Endoplasmic Reticulum Stress in Nonalcoholic Fatty Liver Disease

Michael J. Pagliassotti

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, Colorado 80523; email: pagliasm@cahs.colostate.edu

Keywords
unfolded protein response, obesity, hepatic steatosis, steatohepatitis, inflammation

Abstract
The underlying causes of nonalcoholic fatty liver disease are unclear, although recent evidence has implicated the endoplasmic reticulum in both the development of steatosis and progression to nonalcoholic steatohepatitis. Disruption of endoplasmic reticulum homeostasis, often termed ER stress, has been observed in liver and adipose tissue of humans with nonalcoholic fatty liver disease and/or obesity. Importantly, the signaling pathway activated by disruption of endoplasmic reticulum homeostasis, the unfolded protein response, has been linked to lipid and membrane biosynthesis, insulin action, inflammation, and apoptosis. Therefore, understanding the mechanisms that disrupt endoplasmic reticulum homeostasis in nonalcoholic fatty liver disease and the role of the unfolded protein response in the broader context of chronic, metabolic diseases have become topics of intense investigation. The present review examines the endoplasmic reticulum and the unfolded protein response in the context of nonalcoholic fatty liver disease.
INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is currently a global issue affecting both adults and children (1, 89, 143). NAFLD is a disease syndrome characterized by fatty infiltration (steatosis) of the liver in the absence of chronic alcohol consumption. In some individuals, steatosis progresses to nonalcoholic steatohepatitis (NASH), which is characterized by steatosis, inflammation, apoptosis and fibrosis, and end-stage liver disease (28).

The underlying causes of NAFLD are unclear, although recent evidence has implicated the endoplasmic reticulum (ER) in both the development of steatosis and progression to NASH. Disruption of ER homeostasis, often termed ER stress, has been observed in liver and adipose tissue of humans with NAFLD and/or obesity (4, 19, 35, 96, 116). In addition, the signaling pathway activated by disruption of ER homeostasis, the unfolded protein response (UPR), has been linked to cellular perturbations that are common to obesity and NAFLD (55, 68, 86). Therefore, understanding the mechanisms that disrupt ER homeostasis and lead to activation of the UPR in chronic, metabolic diseases have become topics of intense investigation.

THE ENDOPLASMIC RETICULUM AND THE UNFOLDED PROTEIN RESPONSE

The Endoplasmic Reticulum

The smooth ER produces structural phospholipids and cholesterol as well as significant amounts of triacylglycerol and cholesterol esters that have nonstructural roles (128). The smooth ER is the main site of cholesterol synthesis, although much of this lipid is transported to other cellular organelles. The ER membrane is composed of very low concentrations of cholesterol and complex sphingolipids (128). This loose packing of ER membrane lipids may provide an environment conducive to the insertion and transport of newly synthesized lipids and proteins (128). The requirement for such a specialized lipid environment may be relevant to diseases characterized by abnormal lipid accumulation, such as NAFLD.

Proteins destined for secretion or insertion into membranes require modification, such as glycosylation and disulfide bond formation, that cannot be achieved in the cytosol (71). The ER lumen provides a specialized environment for protein folding and maturation and a unique complement of molecular chaperones and folding enzymes (111). The presence of
ER-associated degradation (ERAD) machinery helps to ensure that improperly folded proteins are retrotranslocated to the cytoplasm and targeted for proteasomal degradation. The UPR monitors and responds to the accumulation of improperly folded proteins in the ER lumen (102).

The Core UPR
In mammalian cells, UPR activation involves three ER-localized proteins (Figure 1): inositol-requiring 1α (IRE1α), double-stranded RNA-dependent protein kinase-like ER kinase (PERK), and activating transcription factor-6α (ATF6α) (103). It is currently thought that in unstressed cells all three proteins are maintained in an inactive state via their association with the ER protein chaperone glucose-regulated protein 78/immunoglobulin-heavy-chain-binding protein (GRP78). Upon ER stress, GRP78 is released and sequestered on unfolded proteins, allowing activation of PERK, IRE1α, and ATF6α (149). PERK activation leads to phosphorylation of the α-subunit of the translation initiation factor eIF2 (p-eIF2α) and subsequent attenuation of translation initiation. Paradoxically, p-eIF2α leads to selective translation of mRNAs containing open reading frames, such as activating transcription factor-4 (ATF4) (49, 112). Increased expression of GADD34 (which also contains open reading frames), a member of the growth arrest and DNA damage family of proteins, is involved in dephosphorylation of eIF2α and therefore reversal of translational attenuation (103). Activation of IRE1α promotes the splicing of X-box-binding protein-1 (XBP1s) mRNA and subsequent transcription of molecular chaperones (e.g., GRP78) and genes involved in ERAD [e.g., ER degradation-enhancing α—like protein (EDEM)] (112). IRE1α also appears to mediate rapid degradation of specific mRNAs, presumably in an effort to reduce production of proteins that require folding in the ER lumen (40, 41). Activation of ATF6α leads to its release from the ER membrane, processing in the Golgi, and entry into the nucleus. Transcriptional targets of ATF6α include protein chaperones and XBP1 (138). Thus, UPR activation initiates a core response that includes transient attenuation of global protein synthesis and upregulation of protein folding and degradation. The fundamental goal of this response is to remove unfolded proteins and restore ER homeostasis.

The Expanded UPR
PERK is one of four protein kinases that can phosphorylate eIF2α; the other three are double-stranded RNA-activated protein kinase (PKR), which is activated in response to viral infection; general control nonderepressible 2 kinase, which is activated in response to amino acid deprivation; and heme-regulated inhibitor kinase, which is primarily expressed in reticulocytes and appears to coordinate globin polypeptide synthesis with heme availability (50). Protein kinase-mediated p-eIF2α regulates not only translation but also the activation of nuclear factor kappa-B (NFκB) via reduction in the abundance of the NFκB inhibitor IκB (88, 112, 139). PERK can also phosphorylate nuclear erythroid 2 p45-related factor 2 (Nrf2), triggering the nuclear import of Nrf2 (17). Thus, PERK-mediated p-eIF2α links the UPR to inflammation, via NFκB and redox balance, via Nrf2.

IRE1α, in addition to catalyzing XBP1 splicing, has functions related to cellular signaling. Activated IRE1α can interact with the adaptor protein TNFR-associated factor 2 (TRAF2) and lead to activation of c-Jun-NH2-terminal kinase (JNK) and NFκB (127). IRE1α activation has also been linked to the activation of p38 mitogen-activated protein kinase and extracellular-regulated kinase (39, 44, 78). These interactions suggest that the IRE1α branch of the UPR not only regulates adaptation to ER stress and cell survival via XBP1 splicing but also activation of signaling pathways involved in inflammation, insulin action, and apoptosis.

ATF6α and XBP1s have been linked to lipid biosynthesis and ER membrane expansion.
via mechanisms that are partially distinct (6, 121). Recent studies have also demonstrated that the transcriptional activity of XBP1s can be modified by acetylation/deacetylation and SUMOylation (13, 131). The ability to modify the transcriptional activity of XBPs is a logical mechanism to regulate the magnitude and/or selectivity of IRE1α-XBP1-mediated outputs.

Physical and functional links between the ER and mitochondria have been demonstrated (8, 15). ER-mitochondrial coupling may promote mitochondrial respiration and be influenced by ER stress and UPR activation (8). Chronic or severe ER stress may, in turn, modify cellular metabolism (132). Mitochondrial energy metabolism may also support ER function (10). Mitochondrial function is closely aligned with the development and/or exacerbation of chronic, metabolic diseases, including NAFLD (69). It is likely that the alignment of mitochondrial function with chronic, metabolic diseases also involves the ER.

Much of what we know about ER stress and the UPR has been derived from studies that utilize pharmacologic agents (tunicamycin, thapsigargin). Much less is known about the UPR in the context of physiologic stressors. In vivo, the diversity of ER stress-mediated UPR signaling likely yields outcomes that are specific to the stress imposed and the needs of the involved cell but may be broadly grouped into three potential outputs: adaptation (ER stress → UPR activation → re-establishment of ER homeostasis); alarm (ER stress → UPR activation → activation of signaling pathways involved in inflammation, antioxidant defense, and/or insulin action → re-establishment of ER homeostasis or mild, chronic ER stress); and apoptosis (ER stress → UPR activation → failure to resolve severe ER stress → cell death) (52).

THE UPR AND THE DEVELOPMENT OF HEPATIC STEATOSIS

NAFLD is characterized by lipid accumulation in the liver in the absence of chronic alcohol consumption or other liver disease (28). Sources of hepatic lipids in NAFLD include dietary chylomicron remnants, free fatty acids released from adipose tissue triglycerides, and de novo lipogenesis (21, 22, 54). In addition, impairments in hepatic fatty acid oxidation and/or very-low-density lipoprotein secretion may also contribute to hepatic lipid accumulation (34, 100).

XBP1 has been linked to hepatic lipogenesis and adipocyte differentiation (55, 115). Conditional disruption of Xbp1 in the liver led to reduced plasma levels of triglyceride, cholesterol, free fatty acids, and liver cell lipogenesis in mice (55). Adipogenesis was also reduced in XBP1-deficient preadipocytes or MEF cells (115). The potential role of the IRE1α-XBP1 pathway in the regulation of lipid biosynthesis will likely depend on the physiologic setting. Recent studies have observed IRE1α-mediated XBP1 splicing in the liver following a meal and in response to hyperinsulinemia (79, 93). In this context, XBP1 may be an important determinant of lipid biosynthesis.

PERK and p-eIF2α also appear to regulate lipogenesis and hepatic steatosis. Targeted deletion of PERK in mammary epithelium reduced free fatty acids in milk, led to growth retardation in suckling pups, and reduced expression of lipogenic genes (3). Whether PERK-mediated regulation of lipogenesis occurs in hepatocytes is presently unknown. GADD34 (PPP1R15a) encodes a regulatory subunit of a phosphatase that selectively dephosphorylates eIF2α. Enforced expression of an active C-terminal fragment of GADD34 in the liver reduced hepatic steatosis and expression of the adipogenic nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) and upstream regulators of PPARγ, CCAAT/enhancer-binding protein-α and -β (C/EBPα, C/EBPβ), in mice fed a high-fat diet (83). Protein kinase–mediated p-eIF2α increases the translation of a subset of genes that include ATF4. The phenotype of ATF4 heterozygous mice includes protection from age-related and diet-induced obesity and diet-induced hepatic steatosis (114). Thus, when these studies are considered together it would
appear that protein kinase–mediated p-eIF2α can regulate the lipogenic transcriptional program and perhaps the development of hepatic steatosis.

ATF6α and sterol regulatory element–binding proteins (SREBPs) are ER-membrane-bound transcription factors that are activated by proteolytic cleavage. At least one study has demonstrated that the nuclear form of ATF6α inhibits the transcriptional activity of SREBP2 by forming a complex with SREBP2 that recruits histone deacetylase-1 (147). The functional consequence of this interaction was to reduce Oil-Red-O staining in liver cells. Thus, all three proximal UPR sensors—PERK, IRE1α, and ATF6α—can regulate lipid stores in the liver. The degree to which the UPR contributes to hepatic steatosis may depend on the cellular event that elicits activation, the relative response of the three proximal UPR sensors, and appropriate downstream protein-protein and/or protein-DNA interactions.

A fundamental function of the UPR is to restore ER homeostasis in response to the accumulation of unfolded proteins by reducing the protein load entering the ER lumen and increasing the capacity of the ER to fold and degrade proteins. The presence of ER stress and activation of the UPR in chronic diseases such as obesity and NAFLD implies that the ability to resolve ER stress has been compromised. A recent study has examined the role of the UPR in hepatic steatosis from this perspective (104). Genetic ablation of eIF2α, IRE1α, or ATF6α resulted in hepatic steatosis in response to chemical induction of ER stress. Steatosis, in this model, appeared to result from impairments in the capacity to oxidize fatty acids and was potentially augmented by impaired lipoprotein secretion. Thus, the UPR may promote lipid homeostasis (i.e., prevent hepatic steatosis) via its ability to maintain or rapidly re-establish ER homeostasis following ER stress. Perhaps development and/or exacerbation of hepatic steatosis in the context of chronic disease involve selective impairments to the UPR that reduce the ability of the UPR to resolve ER stress (104, 151).

Recent studies using GRP78 +/− mice and adenoviral-mediated overexpression of GRP78 in vivo support this concept (48, 144). GRP78 +/− mice were resistant to high-fat-diet-induced insulin resistance, hepatic steatosis, white adipose tissue inflammation, and hyperglycemia (144). It was postulated that GRP78 heterozygosity triggered an adaptive UPR, characterized by upregulation of other ER chaperones (e.g., GRP94) and components of ERAD machinery. The results of this study predict that selective upregulation of protein chaperones in the liver should improve insulin action and hepatic steatosis. Indeed, a recent study demonstrated that selective overexpression of GRP78 in the liver improved ER homeostasis, hepatic steatosis, and insulin action in ob/ob mice (48).

THE UPR AND DISEASE PROGRESSION IN NAFLD

Liver pathology in NASH can include macrovesicular steatosis, inflammation, fibrosis, apoptosis, and necrosis (26, 87). Multiple factors, including insulin resistance, oxidative stress, cytokine-mediated signaling, inflammation, bacterial endotoxin, and excess fatty acids likely function in concert to provoke disease progression in NAFLD.

JNK: A Common Link to Insulin Action, Apoptosis, and Inflammation

Obesity and insulin resistance are thought to play an important role in the pathogenesis of NAFLD (70, 87). ER stress may be linked to insulin resistance via the ability of the UPR to regulate stress kinases, such as JNK and NFκB (11, 86). Activation of JNK can also lead to liver damage and hepatocyte apoptosis (18), the latter being a characteristic feature of NASH and correlated with disease severity (27, 135). Global deletion of JNK1, but not JNK2, reduced hepatic triglyceride accumulation, inflammation, liver injury, and apoptosis in methionine-choline-deficient diet–fed mice (109). In contrast, specific ablation of JNK1
in hepatocytes produced a phenotype that included glucose intolerance, insulin resistance, and hepatic steatosis (105), suggesting that JNK isoforms may have tissue-specific actions that dictate their roles in NAFLD.

Recent studies have also provided evidence that XBP1 may be a central mediator of insulin action and/or glucose homeostasis in the liver (56, 91, 137, 152). Haploinsufficiency of XBP1 resulted in insulin resistance in mice (86), and the ability of XBP1s to translocate to the nucleus was impaired in murine models of obesity (91). It also appears that XBP1s can interact with phosphoinositide 3-kinase (PI3K) (91) and may influence hepatic glucose metabolism independently of insulin signaling, via interaction with Foxo1 (152).

**Inflammation**

It is now well established that the NFκB pathway is an important determinant of the inflammatory response and insulin resistance (118). It is presently unclear whether and how UPR-mediated activation of NFκB is linked to NAFLD. If the formation of the IRE1α-TRAF2 complex is crucial to activation of both JNK and NFκB in NAFLD (44, 127), it will be important to understand how and under what physiologic conditions these two proteins interact with the IRE1α-TRAF2 complex. Alternatively, it will be important to consider whether ER stress-mediated activation of JNK and NFκB is shared among the three proximal UPR sensors, and if so, to identify the mechanism by which this is accomplished. This latter possibility may be particularly relevant given that NFκB can protect hepatocytes from oxidative stress and TNFα-induced cell death (32, 61) as well as steatohepatitis and hepatocellular carcinoma (64). Thus, the ultimate outcome of NFκB activation likely depends on such factors as the duration and magnitude of the stimulus and the interaction of NFκB with other signaling networks.

PKR is an interferon-induced serine/threonine protein kinase that is activated by double-stranded RNA (23). PKR appears to be required for NFκB activation in response to double-stranded RNA, and therefore PKR has been linked to immune and inflammatory responses (24). PKR activity is increased in adipose tissue and liver of murine models of obesity and inhibits insulin signaling directly and indirectly, the latter via activation of JNK (76). The ability of PKR to respond to pathogens, nutrients, and organelle stress and to regulate inflammatory and insulin-signaling pathways suggests that PKR may be a core component of an inflammatory complex (43, 76, 142). PKR can use catalysis-dependent and -independent activities to function both as a pro- and antiapoptotic factor via regulation of NFκB and eIF2α, respectively (24). Thus, it has been proposed that “PKR may serve as a molecular clock to time the sequential events of survival and death following virus infection” (24). It is feasible that other multifunctional, UPR-linked protein kinases employ similar strategies to elicit cell- or stress-selective outcomes.

Regulated intramembrane proteolysis (RIP), the release and transport of ER-resident proteins from the ER membrane to the Golgi for processing, may represent an important link between the ER and inflammation (75, 99). ATF6α and SREBP both undergo RIP prior to their entry into the nucleus (149); thus, RIP is required for the activation of one arm of the UPR and for transcriptional regulation of the lipogenic program in the liver. CREBH, a transcription factor belonging to the CREB/ATF family of transcription factors, is an RIP-regulated, liver-enriched protein that appears to be required for the hepatic synthesis of amyloid P-component and C-reactive protein (63, 150). In addition, free fatty acids increase the expression of CREBH, and proinflammatory cytokines and lipopolysaccharide induce the cleavage of CREBH in the liver in vivo (33, 150). Thus, ER stress in the liver may be linked to systemic inflammation via RIP-mediated mobilization of CREBH.
Oxidative Stress
Protein folding in the ER is linked to the generation of reaction oxygen species and oxidative stress (108, 117). Conversely, cellular oxidative stress can disrupt ER homeostasis (37, 140, 145). Therefore, it is not surprising that the UPR can activate an antioxidant program via the transcription factor Nrf2 (16). Nrf2 deletion results in rapid onset and progression of steatohepatitis in mice provided a methionine-choline-deficient diet (123). In addition, Nrf2-deficient mice were characterized by increased mortality in response to endotoxin- and cecal ligation and puncture-induced septic shock (125). These studies have led to the proposal that Nrf2 also participates in the regulation of the innate immune response. PERK-mediated p-eIF2α also leads to the upregulation of ATF4. Along with Nrf2, ATF4 has been linked to the maintenance of cellular glutathione (17). Recent evidence has also linked the IRE1α-XBP1 branch of the UPR to the regulation of antioxidant defenses (62). Thus, XBP1 may provide protection from oxidative stress; however, whether this regulation occurs in hepatocytes is presently unknown.

The ER and Cell Death
Hepatocyte apoptosis is increased in patients with NASH and correlates with disease severity; therefore, apoptosis has been proposed as a component of disease progression in NAFLD (27, 135). Failure of the UPR to ameliorate ER stress can lead to cell death via several mechanisms. C/EBP homologous protein (Chop) is among the best characterized of the UPR-regulated proapoptotic proteins (85). Chop expression is regulated by ATF4 and perhaps ATF6α, and deletion of Chop provides some protection from ER stress–induced cell death in both cells and animals (65, 81, 85, 101, 120). Chop deficiency delayed the development of ER stress–mediated diabetes in Akita mice and attenuated cholestasis-induced liver fibrosis (84, 124). However, the role of Chop in NAFLD is unclear, as recent evidence demonstrated that methionine-choline-deficient diet-induced liver injury was not reduced in Chop knockout mice (92).

It is possible that ER-mediated calcium release links the ER to alterations in mitochondrial function and oxidative stress in NAFLD. For example, the release of ER calcium and subsequent calcium influx into mitochondria can lead to mitochondrial membrane permeabilization and activation of the intrinsic apoptotic pathway (20). A number of studies have demonstrated that ER stress, ER-localized proteins, and B-cell leukemia/lymphoma 2 protein (Bcl-2) protein family members interacting with ER localized proteins can regulate ER calcium flux (20, 57, 80, 113). Hapatic sarco-endoplasmic reticulum calcium-ATPase (SERCA) activity appears to be reduced in murine models of obesity (29, 90), and modification of the ER membrane lipid bilayer can also influence the activity of SERCA (58). Thus, it is possible that the hepatic milieu in NAFLD modifies ER calcium flux, perhaps via alterations in SERCA.

Autophagy is a cellular degradation process for long-lived proteins and damaged organelles (53, 73, 74). Recent evidence has demonstrated that inhibition of macroautophagy in cultured hepatocytes and mouse liver increased triglyceride storage in lipid droplets (119). ER stress can trigger autophagy via mechanisms that may require calcium-mediated activation of protein kinase Cθ (107, 146). In addition, autophagy is a necessary pathway for the maintenance of structure, mass, and function in pancreatic β-cells (47). One can envision that ER stress–mediated activation of autophagy may be part of the protective, adaptive component of the UPR.

Intestinal Function
Bacterial translocation through the intestinal wall and small intestinal bacterial overgrowth may be involved in the pathogenesis of NASH (136, 141). Recent studies have confirmed the presence of increased plasma endotoxin and intestinal permeability in humans with NAFLD (72, 126). Intestinal secretory cells may be susceptible to ER stress because they produce large
amounts of secretory proteins. It has been proposed that ER stress and UPR activation can lead to intestinal inflammation (25), which may be an important component of disease progression in NAFLD.

Adipose Tissue

Free fatty acids derived from adipose tissue are thought to play an important role in the development of hepatic steatosis (22). It is of interest, therefore, that a recent study in Caenorhabditis elegans suggested that IRE-1 and HSP-4, the nematode IRE1 and GRP78 homologs, respectively, regulate the expression of the fasting-induced lipases, FIL-1 and -2 (46). These lipases were both necessary and sufficient for fasting-induced fat granule hydrolysis. In addition, free fatty acids can induce inflammation in adipose tissue via mechanisms that involve PERK and IKKβ (45). Low levels of adiponectin are linked to many features of the metabolic syndrome and liver fat accumulation (2, 94). Importantly, adipose tissue hypoxia has been linked to ER stress and suppression of adiponectin expression in adipose tissue (42). It is presently unclear whether the UPR can regulate lipolysis in mammalian adipose tissue; however, given the evidence presented above, one can envision a role for this pathway in adipose tissue lipolysis, inflammation, and adipokine release in the context of obesity and NAFLD.

Summary

ER-mediated signals are linked to a number of downstream pathways that contribute to the pathogenesis of NAFLD. The extent to which ER stress and the UPR contribute to disease progression in NAFLD will likely depend on the ability of the UPR to alleviate the insult that led to disruption of ER homeostasis. The scenario most conducive to ER stress–mediated disease progression likely involves chronic insults that provoke continuous ER stress coupled to signals that reduce or impair the UPR’s ability to alleviate those insults.

NUTRIENTS AND UPR ACTIVATION

Several nutrient-related signals can activate the UPR. Elevated circulating free fatty acids are a characteristic feature of NAFLD and are positively correlated with liver disease severity (77). A growing body of evidence has demonstrated that elevated free fatty acids—in particular, long-chain saturated fatty acids—induce ER stress and activation of the UPR in liver cells (5, 82, 134). Glycosylation is an essential ER luminal modification for proper stability, folding, translocation, and function of many proteins (50). The hexosamine biosynthetic pathway plays a key role in glycosylation, and increased flux through this pathway, which can occur under conditions of hyperglycemia and/or hyperlipidemia, has been linked to PERK-dependent ER stress and attenuation of ApoB100 synthesis (97, 106). Oxidative stress has also been linked to the development and progression of a number of chronic diseases including NAFLD (30, 129). Although the oxidation of cysteine residues during disulfide bond formation in the ER may be a significant source of reactive oxygen species and lead to the development of oxidative stress (68), extraluminal sources of pro-oxidants can also induce ER stress and promote the formation of inclusion bodies in liver cells (37, 67).

Apolipoprotein B100 (ApoB100) is an important client protein in the liver. The biogenesis of ApoB100 requires co- and post-translational modification, and studies have identified interactions between newly synthesized ApoB100 polypeptides and ER chaperone proteins, such as GRP78, GRP94, endoplasmic reticulum protein-72, calreticulin, and calnexin (14, 98, 148). Prolonged and/or severe ER stress can reduce ApoB100 secretion; however, it has also been postulated that ApoB100 may serve as a molecular link
between fatty acid–induced ER stress and insulin resistance in hepatocytes (12, 82, 122).

ACTIVATION OF THE UPR IN HUMAN OBESITY AND NAFLD

Activation of the UPR has been observed in liver and/or adipose tissue of dietary and genetic animal models of obesity, many of which include features of NAFLD (5, 86, 130). Currently only one study has compared markers of UPR activation in humans with or without NAFLD (96). In this study, liver samples were obtained from subjects with metabolic syndrome and normal liver histology (controls, \( n = 17 \)), subjects with metabolic syndrome and hepatic steatosis (NAFL, \( n = 21 \)), and subjects with metabolic syndrome and NASH (NASH, \( n = 21 \)). Although livers from NAFL and NASH were characterized by increased p-eIF2\( \alpha \), several other putative markers of UPR activation were not increased (e.g., ATF4, Chop, GADD34, EDEM mRNA). Livers from NASH subjects were additionally characterized by a reduction in the amount of spliced XBP1 mRNA.

Two studies have examined markers of UPR activation in adipose tissue of obese, insulin-resistant subjects (4, 116). In one of these studies, subcutaneous fat biopsies were obtained from the upper thigh in lean (body mass index (BMI) 24 ± 1.2 kg/m\(^2\), \( n = 6 \)) and obese (BMI 33.5 ± 1.6 kg/m\(^2\), \( n = 6 \)) healthy subjects (4). Adipose tissue from obese subjects was characterized by increased protein levels of calnexin, calreticulin, and protein disulfide isomerase as well as increased XBP1s mRNA and phosphorylation of JNK. In the second study, adipose tissue was obtained from 78 healthy, nondiabetic subjects over a spectrum of BMIs (116). Several gene markers associated with the UPR, including GRP78, ATF6\( \alpha \), PERK, XBP1s, EDEM1, calreticulin, and oxygen-regulated protein 150, were significantly correlated to BMI. Correlations with BMI remained significant after controlling for contributions made by macrophages using CD68 gene expression.

One study examined both liver and adipose tissue in morbidly obese subjects (BMI 51.3 ± 3 kg/m\(^2\), \( n = 11 \)) prior to and one year following gastric bypass surgery (35). Subjects lost ~40% of body weight at the one-year follow-up, at which time significant reductions were observed in adipose tissue GRP78 mRNA, XBP1s mRNA, p-eIF2\( \alpha \), and phosphorylation of JNK. Liver samples were characterized by reduced staining for GRP78 and p-eIF2\( \alpha \).

A recent study analyzed hepatic gene networks in morbidly obese patients with NAFLD (BMI 49.6 ± 7.4 kg/m\(^2\), \( n = 24 \), 89% female) or without NAFLD (BMI 48.8 ± 5.9, kg/m\(^2\), \( n = 25 \), 96% female) (31). Three genes associated with the fibrosis pathway (\( \text{COL1A1} \), IL10, and IGFBP3) were upregulated and one gene associated with the UPR (\( \text{HSPA5} \), also known as GRP78) was downregulated in patients with NAFLD compared with patients without NAFLD. Comprehensive analysis of the UPR is required in animal models of chronic disease and humans with obesity and NAFLD to better define UPR activation/inactivation.

ER STRESS AND THE UPR IN NAFLD: A CONCEPTUAL FRAMEWORK

ER stress is typically defined as the accumulation of mis- or unfolded proteins in the ER lumen (50). Activation of the UPR in response to ER stress functions to remove these proteins and restore ER homeostasis. Based on this fundamental view, activation of the UPR or components of the UPR in obesity and NAFLD implies that mis- or unfolded proteins have accumulated in the ER lumen of the liver and/or adipose tissue. This scenario predicts that activation of the UPR in obesity and NAFLD results from an imbalance in the protein load presented to the ER lumen and the ability to fold, degrade, and/or transport these proteins (Figure 2). Experimental support for such a scenario includes studies that have examined ER stress in pancreatic \( \beta \)-cells (110) and lipid-mediated ER stress in Chinese hamster ovary and liver cells (7, 133). It is also
possible that physiologic signals that activate the UPR in a regulatory or chronic fashion may do so through mechanisms that operate in concert with or distinct from the accumulation of unfolded proteins. For example, chronic diseases such as obesity and NAFLD may alter the composition of the ER membrane, which in turn may influence the function of any or all of the proximal UPR membrane-bound sensors (Figure 2). In fact, a recent study demonstrated that membrane factors and unfolded proteins activate IRE1 via different mechanisms in yeast (95). Recent studies have also identified several proteins that directly interact with and/or regulate the activity of IRE1 (36, 38, 60). In addition, there appears to be a sensing mechanism within the lipid bilayer that triggers selective activation of ATF6 (66). It is also possible that cytosolic signals may interact with proximal UPR sensors and lead to selective activation of components of the UPR. Previous studies have identified links between growth factors and PERK (59), and between PI3K signaling and PKR (51), that may be independent of unfolded protein accumulation. Moreover, the basal expression of some ER chaperones appears to be dependent on a mitogenic pathway that is distinct from the ER stress–induced UPR (9).

CONCLUDING REMARKS
The UPR is a robust, highly efficient pathway that functions to remove unfolded proteins from the ER lumen but also interacts with multiple cellular-signaling pathways. The ability to resolve ER stress is closely linked to the magnitude and duration of the UPR. It is therefore hypothesized that in the setting of chronic diseases, the inability to mitigate signals that induce ER stress and/or activate the UPR coupled with signals that impair components of the UPR provide an environment that will promote disease progression.

DISCLOSURE STATEMENT
The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED


Figure 1
Overview of the mammalian unfolded protein response. Accumulation of unfolded proteins provokes release of GRP78, activation of PERK, IRE1α, and ATF6, and subsequent attenuation of protein translation and activation of gene transcription. Abbreviations: ATF6α, activating transcription factor-6α; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GRP78, glucose-regulated protein 78/immunoglobulin-heavy-chain-binding protein; IRE1α, inositol-requiring 1α; p-eIF2α, phosphorylation of the α-subunit of the translation initiation factor eIF2; PERK, protein kinase-like ER kinase; UPR, unfolded protein response.
Figure 2
Hypothetical scenarios for unfolded protein response (UPR) activation. Unfolded proteins, membrane events, and/or cytosolic signals may activate the UPR. Abbreviation: ER, endoplasmic reticulum.
Contents

An Unexpected Life in Nutrition
*Malden C. Nesheim* ....................................................... 1

Endoplasmic Reticulum Stress in Nonalcoholic Fatty Liver Disease
*Michael J. Pagliassotti* ................................................... 17

Modeling Metabolic Adaptations and Energy Regulation in Humans
*Kevin D. Hall* ............................................................ 35

Hypomagnesemia and Inflammation: Clinical and Basic Aspects
*William B. Weglicki* ..................................................... 55

Selenoproteins and Cancer Prevention
*Cindy D. Davis, Petra A. Tsuji, and John A. Milner* ........... 73

The Role of Vitamin D in Pregnancy and Lactation:
Insights from Animal Models and Clinical Studies
*Christopher S. Kovacs* .................................................. 97

Vitamin A Metabolism in Rod and Cone Visual Cycles
*John C. Saari* ............................................................ 125

Lipoprotein Lipase in the Brain and Nervous System
*Hong Wang and Robert H. Eckel* .................................. 147

New Roles of HDL in Inflammation and Hematopoiesis
*Xuewei Zhu and John S. Parks* ..................................... 161

Nutritional Metabolomics: Progress in Addressing Complexity
in Diet and Health
*Dean P. Jones, Youngja Park, and Thomas R. Ziegler* ........ 183

Resolvins: Anti-Inflammatory and Proresolving Mediators Derived
from Omega-3 Polyunsaturated Fatty Acids
*Michael J. Zhang and Matthew Spite* .............................. 203

Visfatin/NAMPT: A Multifaceted Molecule with Diverse Roles
in Physiology and Pathophysiology
*Tuva B. Dahl, Sverre Holm, Pål Aukrust, and Bente Halvorsen* 229

Gene-Environment Interactions in the Development of Type 2
Diabetes: Recent Progress and Continuing Challenges
*Marilyn C. Cornelis and Frank B. Hu* .............................. 245
Mechanisms of Inflammatory Responses in Obese Adipose Tissue  
Shengyi Sun, Yewe Ji, Sander Kersten, and Ling Qi .......................... 261

Bone Metabolism in Obesity and Weight Loss  
Sue A. Shapses and Deeptha Sukumar ........................................... 287

Obesity in Cancer Survival  
Niyati Parekh, Urmila Chandran, and Elisa V. Bandera ......................... 311

Inflammation in Alcoholic Liver Disease  
H. Joe Wang, Bin Gao, Samir Zakhari, and Laura E. Nagy ...................... 343

Lessons Learned from Randomized Clinical Trials of Micronutrient  
Supplementation for Cancer Prevention  
Susan T. Mayne, Leah M. Ferrucci, and Brenda Cartmel ........................ 369

Population-Level Intervention Strategies and Examples for Obesity  
Prevention in Children  
Jennifer L. Foltz, Asbleigh L. May, Brook Belay, Allison J. Nibiser,  
Carrie A. Dooyema, and Heidi M. Blanck ...................................... 391

Type 2 Diabetes in Asians: Prevalence, Risk Factors, and Effectiveness  
of Behavioral Intervention at Individual and Population Levels  
Mary Beth Weber, Reena Oza-Frank, Lisa R. Staimez, Mohammed K. Ali,  
and K.M. Venkat Narayan ........................................................... 417

Indexes

Cumulative Index of Contributing Authors, Volumes 28–32 .................. 441
Cumulative Index of Chapter Titles, Volumes 28–32 .......................... 444

Errata

An online log of corrections to Annual Review of Nutrition articles may be found at  
http://nutr.annualreviews.org/errata.shtml