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Correlation between omental TNF-α protein and plasma PAI-1 in obesity subjects

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Abstract

Background: The role of TNF-α in contributing to obesity-associated cardiovascular and metabolic risk has gained much attention.
Materials and methods: Paired biopsies of omental and subcutaneous fat were collected from 16 lean subjects and 32 central obesity subjects. The expression of TNF-α in omental and subcutaneous fat was quantified by western blotting method, and correlations with plasma PAI-1, homeostasis model assessment insulin resistance (HOMA-IR), and lipid were investigated.
Results: In obese female, TNF-α expression was higher in the omental than in the subcutaneous fat tissue. There was no significant difference in the levels of TNF-α between subcutaneous and visceral fat in obese male. Significant positive correlations were found between omental TNF-α protein and plasma PAI-1 levels in obesity. In obese female subjects, omental TNF-α protein levels showed a close association with most of the parameters studied: fasting glucose ($r=0.541$, $P<0.05$); fasting insulin ($r=0.599$, $P<0.01$); HOMA-IR ($r=0.546$, $P<0.05$); triglycerides ($r=0.469$, $P<0.05$); HDL-cholesterol ($r=-0.759$, $P<0.01$). In obese male population, correlations between omental TNF-α protein levels and fasting glucose ($r=0.762$, $P<0.01$); fasting insulin ($r=0.622$, $P<0.05$); triglycerides ($r=0.650$, $P<0.05$); HDL-cholesterol ($r=-0.880$, $P<0.01$) were found.
Conclusion: Omental TNF-α may play a key role in contributing to cardiovascular risk in central obesity subjects.

Keywords: Tumor necrosis factor-alpha; Obesity; Plasminogen activator inhibitor 1; Omentum; Vascular diseases

1. Introduction

Obesity, especially central obesity, related diseases now present the biggest health challenges worldwide. Many epidemiological studies have shown that visceral obesity is associated with a high risk of obesity-related cardiovascular complications than is peripheral obesity [1,2]. The particular adverse consequences of visceral obesity may, in part, relate to intrinsic differences in the properties of the adipocytes from this depot. For example, visceral adipocytes express higher levels of interleukin-6 [3], interleukin-8 [4], plasminogen activator inhibitor 1 (PAI-1) [5], and angiotensinogen [6] than subcutaneous adipocytes.

The expression of tumor necrosis factor-alpha (TNF-α) in adipose tissue is increased in human obesity [7,8], and TNF-α is proposed as molecular link between obesity and insulin resistance [9]. Furthermore, TNF-α induce endothelial dysfunction in humans with an increased cardiovascular risk due to type 2 diabetes [10]. A compelling body of evidence has recently emerged that indicates that the adipocyte-derived protein TNF-α plays a major role between obesity and atherosclerosis [11]. PAI-1 inhibits fibrin clot breakdown, thereby favoring thrombus formation upon ruptured atherosclerotic plaques [12]. In vitro, TNF-α could increase PAI-1 expression in adipocytes [13]. In humans, circulating PAI-1 levels correlate with atherosclerotic events and mortality, and studies conclude that PAI-1 is an independent risk factor for

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coronary artery disease [14]. Little is currently known about the correlations between TNF-α protein in omental and subcutaneous adipose tissue and plasma PAI-1 levels [15], and from this point to clarify which depot is more associated with cardiovascular diseases.

Therefore, we set out to examine the association between the expression of TNF-α protein in omental and subcutaneous adipose tissue and homeostasis model assessment insulin resistance (HOMA-IR), lipids, and plasma PAI-1 levels in female and male respectively. The results obtained that TNF-α protein expression in omental fat was significantly correlated with plasma PAI-1 levels in both women and men obesity. These data suggest that the role of omental fat may be more associated with cardiovascular complications. The present study provides a strong basis for further investigations about TNF-α antagonist which may unravel innovative therapeutic strategies to improve cardiovascular health in people affected by obesity.

2. Materials and methods

2.1. Subjects

16 lean and 32 central obesity subjects involved in either weight reduction surgery with adjustable gastric banding or cholecystectomy were recruited at surgery department of the First Affiliated Hospital of China Medical University (Shenyang, China). Except for obesity or gallstones, they were all healthy and taking no medication. Subjects had been weight stable for at least 3 months before surgery. Obesity subjects met the criteria for central obesity, BMI $\geq$ 28 kg/m^2, waist circumference $\geq$ 85 cm in male, and waist circumference = 80 cm in female, lean subjects met the criteria for BMI < 24 kg/m^2, adopted from "the guidelines for prevention and control of overweight and obesity in Chinese adults" [16]. Patients were divided into groups of obese (n = 32), and lean (n = 16). The ethics committees of the First Affiliated Hospital of China Medical University approved the clinical investigations, and all subjects gave informed consent.

2.2. Physical measures

Participants in a fasting state underwent anthropometric evaluation. Anthropometric measurements included weight, height, and waist circumferences. Using calibrated electronic scales, weight was obtained while subjects wore light clothing and no shoes; height was measured with a fixed stadiometer. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Waist circumference was measured in orthostatic position at the midpoint between the lateral iliac crest and lowest rib.

2.3. Laboratory tests

Blood samples were collected after an overnight fast of 12 h. Fasting plasma glucose concentration (glucose oxidase method), serum cholesterol (CHOD-PAP method), serum triglycerides (GPO-PAP method), and high-density lipoprotein cholesterol (IRC method) of all the patients were estimated on an analyser (Hitachi 7600-020 Automatic Chemistry Analyzer). Fasting serum insulin concentrations were determined with chemiluminescence method using automated immunoassay system (Bayer Insulin Assay). The homeostasis model assessment insulin resistance (HOMA-IR) index derives an estimate of whole-body insulin sensitivity from fasting glucose and insulin concentrations: HOMA-IR = fasting insulin (mU/l) × fasting plasma glucose (mM) / 22.5 [17].

2.4. Immunomorphologic analysis of adipose tissue

Paired omental and subcutaneous fat biopsies were obtained during gastric surgery. A portion of each fat biopsy was immediately transferred into liquid nitrogen before western blotting analysis. The other part was immediately embedded in O.C.T. Compound (Sakura) in a plastic mold, without air bubbles surrounding the tissue, then dipped promptly in liquid nitrogen and stored at −80 °C. Studies were performed on 15-μm-thick frozen sections (Leica RM1900). Slides were fixed in acetone for 30 min at room temperature. After washing with PBS twice, slides were incubated with 30% H_2O_2 mixed methanol (1:50) for 30 min to cut down the endogenous peroxidase. The slides were blocked with 5% BSA for 20 min after washing. The rabbit anti-human monoclonal TNF-α (Boster Biological Technology Ltd) were added onto the sections and incubated overnight at 4 °C. The antibodies were removed, and the tissue slides were washed thrice, for 2 min each time, with PBS buffer, at room temperature. The biotinated polyclonal antibody against rabbit IgG (Boster Biological Technology Ltd) was added and incubated for an additional 30 min. After washing, an SABC (StreptAvidin Biotin Complex) reagent was added for 30 min of incubation. Amino-ethyl-carbasol chromogen substrate was counterstained (reddish color) for less than 5 min, and a Mayer hematoxylin staining was also carried out for 1 min. The slides were then washed with deionized water for 5 min and left on a bench to air dry. The stained sections were observed under microscope and digital images were captured by a camera (Olympus).The sections were observed under microscope and digital images were captured by a camera (Olympus). Adipocyte diameters were measured in 10 different randomly chosen areas in each processed slide with 40× magnification. Measuring was performed blindly by three independent observers. Adipocyte size was calculated using the formula for ellipsoid body volume $[(a \times b^2) \times 4\pi/3]$ [18]. (a and b are the long and short radii).

2.5. Western blotting analysis

The frozen adipose tissue was powdered in a stainless-steel mortar and pestle with liquid N_2 and homogenized for