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Differences in the redox status of human visceral and subcutaneous adipose tissues – relationships to obesity and metabolic risk

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ABSTRACT

Objective. Metabolic homeostasis depends on adipocyte metabolic responses/processes, most of which are redox-regulated. Besides, visceral and subcutaneous adipose tissues (VAT and SAT, respectively) differ metabolically and in their contribution to metabolic complications, but their redox characteristics in humans are still unknown. To understand the molecular mechanisms of metabolic syndrome development, we analysed the redox characteristics of VAT and SAT in groups with various body weights and metabolic risks.

Material and Methods. Fifty premenopausal women were classified according to body mass index into normal-weight and obese groups, and these groups were further sub-classified into metabolically healthy and metabolically obese ("at risk") based on the homeostasis model assessment of insulin resistance (HOMA-IR) index and the triglyceride, total-, LDL- and HDL-cholesterol levels. Antioxidant components, NADPH oxidase protein and 4-hydroxynonenal (4-HNE) levels were analysed in VAT and SAT.

Results. Compared with the SAT, the VAT showed a higher basal level of glutathione (GSH) and GSH-dependent enzyme activities. Compared with the metabolically healthy normal-weight controls, the obese groups of women showed lower GSH levels in both depots. However, in these groups, additional prooxidative changes (increased NADPH oxidase and 4-HNE and decreased levels of SOD and/or CAT) were observed only in VAT.

Conclusions. Because of the critical role of thiol-redox homeostasis in lipogenesis, interdepot-differences in the GSH-dependent antioxidant part may be connected to the higher metabolic activity found in VAT. Analogously, the lower GSH levels that occur during

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; CAT, catalase; CuZnSOD, copper, zinc superoxide dismutase; GR, glutathione reductase; GSH, glutathione; GSH-Px, glutathione peroxidase; GST, glutathione-S-transferase; MnSOD, manganese superoxide dismutase; SOD, superoxide dismutase; totSOD, total superoxide dismutase; TR, thioredoxin reductase; Trx, thioredoxin; 4-HNE, 4-hydroxynonenal; HOMA-IR, homeostasis model assessment of insulin resistance; BMI, body mass index.

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obesity and the corresponding additional redox imbalance may be signs of VAT metabolic dysfunction that underlie the subsequent metabolic impairment.

1. Introduction

Redox regulation includes the dynamic production, interactions, and neutralisation of different reactive oxygen and nitrogen species for signal transduction [1]. Tissue antioxidant defence plays an important role in maintaining redox homeostasis not only due to its protective role but also because it sets the steady-state level of reactive species [2]. The intracellular redox buffering capacity is primarily provided by superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and glutathione peroxidase (GSH-Px, 1.11.1.9) as well as the glutathione (GSH) and thioredoxin (Trx) systems that include GSH, Trx and the enzymes involved in their redox metabolism. These antioxidant constituents have overlapping but also specific and well-orchestrated actions, which work to maintain redox balance.

The intracellular redox milieu affects different metabolic, endocrine and structural processes, which are integral parts of the functional response of various tissues, including adipose tissue [3,4]. The metabolic function of adipose tissue in buffering glucose and non-esterified fatty acids into safe fat stores is crucial for the overall maintenance of metabolic homeostasis [5]. This adipocentric view of metabolic balance indicates that reducing or overwhelming the adipogenic capacity of adipocytes (e.g., in lipodystrophies or obesity) may contribute to dyslipidemia, insulin resistance and hyperglycaemia. However, it is well established that a higher amount of visceral adipose fat, which can be estimated by an expanded waist or waist/hip circumference ratio, is a better marker of obesity-associated metabolic complications than is obesity *per se* [5,6]. The observation that visceral adiposity is particularly deleterious to health [7] has prompted a detailed examination of different fat depots.

A wealth of clinical, epidemiological and physiological data has shown many intrinsic biological differences in the fat tissues from subcutaneous and visceral depots [8–12]. The most obvious distinction between these two fat depots is that visceral adipocytes are characterised by higher metabolic activity, basal glucose uptake, and responsiveness to lipolytic stimulation and poorer antilipolytic regulation by insulin [13–15]. In obese subjects, visceral and subcutaneous adipose tissues (VAT and SAT, respectively) also show distinctive changes, and the alterations in the VAT fatty acid metabolism and adipogenic genes correlate with insulin resistance markers [16,17]. Moreover, considerable evidence has demonstrated that the increased synthesis of chemokines and proinflammatory cytokines and the decreased adiponectin level are all associated with excess levels of reactive oxidative species in the visceral depot of obese subjects [18].

In this study, we focused on the intrinsic characteristics of visceral and subcutaneous adipose tissues in the antioxidant state and the eventual changes in the tissue redox status for groups that differed by having obesity and/or increased metabolic risk. We examined the protein levels/activities of the most important tissue antioxidant constituents, the protein content of a superoxide generating enzyme (NADPH oxidase) and the active aldehydic product of lipid peroxidation (4-hydroxynonenal) in the VAT and SAT of normal-weight and obese women with different metabolic profiles: metabolically healthy and metabolically obese ("at risk").

2. Methods

2.1. Subjects and sample collection

This study conformed to the standards set by the latest revision of the *Declaration of Helsinki*. The subjects volunteered for the study and signed an informed consent form. All procedures were approved by the Ethics committee of the Clinical Center of Vojvodina. The study group consisted of 30 overweight or obese women and 20 normal-weight women who were hospitalised for elective surgery. The subjects were premenopausal (with regular menses for the last 6 months) with an average age of 42.88 ± 7.17 years and a stable body weight for the last 6 months. The indications for laparotomy were a benign case of one of the following: uterine myomas, cholelythiasis and ovarian cysts. The body mass index (BMI) was used to classify the women as normal weight, overweight or obese [19]. The body composition was assessed using a Tanita Body Composition Analyzer BC-418 MA III (Tanita Corporation, Tokyo, Japan). The waist and hip circumference were measured to assess the fat distribution. To determine the metabolic profile, we used the criteria outlined by Karelis *et al.* [20]. Metabolically healthy individuals, regardless of nutritional level, were identified as those who fulfilled four out of five criteria: homeostasis model assessment of insulin resistance (HOMA-IR) <1.95, triglycerides <1.7 mmol/L, total cholesterol <5.2 mmol/L, LDL cholesterol <2.6 mmol/L and HDL cholesterol >1.1 mmol/L. The subjects were classified as metabolically healthy normal-weight, metabolically obese normal-weight, metabolically healthy obese, and "at risk" obese. The total cholesterol and total triglyceride levels were determined by an enzyme-based method, the HDL-cholesterol levels were determined by the precipitation method with sodium phosphowolframate, and the LDL-cholesterol levels were calculated using the formula of Friedewald *et al.* [21]. The fasting glucose levels were determined by the Dialab glucose GOD-PAP method, and the serum insulin levels were determined by ELISA. The HOMA-IR was used as a measure of insulin resistance (HOMA-IR = fasting glucose (mmol/L) × fasting insulin ((μU/mL)/22.5). Anthropometric measurements, body composition assessment, and blood sampling were performed before surgery. The metabolically obese normal-weight women had significantly higher levels of total and LDL cholesterol than those of the metabolically healthy normal-weight women. Compared