An obese brain and an inflamed body: Central and peripheral consequences of obesity

de Weijer, B.A.M.

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Influx of macrophages and T cells in visceral and subcutaneous adipose tissue of morbidly obese women is not associated with insulin sensitivity


Submitted
Abstract

**Background:** Infiltration and activation of adipose tissue immune cells contribute to low grade inflammation in obesity. Whether the expression profiles of inflammatory markers in different adipose tissue compartments contribute to disturbed metabolic fluxes in insulin target tissues in obese humans is unclear.

**Methods:** mRNA expression profiles of both pro- and anti-inflammatory macrophage and T cell markers as well as mRNA expression of glucose transporter (GLUT) 4 were determined in subcutaneous (SAT) and visceral adipose tissue (VAT) in 20 morbidly obese women undergoing bariatric surgery. Expression profiles were compared to 6 lean controls undergoing elective cholecystectomy. In obese women, insulin sensitivity and insulin-mediated suppression of lipolysis were determined using a hyperinsulinemic euglycemic clamp with stable isotopes.

**Results:** The obese women were insulin resistant, characterized by lower adipose tissue GLUT 4 expression and reduced peripheral insulin sensitivity. Circulating levels of C-reactive protein (CRP) were increased in the obese subjects. Overall, the expression of both pro- and anti-inflammatory markers were increased in SAT and VAT in the obese compared to the lean controls. SAT displayed a predominant pro-inflammatory phenotype, whereas VAT showed higher expression of anti-inflammatory markers. In addition, obese subjects showed higher influx of T cells in both adipose tissue compartments with higher expression of CD25, a marker of activated T cells, in VAT. Despite these distinct inflammatory phenotypes of adipose tissue in obesity, no correlations were observed between any of the inflammatory markers and insulin sensitivity.

**Conclusions:** Compared to lean healthy controls, morbidly obese and insulin resistant women show marked inflammation in both SAT and WAT reflected by increased expression of pro- and anti-inflammatory markers. Analysis of adipose tissue extracted CD11b+ macrophages in the obese subjects further revealed higher VAT expression of anti-inflammatory CD163 and mannose receptor and higher SAT expression of CD11c+. This mixed pattern of activated immunity in obesity was not associated with reduced insulin sensitivity of muscle or adipose tissue.
Introduction

Obesity is associated with a state of low-grade inflammation and activation of inflammatory pathways within adipose tissue (AT) is known to interfere with insulin signalling (1, 2, 3, 4). Lean healthy AT displays an anti-inflammatory environment, characterized by high IL4 and IL10 levels (5, 6) while in obesity, AT shows a change towards a more inflammation prone environment. Adipose tissue macrophages (ATM) are crucial mediators of adipose tissue inflammation and have been connected to AT inflammation in obesity over a decade ago (7, 8). In obesity, the number of ATM is positively correlated with BMI and adipocyte size (7, 9) and ATM are predominantly present in so-called crown-like structures, surrounding necrotic adipocytes where they are supposed to scavenge cell debris and free lipids (10). In obese AT, ATM content can increase approximately 4-fold up to 40-50% of total cell numbers (7) and undergo a phenotypic switch from an alternatively activated anti-inflammatory phenotype towards a more classically activated pro-inflammatory phenotype (11). Moreover, obese ATM show an increase in lysosomal biogenesis (8). Inflammation of adipose tissue is associated with insulin resistance in several rodent obesity models and reduction of inflammation either by genetic manipulations in mice or weight loss show reversal of the insulin resistant state (9, 12). More recently, other immune cells besides ATM including T cells, have been connected to the inflammatory AT phenotype as well. Numbers of regulatory T cells (Tregs), which are of an immuno suppressive nature are reduced in adiposity (13, 14). Tregs are CD4+ cells that express CD25+ and FOXP3, a forkhead transcription factor required for their specific development and function (CD4+CD25+FOXP3 regulatory T cells) (15) and secrete the anti-inflammatory cytokine IL10, which inhibits TNF-α production by macrophages, thereby preventing local tissue damage and dampening inflammation. From murine studies it became clear that depletion of Tregs worsens adipose tissue inflammation and thus insulin resistance (16), whereas expansion of the number of Tregs attenuates inflammation and insulin resistance (14). It has been hypothesized that in lean adipose tissue Tregs are keeping chronic inflammation under control, but during adiposity the influx of inflammatory macrophages and other immune cells outnumbers the Tregs, leading to an inflammatory prone environment.

In humans it remains largely unknown whether adipose tissue inflammation in either subcutaneous (SAT) or visceral adipose tissue (VAT) directly contributes to systemic insulin resistance. Therefore, we studied the inflammatory expression profiles of SAT and VAT and of extracted ATM in morbidly obese women undergoing bariatric surgery and in lean controls undergoing elective cholecystectomy. In addition, we assessed insulin sensitivity of muscle (insulin-mediated glucose uptake) and adipose tissue (insulin-mediated suppression of lipolysis) in the obese women using a hyperinsulinemic euglycemic clamp and stable isotope tracers.
Subjects and methods

Subjects

Twenty morbidly obese women scheduled for Roux-en-Y gastric bypass surgery (RYGB) and six matched healthy lean women scheduled for elective cholecystectomy for benign gallbladder disease were included. Subjects were recruited from the outpatient clinics of the Rijnstate Hospital in Arnhem, the Slotervaart Hospital in Amsterdam and the Medical Center Alkmaar in Alkmaar.

The obese patients were eligible for the study if they were scheduled to undergo RYGB surgery, were older than 18 years, understood the objective of the study, and were competent to give informed consent. Exclusion criteria were: childhood onset obesity, insulin dependent DM2, coagulation disorders, a recent history (6 months or less) of substantial alcohol or drug abuse; the use of antipsychotic medication or antidepressant medication; any somatic illness except for obesity-related conditions (hypertension, dyslipidemia and DM2 treated with oral anti-diabetics). Inclusion criteria for the lean controls were: BMI < 25 kg/m2, scheduled for elective cholecystectomy for benign gallbladder disease. Exclusion criteria were any somatic disease, coagulation disorders, glucose intolerance and use of medication. Glucose tolerance was assessed during a 75 gr oral glucose tolerance test.

The study was approved by the Medical Ethical Committee of the Academic Medical Center (AMC) of the University of Amsterdam. After a complete description of the study had been given, written informed consent was obtained.

Analytical procedures

Hyperinsulinemic euglycemic clamp

The obese women participated in a study on the short term metabolic effects of bariatric surgery (17). Insulin sensitivity was measured using a two-step hyperinsulinemic euglycemic clamp after an overnight fast as described previously (17). In short, intravenous glycerol and glucose isotope tracers were infused after drawing a blood sample for measurement of background enrichments. After equilibration, blood was drawn for glucose and glycerol enrichments to calculate basal endogenous glucose production (EGP) and lipolysis as well as FFA and insulin. Thereafter insulin was infused at a rate of 20 mU · m⁻² · min⁻¹ for two hours and a rate of 60 mU · m⁻² · min⁻¹ for another two hours. After each step of the clamp, blood was drawn for glucose and glycerol enrichments to calculate suppression of basal EGP and lipolysis (step 1) and the rate of glucose disposal (Rd) (step 2) as well as FFA and insulin. To keep euglycemia, exogenous glucose enriched with the glucose isotope tracer was infused simultaneously. The detailed experimental protocol has been published earlier (17).

Body composition

Body composition was measured using bioelectrical impedance analysis (Maltron BF-906, Rayleigh, UK).
Laboratory analysis

Plasma glucose was measured with a glucose oxidase method (EKF Diagnostics, Barleben / Magedeburg, Germany). Free fatty acids (FFA) were measured by an enzymatic colorimetric method (Nefa-C test kit; Wako Chemicals, Neuss, Germany) with an intra-assay variation of 1%, inter-assay variation of 4-15% and a detection limit of 0.02 mmol/L. [6,6-2H2]glucose enrichment (tracer-to-tracee ratio) and [1,1,2,3,3-2H5]glycerol enrichment (tracer-to-tracee ratio) were measured as described earlier (18). Insulin was determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA) using a chemiluminescent immunometric assay with an intra-assay variation of 4–5%, inter-assay variation of 5% and detection limit of 15 pmol/l. C-reactive protein (CRP, ng/mL) was determined using ELISA (R&D systems Europe, Ltd. Abingdon, UK), according to the manufacturer’s instructions.

Adipose tissue biopsies

The surgical procedures were carried out in three medical centers (Rijnstate Hospital, Arnhem, Slotervaart Hospital, Amsterdam and Medical Center Alkmaar, Alkmaar) and performed by experienced surgeons. Adipose tissue biopsies were taken from two adipose tissue compartments (visceral and abdominal subcutaneous) during RYGB surgery in the obese and during laparoscopic cholecystectomy in the lean controls. Samples were taken from similar tissue locations in all patients and at the same time point during surgery after a comparable overnight fast. Haemostasis was checked directly after the biopsies and at the end of the surgical procedure. A part of the adipose tissue samples was collected in DMEM media (DMEM with glutamine, 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) and kept at room temperature for a maximum period of 3 hours. Another part was snapfrozen in liquid nitrogen and stored in -80ºC for subsequent analysis.

Extraction of macrophages from adipose tissue biopsies

Adipose tissue collected in DMEM was thoroughly chopped with a sterile surgical blade and resuspended in 10 ml digestion solution (7 ml Hanks’ Solution, 3 ml 7.5 % BSA, and 20 mg Collagenase type II, Sigma). The digestion was performed at 37 ºC using a shaker at 100 rpm for 20 min. After digestion, the adipocyte fraction was passed through a filter: cell strainer 100µM from falcon (ref 352360) and the remaining solution was centrifuged at 1500 rpm, 4 ºC for 5 min. This pellet was resuspended in 2 ml of selection buffer (PBS, 2 mM EDTA, 0.5 % BSA). CD11b positive cells were subsequently selected using CD11b micro-beads (Miltenyi Biotec) according to the manufacturer’s instructions. The negative fraction from this isolation was collected and referred to as the stroma vascular fraction (SVF).

RNA extraction

Total RNA was extracted from the biopsies using TRIzol reagent (Invitrogen), followed by further extraction using the NucleoSpin RNA II kit according to the manufacturer’s recommendations (Macherey-Nagel GmbH, Duren, Germany). This protocol included a RNase-free DNase step.
RNA concentrations were determined using a Nanodrop Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). RNA integrity was investigated by assessing the RNA integrity number (RIN), using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). The mean RIN was 7. Equal amounts of RNA were used to synthesize cDNA, using oligo-(dT)$_{12-18}$ and random hexamers as primers, and Superscript II reverse transcriptase, according to the manufacturer’s method (Invitrogen). Gene-specific analysis was performed on an iCycler MyiQ single-color real-time PCR detection system using iQ SYBR Green Supermix (Bio-Rad Laboratories). Gene expression levels were normalized to acidic ribosomal protein 36B4, also referred to as P0. Specificity of the primers was verified by evaluation of the amplifications with the use of gel electrophoresis and melting curve analysis. The primers used on adipose tissue were CD68, Macrophage Inflammatory Protein (MIP) II1beta (IL1-b), Mannose receptor (MR), GLUT4 and T cell markers (CD25 and CD4), leptin, adiponectin and PPARy. Primers used on macrophages were IL1b, IL18, MIP1beta, CD11c, MR and CD163. The number of analyzed tissue samples of the obese subjects slightly differ per measurement (between N= 16 and N =20) because of poor quality of some samples.

**Calculations and statistical analyses**

Data were analysed using parametric and non-parametric tests. For the statistical analyses of mRNA expression, the unpaired Mann Whitney U test was used. Correlations were determined using the Spearman’s Rho test. Patient characteristics are presented as mean ± SD. SPSS version 20.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Comparisons were considered statistically significant if the p value was <0.05 and p < 0.1 was considered a trend. Clamp data are presented as median [minimal - maximum]. Endogenous glucose production (EGP) and insulin-mediated peripheral glucose uptake (rate of disappearance [Rd]) were calculated using the modified form of the Steel equation as described previously (19, 20). EGP is expressed as $\mu$mol/kg fat-free mass (FFM)$^{-1}$ and, Rd as $\mu$mol/kg$^{-1}$min$^{-1}$ and $\mu$mol/kg.min. Hepatic and adipose tissue insulin sensitivity were expressed as % insulin-mediated suppression of basal EGP and lipolysis, respectively, during step 1 of the clamp. HOMA-IR was calculated as fasting glucose x fasting insulin divided by 22.5 and quantitative insulin sensitivity check index (QUICKI) as $1/(\log(I_0) + \log(G_0))$.

**Results**

**Study participants**

We included 6 lean and 20 obese women. Their baseline characteristics are shown in Table 1(17). The lean women had a normal fasting glucose and normal HOMA-IR.
INFLUX OF MACROPHAGES AND T CELLS IN VISCERAL AND SUBCUTANEOUS ADIPOSE TISSUE OF MORBIDLY OBESE WOMEN IS NOT ASSOCIATED WITH INSULIN SENSITIVITY

Table 1. Subject characteristics. Data are presented as mean ±SD. BMI=Body mass index.

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>LEAN (N=6)</th>
<th>OBESE (N=20)</th>
<th>P</th>
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<tr>
<td>AGE (YEARS)</td>
<td>36 ± 6.6</td>
<td>41 ± 8.5</td>
<td>0.203</td>
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<td>WEIGHT (KG)</td>
<td>69.7 ± 5.0</td>
<td>127.5 ± 21.5</td>
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<tr>
<td>BMI (KG/M²)</td>
<td>22.1 ± 1.4</td>
<td>45.3 ± 6.2</td>
<td>&lt; 0.001</td>
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<td>HOMA-IR</td>
<td>1.0 ± 0.7</td>
<td>2.8 ± 1.2</td>
<td>0.002</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.39 ± 0.09</td>
<td>0.33 ± 0.03</td>
<td>&lt; 0.002</td>
</tr>
<tr>
<td>INSULIN (PMOL/L)</td>
<td>31.1 ± 19.8</td>
<td>83.1 ± 32</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>GLUCOSE (MMOL/L)</td>
<td>5.0 ± 0.4</td>
<td>5.6 ± 1.1</td>
<td>0.169</td>
</tr>
</tbody>
</table>

Glucose metabolism and lipolysis

As expected, HOMA-IR and QUICKI significantly differed between the lean and obese group showing lower insulin sensitivity in the obese group (table 1). Basal glucose metabolism was assessed in 19 and insulin sensitivity in 17 obese women due to technical difficulties with iv lines. Insulin-mediated peripheral glucose uptake (Rₙ), which is a measure for skeletal muscle glucose uptake (21), was decreased (24.6 (11.5-42.5) μmol kg⁻¹ min⁻¹) in 89% of our subjects (i.e. Rₙ < 37.3 μmol kg⁻¹ min⁻¹ which is our recently reported cut off for normal insulin sensitivity) (22). Basal endogenous glucose production (EGP) was 13.7 [10.3-18.1] μmol/kgFFM*min. Lipolysis measured with labeled glycerol was 3.01 [1.92-5.05] μmol/kg.min in the fasted state and 1.48 (0.76-3.31) μmol/kg.min during the first step of the hyperinsulinemic euglycemic clamp. The mean suppression of lipolysis, a measure for insulin sensitivity of adipose tissue, was 51.5% (12.8 – 67.1).

Adipose tissue mRNA expression of key metabolic genes associated with metabolic health

Downregulation of adipose GLUT4 in obesity is a hallmark of insulin resistance (23). Indeed, in the obese subjects expression of GLUT4 mRNA in SAT and VAT was significantly lower compared to the lean controls (figure 1 a) and correlated with insulin action in muscle SAT (r = 0.58; p = 0.014;) and VAT (r = 0.576; p 0.016). mRNA expression of leptin, a hormone involved in food intake and energy metabolism and known to be higher in obesity in relation to body weight (24) was significantly higher in both adipose compartments in the obese subjects (figure 1 b). Next we measured mRNA expression of adiponectin, an insulin sensitizing and anti-inflammatory adipokine that has been shown to be downregulated in insulin resistant obese people (25) and expression of peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor crucial for adipogenesis (26). Adiponectin was significantly lower in SAT in the obese subjects (figure 1c) while expression of PPARγ was similar in both groups (figure 1d). These data show distinct features associated with obesity and insulin resistance in adipose tissue of the obese subjects.
Inflammatory profiles in SAT and VAT in obese versus lean subjects

First we measured circulating CRP as a measure of whole body low grade inflammation and found CRP to be increased in the obese subjects (obese 9489±6144 ng/mL and lean 1419±521 ng/mL; p < 0.001). To assess the inflammatory phenotype of adipose tissue we measured mRNA expression levels of several pro- and anti-inflammatory markers. Expression of the macrophage marker (CD68) (figure 2a) was significantly increased in SAT in the obese subjects, while the pro-inflammatory chemokine (MIP1beta) (figure 2b), the macrophage marker mannose receptor (MR), which increased expression has been shown on alternative activated macrophages and the anti-inflammatory cytokine IL10 (figures 2c and 2d) were significantly increased in the obese subjects in both SAT and VAT. None of the inflammatory markers correlated with insulin action in muscle or adipose tissue.
INFLUX OF MACROPHAGES AND T CELLS IN VISCERAL AND SUBCUTANEOUS ADIPOSE TISSUE OF MORBIDLY OBESE WOMEN IS NOT ASSOCIATED WITH INSULIN SENSITIVITY

Figure 2. Relative mRNA expression levels of CD68 (a), MIP1beta (b), Mannose Receptor (MR) (c) and IL10 (d) in subcutaneous (SAT) and visceral (VAT) adipose tissue in obese (N=20) and lean (N=6) subjects (* p < 0.05 and # p < 0.001). Data are presented as mean ± SD.

Inflammatory profiles of adipose tissue macrophages (ATM)

To further analyze the inter-compartment differences in inflammatory state of the SAT and VAT resident macrophages in the obese subjects, we isolated CD11b⁺ macrophages and measured expression levels of M1 and M2 markers. IL1b and IL18 are produced by M1 activated macrophages, but were not differentially expressed between SAT and VAT (figure 3 a and b) while CD11c (figure 3 c) showed a higher and MIP1beta (figure 3 d) a lower gene expression in extracted macrophages from SAT. Expression of the anti-inflammatory marker CD163 (figure 3 e) was significantly higher in ATM in VAT, finally the M2 marker MR (figure 3 f) shows a trend towards higher expression in VAT (p=0.071). These data show a more M2-like phenotype in VAT and higher expression of the pro-inflammatory marker CD11c in SAT. ATM expression of M1 and M2 markers were not associated with insulin-mediated peripheral
glucose uptake or suppression of lipolysis, indicative for muscle and adipose tissue insulin sensitivity, respectively.

**Figure 3.** Relative mRNA expression levels of the M1 markers Il1b (a), IL18 (b), CD11c (c) and MIP1beta (d) and the M2 markers Mannose Receptor (MR) (e) and CD163 (f) in extracted macrophages from subcutaneous (SAT) and visceral (VAT) adipose tissue from 19 obese subjects (* p< 0.05 and # p < 0.001). CD11c in VAT, MR in SAT and CD163 in SAT: N=18. Data are presented as mean ± SD.
T-cell markers in SAT and VAT of obese and lean subjects

Since it has been shown recently that T-cells are involved in obesity-associated inflammation, we studied a marker of general T cell influx as well as markers of activated T cells in SAT and VAT by measuring gene expression levels of CD4, CD25 and FOXP3 respectively. CD4 was significantly higher in SAT in the obese subjects compared to the lean controls but it was not differentially expressed between AT compartments (figure 4a). CD25, a surface marker of activated T cells showed higher expression in both adipose compartments in the obese versus the controls (figure 4b) and additionally showed higher expression in VAT within the obese group (p=0.001). FOXP3, a marker of regulatory T cells, expression was not different between lean and obese subjects or between compartments in both groups (data not shown). These data show an overall increased influx of CD4 positive T cells in SAT and VAT in obesity as well as an increase of activated T cells, the latter having the highest expression in obese VAT. Importantly, the expression levels of the different T cell markers were not correlated with insulin sensitivity of muscle or adipose tissue.

Discussion

In this study we show that in obese and insulin resistant women, subcutaneous and visceral adipose tissue is characterized by lower expression of GLUT4 as well as influx and activation of macrophages and activated T cells. Lower GLUT4 expression in adipose tissue was associated with insulin sensitivity while the inflammatory, T cell and macrophage markers were not correlated with peripheral glucose uptake, insulin-mediated suppression of lipolysis and endogenous glucose production, i.e. insulin action in muscle, adipose tissue and liver respectively. More detailed analyses of the expression levels of pro- and anti-inflammatory markers in CD11b+ enriched ATM isolated from the VAT and SAT compartments revealed that subcutaneous ATM display a predominant pro-inflammatory phenotype, whereas visceral
ATM are of a more anti-inflammatory nature. Finally, we show that the influx of activated T cells in adipose tissue in obese subjects is more pronounced in VAT which is in line with the presence of higher anti-inflammatory markers in that compartment since T cells play a pivotal role in reducing inflammation.

There is robust scientific evidence showing that obesity is associated with a state of low-grade inflammation and that inflammatory changes negatively influence insulin sensitivity and adipose tissue function (1, 8, 27, 28) although most studies showing this direct link have been performed in rodents. In line with other published data (29, for review 30), CRP was markedly elevated in the obese subjects compared to the matched lean controls. CRP is mainly produced by the liver, but adipose tissue also contributes to serum CRP levels (27). Whether circulating CRP has a direct impact on insulin sensitivity is unclear and in our cohort no correlation was present between insulin action and serum CRP (data not shown).

Many rodent studies showed a switch from an anti-inflammatory towards a pro-inflammatory environment in adipose tissue during the course of obesity (31, 32). The underlying mechanism explaining this change in phenotype entails multiple pathways including tissue hypoxia, ER-stress, adipocyte necrosis, altered adipokine secretion, upregulation of MCP-1 and abnormal extracellular matrix remodeling leading to fibrosis of adipose tissue (for review 33). In rodents, these inflammatory changes influence adipose tissue function with enhanced lipolysis leading to increased circulating fatty acids (34, 35). Ectopic uptake and accumulation of these fatty acids and their intermediates in insulin sensitive tissues such as liver and skeletal muscle and increased levels of circulating pro-inflammatory proteins (36) result in whole body insulin resistance. We here show that despite extensive inflammatory changes in both visceral and subcutaneous adipose tissue in obese compared to lean subjects no correlation with lipolysis or insulin sensitivity was present. It has been shown that insulin resistance in obesity is mainly predicted by the amount of visceral fat mass (37) and therefore inflammatory changes in that adipose tissue compartment could contribute to this association (38, 39). In addition, in upper body obese women lipolysis rates are higher compared to lower body obese women, suggesting an independent effect of adipose tissue compartment on insulin action in adipose tissue (40).

Furthermore, it has been shown in mice that intra-abdominal transplantation of subcutaneous adipose tissue reversed high fat diet induced inflammation and glucose intolerance, suggesting that subcutaneous adipose tissue protects from metabolic deterioration in the setting of obesity by reducing inflammation (41). Surprisingly, the obese subjects in our study showed a more anti-inflammatory profile with more M2 markers and higher expression of T cell markers in visceral adipose tissue suggesting that as long as inflammation in visceral adipose tissue is predominantly anti-inflammatory in nature, no effect on adipose tissue or muscle insulin sensitivity occurs. On the other hand, lipolysis was assessed as whole body total triglyceride lipolysis and which makes it impossible to distinguish between subcutaneous and visceral adipose tissue lipolysis and, therefore, we cannot exclude a
direct effect of visceral adipose tissue inflammation on visceral adipose tissue lipolysis. Insulin sensitivity of skeletal muscle, reflected as insulin-mediated glucose uptake, was not correlated to any of the inflammatory markers assessed in adipose tissue. This is in line with a more recent study showing that the presence of crown-like structures, i.e. macrophages surrounding necrotic adipocytes in inflamed adipose tissue, in mesenteric but not omental or subcutaneous adipose tissue predicted insulin resistance. However these were elderly men with vascular disease and insulin sensitivity was measured using HOMA index (42).

In the obese subjects, both adipose tissue compartments showed influx of macrophages and activated T cells suggesting that attraction of immune cells into adipose tissue in obesity is not adipose tissue depot-specific. However, activation of the invaded immune cells showed a depot specific difference. Surprisingly, visceral adipose tissue showed less pro-inflammatory features compared to the subcutaneous compartment. Studies on differences in inflammation between VAT and SAT show conflicting results. Secretome analyses in pre-adipocytes from VAT compared to SAT from obese subjects revealed VAT pre-adipocytes having higher chemo-attractant properties (43) but the intracellular protein differences in between compartments were less prominent prominent suggesting an overlap in expression of intracellular inflammatory proteins between AT compartments. Another recent study in elderly men with aortic aneurysm and a broader range in BMI, showed that CD68 was lowest in SAT and that expression of many inflammatory proteins was similar for omental and subcutaneous adipose tissue except for IL-18 and MIF which were higher in omental AT (42). However the current study subjects were female, younger on average and without vascular disease. The more prominent pro-inflammatory profile in SAT in the obese subjects could be explained by lower adiponectin since it has been shown that adiponectin has anti-inflammatory properties (44) and adipose tissue expression levels are correlated to circulating CRP (45). Finally the higher expression levels of markers of activated T cells in VAT might explain the anti-inflammatory phenotype in that compartment because T cells exert anti-inflammatory actions (46). We did not perform FACS analyses, which would have allowed us to quantify the activated T cell regulatory fraction of CD4 positive cells. Future studies are needed to study the potential protective role of T regulatory cells in adipose tissue inflammation and insulin resistance. Furthermore, a future therapy might be directed towards blunting the M1 response without affecting M2 polarization by targeting specific molecules. Additional translational studies from rodents to humans are needed since their inflammatory responses may be substantially different. Moreover, it has yet to be established whether inhibition of the inflammatory response contributes to improvements in glucose homeostasis in humans.

Since our data were analyzed in a cross-sectional manner, we cannot draw conclusions on how changes in degree and type of inflammation over time during further weight gain or weight loss would affect metabolic health. Thus, follow up studies are needed to elucidate whether modulation of specific inflammatory markers or profiles over time predict changes in metabolic health.
We conclude that SAT and VAT of morbidly obese and insulin resistant women is characterized by influx of macrophages and T cells and that VAT is less pro-inflammatory compared to SAT. The latter might be explained by lower expression of adiponectin in SAT and the presence of higher anti-inflammatory T cells in VAT. Despite the presence of inflamed adipose tissue in these women, none of the inflammatory markers in adipose tissue correlated with insulin action in muscle, liver or adipose tissue.

**Acknowledgements**

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**References**


**Supplemental data**

The following primers were used: **CD68** forward primer 5'-GCTGGCTGTGCTTTTCTCG-3'; reverse primer 5'-GTCACCGTGAAGGATGGCA-3' (NM_001251; 197–307); **MR** forward primer 5'-TGCAGAAGCAAACAAAACT-3'; reverse primer 5'-CAGGCCCTTAAGCCAACGAAACT-3'; **MIP-1β/CCL4** forward primer 5'-GCGTGACTG-TCTGCTCTCCTC-3'; reverse primer 5'-ACCACAAAGTTGCGAGGAAGC-3'; human **GLUT4** forward primer 5'-TGCTTCACTGCAAGCTGT-3'; reverse primer 5'-TAACATGGTGAAACCGCGTA-3'; Human CD4 forward primer 5'-CAGATCAAGAGACTCCTCAGTG-3'; reverse primer 5'-GCCTGCTGTGCTCAAATGG-3'; human **CD25** forward primer 5'-CCTGGGACAACCAATGTCAAT-3'; reverse primer TTCTTCAGGTAGGTGTAAC-3'; human **ITGAX/CD11c** forward primer, 5'-GGAGAAAACGTTCTTGCT-3'; reverse primer 5'-GTCAAGTCTTCTGGGAACA-3'; **IL10** forward primer 5'-TGCCCTCAGCAGATGAAGACTT-3'; reverse primer 5'-TCCTCCAGCAAGGACTCTTC-3' (NM_000572; 197–277); **CD163** forward primer 5'-ACATAGACATGATCCTGTCCTTTG-3'; reverse primer 5'-ATTTCCCTGGAATCTCACTT-3'; Human **PPARy** forward primer 5'-GCTGTGCAGGAGATCACAGA-3'; reverse primer 5'-GGGCTCCATAAAGTCAACAA-3'; human **adiponectin** forward primer 5'-AGTTGACCAGGTGTGATGG-3'; reverse 5'-AAGGTCACTAGATGGCAGC-3'; **leptine** forward primer 5'-AAGGTCACTAGATGGCAGC-3'; reverse 5'-TTGGCT CACTGTACTCCG-3'; **IL-1β** forward primer 5'-AGCAAAAGCTTGTGATGTCT-3'; reverse 5'-GGACATGGAACACCACCTTGT-3'; **IL18** forward primer 5'-CCAAGGAAATCGGCTCTATT-3'; reverse 5'-CTTCACAGAGATGTTACAGCCACACT-3'.