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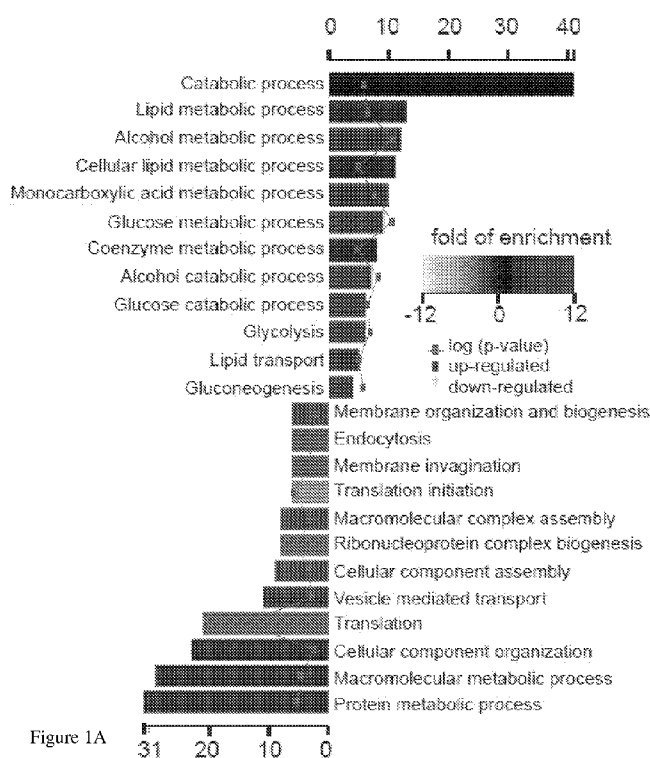


Figure 1A

(57) Abstract: The embodiments of the invention provide for genetic, chemical or dietary interventions that modulate hepatic phospholipid synthesis and/or endoplasmic reticulum (ER) calcium homeostasis function. More specifically, the present invention addresses modulation of the lipid composition of the hepatic stressed ER and/or improvement of the hepatic ER calcium metabolism to reduce ER stress and thus treat type 2 diabetes, fatty liver disease, atherosclerosis, inflammation, and/or dislipidemia.



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NEW TARGETS FOR TREATMENT OF ER STRESS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/454,099 filed March 18, 2011, the contents of which is incorporated herein by reference in its entirety.

FEDERAL FUNDING

[0002] This invention was made with government support under Grants T32 ES7155-24, DK52539 and 1RC4- DK090942, awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

FIELD

[0003] The present invention relates to molecular biology and cell metabolism.

BACKGROUND

[0004] In recent years, the world has seen an alarming increase in metabolic diseases including obesity, insulin resistance, diabetes, fatty liver disease, and atherosclerosis. For example, over twenty million children and adults in the U.S., or 8% of the population, suffer from diabetes. Atherosclerosis is a leading cause of coronary heart disease and stroke, killing more than 600,000 Americans annually; more than 25% of all deaths in the U.S.

SUMMARY

[0005] The embodiments of the invention provide for genetic, chemical or dietary interventions that modulate hepatic phospholipid synthesis and/or endoplasmic reticulum (ER) calcium homeostasis function. More specifically, the present invention addresses modulation of the lipid composition of the hepatic stressed ER and/or improvement of the hepatic ER calcium metabolism to reduce ER stress and thus treat type 2 diabetes, fatty liver disease, atherosclerosis, inflammation, and/or dyslipidemia.

[0006] An embodiment provides for the overall modulation of cellular phospholipid synthesis, in particular correcting the abnormal distribution of PC and PE in the ER and other

cell membranes and organelles, to modulate cellular functions and inflammation. For example, the PC/PE ratio is increased in the ER but decreased in the plasma membrane of obese subjects, therefore there is a clear imbalance regarding phospholipid distribution across different cellular compartments. Modulating the PC/PE ratio balance between cellular organelles is beneficial both on cellular level as well as the body level.

[0007] Another embodiment of the present invention provides for inhibitors of Pemt expression or PEMT activity, comprising genetic, molecular (e.g., drug) and/or specific dietary regimens, that modulate phospholipid synthesis in the ER, and thus regulate calcium homeostasis, glucose homeostasis, and insulin sensitivity. More specifically, down-modulation of hepatic PEMT lowers the hepatic PC/PE ratio from a higher ratio to the lower ratio observed in normal (e.g., non-obese, non-ER stressed) hepatic ER.

[0008] Another embodiment provides for compositions and methods to modulate calcium homeostasis in the ER. More specifically, increased SERCA concentration or activity in the hepatic ER improves calcium homeostasis in the ER, and suppresses glucose production and thus restores normoglycemia. SERCA may be modulated using, for example, liver-specific SERCA agonists, phospholamban inhibitors, vitamin D interventions, as well as other genetic and molecular approaches. Correcting SERCA function is also useful in suppressing hepatic VLDL production and dislipidemia, and thus atherosclerosis. Thus, an embodiment of the invention is a method for treating atherosclerosis or dislipidemia, or suppressing hepatic VLDL comprising modulating expression or activity of hepatic SERCA.

[0009] Yet another embodiment provides for the measurement of ASGAR and HP as diagnostic biomarkers for fatty liver disease and/or liver failure associated with ER stress and abnormal calcium metabolism. In particular, the synthesis of ASGAR and HP are dramatically reduced in the fatty liver as compared with normal liver.

DESCRIPTION OF THE DRAWINGS

[0010] Figs. 1A to 1E present the proteomic and lipidomic landscape of the lean and obese ER. Fig. 1A shows biological pathways associated with significantly regulated proteins in the obese ER proteome. Bar colors indicate the fold enrichment with significance values (negative log of p-values) superimposed. Figs. 1B, 1C show transcript levels of genes involved in lipid metabolism in the lean and obese mouse liver. Fig. 1D shows alterations of liver ER lipidome. Heatmap display of all significant ($p < 0.05$) alterations present between lean and obese

ER lipidomes. The color corresponds to differences in the relative abundance (nmol%) of each fatty acid among individual lipid groups detected in the lean and obese liver ER. Fig. 1E shows the relative abundance of PC and PE in lean and obese liver ER samples. Values are mean $\pm$ SEM (n=6 or each group). * denotes $p < 0.05$, Student's t-test.

[0011] Figs. 2a-2h demonstrate that elevated PC/PE ratio impairs SERCA activity and ER homeostasis. Fig. 2a reflects calcium transport activity of microsomes loaded with PC and PE in vitro. Transcript levels of *Pemt* (Fig. 2b) and corresponding microsomal calcium transport activities (Fig. 2c) of Hepa1-6 cells expressing control (Gfp) or mouse *Pemt* ORF. Fig. 2d shows calcium transport activity (top) and SERCA protein levels (bottom) of microsomes prepared from lean and obese mouse liver. Liver *Serca2b* transcript levels (Fig. 2e) and microsomal calcium transport activities (Fig. 2f), immunoblot (Fig. 2g) and quantitative RT-PCR (Fig. 2h) measurement of ER stress markers in the livers of lean mice expressing either LacZ (control) or *Serca2b* shRNAs. * in Fig. 2h denotes the phosphorylated IRE1 α ; and * in other panels denotes significant difference ($p < 0.05$, $n=4$) by student's t-test. Values are mean $\pm$ SEM.

[0012] Figs. 3a-3l show that suppression of liver *Pemt* expression corrects ER PC/PE ratio, relieves ER stress, and improves systemic glucose homeostasis in obesity. Fig. 3a, PC/PE ratio, and Fig. 3b, calcium transport activity of liver ER from ob/ob mice expressing LacZ (control) or *Pemt* shRNAs. Immunoblot (Fig. 3c) and quantitative PCR (Fig. 3d) measurement of ER stress markers in the liver. Expression of hepatic lipogenesis and gluconeogenesis genes (Fig. 3e), triglyceride content (Fig. 3f), and Hematoxylin & Eosin staining (Figs. 3g and 3h) of liver samples. Plasma glucose (Fig. 3i) and insulin (Fig. 3j) levels in control and *Pemt* shRNA-treated ob/ob mice after 6-hour food withdrawal. Figs. 3k-3l, Plasma glucose levels of control and *Pemt* shRNA-treated ob/ob mice after intraperitoneal administration of either 1 g/kg of glucose (Fig. 3k) or 1 IU/kg of insulin (Fig. 3l). All data are mean $\pm$ EM ($n=4$ for 3a-3e, $n=6$ for 3f-3l); * denotes $p < 0.05$ (one-way ANOVA for data presented in 3k and 3l, and Student's t-test for others).

[0013] Figs. 4a-4i demonstrate exogenous SERCA expression alleviates ER stress and improves systemic glucose homeostasis. Liver *Serca2b* transcript levels (4a) and microsomal calcium transport activities (4b) of control or *Serca2b* overexpressing obese mice. Plasma glucose (4c) Plasma insulin levels (4d), tissue weights (4e) of ob/ob mice as in panel a. Triglyceride content (4f), H&E staining (4g, 4h) and immunoblot analyses (4i) of ER stress markers (IRE1 α and eIF2 α phosphorylation, and CHOP) and secretory proteins (ASGR and HP)

in the obese liver expressing Serca2b compared to controls. All values are mean $\pm$ SEM (n=4 for 4a-4b, n=6 for 4c-4h); * denotes $p < 0.05$ (Student's t-test).

[0014] Figs. 5A-5D present data from ER fractionation and validation. Fig. 5A, is an illustration of ER fractionation procedure for proteomic and lipidomic analyses and polysome profiling. Fig. 5B shows validation of ER fractionation methodology by immunoblot analyses of subcellular markers. PDI: protein disulfide isomerase, CANX: Calnexin, IR: Insulin receptor, H2A: Histone 2A. Fig. 5C is a volcano plot of the fold changes of median spectral counts of proteins from obese and lean samples against the significance of differential expression (log-normalized p-Values). Proteins of interest are highlighted (red: $p < 0.05$, fold of change (obese/lean) ~ 1.5 , average spectral counts ~ 5 ; green: $p < 0.05$, fold of change (lean/obese) ~ 1.5 , average spectral counts ~ 5). Fig. 5D shows immunoblot of differentially regulated proteins identified from the proteomic study for protein lysates prepared from cytosolic and ER fractions of unfasted lean and obese liver. PMSA: Proteasome small subunit a, RPS6: Ribosomal small subunit 6, APOB: Apolipoprotein B, Mtp: Microsomal triglyceride transfer protein; HP: Hepatoglobin; ASGR: Asialoglycoprotein receptor; mEH: Microsomal epoxide hydrolase; MRC1: Mannose receptor, C type 1.

[0015] Fig. 6A-6B show expression of ER stress markers in the obese liver. Fig. 6a, Immunoblot detection of representative ER stress markers in total protein lysates prepared from the liver of lean and ob/ob mice sacrificed at 12 weeks of age after 6 hours of food withdrawal. Fig. 6b, Transcript levels of genes involved in ER-associated protein degradation (ERAD) in the liver of lean and ob/ob mice as determined by quantitative RT-PCR.

[0016] Figs. 7A-7C demonstrate the distinct contributions of dietary fat and de novo lipogenesis to ER lipid composition. Fig. 7A is an illustration of the synthesis of nine classes of lipids detected in the ER lipidome. Dashed lines indicate multiple enzymatic steps. Genes studied herein are colored red. Fig. 7B is a heatmap display of all significant ($p < 0.05$, Student's t-test) alterations present between diet and lean ER lipidomes. The color scheme reflects differences calculated based on the relative abundance (nmol%) of each fatty acid among individual lipid groups detected in the ER of lean liver and the diet. Fig. 7C shows a complete linkage analysis of all twelve ER lipidomes (six lean vs. six obese). The length of each branch correlates with the magnitude of lipidomic differences.

[0017] Figs. 8A-8D show the effect of Pemt knockdown on liver ER lipidome and ER stress in ob/ob mice. Fig. 8A, Transcript levels of Pemt in the liver of ob/ob mice administered

with adenoviral control (LacZ shRNA) or *Pemt* shRNA expressing viruses. Fig. 8B, Heatmap display of the fatty acid composition of ER isolated from the liver of ob/ob mice administered with control and *Pemt* shRNA. The color scheme denotes differences calculated from the relative abundance (nmol%) of each fatty acid among individual lipid groups detected in the ER of control and *Pemt* shRNA liver samples. Fig. 8C, Complete linkage analysis of ER lipidome for samples prepared from control and experimental groups. Fig. 8d, Quantification of immunoblot signals presented in Fig. 3d. Values are mean $\pm$ SEM; n=4; * denotes $p < 0.05$, Student's t-test.

[0018] Figs. 9A-9E demonstrate amelioration of ER stress in the liver of high-fat diet (HFD) induced obese mouse by *Pemt* knockdown. Figs. 9A-9B, Hematoxylin & Eosin staining of liver sections prepared from control (Fig. 9A) as well as *Pemt* shRNA-treated mice after 22 weeks of HFD (Fig. 9B). The white vesicles represent lipid droplets. Fig. 9C, Blood glucose levels of control and *Pemt* shRNA-treated HFD mice. Figs. 9D-9E, Immunoblot and quantification of ER stress markers in the liver of control and experimental HFD mice. Values are mean $\pm$ SEM, n=4; * denotes $p < 0.05$, Student's t-test.

[0019] Figs. 10A-10B show that SERCA2b overexpression improves systematic glucose homeostasis of ob/ob mice. Plasma glucose levels of control and SERCA2b overexpressing ob/ob mice after intraperitoneal administration of either 1IU/kg of insulin (Figure 10A) or 1g/kg of glucose (Figure 10B). All data are mean $\pm$ SEM; * denotes $p < 0.05$ (one-way ANOVA, n=6/group).

[0020] Figs. 11A-11E show detergent-dependent solubilization of SERCA2b proteins from fatty liver samples and comparison of SERCA2b expression in lean with obese animals. Fig. 11a, Immunoblot of total protein lysates as well as ER fractions prepared from the liver of lean and obese mice following two different solubilization methods from the same samples. Liver tissue was first homogenized in lysis buffer containing 1% NP40 and clarified at 200 g for 10 minutes to pellet down cell debris. The whole cell lysate was either further solubilized by the addition of Laemmli buffer (2% SDS, top panel) or clarified by consecutive centrifugations at 16,000g for 10 minutes and 60 minutes (middle panel) as described (see Park et al., 107 PNAS 19230 (2010)), supernatant collected, boiled in Laemmli buffer and loaded on to SDS-PAGE. For the examination of SERCA2b protein levels in the liver ER (bottom panel), ER pellet was resuspended in Laemmli buffer (2% SDS), sonicated for 3 minutes, boiled and clarified by centrifugation at 10,000g for 10 minutes. Figs. 11b-11c, Transcript levels of *Serca2b* in the liver tissues of genetically obese (12 weeks old, 11b) and diet-induced obese (22 weeks of

HFD) mice as compared to age-matched lean controls. Figs. 11d-11e, SERCA2b protein levels in the liver tissues of genetically obese as well as diet-induced obese mice at different ages. The total protein lysates were prepared with Laemmli buffer containing 2% SDS as described in the Examples.

DETAILED DESCRIPTION

[0021] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[0022] As used herein and in the claims, the singular forms include the plural reference and vice versa unless the context clearly indicates otherwise. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.”

[0023] All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0024] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood to one of ordinary skill in the art to which this invention pertains. Although any known methods, devices, and materials may be used in the practice or testing of the invention, the methods, devices, and materials in this regard are described herein.

[0025] The present embodiments address the discovery that there is a fundamental shift in hepatic endoplasmic reticulum (ER) function in obesity: from protein to lipid synthesis and metabolism. The presented invention demonstrates that modulating(i.e., correcting) hepatic

calcium homeostasis and/or ER phospholipid synthesis suppresses hepatic glucose production, increases hepatic lipid oxidation, decreases hepatic VLDL production, and thus improves dislipidemia, and most importantly, normalizes systematic glucose levels and normoinsulinemia. The role of modulating hepatic lipid metabolism and/or calcium homeostasis in restoring systematic normoglycemia and normoinsulinemia, and the role of calcium homeostasis in suppressing hepatic VLDL production and thus dislipidemia (and atherosclerosis) provide novel approaches for treating many liver disease states associated with obesity.

[0026] The ER is the main site of protein and lipid synthesis, membrane biogenesis, xenobiotic detoxification and cellular calcium storage. Perturbation of ER homeostasis leads to stress and the activation of unfolded protein response (UPR). Ron & Walter, 8 Nat. Rev. Mol. Cell. Bio. 519 (2007). Chronic activation of ER stress has been shown to play an important role in the development of insulin resistance and diabetes in obesity. Hotamisligil, 140 Cell, 900 (2010). Mechanisms that lead to chronic ER stress in a metabolic context in general, and obesity in particular, remained a mystery until the present invention. Herein, comparative examination the proteomic and lipidomic landscape of hepatic ER purified from lean and obese mice reveal the mechanisms of chronic ER stress in obesity: Suppression of protein but stimulation of lipid synthesis in the obese ER occurs without significant alterations in chaperone content. Alterations in the ER fatty acid and lipid composition results in the inhibition of sarco/endoplasmic reticulum calcium ATPase (SERCA) activity and ER stress. Correcting the obesity-induced alteration of ER phospholipid composition or hepatic SERCA overexpression in vivo both reduced chronic ER stress and improved glucose homeostasis. Hence, the present inventors have discovered that abnormal lipid and calcium metabolism are important contributors to hepatic ER stress in obesity.

[0027] It has been generally accepted that a surplus of nutrients and energy stimulates synthetic pathways and may lead to client overloading in the ER. It has not been demonstrated, however, whether increased de novo protein synthesis and client loading into the ER and/or a diminished productivity of ER in protein degradation or folding leads to ER stress in obesity. Intriguingly, dephosphorylation of eukaryotic translation initiation factor 2a (eIF2a) in the liver of high-fat-diet fed mice reduced ER stress response (Oyadomari et al., 7 Cell Metab. 520 (2008)), suggesting that additional mechanisms other than translational up-regulation may also contribute to ER dysfunction in obesity.

[0028] To address these mechanistic questions, ER was fractionated from lean and obese liver tissues (Figs. 5A-5B) and then extracted ER proteins for comparative proteomic analysis to

examine the status of this organelle in obesity. A total of 2,021 unique proteins were identified. Among them, 120 proteins were differentially regulated in obese hepatic ER samples (Fig. 5C, Tables 1a and 1b). The differential regulation was validated, when possible, by immunoblot analyses, and the fidelity of the system verified (Fig. 5D). Gene Ontology analysis identified the enrichment of metabolic enzymes, especially ones involved in lipid metabolism, in the obese ER proteome, while protein synthesis and transport functions were over-represented among down-regulated ER proteins (Fig. 1A). Consistently, ER associated protein synthesis was down-regulated in the obese liver as demonstrated by polysome profiling, whereas the expression of genes involved in de novo lipogenesis (Fas, Scd1, Ces3, Dgat2 and Dak2) and phospholipid synthesis (Pcyt1a and Pemt) were broadly up-regulated (Figs. 1B, 1C). Many components of protein degradation pathways were also upregulated, with no broad change in the quantity of ER chaperones (Figs. 6A-6B, Table 1a). Taken together, these data revealed a fundamental shift in hepatic ER function in obesity from protein to lipid synthesis and metabolism.

[0029] The presence of chronic ER stress in obese liver (Figs. 6A-6B) despite reduction in ER-associated protein synthesis led to the hypothesis that ER stress in obesity may not be invoked simply by protein overloading, but is also driven by compromised folding capacity influenced by lipid metabolism. Erbay et al., 15 Nat. Med. 1383 (2009). For example, the ability of palmitate and cholesterol to induce ER stress in cultured cells correlates with their incorporation into the ER. Li et al., 270 J. Biol. Chem. 37030 (2004); Borradaile et al., 47 J. Lipid Res. 2726 (2006).

[0030] Therefore, a quantitative determination of all major lipid species and their fatty acid composition in ER samples isolated from lean and obese liver along with the diet consumed by these animals was undertaken. (Fig. 8A-8D, Table 2). This revealed that the fatty acid composition of ER lipids in the lean mouse liver was distinct from corresponding dietary lipids, suggesting the contribution of a basal level de novo lipogenesis to the biogenesis of ER membranes in vivo (Figs. 6a, 6b; Table 2). Almost all ER derived lipids were composed of significantly higher levels of saturated fatty acids (SFA) whereas their polyunsaturated fatty acid (PUFA) content was much lower than those of corresponding dietary lipids, suggesting that de novo synthesized SFAs are preferred over diet-derived PUFAs as the substrate for the synthesis of hepatic ER lipids. Additionally, the liver ER samples of lean and obese mice also had profoundly different composition of fatty acids and lipids as illustrated by the clear separation of lean and obese ER lipidome in cluster analysis (Fig. 1D). The obese ER was significantly enriched with monounsaturated fatty acids (MUFA, Fig. 1E), a bona fide product of de novo lipogenesis in liver.

[0031] Importantly, the obese ER samples contained a higher level of phosphatidylcholine (PC) as compared to phosphatidylethanolamine (PE) (PC/PE = 1.97 vs. 1.3, $p < 0.05$, Table 2), two of the most abundant phospholipids on the ER membrane. The rise of PC/PE ratio is likely caused by the up-regulation of two key genes involved in PC synthesis and PE to PC conversion: choline-phosphate cytidylyltransferase A (Pcyt1a) and phosphatidylethanolamine N-methyltransferase (Pemt) (Fig. 1C, Fig. 7a), and it is consistent with the essential role of PC for lipid packaging in the form of lipid-droplets or lipoproteins, both of which are increased in obesity. In contrast, the PC/PE ratio in the lean hepatic ER was essentially identical as it is in the diet (Table 2), indicating that the increase of PC/PE ratio in obesity is not due to food consumption, but the result of increased lipid synthesis in the obese liver.

[0032] The desaturation of SFA to MUFA in the obese liver likely has a protective role in reducing lipotoxicity, whereas the decrease of PUFA content in the ER may limit its reducing capacity and contribute to ER stress. Kim, 479 Neurosci. Lett. 292 (2010). The role of PC/PE ratio in regulating hepatic ER homeostasis has not been studied before. Previous biochemical studies have shown that increasing PC content in the membrane inhibits the calcium transport activity of SERCA 5,8. Li et al., 2004; Cheng et al., 261 J. Bio. Chem. 5081 (1986). Consistently, it was found herein that the addition of PC to liver-derived microsomes *in vitro* substantially inhibited SERCA activity (Figs. 2A, 2B). More importantly, overexpression of the PE to PC conversion enzyme, Pemt, in Hepa1-6 cells significantly inhibited microsomal SERCA activity, suggesting changes in the PC/PE balance in a cellular setting can significantly perturb SERCA function (Figs. 2C, 2D). Because calcium plays an important role in mediating chaperone function and protein folding in the ER, and given that SERCA is principally responsible in maintaining calcium homeostasis in this organelle, it was postulated that the increased PC/PE ratio in the ER of obese liver might impair ER calcium retention and homeostasis *in vivo*, thereby contributing to protein misfolding and ER stress. Indeed, as shown herein, microsomes prepared from obese mice livers had significantly lower calcium transport activity than those isolated from lean animals (4.60.2 vs. 5.30.3, $p = 0.046$, Fig. 2e), despite the fact that SERCA protein level was modestly higher in the former: consistent with an inhibitory role of PC/PE ratio on SERCA function.

[0033] Although SERCA dysfunctions have been reported in the muscle of diabetic patients, its role in hepatic ER stress, as shown herein, is novel. Modest defects in SERCA activity have been implicated in the pathology of Darier's disease (Miyauchi et al., 281 J. Biol. Chem. 22882 (2006)). It was found herein that a reduction in SERCA expression *in vivo*

(Fig. 2f) and a concurrent reduction in its calcium transport activity (Fig. 2g) potentially activated hepatic ER stress in lean mice as evident by IRE1a and eIF2a phosphorylation and changes in the expression of Grp78 and Grp94 (Figs. 2h). Therefore, there appears to be little redundancy in the function of SERCA beyond physiological fluctuations to maintain ER homeostasis, and the reduction in calcium transport activity is a potential mechanism of hepatic ER stress in obesity.

[0034] Different but complementary approaches to correct aberrant lipid metabolism caused SERCA dysfunction and the effects on ER homeostasis in the obese liver were examined. If the alteration in PC/PE ratio seen in obese liver is a significant contributor to ER stress, correction of this ratio to lean levels by reducing *Pemt* expression should improve calcium transport defects and produce beneficial effects on hepatic ER stress and metabolism. An adenovirally-expressed shRNA system achieved ~50-70% suppression of the *Pemt* transcript in obese liver (Fig. 3A). As postulated, suppression of *Pemt* led to a decrease of PC content from ~39% to ~33%, which was compensated by an ~7% increase of PE content from ~17% to 24% (Table 3). As a result, the PC/PE ratio is reduced to 1.3 (equivalent to lean ratio), as compared to 2.0 detected in the ER of the obese liver (Fig. 3A). The reduction of PC/PE ratio was accompanied by a significant improvement in the calcium transport activity of the ER prepared from the *Pemt*-knockdown obese mice (Fig. 3B). As the improvement of calcium transport function occurred with few and minor changes in the overall fatty acid composition of ER (Figs. 8A, 8B; Table 3), these results confirmed the rise in PC/PE ratio as an inhibitory factor of SERCA activity in obesity.

[0035] More importantly, hepatic ER stress indicators including the phosphorylation of IRE1a and eIF2a, as well as the expression of C/EBP homologous protein (CHOP), homocysteine-inducible, endoplasmic reticulum stress-inducible protein (HERP) and Der1-like domain family member 2 (DERL2), were all reduced upon suppression of *Pemt* in obese mice (Figs. 3C, 3D; Fig. 8C). Relief of chronic ER stress in the ob/ob mice has been associated with improvement of hepatic steatosis and glucose homeostasis, and *Pemt* knockout mice have been shown to be protected from diet-induced dislipidemia. Ozcan et al., 313 Sci. 1137 (2006); Kammoun et al., 119 J. Clin. Investig. 1201 (2009). It was found herein that genes involved in hepatic lipogenesis (*Fas*, *Scd1*, *Ces3*, *Dgat2*) and lipoprotein synthesis (*ApoA4*) were consistently and significantly down-regulated in the obese liver following suppression of *Pemt* (Fig. 3E). As a result, these mice exhibited a significant reduction in hepatic steatosis and liver triglyceride content (Figs. 3F-3H). Genes involved in glucose production (*G6p*, *Pck1*) in the liver were significantly down-regulated (Fig. 3E), and there were also significant reductions in both hyperglycemia and hyperinsulinemia in obese mice following the suppression of hepatic

Pemt expression (Figs. 3I, 3J). Glucose and insulin tolerance tests revealed significantly enhanced glucose disposal following Pemt suppression (Fig. 3K, 3L). A similar phenotype is also observed upon suppression of hepatic Pemt in the high-fat diet induced obesity with reduced ER stress and improved glucose homeostasis (Figs. 9A-9D). These data are consistent with the phenotype seen in Pemt-deficient mice, which exhibit protection against diet-induced insulin resistance and atherosclerosis. Jacobs et al., 285 J. Biol. Chem. 22403 (2010). Therefore, correcting the PC/PE ratio of ER can significantly improve calcium transport defects, reduce ER stress and improve metabolism, supporting the hypothesis that changes in lipid metabolism contribute to SERCA dysfunction, ER stress and hyperglycemia in both genetic- and diet-induced models of obesity.

[0036] Additionally, over-expression of hepatic Serca in vivo, to overcome the partial inhibition of SERCA activity by PC (Fig. 4a) showed that exogenous SERCA expression in the liver of the ob/ob mice improved the calcium import activity of the ER (Fig. 4b), restored euglycemia and normoinsulinemia within a few days, and markedly improved glucose tolerance (Figs. 4C, 4D; Figs. 10A-10B). Upon Serca expression, liver showed an increase in size but a marked reduction of lipid infiltration (Figs. 4E-4H) and suppression of IRE1a and eIF2a phosphorylation, along with significant reduction in CHOP levels (Fig. 4I). In these liver samples, there was also a marked increase in two secretory proteins that were otherwise diminished in obesity: asialoglycoprotein receptor (ASGR) and haptoglobin (HP) (Fig. 4I). As the folding and maturation of ASGR is sensitive to perturbations of calcium homeostasis in the ER (Lodish & Kong, 265 J. Biol. Chem. 10893 (1990)), the results herein support that exogenously increased SERCA expression restored calcium homeostasis and relieved at least some aspects of chronic ER stress in the obese liver. Taken together, these data reinforced the hypothesis that lipid-driven alterations and the ER calcium homeostasis are important contributors to hepatic ER stress in obesity.

[0037] The chronic activation of ER stress markers has been observed in a variety of experimental obese models as well as in obese humans. Gregor et al., 58 Diabetes 693 (2009). Furthermore, treatment of obese mice and humans with chemical chaperones result in increased insulin sensitivity. Ozcan et al., 2006; Kars et al., 59 Diabetes 1899 (2010). The present systematic, compositional and functional characterization of hepatic ER landscape from lean and obese mice revealed a diametrically opposite regulation of ER functions regarding protein and lipid metabolism and revealed mechanisms giving rise to ER stress. In particular, elevation of the PC/PE ratio in the ER, driven by the up-regulation of de novo lipogenesis in obesity, was linked to SERCA dysfunction and chronic ER stress in vivo. A recent study reported down-

regulation of SERCA protein level in obese liver (Kars et al., 2010), which was not evident in our analysis and appeared to have resulted from the choice of methodology in ER protein preparations (Figs. 11A-11E). Nevertheless, other mechanisms such as oxidative and inflammatory changes associated with obesity can also perturb ER homeostasis by impacting ER calcium fluxes. See, e.g., Park et al., 107 PNAS 19320 (2010); Li et al., 49 Diabetologia 1434 (2006); Cardozo et al., 54 Diabetes 452 (2005).

[0038] The identification of a lipid-driven calcium transport dysfunction and ER stress provides a fundamental framework to understand the pathogenesis of hepatic lipid metabolism and chronic ER stress in obesity. Excessive food intake inevitably stimulates lipogenesis for energy storage, and PC is the preferred phospholipid coat of lipid droplets and lipoproteins. Li et al., 186 J. Cell. Bio. 783 (2009). Therefore, there is a biological need for the synthesis of more PC for packaging and storing the products of hepatic lipogenesis. Also, de novo fatty acid synthesis in the obese liver produces ample amounts of MUFA, which is effectively incorporated into PC but not PE, which further distorts the PC/PE ratio and impairs ER function. The resulting ER stress facilitates the secretion of excessive lipids from liver without ameliorating hyperinsulinemia-induced lipogenesis (Schiller et al., 42 J. Lipid Res. 1501 (2001)), and thus hepatosteatosis and ER stress ensue. As a result relieving ER stress in obesity may ultimately depend on breaking this “lipogenesis-ER stress-lipogenesis” vicious cycle and restoring the ER folding capacity. Therefore, genetic, chemical or dietary interventions that modulate hepatic phospholipid synthesis and/or ER calcium homeostasis function represent a new set of therapeutic opportunities for common chronic diseases associated with ER stress such as obesity, insulin resistance, and type 2 diabetes.

[0039] The interventions that modulate hepatic phospholipid synthesis and/or ER calcium homeostasis function may be used as treatment of hepatic ER stress-associated disease states including type 2 diabetes, dislipidemia, fatty liver disease, inflammation, and/or atherosclerosis. Such treatment may improve a diagnosed condition or make it more manageable, or improve disease symptoms, or correct physiological imbalances associated with hepatic ER stress. Treatment can also include delaying or preventing the onset of hepatic ER stress-associated disease, or preventing recurrence or relapse of hepatic ER stress-associated disease. For example, a treatment of hepatic ER stress improves glucose homeostasis.

[0040] In specific embodiments, the PC/PE ratio of the hepatic ER is modulated by inhibiting (or down-regulating) expression or activity of phosphatidylethanolamine N-methyltransferase (PEMT), encoded by *Pemt*. The modulating includes genetic, chemical or

dietary intervention. An approach to inhibiting expression or activity of PEMT includes (optionally) identifying a cell, cell population or tissue in which modulation (reduction) of the activity or level of PEMT is desired; and contacting said cell, cell population or tissue with an amount of PEMT modulator(s), e.g., PEMT antagonist(s), sufficient to modulate the activity or level of PEMT in the cell, cell population, or tissue. The contacting step may be carried out *ex vivo*, *in vitro*, or *in vivo*. For example, the contacting step may be performed using human cells, or performed in a subject such as a human patient. The PEMT inhibitor may be, for example, an anti-PEMT antibody, a portion of S-adenosyl-L-methionine or phosphatidylethanolamine that acts as a decoy for PEMT, or a small molecule inhibitor of PEMT. The antibody antagonist may be a monoclonal or single specificity antibody, may be human, humanized, chimeric, or *in vitro* generated antibody. The term antibodies also includes any portion of an antibody that binds to a PEMT epitope. An example chemical that inhibits PEMT is rosiglitazone, available as AVANDIA® (rosiglitazone maleate), AVANAMET® (rosiglitazone maleate/metformin HCl) and AVANDARYL® (rosiglitazone maleate and glimepiride) from GlaxoSmithKline. Additional PEMT inhibitors include, for example, 3-deazaadenosine (DZA), bezafibrate and clofibric acid.

[0041] Alternatively, or in combination with PEMT inhibitors, expression of *Pemt* may be inhibited by RNA interference with, e.g., dsRNA, ssRNA, siRNA, shRNA, miRNA, and the like. In a particular embodiment, the RNA interference mediator is a shRNA, or a mixture of shRNAs. An example shRNA effective for inhibiting *Pemt* is presented in Table. 5.

[0042] Similarly, the PC/PE ratio of the hepatic ER can be modulated by inhibiting expression or activity of phosphate cytidyltransferase 1, choline, alpha (also called choline-phosphate cytidyltransferase A), encoded by *Pcyt1a*. The modulating includes genetic, chemical or dietary intervention. The nucleotide sequence of *Pcyt1a* is available, for example, at the National Center for Biotechnology Information (NCBI) website, ID: 5130 (*Homo sapiens*), as is *Pemt*, ID: 10400 (*H. sapiens*).

[0043] Additionally, because modulation of PEMT to down-regulate its expression or function was shown herein to down-regulate the expression of several other genes, additional or alternative modulators of these genes may be useful in the present invention to alleviate hepatic ER stress. Thus, this modulating comprises down-regulating hepatic expression of at least one of a de novo lipogenesis gene such as *Fas*, *Scd1*, *Ces3*, *Dgat2* and *Dak2*; a lipoprotein synthesis gene *ApoA4*; or a gene involved in glucose production such as *G6* and *Pck1*.

[0044] In other specific embodiments, the calcium homeostasis of hepatic ER is modulated by activating (or up-regulating) expression or activity of sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA). The modulating includes genetic, chemical or dietary intervention. An approach to increasing expression or activity of SERCA includes (optionally) identifying a cell, cell population or tissue in which modulation (increase) of the activity or level of SERCA is desired; and contacting said cell, cell population or tissue with an amount of SERCA modulator(s), e.g., SERCA agonist(s), sufficient to modulate the activity or level of SERCA in the cell, cell population, or tissue. The contacting step may be carried out ex vivo, in vitro, or in vivo. For example, the contacting step may be performed using human cells, or performed in a subject such as a human patient.

[0045] Example chemical modulators that increase SERCA activity include nitroxides such as 4-Hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (tempol), ursodeoxycholic acid, and tauroursodeoxycholic acid. Additional SERCA enhancers include, for example, istaroxime, NOS, TUDCA and regucalcin. Alternatively or in concert, SERCA concentration and activity can be increased by genetic means, (i.e., via gene therapy). Example genes encoding SERCA are available at NCBI, ID: 488, ID: 487, ID: 489 (each H. sapiens). Example primers for open reading frame (ORF) cloning are presented in Table 5. The viral vector delivery described herein can be modified for use in humans by techniques known in the art.

[0046] Gene therapy approaches that can be used to increase SERCA expression include lentivirus, herpesvirus, and nonviral vectors. See, e.g., Lam & Dean, Progress & prospects: nuclear import of nonviral vectors, 17 Gene Ther. 439 (2010); Macnab & Whitehouse, Progress & prospects: human artificial chromosomes, 16 Gene Ther. 1180 (2009); Epstein, Progress & prospects: Biological properties & technological advances of herpes simplex virus type 1-based amplicon vectors, 16 Gene Ther. 709 (2009); Brunetti-Pierri & Ng, Progress & prospects: gene therapy for genetic diseases with helper-dependent adenoviral vectors, 15 Gene Ther. 553 (2008); Sinn et al., Gene Therapy Progress & Prospects: Development of improved lentiviral & retroviral vectors - design, biosafety, & production, 12 Gene Ther. 1089 (2005); Flotte, Gene Therapy Progress & Prospects: Recombinant adeno-associated virus (rAAV) vectors, 11 Gene Ther. 805 (2004).

[0047] Additionally or alternatively, SERCA activity can be increased by inhibiting those mechanisms (e.g., lipids, proteins, or pathways) that remove SERCA from the hepatic ER. For example, phospholamban inhibitors can be used to maintain SERCA levels in the hepatic ER.

[0048] Vitamin and mineral supplements along with nutritional support may be useful in concert with any of the treatments discussed herein, including, for example, vitamin D interventions.

[0049] Additionally, the treatment or condition of the hepatic ER can be monitored by measuring expression of hepatic asialoglycoprotein receptor (ASGR) and/or haptoglobin (HP). Monitoring can be achieved using any approach known in the art, including PCR and immunoassay. Phospholamban inhibitors can be used as SERCA activators.

EXAMPLES

Example 1. ER fractionation from obese and lean mice

[0050] Male leptin-deficient (ob/ob) and wild-type littermates in the C57BL/6J background were bred in-house and used for all biochemical experiments. Leptin deficient mice used for adenovirus-mediated expression experiments were purchased from the Jackson Laboratory (strain B6.V-Lepob/J, stock number 000632). All mice were maintained on a 12-hour-light /12-hour-dark cycle in a pathogen-free barrier facility with free access to water and regular chow diet containing 2200 ppm of choline (PicoLab® Mouse Diet 20).

[0051] ER fractionation protocols were adapted from Cox and Emili (1 Nat. Protoc. 1872 (2006)). Briefly, male mice at three months of age (unless otherwise noted) with or without overnight fasting were anesthetized by tribromoethanol and perfused with 20 ml 0.25 M sucrose solution before tissue harvesting. Fresh liver tissue (1.0 g for lean and 1.2 g for obese mice produced an equal amount of ER) was immediately transferred to 10 ml ice cold STM buffer (0.25 M sucrose, 50 mM Tris pH 7.4, 5 mM MgCl₂), chopped into small pieces and homogenized by 6 strokes in a motor-driven, loose-fit, teflon-glass homogenizer at speed setting of 3.5 (Wheaton, NJ). The whole lysates were first cleared by centrifugation at 3000 g for 10 min followed by a series of centrifugations to obtain the final ER pellet. The pellet was washed with 11 ml of ice-cold 0.25M sucrose solution and was subjected to centrifugation to obtain the final ER preparation which was either snap frozen in liquid nitrogen or used directly for biochemical and other analysis.

Example 2. Sample prefractionation by 1D-PAGE

[0052] Aliquots of 20 µl (~100 pg) of the ER protein extract was boiled for 5 min in an equal volume of 2x Laemmli buffer and separated on a 12% SDS-poly-acrylamide gel (15 cm x

15 cm x 1.0 mm). The gel was minimally stained with Coomassie Brilliant Blue and briefly washed in 25% methanol, 7.5% acetic acid and sliced horizontally into 12 bands with roughly similar protein content as estimated from the optical density. See Schmidt et al., 3 Mol. Sys. Bio. 79 (2007). The gel was then cut vertically to separate the protein content of individual lanes. The gel slices were minced with a sterile clean razor blade, transferred into 96-well plates, washed three times with 200 µl of 25 mM ammonium bicarbonate 50% acetonitrile, followed by dehydration with 100 µl HPLC-grade acetonitrile. After removal of acetonitrile, the gel slices were dried completely in a vacuum concentrator (Speed Vac, Thermo, MA) and rehydrated in 200 µl of 50 mM ammonium bicarbonate containing 1 µg/ml trypsin, followed by incubation for 24 hr at 37°C. Protein digests were collected and the gel pieces were further extracted and washed (a) with 200 µl of aqueous 20 mM ammonium bicarbonate pH 8.6; (b) twice with 200 µl of 2% formic acid 50% HPLC-grade acetonitrile; followed by (c) dehydration in 150 µl of 2% formic acid 10% 2-propanol 85% acetonitrile. The combined peptide solutions were filtered using hydrophilic multi-well PTFE filter plates (Millipore, MA) according to the manufacturer's protocol and concentrated to a volume of ~5 µl in a SpeedVac, and resuspended in 60 µl aqueous solvent containing 2% formic acid, 2% acetonitrile. Samples were analyzed by 1D nano-LC ESI tandem mass spectrometry as described herein.

Example 3. Protein identification by 1 D nano-LC tandem mass spectrometry

[0053] *LC MS/MS instrumentation:* A CTC Autosampler (LEAP Technologies, NC) was equipped with two 10-port Valco valves and a 20 µl injection loop. A 2D LC system (Eksigent, CA) was used to deliver the flow rate of 3 µl/min during sample loading and 250 µl/min during nanoflow rate LC separation. Self-packed columns used: a C18 solid phase extraction "trapping" column (250 µm i.d. x 10 mm) and a nano-LC capillary column (100 µm i.d. x 15 cm, 8 µm i.d. pulled tip (NewObjective) both packed with the Magic C18AQ, 3 µm, 200 Å (Michrom Bioresources) stationary phase. A protein digest (10 µl) was injected onto the trapping column connected on-line with the nano-LC column through the 10-port Valco valve. The sample was cleaned up and concentrated using the trapping column, eluted onto and separated on the nano-LC column with a one-hour linear gradient of acetonitrile in 0.1% formic acid. The LC MS/MS solvents were Solvent A: 2% acetonitrile in aqueous 0.1% formic acid; and Solvent B: 5% isopropanol 85% acetonitrile in aqueous 0.1% formic acid. The 85-min-long LC gradient program included the following elution conditions: 2%B for 1 min; 2-35%B in 60 min; 35-90%B in 10 min; 90%B for 2 min; and 90-2%B in 2 min. The eluent was introduced into LTQ Orbitrap (ThermoElectron, CA) mass spectrometer equipped with a nanoelectrospray source

(New Objective, MA) by nanoelectrospray. The source voltage was set to 2.2 kV and the temperature of the heated capillary was set to 180°C. For each scan cycle on full MS scan was acquired in the Orbitrap mass analyzer at 60,000 mass resolution, 6×10^5 AGC target and 1200 ms maximum ion accumulation time was followed by 7 MS/MS scans acquired for the 7-most intense ions for each of the following m/z ranges 350-700, 695-1200, and 1195-1700 amu. The LTQ mass analyzer was set for 30,000 AGC target and 100 ms maximum accumulation time, 2.2 Da isolation width, and 30 ms activation at 35% normalized collision energy. Dynamic exclusion was enabled for 45 sec for each of the 200 ions that had been already selected for fragmentation to exclude them from repeated fragmentation. Each digest was analyzed twice.

[0054] *MS data processing:* The MS data .raw files acquired by the LTQ Orbitrap mass spectrometer were copied to the Sorcerer IDAII search engine (Sage-N Research, Thermo Electron, CA) and submitted for database searches using the SEQUEST-Sorcerer algorithm. The search was performed against a concatenated FASTA protein database containing the forward and reversed human (25H.Sapiens) UniProt KB database downloaded from EMBL-EBI on 10.23.2008 as well as an in-house compiled database with common contaminants. Methionine, histidine, and tryptophane oxidation (+15.994915 atomic mass units, amu) and cysteine alkylation (+57.021464 amu with iodoacetamide derivative) were set as differential modifications. No static modifications or differential posttranslational modifications were employed. A peptide mass tolerance equal to 30 ppm and a fragment ion mass tolerance equal to 0.8 amu were used in all searches. Monoisotopic mass type, fully trypticpeptide termini, and up to two missed cleavages were used in all searches. The SEQUEST output was filtered, validated, and analyzed using Peptide Prophet, Protein Prophet (Institute for Systems Biology, WA) and Scaffold (Proteome Software, OR) software. The balance between reliability and sensitivity of the protein identification data was set by adjusting the estimated false positive peptide identification rate (FPR) to below 0.5%. The FPR was calculated as the number of peptide matches from a “reverse” database divided by the total number of “forward” protein matches, in percentages. The semiquantitative spectral count data sets obtained for all samples were subsequently integrated and processed using the in-house written software ProMerger which allowed us to compare proteomic profiles derived from different samples and perform the downstream pathway analysis.

Example 4. Statistical methods of proteomic analysis

[0055] Spectral counts were computed for each protein in each sample by utilizing high quality MS/MS-based peptide identifications. This example detected differentially abundant

proteins between lean and obese mice, as opposed to absolute protein quantification or cross-protein comparisons of abundance, and this approach ultimately restricted attention to proteins with average spectral count (across samples) greater than 5 for better reliability. *See* Liu et al., 76 Anal. Chem. 4193 (2004). This obviates the need for certain within-protein normalization techniques. *See* Schmidt et al., 2007; Ishihama et al., 4. Mol. Cell. Proteomics 1265 (2005); Lu et al., 25 Nat. Biotech. 117 (2007). Differentially abundant proteins were identified by fit in a Poisson mixed model for each protein. Diggle et al., *in* ANALYSIS OF LONGITUDINAL DATA (Oxford Press, 2002). The Poisson mixed model allows for a principled treatment of discrete-count data and provides a statistically rigorous framework for the identification of differentially abundant proteins accounting for correlation among repeated measures and over-dispersion. A similar approach is followed in Choi et al. (7 Mol. Cell Proteomics 2373 (2008)). This approach relied on fewer modeling assumptions than the Bayesian approach advocated by Choi et al., where variability of abundance is assumed to be constant across proteins -- a strong assumption that generally does not hold in practice. The present approach does not require this assumption. Because it relies on fewer modeling assumptions, it is reasonable to expect that this procedure is, in fact, more robust to model misspecification than that of Choi et al.

[0056] The Poisson mixed model, unlike an ordinary Poisson model, accounts for over-dispersion often present in spectral count data. Indeed, a random intercept term for each mouse in the experiments was applied to account for over-dispersion. Furthermore, in order to adjust for difference in the overall protein abundance in each sample, an offset term was included depending on the total spectral counts (across all proteins) in each sample. Finally, even after including the offset term, there was a substantial differences between the experiments, thus analyses were controlled for an experiment effect. In summary, each protein fit the model described by the equation:

$$\log(\mu_{ijk}) = \log(t_{ijk}) + \alpha + b_j + \gamma_k + \delta x_j$$

where μ_{ijk} is the expected spectral count for the i -th technical replicate from the j -th mouse in experiment k , conditional on the mean zero mouse-specific random effect b_j ; t_{ijk} is the total spectral counts in the sample; γ_k represents the k -th experiment effect; and $x_j = 0$ or 1 according to whether the j -th mouse was from the lean or obese group and δ is the corresponding lean/obese effect. A total of five experiments were conducted. Each was comprised of four mice -- two lean and two obese samples. In one of the experiments, two samples per mouse were available (technical replicates), while in the other four experiments only a single sample per mouse was available. Thus, for each Poisson mixed model fit, a total of 24 observations were

utilized. The parameter of primary interest was δ . For each protein, a p -value was obtained corresponding to δ , and proteins were ranked by these p -values for significance, using the R library lme4 to fit the Poisson mixed models.

Table 1a. Up-regulated proteins in the obese liver ER proteome

Symbol	UniProt Accession	MW (kDa)	Nomenclature	Fold Change	p-val
Acaa1b	Q8VCH0 THIKB_MOUSE	44	acetyl-Coenzyme A acyltransferase 1B	14.0	7.37E-12
Fasn	P19096 FAS_MOUSE	272	fatty acid synthase	8.8	1.04E-07
Oplah	Q8K010 OPLA_MOUSE	138	5-oxoprolinase (ATP-hydrolysing)	7.0	1.21E-02
Pcx	Q3T9S7 Q3T9S7_MOUSE	130	pyruvate carboxylase	7.0	4.00E-04
Apoa4	P06728 APOA4_MOUSE	45	apolipoprotein A-IV	6.0	1.19E-10
Pklr	P53657 KPYR_MOUSE	62	pyruvate kinase liver and red blood cell	5.5	2.22E-06
Aldh3a2	Q5SRE0 Q5SRE0_MOUSE	59	aldehyde dehydrogenase family 3, subfamily A2	5.3	7.74E-10
Tuba1a	P68369 TBA1A_MOUSE	50	tubulin, alpha 1A	5.0	7.22E-03
Tubb2b	Q9CWF2 TBB2B_MOUSE	50	tubulin, beta 2B	5.0	3.46E-10
Gpd1	P13707 GPDA_MOUSE	38	glycerol-3-phosphate dehydrogenase 1 (soluble)	4.5	3.71E-04
Acaca	Q5SWU9 COA1_MOUSE	265	acetyl-Coenzyme A carboxylase alpha	4.3	2.01E-05
Psm1	Q3TXS7 PSMD1_MOUSE	106	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	4.0	2.19E-02
Myh14	Q6URW6 MYH14_MOUSE	229	myosin, heavy polypeptide 14	4.0	9.25E-04
Eno1	P17182 ENO1_MOUSE	47	enolase 1, alpha non-neuron	4.0	1.70E-07
Myh2b	Q3THE2 MLRB_MOUSE	20	myosin, light chain 12B, regulatory	3.4	3.83E-03
Ugp2	Q91ZJ5 UGPA_MOUSE	57	UDP-glucose pyrophosphorylase 2	3.3	5.47E-03
Coasy	Q9DBL7 COASY_MOUSE	62	Coenzyme A synthase	3.0	1.05E-02
Ces3	Q8VCT4 CES3_MOUSE	62	carboxylesterase 3	2.9	3.53E-03
Gstm1	A2AE89 A2AE89_MOUSE	24	glutathione S-transferase, mu 1	2.9	2.27E-06
Pygl	Q9ET01 PYGL_MOUSE	97	liver glycogen phosphorylase	2.7	3.69E-03
Hbb-b1	A8DUK7 A8DUK7_MOUSE	16	hemoglobin, beta adult major chain	2.6	3.52E-02
Dak	Q8VC30 DAK_MOUSE	60	dihydroxyacetone kinase 2 homolog (yeast)	2.6	1.01E-04
Fmo1	P50285 FMO1_MOUSE	60	flavin containing monooxygenase 1	2.5	1.39E-02
Aldob	Q91Y97 ALDOB_MOUSE	40	aldolase B, fructose-bisphosphate	2.5	9.39E-08
Cat	P24270 CATA_MOUSE	60	catalase	2.3	2.81E-02
P4hb	P09103 PDIA1_MOUSE	57	prolyl 4-hydroxylase, beta polypeptide	2.1	1.73E-04
Sds	Q8VB21 SDHL_MOUSE	35	serine dehydratase	2.0	1.79E-02
Gstz1	Q9JJA0 Q9JJA0_MOUSE	16	glutathione transferase zeta 1 (maleylacetoacetate isomerase)	2.0	3.45E-05
Ephx1	P97869 P97869_MOUSE	53	epoxide hydrolase 1, microsomal	2.0	4.24E-08
Maob	Q8BW75 AOFB_MOUSE	59	monoamine oxidase B	1.9	2.80E-06
Cyb5r3	Q9CY59 Q9CY59_MOUSE	34	cytochrome b5 reductase 3	1.8	8.66E-03
Trf	Q92111 TRFE_MOUSE	77	transferrin	1.8	4.52E-03
Cyb5	P56395 CYB5_MOUSE	15	cytochrome b-5	1.8	3.97E-02
Acs15	Q8JZR0 ACSL5_MOUSE	76	acyl-CoA synthetase long-chain family member 5	1.8	1.55E-03
Phb	P67778 PHB_MOUSE	30	prohibitin	1.8	2.86E-02
Aldh1a1	P24549 AL1A1_MOUSE	54	aldehyde dehydrogenase family 1, subfamily A1	1.7	1.38E-03
Slc25a5	P51881 ADT2_MOUSE	33	solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5	1.7	6.42E-03
Atp5h	Q9DCX2 ATP5H_MOUSE	19	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit d	1.7	2.23E-02
Mttp	O08601 MTP_MOUSE	99	microsomal triglyceride	1.7	5.71E-03

Symbol	UniProt Accession	MW (kDa)	Nomenclature	Fold Change	p-val
			transfer protein		
Atp5a1	Q03265 ATPA_MOUSE	60	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	1.7	1.58E-07
Fmo5	P97872 FMO5_MOUSE	60	flavin containing monooxygenase 5	1.6	3.75E-04
Atp5o	Q9DB20 ATPO_MOUSE	23	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, O subunit	1.6	9.15E-03
Etfdh	Q6PF96 Q6PF96_MOUSE	61	electron transferring flavoprotein, dehydrogenase	1.6	3.01E-03
Mvp	Q3THX5 Q3THX5_MOUSE	97	major vault protein	1.6	2.57E-06
Apoe	P08226 APOE_MOUSE	36	apolipoprotein E	1.6	2.01E-08
Mat1a	Q91X83 METK1_MOUSE	44	methionine adenosyltransferase I, alpha	1.5	1.95E-03
Gapdh	P16858 G3P_MOUSE	36	glyceraldehyde-3-phosphate dehydrogenase	1.5	1.90E-02
Rps13	P62301 RS13_MOUSE	17	ribosomal protein S13	1.5	1.67E-02
Flnb	Q80X90 FLNB_MOUSE	278	filamin, beta	1.5	4.31E-03
Myf6	Q60605 MYL6_MOUSE	17	myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	1.5	5.16E-03

Table 1b. Down-regulated Proteins in the obese liver proteome

Symbol	UniProt Accession	MW (kDa)	Nomenclature	Fold Change	p-val
Gnc	A2A 63 A2A 63_MOUSE	11	glucosamine	-19.0	3.92E-09
Eif3f	Q9DCH4 EIF3F_MOUSE	38	eukaryotic translation initiation factor 3, subunit F	-15.5	5.07E-06
Eif2s2	Q99L45 EIF2B_MOUSE	38	eukaryotic translation initiation factor 2, subunit 2 (beta)	-8.5	5.25E-03
Eef1g	Q9D8N0 EF1G_MOUSE	50	eukaryotic translation elongation factor 1 gamma	-8.0	1.08E-02
Eif3g	Q9Z1D1 EIF3G_MOUSE	36	eukaryotic translation initiation factor 3, subunit G	-8.0	7.80E-04
Eif2s3x	A2AAW9 A2AAW9_MOUSE	37	eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked	-7.7	3.06E-05
Egfr	Q01279 EGFR_MOUSE	135	epidermal growth factor receptor	-6.5	2.48E-12
Tdo2	P48776 T23O_MOUSE	48	tryptophan 2,3-dioxygenase	-6.0	3.96E-04
Pfkfb1	P70266 PF261_MOUSE	55	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	-6.0	4.79E-03
Sept9	A2A6U3 A2A6U3_MOUSE	64	septin 9	-5.5	1.62E-02
Eif3e	P60229 EIF3E_MOUSE	52	eukaryotic translation initiation factor 3, subunit E	-5.3	9.44E-05
Eif3m	Q3TI04 Q3TI04_MOUSE	43	eukaryotic translation initiation factor 3, subunit M	-5.0	1.63E-04
Prps1	Q3TI27 Q3TI27_MOUSE	35	phosphoribosyl pyrophosphate synthetase 1	-4.5	3.57E-02
Mrc1	Q61830 MRC1_MOUSE	165	mannose receptor, C type 1	-4.4	2.57E-04
Atp11c	Q9QZW0 AT11C_MOUSE	129	ATPase, class VI, type 11C	-4.2	1.49E-11
Gcn11	Q3U3Z4 Q3U3Z4_MOUSE	118	GCN1 general control of amino-acid synthesis 1-like 1 (yeast)	-4.0	1.52E-03
Eif2s1	Q6ZWX6 EIF2A_MOUSE	36	eukaryotic translation initiation factor 2, subunit 1 alpha	-3.9	1.07E-04
Eif4b	Q8BGD9 EIF4B_MOUSE	69	eukaryotic translation initiation factor 4B	-3.7	6.28E-04
Gstp1	P19157 GSTP1_MOUSE	24	glutathione S-transferase, pi 1	-3.6	1.54E-05
Eif3c	Q8R1B4 EIF3C_MOUSE	106	eukaryotic translation initiation factor 3, subunit C	-3.4	3.84E-05
Dnm2	P39054 DYN2_MOUSE	98	dynamamin 2	-3.2	2.97E-05

Eif3h	Q8BMZ8 Q8BMZ8_MOUSE	7	eukaryotic translation initiation factor 3, subunit H	-3.1	5.95E-05
Eif3i	Q9QZD9 EIF3I_MOUSE	36	eukaryotic translation initiation factor 3, subunit I	-3.1	3.24E-03
Eif3d	O70194 EIF3D_MOUSE	64	eukaryotic translation initiation factor 3, subunit D	-3.1	4.08E-05
Eif3b	Q8CIJ3 Q8CIJ3_MOUSE	109	eukaryotic translation initiation factor 3, subunit B	-3.1	1.50E-09
Actr1b	Q8R5C5 ACTY_MOUSE	42	ARP1 actin-related protein 1 homolog B, contractin beta (yeast)	-3.0	1.89E-02
Cad	Q6P9L1 Q6P9L1_MOUSE	158	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	-3.0	9.82E-04
Abce1	P61222 ABCE1_MOUSE	67	ATP-binding cassette, sub-family E (OABP), member 1	-2.8	1.00E-04
Eif3eip	Q8QZY1 IF3EI_MOUSE	67	eukaryotic translation initiation factor 3, subunit L	-2.8	4.61E-10
Lman1	Q3U944 Q3U944_MOUSE	61	lectin, mannose-binding, 1	-2.8	4.30E-02
Asgr1	P34927 ASGR1_MOUSE	33	asialoglycoprotein receptor 1	-2.7	9.43E-14
Lrp1	Q91ZX7 LRP1_MOUSE	505	low density lipoprotein receptor-related protein 1	-2.7	5.92E-09
Usp9x	A2AD18 A2AD18_MOUSE	291	ubiquitin specific peptidase 9, X chromosome	-2.7	3.96E-02
Eif3a	P23116 EIF3A_MOUSE	162	eukaryotic translation initiation factor 3, subunit A	-2.7	8.68E-09
Scamp3	Q3TDM8 Q3TDM8_MOUSE	35	secretory carrier membrane protein 3	-2.6	3.64E-10
Rps8	P62242 RS8_MOUSE	24	ribosomal protein S8	-2.5	2.38E-04
Cyp2c50	Q91X77 CY250_MOUSE	56	cytochrome P450, family 2, subfamily c, polypeptide 50	-2.5	6.51E-03
Rrbp1	A2AVJ7 A2AVJ7_MOUSE	158	ribosome binding protein 1	-2.5	5.84E-03
Eif3j	Q3UGC7 Q3UGC7_MOUSE	29	eukaryotic translation initiation factor 3, subunit J	-2.4	8.60E-05
Hpx	Q3UKP2 Q3UKP2_MOUSE	51	hemopexin	-2.4	6.30E-04
Atl2	Q6PA06 ATLA2_MOUSE	66	atlastin GTPase 2	-2.2	3.28E-03
Cyp2d9	P11714 CP2D9_MOUSE	57	cytochrome P450, family 2, subfamily d, polypeptide 9	-2.2	3.68E-02
Copb1	Q9JIF7 COPB_MOUSE	107	coatamer protein complex, subunit beta 1	-2.2	1.05E-03
Vps26a	P40336 VP26A_MOUSE	38	vacuolar protein sorting 26 homolog A (yeast)	-2.2	1.11E-04
Ccdc22	Q9JIG7 CCD22_MOUSE	71	coiled-coil domain containing 22	-2.2	2.23E-03
Ugt2b1	Q8R084 Q8R084_MOUSE	60	UDP glucuronosyltransferase 2 family, polypeptide B1	-2.2	1.16E-03
Copa	Q8BTF0 Q8BTF0_MOUSE	139	coatamer protein complex subunit alpha	-2.1	1.98E-04
Pigr	O70570 PIGR_MOUSE	85	polymeric immunoglobulin receptor	-2.1	1.60E-10
Cyp1a2	P00186 CP1A2_MOUSE	58	cytochrome P450, family 1, subfamily a, polypeptide 2	-2.1	2.53E-03
Cct3	P80318 ITCPG_MOUSE	61	chaperonin containing Tcp1, subunit 3 (gamma)	-2.0	1.96E-02
Gnb2l1	P68040 GBLP_MOUSE	35	guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1	-2.0	1.41E-02
Dpp4	P28843 DPP4_MOUSE	87	dipeptidylpeptidase 4	-2.0	5.62E-03
Mup12	A2CEK7 A2CEK7_MOUSE	21	major urinary protein 12	-2.0	1.01E-02
Hp	Q61646 HPT_MOUSE	39	haptoglobin	-2.0	4.50E-04
M6pr	P24668 MPRD_MOUSE	31	mannose-6-phosphate receptor, cation dependent	-2.0	3.18E-03
Ap1m1	P35585 AP1M1_MOUSE	49	adaptor-related protein complex AP-1, mu subunit 1	-2.0	2.44E-03
Eif4a1	P60843 IF4A1_MOUSE	46	eukaryotic translation initiation factor 4A1	-2.0	5.05E-03

Abca6	Q8K441 ABCA6_MOUSE	183	ATP-binding cassette, sub-family A (ABC1), member 6	-1.8	6.82E-03
Anxa11	P97384 ANX11_MOUSE	54	annexin A11	-1.8	2.23E-02
Igf2r	Q07113 IMPRI_MOUSE	274	insulin-like growth factor 2 receptor	-1.8	7.61E-04
Cpne3	Q8BT60 CPNE3_MOUSE	60	copine III	-1.8	1.79E-10
Vps35	Q9EQH3 VPS35_MOUSE	92	vacuolar protein sorting 35	-1.7	6.09E-04
Clint1	Q3UGL3 Q3UGL3_MOUSE	68	clathrin interactor 1	-1.7	2.82E-04
Cope	O89079 COPE_MOUSE	35	coatamer protein complex, subunit epsilon	-1.7	1.11E-02
Dnaja1	P63037 DNJA1_MOUSE	45	DnaJ (Hsp40) homolog, subfamily A, member 1	-1.6	1.66E-03
Rps6	P62754 RS6_MOUSE	29	ribosomal protein S6	-1.6	1.29E-04
Rdh7	O88451 RDH7_MOUSE	36	retinol dehydrogenase 7	-1.6	2.30E-05
Arcn1	Q3U4S9 Q3U4S9_MOUSE	57	archain 1	-1.5	2.85E-02
Aadac	Q99PG0 AAAD_MOUSE	45	arylacetamide deacetylase (esterase)	-1.5	2.90E-02
Ugt2b5	P17717 UD2B5_MOUSE	61	UDP glucuronosyltransferase 2 family, polypeptide B5	-1.5	5.89E-03

Example 5. Bioinformatic analysis of proteomics

[0057] Proteins identified as significantly up- or down-regulated in the obese ER proteome were analyzed by Database for Annotation, Visualization and Integrated Discovery (DAVID, available on the internet at the [ncicrf](http://ncicrf.org) site (*see* Dennis et al., 4 Genome Biol. P3 (2003); Huang et al., 4 Nat. Protoc. 44 (2009)), as plotted in R. Clustering analysis was carried out with the Cluster3.0 program (Eisen et al., 95 PNAS 14863 (1998)), and visualized either in JavaTreeview or MeV (*Id.*; Saeed et al., 411 Meths. Enzymol. 134 (2006)). Functional annotation charts of proteins of interest (absolute median fold change ~1.5, significance of fold change ~0.05, average unadjusted spectral count of 5 across all experiments) were generated using the 'Biological Pathways' subset of Gene Ontology included in the DAVID System using all identified ER proteins as the background set. Biological pathway annotations were manually curated to remove redundant (identical) annotations associated with the same sets of proteins.

Example 6. Quantitative profiling of lipids and fatty acid compositions of ER and statistics

[0058] ER pellets (~50mg) were resuspended in 1 ml of 0.25 M sucrose, 200 µl of which was used for lipid extraction in the presence of authentic internal standards by the method of Folch et al., with chloroform:methanol (2:1 v/v). See Folch et al., 226 J. Biol. Chem. 497 (1957). Individual lipid classes were separated and quantified by liquid chromatography (Agilent Technologies model 1100 Series). To obtain the quantitative composition of fatty acids for each lipid class, the separated lipids were transesterified in 1 % sulfuric acid/methanol at 100°C for 45 minutes and extracted by 0.05% butylated hydroxytoluene/hexane. The resulting fatty acid methyl esters were quantified by gas chromatography (Agilent Technologies model 6890) under nitrogen.

[0059] The nmol% of each fatty acid was computed as the nmole quantity of the individual fatty acid divided by the total nmole amount of fatty acid isolated from each lipid class of each ER sample. The nmole% profile of fatty acids was then averaged in all six lean ER samples to examine the differences in the fatty acid profile that existed among different lipid classes. To identify compositional differences between control and experimental groups, Student's t-tests were performed for all fatty acid/lipid class combinations (26 x 9). The mean difference of nmol% for each fatty acid/lipid class combination with $p < 0.05$ were visualized in MeV34. Complete cluster analyses were performed for the fatty acid compositions of control and experimental groups using the Cluster3.0 program³³ with the following filter setting: 100% present, at least 50% samples with nmole% ~ 2 and (max-min) ~ 1 .

Table 2. Lipid composition of ER prepared from obese and lean mouse liver tissues

Lipid Class (nmol%)	obese mouse						lean mouse						diet #		ob. ave	lean ave	t TEST
	#1	#2	#3	#4	#5	#6	#1	#2	#3	#4	#5	#6	1	2			
Cholesterol	4.071	8.442	1.183	1.691	2.442	1.381	1.488	3.490	6.031	5183	4.367	1.354	0.010	0.010	3.201	3.769	0.696
Ester																	
Diacyl-glycerol	5.617	3.476	1.982	3.470	4.269	1.968	1A54	2557	3577	4 268	1.442	2.468	0.038	0.037	3.464	2.628	0.281
Free cholesterol	17.245	18.169	10.058	15.928	21.385	7.233	9.494	9.155	13.603	18.338	11.266	9528	0.118	0.121	15.118	11.897	0.251
Free fatty acid	8.286	10.921	5.763	15.017	5.356	8.776	5.295	8.452	20.915	12.751	6.277	7.163	0.516	0.005	8.187	10.133	0.466
Triacyl-glycerol	12.320	9.441	11.24	6.651	11386	9.335	9.986	19.135	5.945	7.998	9 542	11.1	0.733	9.732	10.561	9.176	7.45
Phospholipids	52. 461	48.849	69.767	62.251	55.161	71.308	72.283	66.212	49.980	50.813	67.056	68.065	0.995	0.995	59.966	62.393	0.665
Cardiolipin	4.994	3.331	2.680	2.974	3.261	2.731	4.543	3.237	4.469	3.471	3.590	3.779	0.032	6.031	1.323	3.848	0.237
Lysophosphatidylcholine	3.272	4.599	1.776	3.366	3.960	2.014	2.923	1.488	3.953	6.940	2.102	1.529	0.011	0.011	3.164	3.156	0.993
Phosphatidylcholine	26.088	21.077	36.680	36.270	26.469	41.815	31.917	33.044	20.967	17.553	31.225	32 349	0.027	0.026	31.733	27.841	0.394
Phosphatidylethanolamine	12.230	12.416	22.146	13.846	16.414	20.947	27.103	24159	13.067	15.011	4.292	25.826	0.019	0.021	16.338	21.794	0.105
Phosphatidylserine	5.876	0.426	4.485	5.795	503	3.920	5.491	4.284	7.424	7.888	4.247	4.591	5.556	0.046	5.404	5.754	0.675
PC/PE	2.133	1.698	1.747	2.620	1.610	1.996	1.164	1.368	1.695	1.169	1.235	1.252	1.389	1.229	1.967	1.299	0.003
PC/PS	2.081	1.672	4.938	2.389	3.273	5.483	4.992	5.639	1.748	1.915	5.218	5.624	4.195	4.446	3.3136	4.189	0.393

Example 7. Calcium transport assays

[0060] The calcium transport assay for measuring Serca activity was adapted from Moore et al. (250 J. Biol. Chem. 4562 (1975)). Briefly, fresh liver tissues were homogenized in

10 volumes of buffer containing 0.25 M sucrose, 2 mM Tris pH7.4 and 1 mM DTT and EDTA-free protease inhibitor. The ER pellet was obtained after a series of centrifugation as described in the previous section, and then resuspended in 0.25 M sucrose. The same procedure was employed to isolate microsomes from cultured Hepa1-6 cells except that cell pellet was lysed in hypotonic 0.1 M sucrose, 2 mM Tris pH7.4, 1 mM DTT and EDTA-free protease inhibitor. The calcium transport assay was carried out in reaction buffer containing 0.1 M KCl, 30 mM, 5 mM NaN₃, 5 mM MgCl₂, 5 mM K₂C₂O₄, 501&M of CaCl₂ (plus 1 µCi/µmol of ⁴⁵Ca), 1 µM Rethenium Red, 5mM ATP. The reaction was started by the addition of microsomes containing 150 µg proteins for 15 min in a 37°C water bath and stopped by the addition of 0.15 M KCl, 1 mM LaCl₃ and filtered through a 0.2µ HT Tuffryn membrane (PALL Corporation, NY). The calcium transport experiment with lipid overloading was carried out essentially as previously described (Li et al., 2004) except that liposomes were made of egg derived PC and PE by the ethanol injection method (Watanabe et al., 45 J. Electron. Mocrosc. 171 (1996)). The amount of SERCA independent calcium transport was quantified in the presence of 10 µM thapsigargin and subtracted from the calculation.

Example 8. Western blotting, real-time quantitative PCR and molecular cloning

[0061] For the preparation of total cellular proteins, ~0.1g of liver tissues were homogenized in 1ml of a cold lysis buffer containing 50 mM Tris-HCl (pH 7.0), 2 mM EGTA, 5 mM EDTA, 30 mM NaF, 10 mM Na₃VO₄, 10 mM Na₄P₂O₇, 40 mM 3-glycerophosphate, 1% NP-40, and 1% protease inhibitor cocktail. After a brief centrifugation (200 g x 10 min) to pellet down cell debris, 1/5 volume of 6x Laemmli buffer was added into the whole cell lysate, boiled and centrifuged at 10,000 g for 10 min. Protein concentrations were quantified with Bio-Rad Dc Protein Assay (Bio-Rad, CA). Western blotting of protein of interest was done as previously described. Erbay et al., 2009; Ozcan et al., 2006. Total RNA was extracted with Trizol reagent according to manufacturer's recommendations. A total of 2 µg of RNA was used for cDNA synthesis using High Capacity cDNA archiving system (Applied Biosystems). The SYBR real-time PCR system was used to quantify the transcript abundance for genes of interest (Table S6). Either 18S or 28S rRNA was used for internal control.

Example 9. Adenovirus-mediated loss- or gain-of-function experiments

[0062] For *Pemt* knockdown experiments, a series of DNA hairpins specifically targeting the mouse *Pemt* gene were designed by RNAXs (*see* Tafer et al., 26 Nat. Biotech. 578 (2008)), synthesized, cloned into the pENTR/U6 system (Invitrogen, CA) and tested in the

Hepa1-6 cell line. The sequence with best efficacy, and it has 5nt mismatch with the next closest match of genes, were recloned into the pAD/Block-iT-DEST system through recombination, as described, Cao et al., 134 Cell 933 (2008). The *LacZ* shRNA was also cloned into the pAD/Block-iT-DEST system as control. For *Serca2b* over-expression experiment, the open reading frame of human *Serca2b* or *Gfp* (control) was amplified, cloned into pENTR/TOPO vector and then recombined into the pAD/CMV/V5-DEST vector. Adenovirus (serotype 5, Ad5) for the construct of interest was produced and amplified in 293A cells, purified using CsCl column, desalted, and 1×10^{11} virus particles were used for each injection. Adenovirus transductions of mice were performed between 10-11 weeks of age. Blood glucose levels were measured after 6 hr of food withdrawal (9am-3pm) at before and 5 days post-injection and at the time of harvest (9-12 days). For histological analysis, liver tissues were fixed in 10% formalin solution, and sectioned for Hematoxylin and Eosin staining. All oligonucleotide sequences are listed in Table 5.

Samples	Ob/pent shRNAi				ob/lacZ RNAi				pent/ave lacZ.ave				TTEST
	1	2	3	4	1	2	3	4	1	2	3	4	
Lipid Class (nmol%)													
Cholesterol Ester	6.39	1.95	5.11	2.55	1.43	2.22	1.43	3.98	4.00	2.27	0.2017		
Diacylglycerol	1.17	1.45	1.51	1.45	1.79	2.60	1.46	1.53	1.40	1.85	0.1515		
Free cholesterol	6.96	7.83	7.76	6.84	11.42	7.99	6.86	4.01	7.35	7.57	0.8909		
Free fatty acid	2.67	2.59	3.91	3.47	3.37	2.66	2.36	2.26	3.16	2.66	0.2641		
Triacylglycerol	11.69	8.57	9.33	13.46	12.33	11.45	19.90	19.76	10.76	15.86	0.0933		
Phospholipids	71.13	77.61	72.36	72.22	69.67	73.07	67.98	68.46	73.33	69.79	0.1047		
Cardiolipin	9.99	10.12	7.67	8.47	9.26	6.85	7.54	7.73	9.06	7.84	0.1712		
lysophosphatidylcholine	1.77	1.37	1.09	1.25	1.28	1.23	1.15	1.28	1.37	1.23	0.3917		
Phosphatidylcholine	30.07	34.20	32.59	35.16	37.46	41.03	37.96	40.60	33.00	39.26	0.0047		
Phosphatidylethanolamine	24.11	25.46	24.55	21.71	18.09	17.73	17.27	15.65	23.95	17.18	0.0004		
Phosphatidylserine	5.19	6.46	6.48	5.64	3.59	6.22	4.07	3.21	5.94	4.27	0.0660		
PC/PE	1.25	1.34	1.33	1.62	2.07	2.31	2.20	2.60	1.38	2.29	0.0006		
PC/PS	5.79	5.29	5.04	6.23	10.45	6.60	9.34	12.65	5.59	9.76	0.0176		

Table 3

[illegible]

Table 4

Genes	Orientation	Sequence	Usage
18S	forward	AGCCCCTGCCCTTTGTACACA	q-PCR
18S	reverse	CGATCCGAGGGCCCTCACTA	q-PCR
28S	forward	TGTTGACGCGATGTGATTTCTGCC	q-PCR
28S	reverse	AGATGACGAGGCATTTGGCTACCT	q-PCR
Ces3	forward	ATGCGCCTCTACCCTCTGATA	q-PCR
Ces3	reverse	AGCAAATCTCAAGGAGCCAAG	q-PCR
Dak	forward	TCGGGAAAGGGATGCTAACAG	q-PCR
Dak	reverse	CAAGTCCAAAGTTGAGCCGAT	q-PCR
Dgat2	forward	GCGCTACTTCCGAGACTACTT	q-PCR
Dgat2	reverse	GGGCCTTATGCCAGGAACT	q-PCR
Fas	forward	TATCAAGGAGGCCCATTTTGC	q-PCR
Fas	reverse	TGTTTCCACTTCTAAACCATGCT	q-PCR
Herpud1	forward	CTGGGGACTCCTCAAGTGATG	q-PCR
Herpud1	reverse	ACGTTGTGTAGCCAGAGAAGC	q-PCR
LacZ	top	CACCGCTACACAAATCAGCGATTTGAAAAATCGCTGATTTGTGTAG	shRNA
LacZ	bottom	AAAACCTACACAAATCAGCGATTTTTCGAAATCGCTGATTTGTGTAGC	shRNA
Mttp	forward	ATACAAGCTCACGTACTCCACT	q-PCR
Mttp	reverse	TCCACAGTAACACAACGTCCA	q-PCR
Pcyt1a	forward	GATGCACAGAGTTCAGCTAAAGT	q-PCR
Pcyt1a	reverse	TGGCTGCCGTAAACCAACTG	q-PCR
Pcyt2	forward	TGTGTTCACGGCAATGACATC	q-PCR
Pcyt2	reverse	TTCCCGGTA CT CAGAGGCAT	q-PCR
Pemt	forward	TTGGGGATTCTGTGTTTGTGCT	q-PCR
Pemt	reverse	CACGCTGAAGGGAAATGTGG	q-PCR
Ptdss1	forward	GCAGGACTCTGAGCAAGGATG	q-PCR
Ptdss1	reverse	GGCGAAGTACATGAGGCTGAT	q-PCR
Ptdss2	forward	GGATTGCCTTTTCAGTTCACGC	q-PCR
Ptdss2	reverse	AGGTAGAAGGTGTTTCAGCTCTG	q-PCR
Scd1	forward	TTCTTGCGATACACTCTGGTGC	q-PCR
Scd1	reverse	CGGGATTGAATGTTCTTGTGCT	q-PCR
Serca2 (Atp2a2)	forward	CATGCACCGATGGGATTTCTCT	q-PCR
Serca2 (Atp2a2)	reverse	CGCTAAAGTTAGTGTCTGTGCT	q-PCR
Pemt	top	CACCGCCATGTCCCGACACACTAACTCGAGTTAGTGTGTGCGGACATGG	shRNA
Pemt	bottom	AAAACCATGTCCCGACACACTAACTCGAGTTAGTGTGTGCGGACATGGC	shRNA
Serca2b	forward	CACCGCCGTTTGTAATTCTGCTTATCTCGAGATAAGCAGAATTACAAACGGC	shRNA
Serca2b	reverse	AAAAAGCCGTTTGTAATTCTGCTTATCTCGAGATAAGCAGAATTACAAACGGC	shRNA
Serca2b	forward	GCCATGGAGAACGCGCACAC	ORF cloning
Serca2b	reverse	AGACCAGAACATATCGCTAAAGTTAG	ORF cloning

Table 5

CLAIMS

1. A method of treating hepatic chronic endoplasmic reticulum (ER) stress in an obese subject comprising modulating the phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratio in the liver, wherein the subject is suffering from type 2 diabetes, dislipidemia, fatty liver disease, inflammation, or atherosclerosis; and wherein the correcting improves glucose homeostasis.
2. The method of claim 1, wherein modulating is lowering the PC/PE ratio to about 1.3
3. The method of claim 1 or 2, wherein the modulating comprises genetic, chemical or dietary intervention.
4. The method of claim 3, comprising inhibiting expression or function of phosphatidylethanolamine N-methyltransferase, encoded by *Pemt*.
5. A method of treating hepatic chronic endoplasmic reticulum (ER) stress in an obese subject comprising modulating calcium homeostasis in the liver, wherein the subject is suffering from type 2 diabetes, lipodemia, fatty liver disease, inflammation, or atherosclerosis; and wherein the correcting improves glucose homeostasis.
6. The method of claim 5, wherein the modulating comprises genetic, chemical or dietary intervention.
7. The method of claim 5, wherein the modulating comprises increasing hepatic concentration, expression or activity of sarco/endoplasmic reticulum calcium ATPase (SERCA).
8. The method of claim any of the previous claims, wherein the modulating comprises inhibiting *de novo* synthesis of saturated fatty acids and monounsaturated fatty acids in liver.
9. The method of any of the previous claims, further comprising the step of monitoring expression of asialoglycoprotein receptor (ASGR) and/or haptoglobin (HP).
10. The method of any of the preceding claims, wherein said modulating comprises down-regulating hepatic expression of at least one of:

a *de novo* lipogenesis gene selected from *Fas*, *Scd1*, *Ces3*, *Dgat2* and *Dak2*;

a phospholipid synthesis gene selected from *Pcyt1a* and *Pemt*;

a lipoprotein synthesis gene *ApoA4*; or

a gene involved in glucose production selected from *G6* and *Pck1*.

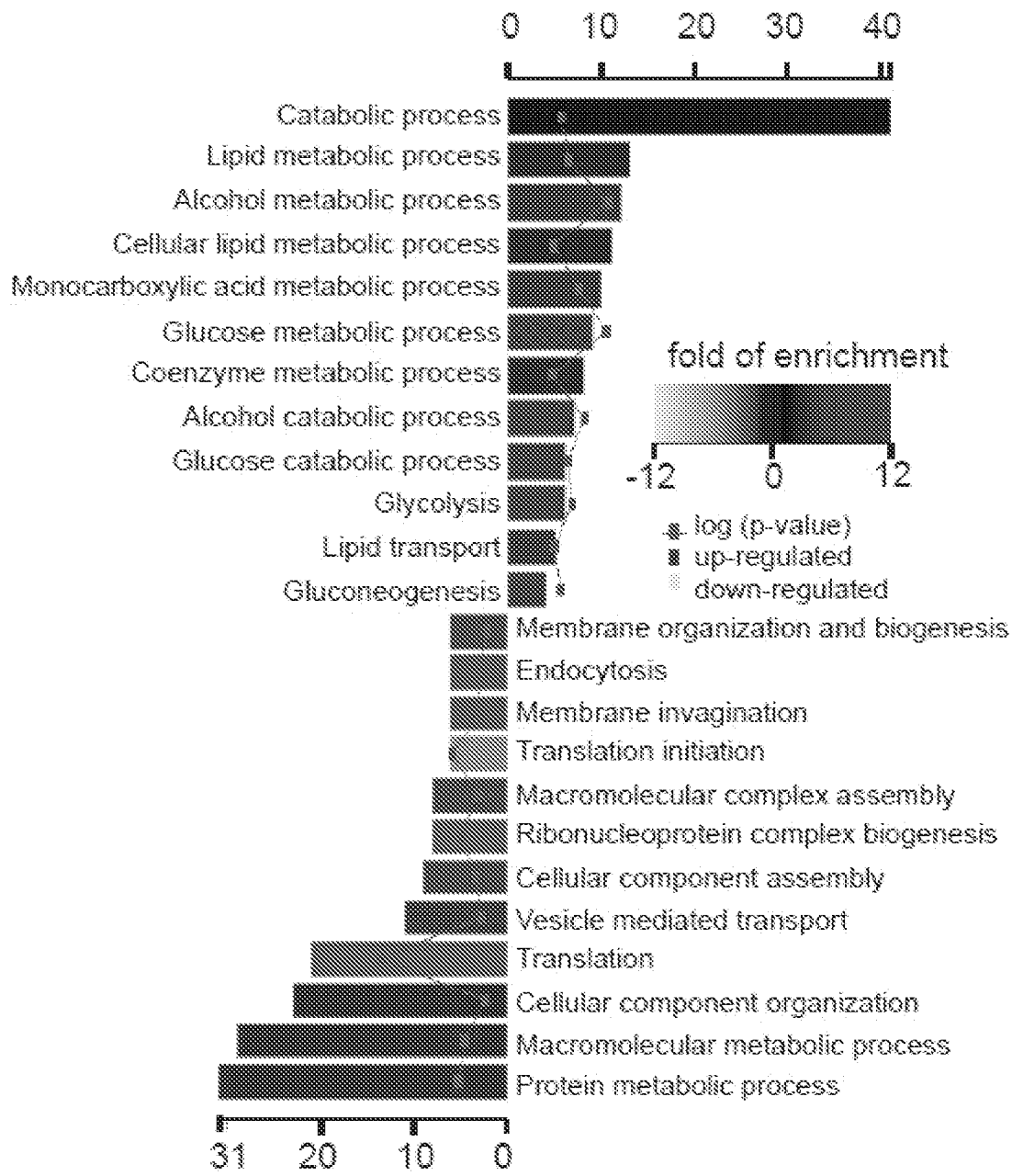


Figure 1A

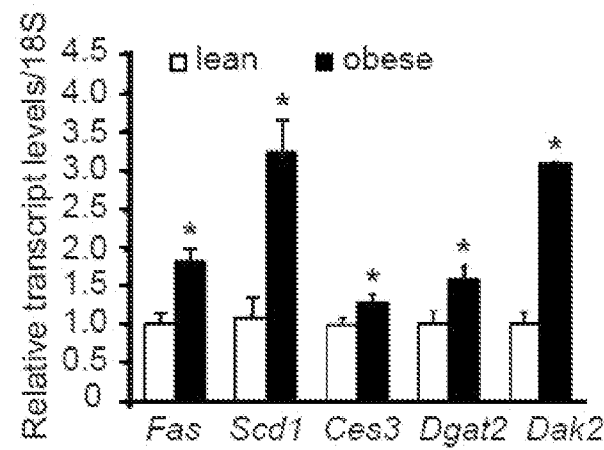


Figure 1B

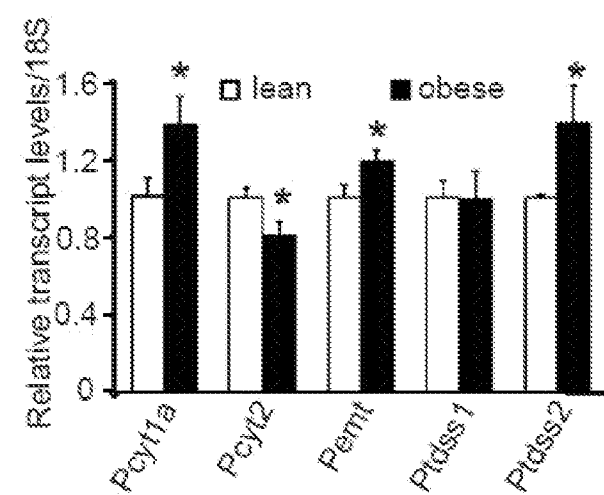


Figure 1C

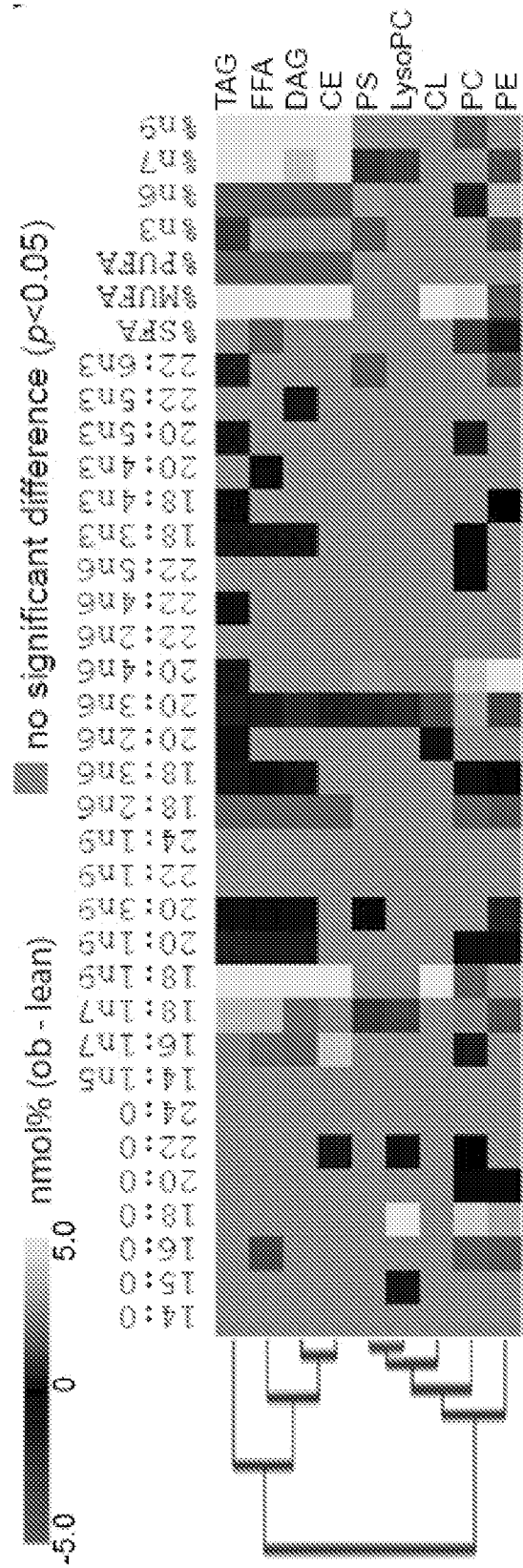


Figure 1D

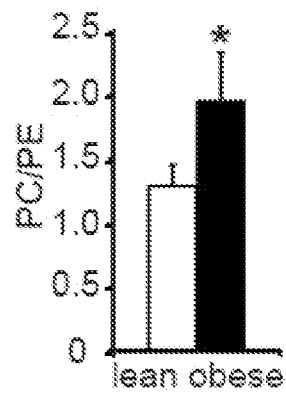
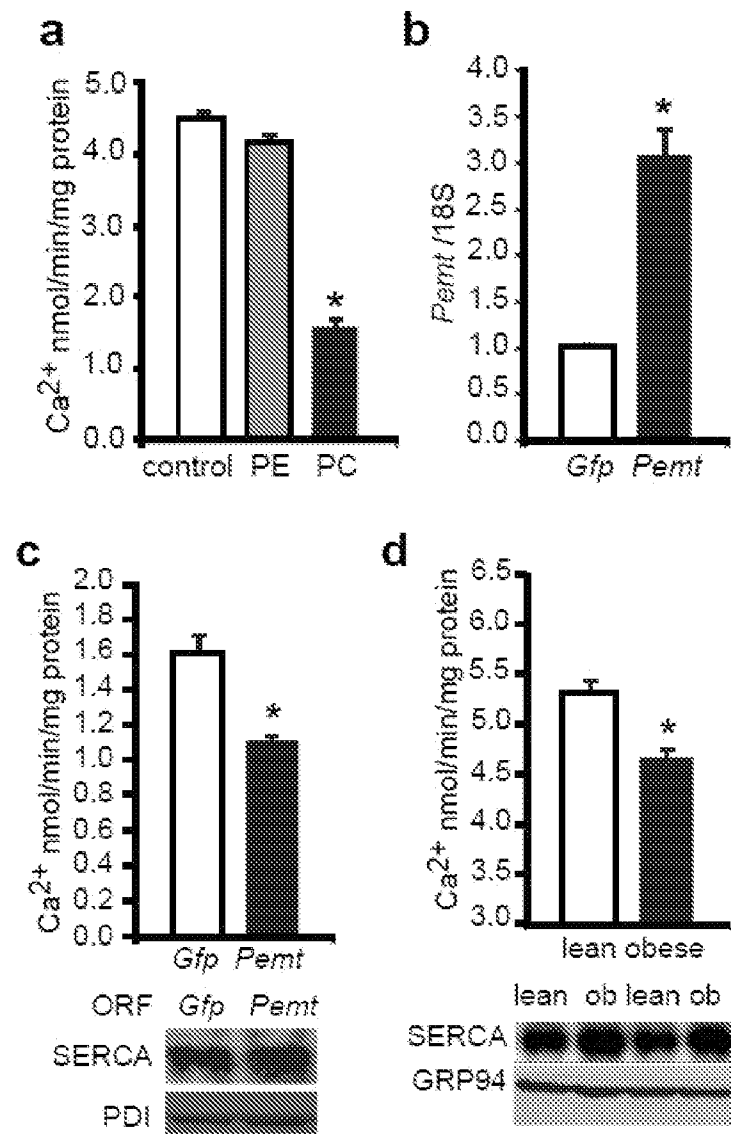
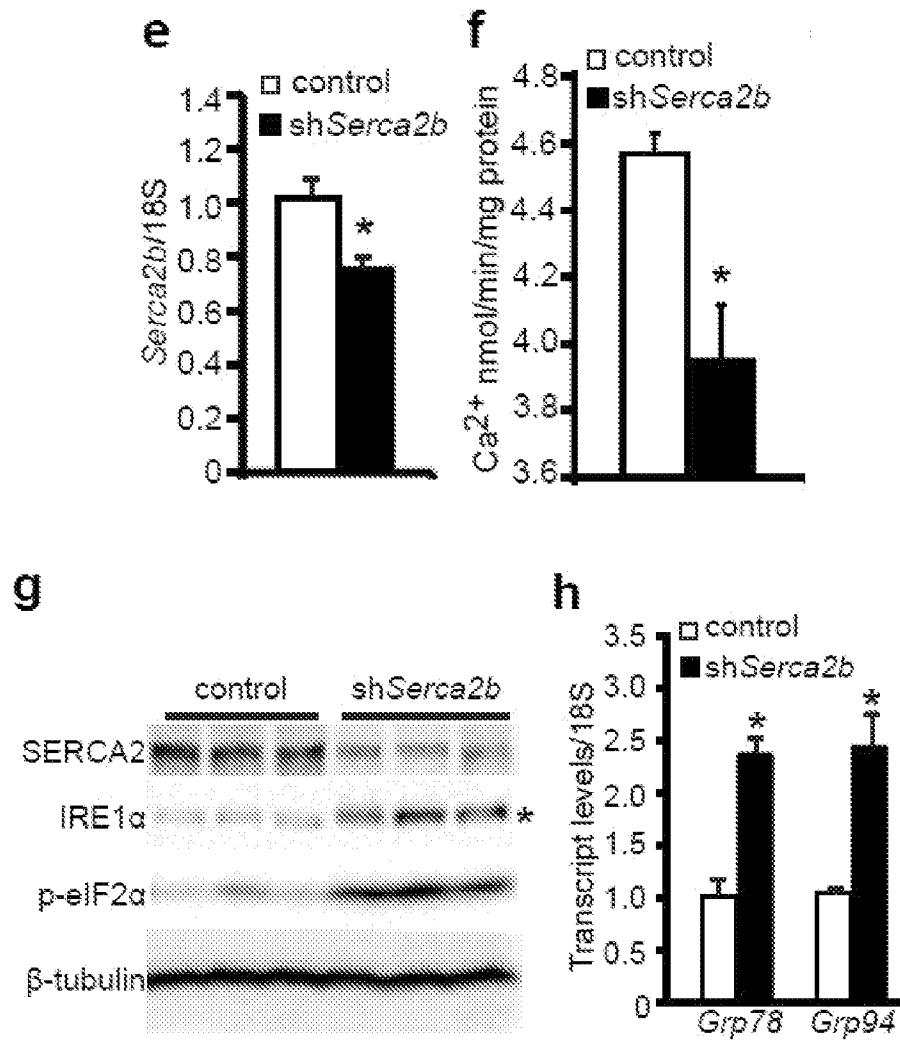


Figure 1E



Figures 2a-2d



Figures 2e-2h

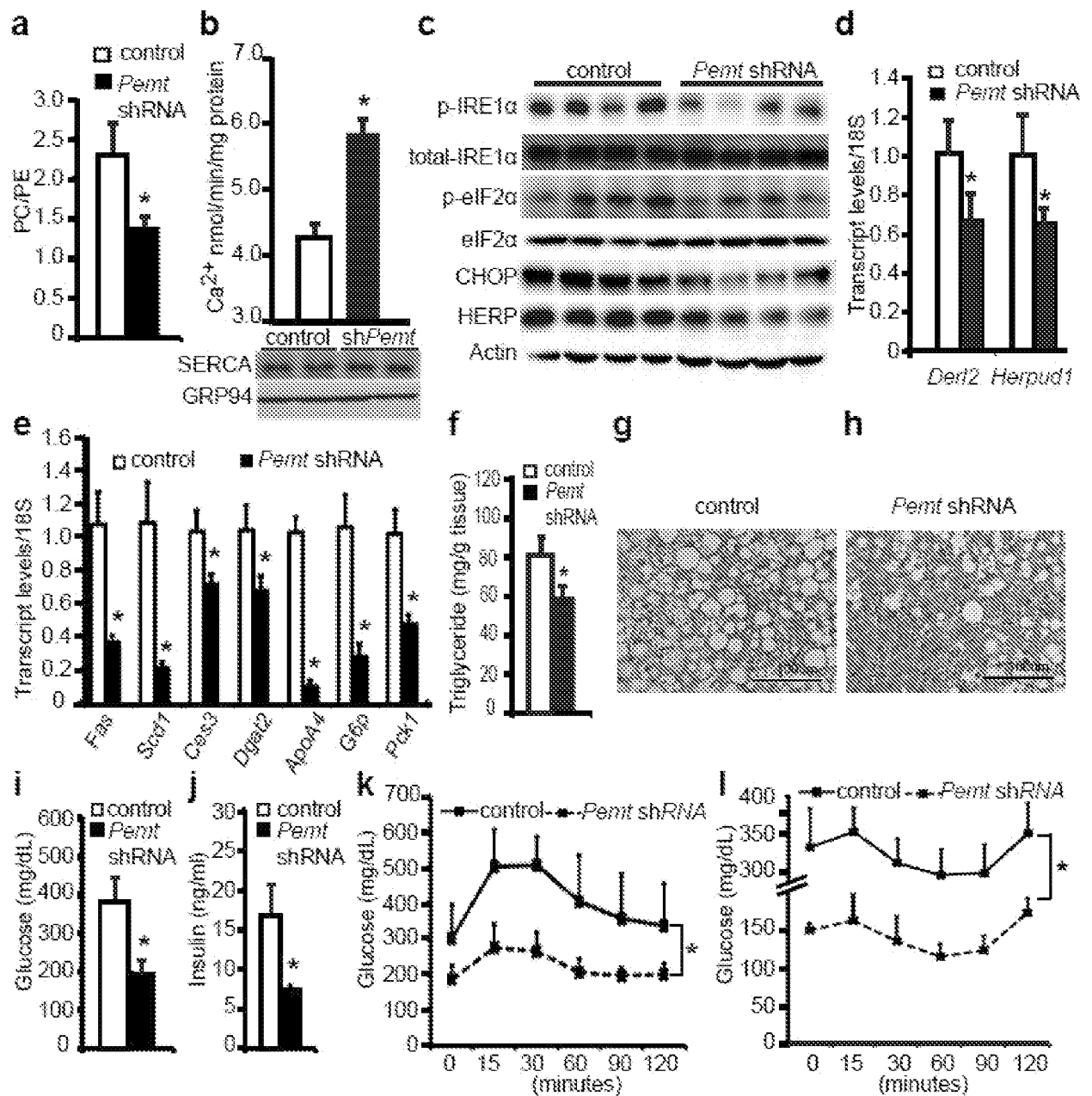


Figure 3

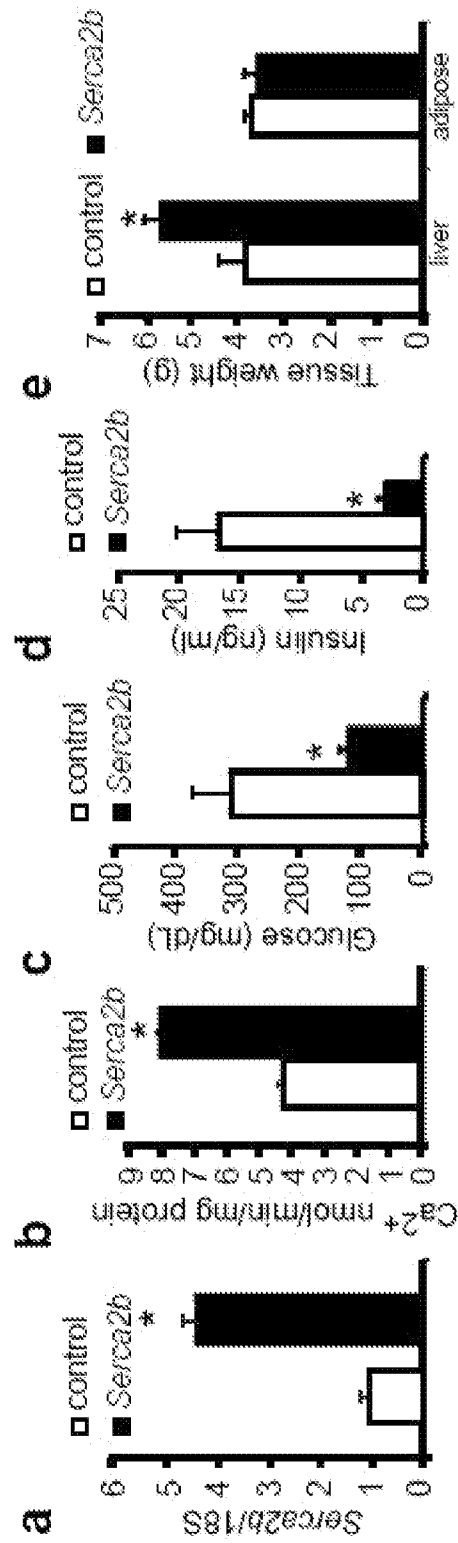


Figure 4a-4e

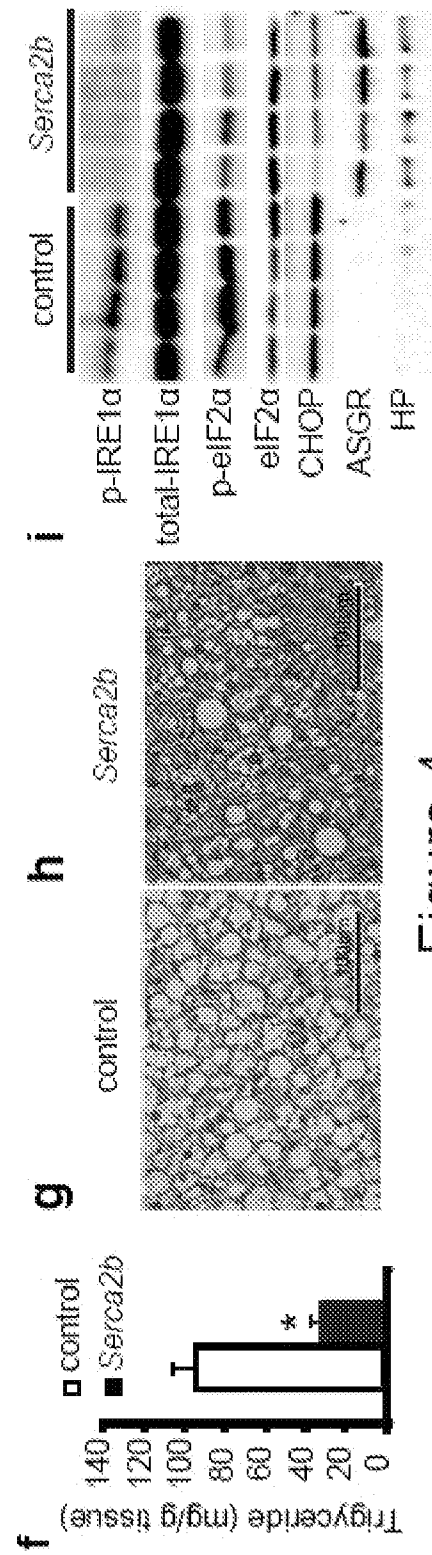


Figure 4

Figures 4f-4i

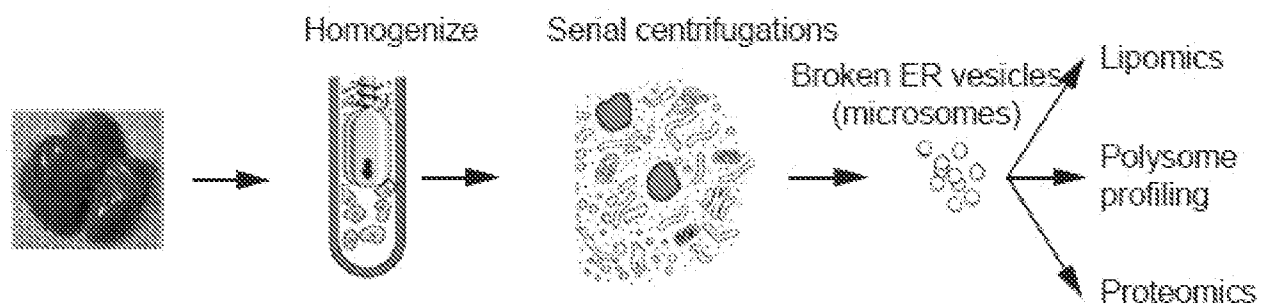


Figure 5A

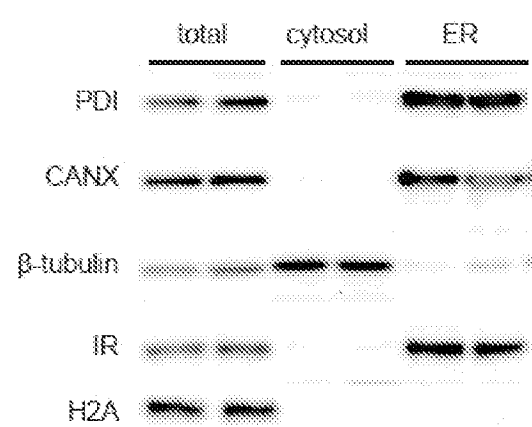


Figure 5B

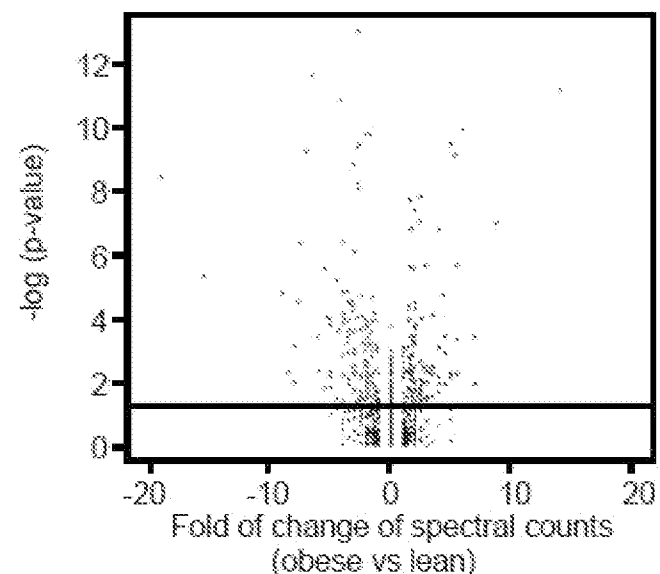


Figure 5C

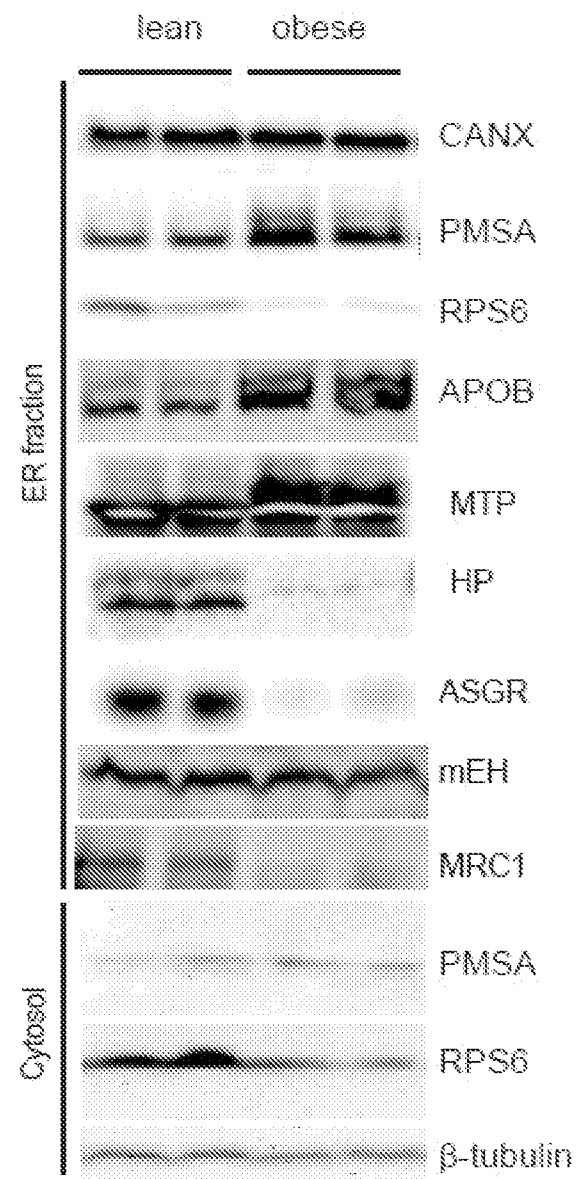


Figure 5D

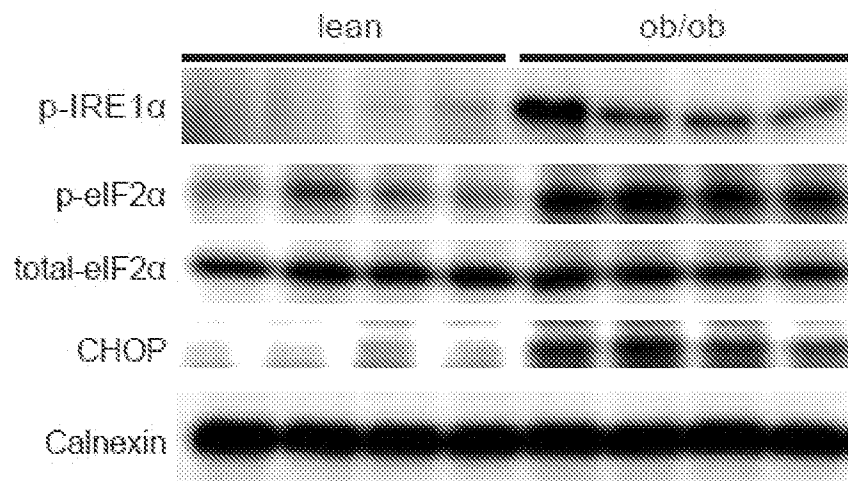


Figure 6A

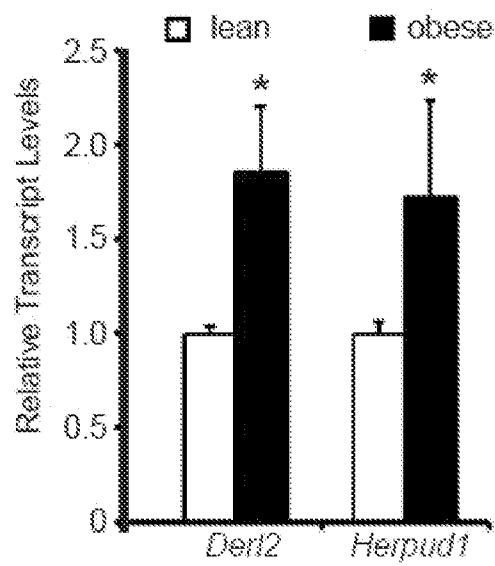


Figure 6B

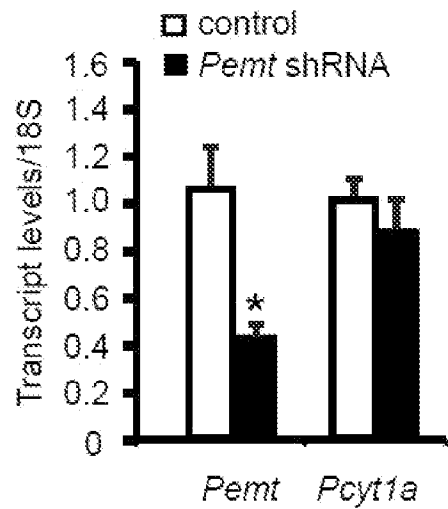


Figure 8A

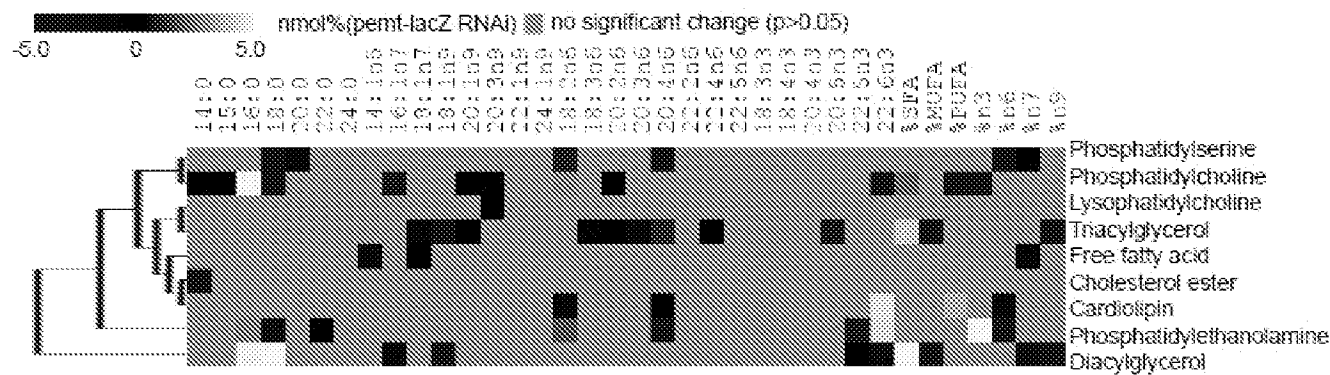


Figure 8B

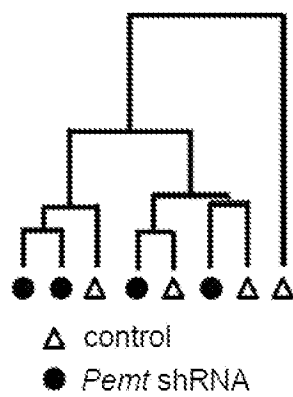


Figure 8C

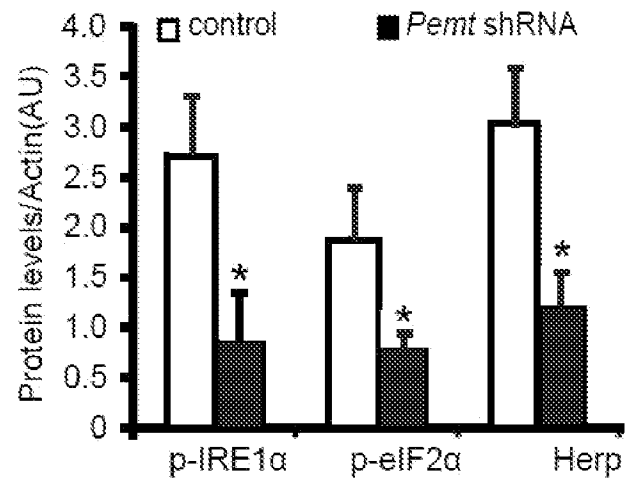
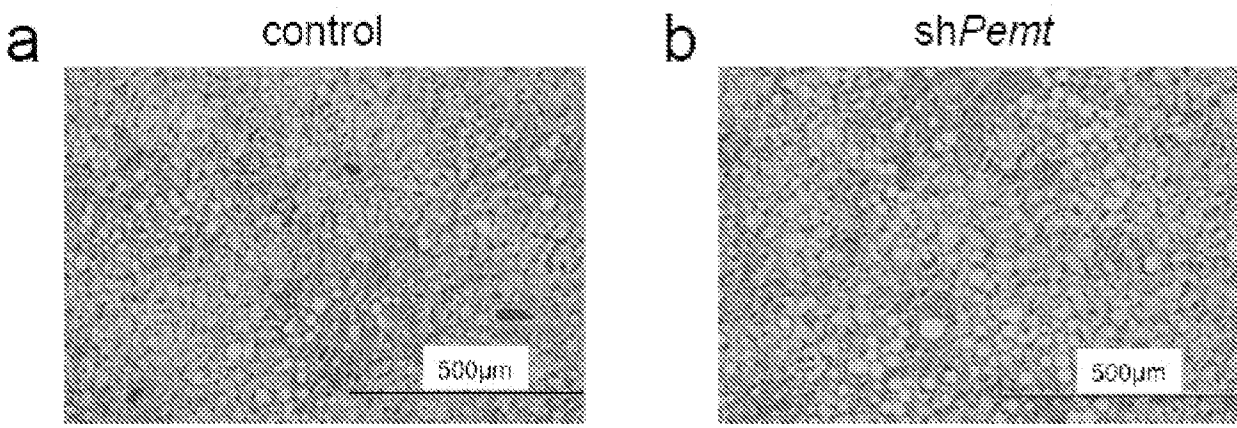
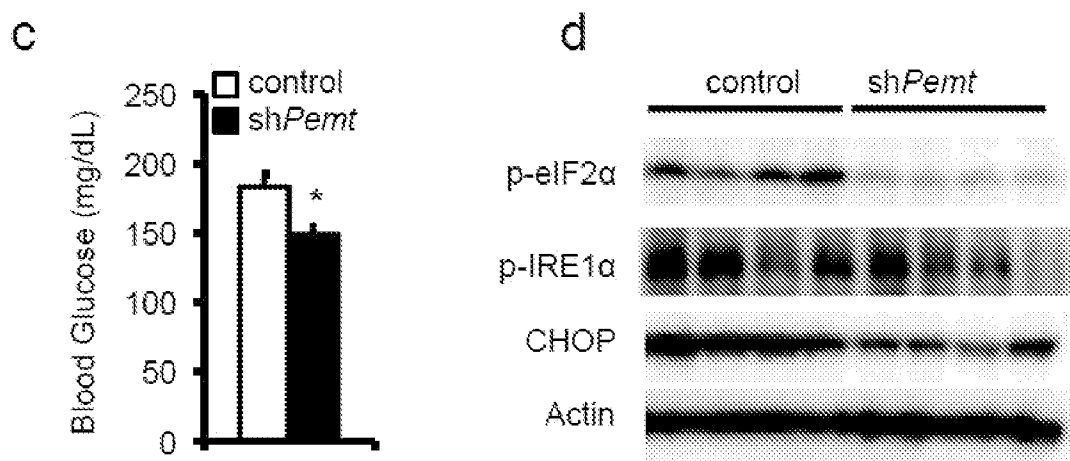


Figure 8D



Figures 9A and 9B



Figures 9C and 9D

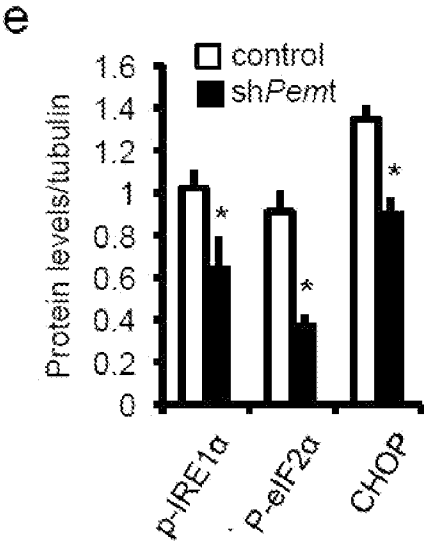


Figure 9e

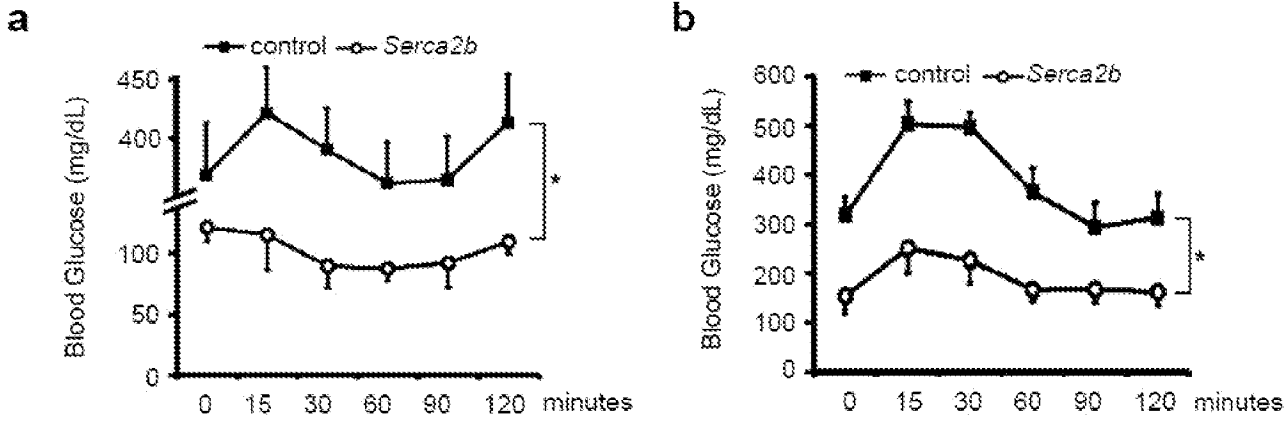


Figure 10

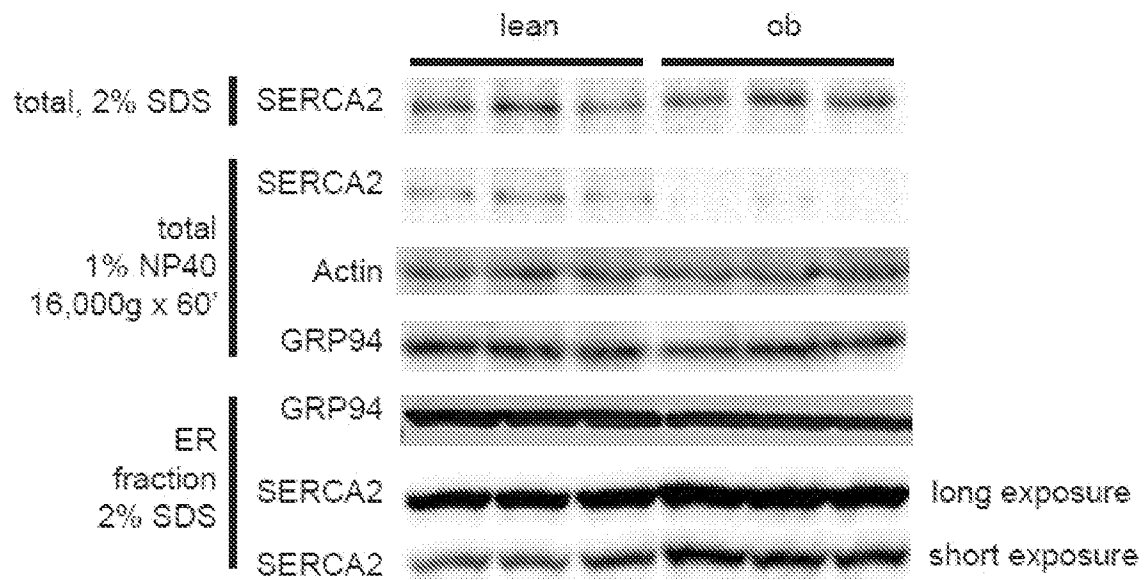
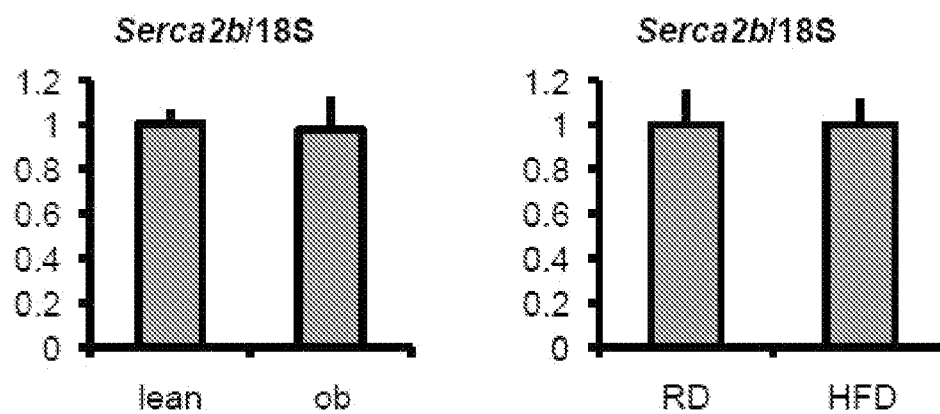


Figure 11A



Figures 11B, 11C



Figure 11D



Figure 11E