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Characterization of the Human Adipocyte Proteome and Reproducibility of Protein Abundance by One-dimensional Gel Electrophoresis and HPLC-ESI-MS/MS

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Abstract

Abnormalities in adipocytes play an important role in various conditions, including the metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease, but little is known about alterations at the protein level. We therefore sought to 1) comprehensively characterize the human adipocyte proteome for the first time, and 2) demonstrate feasibility of measuring adipocyte protein abundances by one-dimensional SDS-PAGE and High Performance Liquid Chromatography - Electron Spray Ionization - tandem Mass Spectrometry (HPLC-ESI-MS/MS). In adipocytes isolated from ~0.5 g subcutaneous abdominal adipose tissue of three healthy, lean subjects we identified a total of 1493 proteins. Triplicate analysis indicated a 22.5% coefficient of variation of protein abundances. Proteins ranged from 5.8 to 629 kDa and included a large number of proteins involved in lipid metabolism, such as fatty acid transport, fatty acid oxidation, lipid storage, lipolysis and lipid droplet maintenance. Furthermore, we found most glycolysis enzymes and numerous proteins associated with oxidative stress, protein synthesis and degradation as well as some adipokines. 22% of all proteins were of mitochondrial origin. These results provide the first detailed characterization of the human adipocyte proteome, suggest an important role of adipocyte mitochondria, and demonstrate feasibility of this approach to examine alterations of adipocyte protein abundances in human diseases.

Keywords

adipocyte; mitochondria; proteomics; human

INTRODUCTION

Adipocytes, the major cell type of adipose tissue, contain the body's largest storage of energy in the form of triglyceride in humans¹. Adipocyte lipid metabolism is highly regulated according to the body's energy demand and involves complex interactions of multiple cellular structures and organelles, including the plasma membrane, mitochondria, endoplasmic reticulum, peroxisomes and lipid droplets. In addition, adipocytes synthesize and secrete bioactive molecules collectively termed adipokines, which can act in an autocrine, paracrine, intracrine and/or endocrine fashion². Both, a reduced ability to take up and retain free fatty acids and abnormalities in the release of adipokines by adipocytes, as it occurs in excess adipose tissue and lack of adipose tissue, can cause metabolic

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Supporting Information Available

Supplementary tables and figure. This material is available free of charge via the Internet at <http://pubs.acs.org>.

derangements such as insulin resistance, type 2 diabetes mellitus (T2DM), and cardiovascular disease³, indicating that normal adipocytes are critical for human wellbeing.

Several studies have examined global gene expression of human adipose tissue and its changes in obesity⁴ and during energy surplus⁵, as well as differences between intra-abdominal or visceral and subcutaneous abdominal adipose tissues^{6–7} using microarray techniques. Moreover, changes in global gene expression during the differentiation of preadipocytes to adipocytes using mesenchymal stem cells or preadipocytes and adipocytes isolated from subcutaneous abdominal fat have been examined^{8–9}. However, gene expression provides only limited information because of its generally poor correlation with protein expression¹⁰.

Presently, some information is available only on the proteome of human adipose tissue^{11–17}, which contains connective tissue matrix, nerve tissue, stromal vascular cells and immune cells¹⁸ in addition to adipocytes, and only from studies using two-dimensional gel electrophoresis (2-DE) in combination with mass spectrometry. 2-DE is very restricted in the detection of low abundance, very hydrophobic, extreme molecular weight and extreme pI proteins. Consequently, the most comprehensive study of the human adipose tissue proteome to date identified only 359 proteins despite the use of antibody arrays in addition to 2-DE¹². Furthermore, 2-DE is relatively inaccurate in protein quantification^{19–21}, significantly limiting the comparison of protein abundances between groups of subjects^{11–17}.

The present study was therefore undertaken to 1) comprehensively characterize the proteome of human adipocytes for the first time, and 2) demonstrate feasibility of measuring adipocyte protein abundances using a large-scale proteomics approach. To this end, we analyzed the proteome of subcutaneous abdominal adipocytes from three subjects and performed triplicate analysis using a combination of one-dimensional gel electrophoresis and HPLC-ESI-MS/MS. Our results show excellent coverage of all major metabolic pathways and cellular processes and structures as well as respectable reproducibility of protein abundance measurements. They hence hold promise for future proteomics studies to further our understanding of human adipocyte biology and abnormalities in various diseases and conditions, such as insulin resistance and T2DM.

MATERIALS AND METHODS

Tissue

Normally discarded and de-identified human adipose tissue samples were obtained from a local plastic surgeon under exemption from the Institutional Review Board at Arizona State University. The samples were obtained from three healthy, lean female subjects (37, 38, 59 years of age; body mass index 20.5, 23.3, 24.3 kg/m², respectively) with normal glucose tolerance undergoing elective abdominoplasty.

Adipocyte Preparation and Staining

Fresh adipose tissues were dissected free from skin and vasculature then minced with scissors into pieces of about 2 mm³. From each adipose tissue sample approximately 0.5 gram was used for the present study. Tissues were digested in 20 ml Krebs-Ringer-Bicarbonate (KRB) isolation buffer (1.2 mM CaCl₂, 4.4 mM KCl, 1.2 mM MgSO₄, 1.5 mM KH₂PO₄, 116 mM NaCl, 29 mM NaHCO₃; pH 7.6) containing 3.5% fatty acid free (FFA-free) bovine serum albumin (BSA; Sigma; St. Louis, MO), 5 mM glucose and 0.75 mg/ml collagenase A (Roche Applied Sciences; Indianapolis, IN) under constant agitation (225 r.p.m) at 37°C in a rotary incubator shaker for 1 hour. The resulting digestion was passed through 1000 micron and 250 micron nylon mesh and the isolated adipocytes were

washed three times with 20 ml Tris wash buffer (20 mM Tris, 2 mM EGTA, 0.5 M sucrose, pH 7.6, preheated to 37°C). An aliquot of intact live adipocytes was stained with Mitotracker Green FM (Invitrogen; Carlsbad, CA; 1 μ M) for 30 min at 37°C. The remaining washed adipocytes were stored at -80°C for proteomic analysis.

Protein Preparation, Electrophoresis and Staining

To prepare adipocyte proteins for proteomic analysis, the adipocyte preparations were homogenized by freeze-thaw ($3 \times$ liquid N₂ and 37°C) followed by the addition of lysis buffer consisting of a final concentration of 50 mM HEPES, pH 7.6, 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM beta-glycerophosphate, 10 mM NaF, 5% SDS, 2 mM Na₃VO₄, 2 mM PMSF, 10 μ g/ml leupeptin and 10 μ g/ml aprotinin. The suspensions were put on ice-water bath for 5 minutes of sonication followed by 30 minutes of incubation at 4°C. The samples were centrifuged at $14,000 \times g$ (10 min, 4°C) and the resulting supernatants were used for proteomic analysis. Protein concentrations were determined using the Coomassie Plus Protein assay (Pierce, IL). In each sample, 40 μ g of adipocyte protein was separated by precast one-dimensional SDS polyacrylamide gel (12%, Bio-Rad, Hercules, CA). Proteins were visualized with Coomassie blue stain (Bio-Rad, Hercules, CA).

In-gel Digestion

The resulting gel lanes from each experiment were cut into 20 slices of approximately equal size, and each slice was cut into 1 mm² pieces prior to digestion. The gel pieces were placed in a 0.6 ml polypropylene tube, washed with 400 μ l of water, destained twice with 300 μ l of 50% acetonitrile (ACN) in 40 mM NH₄HCO₃ and dehydrated with 100% ACN for 15 minutes. After removal of ACN by aspiration, the gel pieces were dried in a vacuum centrifuge at 62°C for 30 minutes. Trypsin (250 ng; Sigma Chemical Co., St. Louis, MO) in 20 μ l of 40 mM NH₄HCO₃ was added and the samples were maintained at 4°C for 15 minutes prior to the addition of 50 μ l of 40 mM NH₄HCO₃. The digestion was allowed to proceed at 37°C overnight and was terminated by addition of 20 μ l 5% formic acid (FA). After incubation at 37°C for an additional 30 minutes and centrifugation for 1 minute, each supernatant was transferred to a clean polypropylene tube. The extraction procedure was repeated using 40 μ l of 0.5% FA, and the two extracts were combined. The sample volume was reduced to ~5 μ l by vacuum centrifugation, and the resulting peptide mixtures were purified by solid-phase extraction (C18 ZipTip; Millipore, Billerica, MA) after sample loading in 0.05% heptafluorobutyric acid:2% formic acid (vol/vol) and elution with 4 μ l 50% acetonitrile:1% formic acid (vol/vol) and 4 μ l 80% ACN:1%FA (v/v), respectively. The two eluates were combined and the samples were dried by vacuum centrifugation. 10 μ l 0.5% FA: 2%ACN (v/v) was added.

Mass Spectrometry

HPLC-ESI-MS/MS was performed on a hybrid linear ion trap (LTQ)-Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer (LTQ FT; Thermo Fisher; San Jose, CA) fitted with a PicoView™ nanospray source (New Objective, Woburn, MA). The mass spectrometer was calibrated weekly according to manufacturer's instructions, achieving mass accuracy of the calibrants within 2 ppm. On-line capillary HPLC was performed using a MichromBioResources Paradigm MS4 micro HPLC (Albany, CA) with a PicoFrit™ column (New Objective; 75 μ m i.d., packed with ProteoPep™ II C18 material, 300 Å). HPLC separations were accomplished with a linear gradient of 2 to 27% ACN in 0.1 % FA in 65 minutes, a hold of 5 minutes at 27% ACN, followed by a step to 50% ACN, hold 5 minutes and then a step to 80%, hold 5 minutes; flow rate, 400 nl/min. A "top-10" data-dependent tandem mass spectrometry approach was utilized to identify peptides in which a full scan spectrum (survey scan) was acquired followed by collision-induced dissociation

(CID) mass spectra of the 10 most abundant ions in the survey scan. The survey scan was acquired using the FTICR mass analyzer in order to obtain high resolution, high mass accuracy data.

Data Analysis and Bioinformatics

Briefly, tandem mass spectra were extracted from Xcalibur “RAW” files and charge states were assigned using the Extract_MSN script that is a component of Xcalibur 2.0 SR2 (Thermo Fisher; San Jose, CA). The fragment mass spectra were searched against the IPI_HUMAN_v3.59 database (80,128 entries, <http://www.ebi.ac.uk/IPI/>) using Mascot (Matrix Science, London, United Kingdom, version 2.2). The false discovery rate was determined by selecting the option to use a “decoy” randomized search strategy that is available in Mascot, v2.2. The search parameters that were used were: 10 ppm mass tolerance for precursor ion masses and 0.5 Da for product ion masses; digestion with trypsin; a maximum of two missed tryptic cleavages; variable modifications of oxidation of methionine and phosphorylation of serine, threonine, and tyrosine. Probability assessment of peptide assignments and protein identifications were made using Scaffold (version Scaffold-2_00_06, Proteome Software Inc., Portland, OR). Only peptides with ≥95% probability were considered. Criteria for protein identification included detection of at least 2 unique identified peptides and a probability score of ≥99%. Proteins that contained identical peptides and could not be differentiated based on MS/MS analysis alone were grouped. The spectra assigned to shared peptides were not used for determining protein abundance. Multiple isoforms of a protein were reported only if they were differentiated by at least one unique peptide with ≥95% probability, based on Scaffold analysis.

Normalized Spectral Abundance Factors and Reproducibility

To determine protein abundance, normalized spectral abundance factors (NSAF) were used²². MS/MS spectra assigned to a protein were normalized to the length of the protein (number of amino acids), resulting in a Spectral Abundance Factor, or SAF, $SAF = \text{SpectrumCount} / \text{NumberAA}$. Each SAF was normalized against the sum of all SAFs in one sample, resulting in the NSAF value. For a protein, i , the normalized spectral abundance

factor, $NSAF$, is calculated by $NSAF_i = SAF_i / \sum_{i=1}^N SAF_i$, where N is the total number of proteins detected in a sample. Thus, NSAF values allow for direct comparison of a protein's abundance between individual runs in a fashion similar to microarray data analysis^{23–24}.

To test reproducibility, adipose tissue from one subject was divided into three sections. Adipocytes were immediately isolated from these sections by digestion as described above. The three adipocyte samples (40 µg of lysate proteins) were subsequently processed on separate days for proteome analysis as described above.

Gene Ontology Annotation

Gene Ontology annotation of human proteins was downloaded from Gene Ontology Annotation (GOA) Databases (<http://www.ebi.ac.uk/GOA>, version 55.0). This GOA human database contains 33,731 distinct proteins and 172,661 GO associations. In addition, GO hierarchy information (version 52) was downloaded from <http://www.geneontology.com>. Human GO associations and GO hierarchy information were assembled into a new database by an in-house script written using MATLAB. IPI IDs (International Protein Index ID), gene names, UniProt and SwissProt IDs of identified proteins were entered into the database to obtain GO associations and GO hierarchy information. Furthermore, gene IDs for identified proteins without subcellular localization information in GOA were manually entered into <http://www.genecards.org> to retrieve that additional information.

Mus Musculus Orthologs Search

To compare our present human adipocyte proteome data to the 3T3-L1 adipocyte proteome data reported by Adachi et al.²⁵, a xref table of mouse to human IPI IDs was downloaded from BioMart version 0.7. Each human IPI ID of the present adipocyte proteome data was mapped to one or more mouse orthologs IPI ID, which were then compared to the 3T3-L1 adipocyte proteome data.

Western Blot Analysis

To confirm protein expression by conventional methods, proteins (40 µg) from adipocyte lysates were separated by SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). Membranes were immunoblotted with the following rabbit anti-human antibodies from Abcam (Cambridge, MA) at 1:1000 dilution unless otherwise stated: glutathione peroxidase 4 (ab16800), integrin alpha 1 (ab90279, mouse anti-human), Annexin A11 (ab91342, 1:500 dilution), resistin (ab78172), IL-6 (ab6672), leptin (ab7208) and TNF-alpha (ab9739). Anti-rabbit/mouse IgG, HRP-linked antibody (cell signaling Technology, Inc., Danvers, MA) was used as a secondary antibody. The specific proteins were then visualized by enhanced chemiluminescence kit (GE Healthcare BioSciences, Piscataway, NJ).

RESULTS

Morphology of Adipocytes and Adipocyte Mitochondria

To examine the purity, integrity and morphology of adipocytes isolated from adipose tissue, aliquots of isolated adipocytes were analyzed by light microscopy (Fig. 1A–D). Furthermore, since adipocyte mitochondria have recently been implicated in playing a role in insulin resistance, adipocyte aliquots were stained with Mitotracker FM Green and analyzed by fluorescence microscopy. As shown in Figure 1, the isolation procedure resulted in highly pure and intact adipocytes appearing as large, round cells (Fig. 1A, C). In approximately 80% of the adipocytes, mitochondria predominantly appeared as punctuate structures distributed throughout the cytoplasm with an increased concentration near the perinuclear region (Fig. 1B); in approximately 20% of the adipocytes, mitochondria predominantly appeared as tubular, reticular structures (Fig. 1D).

Reproducibility of Protein Abundance

To assess reproducibility, three separate portions of adipose tissue from a single subject were individually processed, including adipocyte isolation, protein separation by one-dimensional gel electrophoresis, trypsinization and analysis by HPLC-ESI-MS/MS. Of the 1674 proteins that were detected in these reproducibility experiments, 1203 proteins (72%) were common to all three replicates (Fig. 2A). The false discovery rate was 5.63%, 5.10% and 5.18% at the peptide level or 0.32%, 0.26% and 0.27% at the protein level, respectively. The average coefficient of variation of the normalized spectral abundance factor of these proteins was 22.5%. Power analysis (NQuery Advisor, Statistical Solutions, Saugus, MA) revealed that, using a coefficient of variation of 22.5%, 8 subjects per group is required to detect a two-fold difference with 95% power at the 0.05 level.

Characterization of the Human Adipocyte Proteome

To characterize the human adipocyte proteome, adipocytes isolated from subcutaneous abdominal adipose tissue from three healthy individuals were subjected to 1-DE followed by HPLC-ESI-MS/MS analysis. We identified 1210, 1312 and 1003 unique proteins in adipocytes from these individuals, respectively (Fig. 2B), with a peptide false discovery rate of 5.26%, 5.63% and 5.27%, respectively. Since 2 unique peptides were required for each

protein, the false discovery rate at the protein level was 0.28%, 0.32%, 0.28%, respectively. The total number of unique proteins identified in the isolated adipocytes was 1493. Of these, 874 proteins (59%) were found in all three individuals (Fig. 2B) and 433 had not been detected in 3T3-L1 adipocytes reported by Adachi et al.²⁵, the most comprehensive adipocyte proteome to date. To confirm the expression of proteins in human adipocytes not previously detected in 3T3-L1 adipocytes, Western blot analysis was performed for the expression of a few of these that vary significantly in molecular weight and cellular function, which include GPX4, ANXA11 and ITGA1. All three proteins were readily detected as shown in supplemental Figure 1. A detailed list of all 1493 unique proteins identified by 1-DE and HPLC-ESI-MS/MS together with their IPI ID, amino acid sequence, sequence coverage, and maximal number of unique peptides assigned to each protein is provided in supplemental Table 1. Proteins not previously detected in 3T3-L1 adipocytes are highlighted.

The molecular weight (MW) distribution of the 1493 unique proteins is shown in Fig. 2C. Proteins ranged from 5.8 kDa (ATP synthase subunit epsilon-like protein, mitochondrial) to 629 kDa (Neuroblast differentiation-associated protein AHNAK). Most proteins (more than 1/3rd) had a MW of 20–39 kDa and nearly 80% were below 60 kDa. Twenty-three proteins had a MW <10 kDa and 114 had a MW >100 kDa. Thus, 9.2% of the identified proteins were outside the typical separation limits of two-dimensional gel electrophoresis (10–100 kDa). A similar MW distribution was found in the proteins not previously detected in 3T3-L1 adipocytes²⁵.

The subcellular location of the 1493 proteins was assigned based on the Gene Ontology annotations information from UniProt and Genecards databases (Fig. 2D). Sixty-eight percent of the 1493 identified proteins were assigned to the cytoplasm, representing the predominant subcellular location. In addition, 202 and 271 proteins were attributed to the plasma membrane and nucleus. Of the 1015 cytoplasm proteins, 383, 241, 151 and 62 proteins were assigned to mitochondria, cytosol, endoplasmic reticulum, and Golgi apparatus proteins respectively (Fig. 2D). It is noted that some proteins can be assigned to multiple subcellular locations. Similar proportions of the subcellular location were found for the proteins not previously detected in 3T3-L1 adipocytes²⁵.

Coverage of Adipocyte Protein based on Function

Lipid Metabolism—In adipocytes, lipid metabolism involves the transport of fatty acids across the plasma membrane, fatty acid transport into mitochondria and β -oxidation, fatty acid esterification into triacylglycerol (TAG), lipolysis, and lipid droplet maintenance. The transport of fatty acids from the systemic circulation into adipocytes requires specific fatty acid transporters²⁶, including CD36, the Fatty Acid Transport Protein (FATP) and Fatty Acid Binding Proteins (FABP). Transport of FA and lipids within the cell also requires FABP. In our study, CD36 and two FABP (FABP4 and FABP5) were identified (Figure 3A), of which FABP4 is adipocyte specific.

Activation, intracellular transport and oxidation of fatty acids: The majority of the proteins that are crucial for the activation and transport of fatty acids and subsequent degradation in the β -oxidation pathway were identified (Fig. 3A). These include enzymes involved in the oxidation of long-chain, medium-chain and short-chain fatty acids as well as enzymes required for the oxidation of unsaturated fatty acids. In addition to mitochondrial oxidation, several enzymes of peroxisomal oxidation were identified (Fig. 3A).

TAG synthesis and de-novo lipogenesis: In humans, adipocyte TAG is predominantly formed from fatty acids whereas only a small portion is formed via de novo lipogenesis.

TAG synthesis requires glycerol-3-phosphate, derived from glycolysis, and fatty acyl-CoA to be used as substrates. The latter is formed via esterification of fatty acids by acyl-coenzyme A synthetase enzymes, of which three of them were detected (ACSL1, ACSL3 and ACSL4; Figure 3A). The critical enzymes of TAG synthesis include glycerol-3-phosphate acyltransferases, acylglycerol-3-phosphate acyltransferases and diacylglycerolacyltransferases, which were all detected (Figure 3B). Furthermore, we detected all key enzymes of de novo lipogenesis including acetyl coenzyme A carboxylase (ACACA and ACACB) and fatty acid synthase (FASN)²⁶.

Adipocyte lipolysis: Adipocyte lipolysis is a highly regulated catabolic process leading to the breakdown of triglycerides into fatty acids and glycerol. The majority of proteins/enzymes that are crucial for lipolysis were identified (Fig. 3B), including hormone-sensitive lipase (LIPE), monoglyceride lipase (MGLL), and the recently discovered TAG specific enzyme adipose triglyceride lipase (PNPLA2)²⁷.

In adipocytes, lipolysis is predominantly activated by cAMP dependent pathways^{27–29}. Production of cAMP is increased by the activation of hormone receptors that are coupled to the Gs/Gi family of GTP binding proteins. In addition, activation of lipolysis can be achieved by receptor-coupled G proteins of the Gq family, through molecular mechanisms that involve protein kinase C (PKC)²⁸. Of interest and detected in the present study were Gs/Gi/Gq proteins and their regulatory proteins Gβ/γ, adenylatecyclases associated proteins, protein kinases A and C, and anchoring proteins that mediate subcellular compartmentation of PKA and PKC (Table 1).

Lipid droplet maintenance: Lipid droplets (LDs) represent ~95% of adipocyte volume and are the major cellular organelles for storage of neutral lipids (mainly TAG and sterol esters). LDs are bound by a monolayer of phospholipids³⁰ and are dynamic and heterogeneous in size, location and protein content. LDs are associated with several types of proteins^{31–32}, including structural proteins, lipid-synthesis enzymes, lipases, and membrane-trafficking proteins. The coordination of lipid storage and utilization is in part regulated by the perilipin family of LD coated proteins, containing at least 5 members: perilipin, adipophilin/adipocyte differentiation-related protein (ADRP), S3–12, tail-interacting protein of 47 kilodaltons (TIP47) and oxidative tissue-enriched PAT protein (OXPAT). Perilipin, S3–12 (named KIAA1881 in humans) and TIP47 (named M6PRBP1 in humans) were detected in our study (Table 2), of which the first two are considered adipocyte-specific LD proteins³³.

In addition to being the major cellular organelle for lipid storage, it has recently reported that LDs contain several proteins involved in the trafficking of a variety of intracellular molecules^{31–34}. These include all three small GTPase families (Rab, Arf and Rho), which mediate membrane trafficking. In the present study, a number of members from Rab, Arf and Rho and their regulatory proteins such as EEA1, ARFGAP1 were identified (Table 2). Furthermore, we detected SNAREs, motor proteins, and cytoskeletal components, which are associated with vesicular trafficking pathways and important in the regulation of LD growth^{35–36} (Table 2). Several lipid metabolism enzymes, ER proteins, chaperone proteins as well as signaling proteins reported to be associated with LDs^{31–37} were also identified (Table 2).

Other Major Metabolic Pathways

In the postprandial state, adipose tissue accounts for ~10–15% of systemic glucose uptake in humans³⁸. Most of the glucose taken up by adipocytes enters glycolysis, which plays an important role for triglyceride synthesis by providing glycerol-3-phosphate necessary for fatty acid esterification. The remaining glucose carbons may undergo anaerobic or aerobic glycolysis and enter the citric acid cycle (TCA cycle) for energy production. In the present

study, all enzymes involved in glycolytic pathway and the TCA cycle were found (Fig. 3C–D).

The TCA cycle and fatty acid oxidation produce the energy-rich electron donors, NADH and succinate. Electrons from these donors are passed through the electron transport chain (ETC) to oxygen, which is reduced to water. Proton pumping and adenosine triphosphate (ATP) production are coupled with the electrons transport. This is a multi-step redox process that occurs on the mitochondrial inner membrane. The ETC consists of five complexes, comprising 104 proteins: 46 subunits of complex I (NADH: ubiquinoneoxidoreductase), 4 subunits of complex II (succinate dehydrogenase: ubiquinoneoxidoreductase), 17 subunits of complex III (ubiquinol: cytochrome c oxidoreductase), 18 subunits of complex IV (cytochrome c oxidase), and 19 subunits of complex V (ATP synthase), of which 32, 2, 12, 9, 14 subunits were detected, respectively. Figure 4 lists the individual subunits of each complex that were identified.

Mitochondrial Protein Import

The vast majority of mitochondrial proteins are nuclear-encoded and synthesized as precursors within the cytosolic ribosomes. Mitochondria have developed a complex machinery for protein transport and sorting into different mitochondrial compartments (for review see 39). All mitochondrial protein precursors are channeled towards the TOM complex in the outer mitochondrial membrane, which is comprised of seven proteins. For proteins with complex topology such as the beta-barrel, the SAM complex, formed by 3 proteins, is involved. Proteins destined for the matrix are transferred to the TIM23 complex (4 members), and the associated motor PAM complex (5 members). In the matrix, the presequence signals are removed by processing peptidases (MPPs). Small TIM chaperones (TIM8-TIM13 and TIM9-TIM10 complex) facilitate protein import. In the present analysis, we detected 19 proteins of the mitochondrial protein import machinery. These include four of the TOM complex, three of the SAM complex, two of the TIM23 complex, four of the PAM complex, four of the small TIM complex, and two MPPs (supplemental Table 2).

Oxidative Stress Proteins

At lower concentrations, reactive oxygen species (ROS) can be beneficial, as they are used by the immune system to attack and kill pathogens⁴⁰. ROS are also important modulators of signaling cascades (e.g. glucose transport⁴¹). However, at higher concentrations, ROS are detrimental to DNA integrity and protein function, ultimately leading to cell death⁴². The balance between ROS production and ROS scavenging is therefore critical. Cells are normally able to defend themselves against ROS damage through the use of scavenger enzymes including superoxide dismutases, catalases, glutathione peroxidases and peroxiredoxins. Small molecule antioxidants such as glutathione also play important roles as cellular antioxidants. In the present study we detected three superoxide dismutases, one catalase, three glutathione peroxidases, six peroxiredoxins and ten glutathione S-transferases (Table 3).

Adipocyte Secreted Proteins

Adipocytes synthesize and secrete bioactive molecules collectively termed adipokines, of which many are linked to the immune system. They also include proteins involved in the regulation of blood pressure, vascular haemostasis, and glucose and lipid metabolism². Of interest, in the present 1D-gel and HPLC-ESI-MS/MS study detected adiponectin, an adipokine with anti-inflammatory and insulin-sensitizing properties⁴³, retinol binding protein 4, an antagonist of insulin action⁴⁴, and visfatin, a mediator of innate immunity⁴⁵. However, some of the well-known adipokines, such as leptin, resistin, TNF- α , and IL-6 were not detected and of these, only leptin and IL-6 were detected by Western blots (supplemental

Figure 1). The complete list of adipokines that we detected by 1D-gel and HPLC-ESI-MS/MS is given in Table 4.

Protein Synthesis and Degradation

Ribosomes are molecular machines that make proteins out of amino acids. Eukaryotes have 80S ribosomes, each consisting of a large (60S) and a small (40S) subunit. The large and small subunits are composed of ribosomal RNAs and proteins, and made up of approximately 49 and 33 proteins, respectively⁴⁶. In the present study, 24 proteins from the large and 29 proteins from the small subunits were detected (supplemental Table 3). In addition, we detected 16 proteins from 28S and 39S mitochondrial ribosomes and 18 aminoacyl tRNA synthetases (supplemental Table 3).

Proteasomes are large protein complexes, which degrade unneeded or damaged proteins. The proteasomal degradation pathway is essential for many cellular processes, including the cell cycle, the regulation of gene expression, and responses to oxidative stress. We identified the proteins that make up the 20S proteasome core particle (PSMAs and PSMBs), activators of 20S complex (PSMEs,) and 26S proteasome regulatory subunits (PSMCs and PSMDs) (supplemental Table 3). Enzymes related to ubiquitination and proteasomal degradation were also detected (supplemental Table 3).

Detection of Phosphorylation Sites

Although no attempt was made to enrich phosphopeptides, we detected 24 phosphorylation sites in 21 proteins with 95% confidence using Scaffold analysis (supplemental Table 4). Four of these phosphorylation sites have not been previously reported (sites searched against databases at <http://www.phosphosite.org> and <http://www.phospho.el.eu.org>), including phosphorylated residues on perilipin, galectin-1 and 14-3-3 protein zeta/delta.

DISCUSSION

Adipocytes now are recognized to be complex cells with diverse functions not only involving lipid storage, but also immune responses, blood pressure control, vascular haemostasis, bone metabolism and glucose metabolism², presumably coordinated through the synthesis and release of a variety of adipokines. However, relatively little is known about the physiology or pathophysiology of these processes, especially at the protein level. Thus, in-depth analysis of the human adipocyte proteome, with high reproducibility of measuring protein abundance, may aid further investigation of various human diseases that are related to adipocyte abnormalities. Using a combination of one-dimensional gel electrophoresis and HPLC-ESI-MS/MS, we identified a total of 1493 proteins in 40 µg samples of adipocyte protein isolated from ~0.5 g subcutaneous adipose tissue obtained from three healthy lean subjects. Proteins ranged in size from 5.8 to 629 kDa. Of these proteins, 1105 were assigned to the cytoplasm, 202 to the plasma membrane, and 271 to the nucleus. Of the cytoplasmic proteins, 383, 241, 151, and 62 were assigned to mitochondria, cytosol, endoplasmic reticulum, and the Golgi apparatus, respectively, indicating excellent coverage of various cellular structures and organelles. In addition, most proteins of various major metabolic pathways and cellular functions, ranging from lipid metabolism to oxidative stress, were detected. Measurement of adipocyte protein abundance was reproducible, with a coefficient of variation of 22.5% when we separately processed and analyzed a sample of adipose tissue from one individual in triplicate.

When we compared the present proteome of human adipocytes to that previously reported for 3T3-L1 adipocytes by Adachi et al.²⁵, the largest set for adipocytes to date, we identified 433 “novel” proteins. We confirmed the expression of a few of these by

performing Western blots for GPX4, ANXA11, and ITGA1, which vary significantly in molecular weight and cellular function. GPX4 is a protein that protects against membrane lipid peroxidation and cell death⁴⁷. ANXA11 belongs to a large family of calcium-dependent phospholipid-binding proteins with roles in calcium signaling, apoptosis, and vesicle trafficking. In addition, it is required for midbody formation and completion of the terminal phase of cytokinesis⁴⁸, suggesting a possible role in adipocyte replication. And ITGA1, the alpha subunit of integrin receptors that heterodimerizes with the beta subunit to form a cell surface receptor for collagen and laminin, is involved in cell-cell adhesion. As such it plays an important role in inflammation and fibrosis including macrophage infiltration in various tissue such as kidney⁴⁹, intestine⁵⁰, as well as possibly adipose tissue. It is of note that the proteins detected in the present human study but not in 3T3-L1 adipocytes²⁵ did not have any distinct characteristics, such as molecular weight or subcellular localization. Therefore, our relatively large number of “novel” adipocyte proteins is probably not due to differences in the proteomics approach but reflecting differences in protein abundance in human and 3T3-L1 adipocytes.

Besides other newly recognized functions of adipocytes, lipid storage and metabolism still can be considered to be a major role. As expected, we identified a large number of proteins involved in lipid metabolism, ranging from fatty acid transport proteins through lipolysis and beta-oxidation proteins to proteins involved in lipid droplet maintenance. Adipocyte lipid droplets have recently become an area of intense investigation with the discovery that these organelles are coated with proteins participating in the regulation of lipid turnover and intracellular sorting^{33–51}. In the present study we detected the lipid droplet coat proteins perilipin, S-12 and TIP47, as well as a novel perilipin phosphorylation site. Of particular interest may be perilipin as it becomes more abundant as lipid droplets enlarge and mature⁵². Other potentially important lipid droplet associated proteins are vesicular-trafficking proteins that are involved in movement, growth, and fusion of lipid droplets as well as other vesicles^{34–53}. For example, SNAP23, which we also detected, has recently been shown to be critical for the fusion of lipid droplets in addition to being important for the fusion of glucose transporter 4 (GLUT4) containing vesicles with the plasma membrane in response to insulin stimulation. Evidence suggests that an increased uptake of fatty acids both increases the lipid droplet pool and diverts SNAP23 from the plasma membrane away from the process of insulin-stimulated GLUT4 translocation and glucose uptake, which could represent a novel mechanism to explain the association of lipid accumulation with insulin resistance³⁵.

Adipocyte mitochondria not only play an integral part in fatty acid oxidation but also in triglyceride storage as intracellular ATP is essential for the anti-lipolytic effects of insulin^{54–55}. Abnormalities in mitochondrial mass and/or function may hence impair fatty acid sequestration and be involved in complications associated with elevated plasma FFA concentrations, including insulin resistance, T2DM and cardiovascular disease^{56–58}. This notion is supported by abnormalities in adipocyte/adipose tissue mitochondrial mass, biogenesis and function in insulin-resistant animal models and T2DM individuals^{58–60}, and reversal of most of these abnormalities by treatment with thiazolidinediones (TZD)^{58–60}, which are considered to improve muscle and liver insulin sensitivity largely by redirecting lipids from muscle and liver into adipose tissue^{58–59}. Therefore, characterization and abundance measurement of mitochondrial proteins by proteomic studies could provide new insight into these and perhaps other pathological conditions and diseases.

Similar to previous studies in rodents⁶¹, we found a relatively large number of mitochondria in adipocytes by live cell staining with Mitotracker Green. These mitochondria mostly appeared as punctuate structures, but sometimes were reticular in nature. Mitochondrial proteins, which included enzymes participating in the citric acid cycle and FA metabolism,

most proteins involved in oxidative phosphorylation and protein import as well as several proteins of the mitochondrial ribosome, accounted for 22% of the total number of adipocyte proteins identified. In comparison, it has been estimated that mitochondrial proteins account for only 4.8% of the total human proteome⁶², suggesting an important role of mitochondria in adipocytes.

Another area of potential interest is our identification of many enzymes associated with oxidative stress in adipocytes, including a wide variety of antioxidant enzymes such as SODs, catalase, glutathione peroxidase, peroxiredoxins and glutathione S-transferases (Table 3). In adipocytes, increased ROS may not only be detrimental to DNA integrity and protein function, but also reduce mitochondrial respiration⁶¹ and trigger an inflammatory response that leads to an increased release of pro-inflammatory factors into the systemic circulation⁶¹. Future studies that examine antioxidants in adipocytes in conditions associated with systemic inflammation, such as insulin resistance and cardiovascular disease, may hence provide valuable information.

Adipocytes synthesize and secrete a number of bioactive molecules collectively termed adipokines, which can act in an autocrine, paracrine, intracrine and/or endocrine fashion². A variety of the adipokines are involved in the energy metabolism such as leptin, adiponectin, visfatin, RBP4, resistin and lipocalin⁶³. In the present study, we identified a few of these adipokines including adiponectin, RBP4 and visfatin in addition to several other known human adipocyte secreted proteins^{64–65} (Table 4). However, some of the well-known adipokines were not detected by HPLC-ESI-MS/MS, including leptin, resistin, TNF- α and IL-6, and of those only leptin and IL-6 were detected by Western blots. This is consistent with the lack of their detection in a similar proteome study in 3T3-L1 cells despite much greater protein loading²⁵ and probably due to the low intracellular abundance of these proteins and the fact that they are secreted. Enrichment strategies, such as the use of adipocyte incubation media, may therefore be necessary for more detailed information on the expression of adipokines^{64–66}.

In conclusion, using a combination of one-dimensional gel electrophoresis and HPLC-ESI-MS/MS, we provide the most comprehensive proteome coverage of human adipocytes to date and demonstrate reproducibility of protein abundance measurements. Coverage included a vast number of proteins of various cellular structures and organelles and most proteins of various major metabolic pathways and cellular functions. Therefore, this proteomics approach may be a valuable tool in clinical studies to elucidate changes in adipocyte proteins in human disease associated with adipocyte alterations, such as insulin resistance, type 2 diabetes and cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

FA	formic acid
ACN	acetonitrile
MW	molecular weight
GOA	Gene Ontology annotation
T2DM	type 2 diabetes mellitus

NSAF	normalized spectral abundance factors
IPI	international protein index
TAG	triacylglycerol
LD	lipid droplet
ETC	electron transport chain
ROS	reactive oxygen species
TZD	thiazolidinediones

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Figure 1. Images of adipocytes and adipocyte mitochondria isolated from human subcutaneous abdominal fat tissue

Representative images of living isolated adipocytes stained with MitoTracker Green captured by both light (A, C) and fluorescence (B, D) microscopy. Scale bars = 50 μm.

Figure 2. Characteristics of proteins identified in subcutaneous abdominal adipocytes
 (A) Venn diagram showing number of proteins identified in 3 replicates from the same subject. (B) Venn diagram showing number of proteins detected in 3 subjects. (C) Molecular weight distribution of 1493 proteins detected in 3 subjects. Black bar, grey and white bar represent subject A, B and C respectively. In cases where an identified protein group contained more than one IPI ID, the average molecular weight was used. (D) Subcellular distribution of 1493 identified proteins based on Gene Ontology (GO) annotations.

Figure 3. Coverage of major enzymes involved in lipid and glucose metabolism in human adipocytes

Isoforms and/or subunits of enzymes involved in (A) fatty acid transport and oxidation, (B) triglyceride metabolism, (C) glycolysis, and (D) citric acid cycle. Proteins identified are shown in gray boxes. Associated gene names, maximum observed number of unique peptides, and sequence coverage are presented in adjacent white boxes.

Figure 4. Coverage of proteins involved in oxidative phosphorylation (OXPHOS) in human adipocytes

Gene names from complex I-V are listed under each complex followed by the maximum unique number of peptides identified and sequence coverage. Proteins without the number of peptides and sequence coverage were not detected. Gene names listed under ubiquinone are involved in the biosynthesis of the coenzyme.

Table 1

Proteins involved in lipolysis identified in human subcutaneous abdominal adipocytes.

Protein	Gene name	MW (kDa)	Max. unique peptides	Max. seq coverage (%)
Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	GNAS	111	13	15
Guanine nucleotide-binding protein G(i), alpha-1 subunit	GNAI1	40	9	27
Guanine nucleotide-binding protein G(i), alpha-2 subunit	GNAI2	40	8	26
Guanine nucleotide-binding protein G(k) subunit alpha	GNAI3	41	9	33
Guanine nucleotide binding protein G(q), subunit alpha	GNAQ	42	13	38
Guanine nucleotide-binding protein subunit alpha-11	GNA11	42	7	24
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	GNB1	37	6	20
Guanine nucleotide-binding protein subunit beta-2-like 1	GNB2L1	38	8	32
Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	GNG2	8	4	42
Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	GNG12	8	4	50
Adenylyl cyclase-associated protein 1	CAP1	52	9	25
Adenylyl cyclase-associated protein 2	CAP2	53	7	22
cAMP-dependent protein kinase catalytic subunit alpha	PRKACA	41	9	31
cAMP-dependent protein kinase catalytic subunit beta	PRKACB	46	14	37
cAMP-dependent protein kinase type II-alpha regulatory subunit	PRKAR2A	46	13	45
cAMP-dependent protein kinase type II-beta regulatory subunit	PRKAR2B	46	12	33
Protein kinase C delta type	PRKCD	78	7	14
Protein kinase C delta-binding protein	PRKCDBP	28	15	51
Calmodulin	CALM1	17	12	61
Serine/threonine-protein phosphatase 2B catalytic subunit beta	PPP3CB	59	2	6
A kinase anchor protein 12	AKAP12	182	14	13
PALM2-AKAP2 protein isoform 2	PALM2AKAP2	121	6	10

Table 2

Proteins probably associated with lipid droplets identified in human subcutaneous abdominal adipocytes.

Protein	Gene name	MW (kDa)	Max. unique peptides	Max. seq coverage (%)
<i>Lipid droplet coat proteins</i>				
Perilipin	PLIN	56	32	68
Isoform B of Mannose-6-phosphate receptor-binding protein 1	M6PRBP1	47	3	11
Protein KIAA1881	KIAA1881	141	62	49
<i>Vesicular trafficking</i>				
Ras-related protein Rab-1A	RAB1A	23	13	68
Ras-related protein Rab-1B	RAB1B	22	5	39
Ras-related protein Rab-2A	RAB2A	24	13	65
Ras-related protein Rab-5A	RAB5A	24	4	29
Ras-related protein Rab-5B	RAB5B	24	6	44
Ras-related protein Rab-5C	RAB5C	23	9	59
Ras-related protein Rab-6A	RAB6A	24	4	23
Ras-related protein Rab-7A	RAB7A	23	14	64
Ras-related protein Rab-8A	RAB8A	24	3	16
Ras-related protein Rab-8B	RAB8B	24	4	24
Ras-related protein Rab-9A	RAB9A	23	6	45
Ras-related protein Rab-10	RAB10	23	10	50
Ras-related protein Rab-11B	RAB11B	24	14	73
Ras-related protein Rab-13	RAB13	23	2	14
Ras-related protein Rab-14	RAB14	24	15	68
Ras-related protein Rab-18	RAB18	23	9	45
Ras-related protein Rab-21	RAB21	24	6	36
Ras-related protein Rab-22A	RAB22A	22	5	39
Ras-related protein Rab-23	RAB23	27	3	15
Ras-related protein Rab-33B	RAB33B	26	3	14
Ras-related protein Rab-35	RAB35	23	4	18
Ras-related protein Rab-43	RAB43	23	2	14
Synaptosomal-associated protein 23	SNAP23	23	4	32
SNARE-associated protein Snapin	SNAPIN	15	2	18
Synaptobrevin homolog YKT6	YKT6	22	4	23
Vesicle transport through interaction with t-SNAREs homolog 1B	VTI1B	27	4	20
Syntaxin-4	STX4	34	3	13
Syntaxin-7	STX7	30	6	32
Syntaxin-8	STX8	27	3	18
Syntaxin-11	STX11	33	6	27
Syntaxin-12	STX12	32	6	30
Vesicle-associated membrane protein 3	VAMP3	11	5	41
Early endosome antigen 1	EEA1	162	32	28

Protein	Gene name	MW (kDa)	Max. unique peptides	Max. seq coverage (%)
ADP-ribosylation factor 1	ARF1	21	12	65
ADP-ribosylation factor 4	ARF4	21	4	24
ADP-ribosylation factor 5	ARF5	21	4	28
ADP-ribosylation factor 6	ARF6	20	5	39
ADP-ribosylation factor-like protein 8B	ARL8B	27	10	59
Vesicle-trafficking protein SEC22b	SEC22B	25	18	65
Vacuolar protein sorting-associated protein 26A	VPS26A	38	13	49
Vesicle-fusing ATPase	NSF	83	5	8
RER1 protein	RER1	25	2	18
RAC-beta serine/threonine-protein kinase	AKT2	56	2	5
AP-2 complex subunit beta-1	AP2B1	105	3	6
Clathrin heavy chain 1	CLTC	188	54	43
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	36	17	58
Calcium-binding protein p22	CHP	22	9	58
Flotillin-1	FLOT1	47	13	41
Transmembrane emp24 domain-containing protein 10	TMED10	25	7	33
<i>Lipid metabolism</i>				
Patatin-like phospholipase domain-containing protein 2	PNPLA2	55	2	6
Long-chain-fatty-acid--CoA ligase 1	ACSL1	78	35	43
Long-chain-fatty-acid--CoA ligase 3	ACSL3	80	6	13
Long-chain-fatty-acid--CoA ligase 4	ACSL4	79	5	12
NADH-cytochrome b5 reductase 3	CYB5R3	34	20	63
Acetyl-CoA carboxylase 1	ACACA	260	49	29
Abhydrolase domain-containing protein 5	ABHD5	39	8	36
Phosphatidylinositol phosphatase SAC1	SACM1L	67	12	27
Protein disulfide-isomerase A3	PDIA3	57	29	57
Palmitoyl-protein thioesterase 1	PPT1	34	2	11
<i>Lipid droplet associated signaling protein</i>				
Guanine nucleotide-binding protein G(i), alpha-2 subunit	GNAI2	40	8	26
Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	GNAS	111	13	15
Guanine nucleotide-binding protein G(q) subunit alpha	GNAQ	42	13	38
Transforming protein RhoA	RHOA	22	4	32
Rho-related GTP-binding protein RhoG	RHOG	21	7	38
Rho-GTPase-activating protein 1	ARHGAP1	53	9	30
Caveolin-1	CAV1	20	12	65
Caveolin-2	CAV2	18	3	34
Annexin A2	ANXA2	39	37	77
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	ATP2A2	110	13	19
Cell division control protein 42	CDC42	21	4	26
14-3-3 protein theta	YWHAQ	28	11	42
Peroxisomal protein 1	PRDX1	22	15	52

Protein	Gene name	MW (kDa)	Max. unique peptides	Max. seq coverage (%)
Cytoskeleton				
Neuroblast differentiation-associated protein AHNAK	AHNAK	629	182	42
Alpha-actinin-1	ACTN1	103	37	52
Actin, cytoplasmic 1	ACTB	42	18	51
Tubulin alpha-1C chain	TUBA1C	50	17	49
Vimentin	VIM	54	56	86
Spectrin beta chain, brain 1	SPTBN1	276	130	59
Tubulin beta chain	TUBB	50	6	20
Myosin-Ic	MYO1C	122	54	56
Cofilin-1	CFL1	19	10	49
Band 4.1-like protein 2	EPB41L2	113	12	18
Filamin-A	FLNA	281	65	36
Plectin-1	PLEC1	514	84	22
ER				
Dolichyl-diphosphooligosaccharide—protein glycosyltransferase subunit 1	RPN1	73	18	39
Dolichyl-diphosphooligosaccharide—protein glycosyltransferase subunit 2	RPN2	68	15	38
HSPA5 protein	HSPA5	72	36	47
Peptidyl-prolyl cis-trans isomerase B	PPIB	24	13	51
Transitional endoplasmic reticulum ATPase	VCP	89	22	30
Catalase	CAT	60	28	57
Tripeptidyl-peptidase 1	TPP1	60	7	27
transmembrane emp24 protein transport domain containing 9	TMED9	27	7	26
Chaperones				
DnaJ homolog subfamily B member 11	DNAJB11	41	2	8
Calnexin	CANX	72	16	26
Heat shock protein HSP 90-beta	HSP90AB1	83	14	21
60 kDa heat shock protein, mitochondrial	HSPD1	61	38	68
60S acidic ribosomal protein P0	RPLP0	34	9	41
Elongation factor 1-alpha 1	EEF1A1	50	15	38
Others				
Methyltransferase-like protein 7A	METTL7A	28	5	23
Probable saccharopine dehydrogenase	SCCPDH	47	7	21
Dehydrogenase/reductase SDR family member 1	DHRS1	34	3	14
Polymerase I and transcript release factor	PTRF	43	24	54
Prohibitin-2	PHB2	33	16	58
Atlastin-3	ATL3	61	18	44
ATP synthase subunit alpha, mitochondrial	ATP5A1	60	31	56

Table 3

Proteins involved in oxidative stress identified in human subcutaneous abdominal adipocytes.

Protein	Gene name	MW (kDa)	Max. unique peptides	Max. seq coverage (%)
Superoxide dismutase [Cu-Zn]	SOD1	16	4	31
Superoxide dismutase [Mn], mitochondrial	SOD2	25	12	64
Extracellular superoxide dismutase [Cu-Zn]	SOD3	26	2	10
Catalase	CAT	60	28	57
Glutathione peroxidase 1 isoform 1	GPX1	22	9	44
Phospholipid hydroperoxide glutathione peroxidase, mitochondrial	GPX4	27	9	45
Probable glutathione peroxidase 8	GPX8	24	2	10
Peroxiredoxin-1	PRDX1	22	15	52
Peroxiredoxin-2	PRDX2	22	13	37
Thioredoxin-dependent peroxide reductase, mitochondrial	PRDX3	28	11	34
Peroxiredoxin-4	PRDX4	31	6	29
Peroxiredoxin-5, mitochondrial	PRDX5	22	8	50
Peroxiredoxin-6	PRDX6	25	16	63
Microsomal glutathione S-transferase 1	MGST1	18	5	54
Microsomal glutathione S-transferase 3	MGST3	17	5	40
Glutathione S-transferase kappa 1	GSTK1	25	11	60
Glutathione S-transferase Mu 1	GSTM1	26	8	28
Glutathione S-transferase Mu 2	GSTM2	26	9	41
Glutathione S-transferase Mu 3	GSTM3	27	7	35
Glutathione S-transferase omega-1	GSTO1	28	3	14
Glutathione S-transferase P	GSTP1	23	12	65
Glutathione S-transferase theta-1	GSTT1	27	7	29
Glutathione S-transferase zeta 1	GSTZ1	24	4	32
Heme oxygenase 1	HMOX1	33	5	25
NAD(P)H dehydrogenase, quinone 1	NQO1	31	8	44
NAD(P)H dehydrogenase, quinone 2	NQO2	26	9	54
Amine oxidase [flavin-containing] A	MAOA	60	23	53
Amine oxidase [flavin-containing] B	MAOB	59	20	49
Glutamate--cysteine ligase catalytic subunit	GCLC	73	2	4

Table 4

Adipocyte secreted proteins identified in human subcutaneous abdominal adipocytes.

Protein	Gene name	MW (kDa)	Max. unique peptides	Max. seq coverage (%)
Adiponectin	ADIPOQ	26	3	20
Retinol binding protein 4	RBP4	23	3	16
Isoform 1 of Nicotinamide phosphoribosyltransferase	NAMPT	56	6	22
Laminin subunit beta-2	LAMB2	196	44	30
Laminin subunit gamma-1	LAMC1	178	40	28
Calreticulin	CALR	48	14	34
Cathepsin B	CTSB	38	5	24
Cathepsin D	CTSD	45	10	23
Monocyte differentiation antigen CD14	CD14	40	2	10
Gelsolin	GSN	86	5	10
Nidogen-1	NID1	136	24	25
SPARC	SPARC	35	4	14
Serotransferrin	TF	77	9	15