Published in final edited form as:

Metabolism. 2013 May ; 62(5): 753–760. doi:10.1016/j.metabol.2012.12.001.

Fatty acid-mediated endoplasmic reticulum stress in vivo: Differential response to the infusion of Soybean and Lard Oil in rats

Angela M. Nivala¹, Lauren Reese¹, Melinda Frye², Christopher L. Gentile¹, and Michael J. Pagliassotti¹

¹Departments of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523

²Biomedical Sciences, Colorado State University, Fort Collins, CO 80523

Abstract

Background—In cell systems, saturated fatty acids, compared to unsaturated fatty acids, induce a greater degree of ER stress and inflammatory signaling in a number of cell types, including hepatocytes and adipocytes. The aim of the present study was to determine the effects of infusions of lard oil (enriched in saturated fatty acids) and soybean oil (enriched in unsaturated fatty acids) on liver and adipose tissue ER stress and inflammatory signaling in vivo.

Methods—Lipid emulsions containing glycerol, phosphatidylcholine, antibiotics (Control, n=7) and either soybean oil (Soybean, n=7) or lard oil (Lard, n=7) were infused intravenously into rats over a 4 hour period.

Results—Plasma free fatty acid levels were 0.5 ± 0.1 mM (mean $\pm$ SD) in Control and were increased to 1.0 ± 0.3 mM and 1.1 ± 0.3 mM in Soybean and Lard, respectively. Glucose and insulin levels were not different among groups. Markers of endoplasmic reticulum (ER) stress and activation of inflammatory pathway signaling were increased in liver and adipose tissue from Soybean and Lard compared to Control, but were increased to a greater extent in Lard compared to Soybean.

Conclusions—These data suggest that elevated plasma free fatty acids can induce hepatic and adipose tissue ER stress and inflammation in vivo. In addition, saturated fatty acids appear to be more cytotoxic than unsaturated fatty acids in vivo.

Keywords

Liver; adipose tissue; inflammation; obesity; non-alcoholic fatty liver disease

© 2012 Elsevier Inc. All rights reserved

Correspondence: Michael Pagliassotti, Department of Food Science and Human Nutrition, Colorado State University, 234 Gifford, Fort Collins, CO 80523-1571; Phone: 970-491-1390; Fax: 970-491-3875; michael.pagliassotti@colostate.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions A.M.N., M.F., C.L.G. and M.J.P. are responsible for the conception and design of the study; A.M.N., L.R., C.L.G. and M.J.P. performed the experiments; A.M.N., L.R., M.F. and M.J.P. analyzed the data; A.M.N., C.L.G. M.F. and M.J.P. interpreted the results of the analyses; A.M.N. and M.J.P. wrote the manuscript; L.R., M.F. and C.L.G. edited the manuscript; all authors approved the final version of the manuscript.

Introduction

The initial stage of non-alcoholic fatty liver disease (NAFLD) involves accumulation of triglycerides in the liver. Hepatic lipids are derived from three potential sources: the diet, de novo lipogenesis, and circulating free fatty acids released from adipose tissue. Donnelly et al reported that the latter source accounts for ~60% of the hepatic triglyceride content in NAFLD patients [1]. In addition to their role in the development of NAFLD, fatty acids appear to play an important role in disease progression. Circulating free fatty acids are elevated in patients with NAFLD and are positively correlated with disease severity [2, 3], and experimental suppression of fatty acids improves hepatic insulin sensitivity and reduces liver enzymes in healthy individuals [4]. These data have led to the concept that elevated fatty acids and products of fatty acid metabolism, rather than triglycerides per se, promote hepatotoxicity. Consistent with this notion, hepatic triglycerides are higher in patients with benign steatosis compared to those with non-alcoholic steatohepatitis [5], and esterification of fatty acids into triglycerides prevents saturated fatty acid-mediated toxicity in hepatocytes and reduces liver damage in experimental animals[6–8].

Several studies have linked endoplasmic reticulum dysfunction (ER stress) and activation of the unfolded protein response (UPR) to impairments in glucose homeostasis, insulin action and inflammation [9–11]. ER stress and activation of the UPR have also been observed in the liver and/or adipose tissue in genetic and dietary murine models of obesity, dietary models of NAFLD, and in humans with NAFLD [10, 12–15]. The factors that induce ER stress in chronic disease models or in humans are poorly understood. In cell systems, elevated fatty acids, in particular saturated fatty acids, produce ER stress and activation of the UPR in a number of cell types, including hepatocytes[16–18]. To the best of our knowledge, a direct comparison of the effects of unsaturated and saturated fatty acids on ER stress in vivo has not been performed. This is particularly relevant given that previous human studies have suggested that a) saturated fatty acids have selective effects on hepatic glucose metabolism [19] and b) serum saturated fatty acid-containing triglycerides may be better markers of insulin resistance than total serum triglycerides [20]. Therefore, the aim of this study was to examine the effects of saturated and unsaturated fatty acids on ER stress and inflammatory markers in liver and adipose tissue in vivo. To mimic conditions of increased adipose tissue-derived fatty acids observed in individuals with NAFLD, we measured outcomes in response to short term infusion of either lard oil or soybean oil.

Methods

Animals

Male Wistar Crl(WI)BR rats (Charles River, Wilmington, MA) weighing ~180 g upon arrival were provided free access to a purified high starch diet (Research Diets, Inc., New Brunswick, NJ[21]) and water for 1 wk. Rats were housed individually in a temperature- and humidity-controlled environment with a 12-h light:dark cycle. All procedures were reviewed and approved by the Colorado State University institutional animal care committee.

Surgical Procedures

Catheters were implanted in the carotid artery and jugular vein under general anesthesia as described previously [22, 23]. Experiments were performed following 4–5 days of recovery at which time body weight was >100% of pre-surgery body weight.

Lipid Emulsions

Lipid emulsions were prepared based on the protocols of Stein et al [24]. Briefly, emulsions consisted of glycerol, phosphatidylcholine, penicillin-streptomycin and heparin, and either

no other additions (Control, n=7), soybean oil (Sigma Chemical Co, St. Louis, MO, Soybean, n=7) or lard oil (Sigma, Lard, n=7). The composition of Soybean oil was: 11% 16:0, 4 % 18:0, 23.4% 18:1, 53.2% 18:2 and 8% 18:3 and the composition of Lard oil was: 1.5% 14:0, 24.8% 16:0, 12.3% 18:0, 45.1% 18:1, 10% 18:2 and 0.1% 18:3. Emulsions were heated at 80°C for ~20 min and then sonicated immediately prior to use.

Experimental Procedures

Control, Soybean or Lard intravenous infusions were initiated at a rate of 70 μ l/min per kg and continued for 240 min. The 4 hour duration was chosen based on the volume delivered to the animals and the amount of blood taken over this period of time. Arterial blood samples were taken prior to the start of infusions and every 30 min during infusions. Following the last blood samples animals were deeply anesthetized (sodium pentobarbital, 70 mg/kg iv) and liver and retroperitoneal adipose tissue was removed for processing as described previously [12].

Analyses

Plasma Measures—Glucose was measured with an automated analyzer (Beckman Instruments, Fullerton, CA). Insulin was analyzed by ELISA (Linco Research, St. Charles, MO). Free fatty acids were analyzed using the HR series NEFA kit (Wako Pure Chemical Industries, Osaka, Japan). Plasma free fatty acid composition was determined by gas liquid chromatography (Hewlett-Packard 5890 Series II, Palo Alto, CA), and methylation of the free fatty acids was performed as described by Boberg et al [25].

Western Blot Analysis—Liver and adipose tissue were processed and analyzed as described previously [12, 14]. Primary antibodies included total and phosphorylated RNA-dependent protein kinase-like ER eukaryotic initiation factor-2 α kinase (PERK), tumor necrosis factor- α (TNF α), interleukin-6 (IL6), monocyte chemoattractant protein-1 (MCP1) and haptoglobin (Santa Cruz Biotechnology, Santa Cruz, CA); total and phosphorylated eukaryotic initiation factor-2 α (eIF2 α), total and phosphorylated I κ B kinase β (IKK β ; Cell Signaling, Beverly, MA); matrix metalloproteinase-12 (Mmp12), suppressor of cytokine signaling-3 (SOCS-3), cluster of differentiation 68 (CD68; Abcam, Cambridge, MA); and macrophage inflammatory protein-1 α (MIP1 α ; Lake Forest, CA).

RNA analysis—Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). For PCR analysis of XBP-1, a two-step protocol was used for reverse transcription PCR using SuperScript II reverse transcriptase and Taq polymerase [26]. For Real Time PCR, reverse transcription was performed using 0.5 μ g of DNase-treated RNA, Superscript II RnaseH- and random hexamers as described previously [18]. Target genes were normalized to β -microglobulin or 18S rRNA.

Statistical Analysis

Group comparisons were performed using a one-way ANOVA or the Kruskal-Wallis ANOVA on ranks. Post hoc testing was done with the Student-Newman-Keuls test. Differences were considered significant at $P < 0.05$. All data are reported as the mean $\pm$ standard deviation.

Results

Plasma glucose, insulin and free fatty acids

Plasma glucose and insulin levels were not different among groups (Fig. 1). Plasma free fatty acids levels were increased in Soybean and Lard compared to Control at 90 minutes

and all subsequent time points (Fig. 1). The fatty acid composition of circulating free fatty acids is provided in table 1. The infusion of soybean oil increased the levels of 18:1, 18:2 and 18:3(n-6), whereas the infusion of lard oil increased the levels of 14:0, 16:0, 18:0, 18:1 and 18:2 when compared to control values. The infusion of lard oil also increased the levels of 14:0, 16:0, 18:0 and 18:1 when compared to soybean oil.

ER stress and inflammatory markers in the liver

Disruption of ER homeostasis, collectively termed ER stress, activates the UPR [27]. The UPR is initiated by three ER transmembrane proteins, inositol requiring ER-to-nucleus signaling protein 1 α (IRE1 α), PERK and activating transcription factor-6 (ATF6) [28]. Activation of IRE1 α promotes the splicing of X-box-binding protein-1 (Xbp1) mRNA and subsequent transcription of molecular chaperones (e.g. glucose regulated protein 78 (GRP78)) and genes involved in ER-associated degradation [29]. PERK activation leads to phosphorylation of the α -subunit of the translation initiation factor eIF2 and subsequent attenuation of translation initiation, as well as increased expression of GRP78, C/EBP Homologous Protein (CHOP), a pro-apoptotic gene, and growth arrest and DNA damage-inducible protein 34 (GADD34) [29, 30]. GADD34 mediates dephosphorylation of eIF2 α and therefore reversal of translational attenuation [28]. Activation of ATF6 can also lead to increased expression of both molecular chaperones and CHOP. Phosphorylation of PERK and eIF2 α , XBP1 splicing, and the expression of UPR target genes were increased in Soybean compared to Control and in Lard compared to Soybean and Control (Fig. 2). Phosphorylation of IKK β and the expression of inflammatory genes were increased in Soybean compared to Control and in Lard compared to Soybean and Control (Fig. 2 and 3). The choice of inflammatory genes related to the fact that they were either targets of NFK β and/or were previously demonstrated to be upregulated under conditions of inflammation in the liver [31]. MIP1 α and Mmp12 protein levels were significantly increased in Lard compared to Soybean and Control (Supplemental Figure 1).

ER stress and inflammatory markers in adipose tissue

Phosphorylation of PERK and eIF2 α , XBP1 splicing, and the expression of UPR target genes were increased in Soybean compared to Control and in Lard compared to Soybean and Control (Fig. 4). Phosphorylation of IKK β and the expression of inflammatory genes were increased in Soybean compared to Control and in Lard compared to Soybean and Control (Fig. 4 and 5). The choice of inflammatory genes related to the fact that they were either targets of NFK β and/or were previously demonstrated to be upregulated under conditions of inflammation in adipose tissue [32]. MCP1 and haptoglobin protein levels were significantly increased in Lard compared to Soybean and Control (Supplemental Figure 2).

Discussion

Obesity is characterized by hyperlipidemia, insulin resistance, hepatic steatosis, inflammation and ER stress [1, 14, 15, 33]. Elevated circulating free fatty acids appear to play an important role in the development of insulin resistance and hepatic steatosis [1, 33, 34]. In cell systems, long-chain saturated fatty acids induce ER stress and/or cell death, whereas unsaturated fatty acids typically do not [16, 18, 35, 36]. In the present study, we examined the potential role of saturated and unsaturated fatty acids on markers of ER stress and inflammation in vivo. Our data suggest that the short term infusion of soybean or lard oil increased markers of ER stress and inflammation in the liver and adipose tissue. However, these markers were increased to a greater extent following the infusion of lard oil.

Although the link between obesity and ER stress in liver and adipose tissue has been established in murine models and in humans, the physiologic processes affected by ER stress and the factors that lead to ER stress remain unclear [10, 14, 15]. Two previous in vivo studies demonstrated that elevated fatty acids can induce ER stress in the liver [37, 38]. Infusion of 6 mM albumin-bound oleate resulted in increased phosphorylation of eIF2 α and XBP1 splicing in the liver of mice [37]. Glucose and/or lipid infusions (Liposyn III) at rates equivalent to 17–50% of normal daily calorie intake resulted in increased markers of ER stress in rat liver [38]. Results from the present study support and extend these findings, in that the infusion of soybean oil or lard oil increased markers of ER stress and inflammation in both liver and adipose tissue.

In the present study, lard oil and soybean oil infusions resulted in similar total free fatty acid concentrations, however the former resulted in increased markers of ER stress and inflammation in both liver and adipose tissue. These data suggest that enrichment of the plasma free fatty acid pool with saturated fatty acids may be more deleterious than enrichment with unsaturated fatty acids. In the present study the graded increase in markers of ER stress and inflammatory signaling observed in soybean and lard oil infused rats correlated with the graded increase in the total amount of saturated fatty acids in the circulation, ~200 μ mol/L in control, ~308 μ mol/L in soybean oil and ~547 μ mol/L in lard oil. Thus, under conditions of elevated free fatty acids concentrations, the ratio of saturated to unsaturated fatty acids and/or the absolute amount of saturated fatty acids may be an important determinant of ER homeostasis and inflammatory signaling in the liver and adipose tissue. Several human studies have suggested that saturated fatty acids are more highly correlated to, or more predictive of, metabolic impairments associated with obesity and type 2 diabetes [20, 39, 40]. In a prospective cohort study involving 895 middle-aged normoglycemic men, baseline proportions of serum esterified and non-esterified saturated fatty acids were increased and polyunsaturated fatty acids decreased in those men who, after 4 yrs, had developed impaired fasting glucose [39]. More recently it was demonstrated that serum triglyceride containing saturated and monounsaturated fatty acids (16:0/16:0/18:1 or 16:0/18:1/18:0) may be more precise markers of insulin resistance than total triglyceride concentrations [20]. Thus, even in the mixed esterified and non-esterified free fatty acid composition of the circulation, an increase of saturated fatty acids may be an important component of obesity-associated ER stress.

Chronic, low-grade inflammation is a characteristic feature of obesity and metabolic diseases [41]. Circulating and intracellular lipids can promote or antagonize inflammation and inflammatory pathway signaling [42]. In the present study, markers of inflammation and inflammatory signaling were increased in both the soybean and lard oil groups, although these markers were increased to a greater extent following lard oil infusions. ER stress and activation of the unfolded protein response can lead to the activation of inflammatory signaling [42]. Therefore, it can be postulated that the induction of inflammatory signaling in the present study was mediated by lipid-induced ER stress in liver and adipose tissue. Future studies are required to test this hypothesis.

A fundamental function of the UPR is to alleviate the accumulation of unfolded proteins through the upregulation of protein folding and degradation pathways in the ER and attenuation of global protein synthesis [43]. Based on this fundamental view, activation of the UPR in liver and adipose tissue following lipid infusions implies that mis- or un-folded proteins have accumulated in the ER. It further predicts that the mechanism involved in lipid-mediated ER stress involves an imbalance in the protein load presented to the ER lumen and the ability to fold, degrade, and/or transport proteins. However, the proximal UPR sensors are transmembrane proteins and therefore may also be regulated by membrane-generated signals. A recent study demonstrated that membrane factors and unfolded proteins

activate IRE1 via different mechanisms in yeast[44]. In addition, there appears to be a sensing mechanism within the lipid bilayer that can trigger selective activation of ATF6[45]. It is also possible that cytosolic signals may interact with proximal UPR sensors, via their cytosolic domains, and lead to selective activation of components of the UPR[46, 47]. Given this and the documented role of the UPR in a diverse 246 array of cellular functions (e.g. differentiation, ER and mitochondrial biogenesis, insulin action, glucose and lipid metabolism) it will be important to identify how and why nutrients and growth factors activate the UPR.

The present study utilized intravenous infusions of soybean or lard oil to examine the effects of unsaturated and saturated fats on markers of ER stress and inflammation in vivo. In this regard it must be acknowledged that lard oil, while enriched in 16:0 and 18:0 fatty acids, also contains higher amounts of 18:1 and lower amounts of 18:2 and 18:3 fatty acids compared to soybean oil. Thus, while lard oil infusions resulted in increased markers of ER stress and inflammation in both liver and adipose tissue, we cannot definitively state that this response is solely due to an enrichment in the saturated fatty acid composition of circulating free fatty acids. Given that both infusion protocols resulted in increased markers of ER stress and inflammation in liver and adipose tissue, these data support the notion that elevated free fatty acids may be important determinants of ER stress and inflammation in vivo.

This study has several limitations which must be acknowledged. The unfolded protein response is initiated by three ER membrane-bound proteins, PERK, inositol requiring enzyme 1 α (IRE1 α), and activating transcription factor-6. In the present study, we have only measured activation of PERK and used XBP1 splicing as a surrogate measure of IRE1 α activation[12]. Thus, whether the nutrient stimuli used in the present study also activate ATF6 α is unclear. The interaction between ER stress and inflammation is complex, and the present study does not identify how the infusion of soybean oil or lard oil induces markers of ER stress and or inflammatory signaling. It is also unclear whether the induction of ER stress and inflammatory signaling in response to these infusions are mechanistically linked. Finally, the present study has not identified potential downstream consequences of ER stress and inflammatory signaling, such as insulin resistance. Future studies will be necessary to examine these issues.

Elevated plasma free fatty acids are a characteristic feature of several metabolic disorders, including obesity and type 2 diabetes. Elevated free fatty acids can induce insulin resistance, inflammation and ER stress, therefore they are intimately linked to the development and/or exacerbation of these metabolic disorders [38, 48, 49]. The fatty acid composition of circulating lipids has also been linked to metabolic disorders and to differential effects on glucose metabolism, insulin action and ER stress [19, 20, 50]. Results from the present study suggest that an increase in the amount of saturated free fatty acids, under conditions of elevated plasma free fatty acids, may provoke more ER stress and inflammation in liver and adipose tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants R01-DK072017 (M.J.P.) K01-OD010971 (M.F.) and K01-DK087777 (C.L.G.).

List of Abbreviations

ATF6	activating transcription factor-6
CHOP	C/EBP homologous protein
NAFLD	non-alcoholic fatty liver disease
eIF2α	eukaryotic initiation factor-2 α
ER	endoplasmic reticulum
GADD34	growth arrest and damage-inducible protein 34
GRP78	glucose regulated protein 78
IKKβ	IkappaB kinase β
IRE1	inositol requiring ER-to-nucleus signaling protein 1 α
PCR	polymerase chain reaction
PERK	RNA-dependent protein kinase-like ER eukaryotic initiation factor-2 α kinase
UPR	unfolded protein response
XBP1	X-box binding protein 1

References

1. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest.* 2005; 115:1343–1351. [PubMed: 15864352]
2. Nehra V, Angulo P, Buchman AL, Lindor KD. Nutritional and metabolic considerations in the etiology of nonalcoholic steatohepatitis. *Dig Dis Sci.* 2001; 46(11):2347–2352. [PubMed: 11713934]
3. Diraison F, Moulin P, Beylot M. Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab.* 2003; 29(5):478–485. [PubMed: 14631324]
4. Rigazio S, Lehto H-R, Tuunanen H, Nagren K, Kankaanpää M, Simi C, Borra R, Naum AG, Parkkola R, Knuuti J, et al. The lowering of hepatic fatty acid uptake improves liver function and insulin sensitivity without affecting hepatic fat content in humans. *Am J Physiol Endocrinol Metab.* 2008; 295:E413–E419. [PubMed: 18505832]
5. Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology.* 2007; 46:1081–1090. [PubMed: 17654743]
6. Listenberger LJ, Han X, Lewis SE, Cases S, Farese RV, Ory DS, Schaffer JE. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A.* 2003; 100:3077–3082. [PubMed: 12629214]
7. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, Bhanot S, Monia BP, Li YX, Diehl AM. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology.* 2007; 45(6):1366–1374. [PubMed: 17476695]
8. Li ZZ, Berk M, McIntyre TM, Feldstein AE. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: Role of stearoyl-CoA desaturase. *J Biol Chem.* 2009; 284:5637–5644. [PubMed: 19119140]
9. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P, Saunders T, Bonner-Weir S, Kaufman RJ. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol Cell.* 2001; 7:1165–1176. [PubMed: 11430820]

10. Ozcan U, Cao Q, Yilmaz E, Lee A-H, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun CZ, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action and type 2 diabetes. *Science*. 2004; 306:457–461. [PubMed: 15486293]
11. Hotamisligil GS. Role of endoplasmic reticulum stress and c-Jun NH2-terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes*. 2005; 54(Suppl 2):S73–78. [PubMed: 16306344]
12. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. *Endocrinology*. 2006; 147:943–951. [PubMed: 16269465]
13. Puri P, Mirshahi F, Cheung O, Natarajan R, Maher JW, Kellum JM, Sanyal AJ. Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease. *Gastroenterology*. 2008; 134(2):568–576. [PubMed: 18082745]
14. Boden G, Duan X, Homko C, Molina EJ, Song W, Perez O, Cheung P, Merali S. Increase in endoplasmic reticulum stress-related proteins and genes in adipose tissue of obese, insulin-resistant individuals. *Diabetes*. 2008; 57(9):2438–2444. [PubMed: 18567819]
15. Gregor MF, Yang L, Fabbrini E, Mohammed BS, Eagon JC, Hotamisligil GS, Klein S. Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. *Diabetes*. 2009; 58(3):693–700. [PubMed: 19066313]
16. Borradaile NM, Han X, Harp JD, Gale SE, Ory DS, Schaffer JE. Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J Lipid Res*. 2006; 47:2726–2737. [PubMed: 16960261]
17. Moffitt JH, Fielding BA, Evershed R, Berstan R, Currie JM, Clark A. Adverse physicochemical properties of tripalmitin in beta cells lead to morphological changes and lipotoxicity in vitro. *Diabetologia*. 2005; 48(9):1819–1829. [PubMed: 16094531]
18. Wei Y, Wang D, Topczewski F, Pagliassotti MJ. Saturated fatty acids induce endoplasmic reticulum stress and apoptosis independently of ceramide in liver cells. *Am J Physiol Endocrinol Metab*. 2006; 291:E275–E281. [PubMed: 16492686]
19. Clore JN, Stillman JS, Li J, O'Keefe SJD, Levy JR. Differential effect of saturated and polyunsaturated fatty acid on hepatic glucose metabolism in humans. *Am J Physiol Endocrinol Metab*. 2004; 287:E358–E365. [PubMed: 15082421]
20. Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Bergholm R, Ekroos K, Makkonen J, Taskinen MR, Oresic M, Yki-Jarvinen H. Serum saturated fatty acids containing triacylglycerols are better markers of insulin resistance than total serum triacylglycerol concentrations. *Diabetologia*. 2009; 52(4):684–690. [PubMed: 19214471]
21. Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA. Changes in insulin action, triglycerides, and lipid composition during sucrose feeding in rats. *Am J Physiol*. 1996; 271(5 Pt 2):R1319–1326. [PubMed: 8945970]
22. Popovic V, Popovic P. Permanent cannulation of aorta and vena cava in rats and ground squirrels. *J Appl Physiol*. 1960; 15:727–728. [PubMed: 14434161]
23. Pagliassotti MJ, Shahrokhi KA, Moscarello M. Involvement of liver and skeletal muscle in sucrose-induced insulin resistance: dose-response studies. *Am J Physiol Regul Integr Comp Physiol*. 1994; 266(5):R1637–1644.
24. Stein DT, Stevenson BE, Chester MW, Basit M, Daniels MB, Turley SD, McGarry JD. The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. *J Clin Invest*. 1997; 100(2):398–403. [PubMed: 9218517]
25. Boberg M, Croon LB, Gustafsson IB, Vessby B. Platelet fatty acid composition in relation to fatty acid composition in plasma and to serum lipoprotein lipids in healthy subjects with special reference to the linoleic acid pathway. *Clin Sci (Lond)*. 1985; 68(5):581–587. [PubMed: 3919990]
26. Wang D, Wei Y, Schmoll D, Maclean KN, Pagliassotti MJ. Endoplasmic reticulum stress increases glucose-6-phosphatase and glucose cycling in liver cells. *Endocrinology*. 2006; 147:350–358. [PubMed: 16223860]
27. Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev*. 1999; 13(10):1211–1233. [PubMed: 10346810]

28. Rutkowski DT, Kaufman RJ. A trip to the ER: coping with stress. *Trends Cell Biol.* 2004; 14(1): 20–28. [PubMed: 14729177]
29. Schroder M, Kaufman RJ. ER stress and the unfolded protein response. *Mutat Res.* 2005; 569(1–2):29–63. [PubMed: 15603751]
30. Kaufman RJ. Orchestrating the unfolded protein response in health and disease. *J Clin Invest.* 2002; 110(10):1389–1398. [PubMed: 12438434]
31. Flowers MT, Keller MP, Choi Y, Lan H, Kendzierski C, Ntambi JM, Attie AD. Liver gene expression analysis reveals endoplasmic reticulum stress and metabolic dysfunction in SCD1-deficient mice fed a very low-fat diet. *Physiol Genomics.* 2008; 33(3):361–372. [PubMed: 18381840]
32. Wellen KE, Fucho R, Gregor MF, Furuhashi M, Morgan C, Lindstad T, Vaillancourt E, Gorgun CZ, Saatcioglu F, Hotamisligil GS. Coordinated regulation of nutrient and inflammatory responses by STAMP2 is essential for metabolic homeostasis. *Cell.* 2007; 129(3):537–548. [PubMed: 17482547]
33. Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab.* 2000; 11(9):351–356. [PubMed: 11042464]
34. Shimabukuro M, Zhou Y-T, Levi M, Unger RH. Fatty acid-induced B cell apoptosis: A link between obesity and diabetes. *Proc Natl Acad Sci U S A.* 1998; 95:2498–2502. [PubMed: 9482914]
35. Baldwin AC, Green CD, Olson LK, Moxley MA, Corbett JA. A role for aberrant protein palmitoylation in FFA-induced ER stress and beta cell death. *Am J Physiol Endocrinol Metab.* 2012; 302:E1390–E1398. [PubMed: 22436701]
36. de Vries JE, Vork MM, Roemen TH, de Jong YF, Cleutjens JP, van der Vusse GJ, van Bilsen M. Saturated but not mono-unsaturated fatty acids induce apoptotic cell death in neonatal rat ventricular myocytes. *J Lipid Res.* 1997; 38:1384–1394. [PubMed: 9254064]
37. Ota T, Gayet C, Ginsberg HN. Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress in rodents. *J Clin Invest.* 2008; 118:316–322. [PubMed: 18060040]
38. Boden G, Song W, Duan X, Cheung P, Kresge K, Barrero C, Merali S. Infusion of glucose and lipids at physiological rates causes acute endoplasmic reticulum stress in rat liver. *Obesity (Silver Spring).* 2011; 19(7):1366–1373. [PubMed: 21475143]
39. Laaksonen DE, Lakka TA, Lakka HM, Nyyssonen K, Rissanen T, Niskanen LK, Salonen JT. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. *Diabet Med.* 2002; 19(6):456–464. [PubMed: 12060056]
40. Tremblay AJ, Despres JP, Piche ME, Nadeau A, Bergeron J, Almeras N, Tremblay A, Lemieux S. Associations between the fatty acid content of triglyceride, visceral adipose tissue accumulation, and components of the insulin resistance syndrome. *Metabolism.* 2004; 53(3):310–317. [PubMed: 15015142]
41. Shoelson SE. Obesity, inflammation, and insulin resistance. *Gastroenterology.* 2007; 132:2169–2180. [PubMed: 17498510]
42. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest.* 2005; 115:1111–1119. [PubMed: 15864338]
43. Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev.* 1999; 13:1211–1233. [PubMed: 10346810]
44. Promlek T, Ishiwata-Kimata Y, Shido M, Sakuramoto M, Kohno K, Kimata Y. Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. *Mol Biol Cell.* 2011; 22(18):3520–3532. [PubMed: 21775630]
45. Maiuolo J, Bulotta S, Verderio C, Benfante R, Borgese N. Selective activation of the transcription factor ATF6 mediates endoplasmic reticulum proliferation triggered by a membrane protein. *Proc Natl Acad Sci U S A.* 2011; 108(19):7832–7837. [PubMed: 21521793]
46. Kazemi S, Mounir Z, Baltzis D, Raven JF, Wang S, Krishnamoorthy JL, Pluquet O, Pelletier J, Koromilas AE. A novel function of eIF2alpha kinases as inducers of the phosphoinositide-3 kinase signaling pathway. *Mol Biol Cell.* 2007; 18(9):3635–3644. [PubMed: 17596516]
47. Li Y, Iida K, O'Neil J, Zhang P, Li S, Frank A, Gabai A, Zambito F, Liang SH, Rosen CJ, et al. PERK eIF2alpha kinase regulates neonatal growth by controlling the expression of circulating

- insulin-like growth factor-I derived from the liver. *Endocrinology*. 2003; 144(8):3505–3513. [PubMed: 12865332]
48. Boden G, Cheung P, Stein TP, Kresge K, Mozzoli M. FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. *Am J Physiol Endocrinol Metab*. 2002; 283:E12–E19. [PubMed: 12067837]
49. Hotamisligil GS. Inflammation and endoplasmic reticulum stress in obesity and diabetes. *Int J Obes (Lond)*. 2008; 32(Suppl 7):S52–54. [PubMed: 19136991]
50. Gentile CL, Pagliassotti MJ. The role of fatty acids in the development and progression of nonalcoholic fatty liver disease. *J Nutr Biochem*. 2008; 19(9):567–576. [PubMed: 18430557]

Figure 1. Plasma glucose, free fatty acid (FFA) and insulin concentrations during control (n=7), soybean oil (n=7) and lard oil (n=7) infusions. *, significantly different from Control.

Figure 2. ER stress and inflammatory signaling in the liver. Phosphorylation of PERK and eIF2 α (A), XBP1 splicing (B), Chop, GADD34 and GRP78 gene expression (C) and phosphorylation of IKKB (D) in the liver following control (n=7), soybean oil (n=7) or lard oil (n=7) infusions. *, significantly different from Control; +, significantly different from other two groups.

Figure 3. Inflammatory gene expression in the liver. Interleukin-1 β (IL-1B), macrophage inflammatory protein-1 α (MIP-1a) tumor necrosis factor- α (TNFa), matrix metalloproteinase 12 (Mmp12), and CD68 antigen (CD68) mRNA following Control (n=7), Soybean oil (n=7) or Lard oil (n=7) infusions. *, significantly different from Control; +, significantly different from other two groups.

Figure 4.

ER stress and inflammatory signaling in adipose tissue. Phosphorylation of PERK and eIF2 α (A), XBP1 splicing (B), Chop, GADD34 and GRP78 gene expression (C) and phosphorylation of IKKB (D) in adipose tissue following Control (n=7), Soybean oil (n=7) or Lard oil (n=7) infusions. *, significantly different from Control; +, significantly different from other two groups.

Figure 5.

Inflammatory gene expression in adipose tissue. Interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor- α (TNF α), suppressor of cytokine signaling-3 (SOC-3) and haptoglobin mRNA following Control (n=7), Soybean oil (n=7) or Lard oil (n=7) infusions. *, significantly different from Control; +, significantly different from other two groups.

Table 1

Plasma free fatty acid (FFA) composition in control-, soybean- or lard oil-infused rats.

Plasma FFA	Control	Soybean ($\mu\text{mol/L}$)	Lard
14:0	15.4 $\pm$ 1.5	13.4 $\pm$ 2.3	33.1 $\pm$ 2.8 ⁺
16:0	125.1 $\pm$ 16.9	177.4 $\pm$ 24.2	283.5 $\pm$ 22.4 ⁺
16:1	21.5 $\pm$ 2.3	63.5 $\pm$ 5.7 [*]	60.4 $\pm$ 7.5 [*]
18:0	58.7 $\pm$ 8.3	77.1 $\pm$ 13.9	229.9 $\pm$ 22.1 ⁺
18:1	79.0 $\pm$ 7.7	204.8 $\pm$ 19.1 [*]	264.5 $\pm$ 25.9 ⁺
18:2	82.2 $\pm$ 7.9	341.5 $\pm$ 27.8 [*]	132.2 $\pm$ 12.8 ⁺
18:3 (n-6)	6.1 $\pm$ 0.5	48.7 $\pm$ 5.3 ⁺	10.4 $\pm$ 1.9
20:4	19.8 $\pm$ 2.4	21.1 $\pm$ 2.6	23.2 $\pm$ 3.3
22:6	2.5 $\pm$ 0.3	3.5 $\pm$ 0.4	3.9 $\pm$ 0.4

Values are means $\pm$ SDEV for n=7/group.

^{*} Significantly different from Control,

⁺ Significantly different from other two groups.