Increase in Endoplasmic Reticulum (ER) Stress Related Proteins and Genes in Adipose Tissue of Obese, Insulin Resistant Individuals

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Objective: To examine fat biopsy samples from lean, insulin sensitive and obese, insulin resistant non-diabetic individuals for evidence of ER stress.

Research Design and Methods: Subcutaneous fat biopsies were obtained from the upper thighs of 6 lean and 6 obese non-diabetic subjects. Fat homogenates were used for proteomic (2-D gel and MALDI-TOF-TOF), Western blot, and RT-PCR analysis.

Results: Proteomic analysis revealed 19 differentially upregulated proteins in fat of obese subjects. Three of these proteins were the ER stress related unfolded protein response (UPR), proteins calreticulin (CRT), protein disulfide-isomerase A3 (PDI) and glutathione-S-transferase P. Western blotting revealed upregulation of several other UPR stress related proteins including calnexin (CNX), a membrane bound chaperone and phospho c-jun NH2-terminal kinase 1 (JNK-1), a downstream effector protein of ER stress. RT-PCR analysis revealed upregulation of the spliced form of X box protein-1 (XBP-1s), a potent transcription factor and part of the proximal ER stress sensor, inositol requiring enzyme 1 (IRE 1) pathway.

Conclusion: These findings represent the first demonstration of UPR activation in subcutaneous adipose tissue of obese human subjects. As JNK can inhibit insulin action and activate proinflammatory pathways, ER stress activation of JNK may be a link between obesity, insulin resistance and inflammation.
Obesity is associated with insulin resistance and with a low grade state of inflammation (1). Whereas the cause of neither is completely understood, there is good evidence to show that free fatty acids (FFAs) play an important role in the development of obesity related insulin resistance and inflammation (2). Plasma FFA levels are increased in most obese people (3). Acutely raising plasma FFA levels increases insulin resistance (4) while lowering plasma FFA levels reduces insulin resistance (5). Mechanisms involved in FFA induced insulin resistance include accumulation (in muscle and liver), of lipid and lipid intermediates, including diacylglycerol (DAG), activation of several protein kinase C (PKC) isoforms and reduction in tyrosine phosphorylation of insulin receptor substrate-1/2 (IRS-1/2) (6-8). FFAs also activate the proinflammatory NFκB pathway (6,9), in part, via signaling through toll like receptor 4 (TLR 4) pathways (10). However, not all obese, insulin resistant subjects have elevated plasma FFA levels. It is, therefore, likely that there are other causes for obesity related insulin resistance. One of these appears to be ER stress. Indeed, chronic excessive nutrient intake has been shown to cause ER stress in adipose tissue of ob/ob mice and mice fed high fat diets (11-13). 

The ER is a major site for protein as well as for lipid and sterol synthesis (14,15). Ribosomes attached to the ER membranes release newly synthesized peptides into the ER lumen where protein chaperones and foldases assist in the proper post translational modification and folding of these peptides (14-16). The properly folded proteins are then released to the Golgi complex for final modification before they are transported to their final destination. If the influx of misfolded or unfolded peptides exceeds the ER folding and/or processing capacity, ER stress ensues. Three proximal ER stress sensors have been identified. They are the inositol requiring enzyme 1 (IRE 1), the PKR-like ER protein kinase (PERK) and the activating transcription factor 6 (ATF-6). These sensors trigger activation of pathways, termed the unfolded protein response (UPR) which act to alleviate ER stress. The UPR can achieve this by slowing protein synthesis, and/or by turning up the production of protein chaperones needed for proper protein folding or if unsuccessful, by degrading the unfolded proteins (14,16). So far, however, ER stress has only been reported in some rodent models of obesity (11-13) but not in fat of obese human subjects. We have, therefore, examined fat biopsy samples from lean and obese non-diabetic individuals for evidence of ER stress using proteomic, Western blot and RT-PCR analyses.

**RESEARCH DESIGN AND METHODS**

**Subjects**

Six lean and 6 obese healthy volunteers were studied. Their clinical characteristics are shown in Table 1. None of the participants had a family history of diabetes or other endocrine disorders or were taking medications. Their bodyweights were stable for at least 2 months before the biopsies. Compared to the non-obese volunteers, the obese volunteers were heavier (93.4 vs. 77.4 kg, p < 0.03), had more body fat (40.7 vs. 19.9 kg, p = 0.004), but had the same fat free mass (57.6 vs. 57.6 kg) and were insulin resistant (1/HOMA 0.44 vs. 0.29, p < 0.05). Informed written consent was obtained from all subjects after explanation of the nature, purpose and potential risks of these studies. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

**Experimental Design**

The subjects were admitted to the Temple University Hospital Clinical Research Center on the day before the studies. At ~ 8 AM on the day after admission, a venous blood
sample was obtained and an open fat biopsy was performed by a surgeon.

**Fat Biopsies**

Fat biopsies were obtained from the lateral aspect of the upper thigh (~ 15 cm above the patella) under local anesthesia as described (4). The excised fat was dropped immediately into isopentane, kept at its freezing point (-160°C) by liquid nitrogen. The frozen fat was stored at -80°C until analyzed.

**Sample Preparation for 2-D Gel Analysis**

Frozen adipose tissue from 6 non-obese and 6 obese non-diabetic volunteers was individually processed by grinding with a mortar and pestle cooled with liquid nitrogen. Frozen powders from each individual tissue block were thawed by adding 0.8 ml of cold lysis buffer (7 M urea, 2 M thiourea, 4 % CHAPS, 40 mM Tris, and 60 mM DTT) and sonicated in an ice bath for ~ 1 min (Sonic Dismembrator, Fisher Scientific). Sonicated solutions were centrifuged at 10,000 g for 12 min at 4°C. Supernatants were collected and acetone added to precipitate proteins. Precipitated proteins were resolubilized using the above lysis buffer. Protein concentrations in the extracts were measured in triplicate using a BioRad Bradford-based protein assay with BSA as the standard.

**Two-Dimensional (2D) Gel Electrophoresis**

Adipose tissue lysates were processed individually (6 from each group of subjects). For 1st dimension separation, 50 μg of sample protein was diluted in 125 μL of rehydration buffer and loaded onto an immobilized pH gradient (IPG) strip by overnight passive in-gel rehydration. A global view of the proteome was obtained initially using IPG strips of pI 3-10 (data not shown). To enhance resolution and sensitivity, narrow range IPG strips (pI 4-7 and 6-10) were subsequently used. The rehydration buffer contained 8 M urea, 2% CHAPS, 0.2% carrier ampholytes and 10 mM DTT for pI 4-7 linear and pI 3-10 nonlinear IPG strips. The pI 6-10 IPG strips were rehydrated with the rehydration buffer containing 15 mg/ml Destreak reagent substitute for 10 mM DTT. Isoelectric focusing (IEF) within the strips was performed at 20°C with a Ettan IPGphor system Amersham (Piscataway, NJ) using a total of 12,000 V·h with a maximum of 8,000 V.

For 2 dimensional separation, the IPG strips were soaked for 15 min in 10 mL of an equilibration buffer (6M urea, 30% glycerol, 2% SDS, 1% DTT, and 0.05 M pH 8.8 Tris) followed by 15 min in 10 mL of a second equilibration buffer (with 2.5% iodoacetamide substituted for 1% DTT), and positioned against 10-14% SDS polyacrylamide gels in a BioRad Mini-PROTEAN® 3 System at 200 V for 45 min. Polyacrylamide gels were then fixed twice using 50% methanol, 7% acetic acid, balance water. The resolved protein spots in the gels were visualized with Sypro-Ruby fluorescence total protein stain.

**Image Analysis of 2D Gels**

Fluorescence images of individual gels from the 12 adipose tissue lysates were captured with a FLA-5000 Fluor Imager (Fuji Photo Film Co, Ltd., Tokyo, Japan) and analyzed using PDQuest Software (Version 8.0). After automatic detection of spots by PDQuest software, the files were also inspected manually to assess accuracy of computer generated images. The software calculated individual spot “volumes” in each gel by density/area integration. To control for slight differences in protein loading across gels, the spot volume obtained from each individual fat lysate was automatically calculated by image analysis software and normalized to total spot volume on that gel.

**In-Gel Trypsin Digestion**

Differentially expressed spots were excised using an Xcise™ automated robotic system (Shimadzu Biotech, Columbia, MD). Destaining of excised gel pieces was performed by two 30 min washes with 50% acetonitrile containing 50 mM ammonium
bicarbonate. Following dehydration with 100% acetonitrile, 10 µL of 12.5 ng/µL sequencing grade trypsin (Promega, Madison, WI) was added to the gel pieces and incubated overnight at 37°C. Resulting tryptic peptides were extracted twice with 15 µL (5% formic acid, 50% acetonitrile, balance water) for 20 min and the pooled extracts were desalted with ZipTips C18 (Millipore, Billerica, MA).

**MALDI-TOF-TOF analysis**

The desalted peptides from each spot were mixed 1:1 with matrix solution (1% α-cyano-4-hydroxy cinnamic acid in 50% acetonitrile and 50% 0.1% trifluoroacetic acid) and 1.0 µL volumes were applied to wells of an AnchorChip™ sample target plate used for the Bruker Auto-flex MALDI-TOF-TOF instrument. Peptide mass fingerprints were obtained using the reflective and positive ion mode. Mass spectra were collected from the sum of 100-400 laser shots and mono-isotopic peaks were generated by FlexAnalysis™ software with signal-to-noise ratio of 2:1. Mass peak value calculations used two trypsin auto-digestion peptides with M+H values 842.509 and 2211.104 as internal standards. Proteins were identified by matching the calibrated peptide mass values within SwissProt protein database for Homo sapiens using an in-house version of Mascot Server 2.1 imbedded in Bruker’s Biotool software. Match variances allowed were a mass tolerance of 50 ppm, one missed trypsin cleavage, fixed modification of carbamidomethyl cysteine, and variable modification of methionine oxidation. For the samples that did not produce a "hit" with a confident score, peptide peaks with good signal were further fragmented using “Laser-induced decomposition” to obtain LIFT-TOF/TOF spectra and these MS/MS data alone or combined with the previously produced MS data were used to search against the protein database through the Mascot.

**Western blot analysis**

Proteins (30 to 80 µg) from the same adipose tissue lysates as used for the 2-D gels were separated by 10-14% gradient SDS-PAGE. The separated proteins were transferred to a nitrocellulose membrane in a semi-dry blotting chamber according to the manufacturer’s protocol (Biorad, Hercules, CA).

Blots were blocked with 5% milk in Tris-buffer saline solution (pH 7.6) containing 0.05% Tween-20 (TBS/T), and probed with the following rabbit anti-human antibodies from Santa Cruz Biotechnology (Santa Cruz, CA) at a concentration of 0.4 µg/mL: PDI (SC-20132), calreticulin (SC-11398) and calnexin (SC-11397). In addition, a rabbit anti-serum that detects JNK1 and JNK2 and 3 (Cell Signaling Technology, Danvers, MA) and a rabbit anti-serum that detects total JNK were used. Blots were incubated with primary antibody overnight at 4°C at with gentle shaking and then incubated with a mouse anti-rabbit HRP-conjugated secondary Ab (1:10000) (Biomed Corp., Foster City, CA) for 1 hr at room temperature. Blots were exposed using a chemiluminescent detection method (Enhanced ECL Detection System, GE Healthcare BioSciences Corp., Piscataway, NJ).

**RT-PCR**

Total RNAs were isolated from frozen adipose tissues and real-time RT-PCR was performed with a SYBR Green One-Step qRT-PCR kit (Invitrogen, Carlsbad, CA) and a Light-Cycler (Roche, Indianapolis, IN). Primers for XBP-1s (NM-005080): sense- TTGAGAACCAGGAGTAAAG) and antisense- CTGCACCTGCTGCCGAAGT.

**Statistical Analysis**

2D gel analysis was performed by PDQuest software (Bio-Rad), version 8.0. Each gel from obese and lean fat samples was enumerated, analyzed for spot detection, background subtraction and protein spot volume quantification. Of the six samples per group, the gel image showing the highest
quality of spots and the best protein pattern was chosen as a reference template, and spots in a reference gel were then matched across all gels. Manual corrections were performed to validate the matches automatically generated by the software. Spot volume values were normalized in each gel by dividing the raw quantity of each spot by the total volume of all the spots included in the same gel. For each protein spot, the average spot volume values and its standard deviation in each group were determined. The match spots were subjected to student t test in order to determine the spots that were differentially expressed. Only those spots that show a statistically significant difference with a confidence level of 0.05 were chosen for identification. To test for inter-gel reproducibility, 2D gel analysis was performed in triplicate using one representative sample. Equal amounts of high quality protein spots (345 for pI 4-7) were detected. The average variance (CV) of the normalized spot volume, was determined to be 19.25%.

RESULTS

Proteomic Analysis

Gels with isoelectric focusing ranges of PI 4-7 and 6-10 produced a total of ~ 900 protein spots in each gel (Figure 1). The comparison of all spots visualized yielded 24 spots which were significantly different in lean vs. obese volunteers. Three of these spots represented multiple isoforms of vimentin. The 3 vimentin isoforms were considered as one protein (24-22). Two other spots could not be identified (22-2=20). Of the 20 remaining differentially expressed proteins, 19 were upregulated and 1 (α-enolase) was downregulated in obese vs. lean volunteers (Table 2). The differentially expressed proteins were grouped into the following categories: 1) UPR and stress (7 proteins), 2) energy and FFA metabolism (5 proteins), 3) structural proteins (4 proteins) and 4) protein transport and signaling (4 proteins) (Table 2).

UPR and stress

Levels of expression of the following UPR proteins were overexpressed in 2D gels in adipose tissue from obese volunteers: calreticulin (CRT), a protein chaperone, increased from undetectable to 1019 ± 236 arbitrary units, protein disulfide isomerase A3 (PDI), a protein foldase increased ~ 3-fold, and glutathione-S-transferase P, an antioxidant protein belonging to an UPR regulated pathway increased ~ 1.8-fold (Figure 1, Table 2).

Several cytosolic small heat shock proteins (HSPs) (20 kDa and 27 kDa HSP) and one mitochondrial HSP (60 kDa HSP) were also overexpressed in the adipose tissue of obese volunteers, suggesting presence of cytosolic and mitochondrial stress in addition to ER stress (Table 2).

Other proteins

Several proteins involved in energy and FFA metabolism (ATP synthase subunit beta, perilipin, aldehyde dehydrogenase, α-enolase and carbonic anhydrase 1), structural proteins (myosin light chain polypeptide 6, tropomyosin beta chain, tropomyosin alpha-4 chain and vimentin) and proteins involved in transport and signaling (gamma-synuclein, Rho GDP-dissociation inhibitor 1, 14-3-3 protein gamma and galectin-1) were also differentially overexpressed in fat from obese volunteers (Table 2).

Western blot analysis

Western blotting confirmed upregulation of CRT and PDI and revealed upregulation of calnexin (CNX ~ 1.8-fold), a membrane bound chaperone and phospho-JNK-1 ~ 2.0-fold, a downstream effector protein of the UPR. Phospho-JNK 2/3, however was unchanged (Figure 2).

RT-PCR analysis

Upregulation of the spliced form of the x box binding protein-1 (XBP-1s), a part of the IRE 1/XBP-1 proximal ER stress sensor, and of TNF-α, a pro-inflammatory cytokine, were documented with RT-PCR whereas there
were no differences in IL-1β and IL-6 mRNA in adipose tissue of lean and obese subjects (Figure 3).

**DISCUSSION**

In the present study, we found activation of 1 of the 3 UPR proteins, known to be proximal ER stress sensors (IRE 1/XBP-1) but failed to detect statistically significant activation of the other two ER stress sensors (ATF 6 and PERK) and 2 other UPR proteins (GLP 78 and CHOP) in fat from obese subjects (data not shown). This was not surprising as it has previously been shown that only severe and long lasting ER stress activates all 3 UPR pathways simultaneously, whereas different duration and types of ER stress can activate 1, 2 or all 3 pathways in different tissues (17).

The IRE 1/XBP-1 pathway regulates induction of chaperone synthesis. IRE 1 activation results in dimerization and autophosphorylation of IRE 1. This activates IRE 1’s endonuclease activity and initiates splicing of a 26 base pair segment from the XBP-1 mRNA, creating XBP-1s, a potent transcription factor which targets genes encoding ER chaperones including CRT and CNX and PDI, a foldase which catalyzed disulfide bond formation (14, 17, 18). XBP-1s mRNA, CRT, CNX and PDI were all upregulated in fat of obese compared to non-obese volunteers (Figures 1-3) indicating activation of UPR and suggesting presence of ER stress. The lectin/chaperones CNX and CRT facilitate protein folding by shielding unfolded protein regions from surrounding proteins thus preventing aggregation. In addition, the CNX/CRT cycle is one arm of the quality control machinery in the ER which monitors the glycosylation status of proteins and determines whether a molecule is exported to the Golgi complex or targeted for ER associated degradation (ERAD) (14,19).

In addition, we found phospho-JNK-1 (but not phospho-JNK-2 or phospho-JNK-3) to be markedly upregulated. ER stress has previously been reported to activate JNK to phosho-JNK (primarily to phospho-JNK-1) in cultured cells and animal models (11,20-22). JNK activation is coupled to ER stress induced IRE-1 activation through recruitment of TRAF-2 (tumor necrosis factor receptor-associated factor 2) and ASK-1 (apoptosis signal-regulating kinase 1) to the cytosolic leaflet of the ER membrane (22). The complex of these 3 proteins phosphorylates and activates JNK. JNK activation has been shown to produce insulin resistance and inflammation while JNK inhibition improved insulin sensitivity in fat, liver and muscle of several animal models of obesity (11).

Proteosome analysis also revealed a 3.5-fold increase in gamma-synuclein. Gamma-synuclein belongs to a family of small unfolded proteins with unknown physiological functions. Gamma synuclein has been detected in various cancers, particularly in advanced stages of breast and ovarian cancer (23). In vitro, gamma-synuclein inhibits normal metastatic checkpoint control, promotes cancer invasion and metastasis and is being used as a marker for assessing breast cancer progression (24). The observed large upregulation of γ-synuclein in fat from obese individuals is interesting in view of the recognized increase in cancer risk in obesity (25,26).

Pertubation of ER function was not restricted to the ER but involved other sections of the protein secretory pathway as indicated by upregulation of several cytosolic protein chaperones including 3 small heat shock proteins (the 20 kDa HSP beta-6, the 27 kDa HSP beta-1 and the HSP beta-5). In addition, it involved mitochondria as evidenced by upregulation of the mitochondrial 60 kDa heat shock protein (chaperonin) which participates in the folding of newly synthesized proteins which are transported from the cytosol to mitochondria. (14).

White adipose tissue hypoxia associated with increased expression of ER stress
markers has been reported in mice (27,28). To explore a possible role of adipose tissue hypoxia in the observed UPR, we have determined expression of hemoxygenase 1 (hemox-1). This gene has been shown to be dramatically upregulated in fat of hypoxic ob/ob mice (28). There was, however, no difference in the hemox-1 mRNA/18srRNA ratios in subcutaneous fat from lean and obese subjects (0.5 vs. 0.5, NS).

It has been shown, in vitro and in animal models, that nutrient excess, (the cause for obesity) produces ER stress (11,12) and it has been speculated that the reason for ER stress in response to nutrient excess may be increased demand for lipid and protein synthesis and for structural changes in the organs primarily involved in metabolizing and storing excess nutrients, i.e., the adipose tissue and the liver (12). Compatible with this interpretation, we found overexpression of several structural proteins and proteins related to energy and fat metabolism and protein transport. Moreover, an increase in reactive oxygen species (ROS), which frequently accompanies nutrient excess, can cause ER stress (29) and activate the UPR (30).

ER stress has been demonstrated to trigger activation of several serine/threonine kinases including JNK and IKK and is a major source for the production of reactive oxygen species (ROS) which not only produce insulin resistance but also stimulate synthesis and release of proinflammatory cytokines and chemokines such as TNF-α, IL1-β, IL-6 and MCP-1 (11 and this paper). Thus, the ER might be a proximal site that senses nutritional excess and translate that excess into metabolic and inflammatory responses (12).

Finally, all our fat biopsies were from thigh subcutaneous adipose tissue and thus the results may not be representative of fat in other locations.

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Statement of responsibility. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.
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## Table 1 – Study Subjects

|                  | Lean          | Obese         | P   |
|------------------|---------------|---------------|-----|
| Gender (M/F)     | 4M/2F         | 3M/3F         |     |
| Age (yr)         | 36 ± 4        | 44 ± 4        | NS  |
| Weight (kg)      | 77.4 ± 3.4    | 93.4 ± 5.6    | 0.03|
| Height (cm)      | 180.0 ± 2.7   | 167 ± 4.5     | 0.03|
| BMI (kg/m$^2$)   | 24.0 ± 1.2    | 33.5 ± 1.6    | 0.02|
| Fat (kg)         | 19.9 ± 2.9    | 40.7 ± 5.9    | 0.004|
| FFM (kg)         | 57.6 ± 3.5    | 57.6 ± 5.7    | NS  |
| 1/HOMA           | 0.441 ± 0.126 | 0.291 ± 0.02  | 0.05|
| FBG (mg/dl)      | 97.1 ± 2.4    | 93.5 ± 1.2    | NS  |
| FFA (μmol/l)     | 481 ± 81      | 424 ± 37      | NS  |
Table 2. Proteins Differentially Expressed in 2D Gels

| Spot # | Protein ID                      | Swiss protein accession # | Mouse Score | Peptides matched | Normalized spot volumes ± SD Lean (n=6) | Normalized spot volumes ± SD Obese (n=6) | P        |
|--------|--------------------------------|---------------------------|-------------|-----------------|-----------------------------------------|------------------------------------------|---------|
|        | **UPR and Stress**             |                           |             |                 |                                         |                                         |         |
| 3      | Calreticulin                   | P27797                    | 66          | 7               | 0                                       | 1019 ± 236                               | < 0.001 |
| 23     | Protein disulfide-isomerase A3 | P30101                    | 61          | 6               | 421 ± 328                               | 1170 ± 63                                | < 0.01  |
| 20     | 20 kDa Heat-shock protein beta-6 | P014558                   | 76          | 5               | 2534 ± 1157                             | 7530 ± 248                               | 0.001   |
| 21     | 27 kDa Heat-shock protein beta-1 | P04792                    | 78          | 8               | 4242 ± 1438                             | 8140 ± 1643                              | 0.001   |
| 25     | Heat-shock protein beta-5      | P02511                    | 74          | 9               | 4162 ± 558                              | 8967 ± 2918                              | 0.049   |
| 16     | 60 kDa heat-shock protein      | P10809                    | 69          | 6               | 600 ± 558                               | 1868 ± 56                                | < 0.001 |
| 19     | Glutathione S transferase P    | P09211                    | 107         | 8               | 2231 ± 698                              | 3936 ± 390                               | 0.013   |
|        | **Energy and FFA Metabolism**  |                           |             |                 |                                         |                                         |         |
| 12     | ATP synthase subunit beta      | P06576                    | 138         | 11              | 2413 ± 801                              | 5499 ± 1251                              | 0.01    |
| 15     | Perilipin                      | 060240                    | 57          | 6               | 1602 ± 381                              | 3960 ± 1019                              | 0.007   |
| 22     | Aldehyde dehydrogenase         | P05091                    | 87          | 9               | 1341 ± 161                              | 4088 ± 799                               | 0.001   |
| 24     | Alpha-enolase                  | P06733                    | 65          | 7               | 803 ± 214                               | 293 ± 74                                 | 0.018   |
| 26     | Carbonic anhydrase 1           | P00915                    | 108         | 11              | 2463 ± 774                              | 4846 ± 523                               | 0.012   |
|        | **Structural Proteins**        |                           |             |                 |                                         |                                         |         |
| 1      | Myosin light chain polypeptide 6 | P60660                    | 60          | 6               | 1792 ± 528                              | 3837 ± 284                               | 0.002   |
| 2      | Tropomyosin beta chain         | P07951                    | 58          | 6               | 1141 ± 443                              | 4072 ± 531                               | 0.03    |
| 4      | Tropomyosin alpha-4 chain      | P67936                    | 94          | 8               | 770 ± 355                               | 3429 ± 799                               | 0.002   |
| 7-9    | Vimentin                       | P08670                    | 204         | 19              | 3136 ± 1418                             | 11005 ± 3353                             | 0.007   |
|        | **Protein transport and signaling** |                       |             |                 |                                         |                                         |         |
| 6      | Gamma-synuclein                | 076070                    | 66          | 4               | 488 ± 465                               | 1714 ± 670                               | 0.034   |
| 11     | Rho GDP-dissociation inhibitor 1 | P52565                    | 75          | 5               | 2163 ± 612                              | 3168 ± 286                               | 0.049   |
| 5      | 14-3-3 protein gamma           | P61981                    | 78          | 6               | 875 ± 525                               | 3619 ± 1005                              | 0.005   |
| 10     | Galectin-1                     | P09382                    | 113         | 10              | 9030 ± 1387                             | 25049 ± 4175                             | 0.001   |
Expression levels of the UPR proteins calreticulin and protein disulfide isomerase A3 (PDI) in adipose tissue of one lean and one obese subject (upper panels).

Differentially expressed proteins in fat homogenates from one lean and one obese subject. Proteins were separated by isoelectric focusing and molecular weight (2DG) as described in Methods. The sub-proteome from each sample was assessed using Pi ranges of 4-7 (middle panels) and 6-10 (lower panels). The proteins were stained with spyro-ruby and images compared by PD Quest software. The numbers correspond to the spot numbers in Table 2. The arrows indicate upregulated proteins in the fat of obese subjects.
Figure 2
Protein abundance (by Western blots) of calnexin/β-actin, calreticulin/β-actin, PDI, phospho-JNK-1/JNK-1 and phospho-JNK-2,3/JNK-2,3 in subcutaneous adipose tissue from 4 lean (insufficient fat was available for Western analysis from 2/6 lean subjects) and 6 obese non-diabetic subjects. Inserts show representative Western blots.
Figure 3
Messenger RNA (mRNA) corrected for 18s ribosomal RNA (18sr RNA) of sXBP-1, TNF-α, IL-1β and IL-6 in 6 lean and 6 obese non-diabetic subjects.