression of specific genes significantly quantitatively differs between isolated adipocytes and whole adipose tissue.

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TABLE 1. Clinical, hormonal and metabolic characteristics of the study subjects.

	Controls (n = 12)	Obese (n = 12)
Age (yr)	48.9 ± 2.6	44.9 ± 2.7
Body mass index (kg/m <sup>2</sup> )	23.6 ± 0.7	41.0 ± 1.9*
C-reactive protein (mg/l)	5.8 ± 1.5	21.9 ± 4.8*
Fasting serum insulin (mIU/l)	9.6 ± 1.7	38.8 ± 10.4*
Fasting serum glucose (mmol/l)	4.2 ± 0.3	6.5 ± 0.7*
HOMA-IR	2.0 ± 0.4	11.9 ± 2.9*
HbA1c (%)	3.9 ± 0.1	4.3 ± 0.5
Cholesterol total (mmol/l)	5.2 ± 0.3	4.3 ± 0.2
Triacylglycerides (mmol/l)	1.2 ± 0.2	1.6 ± 0.2
Leptin (ng/ml)	12.9 ± 2.2	48.4 ± 4.7*
Resistin (ng/ml)	7.9 ± 1.1	10.3 ± 1.2
Adiponectin (µg/ml)	21.9 ± 2.1	13.3 ± 1.5*

Values represent mean ± SEM.

\*p < 0.05 vs. non-obese controls.

TABLE 2. Fold changes of the gene expression levels in subcutaneous and visceral adipocytes and subcutaneous and visceral whole adipose tissue from obese (n = 12) relative to normal-weight subjects (n = 12). The mean value of the expression of the gene of interest in normal-weight subjects was taken as 100% or 1.0, respectively.

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TABLE 3. Comparison of the relative expression of studied genes in subcutaneous isolated adipocytes vs. visceral isolated adipocytes, in subcutaneous isolated adipocytes vs. whole subcutaneous adipose tissue, in visceral isolated adipocytes vs. whole visceral adipose tissue and in whole subcutaneous adipose tissue vs. whole visceral adipose tissue from obese (n = 12) and normal-weight subjects (n = 12).

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TABLE 2. Fold changes of the gene expression levels in subcutaneous and visceral adipocytes and subcutaneous and visceral whole adipose tissue from obese (n = 12) relative to normal-weight subjects (n = 12). The mean value of the expression of the gene of interest in normal-weight subjects was taken as 100% or 1.0, respectively.

gene symbol	gene name	Subcutaneous adipocytes		Visceral adipocytes		Whole subcutaneous adipose tissue		Whole visceral adipose tissue	
		fold change	t-test (P value)	fold change	t-test (P value)	fold change	t-test (P value)	fold change	t-test (P value)
ADIPOQ	adiponectin	1.04	0.82	<b>0.70</b>	<b>0.01</b>	0.95	0.55	0.82	0.39
GHRL	ghrelin/obestatin preprohormone	1.98	0.53	3.8	0.29	1.37	0.21	1.24	0.39
LEP	leptin	1.00	0.31	1.06	0.31	1.48	0.11	1.50	0.09
ADIPOR1	adiponectin receptor 1	0.63	0.38	0.75	0.38	1.17	0.42	1.09	0.74
ADIPOR2	adiponectin receptor 2	0.77	0.35	0.99	0.65	0.87	0.39	1.12	0.48
LEPR	leptin receptor	2.65	0.68	0.58	0.18	1.32	0.63	1.27	0.59
RETN	resistin	UD		UD		2.49	0.16	0.99	0.98
PBEF1	visfatin	0.61	0.25	0.77	0.17	1.19	0.54	0.86	0.53
IL1B	interleukin 1, beta	0.58	0.91	19.5	0.71	0.89	0.64	2.09	0.11
IL6	interleukin 6	0.51	0.68	6.77	0.20	0.48	0.48	<b>3.58</b>	<b>0.04</b>

IL10	interleukin 10	UD		UD		1.72	0.12	1.86	0.23
TNFA	tumor necrosis factor, alpha	0.97	1.00	UD		0.72	0.48	1.23	0.39
CCL3	chemokine (C-C motif) ligand 3	<b>23.02</b>	<b>0.04</b>	14.78	0.55	2.72	0.23	3.47	0.81
MCP1	monocyte chemoattractant protein 1	0.57	0.46	2.07	0.95	0.79	0.89	1.35	0.65
CD14	CD14 molecule	0.88	0.55	0.64	0.36	1.11	0.58	<b>0.67</b>	<b>0.02</b>
PTPRC	protein tyrosine phosphatase, receptor type, C	0.66	0.17	1.03	0.70	1.43	0.13	1.15	0.53
ICAM1	intercellular adhesion molecule 1 (CD54)	0.34	0.13	1.34	0.47	0.51	0.58	1.12	0.64
SELE	selectin-E (endothelial adhesion molecule 1)	0.47	0.51	3.71	0.18	0.43	0.65	3.27	0.88
SELP	selectin P (granule membrane protein 140kDa, antigen CD62)	1.05	0.46	1.27	0.22	1.15	0.57	1.34	0.21
VCAM1	vascular cell adhesion molecule 1	0.46	0.36	0.87	0.39	1.19	0.29	0.99	0.98
ANGPT2	angiopoietin 2	2.05	0.34	8.87	0.23	<b>2.02</b>	<b>0.02</b>	1.02	0.93
AGT	angiotensinogen	1.58	0.79	0.32	0.95	1.37	0.69	0.71	0.89
FIGF	c-fos induced growth factor (vascular endothelial growth factor D)	<b>1.76</b>	<b>0.04</b>	1.03	0.73	0.78	0.24	0.71	0.25
HGF	hepatocyte growth factor	0.69	0.50	2.32	0.24	1.04	0.84	1.15	0.56
VEGF	vascular endothelial growth factor	<b>0.49</b>	<b>0.03</b>	<b>0.37</b>	<b>0.002</b>	0.73	0.138	0.68	0.15
VEGFC	vascular endothelial growth factor C	1.74	0.09	<b>2.58</b>	<b>0.04</b>	1.31	0.29	1.04	0.84
PPARA	peroxisome proliferator-activated receptor alpha	<b>0.42</b>	<b>0.008</b>	0.93	0.26	0.74	0.83	0.64	0.36

PPARD	peroxisome proliferator-activated receptor delta	1.04	0.85	1.66	0.64	0.89	0.74	0.69	0.89
PPARG	peroxisome proliferator-activated receptor gamma	0.66	0.59	0.65	0.16	1.04	0.87	0.58	0.12
SREBF1	sterol regulatory element binding transcription factor 1	<b>0.28</b>	<b>0.003</b>	0.26	0.11	<b>0.61</b>	<b>0.03</b>	0.71	0.23
TCF8	transcription factor 8 (represses interleukin 2 expression)	1.62	0.09	2.75	0.06	1.14	0.44	1.09	0.68
SLC2A4	facilitated glucose transporter, member 4	<b>0.29</b>	<b>0.001</b>	0.51	0.14	<b>0.40</b>	<b>0.02</b>	0.55	0.21
IRS1	insulin receptor substrate 1	0.65	0.20	0.82	0.21	0.84	0.19	<b>0.69</b>	<b>0.04</b>
FASN	fatty acid synthase	<b>0.42</b>	<b>0.04</b>	0.29	0.88	<b>0.23</b>	<b>0.004</b>	0.57	0.11
LPL	lipoprotein lipase	1.24	0.43	0.87	0.59	1.05	0.85	0.77	0.79
AQP7	aquaporin-7	0.88	0.47	0.79	0.29	0.89	0.59	0.84	0.51
RBP4	retinol binding protein 4	0.94	0.79	0.67	0.33	1.44	0.51	0.84	0.33
SERPINE1	serpin peptidase inhibitor (plasminogen activator inhibitor type 1)	3.28	0.24	0.34	0.27	1.93	0.12	1.92	0.44
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)	1.11	0.65	<b>0.47</b>	<b>0.04</b>	1.29	0.32	0.92	0.72
CAT	catalase	1.59	0.92	1.12	0.66	1.08	0.71	0.85	0.51
SOD1	superoxide dismutase 1, soluble	0.97	0.92	0.60	0.17	1.28	0.75	0.81	0.08

UD = undetectable

TABLE 3. Comparison of the relative expression of studied genes in subcutaneous isolated adipocytes vs. visceral isolated adipocytes, in subcutaneous isolated adipocytes vs. whole subcutaneous adipose tissue, in visceral isolated adipocytes vs. whole visceral adipose tissue and in whole subcutaneous adipose tissue vs. whole visceral adipose tissue from obese (n = 12) and normal-weight subjects (n = 12).

Gene	Function	obese (n = 12)				control subjects (n = 12)			
		subcutaneous adipocytes/ visceral adipocytes	subcutaneous adipocytes/ subcutaneous adipose tissue	visceral adipocytes/ visceral adipose tissue	subcutaneous adipose tissue/ visceral adipose tissue	subcutaneous adipocytes/ visceral adipocytes	subcutaneous adipocytes/ subcutaneous adipose tissue	visceral adipocytes/ visceral adipose tissue	subcutaneous adipose tissue/ visceral adipose tissue
ADIPOQ	hormone, energy metabolism, insulin sensitivity		0.53*	0.56*			0.49*		
GHRL	hormone, energy metabolism							5.20*	
LEP	hormone, energy metabolism, insulin sensitivity	1.43*	0.41***	0.39***		1.51*	0.61*	0.56*	1.37*
ADIPOR1	receptor, molecular and biological function unclassified		0.19*	0.26*			0.35*	0.37*	
ADIPOR2	receptor, molecular and biological function unclassified		3.21*	3.27*			3.64*	3.69*	
LEPR	receptor, molecular and biological function		0.64*	0.35*		0.33*	0.32***		0.81*

	unclassified								
RETN	signaling molecule, cytokine, interleukin								
PBEF1	signaling molecule, cytokine	2.29*	3.77***			2.9*	7.36***	3.17*	
IL1B	signaling molecule, cytokine, interleukin			25.31*				2.71*	
IL6	signaling molecule, cytokine, interleukin			40.19***			1.05*	21.24*	
IL10	signaling molecule, cytokine, interleukin								
TNFA	signaling molecule, cytokine								
CCL3	chemokine, signalling molecule	13.62*	22.8*			4.01*			
MCP1	chemokine, signaling molecule					1.34*			
CD14	membrane-associated GPI-linked protein, macrophage marker		1.84*	2.49*			2.32***	2.63***	0.69***
PTPRC	protein tyrosin phosphatase, leukocyte marker		0.24*						
ICAM1	receptor, cell adhesion molecule								
SELE	cell-adhesion molecule			8.01*					
SELP	cell-adhesion molecule	0.15*	0.31*	2.36*			0.34*		
VCAM1	cell-adhesion molecule		0.21*	0.19***					
ANGPT2	signal transduction, cell communication, development						0.41*		
AGT	protein metabolism, proteolysis	0.43*							

FIGF	signaling molecule, growth factor				2.46*		0.23***		2.26*
HGF	signaling molecule, growth factor		0.55*						
VEGF	signaling molecule, growth factor	1,88***	1.84***				2.72***	1.97*	
VEGFC	signaling molecule, growth factor		0.27***	0.50*			0.19***	0.21*	
PPARA	transcription factor, nuclear hormone receptor						1.81*		
PPARD	transcription factor, nuclear hormone receptor						0.35*		1.44*
PPARG	transcription factor, nuclear hormone receptor				1.86*				
SREBF1	transcription factor, regulation of lipid, fatty acid and steroid metabolism		1.63*				3.54*		
TCF8	transcription factor, nucleic acid metabolism		0.46***				0.32***	0.24***	
SLC2A4	carbohydrate transporter, carbohydrate metabolism								
IRS1	glucose homeostasis, intracellular insulin signaling cascade		2.19*	2.74*			2.83***	2.30***	
FASN	synthase, fatty acid metabolism		2.51*						
LPL	hydrolase, lipase, lipid, fatty acid and steroid metabolism		0.59*	1.66*			0.52***		
RBP4	transfer/carrier protein, coenzyme and prosthetic group metabolism				2.43*		1.63*		
SERPINE	serine protease inhibitor	24.29*			2.44*				

1									
UCP2	mitochondrial carrier protein		0.24***	0.49***		0.25***	0.28***		
CAT	oxidoreductase, peroxidase								
SOD1	oxidoreductase								

\* $p < 0.05$  vs. localisation

\*\*\*  $p < 0.005$  vs. localisation

Free fields = not significant