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FORUM REVIEW ARTICLE

Endoplasmic Reticulum Stress and the Unfolded Protein Response in Nonalcoholic Fatty Liver Disease

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Abstract

The underlying causes of nonalcoholic fatty liver disease (NAFLD) are unclear, although recent evidence has implicated the endoplasmic reticulum (ER) in both the development of steatosis and progression to nonalcoholic steatohepatitis. Disruption of ER homeostasis, often termed "ER stress," has been observed in liver and adipose tissue of humans with NAFLD and/or obesity. Importantly, the signaling pathway activated by disruption of ER homeostasis, the unfolded protein response, has been linked to lipid biosynthesis, insulin action, inflammation, and apoptosis. Therefore, understanding the mechanisms that disrupt ER homeostasis in NAFLD and the role of ER-mediated signaling have become topics of intense investigation. The present review will examine the ER and the unfolded protein response in the context of NAFLD. *Antioxid. Redox Signal*. 15, 505–521.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is an emerging obesity-related disorder 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 (39). NAFLD is now recognized as the most common cause of chronic liver enzyme elevations and cryptogenic cirrhosis (16). The prevalence of NAFLD has nearly doubled since 1980, and current estimates indicate that NAFLD may affect up to 25% of the general population and 80% of individuals with obesity (175). NAFLD has also emerged as a common pediatric problem, afflicting 3%–9% of all children in the United States and up to 50% of obese children (122).

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 (9, 26, 45, 127, 146). Importantly, the signaling pathway activated by disruption of ER homeostasis, the unfolded protein response (UPR), has been linked to lipid biosynthesis, insulin action, inflammation, and apoptosis (44, 52, 65). Therefore, understanding the mechanisms that disrupt ER homeostasis in NAFLD and the role of ER-mediated signaling in NAFLD have become topics of intense investigation. The present review will examine the ER in the context of NAFLD.

The ER and the UPR

Endoplasmic reticulum

In general, the largest membrane in a eukaryotic cell encloses the ER, an array of tubules (cisternae) that forms a three-dimensional network (reticulum) stretching from the nuclear envelope to the cell surface. The smooth ER, which lacks ribosomes, produces structural phospholipids and cholesterol, as well as significant amounts of triacylglycerol and cholesterol esters that have nonstructural roles (167). The smooth ER is the main site of cholesterol synthesis, although much of this lipid is transported to other cellular organelles. Thus, the ER membrane is comprised of very low concentrations of cholesterol and complex sphingolipids (167). This loose packing of ER membrane lipids may provide an environment conducive to the insertion and transport of newly synthesized lipids and proteins (167). The requirement for such a specialized lipid environment within the ER has implications in diseases characterized by abnormal lipid accumulation, such as NAFLD.

All eukaryotic cells contain a significant amount of rough ER, the site for protein folding and maturation. Proteins destined for secretion or insertion into membranes require modification, such as glycosylation and disulfide bond formation, that cannot be achieved in the cytosol (88). The ER lumen provides a specialized environment for protein folding and

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maturation that is characterized by high concentrations of calcium, a low ratio of reduced glutathione to oxidized glutathione (1:1 to 3:1), and a unique complement of molecular chaperones and folding enzymes (141). 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 ability of the ER lumen to match folding and degradation to the rate of entry of newly synthesized proteins is monitored by the UPR, a highly conserved quality control system that functions to restore ER homeostasis following periods of stress.

Canonical UPR

In mammalian cells, the UPR (Fig. 1) is initiated by three ER-localized proteins: inositol-requiring transmembrane kinase and endonuclease 1α (IRE1α), double-stranded RNA (dsRNA)-dependent protein kinase-like ER kinase (PERK), and activating transcription factor-6 (ATF6) (133). Each of these transmembrane proteins has an ER-luminal domain to sense unfolded proteins, a transmembrane domain for targeting to the ER membrane, and a cytosolic domain to transmit signals to the transcriptional and/or translational apparatus (182). It is currently thought that in un-stressed cells, all three proteins are maintained in an inactive state *via* their association with the ER protein chaperone glucose-regulated protein 78 (GRP78)/immunoglobulin-heavy-chain-binding protein. It has been postulated that upon ER stress, GRP78 is released and sequestered on unfolded proteins, thereby allowing activation of PERK, IRE1α, and ATF6 (194). PERK activation leads to phosphorylation of the α-subunit of the translation initiation factor eukaryotic initiation factor 2α (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*) (62, 142). Increased expression of growth arrest and DNA damage-inducible protein 34 (*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 (133). 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*]) (142). ATF6 is a bZIP-domain containing transcription factor belonging to the cAMP-response element binding protein (CREB)/ATF family of transcription factors. Activation of ATF6 leads to its release from the ER membrane, processing in the Golgi, and entry into the nucleus. Transcriptional targets of ATF6 include protein chaperones and *XBP1* (182). Thus, activation of the UPR initiates a spectrum of responses that transiently attenuate global protein synthesis and enhance the capacity for protein folding and degradation. These responses, in turn, attempt to ameliorate ER stress by restoring the balance between the protein load imposed on the ER lumen and the ability to fold and degrade entering proteins.

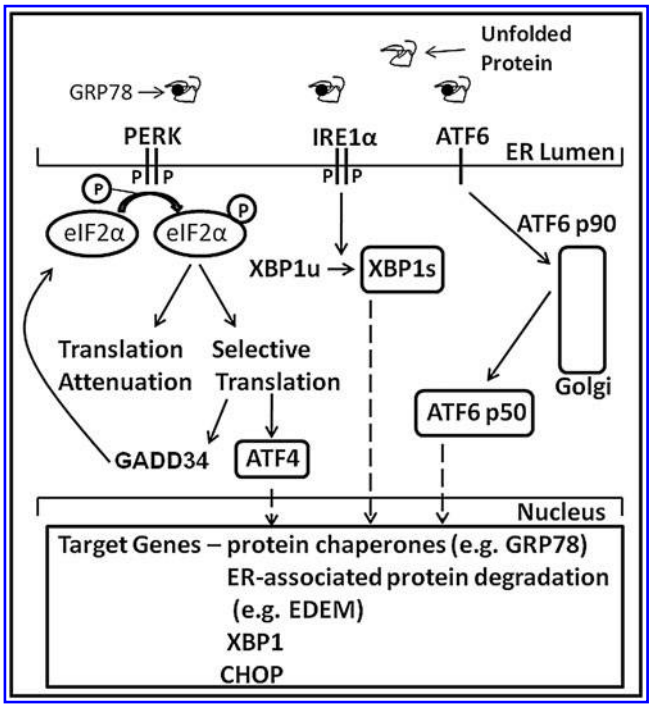

FIG. 1. Overview of the mammalian unfolded protein response (UPR). The presence of unfolded proteins in the endoplasmic reticulum (ER) lumen leads to dimerization and autophosphorylation of protein kinase-like ER kinase (PERK) and (IRE1α), and the release and proteolytic cleavage of activating transcription factor 6 (ATF6) in the Golgi. PERK-mediated phosphorylation of eukaryotic initiation factor 2α (eIF2α) leads to transient attenuation of translation but selective translation of mRNAs containing upstream open reading frames, such as activating transcription factor 4 (*ATF4*). Increased transcription and translation of GADD34 subsequently leads to dephosphorylation of eIF2α and resumption of translation. Activation of IRE1α leads to the splicing of *XBP1*. Spliced X-box binding protein 1 (XBP1s), ATF4, and the cleaved form of ATF6 lead to transcriptional activation of a number of gene targets related to protein folding and ER-associated degradation (see text).

An expanded view of the UPR

PERK is one of four protein kinases that can phosphorylate eIF2α (Fig. 2A); the other three are dsRNA-activated protein kinase (PKR), which is activated in response to viral infection, general control nonderepressible 2 kinase (GCN2), which is activated in response to amino acid deprivation, and heme-regulated inhibitor kinase (HRI), which is primarily expressed in reticulocytes and appears to coordinate globin polypeptide synthesis with heme availability (63). Protein kinase-mediated phosphorylation of eIF2α regulates not only translation but also the activation of nuclear factor kappa-*β* (NFκB), *via* reduction in the abundance of the NFκ*β* inhibitor (Iκ*β*) (121, 142, 183). Further, PERK can phosphorylate nuclear erythroid 2 p45-related factor 2 (Nrf2) triggering the dissociation of Nrf2/Keap1 complexes and subsequent nuclear import of Nrf2 (22). Thus, activation of this branch of the UPR links disruption of ER homeostasis to both inflammation, *via* NFκ*β* and redox balance, *via* Nrf2.

IRE1α, in addition to catalyzing *XBP1* splicing, has additional functions related to cellular signaling (Fig. 2B). Activated IRE1α can interact with the adaptor protein TNFR-associated factor 2 (TRAF2) and lead to activation of c-Jun-NH_2-terminal kinase (JNK) and NFκ*β* *via* apoptosis

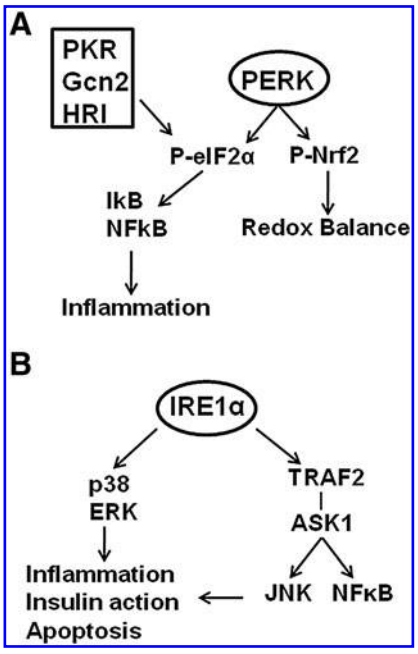

FIG. 2. Protein kinase-mediated phosphorylation of eIF2α and additional downstream targets of p-eIF2α and IRE1α. (A) Phosphorylation of eIF2α can be mediated by four protein kinases: double-stranded RNA-activated protein kinase (PKR), general control nonderepressible 2 kinase (Gcn2), heme-regulated inhibitor kinase (HRI), and PERK. Phosphorylation of eIF2α is linked to the inflammatory response *via* activation of nuclear factor kappa-*β* (NFκ*β*). PERK-mediated phosphorylation of nuclear erythroid 2 p45-related factor 2 (Nrf2) links PERK activation to the regulation of redox balance (see text). **(B)** Activation of IRE1α can lead to activation of stress kinases, including p38 mitogen-activated protein kinase (MAPK), extracellular-regulated kinase (ERK), c-Jun-NH_2-terminal kinase (JNK), and NFκ*β* (see text).

signaling-regulating kinase 1 (ASK1) (166). IRE1α activation has also been linked to the activation of p38 mitogen-activated protein kinase (p38 MAPK) and extracellular-regulated kinase (ERK) (50, 54, 104). These interactions suggest that the IRE1α branch of the UPR regulates not only adaptation to ER stress and cell survival *via XBP1* splicing but also activation of signaling pathways involved in inflammation, insulin action, and apoptosis.

Several proteins that share sequence homology with ATF6α, including Tisp40 (CREB4 and CREBSL4), BBF2H7 (CREB3L2), Luman (CREB3), old astrocyte specifically induced substance (CREB3L1), and CREBH (CREB3L3), are similarly anchored to the ER membrane and activated *via* regulated intramembrane proteolysis (RIP) in response to ER stress (7, 98, 111). Although each of these proteins appears to be mobilized in response to ER stress their unique tissue distributions suggest that ER stress-mediated RIP may be a mechanism to achieve tissue/cell-specific outcomes. Thus, it appears likely that future studies related to ER stress-mediated RIP will expand the role of the UPR in cellular signaling.

It is important to emphasize that much of what we know about the UPR has been derived from studies that utilize pharmacologic agents (tunicamycin and thapsigargin) to induce severe, protracted ER stress and cell death. Much less is known about the UPR in the context of physiologic stressors that provoke ER stress. *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) (65). In light of this complexity we will first review studies that link ER stress and/or the UPR to the pathogenesis of NAFLD and then discuss the signals that might provoke activation of the UPR in NAFLD, novel roles for the UPR in this disease and the physiologic implications of these studies.

The UPR and the Development of Hepatic Steatosis

NAFLD is characterized by lipid accumulation in the liver (≥10% of liver weight) in the absence of chronic alcohol consumption or other liver disease (39). Sources of hepatic lipids in NAFLD include dietary chylomicron remnants, free fatty acids released from adipose tissue triglycerides, and *de novo* lipogenesis. Recent work has emphasized the latter two mechanisms as the principle contributors to hepatic fat accumulation in humans with NAFLD (31, 32, 67). Several recent studies have linked the UPR to the regulation of lipogenesis and hepatic steatosis.

IRE1α-XBP1 pathway

The downstream targets of IRE1α activation include splicing of homologous to ATF/CREB1 (*HAC1*) mRNA in yeast and *XBP1* mRNA in mammalian cells (63). IRE1α-mediated splicing of HAC1 mRNA produces Hac1p, whereas IRE1α-mediated splicing of XBP1 produces XBP1s, both of which are critical to membrane lipid synthesis in yeast and mammalian cells, respectively (18, 153). In fact, the enforced expression of XBP1s in NIH-3T3 fibroblasts was sufficient to induce the synthesis of phosphatidylcholine, the primary phospholipid in the ER membrane (153). To investigate the role of XBP1 in the postnatal liver, Lee *et al.* utilized mice bearing an inducible, conditional disruption of the *Xbp1* gene (71). The phenotype of mice with conditional disruption of *Xbp1* in the liver included reduced plasma levels of triglyceride, cholesterol, and free fatty acids. Primary hepatocytes isolated from these mice were characterized by reduced incorporation of [^{14}C] acetate into fatty acids and sterols, suggesting that XBP1 was required for *de novo* lipogenesis. Chromatin immunoprecipitation assays, using liver extracts from mice fed a high fructose diet, demonstrated that XBP1 was able to bind to promoter regions of several lipogenic genes. The IRE1α-XBP1 pathway has also been linked to adipogenesis in mouse embryonic fibroblasts and 3T3-L1 cells (145). Thus, XBP1 splicing may, under the appropriate conditions, promote hepatic lipogenesis (Fig. 3A) and perhaps contribute to the development of hepatic steatosis.

PERK-eIF2α pathway

PERK and phosphorylation of eIF2α also appear to regulate lipogenesis and hepatic steatosis. Targeted deletion of *PERK* in mammary epithelium reduced free fatty acids in mouse milk

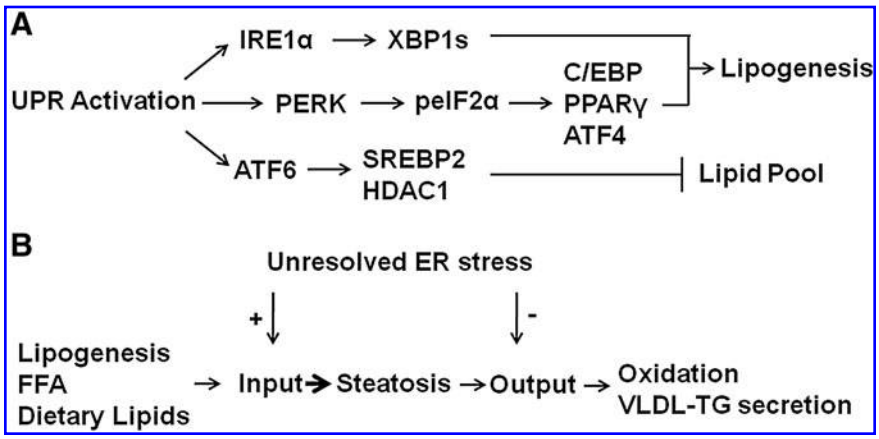

FIG. 3. The UPR is linked to regulation of lipogenesis and hepatic lipid stores. (A) The IRE1α-XBP1 and the PERK-peIF2α pathways can upregulate the lipogenic gene program. In contrast, interactions among ATF6, sterol regulatory element binding protein 2 (SREBP2), and histone deacytelase-1 (HDAC1) can limit lipogenesis. **(B)** Inability to resolve ER stress may promote hepatic steatosis *via* upregulation of pathways that contribute to lipid input and downregulation of lipid output pathways.

and led to growth retardation in suckling pups (8). Further analysis revealed that PERK deletion resulted in reduced expression of several lipogenic genes, including sterol regulatory element binding protein 1 (*SREBP1*). This study led to the hypothesis that PERK-mediated phosphorylation of eIF2α promotes SREBP1 activation during mid-lactation *via* depletion of insulin-induced gene 1 (INSIG1) protein, an ER-localized protein that anchors the SREBP-SREBP cleavage-activating protein (SCAP) complex in the ER membrane (72). 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 (phosphoserine 51). Enforced expression of an active C-terminal fragment of GADD34 from a liver-specific albumin promoter was used to study the role of p-eIF2α-mediated signaling in the mouse liver (114). The presence of the transgene resulted in a number of metabolic adaptations, including reduced hepatic steatosis in mice fed a high-fat diet. The reduction in hepatic steatosis was associated with lower 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 and C/EBPβ). Protein kinase-mediated phosphorylation of 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 (144). Thus, when these studies are considered together it would appear that protein kinase-mediated eIF2α phosphorylation can regulate the lipogenic transcriptional program and perhaps contribute to the development of hepatic steatosis (Fig. 3A).

ATF6

ATF6 and SREBPs are both ER-membrane-bound transcription factors that are activated by proteolytic cleavage. At least one study has demonstrated that the nuclear form of ATF6 inhibits the transcriptional activity of SREBP2 by forming a complex with SREBP2 that recruits histone deacytelase-1 (HDAC1) (192). The functional consequence of this interaction was to reduce Oil-Red-O staining in liver cells (Fig. 3A). 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 relative activation of the three proximal UPR sensors, PERK, IRE1α, and ATF6, coupled with appropriate downstream protein–protein and/or protein–DNA interactions.

Homeostatic model

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 (134). 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 after ER stress. Perhaps development and/or exacerbation of hepatic steatosis involves selective impairments to the UPR that reduce the ability of the UPR to resolve ER stress (Fig. 3B). Further work is necessary to investigate this hypothesis using physiologic models of hepatic steatosis.

Recent studies using $GRP78^{+/-}$ mice and adenoviral-mediated overexpression of *GRP78 in vivo* support the homeostatic model concept (61, 189). $GRP78^{+/-}$ mice were resistant to high-fat-diet-induced insulin resistance, hepatic steatosis, white adipose tissue inflammation, and hyperglycemia (189). It was postulated that GRP78 heterozygosity triggered the adaptive UPR *via* upregulation of other ER chaperones (*e.g.*, GRP94) and components of the ERAD machinery, improvement of ER homeostasis, and attenuation of the deleterious consequences of a high-fat diet. 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 (61).

The UPR and Disease Progression in NAFLD

Liver pathology in NASH can include macrovesicular steatosis, inflammation, fibrosis, apoptosis, and necrosis (5,

37, 48, 112, 120, 156). Multiple factors, including insulin action, oxidative stress, cytokine-mediated signaling, inflammation, bacterial endotoxin, and excess fatty acids likely function in concert to provoke disease progression in NAFLD. In this section we will consider potential ways in which the UPR, *via* interactions with some of these factors, may contribute to the development of NASH and progressive liver disease.

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 (12, 23, 87, 120, 152). An elegant study identified ER stress as a molecular link between obesity and deterioration of insulin action in liver and adipose tissue (117). In this study, obesity-induced ER stress reduced insulin signaling *via* an IRE1α-dependent activation of JNK, a protein kinase that can interfere with insulin signaling *via* serine phosphorylation of insulin-receptor substrate-1 (51, 53, 166). Notably, activation of JNK can also lead to liver damage and hepatocyte apoptosis (17, 24, 68, 105), the latter of which is a characteristic feature of NASH and correlates with disease severity (38, 176). In addition, the expression of proinflammatory cytokines was reduced in mice lacking JNK (52, 164), leading to the inference that IRE1α-mediated activation of JNK may also lead to a pro-inflammatory state. Global deletion of *JNK1*, but not *JNK2*, reduced hepatic triglyceride accumulation, inflammation, liver injury, and apoptosis in methionine-choline diet-fed mice (140). In contrast, specific ablation of *JNK1* in hepatocytes produced a phenotype that included glucose intolerance, insulin resistance, and hepatic steatosis (135), suggesting that JNK isoforms may have tissue-specific actions that dictate their roles in NAFLD. Thus, more work is needed to elucidate the role of JNK in NAFLD, how and under what conditions IRE1α regulates JNK, the cell types responsible for IRE1α-JNK-mediated outcomes (25), and the molecular mechanisms that distinguish ER lumen-mediated regulation of JNK from that which occurs from signals originating in the cytosol or plasma membrane. Clearly, JNK represents an important nexus for control of insulin action, inflammation, and cell death. The ability of IRE1α to regulate JNK activity suggests that ER stress and the UPR play an important role in disease progression in NAFLD.

Inflammation

The liver plays a critical role in the production of pro-inflammatory mediators, such as C-reactive protein (CRP), amyloid P-component, fibrinogen, and interleukin-6 (IL-6). Low-level activation of NFκβ in the liver was sufficient to increase the hepatic production of these pro-inflammatory mediators to an extent that was equivalent to what occurred in wild type mice fed a high-fat diet (14). Thus, it is now well appreciated that the NFκβ pathway is an important determinant of the inflammatory response and subsequent changes in insulin action (149). UPR-mediated signaling can lead to activation of NFκβ *via* the PERK, IRE1α, and/or ATF6 pathways (52, 142, 186). However, the importance of UPR-mediated signaling to the activation of NFκβ in the context of obesity and NAFLD are presently unknown. If the formation of the IRE1α-TRAF2 complex is crucial to activation of both JNK and NFκβ in NAFLD (54, 166), 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 NFκβ 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 NFκβ can protect hepatocytes from oxidative stress and TNFα-induced cell death (42, 80), as well as steatohepatitis and hepatocellular carcinoma (83). Thus, the ultimate outcome of NFκβ activation likely depends on such factors as the duration and magnitude of the stimulus, and the interaction of NFκβ with other signaling networks.

PKR is an interferon-induced serine/threonine protein kinase that is activated by dsRNA and, as previously described (Fig. 2A), is one of four protein kinases that can phosphorylate eIF2α in response to ER stress (33). PKR appears to be required for NFκβ activation in response to dsRNA, and therefore PKR has been linked to immune and inflammatory responses (34). 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 (101). The ability of PKR to respond to pathogens, nutrients, and organelle stress and to regulate inflammatory and insulin signaling pathways suggest that PKR may be a core component of an inflammatory complex (52, 101, 188). PKR exemplifies the complexity of UPR signaling and its downstream outcomes. For example, dsRNA-activated PKR can use catalysis-dependent and -independent activities to function both as a pro- and anti-apoptotic factor, *via* regulation of NFκβ and phosphorylation of eIF2α, respectively (34). 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" (34). It is feasible that other multifunctional, UPR-linked protein kinases employ similar strategies to elicit cell- or stress-selective outcomes.

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 (97, 130). The three-proximal UPR sensor, ATF6α, and the lipogenic transcription factor, SREBP, both undergo RIP before their entry into the nucleus (194); 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 CRP (82, 195). In addition, pro-inflammatory cytokines and lipopolysaccharide (LPS) induce the cleavage of CREBH in the liver *in vivo* (195). Thus, ER stress in the liver may be linked to systemic inflammation *via* RIP-mediated mobilization of CREBH.

Oxidative stress

Oxidative stress is thought to be an important pathogenic event in NAFLD. The ER provides a unique oxidizing environment for protein folding and disulfide bond formation. Each disulfide bond formed during oxidative protein folding produces a single reactive oxygen species. It has been estimated that secretory cells produce 3–6 million disulfide bonds

per minute; thus, protein folding in the ER is intimately linked to the generation of reaction oxygen species and potentially oxidative stress (138, 148). Conversely, cellular oxidative stress can disrupt ER homeostasis and induce ER stress (47, 184, 190). Therefore, it is not surprising that the UPR can activate an antioxidant program *via* the transcription factor Nrf2 (21). *Nrf2* belongs to the Cap 'n' Collar family of basic leucine zipper transcription factors and regulates the inducible expression of antioxidant response element-containing genes (21). Nrf2 is highly expressed in the liver and kidney and is a substrate of the proximal UPR sensor PERK (96). Importantly, *Nrf2* deletion results in rapid onset, and progression of steatohepatitis in mice provided a methionine-choline-deficient diet (155). In addition, *Nrf2*-deficient mice were characterized by increased mortality in response to endotoxin-induced and cecal ligation and puncture-induced septic shock (162). These studies have led to the proposal that Nrf2 participates in the regulation of the innate immune response. As noted, PERK-mediated phosphorylation of eIF2α also leads to the upregulation of *ATF4*. Along with Nrf2, this transcription factor has been linked to the maintenance of cellular glutathione (22). Thus, the PERK arm of the UPR appears to play a critical role in the defense against oxidative stress and the downstream substrate Nrf2 has been directly linked to steatohepatitis. In addition to the PERK arm of the UPR, recent evidence has also linked the IRE1α-XBP1 branch of the UPR to the regulation of antioxidant defenses (81). In this study, hydrogen peroxide-mediated cell death occurred more extensively in mouse embryonic fibroblast cells deficient in *XBP1*. *XBP1* deficiency resulted in reduced catalase expression, and overexpression of *XBP1* restored catalase expression in *XBP1*-deficient cells. 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 (38, 176). 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 pro-apoptotic proteins (116). Chop expression is regulated by ATF4 and perhaps ATF6, and deletion of *Chop* provides some protection from ER stress-induced cell death in both cells and animals (84, 109, 116, 132, 151). *Chop* deficiency delayed the development of ER stress-mediated diabetes in Akita mice and attenuated cholestasis-induced liver fibrosis (115, 157). 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 (125). The Chop protein is unstable when compared to protein chaperones, such as GRP78. Therefore, the role of Chop as a pro-apoptotic protein may be determined by the level of Chop expression, the presence of factors that increase its stability and/or protein–protein interactions that direct cell-specific effects (89, 132, 180).

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 (30). 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 (30, 74, 107, 143). Although the mechanisms involved in the regulation of ER calcium are not fully understood available data implicate the inositol triphosphate (IP3) receptor and truncated variants of the sarcoendoplasmic reticulum calcium-ATPase (74, 107). It also appears that the ER and mitochondria physically and functionally interact, in part, *via* tethers that link both smooth and rough ER to mitochondria (20, 131). A reduction in the length of these tethers has been observed in response to apoptotic agents (20). Although it is unclear whether ER calcium influences disease progression and apoptosis in NAFLD, it is possible that the hepatic milieu in NAFLD modifies ER calcium flux perhaps *via* alterations in IP3 receptor function and or ER-mitochondrial interactions.

Murine caspase-12 and human caspase-4 are members of the IL-1β-converting enzyme subfamily of caspases that are 48% identical at the amino acid level and have predicted structures that are consistent with initiator caspases (69, 108). Both of these caspases appear to be ER-localized and cleaved in response to ER stress. *Caspase-12* knockout mice and human cell lines in which caspase-4 was knocked down were protected from ER stress-induced apoptosis (99, 100). Whether these caspases play a functional role in ER stress-mediated apoptosis is controversial, in part due to the fact that caspase-4 belongs to a group of caspases that are best known for proteolytic activation of cytokines and not apoptosis (108, 147).

As noted above, IRE1α, *via* interaction with TRAF2, can mediate activation of the pro-inflammatory and pro-apoptotic protein, JNK (166). In addition, IRE1α appears to be a platform for the interaction of several Bcl-2 proteins, including the pro-apoptotic proteins Bcl-2-associated X protein (Bax) and Bcl-2 antagonist/killer (Bak) (49, 65, 107, 143). Although there are clearly a number of potential mechanisms linking the ER to the apoptotic program, the extent to which these mechanisms play a role in NAFLD remains unclear. It is also important to emphasize that cell death linked to persistent ER dysfunction can proceed independently of caspase activity, and that cell death in response to ER stress can involve caspase-dependent apoptosis and caspase-independent cell necrosis (65).

Autophagy, in principle, is a cellular degradation process for long-lived proteins and unnecessary or damaged organelles (66, 93, 94). At least three forms of autophagy have been identified, chaperone-mediated autophagy, microautophagy, and macroautophagy, that have distinct physiologic functions and modes of cargo delivery to the lysosome (73). Recent evidence has demonstrated that inhibition of macroautophagy in cultured hepatocytes and mouse liver increased triglyceride storage in lipid droplets (150). ER stress can trigger macroautophagy *via* mechanisms that may require calcium-mediated activation of protein kinase Cθ (137, 191). In addition, autophagy is a necessary pathway for the maintenance of structure, mass, and function in pancreatic β-cells (60). One can envision that ER stress-mediated activation of autophagy may be part of the protective, adaptive component of the UPR. The extent to which autophagy contributes to the

development of hepatic steatosis or disease progression in NAFLD requires further study.

Summary

There are a number of viable candidate mechanisms that link unresolved ER stress and the UPR to insulin resistance, inflammation, and apoptosis (Fig. 4). In addition, the process of protein folding in the ER lumen can generate reactive oxygen species and the UPR has the ability to mount a protective response against the development of oxidative stress (Fig. 4). Thus, ER-mediated signals are linked to a number of downstream pathways that contribute to the pathogenesis of NAFLD. However, whether ER stress and the UPR contribute to disease progression in NAFLD will ultimately 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. Next we will consider potential factors that elicit activation of the UPR and are relevant to NAFLD.

Determinants of UPR Activation in NAFLD: Steatosis

The upstream signals that mediate the putative link between the UPR and lipogenesis are presently unclear. Recent studies have demonstrated that the postprandial environment can elicit IRE1α activation, XBP1 splicing, and phosphorylation of eIF2α (71, 114, 126). It is possible that the postprandial environment provokes ER stress by transiently increasing protein synthesis above the capacity for protein folding and degradation. Alternatively, it is possible that the postprandial environment activates selective proximal UPR sensors that allow for the regulation of lipogenesis independent of ER stress *per se*. In support of this latter concept, hepatic XBP1s was induced in mice fed a 60% fructose diet in the absence of changes in GRP78 or Chop (71). Previous studies have also identified novel links between PERK and the expression of growth factors (75), and between PKR and phosphoinositide-3 kinase signaling (64), that may be independent of unfolded protein accumulation. Moreover, the basal expression of at least some ER chaperones appears to be dependent on a mitogenic pathway that is distinct from the ER stress-induced UPR (11). The critical role of the UPR in ER membrane biogenesis may require such independent regulation (106).

It is certainly possible that individual UPR sensors or downstream signaling components may be regulated by signals that do not necessarily involve ER stress (179). Although PERK and IRE1α share functionally similar ER-luminal sensing domains and both appear to be simultaneously activated by chemically induced ER stress, they may be selectively engaged *in vivo* (50). In particular, recent studies have identified several proteins that directly interact with and/or regulate the activity of IRE1α (46, 49, 78). This has led to the postulate that IRE1α activity in mammalian cells is determined by the formation of a complex protein platform, discussed above, that is assembled at the ER membrane (Fig. 5). Thus, the activity of IRE1α and its ability to interact with and regulate downstream pathways may be dependent on the composition of the IRE1α protein platform. In addition, the transcriptional response to increased splicing of *XBP1* may be modulated by protein interactions with XBP1s that are dependent on the cellular environment (123, 178, 185). Indeed, recent work has demonstrated that the nuclear localization of XBP1s requires the interaction of XBP1s with the p85 subunit of phosphoinositide 3-kinase (123, 178). Ultimately, what may be a critical determinant of UPR-mediated hepatic steatosis is the ability to turn off postprandial activation of IRE1α or related UPR components. In this regard, mammalian target of rapamycin (mTOR) has been linked to the postprandial activation of the IRE1α-XBP1 pathway in the liver (126). In addition, chronic activation of mTOR can provoke UPR-mediated inhibition of insulin signaling and susceptibility to apoptosis (118). Thus, the ability to regulate mTOR may play an important role in UPR-mediated regulation of lipogenesis and subsequent development of hepatic steatosis. Recent findings have also

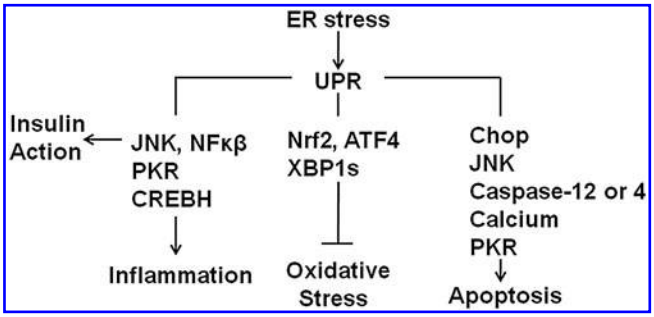

FIG. 4. The UPR is linked to insulin action, inflammation, oxidative stress, and apoptosis. The UPR is linked to insulin action *via* interactions with JNK, to inflammation *via* PKR, regulated intramembrane proteolysis of CREBH, and interactions with JNK, to protection from oxidative stress *via* phosphorylation of Nrf2, splicing of XBP1 and selective translation of ATF4, to apoptosis *via* interactions with JNK, upregulation of C/EBP homologous protein (Chop), activation of caspase-12, and extrusion of luminal calcium (see text).

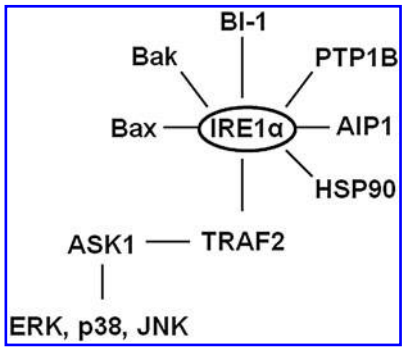

FIG. 5. IRE1α can interact with a diverse set of proteins. The activity of IRE1α can be modified by a number of interacting proteins, including B-cell leukemia/lymphoma 2 protein (Bcl-2)-associated X protein (Bax), Bcl-2 antagonist/killer (Bak), Bax inhibitor (BI)-1, protein tyrosine phosphatase-1B (PTP1B), apoptosis signaling-regulating kinase 1 (ASK1)-interacting protein 1 (AIP1), and HSP90. In turn, IRE1α, *via* interactions with TNFR-associated factor 2 (TRAF2), can modify the activity of ERK, p38, JNK, and NFκ*β* (see text).

linked the IRE1α-XBP1 pathway to the regulation of hepatic lipid metabolism by the circadian clock (19). This disruption of the circadian clock may represent another important link between the UPR and hepatic steatosis.

Determinants of UPR Activation in NAFLD: Disease Progression

Fatty acid composition

A large portion of the elevated hepatic triglyceride stores in NAFLD appear to arise from re-esterification of circulating free fatty acids (32). Elevated circulating free fatty acids are a characteristic feature of NAFLD and are positively correlated with liver disease severity (103). 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 (113, 173). In addition, *in vivo* studies have demonstrated that a reduction in the ability to desaturate fatty acids or a dietary-induced increase in the amount of saturated fatty acids in the liver provoke ER stress, apoptosis, and/or liver injury (76, 170). Saturated fatty acids disrupt ER homeostasis and induce apoptosis in liver cells *via* mechanisms that do not appear to involve ceramide accumulation (173). It is possible that the link between circulating and intrahepatic fatty acids and ER stress is mediated by changes in intracellular phospholipid pools (161, 181). Several studies suggest that saturated fatty acids disrupt ER homeostasis *via* selective, structural effects to the ER (10, 13, 95, 165). The highly unsaturated, fluid environment of the ER membrane, and ER membrane-bound UPR sensor proteins may be particularly susceptible to even minor increases in the amount of saturated fatty acids (167). Another mechanism by which saturated fatty acids may disrupt ER homeostasis is through reduced ER calcium stores in liver cells, perhaps *via* compromised calcium-dependent ER protein chaperone function (124, 172). Finally, recent data have demonstrated that saturated fatty acids increase the expression of phosphotidylinositol 3-kinase interacting protein 1, and SiRNA-mediated knockdown of this protein protected HepG2 cells from palmitate-mediated ER stress (27). However, it is presently unclear whether fatty acid composition plays a significant role in disease progression in human NAFLD (3, 29, 35, 43, 102).

Apolipoprotein B100

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, glucose-regulated protein-94 (GRP94), endoplasmic reticulum protein-72 (ERp72), calreticulin, and calnexin (15, 129, 193). 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 (113, 154). These data suggest a scenario in which fatty acids stimulate ApoB100 synthesis to an extent that exceeds the ability of the ER lumen to modify this protein, leading to ER stress and activation of stress signaling proteins (*e.g.*, JNK and glycogen synthase kinase-α) that can influence insulin action, inflammation, and/or apoptosis.

Hexosamine biosynthetic pathway

Glycosylation is an essential ER luminal modification for proper stability, folding, translocation, and function of many proteins (63). 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 (128, 136). Overexpression of glutamine:fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme of the hexosamine biosynthetic pathway induced ER stress, lipid accumulation, and upregulation of genes associated with inflammatory pathways in HepG2 cells (136). Treatment of cells with a GFAT antagonist blocked these responses to GFAT overexpression. Thus, one can envision a scenario in which increased flux of fatty acids and glucose may compromise the functional capacity of the ER lumen *via* direct effects on ApoB100 processing and maturation.

Oxidative stress

Changes in nutrient flux in NAFLD may also influence the functional capacity of the ER *via* affects on redox balance. Oxidative stress has been implicated in many diseases associated with protein misfolding, such as Prion disease, Alzheimer disease, and Parkinson disease (86). Oxidative stress has also been linked to the development and progression of a number of chronic diseases, including diabetes, obesity, and NAFLD (40, 169). 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 (86), extraluminal sources of prooxidants can also induce ER stress and promote the formation of inclusion bodies in liver cells (47, 85). The ability of free fatty acids, as well as TNFα, to provoke ER stress and UPR activation may be linked, at least in part, to the generation of reactive oxygen species outside of the ER lumen (6, 79, 138, 184).

Bacterial endotoxin

Bacterial translocation through the intestinal wall and small intestinal bacterial overgrowth may be involved in the pathogenesis of NASH (177, 187). Recent studies have confirmed the presence of increased plasma endotoxin and intestinal permeability in humans with NAFLD (91, 163). It is perhaps relevant that LPS, contained in the cell wall of gram-negative bacteria, induces ER stress and activation of the UPR in the normal liver and more sustained activation of the UPR in the liver of cirrhotic rats (160, 195). It is also interesting that LPS, as well as several cytokines (IL-6, IL-1β), also induce the cleavage of the proinflammatory CREBH protein from the ER membrane (195). Thus, an environment characterized by hyperlipidemia, elevated cytokines, and endotoxin can promote ER stress in the liver and also potentially provoke systemic inflammation *via* mobilization of CREBH.

Novel Roles for the UPR in NAFLD

Although hepatic iron accumulation in NAFLD may be mild, some studies have found an association between iron overload and disease progression (1, 92). In addition, hepatic iron overload can induce oxidative stress. Two recent studies

have demonstrated that the UPR can regulate hepatic iron metabolism (110, 168). Hepcidin, a defensin-like peptide, is a critical humoral regulator of innate immunity and host defense, and regulates iron stores in the liver *via* the iron export protein, ferroportin. It appears that hepcidin expression is regulated by the UPR in a biphasic manner, reduced initially and then increased if ER stress is sustained, *via* both CREBH and Chop. These data lend further support to the notion that the role of the UPR extends beyond protein quality control and now link ER stress to the regulation of hepatic iron metabolism.

Free fatty acids derived from adipose tissue are thought to play an important role in the development of hepatic steatosis (32). A recent study provided strong evidence that adipocyte apoptosis may be a key early event for macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in mice and humans (2). ER stress is present in adipose tissue of humans with obesity and NAFLD (9, 146). It is of interest, therefore, that a recent study in *C. elegans* suggested that IRE1 and HSP-4, the nematode IRE1 and GRP78 homologs, respectively, regulate the expression of the fasting-induced lipases, FIL-1 and −2 (59). These lipases were both necessary and sufficient for fasting-induced fat granule hydrolysis. It is presently unclear whether the UPR can regulate lipolysis in mammalian adipose tissue; however, one can envision a role for this pathway in adipose tissue lipolysis and apoptosis in the context of obesity and NAFLD.

ER Stress, the UPR, and Human NAFLD

There is currently only one study that has compared markers of ER stress in humans with or without NAFLD (127). 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 phosphorylation of eIF2α, several other markers of ER stress were not increased, including ATF4 mRNA and protein, *Chop* mRNA, *GADD34* mRNA, unspliced *XBP1* mRNA, and *EDEM* mRNA. Livers from NASH subjects were additionally characterized by a reduction in the amount of XBP1s and increased phosphorylation of JNK.

Two studies have examined ER stress markers in adipose tissue of obese, insulin-resistant subjects (9, 146). 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 (9). 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 BMI (146). Several gene markers associated with the UPR, including *GRP78*, *ATF6*α, *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, $n=11$) before and 1 year after gastric bypass surgery (45). Subjects lost ~40% of body weight at the 1 year follow-up at which time significant reductions were observed in adipose tissue *GRP78* mRNA, *XBP1s* mRNA, phosphorylation of eIF2α, and phosphorylation of JNK. Liver samples were characterized by reduced staining for GRP78 and phosphorylated eIF2α. Thus, weight loss appears to modulate the levels of several gene and protein markers of ER stress in liver and adipose tissue.

A recent study analyzed hepatic gene networks in morbidly obese patients with (BMI 49.6 ± 7.4 kg/m^2, $n=24$, 89% female) or without (BMI 48.8 ± 5.9 kg/m^2, $n=25$, 96% female) NAFLD (41). Three genes associated with the fibrosis pathway (*COL1A1*, *IL-10*, and *IGFBP3*) were upregulated and one gene associated with the UPR (*HSPA5*, also known as *GRP78*) was downregulated in patients with NAFLD compared to patients without NAFLD.

These results emphasize several limitations in our current understanding of ER stress in the context of human obesity and NAFLD. These include the lack of lean individuals with normal liver histology as a comparison group, limited tissue access, heavy reliance on gene expression analysis for determination of ER stress and UPR activation, and limited analysis of proteins associated with the UPR. Future *in vivo* studies in both animal models of chronic disease and humans with obesity and NAFLD need to carefully and comprehensively examine the UPR in liver and adipose tissue at multiple time points and under both fasted and fed conditions. Such data will allow us to better define ER stress and understand the UPR within a physiologic context.

Chemical Chaperones and NAFLD

Several studies have demonstrated that chemical chaperones alleviate ER stress in model systems used to study lysosomal storage disease, hereditary hemochromatosis, and cholangiocarcinoma (4, 28, 171). Oral administration of 4-phenyl butyric acid (PBA) or taurine-conjugated ursodeoxycholic acid (TUDCA) to ob/ob mice normalized blood glucose levels improved insulin sensitivity, reduced hepatic steatosis, normalized liver enzymes, and reduced biochemical markers of ER stress in liver and adipose tissue (119). PBA and TUDCA have also proven effective in reducing fatty acid-induced ER stress in liver cells (113, 125). The role and effectiveness of chemical chaperones in human NAFLD is presently unclear. Three trials, one that studied 24 patients over a 12-month period, one that studied 15 patients over a 3-month period, and one that studied 29 patients for 6 months, suggested that UDCA treatment (10–15 mg/kg/day) produced beneficial effects by reducing liver enzymes (36, 70, 139). In contrast, two trials, one that studied 14 women for a 1.5-month period and one that studied 80 patients for a 24-month period, suggested that UDCA (13–15 mg/kg/day) did not have a significant effect on biochemical or imaging markers of liver disease compared to weight reduction or placebo, respectively (77, 90). Future trials using chemical chaperones that can enhance protein-folding capacity should assess ER stress markers, taking into considerations the limitations noted in the previous section.

Concluding Remarks

Nutrient excess is a critical mediator of obesity and local organ/tissue lipid accumulation. Accumulating evidence

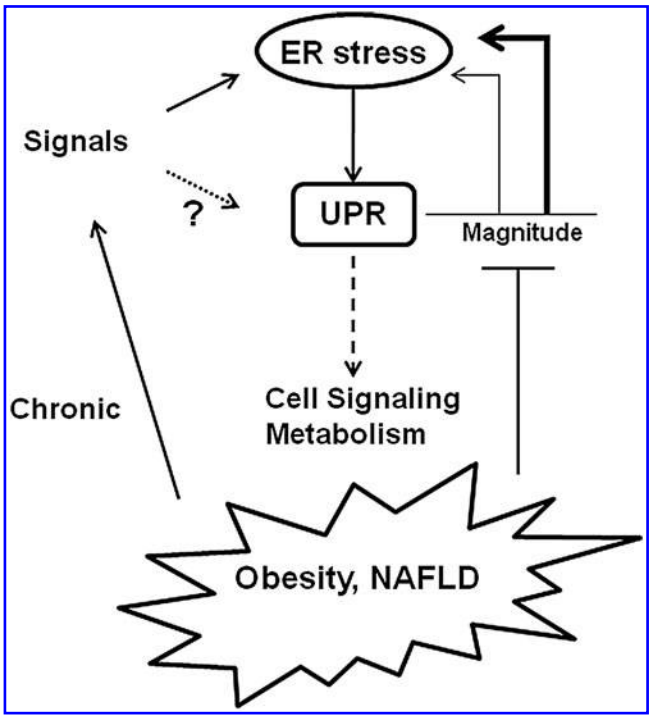

FIG. 6. Hypothetical model for the UPR in chronic diseases. Signals that lead to ER stress activate the UPR. The ability of the UPR to alleviate ER stress is linked to the magnitude of the UPR (*thin line* represents a lower magnitude response; *thicker line* represents higher magnitude response). The UPR is also linked to multiple cell signaling and metabolic pathways. In chronic diseases like obesity and nonalcoholic fatty liver disease (NAFLD) chronic signals induce ER stress, and perhaps activation of the UPR independently of ER stress. In addition, obesity and/or NAFLD may be characterized by signals that impair the UPR, thus dampening the magnitude of the response and the ability to alleviate ER stress (see text).

suggests that the ER plays an important role in nutrient sensing and can interact with multiple metabolic pathways, in part, *via* the UPR. These features, coupled with the apparent activation of components of the UPR in obesity and NAFLD, suggest that disturbances in ER homeostasis likely contribute to the development and/or progression of NAFLD. Elegant studies employing selective genetic manipulation and cell-based systems have clearly demonstrated that both proximal sensors/initiators of the UPR and downstream responses can participate in a diverse array of cellular functions, including differentiation, ER and mitochondrial biogenesis, insulin action, glucose and lipid metabolism, inflammation, and apoptosis. However, much less is known about the role and regulation of ER homeostasis and the UPR in the setting of chronic metabolic diseases (50, 52, 55, 133) or multiple liver diseases (56–58, 158, 159, 174) *in vivo*. For example, what are the primary signals that provoke ER stress *in vivo*, and are these signals tissue/organ specific? Can activation of the UPR *in vivo* occur *via* signal-mediated activation of a proximal UPR sensor independently of the accumulation of unfolded proteins? How is the diverse set of outputs controlled by the UPR regulated *in vivo* and in response to chronic stress? Finally, since ER stress is defined by the activation of the UPR, and not all components of the UPR appear to be activated or increased in human NAFLD, should our focus be on potential impairments in the UPR and how these are linked to disease progression?

In conclusion, the UPR is a robust, highly efficient pathway that functions to remove unfolded proteins from the ER lumen but also interacts with multiple obligatory cellular signaling pathways (Fig. 6). 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 the magnitude of the UPR will provide an environment that will promote disease progression.

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Abbreviations Used

AIP1 = ASK1-interacting protein 1
ApoB100 = apolipoprotein B100
ASK1 = apoptosis signaling-regulating kinase 1
ATF4 = activating transcription factor 4
ATF6 = activating transcription factor 6
Bak = Bcl-2 antagonist/killer
Bax = Bcl-2-associated X protein
BBF2H7 = BBF human homolog on chromosome 7
Bcl-2 = B-cell leukemia/lymphoma 2 protein
BI = Bax inhibitor
BMI = body mass index
C/EBP = CCAAT/enhancer-binding protein
Chop = C/EBP homologous protein
CREB = cAMP-response element binding protein
CRP = C-reactive protein
dsRNA = double-stranded RNA
EDEM = ER degradation-enhancing α-like protein
eIF2α = eukaryotic initiation factor 2α
ER = endoplasmic reticulum
ERAD = ER-associated degradation
ERK = extracellular-regulated kinase
ERp72 = endoplasmic reticulum protein-72
GADD34 = growth arrest and DNA damage-inducible protein 34
Gcn2 = general control non-derepressible 2 kinase
GFAT = glutamine:fructose-6-phosphate amidotransferase
GRP78 = glucose-regulated protein 78
GRP94 = glucose-regulated protein 94
HAC1 = homologous to ATF/CREB1
HDAC1 = histone deacytelase-1
HRI = heme-regulated inhibitor kinase
Iκβ = NFκβ inhibitor
IL-6 = interleukin-6
INSIG1 = insulin-induced gene 1
IP3 = inositol triphosphate
IRE1α = inositol-requiring transmembrane kinase and endonuclease 1α
JNK = c-Jun-NH_2-terminal kinase
LPS = lipopolysaccharide
MAPK = mitogen-activated protein kinase
mTOR = mammalian target of rapamycin
NAFLD = nonalcoholic fatty liver disease
NASH = nonalcoholic steatohepatitis
NFκβ = nuclear factor kappa-β
Nrf2 = nuclear erythroid 2 p45-related factor 2
PBA = 4-phenyl butyric acid
PERK = protein kinase-like ER kinase
PKR = double-stranded RNA-activated protein kinase
PPAR = peroxisome proliferator-activated receptor
PTP1B = protein tyrosine phosphatase-1B
RIP = regulated intramembrane proteolysis
SCAP = SREBP cleavage-activating protein
SREBP = sterol regulatory element binding protein
Tisp40 = transcript induced in spermiogenesis-40
TRAF2 = TNFR-associated factor 2
TUDCA = taurine-conjugated ursodeoxycholic acid
UPR = unfolded protein response
XBP1s = spliced X-box binding protein 1

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