The contribution of omental adipose tissue to adipokine concentrations in patients with the metabolic syndrome

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Manuscript submitted 26th March, 2007
Manuscript accepted 15th July, 2007


Abstract

Purpose: To examine differences in peripheral vascular endothelial growth factor (VEGF), interleukin-6 (IL6) and cortisol concentrations between patients with both visceral obesity and metabolic syndrome, and lean controls. In a subsample of metabolic patients underwent abdominal surgery, the adipokine concentrations were measured in venous blood from the omentum to determine information on some processes of synthesis.

Methods: Forty-two healthy lean controls and 46 overweight-obese patients with central adiposity and stigmata of metabolic syndrome were studied. In a subsample of 11 metabolic patients undergoing non-bariatric surgery, blood samples from omental and peripheral veins were taken intraoperatively to determine VEGF, IL6 and cortisol concentrations.

Results: Median levels (range) of peripheral VEGF and IL6 were higher in patients than in controls [31.5 (3-112) pg/mL vs 21.35 (9-41.9) pg/mL (P<0.05); and 5.50 (1.40-13) pg/mL vs 1.15 (0.3-1) pg/mL (P < 0.0001)]. On the other hand, concentrations of VEGF and IL6 from the omental and peripheral veins were similar in the surgery sub-group. Peripheral cortisol concentrations were not higher in patients than in controls, nor were omental concentrations different from the peripheral. Omental and peripheral VEGF and cortisol values were correlated, whereas no association was found between omental and peripheral IL6.

Conclusions: In the presence of abdominal obesity, VEGF and IL6 concentrations are increased in the systemic circulation. The contribution of visceral adipose tissue to circulating levels of VEGF and IL6 was modest.

Keywords: Vascular endothelial growth factor, interleukin-6, cortisol, metabolic syndrome, central obesity, omental vein blood.

Obesity, particularly abdominal obesity, is a central component of the metabolic syndrome (MS)¹-³ and insulin resistance (IR).⁴ There is uncertainty regarding the relative importance of subcutaneous and visceral adipose tissue.
In a recent study, mesenteric fat thickness measured by sonography (confirmed by magnetic resonance imaging, MRI) was more strongly associated with some cardiovascular risk factors than subcutaneous fat. However, the latter correlated better with the reduced high density lipoprotein (HDL)-cholesterol in men. In other reports, fat accumulation in the visceral depot and the liver were highly correlated with the development and severity of IR. In one study, where insulin sensitivity was determined by the glucose clamp method and abdominal adipose tissue volumes were measured by MRI, subcutaneous abdominal adiposity was more important than visceral adiposity.

Among the adipokines studied, adiponectin, leptin and resistin, vascular endothelial growth factor (VEGF) plays an important role, as the likely link between vascular and metabolic abnormalities. In another report, serum VEGF concentrations were elevated in overweight and obese individuals. High VEGF levels in overweight subjects may indicate some degree of IR, since insulin suppresses VEGF expression. Furthermore, adipose tissue, a major endocrine organ, influences not only vascular homeostasis but also the immune responses. The immune-regulating and acute phase-inducing cytokine interleukin-6 (IL6) is to a large extent secreted from adipose tissue during non-inflammatory conditions in humans, and correlates with BMI.

Also, local production of active cortisol from inactive cortisone, driven by 11 beta-hydroxysteroid dehydrogenase type 1 (11ß-HSD-1), is exaggerated in adipose tissue of obese mice, and that its inhibition ameliorates MS. Patients with Cushing’s syndrome develop a reversible visceral obesity. Transgenic mice over-expressing 11ß-HSD-1 selectively in adipose tissue have increased adipose levels of corticosterone, develop visceral obesity and are glucose intolerant. Studies in obese Zucker rats demonstrated increased 11ß-HSD-1 activity in omental adipose tissue. Central obesity is of paramount importance in determining the metabolic abnormalities underlying the complex atherogenic process. In vitro and animal-model-based studies have demonstrated that omental adipose tissue is not a pure depot tissue, but possibly the site of synthesis of various adipokines but, as yet, there is scant in vivo data.

Intra-abdominal adipose tissue depot is made up of visceral intraperitoneal fat, primarily omentum and mesenteric fat, and retroperitoneal fat masses separated by a delineation along the dorsal margin of the intestine and the ventral surface of the kidney. Omental adipose tissue is not easily evaluated in vivo, as access can only be gained during laparotomy. The first aim of our study was to look for possible differences in peripheral VEGF, IL6 and cortisol concentrations between patients with visceral obesity and MS, and lean controls. Second, in a sub-group of metabolic patients who undergoing abdominal surgery, we assessed the relative contribution of the visceral depot in determining adipokine synthesis.

Patients and Methods

The study was approved by the Research and Ethics Committee of our Medical School and all participants gave written informed consent before the study, in accordance with the Helsinki Declaration.

Study design

Forty-six patients with visceral adiposity and MS were selected from among 186 consecutive obese or overweight patients referred to our Metabolic outpatient-clinic. In the 3-year follow up, 11 of these patients underwent abdominal surgery for different illnesses. None had any evidence of metastatic neoplasia, and none underwent bariatric surgery. In this sub-group we compared the concentrations of VEGF, cortisol and IL6 in blood samples from an omental vein (visceral fat), and from the systemic circulation. A group of 42 lean subjects was chosen as control. All participants were weight stable. None of the female participants was receiving hormone replacement or contraceptive therapy.

Characteristics of population

A diagnosis of MS was made when at least three of the criteria of the National Cholesterol and Education Program, Adult Treatment Panel III report (ATP III,
2001) were met. The BMI was calculated as body weight, divided by height squared. Subjects were classified as being either overweight-obese or normal weight on the basis a BMI ≥ 25.0 or < 25.0 kg/m², respectively. Central obesity was identified by waist circumference > 102 cm in men or > 88 cm in women. Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. The group of lean controls comprised subjects without central obesity and with stable normal weight. All patients were characterized by overweight-obesity with visceral adiposity and MS.

**Surgery**

In patients undergoing surgery, anesthesia was induced with thiopentone followed by succinylcholine and fentanyl. Ceftriaxone and metronidazole were given intravenously. After tracheal intubation, anesthesia was maintained with isoflurane. A lateral omental vein above the transverse colon was cannulated with a 22-gauge catheter in a retrograde fashion. The cannula was kept patent with saline infusion. Oxygenation and blood pressure were maintained at optimum levels without the use of any drugs that may effect glucose metabolism. Simultaneously, a blood sample was taken from a superficial forearm vein of the antecubital fossa. The blood was rapidly centrifuged at 4°C, and the serum was frozen at –40°C for subsequent analysis.

**Specific Assays**

Cortisol, µg/dL, was measured with an Immulite analyzer using a commercial kit, as recommended by the manufacturer (Diagnostic Products Corp., Los Angeles, CA, USA). The range of normal values obtained in 150 subjects was 5-25 µg/dL.

VEGF concentration, pg/mL, was assessed by an Enzyme Immunometric Assay method for quantitative detection of soluble human IL6 (Bender MedSystems GmbH, Vienna Austria, Europe). Sensitivity was 0.92 pg/mL; overall intra- and inter-assay coefficients of variation were 6.2% and 7%, respectively.

The adipokine concentrations were measured on peripheral blood in all 46 metabolic patients and 42 lean controls at study entry. In the surgery sub-group, the peripheral concentrations were re-measured during surgery and compared with the central samples.

**Statistics**

As the values of VEGF, cortisol and IL6 were not normally distributed, data analysis was performed using non-parametric tests including the Wilcoxon test for paired samples and Spearman’s coefficient of rank correlation (rho). When dealing with values belonging to different populations, i.e., controls and all patients, the Mann-Whitney test for independent samples was used. Values are expressed as median and range. A two-tailed probability, \( P < .05 \) was considered to be significant.

**Results**

Demographic, clinical and laboratory data of the 11 metabolic patients who underwent surgery are reported in Table 1. The characteristics of the whole study population are shown in Table 2. The median (range) peripheral concentration of VEGF in the population of metabolic patients, 31.5 (3-112) pg/mL, was higher than in the lean controls, i.e., 21.35 (9-41.9) pg/mL (\( P = 0.0022 \), Figure 1a).

In the surgery sub-group, no difference was found between the concentrations of VEGF in omental, 25 (6-18) pg/mL, and peripheral blood, 26 (4-84) pg/mL, \( P = 0.58 \), Figure 1b. There was a correlation between VEGF concentrations in blood samples from visceral fat and the general circulation (surgery sub-group), (\( P < 0.0075 \), Figure 1c). The median peripheral concentration of IL6 in the 46 metabolic patients was 5.50 (1.40-13.00) pg/mL, which was different from controls, 1.15 (0.3-11) pg/mL (\( P < 0.0001 \), Figure 2a).

In the surgery sub-group the median concentration of IL6 in omental vein blood was 4.0 (1.60-6.60)
pg/mL and in peripheral samples 5.0 (3.20-8.0) pg/mL ($P = 0.57$, Figure 2b). No association of IL6 concentrations was found in blood samples from visceral fat (omental) and the general circulation (Figure 2c).

The median (range) peripheral level of cortisol in the population of metabolic patients was 15.5 (0.4-37.3) μg/dL, overlapping that in the lean population [15.65 (0.4-27.8), $P = 0.81$, Mann-Whitney test (independent samples)]. Furthermore, in the surgery sub-group the median concentrations of cortisol in omental venous blood, 18.2 (0.5-30.8) μg/dL, were similar to those in peripheral samples, 15.5 (0.4-37.3) μg/dL (Figure 3a), and comparable to the median levels of the 42 lean controls and the remaining 35 meta-
FIGURE 1a. Peripheral vascular endothelial growth factor (VEGF) (pg/mL) of the 42 lean controls and the 46 patients with central obesity. Box-and-whisker plots (median and range of values), \( P = 0.0022 \)

FIGURE 1b. Peripheral vascular endothelial growth factor (VEGF) (pg/mL) from omental (Cen) and peripheral (Per) veins in the eleven metabolic patients with central obesity. Box-and-whisker plots (median and range of values), \( P = \text{NS} \).

FIGURE 1c. Scatter diagram and regression between vascular endothelial growth factor (VEGF) pg/mL values from omental (Cen) and peripheral (Per) veins in the eleven metabolic patients with central obesity, \( P < 0.0075 \), \( \rho = 0.84 \).

FIGURE 2a. Peripheral interleukin-6 (IL6) (pg/mL) of the 46 patients with central obesity and the 42 lean controls, \( P = 0.0001 \).

FIGURE 2b. Interleukin-6 (IL6) (pg/mL) in blood from omental (Cen) and superficial forearm (Per) veins in the 11 metabolic patients who underwent surgery, \( P = 0.57 \).

FIGURE 2c. Scatter diagram with the regression line between interleukin-6 (IL6) (pg/mL) values in the blood of omental (Cen) and superficial forearm (Per) veins, in the surgery sub-group of eleven metabolic patients, \( P = 0.22 \), \( \rho = 0.39 \).
bolic patients, 15.5 (0.4-37.3) μg/dL (Figure 3b). There was a strong association between the cortisol concentrations found in blood samples from visceral fat and in the general circulation in the sub-group of surgery patients ($P = 0.0034$, Figure 3c). A weak correlation ($P = 0.002$, Figure 4) was found between peripheral VEGF and IL6 levels in the study population as a whole (42 lean controls and 46 metabolic patients).

**Discussion**

Similar concentrations of VEGF, IL6 and cortisol were detected in the systemic and omental circulations of the patients undergoing non-bariatric surgery. Peripheral VEGF and IL6 concentrations in the whole population were higher than in controls. This confirms an expansion of the capillary bed in regional adipose depots and a chronic, low-grade inflammation state. The association between peripheral VEGF and IL6 may reflect a similar behaviour and, probably, a common site of release, i.e., adipose tissue.

While there is general agreement on the strong association between central or visceral obesity and cardiovascular risk factors, particularly dyslipidemia and hyperinsulinemia, the relative importance of deep vs. subcutaneous abdominal fat has been examined by only a few reports. Recent studies have demonstrated the role of visceral fat in producing adipokines, both in animal models and when applying imaging procedures. A previous study, in obese mice, showed that the mRNA expression levels of VEGF in visceral fat were enhanced more than in the subcutaneous abdominal region. In a study using computed tomography at the umbilical level there was a correlation between serum VEGF and BMI, and visceral fat mass in overweight-obese individuals. Similarly, in a study of obese bariatric surgery patients, in which fragments of subcutaneous and visceral adipose tissue were taken and placed in serum-free medium organ culture, IL6 release in the incubation medium was 3-fold higher in omental than in subcutaneous abdominal adipose tissue. Subsequent research, in primary culture of human adipose tissue from obese patients who had undergone abdominoplasty or laparoscopic gastric bypass, showed greater release of VEGF and IL6 from visceral depot than from subcutaneous abdominal tissues.

Peripheral cortisol values were similar to controls. Although this could be an atypical result, all patients met the ATP III criteria. Such a lack of increase has
been reported.\textsuperscript{23} and a correlation established between metabolic stigmata and cortisol concentrations within the normal range. The glucocorticoids (GC) produced as part of the inflammatory response enhance some of the IL6 effects, such as acute phase protein synthesis, but they down-regulate IL6 expression, providing a negative feedback pathway on the inflammatory response in vivo. Our data support this inverse link, as we found high IL6 levels and low or normal values of serum cortisol in our overweight-obese population. The features of MS are also found in patients with increased circulating GC, or Cushing’s syndrome, although patients with MS do not exhibit increased circulating GC levels. It has been suggested that MS may result from increased intracellular GC tone, as may occur with elevated 11ß-HSD1 activity. Several recent experiments in mice support this hypothesis. Overexpression of 11ß-HSD1 in murine adipose tissue leads to a MS–like phenotype, including increased central obesity, hypertension, impaired glucose tolerance and hypertriglyceridemia.\textsuperscript{24, 25} Our data are consistent with this hypothesis.

This study suffers from a methodological bias, since the number of subjects who underwent surgery is small and unbalanced toward a female prevalence. Further studies on a larger scale are needed to clarify the contribution of visceral and subcutaneous abdominal adipose tissues in producing various adipokines.

In conclusion, this study has shown high peripheral VEGF and IL6 titres in the presence of central obesity. On the basis of this \textit{in vivo} model, the contribution of visceral adipose tissue to the circulating levels of VEGF and IL6 is likely reduced.

References


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