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Important mitochondrial proteins in human omental adipose tissue show reduced expression in obesity

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ABSTRACT

Impaired mitochondrial function is important in obesity and the development of insulin resistance and diabetes. The aim of this study was to identify human adipocyte-derived mitochondrial proteins associated with obesity. Mitochondrial proteins from 20 abdominal omental adipose tissue biopsies (13 obese and 7 control subjects) were separated by anion-exchange chromatography coupled to SDS-PAGE. Protein contents were compared and identified by MALDI-TOF-TOF mass spectrometry. Proteins of interest were validated, verified and quantified using immuno dot blot assays in a total of 76 mitochondrial preparations from both obese and non-obese patients. Mass spectrometric comparison of 20 mitochondrial proteomes yielded 62 proteins that were differentially expressed in adipose tissue of obese subjects. The immunological quantification of 12 mitochondrial proteins from 76 omental adipose tissue biopsies revealed four proteins, citrate synthase, HADHA, LETM1 and mitofilin inversely being associated with BMI, and mitofilin being inversely correlated with gender.

Biological significance
The finding that obese human subjects have reduced levels of important mitochondrial proteins in adipocytes of omental adipose tissue as compared to non-obese controls gives new insights in the impairment of mitochondrial function in this specialized compartment of human adipose tissue in obesity and may eventually lead to the definition of valuable obesity markers.

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1. Introduction

The frequencies of obesity and related comorbidities such as diabetes, hypertension, dyslipidemia and coronary heart disease are rapidly increasing worldwide. Lifestyle changes meet metabolic and genetic conditions in susceptible humans that lead to inappropriate storage of energy mainly as triglycerides in human adipose tissues [1]. The human adipose tissue shows compartmentalization: Subcutaneous adipose tissue mainly serves as a store of energy, while an increase of omental adipose tissue in obese subjects caused by nutrient excess is accompanied by an elevation of proinflammatory cytokines (e.g. tumour necrosis factor-alpha, interleukin-6) and leptin and by a reduction of hormonal adipokines (e.g. adiponectin, visfatin) [1–3]. The intra-abdominal and omental fat depots are thought to store fat less efficiently than peripheral fat tissues, with the consequence of fat redistribution to the liver and muscle, giving rise to insulin resistance in these tissues [4–6]. These observations are supported by proteome analysis of whole adipocytes of the two major types of fat depots in man; they revealed higher metabolic activity as well as increased cell stress in the omental compared to the subcutaneous fat [7] and a reduction of proteins involved in metabolism in human omental adipose tissue [8].

Analysis of the mitochondrial proteome of omental fat therefore may be of particular interest. Generally, such analyses are a prerequisite for the understanding of potential association of mitochondrial dysfunction with certain disease states [9]. As mitochondria control ATP production, energy expenditure and the generation of ROS, impairment of mitochondrial function in obesity (e.g. by changes in proinflammatory cytokines, adipokines and ensuing changes of lipid and glucose metabolism) [10,11], morphological changes of mitochondria and altered mitochondrial DNA, RNA or protein levels are all important aspects for the development of various types of pathologies [12–18]. Currently, over 160 diseases have been linked with mutations in mitochondria-localized proteins [19].

Whereas the cellular proteome of adipocytes in human subcutaneous [7,20] and omental fat [7] has been analysed in detail, the mitochondrial proteome of human adipocytes has not yet been studied. The first human tissue chosen for mitochondrial proteome analysis was human placenta [21]; later similar studies were extended to skeletal muscle and heart tissue [22,23,24] or cell lines [17,25]. A number of other mitochondrial proteome analyses have been carried out, e.g. in mouse and rat tissues (reviewed in [26,27]). Like for these tissues, the identification and understanding of the role of specific mitochondrial proteins in adipose tissues associated with metabolic disorders are of particular interest for the development of novel therapeutic strategies.

In previous studies we have shown that obesity is associated with an increase in mitochondrial DNA-content [28] and a decreased protein level and enzyme activity of citrate synthase in human omental fat [29]. We now extended our studies on changes of specific proteins in the whole proteome of human mitochondria in omental adipose tissue of obese subjects.

2. Methods and procedures

2.1. Patients and tissue samples

A total of 76 obese and non-obese non-diabetic patients undergoing surgery at the St. Claraspital Basel, Switzerland, were included in this study. The 76 biopsies allowed sorting of the samples according to BMI, gender and age of patients (Table 1). C-reactive protein was significantly higher in the obese patients, other metabolic parameters (fasting glucose, ASAT) showed a non-significant tendency to higher values in the obese group, indicative for the well-known metabolic abnormalities in obesity like subclinical inflammation, insulin resistance and fatty liver. The study was approved by the local ethics committee and the patients participated on a voluntary basis after having given written informed consent. Omental adipose tissue biopsies of at least the size of a chicken egg were collected during bariatric or conventional visceral surgery and immediately processed by purifying the adipocytes and isolating their mitochondria (Fig. 1).

2.2. Purification of adipocytes

Minced human adipose tissue was resuspended in modified Krebs Ringer buffer (MKRB) (5 mM D-glucose, 2% BSA, 100 mM Hepes, 100 mM KCl, 123 mM NaCl, 1.3 mM $CaCl_2$) containing collagenase type 2 (1 mg/ml) and subjected to digestion of connective tissue on an orbital shaker at 37 °C. After digestion, cells were separated from connective tissue by serial filtrations through a tea strainer equipped with fine nylon mesh. Adipocytes were separated from other cells by mild centrifugation, leaving adipocytes on top of the cell suspension. Isolated adipocytes were washed three times with MKRB, followed by a PBS wash step.

2.3. Isolation of mitochondria

Purified adipocytes were resuspended in homogenization buffer (HB) (200 mM mannitol, 50 mM sucrose, 1 mM EDTA, 20 mM Hepes, pH 7.4). Lysis was performed with two soft ultrasonic pulses. After centrifugation at 700 *g* for 11 min, the post-nuclear supernatant was collected and further centrifuged at 7000 *g* for 19 min. Subsequently, the pelleted mitochondria were resuspended in HB and recentrifuged at 7000 *g* for 19 min. Finally, the mitochondria were resuspended in HB and the protein concentration was measured using bicinchoninic acid (BCA) protein assay. The protein yields obtained from mitochondrial preparations were related to the mass of the biopsy. More than 1 mg of enriched mitochondrial protein was obtained from 20 clinical samples and less than 1 mg from the remaining 56 samples. A more rigorous purification of mitochondria from these samples was prevented by limitations in sample availability.

2.4. Mitochondrial proteome analysis

Comparative proteome analysis was performed on the subset of 20 patients with a higher protein yield, which were divided into 7 non-obese (BMI < 30 kg/m^2) and 13 obese patients (BMI ≥ 30 kg/m^2) (Table 1).