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Mature adipocyte proteome reveals differentially altered protein abundances between lean, overweight and morbidly obese human subjects

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ABSTRACT

Overweight (OW) and obese individuals are considered to be graded parts of the scale having increasing weight as a common feature. They may not, however, be part of the same continuum and may differ metabolically. In this study we applied an untargeted proteomic approach to compare protein abundances in mature adipocytes derived from the subcutaneous adipose tissue of overweight and morbidly obese female subjects to those of lean age matched controls. Mature adipocytes were isolated from liposuction samples of abdominal subcutaneous adipose tissue collected from both lean (L; n = 7, 23.3 ± 0.4 kg/m^2; mean BMI ± SD), overweight (OW; n = 8, 27.9 ± 0.6 kg/m^2; mean BMI ± SD) and morbidly obese (MOB; n = 7, 44.8 ± 3.8 kg/m^2; mean BMI ± SD) individuals. Total protein extracts were then compared by two-dimensional difference in gel electrophoresis (2D DIGE). One hundred and ten differentially expressed protein spots (i.e., fitting the statistical criteria ANOVA test, $p < 0.05$; fold-change ≥1.5) were detected, and of these, 89 were identified by MALDI-TOF mass spectrometry. Of these, 66 protein spots were common to both groups whereas 23 were unique to the MOB group. Significant differences were evident in the abundances of key proteins involved in glucose and lipid metabolism, energy regulation, cytoskeletal structure and redox control signaling pathways. Differences in the abundance of some chaperones were also evident. The differentially abundant proteins were investigated using Ingenuity Pathway Analysis (IPA) to establish their associations with known biological functions. The network identified in the OW group with the highest score relates cell-to-cell signaling and interaction; in contrast, in the MOB group the major interacting pathways are associated with lipid metabolism, small molecule biochemistry and cancer. The differences in abundance of the differentially regulated proteins were validated by immunoblotting. These findings provide insights into metabolic differences in OW and MOB individuals.

1. Introduction

Obesity is a complex, multifactorial condition that involves appetite regulation and energy metabolism. It is characterized by the deposition of excess energy as neutral fats, notably triglycerides, within white adipose tissue (WAT). Deposition occurs at various locations, but primarily in the subcutaneous and visceral adipose tissues. The American Medical Association recently recognized obesity as a disease state, based on its increasing global prevalence and its impact on an individual's overall health. Most notably, obesity leads to serious clinical comorbidities such as insulin resistance, atherosclerosis, cardiovascular disease, neurodegenerative disorders and some forms of cancer (Grundy, 2000).

Abbreviations: AFABP, adipocyte fatty acid-binding protein; APOA-I, apolipoprotein A-I; ATP, adenosine triphosphate; BMI, body mass index; CVD, cardiovascular disease; 2D-DIGE, 2 dimensional difference in-gel electrophoresis; ECM, extracellular matrix; ETC, electron transport chain; ECHM, enoyl CoA hydratase mitochondrial; EFABP, epidermal fatty acid binding protein; GLUT 4, glucose transporter 4; Hb, hemoglobin; IEF, isoelectric focusing; IPA, Ingenuity Pathway Analysis; LDL, low-density lipoprotein; L, lean; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; MOB, morbidly obese; OW, overweight; SCAT, subcutaneous adipose tissue; SD, standard deviation; SCAD, short chain acyl-coenzyme A dehydrogenase; TCA, tricarboxylic citric acid cycle; TOF, time-of-flight; VAT, visceral adipose tissue; WAT, white adipose tissue.

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While it is generally considered that there is a continuum in weight gain from lean to severe (or morbid) obesity, the spectrum is typically segregated into separate groups based on body mass index (BMI): i.e., lean (BMI 17.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2), obese (BMI 30–39.9 kg/m^2) and severe or morbidly obese (BMI ≥40 kg/m^2). Any metabolic, biological or physiologic differences observed in the obese groups (i.e., obese and morbidly obese) are projected to occur in the overweight group and are thought to differ only in degree. In fact, the terms overweight and obese are often used interchangeably (Greenberg and Obin, 2006). Overweight and obese states are therefore typically considered to be metabolically equivalent with the only distinction being in the magnitude of differences in body weight or size.

Proteomic and gene studies have aimed to differentiate between the lean and obese populations at a molecular level (Mardinoglu et al., 2013; Perez-Perez et al., 2012a), but we know of no studies that have attempted to study overweight individuals separately. There is, however, evidence to suggest that there may be unique and profound changes associated with the obese states that are not seen in the overweight population. These changes may be responsible for the increased risks associated with significant weight gain and this study aimed to establish and identify these differences for the first time.

With this objective in mind, we segregated individuals into lean (L), overweight (OW) and morbidly obese (MOB) groups and used a powerful and quantitatively precise proteomic strategy, combined with network analysis, to identify the metabolic and regulatory pathways that may vary with pronounced changes in weight.

2. Materials and methods

2.1. Ethical considerations and informed consent

Prior to implementation, all procedures and protocols were reviewed and approved by the Institutional Review Board, College of Medicine, King Saud University. Written, informed consent was obtained from all participants. This study was conducted at the Obesity Research Center, College of Medicine, King Saud University, Riyadh, Saudi Arabia.

2.2. Study design

Subcutaneous abdominal tissue (SCAT) fat was obtained by liposuction from L, OW and MOB female patients. Subjects were classified into each group on the basis of their BMI: i.e., lean/control (n = 7; BMI < 25 kg/m^2; group L), overweight (n = 8; BMI 25–29.9 kg/m^2; group OW) and morbidly obese (n = 7; BMI ≥ 40 kg/m^2; group MOB). Total protein was isolated from the adipocytes, the constituent proteins were separated and quantified by 2-dimensional difference in-gel electrophoresis (2D-DIGE), and then identified by matrix assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS). Differentially abundant proteins were entered into the Ingenuity Pathway Analysis (IPA) software program (Ingenuity Systems, Inc., USA) to predict their probable associations with known biological pathways and functions.

2.3. Subjects and biological samples preparation

SCAT samples were collected from otherwise healthy, non-diabetic female subjects, free from other comorbidities who were undergoing elective liposuction surgery. The participants were age matched and grouped according to differences in their BMI: i.e., lean (L; n = 7, 23.3 ± 0.4 kg/m^2; mean BMI ± SD), overweight (OW; n = 8, 27.9 ± 0.6 kg/m^2; mean BMI ± SD) and morbidly obese (MOB; n = 7, 44.8 ± 3.8 kg/m^2; mean BMI ± SD) (see Appendix: Supplementary Table S1). Samples were processed on the day of harvesting according to the previously published method (McTernan et al., 2002). Briefly, the lipo-aspirates were digested with collagenase class 1 (2 mg/mL, in 1× HBSS, for 30 min at 37 °C) in a shaking water bath. The disrupted tissue was filtered through a sterile double-layered cotton mesh and the mature adipocytes were separated from the stromo-vascular fraction (SVF) by centrifugation (360 × *g*, 5 minutes, room temperature (RT)). The floating layer of mature adipocytes was removed from the collagenase-dispersed preparation and from the SVF pellet, washed three times in Dulbecco's modified eagle's medium, and stored at –80 °C until analysis.

2.4. Protein extraction

Proteins were extracted from floating mature adipocytes as previously described (McTernan et al., 2002). Briefly, floating mature adipocytes (1 mL) were re-suspended in lysis buffer (1.5 mL, pH 8.8, 30 mM Tris buffer containing 7 M urea, 2 M thiourea, 2% Chaps, 1× protease inhibitor mix), the samples were gently rocked (20 minutes, RT) and then sonicated (Microsonicator, Qsonica Sonicators, USA; 30% pulse, two intervals of 1 minute each, separated by a 1 minute gap). Fifty mM dithiothreitol (DTT) was then added and the protein extracts centrifuged (20,000 × *g*, 40 minute, 4 °C). The pellet was removed and the solubilized protein in the supernatant was precipitated using a 2D clean-up kit according to the manufacturer's protocol (GE Healthcare, USA).

2.5. Protein labeling with cyanine dyes

The protein pellets were solubilized in labeling buffer (7 M urea, 2 M thiourea, 30 mM Tris–HCl, 4% CHAPS, pH 8.5). Insoluble material was pelleted by centrifugation (12,000 × *g*, RT, 5 min), protein concentrations were determined in triplicate using the 2D-Quantkit (GE Healthcare, USA), and the pH of the samples was adjusted to 8.5 using NaOH (100 mM). Proteins were labeled (400 pmol of CyDye™ DIGE Fluor dyes, GE Healthcare, UK) in 1 μL of DMF and then mixed with sample containing 50 μg of protein. Samples were incubated on ice for 30 min in the dark. The labeling reaction was terminated by adding 1 μL of 10 mM lysine. Each sample was covalently labeled with either Cy3 or Cy5. A mixture of equal amounts of protein isolated from each and every sample in the experiment was labeled with Cy2 and used as an internal standard (see Appendix: Supplementary Table S2).

2.6. Two-dimensional electrophoresis and image scanning

For the first dimension of separation, 11 Immobiline dry strips (24 cm, pH 3–11; GE Healthcare, Sweden) were passively rehydrated (30 V, 12 hours), followed by isoelectric focusing using an Ettan IPGphor IEF unit (GE Healthcare, Sweden). Focusing was performed at 20 °C and 50 μA per strip according to the following step and hold sequence: i.e., (1) 500 V for 1 h, (2) 1000 V for 1 h, (3) 8000 V for 3 h, (4) 8000 V for 45,000 Vhrs. IPG strips were then stored at –80 °C until the next step could be performed. Immediately prior to the second dimension separation the IPG strips were equilibrated, first with dithiothreitol (15 minutes, RT, gentle stirring, 5 mM Tris–HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 65 mM). Strips were then equilibrated for 15 minutes in the same solution containing 250 mM iodoacetamide. Polyacrylamide gradient gels (5–20%) were prepared on low fluorescence glass using a 2-D Optimizer (Nextgen Sciences, UK). Second dimension sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) followed (Ettan DALT six vertical units, GE Healthcare, Sweden; 15 °C, 1 W per gel for 1 h and then 2 W per gel) until the bromophenol blue dye front reached the bottom of the gel. Gels were then scanned (Typhoon Imager (Trio)

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