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

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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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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