The influence of obesity and different fat depots on adipose tissue gene expression and protein levels of cell adhesion molecules

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Short title: Adhesion molecules in obesity

Summary

Increased circulating adhesion molecules in patients with obesity play an important role in the development of endothelial dysfunction/atherosclerosis. The aim of this study was to assess the contribution of various fat depots to adhesion molecules production in obesity.

12 women with 1st and 2nd degree of obesity, 13 women with 3rd degree of obesity and 14 lean age-matched women were included into study. Circulating levels of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin were measured by Luminex kits. mRNA expression of ICAM-1, VCAM-1, E-selectin, monocyte chemoattractant protein-1 (MCP-1), and CD68 in subcutaneous (SAT) and visceral adipose tissue (VAT) was measured by RT-PCR; ICAM-1 and VCAM-1 protein levels by Luminex kits, normalized to protein content. Obesity increased ICAM-1 and VCAM-1 mRNA expression and protein levels and CD68 mRNA expression in VAT. Expression of E-selectin and MCP-1 did not significantly differ between groups. Expression of ICAM-1 and VCAM-1 positively correlated with expression of CD68 in both adipose depots. In VAT, ICAM-1 and VCAM-1 expression and protein levels positively correlated with BMI. Obesity was associated with increased adhesion molecules mRNA expression and protein levels in VAT, but not in SAT. Increased adhesion molecules production in visceral fat may provide a novel direct link between visceral adiposity and increased risk of cardiovascular complications.

Key words: adhesion molecules, atherosclerosis, obesity, adipose tissue, gene expression

Introduction

Endothelial dysfunction represents an early phase of vascular changes that eventually lead to atherosclerosis with all its unfavourable complications (Blankenberg *et al.* 2003; Blann 2003). Numerous studies have found that the presence of obesity in particular in combination with other metabolic abnormalities commonly referred to as metabolic or insulin resistance syndrome markedly increases the risk of atherosclerosis and its complications (Meigs 2004; Haffner 2006). Increasing evidence suggests that an ongoing subclinical inflammation is closely involved in the pathogenesis of obesity and its associated complications such as insulin resistance, dyslipidemia and atherosclerosis (Devaraj *et al.* 2004). In the early stages of atherosclerosis biomarkers of endothelial dysfunction, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, are increased in response to subclinical inflammation and play an important role in the formation of atherosclerotic plaque (Blankenberg *et al.* 2003). Numerous studies documented increased circulating levels of soluble adhesion molecules in obesity (Price and Loscalzo 1999; Pontiroli *et al.* 2004), but the mechanisms are still not completely clear (Ferri *et al.* 1999; Pontiroli *et al.* 2004).

It is now generally accepted that adipose tissue secretes a variety of factors that exert important roles in the local and systemic regulation of numerous metabolic and inflammatory processes (Frayn *et al.* 2003; Rajala and Scherer 2003; Haluzik *et al.* 2004). Dysregulated endocrine function of adipose tissue, in particular in visceral compartment, triggers obesity-associated chronic low-grade inflammation (Trayhurn 2005; Murdolo and Smith 2006) and contributes to the development of obesity-related metabolic complications, including atherosclerosis (Rajala and Scherer 2003; Gable *et al.* 2006). Increased expression of chemokines, e.g., monocyte chemoattractant protein-1 (MCP-1) (Trayhurn 2005) and adhesion molecules, e.g., ICAM-1 (Brake *et al.* 2006), by adipocytes and macrophages

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accumulated in adipose tissue of obese subjects may participate in the development of atherosclerosis.

Although the increase in adhesion molecules circulating levels in obesity is well documented, the contribution of subcutaneous and visceral fat depots to the circulating pool of adhesion molecules is uncertain. Here we tested the hypothesis that both subcutaneous and visceral adipose tissue contribute to the production of adhesion molecules and that this production might be increased in obese patients relative to lean control subjects. To this end, we examined mRNA expression and protein levels of ICAM-1, VCAM-1 and E-selectin in subcutaneous (SAT) and visceral adipose tissue (VAT) of obese and lean healthy women and studied the relationship of local and systemic levels of these adhesion molecules with anthropometric, hormonal and biochemical parameters.

Patients and Methods

Study subjects

Twenty-five obese women and fourteen normal-weight age-matched control women (C; body mass index (BMI): 23.8 ± 0.6 kg/m^2) were included in the study. Obese women were divided into two subgroups; women with BMI 30-40 kg/m^2 (obese group 1; BMI: 35.7 ± 0.9 kg/m^2) and BMI > 40 kg/m^2 (obese group 2; BMI: 46.2 ± 0.8 kg/m^2). In obese group 1, six patients had elevated fasting plasma glucose levels ($5.6 - 7.0$ mmol/l) at the time of examination, one patient had type 2 diabetes mellitus treated by diet. In obese group 2, two patients had elevated fasting plasma glucose levels and four patients had type 2 diabetes mellitus treated by diet or peroral antidiabetics. Eight patients of each obese group were taking antihypertensive medication. Two obese subjects were treated with statins. At the time of examination blood pressure of all patients was within the normal range ($\leq 140/90$ mmHg). Control subjects had normal fasting plasma glucose levels and no history of diabetes. All

patients were examined while hospitalized at the General University Hospital, Prague. The SAT and VAT samples 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. Patients with coronary heart disease, stroke, renal or liver failure or acute infection were excluded from the study. 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 surgery. Blood samples for biochemical and hormonal measurements were withdrawn between 07:00 and 08:00 h after an overnight fasting at basal state (at the day of operation before the start of anesthesia). Serum samples were stored in aliquots at $-70°C$ until further analysis.

Samples of SAT and VAT for mRNA expression analysis were obtained from abdominal region at the beginning of surgery, from the tissue that has not been previously traumatized mechanically or by cauterization to avoid the influence of local tissue damage on studied parameters. The tissue samples were collected to RNA stabilization reagent (RNAlater, Qiagen, Germany) and stored at $-70°C$ until further processing.

Hormonal and biochemical assays

Serum concentrations of soluble E-selectin, ICAM-1 and VCAM-1 were measured using HumanCardiovascularDisease LINCOplex Kit on Luminex®200 instrument (Linco Research, USA). Sensitivity was 79 pg/ml for E-selectin, 16 pg/ml for VCAM-1, 9 pg/ml for

ICAM-1; intra- and interassay variability < 12.0 % and 16.0 %, respectively. Serum insulin concentrations were measured by commercial RIA kit (CisBioInternational, France), C-reactive protein (CRP) levels by Ultra-Sensitive CRP ELISA kit (Diagnostic Systems Laboratories, Inc., USA). Biochemical parameters were measured in the Department of Biochemistry of General University Hospital by standard laboratory methods.

HOMA-R (homeostasis model assessment of insulin resistance) index was calculated as previously described (Matthews *et al.* 1985).

Determination of mRNA expression and protein levels

Total RNA was extracted from 60 – 100 mg of subcutaneous and visceral adipose tissue by homogenization and isolation, as described previously (Lacinova *et al.* 2008). Concentration and purity of RNA were determined by spectrophotometer (BioPhotometr Eppendorf AG, Hamburg, Germany). The integrity of the RNA was checked by visualization of 18S and 28S ribosomal bands on 1 % agarose gel with an ethidium bromide.

0.1 – 1 μg of total RNA was used for reverse transcription to synthesize the first strand cDNA, further used for determination of gene expression of E-selectin, ICAM-1, VCAM-1, MCP-1 and CD68 (monocyte/macrophage marker), and 18S RNA by RT-PCR using TaqMan®Universal PCR MasterMix, NO AmpErase® UNG and specific TaqMan Gene expression Assays (Applied Biosystems, Foster City, CA).

All PCRs for each gene were amplified separately. Controls with no template cDNA 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 treshold cycle (C_T) values. Results were normalized to 18S RNA. Relative gene expression was calculated using the formula $2^{-\Delta\Delta(CT\ cytokine\ -\ CT\ 18S\ RNA)}$.

Approximately 100 – 200 mg adipose tissue (SAT and VAT) from each subject was homogenized in 250 µl of ice-cold homogenization buffer. The homogenate was centrifuged at 3,000 x g for 15 min at 4˚C, the fat cake was discarded and the homogenate was centrifuged again at 14,000 x g for 20 min at 4˚C. The supernatant was stored in aliquots at – 70˚C. ICAM-1 and VCAM-1 protein levels in adipose tissue homogenates were measured using HumanCardiovascular LINCOplex Kit on Luminex®200 instrument (Linco Research, USA) and normalized to protein content (1 mg). Total protein concentration of each sample was estimated by the Bio-Rad Laboratories, Inc., protein dye reagent according to the manufacturer's protocol (Hercules, CA, USA).

Statistical analysis

The statistical analysis was performed on SigmaStat software (SPSS Inc., Chicago). Results are expressed as means ± standard error of mean (SEM). Differences of gene expression and serum parameters between obese and non-obese were evaluated using unpaired t-test, Mann-Whitney Rank Sum Test or One-Way ANOVA, as appropriate. The relationships between the data were calculated by Pearson or Spearman correlation test. Statistical significance was assigned to p < 0.05.

Results

Patients' characteristics

Anthropometric, biochemical and hormonal characteristics of the subjects are summarized in Table 1. The groups studied were age-matched. Both obese subgroups had markedly increased BMI, CRP levels, HOMA index, blood glucose and insulin levels, and significantly reduced HDL-cholesterol relative to control group (C). Glycated hemoglobin levels, LDL-cholesterol levels, systolic and diastolic blood pressure did not significantly

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differ between C and obese subgroups. Total cholesterol levels were significantly reduced in obese group 2 (BMI > 40 kg/m^2) in comparison to C group. Serum triglycerides levels were significantly increased in obese group 1, but not in obese group 2, reative to controls.

Serum concentrations of soluble adhesion molecules

Serum concentration of E-selectin and ICAM-1 were significantly increased in obese group 2 in comparison to control women. Levels of soluble VCAM-1 did not differ among the groups studied (Table 1).

mRNA expression and protein levels of adhesion molecules in subcutaneous and visceral adipose tissue

In SAT, mRNA expression of E-selectin, ICAM-1 and VCAM-1 did not significantly differ between obese and lean women (Figure 2A,B,C). Similarly, no significant differences in ICAM-1 and VCAM-1 protein levels in SAT were found (Figure 1A,B).

In VAT, mRNA expression and protein levels of ICAM-1 and VCAM-1 were significantly increased in both obese groups relative to C group. mRNA expression of E-selectin in VAT tended to be higher in both obese subgroups, but didn't reach the statistical significance (Figure 1 and 2).

Expression of CD68 was significantly increased in VAT, but unchanged in SAT, of both obese subgroups relative to C group (Figure 3A). Expression of MCP-1 did not significantly differ between groups in any fat depot (Figure 3B).

The relationship of adhesion molecules with other studied anthropometric, hormonal, and biochemical parameters

The relationship of studied adhesion molecules with other parameters was calculated in a combined population of all three groups. Serum soluble E-selectin concentration positively correlated with BMI, glucose levels, HOMA index, VCAM-1 and ICAM-1 levels, and systolic blood pressure. Soluble ICAM-1 concentration positively correlated with glycated hemoglobin levels, systolic blood pressure and soluble VCAM-1 levels. Serum soluble VCAM-1 concentration positively correlated with HDL-cholesterol levels (data not shown).

In SAT, protein levels of ICAM-1 significantly positively correlated with its circulating levels. SAT expression of ICAM-1 and VCAM-1 significantly positively correlated with CD68 mRNA expression. Expression of VCAM-1 and E-selectin in SAT significantly positively corelated with serum insulin levels (Table 2).

In VAT, mRNA expression as well as protein levels of ICAM-1 and VCAM-1 significantly positively correlated with BMI. Expression of VCAM-1, ICAM-1, and E-selectin significantly positively correlated with expression of CD68 in VAT. mRNA expression of E-selectin and ICAM-1 in VAT positively correlated with glycated hemoglobin and MCP-1 expression in VAT. Protein levels of ICAM-1 in VAT significantly positively correlated with triglyceride and glucose levels. mRNA expression of CD68 positively correlated with BMI in both SAT and VAT (Table 2).

Discussion

The most important finding of this study are the differences in mRNA and protein abundance of adhesion molecules in adipose tissue of obese and lean women. The expression and protein levels of ICAM-1 and VCAM-1 were significantly higher in VAT, but not in SAT, of obese women relative to lean women, whereas the expression of E-selectin and MCP-1 did not significantly differ between the groups studied in any adipose depot. mRNA

expression of ICAM-1 and VCAM-1 in VAT was significantly related to the expression of macrophage-related marker CD68 and to BMI.

Circulating levels of soluble adhesion molecules are markers of endothelial activation (Pigott *et al.* 1992; Bosanska *et al.* 2008), being elevated in obese individuals in majority (Ferri *et al.* 1999; Pontiroli *et al.* 2004), but not all (Matsumoto *et al.* 2002) previously published studies. Here we found significantly increased levels of soluble E-selectin and ICAM-1, but not VCAM-1, in obese women with BMI > 40 kg/m^2 relative to lean controls. The lack of difference in circulating adhesion molecules between obese women with BMI 30-40 kg/m^2 and lean women may be partially explained by their lower BMI value as compared with obese group 2 (Ferri *et al.* 1999; Troseid *et al.* 2005), possibly also by antihypertensive medication of obese subjects (Boulbou *et al.* 2005) or estrogen influence due to differences in pre- and postmenopausal status (Oger *et al.* 2001; Hemelaar *et al.* 2005; Shifren *et al.* 2008).

We confirmed previous findings (Tsakadze *et al.* 2004) that adhesion molecules are expressed in human adipose tissue which may thus be a potential source of circulating adhesion molecules in obesity. Although we found a significant relationship only between ICAM-1 protein levels in SAT and its circulating levels, accumulating evidence from previous studies suggests that circulating levels of adhesion molecules reflect the combination of their production by endothelial or immunocompetent cells outside the adipose tissue and by various cells within the adipose tissue (Blankenberg *et al.* 2003; Brake *et al.* 2006; Sengenes *et al.* 2007). Furthermore, individual adhesion molecules could have specific roles in the adhesion pathway and specific predominant sources and mode of expression (Blankenberg *et al.* 2003; Marchesi *et al.* 2007).

One of possible roles of adhesion molecules production in adipose tissue may lie in the paracrine regulation of local inflammatory processes rather than in their systemic effects. Adhesion molecules in adipose tissue may contribute to the recruitment and activation of

macrophages in obesity (Brake *et al.* 2006). Significant association of adhesion molecules expression in VAT with BMI and CD68 could partially explain the association of visceral obesity and macrophage infiltration in adipose tissue with endothelial dysfunction. Endothelial dysfunction in the capillary and arteriolar tissue microcirculation is one of the key steps in the evolution of tissue insulin resistance (Serne *et al.* 2002). The increase in ICAM-1 and VCAM-1 expression in VAT may thus be linked to development of type 2 diabetes and atherosclerosis, which are increasingly linked to chronic inflammation. Our results further underline the possible role of VAT in numerous pathophysiological processes related to obesity and provide a novel direct link to explain the mechanism of association of abdominal obesity with cardiovascular diseases (Eckel and Krauss 1998).

We have previosuly shown that in contrast to VAT, the expression of adhesion molecules in SAT was regulated differently (Bosanska *et al.* 2008) and was decreased in obese in comparison to lean women. Diet induced weight reduction led to a significant increase of ICAM-1 and VCAM-1 mRNA expression in SAT. Overall, our data do not confirm the direct role of SAT adhesion molecules production in the development of endothelial dysfunction in obese patients. However, we cannot exclude that the negative results with respect to differences in adhesion molecules production in SAT were affected by relatively low number of patients included.

Although MCP-1 is a potent chemoattractant for monocytes and a marker of the inflammatory activity in patients at risk for atherosclerotic disease (Martinovic *et al.* 2005), we did not find any significant differences in mRNA expression of MCP-1 between obese and lean subjects in any adipose tissue depot. This finding may indicate that other factors than MCP-1 (i.e. adhesion molecules themselves) could play a crucial role in monocyte infiltration of adipose tissue in obese patients.

Previously published studies have shown a direct connection between CD68 mRNA abundance and higher counts of infiltrating macrophages in the adipose tissue of obese subjects (Harman-Boehm *et al.* 2007; Apovian *et al.* 2008). Reports of proportional increase of CD68 in humans with adiposity (Weisberg *et al.* 2003) are in agreement with the direct relationship of CD68 with BMI found in our study. Recently, macrophages have been found to be a major source of platelet-derived growth factor in adipose tissue, the factor induced by local hypoxia that may thus stimulate angiogenesis in adipose tissue (Pang *et al.* 2008). Moreover, in the state of positive energy balance, activated endothelial cells as well as growing fat cells may produce factors that directly or indirectly induce angiogenesis, as more intense vascularisation is needed, e.g. for maintaining chronic inflammatory state (Arner 2007; Sengenes *et al.* 2007). However, the role of the macrophage infiltration remains to be fully understood, the question still remains whether adipocytes or immunocompetent cells residing in adipose tissue are the main sources of increased production of adhesion molecules in VAT of obese subjects. We have previously shown that both isolated adipocytes and stroma-vascular fraction produce adhesion molecules (Dolinkova *et al.* 2008). Expression of VCAM-1 was 5-times lower in visceral adipocytes than in whole VAT, whereas expression of E-selectin was 8-times higher in visceral adipocytes than in whole VAT in obese (Dolinkova *et al.* 2008). Therefore, enhanced release of some adhesion molecules (e.g., VCAM-1) in VAT as compared with SAT could be due to its production by both non-adipose cells such as macrophages or other immunocompetent cells, and adipocytes (Fain *et al.* 2007).

We conclude that obesity is associated with increased mRNA expression and protein levels of adhesion molecules, including ICAM-1 and VCAM-1, in VAT, but not in SAT. The expression of adhesion molecules in VAT of obese is strongly related to BMI and the degree of tissue infiltration by macrophages. Increased adhesion molecules production in visceral fat

may provide a novel direct link between visceral adiposity and increased risk of cardiovascular complications. 13

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Table 1. Anthropometric, hormonal, and biochemical characteristics of the obese patients and healthy control women.

	Control group (n=14)	Obese group 1 BMI 30-40 (n=12)	Obese group 2 BMI > 40 (n=13)
Age (years)	49.9 ± 2.9	48.7 ±2.4	43.5 ± 2.3
BMI (kg/m^2)	23.8 ± 0.6	35.7 ± 0.9 **	46.2 ± 0.8 ** °°
C-reactive protein (mg/l)	3.0 ± 0.7	23.3 ± 6.3 *	20.2 ± 3.4 **
Blood glucose (mmol/l)	4.1 ± 0.25	5.6 ± 0.2 **	6.6 ± 0.9 *
Glycated hemoglobin HbA1c (%) (IFCC)	3.8 ± 0.06	3.8 ± 0.15	4.7 ± 0.4
Total cholesterol (mmol/l)	5.1 ± 0.3	4.7 ± 0.15	4.4 ± 0.2 *
LDL-cholesterol (mmol/l)	3.4 ± 0.2	2.9 ± 0.16	2.8 ± 0.2
Triglycerides (mmol/l)	1.1 ± 0.15	1.8 ± 0.2 *	1.5 ± 0.14
HDL-cholesterol (mmol/l)	1.2 ± 0.06	0.9 ± 0.05 **	0.9 ± 0.08 *
Insulin (mIU/l)	8.6 ± 1.2	20.8 ± 3.5 *	21.6 ± 5.9 *
HOMA index	1.4 ± 0.2	4.7 ± 1.2 *	4.9 ± 1.4 *
Systolic blood pressure (mmHg)	121 ± 3.5	129 ± 3.6	130 ± 4.4
Diastolic blood pressure (mmHg)	74 ± 2.4	82 ± 2.2	82 ± 3.6
Soluble E-selectin (ng/ml)	14.7 ± 1.4	16.4 ± 1.7	25.1 ± 3.2 *
Soluble ICAM-1 (ng/ml)	147.1 ± 9.5	155.1 ± 10.9	194.4 ± 17.6 *
Soluble VCAM-1 (ng/ml)	891.2 ± 78.2	908.1 ± 36.3	965.5 ± 70.3

BMI = body mass index; HOMA = homeostasis model assessment of insulin resistance; ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1

* $p<0.05$ resp. ** $p<0.001$ vs. Control group; °° $p<0.001$ Obese group 2 (BMI > 40 kg/m^2) vs. Obese group 1 (BMI 30-40 kg/m^2)

Table 2. Relationship of the mRNA expression and protein levels of adhesion molecules in adipose tissue to their circulating soluble forms and other studied anthropometric, hormonal, and biochemical parameters calculated in a combined population of all three groups (n = 39).

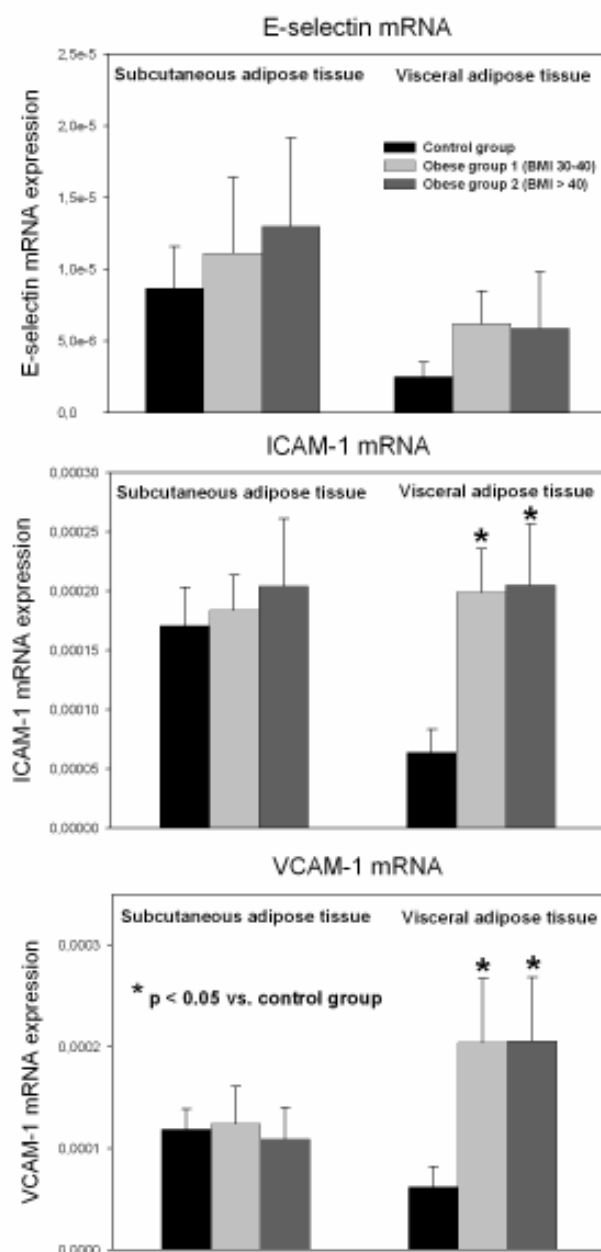|		Subcutaneous adipose tissue					Visceral adipose tissue				
	BMI	ICAM-1 protein	VCAM-1 protein	E-selectin mRNA	ICAM-1 mRNA	VCAM-1 mRNA	ICAM-1 protein	VCAM-1 protein	E-selectin mRNA	ICAM-1 mRNA	VCAM-1 mRNA
BMI	-	NS	NS	NS	NS	NS	r = 0.35 p = 0.04	r = 0.39 p = 0.01	NS	r = 0.41 p = 0.01	r = 0.38 p = 0.03
Soluble E-selectin	r = 0.43 p = 0.006	NS	NS	NS	NS	NS	NS	NS	NS	r = 0.36 p = 0.04	NS
Soluble ICAM-1	NS	r = 0.36 p = 0.02	NS	NS	NS	NS	NS	NS	NS	NS	NS
Soluble VCAM-1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Triglycerides	NS	NS	NS	NS	NS	NS	r = 0.36 p = 0.04	NS	NS	NS	NS
Glucose	r = 0.52 p = 0.001	NS	NS	NS	NS	NS	r = 0.53 p = 0.002	NS	NS	NS	NS
Glycated hemoglobin	NS	NS	NS	NS	NS	NS	NS	NS	r = 0.52 p = 0.009	r = 0.36 p = 0.04	NS
Insulin	r = 0.54 p = 0.008	NS	NS	r = 0.71 p < 0.001	NS	r = 0.53 p = 0.02	NS	NS	NS	NS	NS
CD68 mRNA s.c.	r = 0.43 p = 0.02	NS	NS	NS	r = 0.43 p = 0.03	r = 0.44 p = 0.03	NS	NS	NS	NS	NS
CD68 mRNA visc.	r = 0.45 p = 0.007	NS	NS	NS	NS	NS	NS	NS	r = 0.54 p = 0.008	r = 0.79 p<0.001	r = 0.73 p<0.001
MCP-1 mRNA visc.	NS	NS	NS	NS	NS	NS	NS	NS	r = 0.49 p = 0.03	r = 0.46 p = 0.01	NS

Data are from Spearman or Pearson correlation. BMI = body mass index; ICAM-1 = intercellular adhesion molecule; VCAM-1 = vascular cell adhesion molecule; MCP-1 = monocyte chemoattractant protein-1, s.c. = subcutaneous, visc. = visceral, NS = non-significant

Figure 1A,B. Protein levels of intercellular adhesion molecule-1 (ICAM-1) (A) and vascular cell adhesion molecule (VCAM-1) (B) in subcutaneous and visceral adipose tissue of control lean women (n = 14), obese group 1 (BMI 30-40 kg/m^2; n = 12) and obese group 2 (BMI > 40 kg/m^2; n = 13).
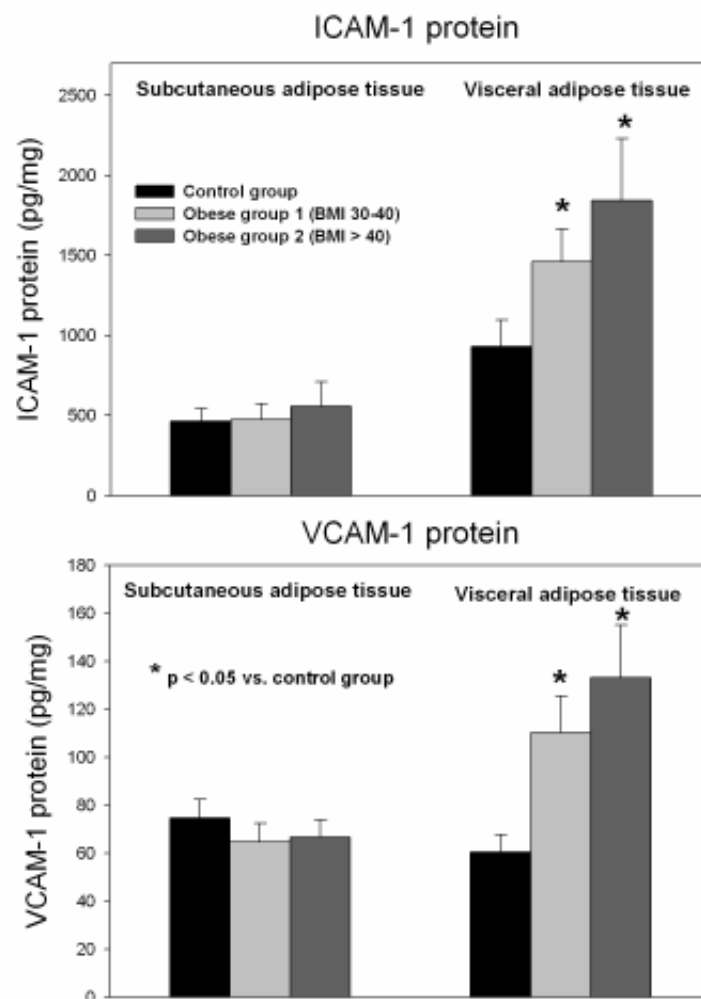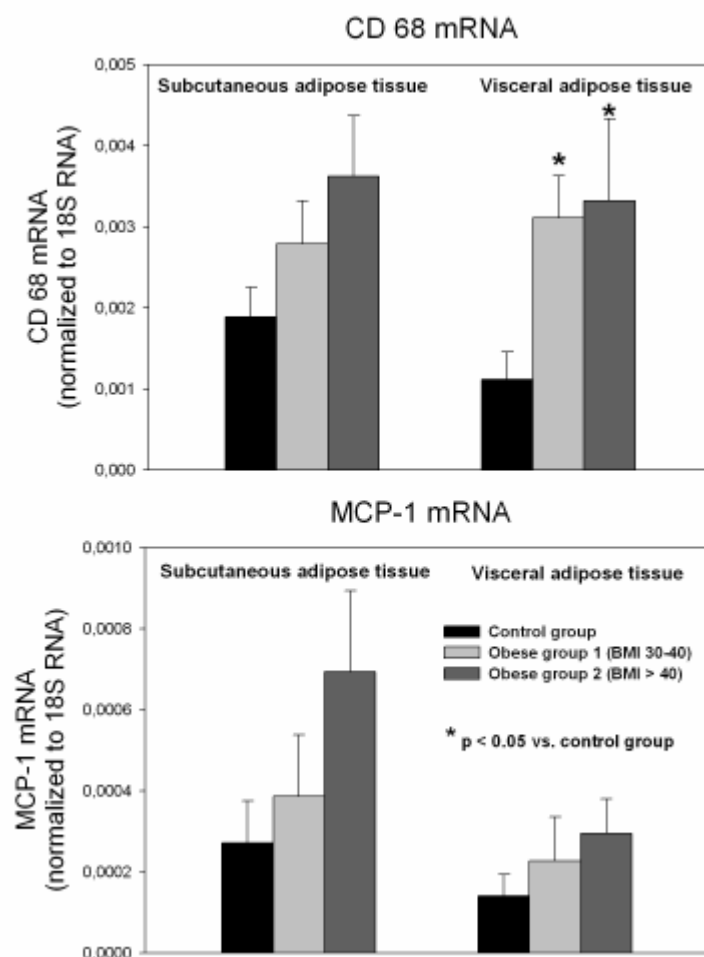

Bosanska et al., Figure 1

Figure 2A,B,C. mRNA expression of E-selectin (A), intercellular adhesion molecule-1 (ICAM-1) (B) and vascular cell adhesion molecule (VCAM-1) (C) in subcutaneous and visceral adipose tissue of control lean women (n = 14), obese group 1 (BMI 30-40 kg/m^2; n = 12) and obese group 2 (BMI > 40 kg/m^2; n = 13).

Bosanska et al., Figure 2

Figure 3A,B. mRNA expression of CD68 and monocyte chemoattractant protein-1 (MCP-1) in subcutaneous and visceral adipose tissue of control lean women (n = 14), obese group 1 (BMI 30-40 kg/m^2; n = 12) and obese group 2 (BMI > 40 kg/m^2; n = 13).

Bosanska et al., Figure 3