**T-lymphocyte Infiltration in Visceral Adipose Tissue**

**A Primary Event in Adipose Tissue Inflammation and the Development of Obesity-Mediated Insulin Resistance**

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**Background**—Adipose tissue inflammation may play a critical role in the pathogenesis of insulin resistance (IR). The present study examined the role of lymphocytes in adipose tissue inflammation and IR.

**Methods and Results**—In a mouse model of obesity-mediated IR, high-fat diet (HFD) induced IR already after 5 weeks, which was associated with a marked T-lymphocyte infiltration in visceral adipose tissue. In contrast, recruitment of macrophages was delayed with an increase of MAC3-positive staining and F4/80 mRNA expression after 10 weeks of HFD, suggesting a dissociation of macrophage invasion into adipose tissue and IR initiation. In patients with type 2 diabetes, lymphocyte content in adipose tissue biopsies significantly correlated with waist circumference, a marker of IR. Immunohistochemical staining of human adipose tissue revealed the presence of mainly CD4-positive lymphocytes as well as macrophage infiltration. Most macrophages were HLA-DR–positive, reflecting activation through IFNγ, a cytokine released from CD4-positive lymphocytes.

**Conclusions**—Proinflammatory T-lymphocytes are present in visceral adipose tissue and may contribute to local inflammatory cell activation before the appearance of macrophages, suggesting that these cells could play an important role in the initiation and perpetuation of adipose tissue inflammation as well as the development of IR. *(Arterioscler Thromb Vasc Biol. 2008;28:1304-1310)*

**Key Words:** inflammation ■ insulin resistance ■ monocytes ■ lymphocytes

Inflammation in adipose tissue contributes to the inflammatory state in obese and insulin-resistant patients and has been considered to represent a common soil for the development of both diabetes mellitus and arteriosclerosis. Previous studies have shown that macrophage infiltration is of critical importance in adipose tissue inflammation and the development of insulin resistance (IR). These cells are attracted by chemokines like monocyte chemoattractant protein-1 (MCP-1) and contribute to local inflammation through the release of inflammatory mediators such as tumor necrosis factor (TNF)α. In high-fat diet (HFD)-fed obese mice, it has been shown that infiltration of macrophages into adipose tissue coincides with the occurrence of hyperinsulinemia, an indirect measure of IR. The important role of adipose tissue macrophages in the pathogenesis of IR has further been supported by recent data in C-C motif chemokine receptor 2 (CCR2)-deficient mice. CCR2 regulates monocyte and macrophage recruitment into peripheral tissue via interaction with MCP-1. CCR2−/− mice exhibited a reduction in adipose tissue macrophage content which was associated with an improvement, but not complete restoration of systemic glucose homeostasis and insulin sensitivity, suggesting that other inflammatory cells may play a role in this context. Indeed, Wu et al demonstrated the presence of CD3-positive T-lymphocytes in human adipose tissue and described the expression of RANTES, a T-cell specific chemokine, and its respective receptor CCR5 in visceral adipose tissue of morbidly obese patients. Experimental data suggested that these...
T-lymphocytes may also play a role in the development of IR during obesity. However, the role of different subtypes of CD3-positive lymphocytes, namely CD4- and CD8-positive cells, in adipose tissue inflammation is largely unexplored. A recent study in mice reported mainly CD8-positive lymphocyte infiltration in hypoxic areas within the adipose tissue. Furthermore, the role of adipose T-lymphocytes as early contributors of obesity-mediated IR and their relationship to adipose tissue macrophage invasion has not yet been studied.

Some of the processes involved in adipose tissue inflammation resemble inflammatory processes in atherogenesis. Inflammation during arteriosclerotic lesion development is also characterized by endothelial cell activation as well as monocyte/macrophage and lymphocyte infiltration. These lymphocytes are mainly CD4-positive lymphocytes which express proinflammatory TH1-cytokines like IFNγ and orchestrate the inflammatory response in the vessel wall by activating other cells. To date, it remains unclear whether TH1-cytokines may play a similar role in adipose tissue inflammation.

Therefore, the current study examined whether T-lymphocyte infiltration may be a primary event in adipose tissue inflammation and obesity-mediated IR and analyzed the role of CD4-positive lymphocytes in human adipose tissue.

**Methods**

**Animal Model**

Male C57BL/6J mice, 4 to 5 weeks of age, were purchased from Harlan Winkelmann (Borchen, Germany). All mice were housed in a temperature controlled (25°C) facility with a 12-hour light/dark cycle. Mice were fed with a HFD (60% kcal from fat; Altromin) for 10 weeks. Mice on a low-fat diet (10% kcal from fat) served as controls. At baseline (week 0), 5, and 10 weeks on HFD animals were metabolically phenotyped including an intraperitoneal glucose tolerance test (ipGTT) using a dose of 1g/kg body weight insulin (Actrapid, Novo Nordisk) intraperitoneally. Tail vein blood was used for glucose quantification with a Glucometer (Precision Xtra, Abbott) during ipGTT and ITT. Afterwards, animals were euthanized and organs were dissected at week 0, week 5, and week 10. All animal procedures were in accordance with institutional guidelines and were approved.

**Adipose Tissue Biopsies**

Subcutaneous adipose tissue biopsies were taken from 54 patients with type 2 diabetes mellitus after written informed consent. Patient characteristics are shown in Table 1 (see supplemental data online at atvb.ahajournals.org). The study was approved by the local ethic committee. RNA was isolated using a standard procedure, and qRT-polymerase chain reaction (PCR) was performed as described below.

**Statistical Analysis**

Results of the experimental studies are reported as mean±standard deviation (SD). Differences were analyzed by 1-way-ANOVA followed by the appropriate posthoc test. A probability value <0.05 was regarded as significant. Correlation analyses in the clinical study were performed using Pearson’s test.

For additional methods, please see the supplemental materials (available online at http://atvb.ahajournals.org).

**Results**

**Adipose Tissue T-Lymphocytes Infiltration Coincides With Early IR in Obese Mice**

To characterize the role of T-lymphocytes in the pathogenesis of obesity-mediated IR, we studied insulin/glucose metabolism and analyzed the presence of lymphocytes and macrophages in adipose tissue in HFD-fed obese mice. Male C57BL/6J mice were fed with a LFD or HFD, and after 10 weeks total body weight was 26.4±0.7 g and 30.4±2.3 g, respectively. HFD led to an increase in gonadal fat by 168% compared to LFD. Metabolic characterization, immunohistological analysis, and gene expression analysis was performed at baseline and after 5 and 10 weeks of diet. HFD-fed mice exhibited impaired glucose tolerance already after 5 weeks of diet (Figure 1A and 1B). Consistently, insulin sensitivity was reduced at this early time point (Figure 1C and 1D) indicating an early deterioration of insulin and glucose metabolism in this model.

In parallel to the early impairment of systemic IR, immunohistochemical staining of gonadal fat demonstrated that T-lymphocytes are already present in adipose tissue 5 weeks after initiation of high fat feeding (Figure 2A and 2B). Quantification of adipose tissue T-lymphocytes by analysis of CD3 mRNA expression using qRT-PCR confirmed an early lymphocyte infiltration into adipose tissue which was further enhanced after 10 weeks of HFD (Figure 2E). qRT-PCR of adipose tissue from mice fed a LFD for 10 weeks did not reveal an increase in CD3 mRNA expression. In accordance to the potential chemotactic role for SDF-1α in human adipose tissue, SDF-1α mRNA expression was significantly upregulated after 5 and 10 weeks of diet parallel to lymphocyte deposition (Figure 2F). qRT-PCR of adipose tissue from ob/ob mice also show increased CD3 mRNA expression compared to control mice (see supplemental Figure I). Taken together, these data demonstrate that the occurrence of IR and...
glucose intolerance in obese mice is closely associated with an early adipose tissue infiltration by T-lymphocytes. Accumulation of Macrophages in Adipose Tissue Occurs During Late Stages of IR

Next we examined the presence of macrophages in gonadal adipose tissue during the development of IR. Despite the presence of IR/glucose intolerance after 5 weeks of diet, immunohistological staining of fat tissue for the macrophage marker MAC3 was absent at this early time point and could only be detected after 10 weeks (Figure 3A through 3D). Consistently, mRNA expression levels of the macrophage marker F4/80 and the monocyte chemoattractant MCP-1 were low at 5 weeks and significantly increased after 10 weeks of diet (Figure 3F and 3G). LFD did not increase F4/80 expression after 10 weeks (Figure 3F). These data indicate that macrophage infiltration into adipose tissue occurs at late stages of obesity-mediated IR and is obviously dissociated from the initiation of an impaired insulin response in obese mice.

Adipose Tissue Lymphocyte Infiltration Correlates With Waist Circumference in Patients With Type 2 Diabetes Mellitus

Because our animal data suggested a potential role of adipose tissue lymphocyte infiltration during the development of IR, we next examined lymphocyte content in subcutaneous adipose tissue biopsies from 54 patients with type 2 diabetes (supplemental Table I) and its association to waist circumference as a marker of insulin resistance.8–11 Waist circumference significantly correlated with CD3 mRNA expression as well with the expression of IFNγ mRNA, a proinflammatory cytokine derived from lymphocytes (Figure 4A and 4B). Interestingly, in our population we did not find a significant correlation of waist circumference with the expression of the macrophage marker CD68 (Figure 4C). These data suggest that an association of IR in diabetic patients with adipose tissue lymphocyte infiltration.

Human Adipose Tissue Contains CD4-Positive Lymphocytes

To further characterize the lymphocyte population in human adipose tissue, we performed immunohistochemical staining of visceral adipose tissue specimens of 5 individuals. We found adipose tissue macrophages and CD4-positive lymphocytes in all subjects with localization in both the stroma as well as the vascular component of the adipose tissue. CD8-positive lymphocytes were not detectable by immunohistochemical staining, but using RT-PCR we have been able to detect moderate CD8 expression (data not shown), suggesting the presence of some CD8-positive cells within the adipose tissue. Staining of sections with isomatched IgG at similar concentrations showed no immunoreactivity, thus affirming the specificity of the detected signals (Figure 5A through 5I).

To further investigate whether CD4-positive lymphocytes within the adipose tissue may stimulate macrophages, we analyzed the expression of HLA-DR, a cell activation marker reflecting stimulation by the lymphocyte-derived TH1-cytokine IFNγ. As shown in Figure 5K and 5L, a subgroup of macrophages within the adipose tissue—in addition to other cells—expressed HLA-DR, suggesting that these cells could have been activated by IFNγ. Indeed, RT-PCR demonstrated IFNγ mRNA expression in fat samples of all 5 subjects, suggesting the presence of this mainly lymphocyte-derived TH1-cytokine in human visceral adipose tissue (data not shown).

Next we examined whether CD4-positive lymphocyte content in visceral adipose tissue correlates with body weight. We found a significant correlation (r=0.515; P=0.02) of BMI with CD4-positive staining in visceral adipose tissue of 19 patients (13 with type 2 diabetes, 6 patients without diabetes), suggesting that the number of CD4-positive lymphocytes increases with body weight (supplemental Figure II).
Expression of SDF-1α in Human Adipose Tissue

Given the presence of CD4-positive lymphocytes in visceral adipose tissue we next examined the expression of T-cell specific chemokines in human adipose tissue specimen. Immunohistochemical staining revealed the expression of SDF-1α protein (supplemental Figure III) and RANTES (data not shown) whereas other chemokines like IP-10, MIG, and I-TAC were not detectable (data not shown). Staining of parallel sections revealed SDF-1α expression in preadipocytes/adipocytes as well as in other cells like endothelial cells (supplemental Figure IIIA to IIIF). RT-PCR confirmed these data, showing the expression of SDF-1α mRNA in human visceral adipose tissue (data not shown).

To investigate whether preadipocytes or adipocytes within the visceral adipose tissue may be a source of SDF-1α, isolation of human preadipocytes and subsequent differentiation into adipocytes was performed. SDF-1α mRNA expression was analyzed during the time course of differentiation using qRT-PCR. As shown in supplemental Figure IIG, human preadipocytes express SDF-1α mRNA, and this expression is significantly downregulated during adipocyte differentiation to levels of 28±15% in differentiated compared to undifferentiated cells. Similarly, undifferentiated human SGBS preadipocytes express both SDF-1 mRNA (data not shown) and protein, whereas differentiation of these cells toward a more mature adipocyte cell type leads to a significant downregulation of SDF-1α release with a minimal expression after 6 days of differentiation (supplemental Figure IIIH).

To examine the functional relevance of SGBS preadipocyte-derived SDF-1α, isolated human CD4-positive lymphocytes were incubated with conditioned media from preadipocytes and ICAM3 translocation to the uropod was assessed as a read-out for migrating cells. Supernatants from SGBS preadipocytes contained SDF-1α (data not shown) and significantly enhanced ICAM3 translocation compared to control media indicating the induction of cell migration by these supernatants (supplemental Figure III I).

IFNγ Increases MCP-1 Secretion From SGBS Preadipocytes

Because lymphocyte infiltration of adipose tissue preceded the recruitment of macrophages in our animal model of IR and given the presence of lymphocyte-derived IFNγ in human adipose tissue, we next asked whether IFNγ may influence the production/secretion of MCP-1 and SDF1α in SGBS preadipocytes. Treatment of SGBS cells with IFNγ significantly increased the release of the monocyte chemotractant MCP-1 from 2259±1051 ng/mL to 6542±3150 ng/mL (P<0.01; n=7) (supplemental Figure IV A), but did not affect secretion of the T-cell chemokine SDF-1α (1703±774 ng/mL in control cells versus 1503±1070 ng/mL in IFNγ-stimulated cells; n=8; P=n.s.; supplemental Figure IVB). Similar data were obtained after IFNγ-stimulation of isolated human adipocytes. IFNγ significantly increased MCP-1 mRNA expression but had no effect on the mRNA expression of SDF-1α (supplemental Figure IVC and IVD). These data together with our animal studies raise the hypothesis that lymphocytes once attracted into the adipose tissue may stimulate preadipocytes to release MCP-1, thus facilitating the attraction of monocytes.

Discussion

The present study demonstrates an early lymphocyte infiltration during the development of IR in a mouse model of HFD-induced obesity. In addition, our data show a correlation of adipose tissue lymphocyte content with waist circumference in patients with type 2 diabetes, and suggest the...
presence of activated proinflammatory CD4-positive lymphocytes in human adipose tissue. Early work has already indicated the presence of CD3-positive T-lymphocytes in human adipose tissue, and more recent studies showed high numbers of T-cells in adipose tissue of diet-induced obese insulin-resistant mice. Still, others reported that they did not find an increase in adipose tissue lymphocyte content in a different mouse model, but experimental differences could account for this discrepancy. Our study extends the knowledge on the role of these cells by demonstrating that T-lymphocyte infiltration into the adipose tissue precedes the recruitment of macrophages during the development of IR. In our mouse model of IR, 5 weeks of HFD significantly reduced insulin sensitivity as demonstrated in insulin tolerance tests. Both immunohistochemical staining as well as qRT-PCR analyses demonstrated the presence of CD3-positive lymphocytes in visceral adipose tissue at this time point, whereas macrophages were not detectable after 5 weeks of diet. These cells could be found in the adipose tissue after 10 weeks of diet, suggesting an early infiltration of lymphocytes followed by the recruitment of monocyte/macrophages. The early presence of T-lymphocytes in visceral adipose tissue at the time of manifest IR raises the hypothesis that these cells may orchestrate the inflammatory process in adipose tissue with subsequent development of IR. Interestingly, CD3 mRNA expression in adipose tissue biopsies from patients with type 2 diabetes mellitus significantly correlated with waist circumference, suggesting an association of IR in diabetic patients with adipose tissue lymphocyte infiltration. Moreover, immunohistochemical staining of human visceral adipose tissue as well as RT-PCR characterized these lymphocytes suggesting that most of these cells are CD4-positive with only a few CD8-positive cells. Interestingly, the number of CD4-positive lymphocytes in visceral adipose tissue correlated with BMI, suggesting that adipose tissue infiltration of these cells increases with body weight. CD4-positive lymphocytes can either differentiate to TH1-cells, releasing proinflammatory cytokines like IFNγ and TNFα, or to antiinflammatory TH2-cells with the expression of interleukin (IL)-10 or IL-4. Despite some reports on IFNγ expression in other cells like macrophages and dendritic cells, T-lymphocytes are thought to be the major source of this cytokine. Therefore, the detection of IFNγ mRNA in our study suggests the presence of proinflammatory TH-1 cells in human visceral adipose tissue. Moreover, IFNγ is functionally active within the adipose tissue as shown by the expression of HLA-DR, an indicator of IFNγ-mediated cell activation. HLA-DR positive cells are mostly macrophages, suggesting an inflammatory activation of these cells by T-lymphocytes, as previously shown in atherogenesis. The fact that the visceral adipose tissue biopsies used in our study stem from the omentum of colon cancer patients may represent a potential confounder. Still, the increase of CD4-positive cell infiltration with BMI and the fact that biopsies were taken from areas distal of the tumor make this potential confounder an unlikely explanation for our findings.

T-cell recruitment to sites of inflammation is usually mediated by chemokines released from endothelial cells, stromal cells, or macrophages. In preadipocytes and adipocytes, several groups have shown the expression of T-cell specific chemokines like RANTES, MCP-1, or
IP-10, 17, 18 We have also been able to detect RANTES in human adipose tissue, but additionally demonstrate the expression of SDF-1α, another chemotactic protein for lymphocytes. 19 Immunohistochemical data suggest that SDF-1 within the adipose tissue stems from both endothelial cells as well as from preadipocytes/adipocytes. In our mouse model of HFD-induced IR adipose tissue SDF-1α expression is paralleled by the early recruitment of CD3-positive T-lymphocytes, and our in vitro data in human adipocytes and cells of the SGBS adipose cell line show a downregulation of SDF-1α during adipocyte differentiation. Future work is warranted to examine whether a longer duration of high-fat diet may also lead to a decrease in SDF-1α expression attributable to adipocyte differentiation. Interestingly, stimulation of SGBS preadipocytes with IFNγ induced the release of MCP-1, a chemokine known to be critical for monocyte recruitment into the adipose tissue. These data suggest a differential expression of various chemokines during differentiation and activation of preadipocytes/adipocytes. Recent work suggests that conditioned media from adipocytes may contribute to angiogenesis,20 and as such an increase in the number of endothelial cells could lead to an enhanced release of SDF-1α. However, future studies are needed to determine to what extent the expression of SDF-1α from endothelial cells or preadipocytes/adipocytes contributes to lymphocyte recruitment.

Our study raises the hypothesis that T-lymphocytes may be recruited during early adipose tissue inflammation by preadipocyte/adipocyte-derived chemokines like SDF-1α, and that these cells may then govern the local inflammatory process through the release of proinflammatory TH1 cytokines such as IFNγ. On the one hand, T-cell–derived IFNγ could promote the recruitment of monocytes by inducing MCP-1 secretion from preadipocytes, and on the other hand it could activate other cells like macrophages. Recently, functional differences between distinct adipose tissue macrophage populations have been described:21 The “classically” activated M1 class, present in adipose tissue from obese insulin-resistant mice, exerts mainly proinflammatory actions; and the “alternatively” activated M2 class which has been found in fat from insulin sensitive, lean mice, and which have high levels of antiinflammatory cytokines. Future studies are needed to further characterize the effect of T-lymphocyte–derived cytokines on adipose tissue macrophage differentiation during the development of IR.

In summary the present study identifies the deposition of activated CD4-positive lymphocytes in human adipose tissue as well as a primary T-lymphocyte infiltration during the development of IR in a mouse model of HFD-induced obesity. The association of an early T-lymphocyte occurrence in adipose tissue and the parallel initiation of IR in diet-induced obesity suggest a potential pathophysiological role of this cell type in the development of HFD-mediated IR, but future studies are warranted to prove this hypothesis.

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Disclosures
None.

References
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Methods

Immunohistochemical staining

Serial Cryostat sections of human visceral fat of patients who underwent elective abdominal surgery were cut, airdried onto microscope slides, and fixed in acetone at -20°C for 5 minutes. Standard immunohistochemical staining was performed with the following antibodies: mouse anti-human CD4 antibody (Dako), mouse anti-human CD8 antibody (Dako), mouse anti-human CD68 antibody (Dako), anti-HLA-DR antigen beta chain antibody (Dako), goat anti-human SDF1 antibody (Abcam). For immunohistochemical studies in mice, epididymal adipose tissue was fixed in 4% formalin, embedded in paraffin and stained with the corresponding antibodies. The following antibodies were used: Lymphocytes were identified by staining with an anti-mouse CD3 antibody (Serotec, MCA 1477) and macrophages were detected by an anti-mouse MAC3 antibody (BD).

Visceral adipose tissue biopsies

To assess the correlation of adipose tissue CD4-positive lymphocyte content with BMI, we obtained visceral adipose tissue from 19 patients (13 patients with diabetes, 6 patients without diabetes) who underwent elective abdominal surgery. Tissue sampling was approved by the local ethic committee of the University of Ulm. Immunohistochemical staining was performed as described above. Computer-assisted image analysis was used to quantify staining on sections using Image-Pro Plus software (Mediacybernetics, Silver Spring, MD).

Isolation and culture of human preadipocytes

Stromal cells from human adipose tissue were prepared and cultured as described previously. Briefly, after removing all fibrous material and visible blood vessels, adipose tissue samples were cut into small pieces and digested in phosphate buffered saline (PBS) containing 200 U/ml crude collagenase and 20 mg/ml bovine serum albumin, pH 7.4, for 90 min. Stromal cells were sedimented, treated with an erythrocyte lyzing buffer (154 mmol/l NH4Cl, 5.7 mmol/l K2HPO4, and 0.1 mmol/l EDTA) for 10 min and filtered twice through meshes with a pore size of 150 µm and 70 µm. Afterwards sedimentation cells were resuspended in Dulbecco’s Modified Eagle’s/Ham’s F-12 (DMEM/F-12) medium (w/w, 1:1) supplemented with 10 % fetal calf serum and inoculated into culture dishes at a density of 5 x 10⁵ cells/cm². They were cultured with a proliferation mixture containing 10 ng/ml EGF, 1 ng/ml bFGF and 8.7 µm insulin, and 2.5% FBS. After confluence, medium was changed either to an adipogenic cocktail containing 0.5 mM IBMX and 2 µM rosiglitazone for 72 hrs. or cultured without these supplements to obtain undifferentiated cells.
**Cell culture**

SGBS preadipocytes were cultured and differentiated as described before\(^8\). In some experiments SGBS preadipocytes were grown to confluence in 6-well plates, stimulated with IFN\(\gamma\) for 72 h before SDF-1\(\alpha\) and MCP-1 protein content in cell-free supernatants was assessed by ELISA (R&D Systems).

Human CD4-positive lymphocytes were isolated and cultured as described before\(^9\).

To evaluate the effect of preadipocyte supernatants on lymphocyte migration, conditioned media was lyophilized and resuspended in lymphocyte media. Media was then added to human CD4-positive lymphocytes and ICAM3 translocation at the uropod of the cells was determined by immunofluorescence staining after 3 hours.

**RT-PCR and qRT-PCR**

Total RNA from human subcutaneous adipose tissue was isolated using a column-based RNA isolation kit (Qiagen). Equal amount of total RNA was reverse-transcribed into cDNA at 37°C for 1 h. Amplification of CD3\(\gamma\) cDNA used the following primers: forward: 5’-TCATTGCTGGACAGGATGGA-3’; reverse: 5’-GGGCTGGTAGAGCTGGTCATT-3’; CD68: forward 5’-GAACCCCAACAAAAACCAAG-3’; reverse: 5’-GATGAGAGGCAGCAAGATG-3’; Interferon gamma: assay-on-demand from Applied Biosystems: Hs00174143_m1. Real-time PCR of mouse adipose tissue was performed as previously described using an ABI 7000 and Stratagene 3000 MXP PCR cycler with either the SybrGreen or FAM-TAMRA detection system\(^6\). Real-time PCR for CD3, SDF1\(\alpha\), F4/80, and MCP-1 was performed by using assays-on-demand from Applied Biosystems. Quantification of SDF-1\(\alpha\) mRNA expression of human preadipocytes was performed by real-time PCR in optical twin.tec 96-well microtiter plates (Eppendorf, Hamburg, Germany) using the mastercycler ep realplex\(^4\) system (Eppendorf). And the following primers: (SDF-1 forward: 5’-TCTCAAAATTCTCAACACTCCA AACT-3’; SDF-1 reverse: 5’-GCACACTTGCTGTGTGTGTGTCTTC-3’)]. MCP-1 PCR was performed using a TaqMan ® Gene Expression (# Hs00234140_m1) and target sequences were normalized for GAP-DH expression (# Hs 99999905_m1) (ABI).
# Table I: Characteristics of patients

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<td>Age (mean; min / max)</td>
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<tr>
<td>Gender (m / f)</td>
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<tr>
<td>BMI (mean; min / max)</td>
<td>28.9 (22 / 48) kg / m²</td>
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<tr>
<td>Waist circumference (mean; min / max)</td>
<td>102.6 (82 / 136) cm</td>
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<tr>
<td>HbA1c (mean; min / max)</td>
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## Medication:

<table>
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<th>Medication</th>
<th>Count (% of total)</th>
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<td>ACE-inhibitor</td>
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<tr>
<td>Diuretics</td>
<td>29 (53.7%)</td>
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<tr>
<td>Calcium antagonist</td>
<td>13 (24.1%)</td>
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<tr>
<td>Beta-blocker</td>
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<tr>
<td>Metformin</td>
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<tr>
<td>Sulfonylurea</td>
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<td>Glinides</td>
<td>2 (3.7%)</td>
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<tr>
<td>Acarbose</td>
<td>3 (5.6%)</td>
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<tr>
<td>Statins</td>
<td>44 (81.5%)</td>
</tr>
<tr>
<td>ASS</td>
<td>43 (79.6%)</td>
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Figure I: qRT-PCR reaction of the lymphocyte marker CD3 in murine visceral adipose tissue from wild type mice and ob/ob mice. Bars represent mean ± SD; n=3.

Figure II: CD4-positive lymphocyte content in visceral adipose tissue correlates with body weight. Omental adipose tissue from 19 patients with elective abdominal surgery was stained for CD4-positive
cells. Immunohistochemical staining for CD4 was quantified using computer-assisted image analysis. Data are expressed as % positive staining of total section area.

**Figure III**

![Figure III](image_url)

Figure III: SDF-1α expression in human adipose tissue. A and B: Immunohistochemical staining for SDF-1α in human visceral adipose tissue (B: high power view); positive staining is indicated by an arrow. C: Negative control staining employed type and isomatched IgG antibodies. D. and E: Parallel sections stained for the endothelial marker CD31 (E: high power view). F: negative control. White
arrows indicate colocalisation of SDF1α and CD31. Similar results were obtained in adipose tissue biopsies of 5 different subjects. G: SDF-1α mRNA expression in human preadipocyte and adipocytes. Human preadipocytes were differentiated to adipocytes and RT-PCR for SDF-1α mRNA was performed at different time points. Dots represent mean±SEM compared to baseline; * p<0.05; ** p<0.01; n=3. H: SDF-1α protein secretion from human SGBS adipocytes. SGBS preadipocytes were differentiated to mature adipocytes and SDF-1α protein release was determined by ELISA in cell-free supernatants. Bars represent mean±SD; n=3; * p<0.05; ** p<0.01. I. Conditioned media from SGBS preadipocytes induces ICAM3 translocation in human CD4-positive lymphocytes. Supernatants from SGBS preadipocytes were collected after 72h, lyophilized and resuspended in lymphocyte media. Isolated human CD4-positive lymphocytes were then incubated with this media or control media for 30 min before ICAM3 translocation was assayed using immunofluorescence staining. ICAM3 translocation at the uropod of migrating cells is indicated by the arrow. Right panel shows statistical analysis of cells positive for ICAM3 translocation as % of DAPI-positive cells; data were normalized to control. Bars represent mean±SD; n=4; * p<0.05 compared to cells incubated with control media.

Figure IV
Figure IV: IFNγ induces MCP-1 secretion from SGBS preadipocytes. SGBS preadipocytes were stimulated with IFNγ (1000 U/mL) for 72 h. MCP-1 (A) and SDF-1α (B) protein content was measured in cell free supernatants using ELISA. Bars represent mean±SD; n=7 (MCP-1); n=8 (SDF-1a); * p<0.05 compared to unstimulated cells. C and D: Isolated human preadipocytes were stimulated with IFNγ (1000 U/mL) and MCP-1 as well as SDF-1 mRNA expression was assessed by RT-PCR. Bars represent mean±SD; n=3; * p<0.05