

Gender Differences in Tumor Necrosis Factor α and Leptin Secretion from Subcutaneous and Visceral Fat Tissue

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Received July 24, 2003

Accepted October 28, 2003

Summary

Tumor necrosis factor α (TNF α) and leptin concentrations were determined in the abdominal subcutaneous and visceral (omental) adipose tissue of patients undergoing elective open-abdominal surgery and compared with their body mass index. The concentration of leptin did not differ significantly between women and men, being high in subcutaneous fat tissue and low in visceral fat tissue. TNF α concentration in subcutaneous fat tissue was approximately the same in both genders, but it was significantly lower in visceral fat tissue of women and unchanged in visceral fat tissue of men. A significant correlation between BMI and leptin was found in the two fat tissue compartments of both genders, but the correlation between BMI and TNF α was found only in subcutaneous fat tissue of women.

Key words

Tumor necrosis factor α • Leptin • Subcutaneous fat tissue • Visceral fat tissue

Introduction

Many studies have shown the detrimental influence of growing intra-abdominal (visceral, omental) and abdominal subcutaneous fat depots on metabolic processes associated with high insulin resistance, metabolic syndrome and increased cardiovascular mortality (Fujioka *et al.* 1987, Pouliot *et al.* 1992, Park *et al.* 1991, Després 1993, Goodpasture *et al.* 1997). These metabolic consequences are at least partly conditioned by the abundant production of several factors expressed in adipocytes, including leptin and TNF α .

In human, a strong positive correlation was observed between serum leptin levels and the amount of body fat and adipocyte leptin mRNA (Caro *et al.* 1996, Maffei *et al.* 1995). Leptin mRNA was greater in

subcutaneous than in omental adipocytes and the subcutaneous-omental ratio of leptin mRNA was markedly higher in women than in men (Montague *et al.* 1997).

Adipocytes are also a source of the cytokine TNF α . Obese individuals express 2.5-fold more TNF α mRNA in subcutaneous fat tissue than lean controls, with a significant correlation between TNF α mRNA and BMI (Kern *et al.* 1995, Hotamisligil *et al.* 1995). A strong positive correlation was observed between TNF α mRNA expression in fat tissue and the level of insulin (Dandona *et al.* 1996). The decreased insulin action probably depends on TNF α -induced serine phosphorylation of insulin-receptor-substrate-(IRS)-1 (Hotamisligil *et al.* 1998).

In our previous paper (Lichnovská *et al.* 2002),

we have found significant gender differences in factors determining insulin resistance in non-diabetic elderly men and postmenopausal women of similar age. In addition to a significant association with serum leptin in both genders, the insulin resistance in women was associated with serum triglycerides, TNF α and decreased HDL-cholesterol (but not with BMI), while in men with insulin resistance there was only a distinct influence of BMI and decreased HDL-cholesterol (but not of TNF α).

The aim of this study was 1) to determine the leptin and TNF α content in abdominal subcutaneous and abdominal visceral (omental) fat tissues of elderly men and women, 2) to correlate them with body mass index, and 3) to elucidate some new aspects of gender differences in the metabolic role of various fat compartments.

Methods

Abdominal subcutaneous and intra-abdominal (visceral, omental) tissue biopsies were obtained from patients undergoing elective open-abdominal surgery. All patients were fasted for at least 6-12 h during the night before the operation, and all underwent general anesthesia and the surgery in the morning. Biopsies were obtained from 5 women (age 59.2 \pm 16.9 years, BMI 26.6 \pm 3.6 kg/m²) and 8 men (age 67.1 \pm 5.5 years, BMI 25.5 \pm 2.6 kg/m²). None of the patients had diabetes or severe systemic illness, and none of them took any medication known to influence adipose mass or metabolism. The study was approved by the Faculty of Medicine Ethics Board and informed consent was obtained from each patient.

The biopsies were immediately placed in liquid nitrogen and kept under these conditions until chemical analysis. After all biopsies had been collected, protein extraction from small pieces (about 40 mg) of fat tissues was performed by 30 min shaking in 1 ml ice-cold Bug Buster Protein Extraction Reagent (Novagen, Inc., Merck Eurolab GmbH, Darmstadt). Extracts were centrifuged and assayed for protein (Bradford 1978), TNF α (Human Tumor Necrosis Factor-alpha, Ultrasensitive, ELISA Kit, Biosource International, Camarillo, CA, U.S.A.) and leptin (Human Leptin ELISA, Clinical Range, BioVendor Laboratory Medicine, Inc., Brno, Czech Republic). TNF α and leptin content in fat tissues was related to the protein.

Data were expressed as means \pm S.D. Student's t-test and two-way ANOVA were used to compare the contents of TNF α and leptin in various fat tissue

compartments of both genders. Pearson's test was used to calculate the correlation coefficients between BMI, TNF α and leptin values. A significance limit of 0.05 was used. Statistical analysis was performed using STATISTICA software (StatSoft CR, Czech Republic).

Results

TNF α content in subcutaneous fat tissue of women did not differ significantly from men, but was significantly higher than TNF α in visceral fat tissue in women. In men, TNF α content in visceral fat tissue did not differ from subcutaneous fat tissue (Fig. 1). The content of leptin in subcutaneous fat compartment did not differ significantly between women and men (Fig. 2). Leptin content in visceral fat tissue was substantially lower than in subcutaneous fat tissue in both women and men.

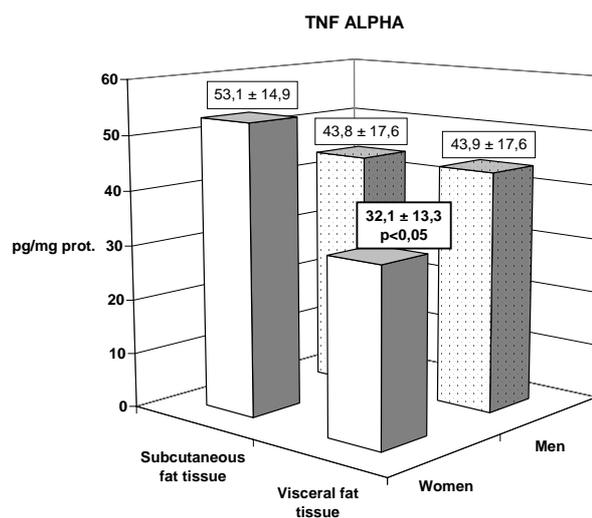


Fig. 1. TNF α concentrations in various fat tissue compartments of women and men. Data are means \pm SD. Significant differences between subcutaneous and visceral fat tissues are bold faced.

While BMI correlated with leptin content in subcutaneous and visceral fat tissue in both genders, the correlation between BMI and TNF α was significant only in subcutaneous fat tissue of women (Fig. 3), where the content of leptin in subcutaneous fat tissue also correlated with TNF α level in this fat tissue compartment.

Thus, while the level of leptin did not differ substantially between women and men, being high in subcutaneous fat tissue and low in visceral fat tissue, the content of TNF α in subcutaneous fat tissue was approximately the same in both genders, but it was lower

in visceral fat tissue of women and unchanged in visceral fat tissue of men. The association between BMI and leptin was expressed in both fat tissue compartments, irrespective of gender, but the association between BMI and TNF α was found only in female subcutaneous fat tissue.

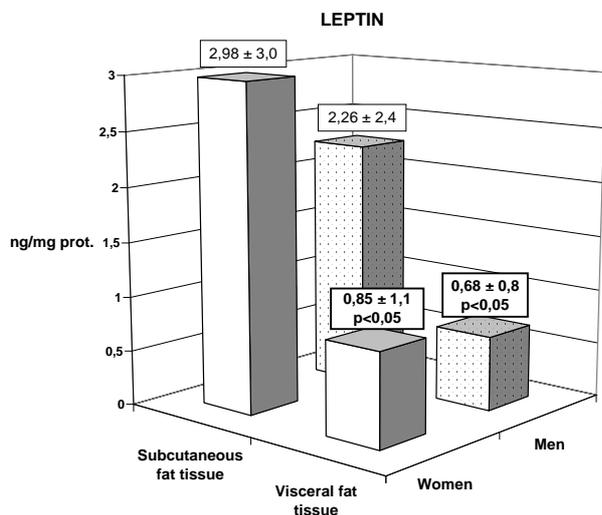


Fig. 2. Leptin concentrations in various fat tissue compartments of women and men. Data are means \pm SD. Significant differences between subcutaneous and visceral fat tissues are bold faced.

Discussion

Our data confirm the findings of Van Harmelen *et al.* (1998) that leptin secretion rate in women is substantially higher in subcutaneous than in the omental fat tissue, and that in men there is a positive correlation between BMI and leptin secretion rates in abdominal subcutaneous and visceral fat tissue. Serum leptin concentrations in humans exhibit a sexual dimorphism, with serum levels being higher in women than in men, which could reflect a significantly higher subcutaneous-omental fat ratio and the different sex steroid milieu in women than in men for the same BMI (Tritos and Mantzoros 1997).

Preliminary data have indicated a trend for higher expression of TNF α in subcutaneous than omental adipose tissue obtained from obese subjects of both genders (Hube *et al.* 1999). According to Bertin *et al.* (2000) plasma TNF α level was similar in men and women and was not related to age and fasting glycemia. A relationship was highlighted with changes of BMI, but it was only dependent on the intraabdominal fat mass as assessed by the waist-to-hip circumference ratio and the

visceral adipose tissue area. Given the well established link between omental adiposity and insulin resistance in humans, if adipocyte TNF α expression was linked to insulin resistance (Hotamisligil *et al.* 1995), there should be evidence for a site-related TNF α expression in isolated human adipocytes that has not been found (Montague *et al.* 1998). On the contrary, in contrast to the marked site-related expression of leptin, TNF α was not differentially expressed in human subcutaneous and omental adipocytes.

Our results differ from some of these findings. The content of TNF α in visceral fat tissue was the same in men as in the subcutaneous fat tissue, while it was significantly lower in women (Fig. 2). The subcutaneous abdominal tissue seems to be a main source of TNF α in women, while in men no difference between these tissues was found. In addition, the expression of TNF α in subcutaneous fat tissue correlated with BMI in women only.

Cnop *et al.* (2002) examined in 174 lean insulin-sensitive, lean insulin-resistant, and obese insulin-resistant subjects of both genders in order to find how fat distribution contributes to insulin sensitivity. In both genders fasting leptin levels were strongly associated with subcutaneous fat area but not with intra-abdominal fat. The insulin sensitivity in non-obese subjects was not dependent on BMI, but was strongly associated with intra-abdominal fat. Accumulation of intra-abdominal fat correlated with insulin resistance, whereas subcutaneous fat deposition correlated with circulating leptin levels. The concurrent increase in these metabolically distinct fat compartments was a major explanation for the association between insulin resistance and elevated circulating leptin concentrations in lean and obese subjects.

Our results are not fully consistent with these findings. In both genders the growing BMI of our subjects was associated with an increasing expression of leptin in both subcutaneous and visceral fat. According to our previous results, the insulin sensitivity was dependent on serum leptin concentrations in both genders and also on BMI in men (Lichnovská *et al.* 2002). The role of the subcutaneous fat compartment in insulin resistance, which is undoubtedly the main source of leptin in both genders, seems to be apparent. Gender differences exist in the association between BMI and TNF α in fat tissue compartments. In contrast to men, a higher concentration of TNF α in subcutaneous fat tissue in relation to visceral fat tissue was found in women. This is consistent with the finding of Hube *et al.* (1999) that in obese women

subcutaneous fat depot exhibits a 1.67 fold higher TNF α mRNA expression relative to omental fat depot. The level of TNF α correlates with BMI only in the female subcutaneous fat tissue. Studies that failed to find correlation between BMI and TNF α concentration in

adipose tissue were performed predominantly in men or did not take into account different genders and different fat tissue localization. Our results seem to stress a significant role of subcutaneous fat tissue as a source of insulin-desensitizing cytokine TNF α in women.

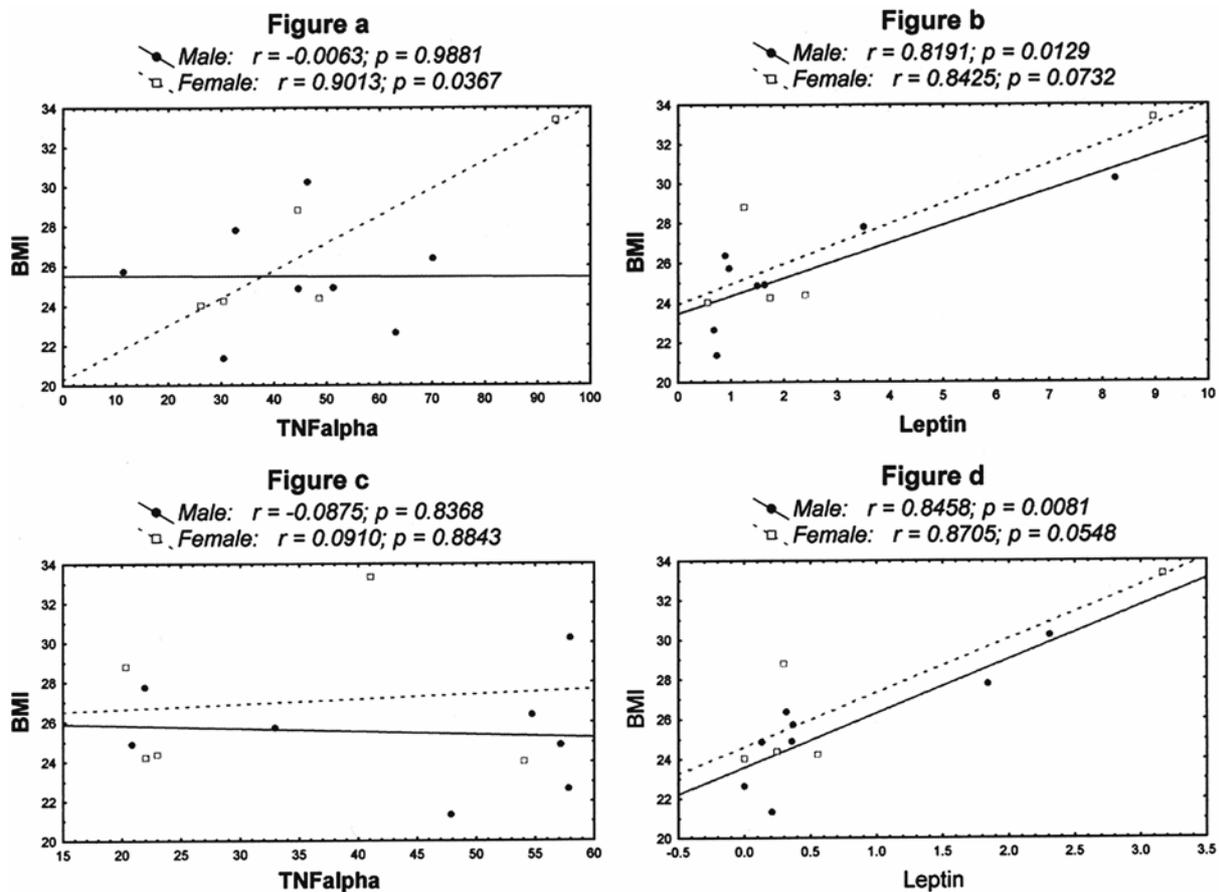


Fig. 3. The correlations between BMI and the cytokines TNF α and leptin, respectively, in subcutaneous (panels a and b) and visceral (panels c and d) fat tissues of females and males. Values of TNF α are presented in pg/mg protein, values of leptin in ng/mg of protein.

Acknowledgements

Authors would like to thank Lenka Surzynová for technical help. This work was supported by grant MSM 151100005 of the Ministry of Education, Youth and Physical Training, Czech Republic, and by grant OC

B5.10 of the European Cooperation in the field of Scientific and Technical Research (COST) in Brussels.

These results were presented in preliminary form at the Meeting of the Czech and Slovak Physiological Societies in Plzeň, February 5-7, 2003 (Orel *et al.* 2003).

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