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# Vascular Endothelial Growth Factor Is Induced by the Inflammatory Cytokines Interleukin-6 and Oncostatin M in Human Adipose Tissue In Vitro and in Murine Adipose Tissue In Vivo

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**Objectives**—It is believed that adipose tissue acts as an endocrine organ by producing inflammatory mediators and thereby contributes to the increased cardiovascular risk seen in obesity. A link between adipose tissue mass and angiogenesis has been suggested. Vascular endothelial growth factor (VEGF) seems to be implicated in this process. Members of the glycoprotein (gp)130 ligand family regulate VEGF expression in other cells.

**Methods and Results**—We used tissue explants as well as primary cultures of preadipocytes and adipocytes from human subcutaneous and visceral adipose tissue to investigate whether the gp130 ligands oncostatin M (OSM), interleukin-6 (IL-6), leukemia inhibitory factor (LIF), and cardiotrophin-1 (CT-1) regulate VEGF expression in human adipose tissue. Human subcutaneous and visceral adipose tissue responded to treatment with IL-6 and OSM with a significant increase in VEGF production. Human preadipocytes were isolated from subcutaneous and visceral adipose tissue. Adipocyte-differentiation was induced by hormone-supplementation. All cell types responded to IL-6 and OSM with a robust increase in VEGF protein production and a similar increase in VEGF-specific mRNA. Furthermore, IL-1 $\beta$  synergistically enhanced the effect of OSM on VEGF production. AG-490, a JAK/STAT inhibitor, abolished the OSM-dependent VEGF induction almost completely. In mice, IL-6 and OSM increased serum levels of VEGF and VEGF mRNA and vessel density in adipose tissue.

**Conclusion**—We speculate that the inflammatory cytokines IL-6 and OSM might support angiogenesis during adipose tissue growth by upregulating VEGF. (*Arterioscler Thromb Vasc Biol.* 2007;27:1587-1595.)

**Key Words:** obesity ■ angiogenesis ■ inflammation ■ cytokines

Obesity is the primary metabolic disorder in Western industrialized countries. Obese patients are at higher risk of developing cardiovascular diseases, and several studies suggest obesity as an independent risk factor. Obesity is characterized by an increased adipose tissue mass with increased size and number of mature adipocytes. Adipose tissue—besides its role in energy storage—is now also seen as an endocrine organ that produces and secretes a variety of cytokines, hormones, and other proteins such as interleukin (IL)-6, IL-8, leptin, resistin, and plasminogen activator inhibitor (PAI)-1.<sup>1</sup> Elevated plasma levels of several adipose tissue-derived factors, also called adipokines, such as IL-6 and PAI-1 are found in obese patients and are associated with the development and progression of cardiovascular diseases.<sup>2,3</sup> It should be noted that extensive vascularization is

required for adipose tissue to act as an endocrine organ efficiently. Thus a complex relationship between adipose tissue growth, vascularization, and adipokines and thus possibly also cardiovascular diseases seems to exist. In fact, a recent study found that adipose tissue mass could be regulated through the vasculature.<sup>4</sup> Angiogenesis—the formation of new blood vessels from preexisting vessels—provides efficient supply with nutrients and oxygen and thus is crucial for tissue development and growth. In the case of adipose tissue, also differentiation of preadipocytes into adipocytes seems to be dependent on new blood vessel formation.<sup>5</sup> Treatment with different angiogenesis inhibitors resulted in reversible weight reduction and adipose tissue loss.<sup>4,6,7</sup> Vascular endothelial growth factor (VEGF), a key agonist of angiogenesis and major permeability factor, is expressed and

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From the Departments of Internal Medicine II (G.R., C.K., S.D., S.P., K.R., P.J.H., S.P.K., W.S.S., T.W.W., G.M., J.W.) and Surgery (M.F.), Medical University Vienna; Ludwig Boltzmann Cluster for Cardiovascular Research (S.P.K., J.W.), Vienna; the Department of Vascular Biology and Thrombosis Research (J.M.B., P.U., J.Z.), Medical University Vienna; and the 2nd Department of Surgery (R.R.) and the 3rd Medical Department for Cardiology and Emergency Medicine (A.F., K.H.), Wilhelminenhospital, Vienna, Austria; and the Department of Cell Biology (V.Z.), Institute Cochin, Paris, France.

Correspondence to Johann Wojta, Department of Internal Medicine II, Medical University Vienna, Waehringer Guertel 18–20, A-1090 Vienna, Austria. E-mail johann.wojta@meduniwien.ac.at

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secreted by adipose tissue, preadipocytes, and adipocytes.<sup>8,9</sup> A recent article showed that overexpression of VEGF is associated with increased angiogenesis during rebound weight gain after restricted food intake.<sup>10</sup>

Members of the glycoprotein 130 (gp130) receptor/gp130 ligand family have been shown to play a direct and indirect role in angiogenesis in different tissues.<sup>11,12</sup> For example, IL-6 increases the expression of VEGF in myeloma cells, gastric carcinoma, epidermoid, and cervical cancer.<sup>12–15</sup> Another gp130 ligand, namely oncostatin M (OSM), mainly produced by activated T lymphocytes, monocytes, and macrophages and involved in the regulation of inflammation, tissue remodeling, and cell growth, upregulates VEGF in cardiac myocytes, astroglia cells, and smooth muscle cells as shown by us and others.<sup>16–18</sup> Furthermore we could demonstrate recently that human preadipocytes and adipocytes express receptors for gp130 ligands.<sup>19</sup>

In the present study we investigated the impact of the gp130 ligands OSM, IL-6, leukemia inhibitory factor (LIF), and cardiotrophin-1 (CT-1) on VEGF expression in human adipose tissue *in vitro* and *ex vivo*. These effects were studied in subcutaneous and visceral white adipose tissue and in the human brown adipose tissue derived cell-line PAZ6. Furthermore we determined possible effects of IL-6 and OSM on the expression of VEGF in adipose tissue in mice *in vivo*.

## Materials and Methods

Primary cultures of human visceral and subcutaneous preadipocytes, adipocytes, and *ex vivo* cultures were prepared from adipose tissue and characterized as described previously.<sup>19</sup> The human brown adipose tissue–derived cell line PAZ6 was treated under the same conditions as freshly isolated preadipocytes.<sup>20</sup>

Mice were injected with recombinant murine (rm) OSM or rm IL-6. Serum was stored at  $-80^{\circ}\text{C}$ . Organs were perfused with saline and epididymal and visceral adipose tissue were excised and stored in liquid nitrogen ( $\text{LN}_2$ ) or embedded in Tissue-Tek OCT Compound and stored at  $-20^{\circ}\text{C}$ .

VEGF was determined by specific enzyme-linked immunosorbent assays (ELISAs). Real-time-polymerase chain reaction (PCR) was performed using LightCycler-RNA Master SYBR Green I (Roche) according to the manufacturers instructions. Primers were designed using the LightCycler Probe Design Software Version 1.0 (Roche) and the Primer3 Software (<http://frodo.wi.mit.edu/>).

Mouse adipose tissue sections were stained with anti-CD31 goat IgG and with Alexa Fluor 546 donkey anti-goat IgG.

Data were compared statistically by ANOVA. Values of  $P < 0.05$  were considered significant.

For details please see supplemental data, available online at <http://atvb.ahajournals.org>.

## Results

### Effects of gp130 Ligands on VEGF Secretion by Ex Vivo Culture of Adipose Tissue

Human adipose tissue fragments of subcutaneous and visceral fat were incubated with the gp130 ligands OSM (100 ng/mL), IL-6 (100 ng/mL), LIF ( $10^4$  U/mL), and CT-1 (100 ng/mL), respectively, for 48 hours. In subcutaneous and visceral adipose tissue, OSM induced a significant increase of VEGF production up to 6.5-fold and 9-fold, respectively. IL-6 also significantly increased VEGF secretion up to 2.5-fold in subcutaneous and visceral fat. In both tissue types, LIF led

only to a minor increase of VEGF (1.5-fold, not significant), whereas CT-1 had no effect (Figure 1A and 1B).

### Effects of gp130 Ligands on VEGF Production by Preadipocytes and Adipocytes

Preadipocytes of subcutaneous and visceral adipose tissue were treated with OSM (100 ng/mL), IL-6 (100 ng/mL), LIF ( $10^4$  U/mL), and CT-1 (100 ng/mL), respectively, for 48 hours. VEGF secretion was significantly increased by OSM and IL-6 in both subcutaneous and visceral adipose cell cultures up to 6.5-fold and 7-fold (OSM) and up to 3-fold (IL-6). LIF significantly upregulated VEGF by 3-fold in subcutaneous and visceral preadipocytes. CT-1 led to a minor increase of VEGF production in visceral preadipocytes and had no effect in subcutaneous preadipocytes (Figure 1C and 1D).

We stimulated adipocytes of subcutaneous and visceral adipose tissue under the same conditions to investigate a possible cell-dependent effect within the adipose tissue. OSM and IL-6 induced a significant increase of VEGF secretion in subcutaneous adipocytes up to 4.5-fold and 2-fold, respectively, and up to 15.5-fold and 3.5-fold in visceral adipocytes. LIF led to a minor increase (1.5-fold) in adipocytes of both tissue types, whereas CT-1 induced VEGF production up to 3-fold in visceral adipocytes, but had no effect in subcutaneous adipocytes (Figure 1E and 1F).

### OSM and IL-1 $\beta$ Induce VEGF Synergistically

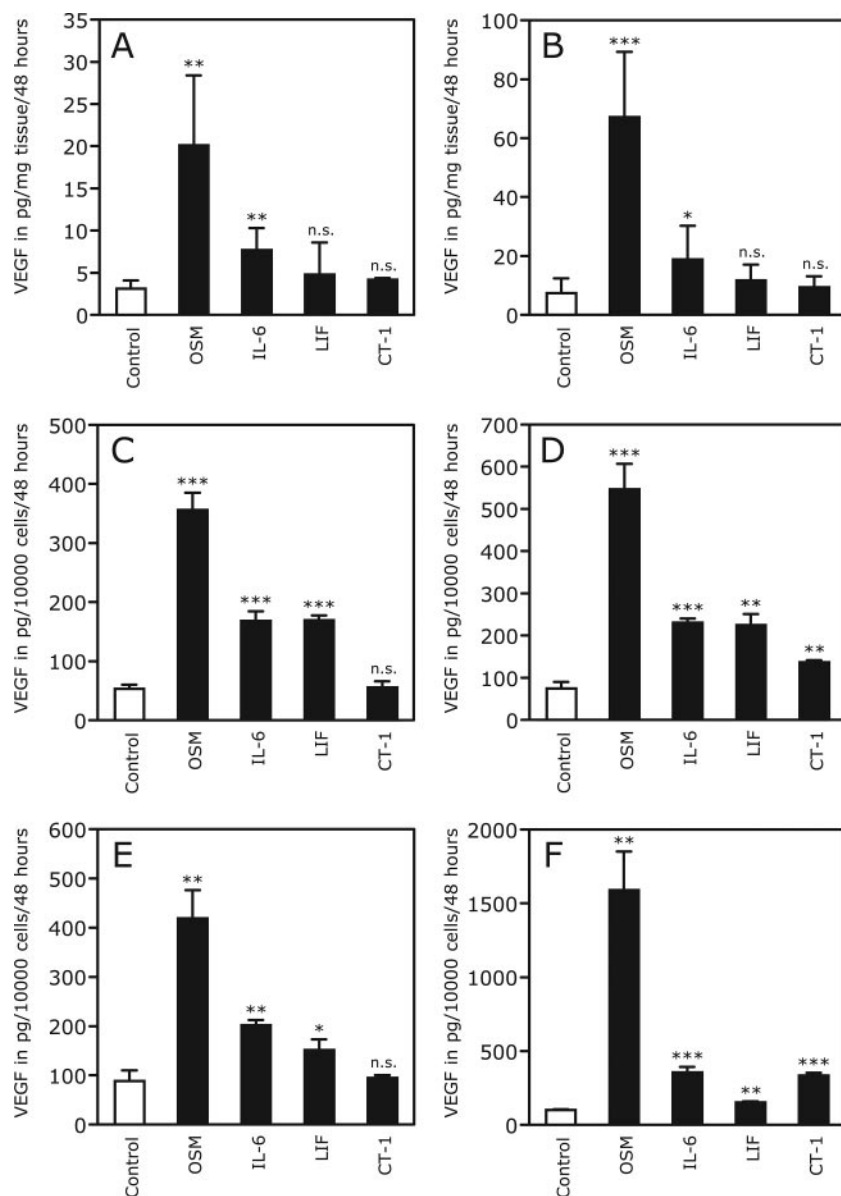
The inflammatory cytokine IL-1 $\beta$  is expressed in and secreted by human adipose tissue.<sup>21</sup> Combined treatment with OSM and IL-1 $\beta$  has been shown to synergistically upregulate VEGF release in astroglia cells and smooth muscle cells.<sup>17,18</sup> Visceral and subcutaneous preadipocytes, adipocytes, and adipose tissue explants were stimulated with OSM (100 or 10 ng/mL) and IL-1 $\beta$  (1 ng/mL) for 48 hours. IL-1 $\beta$  enhanced significantly OSM-induced VEGF secretion in all cell and tissue types (Figure 2A through 2F). These results were confirmed on the level of mRNA (supplemental Table I).

### Dose-Dependent Effect of OSM and IL-6

When preadipocytes and adipocytes of subcutaneous and visceral fat were treated with increasing concentrations of OSM (0.1 to 100 ng/mL) or IL-6 (0.1 to 100 ng/mL) for 48 hours, VEGF secretion was stimulated in a dose-dependent manner. The maximum response to OSM and IL-6 was observed at a concentration of 100 ng/mL, respectively (Figure 3A through 3F).

### Effects of gp130 Ligands on VEGF mRNA

Preadipocytes and adipocytes of visceral and subcutaneous adipose tissue were incubated with OSM (100 ng/mL), IL-6 (100 ng/mL), LIF ( $10^4$  U/mL), and CT-1 (100 ng/mL), respectively, for 8 hours. As shown in supplemental Table I, OSM and IL-6 induced a significant increase of VEGF-specific mRNA in all cell types. LIF upregulated VEGF mRNA in subcutaneous preadipocytes and adipocytes and in visceral preadipocytes, but had no effect in visceral adipocytes. VEGF mRNA was increased by CT-1 in subcutaneous



**Figure 1.** gp130 ligands increase VEGF production in human subcutaneous and visceral adipose tissue ex vivo and in preadipocytes and adipocytes in vitro. Pieces of human subcutaneous (A) and visceral (B) adipose tissue and human preadipocytes (C and D) and adipocytes (E and F) of subcutaneous (C and E) and visceral (D and F) adipose tissue were prepared and incubated for 48 hours as described under Materials and Methods in the absence or presence of rh OSM (100 ng/mL), rh IL-6 (100 ng/mL), rh LIF ( $10^4$  U/mL), or rh CT-1 (100 ng/mL), respectively. Conditioned media were collected and VEGF was determined as described under Materials and Methods. Values represent mean values  $\pm$  SD of 3 independent determinations. Experiments were performed 3 times with adipose tissue obtained from 3 different donors and 3 times for each cell type prepared from adipose tissue obtained from 3 different donors with similar results. A representative experiment is shown. \*\*\* $P < 0.0005$ , \*\* $P < 0.005$ , \* $P < 0.05$ ; n.s., not significant.

preadipocytes and visceral preadipocytes and adipocytes, but not in subcutaneous adipocytes.

#### Effects of gp130 ligands on VEGF Expression by PAZ-6 Preadipocytes and Adipocytes

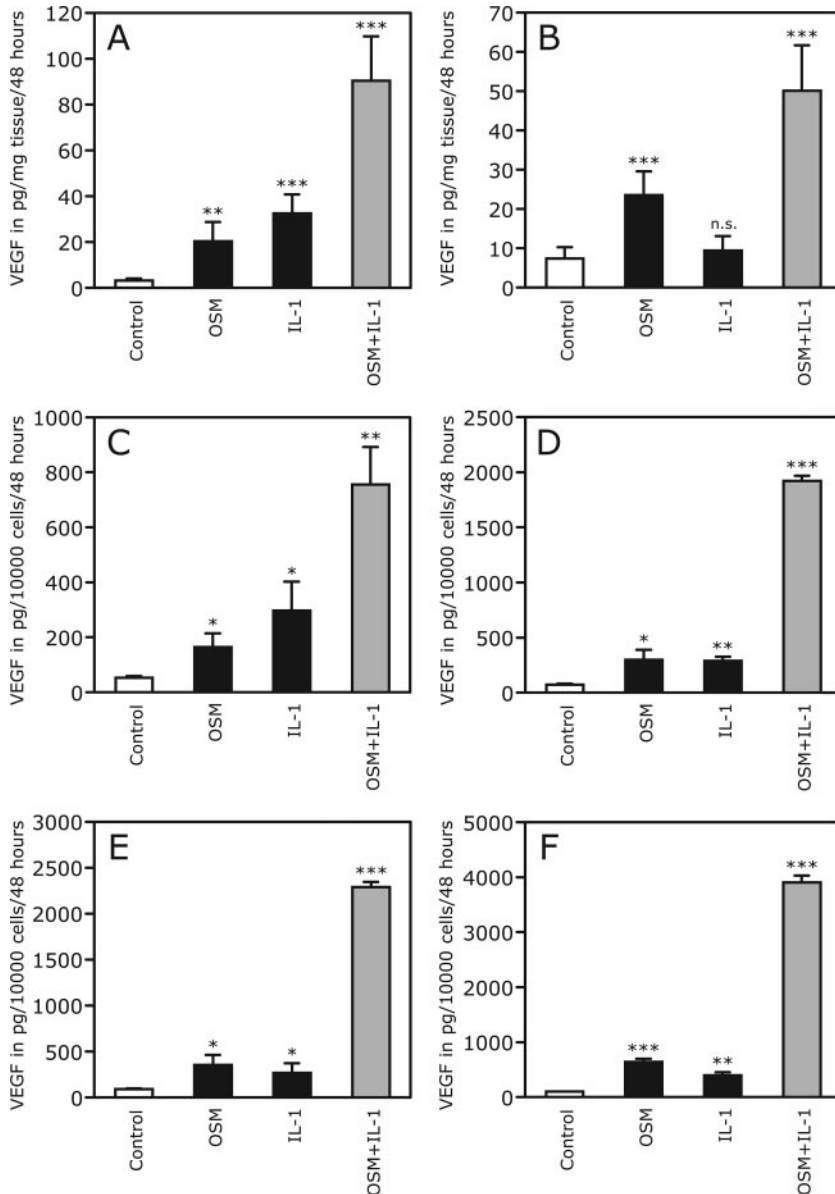
We stimulated preadipocytes and adipocytes of a human brown adipose tissue cell line, PAZ-6, with OSM (100 ng/mL), IL-6 (100 ng/mL), LIF ( $10^4$  U/mL), and CT-1 (100 ng/mL). OSM, IL-6, and CT-1 significantly increased VEGF secretion in both PAZ-6 preadipocytes and adipocytes up to 4-fold and 3.5-fold (OSM), up to 3.5-fold and 2-fold (IL-6), and up to 3-fold and 2-fold (CT-1). LIF led to a minor increase of VEGF in PAZ-6 preadipocytes (1.5-fold) and adipocytes (1.5-fold, n.s.; Figure 4A and 4B). The effect of OSM and IL-6 on VEGF secretion was dose-dependent with a maximum response at a concentration of 100 ng/mL, respectively (Figure 4C through 4F). All gp130 ligands induced a significant increase of VEGF mRNA in PAZ-6 preadipocytes and adipocytes, except for LIF in PAZ-6 adipocytes (supplemental Table I).

#### OSM Upregulates VEGF Secretion Independent of IL-6 in Adipocytes

To investigate a possible autocrine effect of IL-6 produced by adipocytes on OSM-induced VEGF production, subcutaneous adipocytes were stimulated with OSM (100 ng/mL) or IL-6 (100 ng/mL) for 24 hours in absence or presence of an IL-6 neutralizing polyclonal goat antibody (4  $\mu$ g/mL, R&D Systems). As shown in Figure 5A, increased VEGF secretion by OSM in subcutaneous adipocytes was not abolished by neutralizing IL-6 whereas increased secretion of VEGF by IL-6 was reduced significantly by this antibody.

#### OSM Increased VEGF Release in Human Adipose Tissue Cells via JAK/STAT Pathway

It is known that gp130-130 ligand interaction activates Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen activated protein kinase (MAPK) and the phosphatidylinositol 3 kinase (PI3K)-Akt pathways. We and others could show that OSM induction of VEGF is mediated



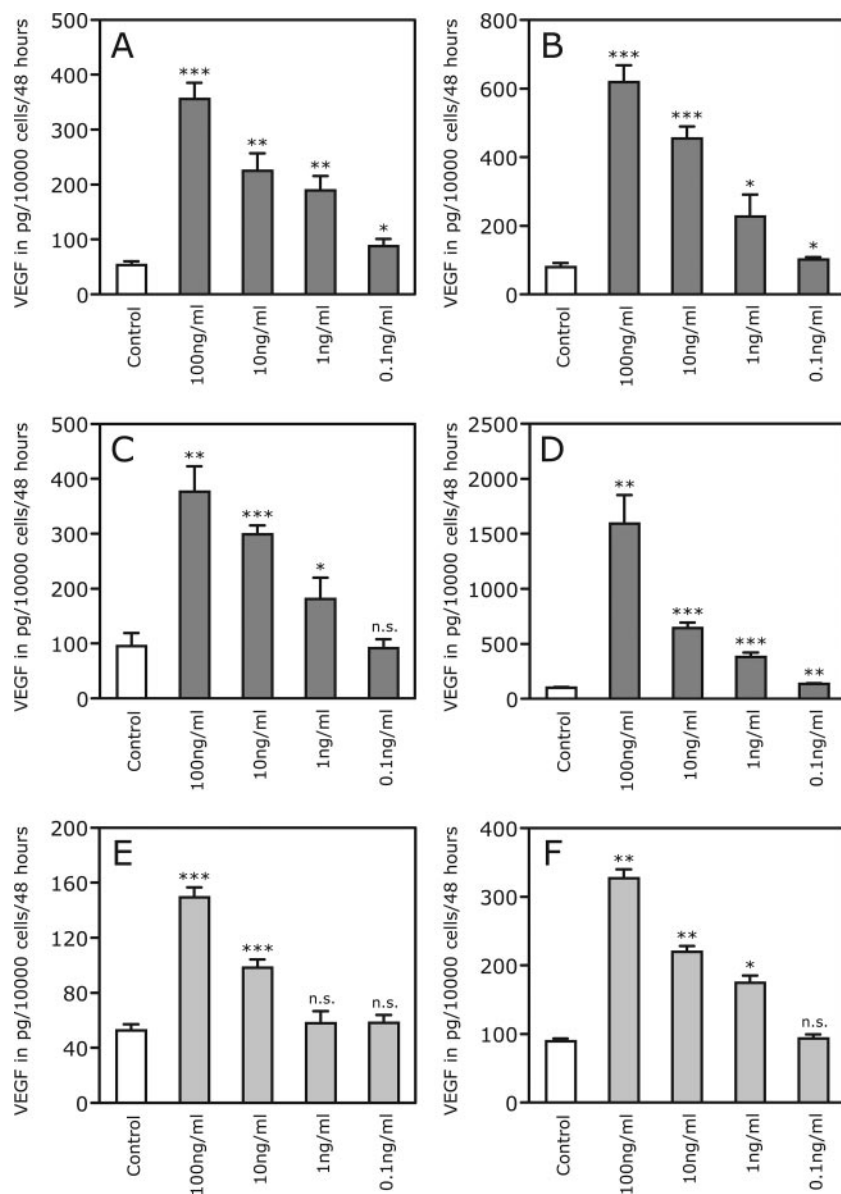
**Figure 2.** OSM and IL-1 $\beta$  induce VEGF synergistically in human subcutaneous and visceral adipose tissue ex vivo and in preadipocytes and adipocytes in vitro. Pieces of human subcutaneous (A) and visceral (B) adipose tissue and human preadipocytes (C and D) and adipocytes (E and F) of subcutaneous (C and E) and visceral (D and F) adipose tissue were prepared and incubated for 48 hours as described under Materials and Methods in the absence or presence of rh OSM (100 ng/mL in A and B or 10 ng/mL in C, D, E, and F), rh IL-1 $\beta$  (1 ng/mL), and a combination of both cytokines at the respective concentration. Conditioned media were collected and VEGF was determined as described under Materials and Methods. Values represent mean values  $\pm$  SD of 3 independent determinations. Experiments were performed 3 times with adipose tissue obtained from 3 different donors and 3 times for each cell type prepared from adipose tissue obtained from 3 different donors with similar results. A representative experiment is shown. \*\*\* $P$  < 0.0005, \*\* $P$  < 0.005, \* $P$  < 0.05; n.s., not significant.

by STAT-3 in cardiac myocytes, astroglia cells, and human airway smooth muscle cells.<sup>16–18</sup> To investigate which of these pathways was involved in OSM-induced VEGF expression in adipose tissue we preincubated visceral preadipocytes for 60 minutes with a JAK/STAT inhibitor, AG-490, with a PI3K inhibitor, LY294002, with a MEK-1 inhibitor, PD98059, and with an inhibitor of p38MAPK, SB202190 (all at 30  $\mu$ mol/L, Calbiochem/Merck). Thereafter medium was removed and cells were treated with fresh DMEM/F-12 in the presence or absence of OSM at a concentration of 100 ng/mL for 24 hours. The subsequent ELISA showed that OSM-mediated induction of VEGF was highly dependent on the JAK/STAT pathway. The OSM-mediated increase of VEGF was reduced by 78% on treatment with AG-490 in visceral preadipocytes whereas LY294002 reduced VEGF secretion by 27%, and PD98059 and SB202190 by 42% under these conditions (Figure 5C). No evidence of a toxic effect of the

inhibitors was observed during the incubation, as assessed by morphology and by measuring lactate dehydrogenase leakage as described previously.<sup>19</sup> Similar results were seen when subcutaneous adipocytes were treated with OSM and different inhibitors as described above (Figure 5B).

### VEGF mRNA in Adipose Tissue and VEGF Serum Levels Are Upregulated by OSM and IL-6 in Mice In Vivo

After intraperitoneal injection of OSM or IL-6 as described under Materials and Methods, a significant increase of VEGF mRNA levels in adipose tissue in these mice was observed. In epididymal adipose tissue VEGF mRNA levels increased up to 4.5-fold and 2.5-fold and in visceral adipose tissue an up to 2-fold and 1.5-fold increase was seen (Figure 6A and 6B). In addition, serum levels of VEGF in such treated mice were significantly increased as shown in Figure 6C.



**Figure 3.** OSM and IL-6 increase VEGF production in human subcutaneous and visceral preadipocytes and adipocytes dose-dependently. Human preadipocytes (A, B, and E) and adipocytes (C, D, and F) were prepared from subcutaneous (A, C, and E) and visceral (B, D, and F) adipose tissue as described under Materials and Methods and were incubated for 48 hours in the absence or presence of rh OSM (A-D) or rh IL-6 (E and F) at the indicated concentrations. Conditioned media were collected and VEGF was determined as described under Materials and Methods. Values represent mean values  $\pm$ SD of 3 independent determinations. Experiments were performed 3 times for each cell type prepared from adipose tissue obtained from 3 different donors, respectively, with similar results. A representative experiment is shown. \*\*\* $P < 0.0005$ , \*\* $P < 0.005$ , \* $P < 0.05$ ; n.s., not significant.

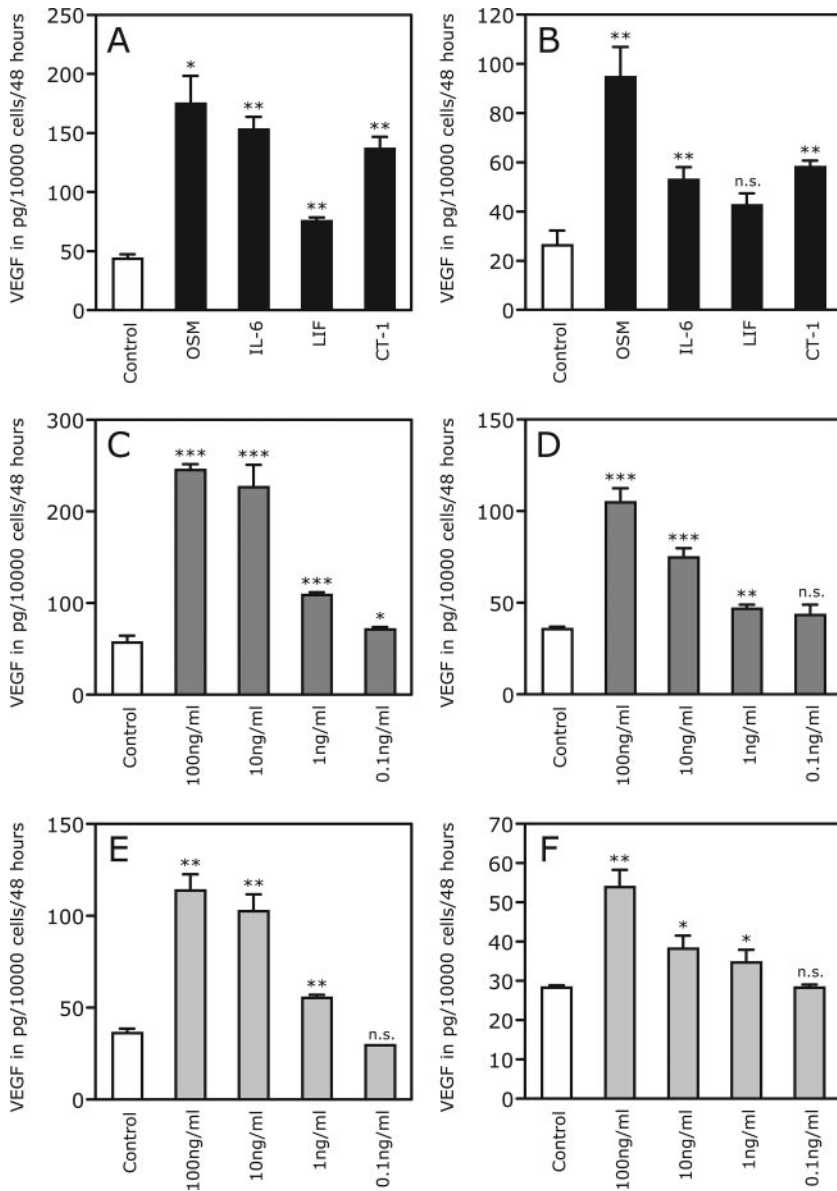
### Expression of CD31 Positive Cells in Mouse Adipose Tissue Is Increased by OSM and IL-6 In Vivo

Immunohistochemistry with an anti-CD31 antibody, a specific endothelial cell marker, revealed a significant increase of CD31-positive cells in retroperitoneal adipose tissue, when mice were injected with OSM or IL-6 intraperitoneally over a period of 18 days (Figure 6D). The presence of adipose tissue was verified by staining with Sudan III and hematoxylin (Figure 6E). Figure 6F shows a typical example of CD31 staining.

### Discussion

Earlier studies have demonstrated that VEGF is expressed in adipose tissue and that its expression is upregulated during adipocyte differentiation and modulated by hypoxia, norepinephrine, dexamethasone, and insulin.<sup>8,22-24</sup> Furthermore increased VEGF mRNA and protein levels were found in adipose tissue of obese animal models.<sup>9</sup> In the present study

we provide evidence that VEGF is produced ex vivo by human subcutaneous and visceral adipose tissue explants. VEGF production in these explants was significantly increased by the gp130 ligands IL-6 and OSM whereas LIF and CT-1 had no effect. Furthermore we show that primary human preadipocytes and adipocytes isolated from visceral as well as from subcutaneous adipose tissue constitutively express VEGF. We published previously that human preadipocytes and adipocytes of subcutaneous and visceral adipose tissue express gp130, the common receptor unit for all gp130 ligands, and the specific receptor subunit for IL-6 receptor, LIF receptor, and OSM receptor.<sup>19</sup> Here we show that similar to the results obtained with adipose tissue explants, OSM and IL-6 led to a robust increase of VEGF protein in all cell types studied. In contrast to adipose tissue explants also LIF significantly increased the production of VEGF in preadipocytes and adipocytes of visceral and subcutaneous origin, whereas CT-1 only upregulated VEGF production in visceral adipose tissue cells. It should be emphasized that the OSM-



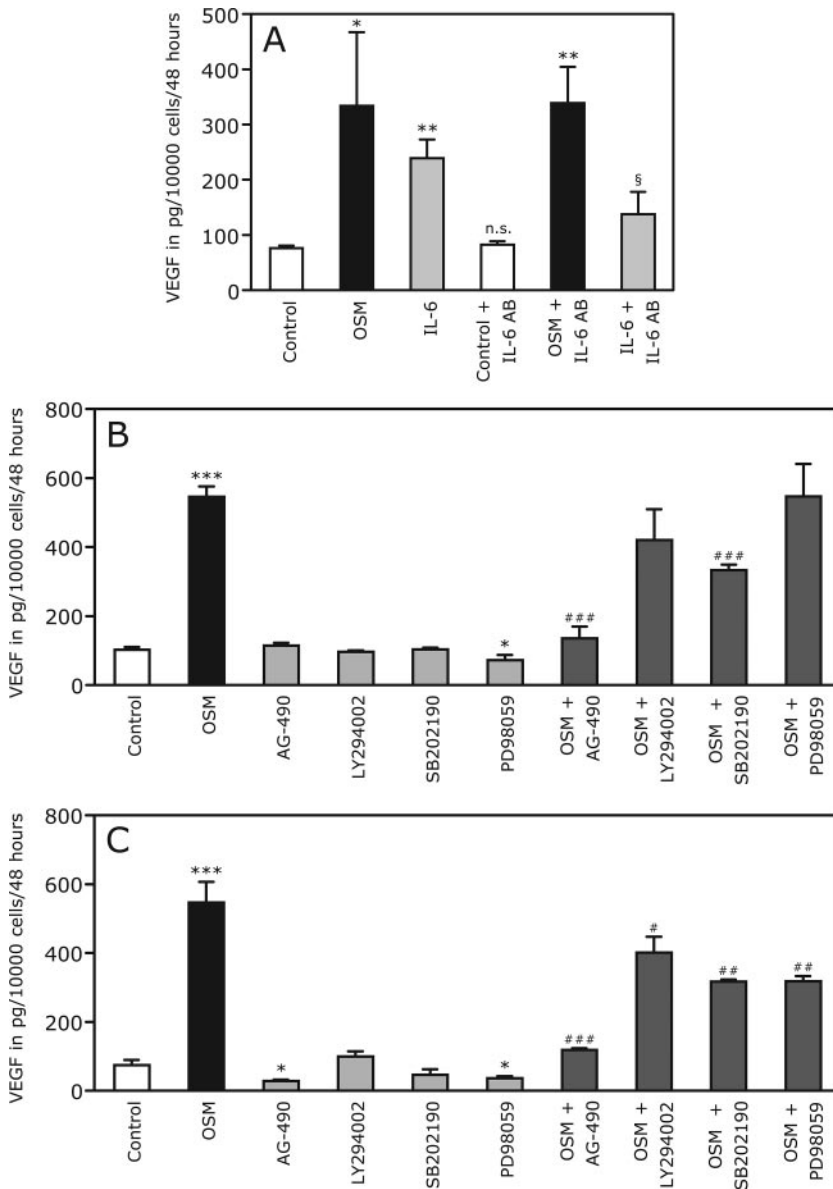
**Figure 4.** gp130 ligands increase VEGF production in PAZ-6 preadipocytes and adipocytes. PAZ-6 preadipocytes (A, C, and E) and adipocytes (B, D, and F) were prepared as described under Materials and Methods and were incubated for 48 hours in the absence or presence of rh OSM (100 ng/mL), rh IL-6 (100 ng/mL), rh LIF (10<sup>4</sup> U/mL), or rh CT-1 (100 ng/mL), (A and B), respectively, with increasing concentrations of rh OSM (C and D) and with increasing concentrations of rh IL-6 (E and F). Conditioned media were collected and VEGF was determined as described under Materials and Methods. Values represent mean values ± SD of 3 independent determinations. Experiments were performed 3 times with similar results. A representative experiment is shown. \*\*\**P*<0.0005, \*\**P*<0.005, \**P*<0.05; n.s., not significant.

induced increase in VEGF production by adipocytes and preadipocytes was further significantly amplified in the presence of IL-1β, another well-known inflammatory cytokine. In correlation with the data obtained on the protein level, IL-6 and OSM also increased VEGF-specific mRNA levels in all subcutaneous and visceral cells as shown by real-time-PCR whereas VEGF-specific mRNA was upregulated by LIF only in subcutaneous preadipocytes and adipocytes and in visceral adipocytes and by CT-1 only in subcutaneous preadipocytes and visceral preadipocytes and adipocytes. We also provide evidence that the effect of OSM on VEGF production is independent of autocrine IL-6 production in these cells, because it was not affected by neutralizing anti-IL-6 antibodies. Using the JAK/STAT inhibitor AG-490 we could show that the effect of OSM on VEGF release was mainly mediated by activating the JAK/STAT pathway. This is in line with previous papers demonstrating an OSM-induced upregulation of VEGF in a STAT-3-dependent manner in human adult

cardiac myocytes, human astrogloma cells, and human airway smooth muscle cells.<sup>16-18</sup>

Our in vitro evidence for a role of particular gp130 ligands in the regulation of VEGF expression by adipocytes is further supported by our findings that IL-6 and OSM upregulate the levels of mRNA specific for VEGF in adipose tissue in mice in vivo. This increase of VEGF mRNA levels in adipose tissue was accompanied by a concomitant increase in VEGF serum levels in these animals. Furthermore it should be emphasized that a significant increase in blood vessel density in adipose tissue samples obtained from these mice was observed.

In addition to our results obtained with white adipose tissue we could also show that IL-6 and OSM dose-dependently increase VEGF production in preadipocytes and adipocytes of the brown adipose tissue cell line PAZ-6. Brown adipose tissue exhibits a completely different capacity of metabolic activities compared with white adipose tissue. In that respect

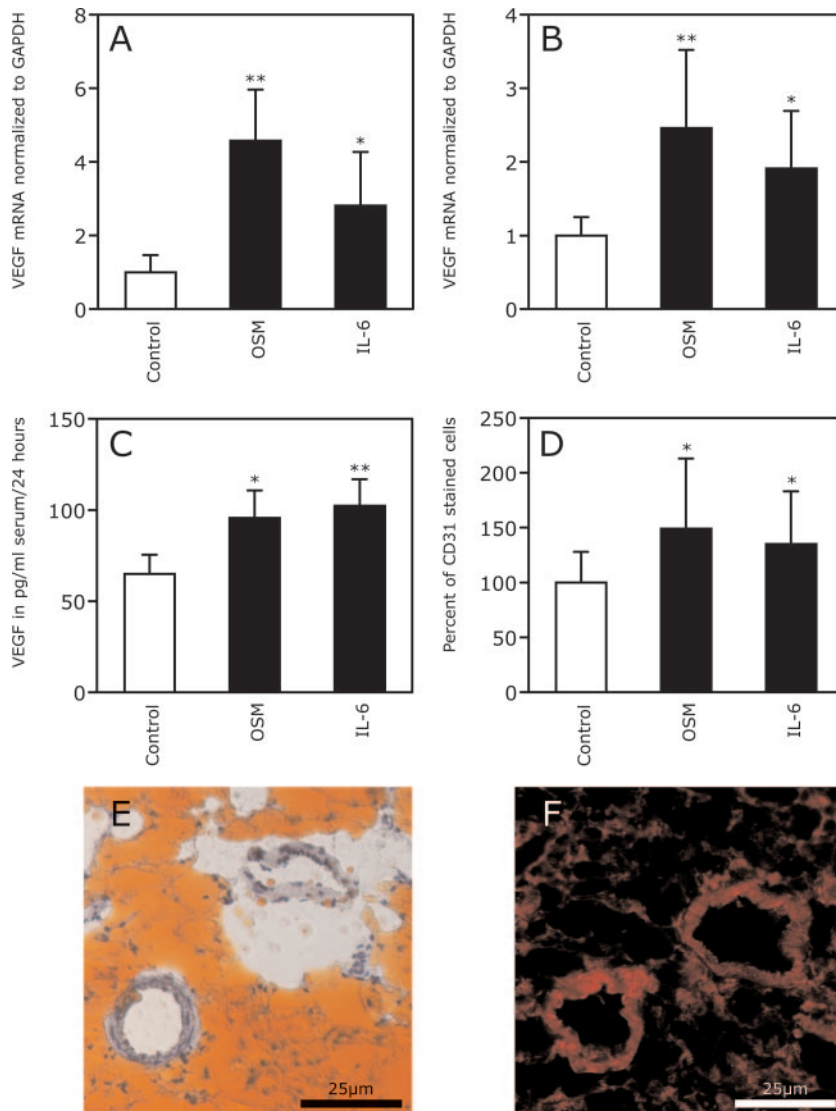


**Figure 5.** VEGF upregulation by OSM in human adipose tissue cells is independent of IL-6 and mainly induced via JAK/STAT pathway. Human subcutaneous adipocytes were prepared from subcutaneous adipose tissue as described under Materials and Methods and were incubated for 24 hours in the absence or presence of rh OSM (100 ng/mL) or rh IL-6 (100 ng/mL) without and with an IL-6-neutralizing polyclonal goat antibody (4  $\mu$ g/mL; A). Human subcutaneous adipocytes (B) and human visceral preadipocytes (C) were prepared from visceral and subcutaneous adipose tissue as described under Materials and Methods and were preincubated for 60 minutes with JAK/STAT inhibitor AG-490, PI3K inhibitor LY294002, MEK-1 inhibitor PD98059, and p38MAPK inhibitor SB202190 (all at 30  $\mu$ mol/L). Thereafter medium was removed, fresh medium with or without OSM (100 ng/mL) was added, and the cells were incubated for 24 hours. Conditioned media were collected and VEGF was determined as described under Materials and Methods. Values represent mean values  $\pm$ SD of 3 independent determinations. Experiments were performed 3 times for each cell type prepared from adipose tissue obtained from 3 different donors, respectively, with similar results. A representative experiment is shown. \*\*\* $P$ <0.0005, \*\* $P$ <0.005, \* $P$ <0.05 compared with control, ### $P$ <0.0005, ## $P$ <0.005, # $P$ <0.05 compared with OSM, § $P$ <0.05 compared with IL-6 and not significant compared with control. n.s., not significant; IL-6 AB: IL-6 neutralizing antibody.

it should be noted that Burysek et al<sup>25</sup> reported an up to 40-fold increase of IL-6 in brown adipocytes stimulated with norepinephrine, a main inducer of thermogenesis. The authors of this article speculated that IL-6 produced by brown adipose tissue could play a paracrine role in the regulation of thermogenesis in this tissue. We could show in the present work that IL-6 stimulates the expression of VEGF in PAZ-6 preadipocytes and adipocytes up to 3.5-fold. We speculate that elevated levels of IL-6 and local accumulation of this cytokine might improve vascularization by upregulating VEGF and thus could support the increased blood flow required for extensive oxygen delivery to brown adipose tissue during thermogenesis.

As discussed above, evidence from studies showing that angiogenesis inhibitors caused adipose tissue loss and a reduction in weight and prevented diet-induced and genetic obesity in mice, supports the notion that adipose tissue mass and thus obesity can be regulated through neovascularization.<sup>4,6</sup> Our findings provide a possible link between the

gp130/gp130 ligand system and the local expression of VEGF in adipose tissue. Increased levels of VEGF in adipose tissue could result in better vascularization, necessary for proper function of adipose tissue as an endocrine organ. Subsequently, increased VEGF levels, induced by gp130 ligands in the fat, could then indirectly lead to elevated levels of adipokines associated with the development of cardiovascular disease such as PAI-1 and IL-6.<sup>2,3</sup> On the other hand, IL-6 and OSM by acting on adipose tissue could also contribute to increased serum levels of VEGF seen in obese patients.<sup>26,27</sup> A possible link between increased plasma levels of VEGF and the development of cardiovascular disease is discussed after two reports showed that administration of VEGF in mice and rabbits induced the progression of atherosclerotic plaques.<sup>28,29</sup> It should be mentioned, however, that the impact of adipose tissue VEGF to the serum VEGF level is still controversially discussed. Here we show that the inflammatory mediators IL-6 and OSM upregulate the expression of VEGF in human and murine adipose tissue and



**Figure 6.** Effect of OSM and IL-6 on VEGF production and CD31 expression in mouse adipose tissue in vivo. Mice were injected with rm OSM (100 ng in 200  $\mu$ L 0.9% NaCl), rm IL-6 (100 ng in 200  $\mu$ L 0.9% NaCl), or 200  $\mu$ L 0.9% NaCl intraperitoneally, re-injected using the same procedure after 12 hours, and euthanized after 24 hours. Epididymal (A) and visceral (B) adipose tissue and blood samples (C) were collected and processed as described under Material and Methods. VEGF mRNA and VEGF antigen, respectively, were determined as described under Material and Methods. VEGF mRNA levels were normalized according to the respective GAPDH mRNA levels and are given as percent of control, which was set as 100%. In a separate experiment, rm OSM (100 ng in 200  $\mu$ L 0.9% NaCl), rm IL-6 (100 ng in 200  $\mu$ L 0.9% NaCl), or 200  $\mu$ L 0.9% NaCl were daily injected intraperitoneally. Animals were euthanized after 18 days and retroperitoneal adipose tissue was collected. CD31 antigen was visualized in cryosections and CD31-stained cells were quantified as described under Material and Methods and are given as percent of control, which was set as 100% (D). The presence of adipose tissue was verified by staining with Sudan III and hematoxylin (E). F shows a typical example of CD31 staining. Values represent mean values  $\pm$  SD. Experiments were performed with 5 different mice. \*\* $P$ <0.005, \* $P$ <0.05.

that both mediators increase serum levels of VEGF in mice. Whereas plasma levels of IL-6 are elevated in obese patients, no such data are yet available for OSM.<sup>2</sup> In that respect it should be noted that IL-6 concentrations in the interstitial fluid of human subcutaneous adipose tissue are markedly higher than in the peripheral blood and IL-6 is also upregulated during adipocyte differentiation and correlates with adipocyte cell size.<sup>30,31</sup> Furthermore, it should be emphasized that macrophages are known to accumulate in adipose tissue and are thought to contribute to elevated levels of inflammatory mediators such as IL-6 also locally in the fat.<sup>26,27</sup> Macrophages, however, also produce OSM and thus it is tempting to speculate that these cells by producing IL-6 and OSM in adipose tissue might contribute to the upregulation of VEGF in obese patients.<sup>32,33</sup>

In conclusion, we present evidence for the first time for a link between selected gp130 ligands and VEGF production in adipose tissue. We show that the gp130 ligands and inflammatory mediators IL-6 and OSM significantly upregulate the expression of VEGF in human preadipocytes and adipocytes of visceral and subcutaneous origin in vitro and in murine

adipose tissue in vivo. We speculate that these cytokines, by upregulating VEGF in adipose tissue, could contribute to vascularization preceding adipose tissue growth, subsequently resulting in elevated levels of adipokines linked to cardiovascular disease on the one hand and on the other hand possibly to increased serum levels of VEGF in obese patients. Thus we hypothesize that the link between particular gp130 ligands and VEGF in adipose tissue could contribute to the increased cardiovascular risk associated with obesity.

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### Disclosures

None.

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