Nutrition, Metabolism & Cardiovascular Diseases (2014) **24**, 1137–1143

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Pro- and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity

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Received 17 February 2014; received in revised form 15 April 2014; accepted 30 April 2014
Available online 27 May 2014

KEYWORDS
Adipose tissue;
Gene expression;
Obesity;
Inflammation

Abstract *Background and aims:* Pro-inflammatory molecules produced by adipose tissue have been implicated in the risk of cardiovascular (CV) disease in obesity. We investigated the expression profile of 19 pro-inflammatory and seven anti-inflammatory genes in subcutaneous adipose tissue (SAT) and in visceral adipose tissue (VAT) in 44 severely obese individuals who underwent bariatric surgery.

Methods and results: SAT and VAT expressed an identical series of pro-inflammatory genes. Among these genes, 12 were significantly more expressed in SAT than in VAT while just one (IL18) was more expressed in VAT. The remaining genes were equally expressed. Among pro-inflammatory cytokines, both IL6 and IL8 were about 20 times more intensively expressed in SAT than in VAT. The expression of nine genes was highly associated in SAT and VAT. Only for three pro-inflammatory cytokines (IL8, IL18, SAA1) in SAT the gene expression in adipose tissue associated with the circulating levels of the corresponding gene products while no such an association was found as for VAT.

Conclusions: The expression of critical pro-inflammatory genes is substantially higher in SAT than in VAT in individuals with morbid obesity. The variability in circulating levels of pro-inflammatory cytokines is, in small part and just for three pro-inflammatory cytokines, explained by underlying gene expression in SAT but not in VAT.

These results point to a compartment-specific adipose tissue contribution to inflammation in obesity and indicate that abdominal SAT contributes more than VAT to the pro-inflammatory milieu associated with severe obesity.

Introduction

Adipose tissue is distributed throughout the body in discrete fat compartments which broadly cluster into two regions, a central and a peripheral one [1]. The central region includes subcutaneous adipose tissue (SAT) of the thorax and the abdomen as well as intra-thoracic and

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http://dx.doi.org/10.1016/j.numecd.2014.04.017
0939-4753/

intra-abdominal visceral adipose tissue (VAT), while peripheral fat consists of subcutaneous fat depots in the arms and the legs. The topography of adipose tissue accumulation is considered relevant for the risk of developing the metabolic and haemodynamic sequels of insulin resistance, including type 2 diabetes, dyslipidaemia and hypertension [2] but the issue remains controversial. Waist-to-hip circumference ratio, an established metric of abdominal obesity, consistently associates with hyperinsulinaemia, glucose intolerance, type 2 diabetes, dyslipidaemia, hyperuricaemia and cardiovascular (CV) disease [3]. However, a large waist-to-hip ratio may encompass both increased SAT and VAT depots and therefore this metric does not allow a distinction of the underlying links of visceral and subcutaneous fat with hyperinsulinaemia and attendant metabolic alterations. The issue is of relevance because VAT is generally held as the main determinant of metabolic risk [4] while SAT is considered either neutral or protective as for the same risk [5]. In VAT, free fatty acids (FFAs) generated by enhanced lipolysis directly augment lipid synthesis and gluconeogenesis in the liver, thereby triggering insulin resistance, hypertension and atherosclerosclerotic complications [4]. However, visceral fat is just a minor segment of total fat depots (<1/5 of whole body fat tissue) contributing to about 15% of the whole body FFA pool which is composed of mainly non-splanchnic adipose tissue [3,6].

Adipose tissue is also an abundant source of inflammatory cytokines and an excess of fat mass has been associated with a chronic subclinical inflammatory state [7]. It is recognized that important differences exist in the gene expression profile of abdominal SAT and VAT [8–10] and that these two fat depots independently enhance the risk of CV complications [11]. However, with respect to inflammatory genes, only few studies explored a large set of pro-inflammatory and anti-inflammatory cytokines [12,13] and the results are controversial. Further studies encompassing multiple inflammatory genes in SAT and VAT are of obvious relevance to clarify the relative role of these two adipose tissue compartments in fat-dependent inflammatory mechanisms in human obesity. With this background in mind, we compared the expression profiles of 19 major pro-inflammatory and seven anti-inflammatory genes in SAT and VAT in 44 severely obese individuals.

Methods

The protocol of the study was approved by the local ethical committee and all subjects gave informed, written consent to their participation into the study.

Subjects

The study population was recruited from the Division of General Surgery 1 of Brescia and included 44 incident obese patients who underwent bariatric surgery (biliopancreatic diversion in 11; gastric bypass in 10; mini-gastric bypass in 22; abdominal plastic in one).

Laboratory measurements

Blood sampling was performed early in the morning after an overnight fast and plasma was stored at −80 °C until batch analyses. Serum glucose, cholesterol, triglycerides, albumin, haemoglobin, urea, uric acid, bilirubin, GOT (Glutamic Oxaloacetic Transaminase), GPT (Glutamate Pyruvate Transaminase), creatinine and C-reactive protein measurements were made using standard methods implemented in a multichannel analyser in the routine clinical laboratory. Insulin (MP Biomedicals, NY, USA) as well as adiponectin and leptin (Linco Research, St. Charles, MO, USA) were measured by radioimmunoassay kits. Enzyme-linked immunosorbent assays (ELISAs) were applied to measure plasma levels of IL1β, IL6, tumour necrosis factor-alpha (TNFα), IL18, resistin, PAI, VCAM1 (R&D Systems, Inc., Minneapolis, USA), IL8, SAA1 (Invitrogen, Carlsbad, CA, USA) and visfatin (Adipogen International, Inc., San Diego, CA, USA).

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according the formula HOMA-IR = [fasting insulin concentration (μU/mL) × fasting glucose concentration (mmol/L)]/22.5.

Adipose tissue sampling and gene expression analysis

SAT and VAT from abdominal region were harvested at the beginning of surgical intervention and the adipose samples were collected in RNAlater (Ambion, Life Technologies, Austin, TX, USA) and stored at −80 °C until processing for RNA extraction. Total RNA was isolated from approximately 80 mg frozen SAT and VAT by means of the RNeasy Lipid Tissue Mini Kit (Qiagen Sciences, Germantown, MD, USA), according to the manufacturer's instructions. Total RNA was treated with the DNA-free kit (Ambion, Austin, TX, USA) to digest contaminating genomic DNA. The concentration of the RNA samples was determined spectrophotometrically (NanoDrop ND-1000, Thermo Fisher Scientific Inc.). Single-stranded complementary DNA (cDNA) was synthesized using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Pre-validated TaqMan Gene Expression Assays from Applied Biosystems were used to quantify the expression of pro-inflammatory genes (IL6, IL6R, IL8, CXCR1, CXCR2, TNFα, IL1β, IL1R1, TGFβ, MCP1, IL18, PAI, SAA1, TLR4, ICAM1, VCAM1, Visfatin, Resistin and Leptin) and anti-inflammatory genes (IL2, IL4, IL10, IL13, SOCS3, CD163 and Adiponectin). The reverse transcription polymerase chain reaction (RT-PCR) was performed by a 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA). All genes were run in duplicate and negative controls were introduced in each plate. Target genes were considered unexpressed if the threshold cycle (Ct) value ≥38. All values were normalized to glyceraldehyde-3-phosphate dehydrogenase (GADPH) gene expression to correct for variation in RNA amounts and efficiency of reverse transcription. The relative quantification value of the target genes was calculated using the comparative Ct method, expressed as $2^{-[\mathrm{delta}][\mathrm{delta}]\mathrm{Ct}}$ (fold difference), and reported as arbitrary units (AU).

Gene expression in pooled samples

To preliminary test the gene expression of the 26 target genes, we performed a pooling analysis using a SAT and VAT pool. Each pool was built using an identical quantity of SAT and VAT mRNA from every patient. Pooled mRNA was reverse transcribed and the resulting cDNA was amplified. The gene expression of the target genes in the two pools was compared and only those differentially expressed in SAT and VAT were further analysed on individual basis. To identify differentially expressed target genes, we adopted

a conservative approach consisting of a difference in SAT/VAT gene expression ratio more than 50%.

Histological analysis of adipose tissue samples

SAT and VAT samples obtained from 14 patients were formalin-fixed and paraffin-embedded. Four-micron tissue sections were stained for haematoxylin and eosin and immunostained using a Bond MaxTM autostainer (Menarini Diagnostics, Florence, Italy). Standard immunoperoxidase staining protocols for CD45 – a pan-leukocyte antigen – (Clone RP2/18 and RP2/22, Leica Microsystems, Newcastle upon Tyne, UK) and CD163 – an antigen for a macrophage subpopulation of major relevance for the anti-inflammatory response – (Clone 10D6, Thermo Scientific, Fremont, CA, USA) were followed. In all the adipose tissue samples, cell automatic counting on CD163 stained sections was performed on digitalized slides (Aperio Scanscope, CA, USA) by analysing the whole section using IHC Nuclear algorithm. Data were expressed as number of cells/cm [2].

Statistical analysis

Data are expressed as mean ± standard deviation (SD; normally distributed data), median and inter-quartile range (non-normally distributed data) or as percent frequency, as appropriate. Within-group comparisons were made by the Wilcoxon rank test. The association between two continuous variables considered simultaneously was assessed by Pearson product moment correlation coefficients (r) and P values. Variables having a positively skewed distribution were log transformed (Ln) before the correlation study. The correlation coefficient was calculated with and without the exclusion of the outliers as identified by Mahalanobis distance test [14]. The agreement between gene expression in VAT and SAT was investigated by calculating the shared variance (r^2) of tested genes in visceral and subcutaneous fat. Because our study focuses on a specific aetiological hypothesis and on a strong a priori we did not account for multiple testing [15].

Study power

In a previous paper on severe obese women [13], the ratio between SAT and VAT gene expression of a major inflammatory biomarker (IL-6) was reported to be 3. With this background in mind, we calculated that by enrolling at least 44 obese individuals (including a 10% attrition rate) our study will achieve 80% power to detect as significant (alpha-error = 0.01) a ratio ≥3 in IL-6 gene expression between SAT and VAT. We assumed that the ratio in the gene expression between SAT and VAT of all inflammatory and anti-inflammatory molecules considered in the study was equal or greater than that calculated for IL-6.

Results

Demographic, somatometric and clinical characteristics of the patients

Demographic, somatometric and clinical characteristics of the patients are reported in Table 1.

The mean age of the patients was 41 ± 9 years (11 M and 33 F). Obesity was of grade I (body mass index (BMI) ranging from 30.0 to 34.9 kg/m^2) in four cases (9%), of grade 2 (BMI ranging from 35.0 to 39.9 kg/m^2) in 12 cases (27%) and of grade III in the remaining 28 cases (64%). The median value of glycaemia was 99 mg/dL and only four were diabetic (three on oral hypoglycaemic drugs and one on insulin treatment). Serum cholesterol was on average 199 mg/dL and was above the upper limit of the normal range (200 mg/dL) in 22 cases. Blood pressure (BP) was 127 ± 8/79 ± 8 mmHg. No patient had a BP exceeding 140/90 mmHg and only three were on anti-hypertensive treatment (two on mono-therapy with sartans or β-blockers or angiotensin-converting enzyme inhibitors and the remaining one on triple therapy with a β-blocker, an angiotensin-converting enzyme inhibitor and a diuretic). Six patients were habitual smokers. None of the patients was suffering from cancer, thyroid disease, liver disease or acute infections.

Table 1 Main demographic, somatometric and clinical characteristics of the study patients.

	(n = 44)
Age (years)	41 ± 9
Male sex n (%)	11 (25)
BMI (kg/m^2)	43 ± 7
Diabetics n (%)	4 (9)
Smokers n (%)	6 (14)
On-anti-hypertensive treatment n (%)	3 (7)
On anti-diabetic treatment n (%)	4 (9)
Systolic BP (mmHg)	127 ± 8
Diastolic BP (mmHg)	79 ± 6
Total cholesterol (mg/dL)	199 ± 40
Triglycerides (mg/dL)	121 (77–169)
Haemoglobin (g/dL)	13.1 ± 1.7
Albumin (g/dL)	4.4 ± 0.2
Glucose (mmol/L)	99 (92–115)
Insulin (μUI/mL)	37 (31–53)
HOMA-IR (μU/mL mmol/L)	1.26 ± 0.8
Azotaemia (mg/dL)	33 ± 7
Uric acid (mg/dL)	5.6 ± 1.6
Total bilirubin (mg/dL)	0.4 (0.3–0.7)
GOT (UI/L)	16.5 (12.2–25.7)
GPT (UI/L)	30.0 (21.2–44.0)
CRP (mg/L)	6.4 (3.3–15.6)
Creatinine (mg/dL)	0.69 ± 0.15

Data are expressed as mean ± SD, median and inter-quartile range or as percent frequency, as appropriate.

Preliminary gene expression analysis in pooled samples

From a total number of 26 cytokines tested in pooled samples (Fig. 1), 13 genes including 12 pro-inflammatory genes (IL6, IL8, CXCR1, CXCR2, TNFα, IL1β, IL18, PAI, SAA1, visfatin, resistin and leptin) and just one anti-inflammatory gene (adiponectin) resulted to be differentially expressed in SAT compared with VAT. Ten genes were equally expressed (IL1R1, IL6R, IL10, TGFβ, MCP1, ICAM1, VCAM1, TLR4, SOCS3 and CD163) in SAT and VAT and three anti-inflammatory genes were unexpressed (IL2, IL4 and IL13) in both fat compartments (Fig. 1).

Gene expression analysis at individual level

On the basis of findings in pooled samples, we undertook detailed analyses in individual patients.

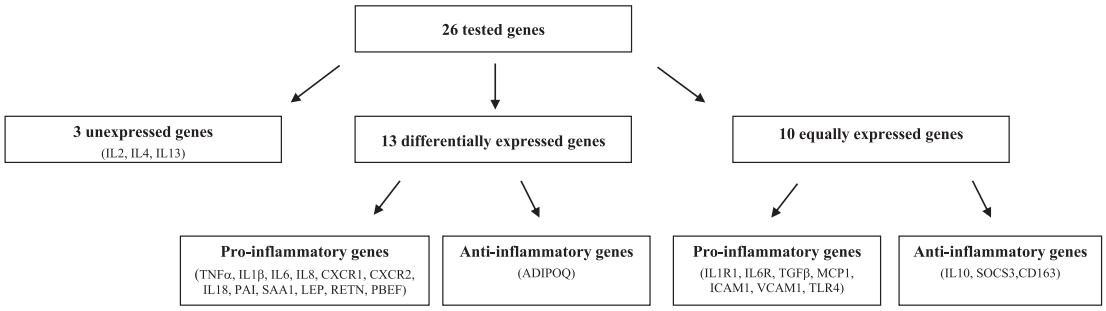

Figure 1 Flowchart representing the process for analysing pro- and anti-inflammatory gene expression in paired samples of SAT and VAT from 44 severely obese individuals. List of adipose tissue pro- and anti-inflammatory genes tested: Pro-inflammatory genes ($n = 19$) – IL1β: Interleukin-1 beta; IL1R1: Interleukin 1 receptor; TNFα: Tumour necrosis factor alpha; IL6: Interleukin 6; IL6R: Interleukin 6 receptor; TGFβ: Transforming growth factor beta; IL8: Interleukin 8; CXCR1: Interleukin 8 receptor 1; CXCR2: Interleukin 8 receptor 2; IL18: Interleukin 18; MCP1: Monocyte chemoattractant protein 1; SAA1: Serum amyloid A1; TLR4: Toll-like receptor 4; ICAM1: Intercellular adhesion molecule 1; VCAM1: Vascular cell adhesion molecule 1; PAI: Plasminogen activator inhibitor; LEP: Leptin; RETN: Resistin; PBEF: Pre-B cell colony-enhancing factor/visfatin. Anti-inflammatory genes ($n = 7$) – IL2: Interleukin 2; IL4: Interleukin 4; IL10: Interleukin 10; IL13: Interleukin 13; SOCS3: Suppressor of cytokine signalling 3; CD163: Cluster of differentiation 163; ADIPOQ: Adiponectin.

Among the 13 differentially expressed genes, adiponectin, leptin, resistin and visfatin gene expressions were from 1.6- to 5.1-fold higher in SAT than in VAT (Table 2) and this was also true for all, but one inflammatory cytokine (IL18) which, instead, was 12-fold more expressed in VAT (Table 2). The expression of IL6 and IL8 genes was about 20 times higher in SAT than in VAT. Of note, the expression level of seven pro-inflammatory genes (IL1β, IL6, IL8, CXCR1, CXCR2, resistin and visfatin) was strongly related (r^2 ranging from 0.24 to 0.35 and $P \leq 0.001$) in SAT and VAT while the remaining two genes (IL18 and PAI) showed much weaker associations ($r^2 = 0.10$ and $r^2 = 0.12$, respectively) (Fig. 2). No relationship was found between SAT and VAT gene expression for the remaining four genes (adiponectin, leptin, TNFα and SAA1) ($P \geq 0.20$). A separate analysis by gender fully confirmed these results (data not shown).

Functional link between expression of pro- and anti-inflammatory genes and circulating molecules

Eleven gene products (TNFα, IL1β, IL6, IL8, IL18, SAA1, PAI, leptin, adiponectin, resistin and visfatin) were measured in plasma. Among these, only four inflammatory cytokines (IL8, IL18, SAA1 and adiponectin) correlated with the corresponding gene expression in SAT or VAT. Plasma IL18 and SAA1 were directly related to the corresponding SAT gene expression while IL8 associated inversely with the corresponding gene expression (Fig. 3). No relationship was observed between plasma levels of these three pro-inflammatory molecules and VAT gene expression of the corresponding genes (Fig. 3). Among anti-inflammatory cytokines, only adiponectin gene expression in VAT showed an association with the corresponding gene product levels in plasma (Fig. 3).

Table 2 Gene expression measurements of pro and anti-inflammatory cytokines in paired samples of SAT and VAT.

Gene	Symbol	SAT median (IQR)	VAT median (IQR)	SAT/VAT	*P* value
Pro-inflammatory genes					
Tumour necrosis factor α	TNF α	1.05 (0.71–1.57)	0.64 (0.36–1.20)	1.6	0.006
Interleukin 1 β	IL 1β	0.46 (0.16–0.97)	0.09 (0.04–0.41)	5.1	0.001
Interleukin 6	IL6	0.48 (0.06–1.00)	0.02 (0.003–0.325)	19.2	<0.001
Interleukin 8	IL8	0.51 (0.14–0.99)	0.02 (0.003–0.305)	20.4	<0.001
Interleukin 8 receptor, type 1	CXCR1	0.23 (0.07–0.63)	0.05 (0.01–0.16)	4.6	<0.001
Interleukin 8 receptor, type 2	CXCR2	0.21 (0.10–0.83)	0.06 (0.01–0.20)	3.5	<0.001
Interleukin 18	IL18	0.69 (0.50–1.03)	8.40 (3.50–15.20)	0.08	<0.001
Serum amyloid A1	SAA1	1.00 (0.70–1.54)	0.63 (0.34–1.09)	1.6	0.03
Plasminogen Activator Inhibitor	PAI	1.07 (0.51–3.97)	0.31 (0.07–1.12)	3.5	<0.001
Leptin	LEP	0.77 (0.60–1.00)	0.24 (0.10–0.42)	3.2	<0.001
Resistin	RETN	0.71 (0.43–1.30)	0.25 (0.16–0.42)	2.8	<0.001
Visfatin	PBEF	0.51 (0.25–1.11)	0.10 (0.04–0.52)	5.1	<0.001
Anti-inflammatory genes					
Adiponectin	ADIPOQ	2.02 (1.19–3.13)	0.96 (0.58–1.76)	2.1	0.001

Gene expression measurements (3rd and 4th columns) are expressed as arbitrary units and reported as median (inter-quartile range). The SAT/VAT ratio (5th column) represents the fold difference in cytokine gene expression measurements between SAT and VAT. In the last column, the *P* value (Wilcoxon rank-sum test) of the difference between SAT and VAT gene expression measurements is also given.

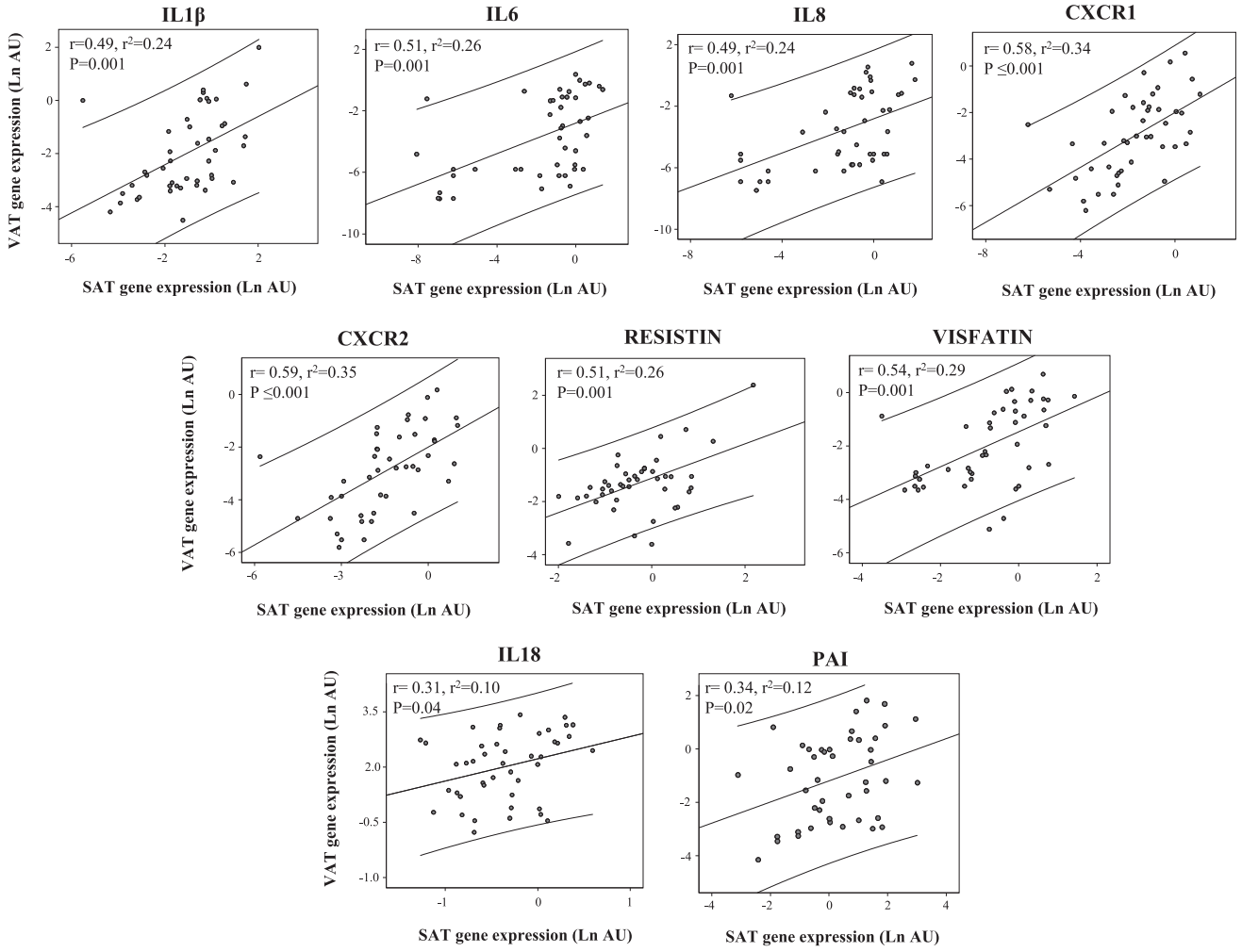

Figure 2 Correlation between cytokines gene expression in SAT and VAT. Gene expression measurements are expressed as arbitrary, Log-transformed units (Ln AU). Data are Pearson correlation coefficients (r), r^2 and P value.

Immune cells counting in SAT and VAT

Given the specific involvement of immune cells in obesity-related inflammation, in a subgroup of 14 patients we counted CD163 + macrophages (a subpopulation of major relevance for the anti-inflammatory response) in SAT and VAT. We found that there was no difference in the number of CD163 + macrophages between SAT and VAT (3418 ± 1353 n/cm^2 vs. 3732 ± 1396 n/cm^2, $P = 0.54$), indicating that differences in the inflammatory status of the two fat compartments do not depend on the number of these cells.

Discussion

In this study, we quantified the gene expression of a large set of pro- and anti-inflammatory cytokines in abdominal SAT and VAT in severe obesity. The vast majority of pro-inflammatory genes were more expressed in SAT than in VAT whereas just one pro-inflammatory gene was more expressed in VAT, suggesting a stronger contribution of subcutaneous adipose compartment to the low-grade obesity-related inflammation.

Gene expression in SAT and VAT

Central adiposity is more strongly associated with adverse CV outcomes than peripheral adiposity [3]. Although this risk excess is traditionally attributed to visceral fat [4], the predominant component of fat mass in central adiposity is subcutaneous rather than visceral [16].

We found that SAT and VAT express the same set of inflammatory cytokines in obese patients and that SAT, rather than VAT, is the fat compartment expressing the higher pro-inflammatory profile. This was true for fundamental fat cytokines such as TNFα and IL6, the expression levels of these cytokines being from 1.6- to 20-fold greater in this fat compartment than in VAT (Table 2). These findings are in accordance with previous studies focusing on TNFα and IL6 [10,13,17] and add weight to the contention that IL1β gene expression is upregulated [10,13], rather than down-regulated [18], in SAT of obese patients. Furthermore, for the first time we show that IL8 is upregulated in SAT and that the gene expression of this chemokine is more than 20 times higher in SAT than in VAT. Such a remarkable increase of IL8 mRNA in SAT was parallelled by a significant increase of the

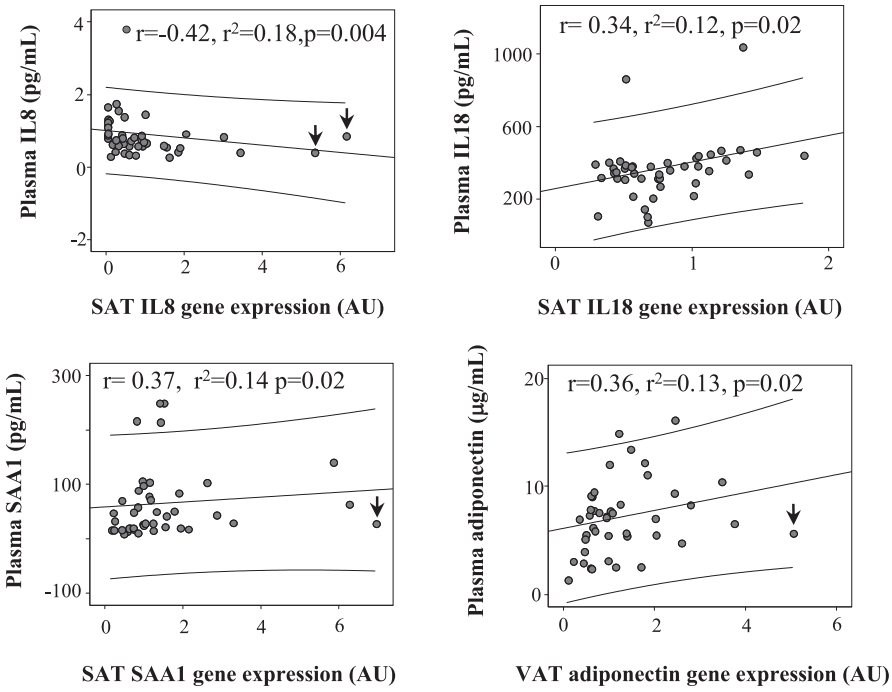

Figure 3 Correlation between plasma levels of cytokines and the corresponding gene expression measurements in SAT or VAT. The arrows indicate the outliers identified by Mahalanobis distance test (see Ref. [14]). The strength of these associations did not materially change after the inclusion of the outliers (all $P \leq 0.02$).

gene expression of its corresponding receptors, that is, CXCR1 and CXCR2, in the same fat compartment (Table 2), further suggesting an augmented role for IL8 signalling in SAT than in VAT in obese individuals. We also document an upregulated expression in SAT of other two important pro-inflammatory molecules such as SAA1 (Table 2) which is involved in early response to injury, and PAI, which is responsible for the negative regulation of the fibrinolytic system.

Leptin, Resistin and Visfatin are potent pro-inflammatory peptides. Consistently with previous studies [10,12], we observed a threefold higher leptin and resistin gene expression in SAT. We found a similar pattern for visfatin, an insulin-mimetic peptide typically expressed in VAT. Of note, adiponectin, an anti-inflammatory cytokine, followed the same pattern, being twice more expressed in SAT than in VAT. IL18 was the sole pro-inflammatory cytokine showing a reverse expression pattern, being upregulated in the visceral rather than in the subcutaneous fat compartment. IL18 is a pleiotropic molecule promoting Th1 cell differentiation, cell-mediated cytotoxicity and inflammation which also induces the synthesis of the anti-inflammatory cytokine IL10 [19] and limits the release of chemokines such as IL8 [20]. IL18-deficiency in mice causes hyperphagia, obesity, diabetes and atherosclerosis by massive fat deposition in the arterial walls [21]. Thus, IL18 downregulation in SAT is in keeping with the hypothesis that the overall expression profile of cytokines in SAT denotes a more pro-atherogenic, metabolically adverse attitude.

Although in our study the expression of pro-inflammatory genes was systematically upregulated in SAT, we found a strong and positive correlation between SAT and VAT gene expression for the majority of the pro-inflammatory genes studied (i.e., IL1β, IL6, IL18, IL8, CXCR1, CXCR2, resistin, visfatin and PAI) indicating that, though at different rates, the two main fat compartments undergo qualitatively similar changes in the expression profile of inflammatory cytokines.

Monocytes/macrophages typically accumulate in adipose tissue and are primarily responsible for the release of inflammatory mediators in this tissue [13,22]. Macrophage infiltration is of comparable extent in SAT and VAT [23] and we show that the number of M2 macrophages, that is, CD163 + macrophages with anti-inflammatory potential, is identical in SAT and in VAT suggesting that in obesity a higher transcriptional activity rather than an expansion of M2 macrophages pool explains the increased pro-inflammatory gene expression of subcutaneous fat compartment.

Gene expression profile and circulating gene products

Circulating levels of inflammatory cytokines such as TNFα [24], IL6 [25], IL8 [26], IL1 [27], IL18 [28], SAA1 [25], leptin [29] and resistin [30] are potent predictors of adverse cardiovascular outcomes. Adipose tissue cytokines mainly act as autacoids in the fat compartment. Interestingly, we found a strong association between adipose tissue gene expression and the corresponding plasma levels for four inflammatory cytokines (IL8, IL18, SAA1, adiponectin). Specifically, we found a positive correlation between SAT gene expression and plasma levels of IL18 and SAA1 and an inverse one in the same fat compartment between gene

expression and plasma levels of IL8 (Fig. 3). Adiponectin plasma levels were positively associated with adiponectin gene expression only in VAT (Fig. 3). Overall, these findings provide circumstantial evidence that the adipose tissue *in vivo* may contribute to regulate circulating levels of, at least, some cytokines.

A potential limitation in our study is that because patients in this series had a low prevalence of diabetes and hypertension, selection bias cannot be excluded.

In conclusion, we show compartment-specific adipose tissue changes in inflammation-related genes in obesity and support the hypothesis that abdominal SAT contributes to the pro-inflammatory burden of severe obesity more than VAT, an observation also in keeping with the association of three pro-inflammatory cytokine genes with the corresponding gene product plasma levels. Whether the augmented pro-inflammatory profile of SAT in obese patients predicts CV events warrants further studies in this high-risk population.

Disclosure

All the authors declare no conflict of interest.

Financial support

This study was supported by grants of the CNR and is part of the Syskid project which is supported through European Union's FP7, Grant agreement number HEALTH-F2-2009-241544.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.numecd.2014.04.017.

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