thematic review

Thematic review series: Adipocyte Biology

Adipocyte stress: the endoplasmic reticulum and metabolic disease

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Abstract In the context of obesity and its related maladies, the adipocyte plays a central role in the balance, or imbalance, of metabolic homeostasis. An obese, hypertrophic adipocyte is challenged by many insults, including surplus energy, inflammation, insulin resistance, and considerable stress to various organelles. The endoplasmic reticulum (ER) is one such vital organelle that demonstrates significant signs of stress and dysfunction in obesity and insulin resistance. Under normal conditions, the ER must function in the unique and trying environment of the adipocyte, adapting to meet the demands of increased protein synthesis and secretion, energy storage in the form of triglyceride droplet formation, and nutrient sensing that are particular to the differentiated fat cell. When nutrients are in pathological excess, the ER is overwhelmed and the unfolded protein response (UPR) is activated. Remarkably, the consequences of UPR activation have been causally linked to the development of insulin resistance through a multitude of possible mechanisms, including c-*jun* N-terminal kinase activation, inflammation, and oxidative stress.▆▆ This review will focus on the function of the ER under normal conditions in the adipocyte and the pathological effects of a stressed ER contributing to adipocyte dysfunction and a thwarted metabolic homeostasis.—Gregor, M. F., and G. S. Hotamisligil. **Adipocyte stress: the endoplasmic reticulum and metabolic disease.** *J. Lipid Res.* 2007. **48:** 1905–1914.

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The adipocyte and the adipose tissue it inhabits have begun to fascinate researchers as a result of rapidly accumulating new knowledge of their function and contribution to whole body metabolic homeostasis. Adipose tissue is highly specialized to store lipid and/or release energy from lipid stores in response to a variety of signals. Adipose tissue also functions as an endocrine organ, secreting specific hormones or adipokines, which act as potent messengers to distant organs such as muscle, liver, and brain, with the purpose of maintaining the body's energy balance and metabolic health.

Recent interest in the study of adipocytes has burgeoned as a result of the increasing incidence of obesity worldwide. Approximately 1.1 billion adults are overweight and 400 million adults are obese (body mass index $\geqslant$ 30) (1). All parts of the earth, developed and now developing countries as well, face an alarming obesity epidemic and the emergence of a cluster of associated pathologies (2). Because of increased urbanization, adaptation of the Western diet, and sedentary lifestyle, obesity rates have tripled in the past two decades in areas such as India, China, and Southeast Asia. As a consequence, the prevalence of diabetes is also predicted to increase 150% or more by the year 2030 in these countries (2). Therefore, it is imperative to take action on multiple levels to prevent this global epidemic and to encourage the involvement of individuals and communities as well as medical, pharmaceutical, and food industries. Understanding the mechanisms underlying obesity and its associated disease cluster is also of great significance, as the need for new and more effective therapeutic strategies is more urgent than ever.

The massive expansion of adipose tissue that occurs in obesity is associated with numerous pathologies, including

Abbreviations: ASK1, apoptosis signal-regulating kinase 1; ATF-6, activating transcription factor-6; CHOP, C/EBP homologous protein; EDEM, ER degradation-enhancing α-mannosidase-like protein; eIF2α, eukaryotic translational initiation factor 2α; ER, endoplasmic reticulum; ERdj4, endoplasmic reticulum-resident DNAj homolog 4; ERO1, ER redox control for endoplasmic reticulum oxidoreductin; GADD34, growth arrest and DNA damage-inducible protein; IKB, inhibitor of NKκB; IKK-NFκB, IKB kinase nuclear factor κB; IL-6, interleukin-6; IRE-1, inositol-requiring enzyme-1; IRS-1, insulin receptor substrate 1; JNK, c-*jun* N-terminal kinase; mTOR, mammalian target of rapamycin; NFκB, nuclear factor κB; ORP150, oxygen-regulated protein; PBA, phenyl butyric acid; PDI, protein disulfide isomerase; PERK, PKR-like eukaryotic initiation factor 2α kinase; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; SREBP, sterol-regulatory element binding protein; TNF-α, tumor necrosis factor-α; TG, triglyceride; TRAF2, tumor necrosis factor receptor-associated factor 2; Trb3, Tribbles 3/SKIP 3; TUDCA, taurine-conjugated ursodeoxycholic acid; UPR, unfolded protein response; XBP-1, X-box protein 1.

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insulin resistance, type 2 diabetes, cardiovascular disease, and cancer. Increased adiposity, and perhaps especially visceral adiposity, is well correlated with an increased risk of insulin resistance and the development of type 2 diabetes (3). It is thought that this excessive or disproportionate gain of adipose tissue may be causal to its dysfunction at many levels. One locus that has emerged as a central mediator of this dysfunction is cellular inflammation (3). Inflammatory pathways [such as c-*jun* N-terminal kinase (JNK) and nuclear factor κB (NFκB) signaling] are upregulated in obese adipose tissue, leading to increased expression of downstream cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 MCP-1, among others. Many of these proinflammatory mediators have been shown to be detrimental to proper insulin signaling, and inhibition of obesity-induced inflammation can improve insulin sensitivity in mice and humans (4–7). Indeed, insulin-resistant adipose tissue is burdened with chronic inflammation as well as other possible insults, such as hypoxia, oxidative stress, and mechanical stress attributable to hypertrophy. These insults cumulatively result in organelle dysfunction, particularly in mitochondria and the endoplasmic reticulum (ER). It is hypothesized that a saturated or, perhaps, dysfunctional adipose tissue must send its lipid load elsewhere for storage, thereby causing other organs to become a "sink" for lipids. This phenomenon, also known as "lipotoxicity," may disrupt the normal function of recipient sites, contributing to pathology in, for example, the liver and muscle tissues as well as pancreatic islets. Thus, it follows that adipose dysfunction may be at the center of obesity-related pathologies, and an understanding of its pathology will be crucial to the development of effective preventive and therapeutic strategies. Many genetic models targeting key metabolic pathways in an adipose tissue-restricted manner in mice provided critical proof for this mechanistic concept. For example, loss of Glut4, insulin receptor, or peroxisome proliferator-activated receptor γ (PPARγ) in adipocytes resulted in alterations in whole body insulin sensitivity, clearly demonstrating the impact of adipose tissue on systemic energy balance as well as glucose and lipid metabolism (8–10).

As stated above, various processes have been implicated in the development of the insulin-resistant adipocyte. Inflammation and oxidative stress are two processes shown to be present in obese adipose tissue and causative for insulin resistance (3, 4, 7). These processes are highly integrated and likely to work in vicious cycles, reflecting a shortcoming in the adaptive capacity of cells to cope with chronic metabolic surplus. An emerging concept to explain the vast array of maladaptive responses is the presence of organelle dysfunction in obesity, affecting the mitochondria and ER (11, 12). Indicative of stress to this latter organelle, the unfolded protein response (UPR) was recently shown to be activated in obese, insulin-resistant tissues in experimental models. Notably, this stress was most prominent in the adipose tissue and contributed to its dysfunction (3, 11).

In this review, we hope to stimulate thinking with regard to the impact of the ER in adipocyte biology, the effects of ER stress on adipocyte function, and, consequently, the implications of adipose tissue stress on whole body metabolic health.

THE ER: REGULATOR OF LIPID, CHOLESTEROL, AND PROTEIN METABOLISM

The ER is a specialized cytosolic organelle in which various metabolic signals and pathways are integrated to regulate lipid, glucose, cholesterol, and protein metabolism. The ER is a principal site of protein synthesis, and together with the Golgi apparatus, it facilitates the transport and release of correctly folded proteins. Ribosomes attached to the ER membrane translate de novo peptides into the luminal space. Within the lumen of the ER, protein chaperones such as BiP (GRP78), calnexin, and calreticulin assist in the proper folding of de novo peptides and prevent the aggregation of unfolded or misfolded precursors. Once conformationally sound, the proteins are released to the Golgi for final modifications (such as oligosaccharide processing) and transported to their cellular destinations. Cells that are specialized for a high secretory capacity, such as plasma cells, liver cells, and pancreatic β cells, are known to expand and adopt their ER capabilities to meet the increased demand of protein synthesis (13). As acknowledged in recent years, the adipocyte also acts as a potent endocrine cell, undergoing transformation during differentiation from a fibroblast-like preadipocyte into a mature cell secreting prodigious amounts of peptide and lipid mediators (**Fig. 1**) (14, 15). Many abundant adipokines, such as leptin, adiponectin, RBP4, and even the fatty acid binding protein ap2, are found at high concentrations in serum (16, 17). Given the capacity of the adipocyte for protein (and lipid) synthesis and secretion, it follows that the ER of a differentiated adipocyte is challenged and may be enhanced to meet the increased demand. In the case of obesity, this expansion may be even more dramatic. Further study in this area is needed to elucidate the role of the ER in adipocyte protein synthesis and secretion.

In addition to protein synthesis, the ER is also the site of triglyceride (TG) droplet formation (the nomenclature also refers to the TG droplet as the lipid body or lipid droplet) (18, 19). In response to fatty acid accumulation within the cell, TG formation occurs as an energy storage and lipid-neutralizing mechanism. Three fatty acids and one glycerol molecule are joined together to form TG by enzymes resident in the ER membrane, and TG molecules (along with cholesterol) are stored in droplet form: the spherical structure surrounded by a single phospholipid membrane layer and associated proteins. TG and, subsequently, droplet formation is thought to occur in the ER. Indeed, one theory of TG droplet formation states that the droplet originates between the two membranes of the ER and eventually buds off, carrying the outer cytoplasmic layer of the ER membrane with it, thus explaining the phospholipid monolayer surrounding the droplet (9). In contrast, a second theory has proposed that TG droplet formation occurs outside the ER, but with the ER
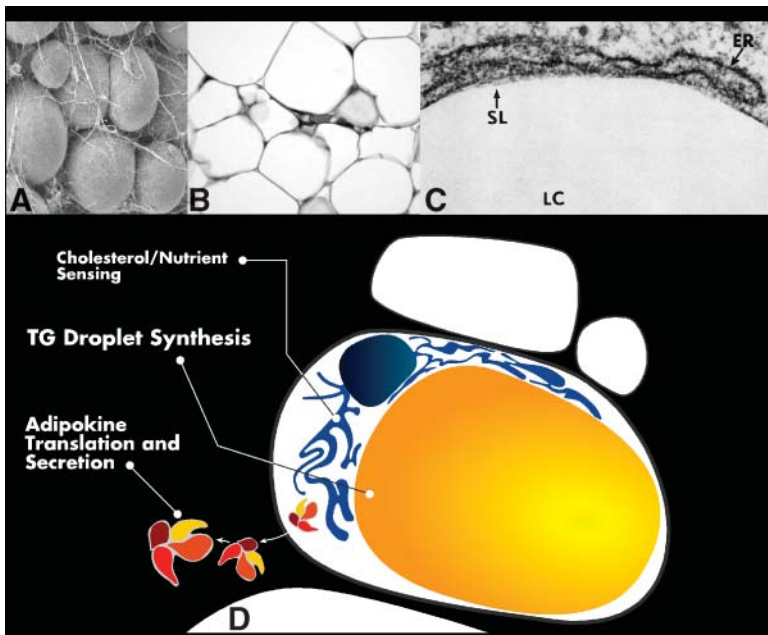
Fig. 1. The adipocyte in three perspectives: focus on the endoplasmic reticulum (ER). Adipocyte morphology in three perspectives illustrating its organization. A: Scanning electron micrograph of mouse adipose tissue, courtesy of Tae-Hwa Chun and Stephen Weiss (88). B: Hematoxylin and eosin-stained section of mouse adipose tissue, courtesy of Drs. Steven Shoelson and Ali Nayer. C: Electron micrograph of 3T3-L1 adipocyte ER surrounding a lipid droplet (LC, lipid core; SL, surface layer), reproduced with permission from Blanchette-Mackie et al. (21). D: Adipocyte ER functions include protein translation, triglyceride (TG) droplet synthesis, and cholesterol and nutrient sensing. Notably, the architecture of the tissue as well as the amount of lipid storage makes the adipocyte a unique and challenging environment for the function of the ER. Artistic design by Deniz Hotamisligil.

facilitating synthesis and surrounding the newly formed droplet as an egg is surrounded by an egg cup (20). Indeed, reports showing organelle spatial relationships of the adipocyte note the presence of the ER surrounding lipid droplets (21, 22) (Fig. 1). Regardless of the model, ER membranes are integral components of lipid droplets. Furthermore, multiple studies isolating lipid droplets from cells have shown the presence of the ER chaperone protein BiP on the droplet (23, 24), supporting its ER origin. Interestingly, the adipocyte, more than any other cell type, is uniquely equipped to store copious amounts of TG and cholesterol in this droplet form, as seen in TG droplet accumulation during differentiation. Release of fatty acids from TG storage by lipolysis also occurs at the TG droplet and is well correlated with adipocyte size (i.e., the larger the cell, the greater the rate of lipolysis) (25). It is unknown what role if any the ER plays in the regulation of droplet number, size, or lipolysis, parameters that are all increased in adipocytes. Given the ER origin of the TG droplet and the proximity of the two organelles within the cell, it would be fascinating if the functional interaction and regulation of the TG droplet by the ER persisted after its initial formation.

A third metabolically relevant function of the ER is its role in cholesterol and nutrient sensing. Cellular levels of cholesterol are regulated through the sterol-regulatory element binding protein (SREBP) family of transcription factors (26). The SREBPs, consisting of three isoforms in mammals, SREBP1a, -1c, and -2, are resident in the ER membrane in an inactive state and retained in precursor form by SCAP proteins (SREBP cleavage-activating proteins). In response to low sterol levels (in the case of SREBP1a and -2) or insulin signaling (in the case of SREBP1c), SREBPs are released from binding to the SCAP proteins and translocate to the Golgi, where they undergo two successive cleavages resulting in the generation of an activated transcription factor. The activated SREBP then enters the nucleus and acts upon target genes to upregulate cholesterol (SREBP1a and -2) or lipid (SREBP1c) synthesis. SREBP1c expression is induced/required during differentiation in adipocytes and is highly expressed in adipose tissue (27, 28). SREBP1c is also downregulated in the adipose tissue of obese and insulin-resistant mouse models and human patients (29–31). This shutdown of lipid biosynthesis may be attributable to the sensing of dietary lipid overload by the hypertrophic (obese) fat cell and may act as a feedback mechanism to maintain homeostasis. One intriguing possibility is that this sensing could occur in the ER. Not only cholesterol but also other nutrients, such as amino acids and glucose, are "sensed" by the ER. The nutrient-responsive mammalian target of rapamycin (mTOR) pathway upregulates protein synthesis, which would naturally increase protein translation and folding in the ER (32). Although not proven, it is possible that nutrient overload could engage this pathway and lead to a translational demand overwhelming the ER. In support of this, mTOR activity is increased in obesity (33), and this augmented signaling may play a role in the abnormal insulin action associated with the obese state (34, 35). Therefore, it is possible that the obesity-related increase in mTOR activity itself may contribute to an ER stress response. Finally, the ER is exquisitely sensitive to glucose availability. Therefore, nutrient and energy deprivation, or the excess of nutrients, may be perceived by the ER via its stress pathways and lead to the mounting of its adaptive responses, commonly referred to as the unfolded protein response (UPR). We now turn to a more detailed description of this important adaptive response.

STRESS IN THE ER: THE UPR

Given the fundamental roles of the ER in integrating multiple metabolic signals and maintaining cellular

homeostasis, it is of paramount importance to the cell to sustain proper ER function. Therefore, under conditions of cellular stress leading to an impairment of ER function, proteins are unable to fold properly and accumulate in the ER lumen. It is to these unfolded or misfolded proteins that the ER has evolved a coping system known as the UPR (36–38) (**Fig. 2**). Cellular stresses that may elicit UPR activation include, as mentioned previously, glucose and energy deprivation, increased protein synthesis, and also inhibition of protein glycosylation, imbalance of ER calcium levels, and the presence of mutant or misfolded proteins. How does the ER sense the imbalance between fluctuations in demand and its folding capacity? The UPR functions via signaling through three arms or branches, denoted for the three stress-sensing proteins found in the ER membrane: PKR-like eukaryotic initiation factor 2α kinase (PERK), inositol-requiring enzyme-1 (IRE-1), and activating transcription factor-6

(ATF-6). These three transmembrane proteins are normally bound by the ER chaperone BiP in their intraluminal domains. When client proteins (also bound by BiP) begin to exceed ER capacity, less BiP is available for binding to the UPR sensors. Without BiP binding, PERK and IRE-1 auto-oligomerize and undergo autophosphorylation, leading to the activation of downstream signaling. ATF-6 is released to the Golgi, where, in the same manner as the SREBPs, it undergoes two subsequent cleavages to produce an active transcription factor.

What effects does an activated UPR have on cell function? One result of PERK activation is selective attenuation of protein translation through inhibitory phosphorylation of eukaryotic translational initiation factor 2α (eIF2α) at serine 51. This phosphorylation also results in an increased alternative translation of ATF-4, which induces the expression of many genes, including those involved in apoptosis [C/EBP homologous protein (CHOP)]. ER redox
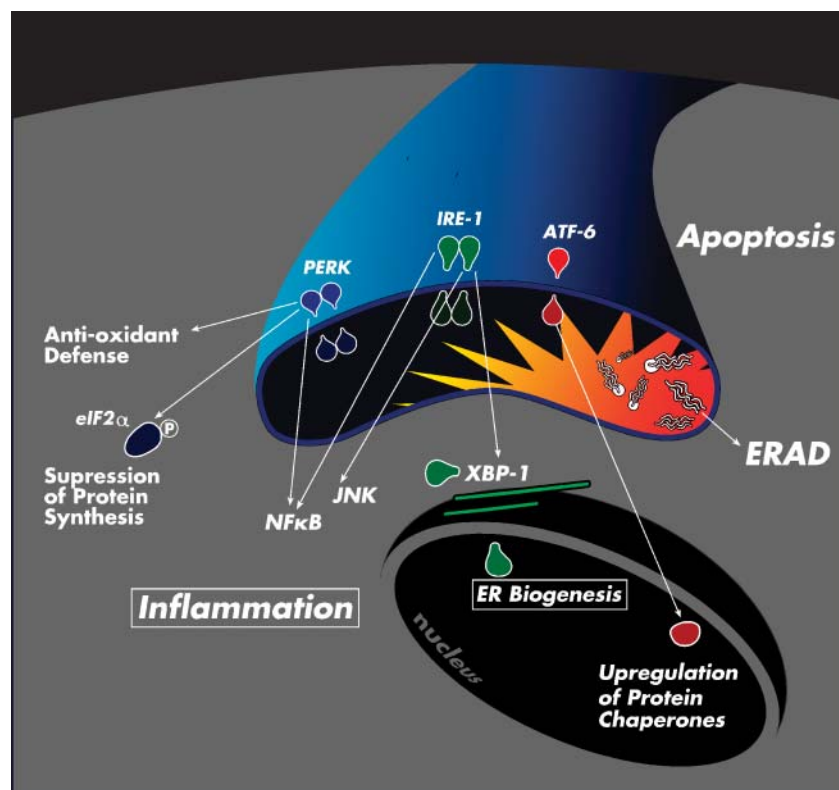
Fig. 2. Stress-sensing response of the ER. Under stress conditions, the three branches of the unfolded protein response (UPR) are activated. PERK (for PKR-like eukaryotic initiation factor 2α kinase) phosphorylation leads to the inhibition of protein synthesis via phosphorylation of eIF2α (for eukaryotic translational initiation factor 2α) and the initiation of an antioxidant response via the transcription factor Nrf2 (for nuclear erythroid 2 p45-related factor 2). Upon splicing by activated IRE-1α (for inositol-requiring enzyme-1α), XBP-1 (for X-box protein 1) also regulates transcription and induces genes involved in ER biogenesis and secretion. Importantly, inflammatory pathways are also activated by the UPR. Nuclear factor κB (NFκB) signaling may be upregulated via PERK or IRE-1α, and the c-*jun* N-terminal kinase (JNK) pathway is also activated by IRE-1α. Both of these pathways have been implicated as causative in the development of insulin resistance. Upregulation of chaperones to assist in protein folding occurs via ATF-6 (for activating transcription factor-6) action in the nucleus, and ER-associated degradation (ERAD) is induced to reduce the unfolded protein load within the ER. If the ER cannot recover from the challenge of the stress, apoptosis signaling will occur from the UPR via multiple mechanisms (details not shown here). Artistic design by Deniz Hotamisligil.

control [endoplasmic reticulum oxidoreductin (ERO1)], and the negative feedback release of eIF2α inhibition [growth arrest and DNA damage-inducible protein (GADD34)]. PERK signaling also results in an antioxidant response mediated by the activated transcription factor Nrf2 (nuclear erythroid 2 p45-related factor 2).

In addition to selective inhibition of de novo protein synthesis, the UPR also induces the transcription of chaperones to assist with the unfolded protein load. Activated ATF-6 translocates to the nucleus and upregulates the gene expression of chaperones such as BiP, calreticulin, and GRP94. The process of ER-associated degradation is also upregulated at this time to facilitate the clearance and degradation of excess client proteins from the ER lumen. ATF-6 induces the expression of ER degradation-enhancing α-mannosidase-like protein (EDEM), which is involved in this process. ATF-6 also upregulates X-box protein 1 (XBP-1) mRNA, which is further processed and specially regulated by the IRE-1 response arm.

IRE-1 activation by the UPR also contributes to the increase in protein chaperone content as well as to ER biogenesis and enhanced secretory capacity via the action of XBP-1. IRE-1α, acting as an endoribonuclease, cleaves a 26 bp segment out of the mRNA of XBP-1, creating a spliced mRNA that translates an active form of the transcription factor (XBP-1s). XBP-1s, in turn, induces the expression of protein chaperones, as well as proteins involved in ER biogenesis and secretion (e.g., EDEM, endoplasmic reticulum-resident DNAj homolog 4 (ERdj4), protein disulfide isomerase (PDI), and ER proteins), and acts as one of the major pathways regulating ER function and folding capacity.

The processes described thus far in the UPR have the end goals of recovery and survival of the cell. However, if the ER stress is not relieved, the UPR may also induce cell death via apoptosis. Although the pathways leading to apoptosis under ER stress conditions are not fully clear, CHOP induction, caspase-12 activation from the ER membrane, and IRE-1α activation of JNK, as well as regulation of the proapoptotic Bcl-2 family of proteins, are thought to play important roles (37, 39).

ER stress-induced IRE-1α phosphorylation leads to the recruitment of tumor necrosis factor receptor-associated factor 2 (TRAF2) and apoptosis signal-regulating kinase 1 (ASK1) to the cytosolic leaflet of the ER membrane (40, 41). This complex of three proteins phosphorylates and consequently activates JNK. JNK activity may lead to a variety of downstream effects depending on the cellular context, some of which include apoptosis, cell survival, inflammation, and insulin resistance. For the purpose of this discussion, we will focus on the actions of JNK in regulating metabolism, primarily through the processes of inflammation and insulin receptor signaling. Obesity leads to marked activation of JNK in metabolically active tissues such as liver, muscle, and adipose tissues (42). JNK activity is also detrimental for pancreatic islet function and survival (43). In the cytoplasm, JNK1 acts to inhibit insulin signaling through the phosphorylation of the insulin receptor substrate 1 (IRS-1) on serine 307 (44). JNK-mediated serine phosphorylation of IRS-1 inhibits insulin

receptor signaling through several mechanisms, including the loss of its ability to serve as a substrate, disruption of insulin receptor-IRS-1 interaction, and, when the stress is severe and prolonged, IRS-1 degradation. Indeed, mice with a genetic deficiency of JNK1 display marked protection from diet-induced obesity and insulin resistance (42). In the nucleus, JNK phosphorylates the transcription factor PPARγ, a major regulator of glucose and lipid homeostasis in the adipocyte and a major effector of insulin sensitivity in humans and mice (45, 46). This phosphorylation may inhibit PPARγ activity and negatively affect insulin sensitivity in the cell. However, further study is needed to elucidate this interaction and its functional consequences in vivo.

THE UPR AND THE INFLAMMATORY RESPONSE

ER stress and the UPR are linked to major inflammatory and stress-signaling networks via several distinct mechanisms, including the activation of JNK-AP-1 and IKB kinase nuclear factor κB (IKK-NFκB) pathways and the production of reactive oxygen species (ROS). Interestingly, these are also the pathways and mechanisms that play a central role in obesity-induced inflammation and metabolic abnormalities. For example, JNK activation by IRE-1α during ER stress is one key pathway to increased inflammation. In the nucleus, JNK upregulates the expression of inflammatory genes through activation of the AP-1 transcription factor complexes (47). Indeed, the beneficial metabolic effects observed in the JNK1-deficient mouse may be mediated, at least in part, through the suppression of inflammatory cytokines, as JNK-deficient animals display decreased levels of TNF-α, IL-6, and MCP-1 (among others) compared with wild-type mice on a high-fat diet (48). IRE-1α can also activate the IKK-NFκB pathway, which is critical in the induction of multiple inflammatory genes such as TNF-α and IL-6 and is also implicated in insulin resistance (7, 49, 50). The NFκB pathway may also be activated through PERK signaling during the UPR. PERK-mediated phosphorylation of eIF2α results in the inhibition of translation of the inhibitor of NFκB (IκB) protein, the major negative regulator of NFκB, thus allowing the activation of NFκB and the induction of its proinflammatory targets (50–52). In vitro, induction of the UPR in various cell types has been reported to cause increased expression of inflammatory genes, including IL-8, IL-6, MCP-1, and TNF-α (53, 54). UPR-mediated upregulation of acute phase response genes through an ER-resident transcription factor in liver has also been reported (55). Prolonged activation of the UPR may also generate oxidative stress, causing a toxic accumulation of ROS within the cell. This occurs as a result of the UPR-stimulated upregulation of chaperone proteins involved in disulfide bond formation in the ER lumen. The enzymes responsible for forming disulfide bonds (Ero1p and Erv2p) perform oxidation-reduction reactions that use molecular oxygen as the final electron recipient (56). This reduced molecular oxygen accumulates during UPR-increased protein folding and

acts as a cellular toxic ROS (57). The UPR has even evolved to anticipate this increase in ROS, as one of the three branches (PERK) activates an antioxidant program through the transcription factor Nrf2, keeping toxic species under control. In addition, it is well appreciated that toxic ROS levels may also elicit an inflammatory response, thus making yet another connection between UPR activation and inflammation.

The close link between ER stress and inflammation is important given the role of inflammation in obesity and insulin resistance (3). As described above, obese adipose tissue is characterized by a chronic, increased inflammatory response, and various inflammatory pathways have been implicated in the development of insulin resistance. Finally, adipocyte death may be a contributor to the inflammation in obese adipose tissue (58), and ER stress may play a role in this death via its ability to engage apoptotic pathways. Therefore, whether through direct activation of inflammatory pathways or indirectly through adipocyte death, the intriguing possibility remains that ER stress is a cause of obesity-induced inflammation. Studies are under way to address this possibility.

ER STRESS IN THE ADIPOCYTE

Given the evidence that the UPR is a source of stress signaling, inflammation, and JNK activation and the fact that these two events are strongly linked to the inhibition of insulin signaling, we hypothesized that one cause of insulin resistance in the obese state is the presence of ER stress within the expanded adipose tissue. Indeed, in adipose tissue of mice fed a high-fat diet for 16 weeks, indicators of ER stress such as PERK phosphorylation and JNK activity are significantly increased compared with mice fed a regular diet (11). In ob/ob mice, which become severely obese as a result of a mutation in the leptin gene, adipose tissue displays signs of ER stress, including increased levels of phosphorylated PERK and IRE-1α, compared with wild-type mice. These markers of UPR activation in ob/ob adipose tissue were also accompanied by an increase in JNK activity and XBP-1 splicing (11, 59). Genetically, ER stress may be caused by an insufficiency of the XBP-1 transcription factor (11). As homozygote XBP-1 null mice are not viable, XBP-1 heterozygous mice were investigated for the effects of ER stress on metabolic homeostasis (11). Remarkably, on a high-fat diet, XBP-1$^{+/-}$ mice developed hyperinsulinemia, hyperglycemia, and impaired glucose and insulin tolerance compared with wild-type controls. Body weight increased in XBP-1$^{+/-}$ mice compared with wild-type mice, and notably, the adipose tissue of XBP$^{+/-}$ mice displayed increased phosphorylation of PERK and IRE-1α and increased JNK activity, coupled with a loss of insulin sensitivity.

In complement, recent studies described the function of the ER chaperone protein oxygen-regulated protein 150 (ORP150) in mouse metabolic homeostasis (60, 61). ORP150 is induced by the UPR and plays a protective role during ER stress. Loss of ORP150 expression in either whole body or liver alone resulted in impaired glucose tolerance and decreased insulin-stimulated signaling through IRS-1. Conversely, overexpression of ORP150 in an obese or diabetic model yielded improved glucose tolerance and enhanced insulin signaling.

A third genetic model of ER stress reported by Scheuner et al. (62) utilized a mouse harboring a point mutant of eIF2α (eIF2s1+/tm1rjk, involving a serine→alanine substitution at serine 51) such that inhibitory phosphorylation of the protein could not occur. With the inability to halt protein synthesis, the UPR is proposed to be activated by the unfolded protein overload. Under high-fat diet conditions, heterozygote mutant mice become obese and develop a type 2 diabetic phenotype. Although pancreatic ER dysfunction may play the primary role in this phenotype, it is interesting that the mutant mice display a marked increase in body weight attributable to increased adiposity compared with wild-type mice (double the percentage of body fat) with no associated change in food intake. A similar body weight phenotype was also observed in XBP-1$^{+/-}$ mice. Intriguingly, it may be that already-stressed adipose tissue responds to ER stress by expanding itself even further.

In the study of adipocytes and ER stress in vitro, few data are available. One interesting observation comes from the story of Tribbles 3/SKIP 3 (Trb3) in adipocytes. Trb3 is a putative protein kinase that was shown to inhibit the downstream effects of insulin signaling in the liver through decreased Akt activity (63). Interestingly, the UPR-induced transcription factors ATF-4 and CHOP were shown to up-regulate Trb3 mRNA through binding to its promoter (64, 65). Buse and colleagues (66) then demonstrated that UPR activation by glucose deprivation or tunicamycin treatment in 3T3-L1 adipocytes induced CHOP and, subsequently, Trb3 mRNA. It may be the case that in adipocytes, a UPR-induced Trb3 could reduce Akt activation and subsequently affect adipocyte function, offering an additional pathway connecting UPR activation and insulin resistance. A second interesting lead may come from a study investigating the effects of human immunodeficiency virus protease inhibitors on 3T3-L1 adipocytes and HepG2 liver cells (67). Using microarray comparisons, the authors showed that the protease inhibitors induced ER stress genes in both cell types. Coupled with this, lipogenic genes were downregulated in the adipocyte but upregulated in the hepatocyte. This is a noteworthy observation given its similarity to the in vivo obese, type 2 diabetic state, in which lipogenesis is decreased in the adipose tissue but increased in the liver, with both tissues displaying ER stress (67). Along these lines, another study using human immunodeficiency virus proteasome inhibitors in 3T3-F442A adipocytes reported decreased nuclear translocation of SREBP1c upon exposure to these drugs (68). Given that SREBP1c increases lipogenic gene expression, this result may be indicative of dysfunction in the ER leading to decreased lipid synthesis.

To date, there are many unexplored areas of adipocyte function with regard to ER stress. Many important questions remain, including the mechanism of ER stress on

adipocyte insulin signaling and glucose uptake. Does ER stress cause an inflammatory response in the adipocyte? Does a compromised ER affect TG droplet formation or the lipogenic/lipolytic balance? Systemically, what effect does ER stress in the adipose tissue have on whole body homeostasis? The answers to these questions are vital pieces in understanding the role of the ER and how it relates to obesity.

CAUSES OF ER STRESS IN OBESITY AND IN THE ADIPOCYTE

The significant presence of ER stress in obese and insulin-resistant adipose tissue leads us to the important question of etiology: what are the origins of ER stress in obesity, particularly in adipose tissue? The decisive answer is unknown, but several possibilities exist. First, the UPR may be induced by the increased demand for protein synthesis under nutrient excess and expansion. Second, the excess nutrients themselves may serve as signals inducing ER stress. Serum FFA levels are increased in obesity, and studies have shown that FFAs can induce the UPR in hepatocytes, cardiomyoblasts, pancreatic β-cells, and macrophages (69–73; E. Erbay and G. S. Hotamisligil, unpublished observations). The effect of FFAs on ER function in the adipocyte remains to be investigated. However, it has been shown that FFAs can induce JNK activation and subsequent insulin resistance in 3T3-L1 adipocytes (74), and this may provide a possible link to ER stress if the UPR is responsible for activating JNK in this context. A third possibility for the cause of ER stress in obesity is glucose deprivation, a known condition of UPR activation in many cell types, including adipocytes (66). Glucose deprivation may occur in obese adipocytes as a result of cellular insulin resistance. Once insulin signaling has been inhibited, glucose uptake is reduced and the cell may be stressed by low-glucose conditions or abnormal fluxes. Second, the tissue environment may be glucose- or nutrient-deprived. Studies in the rat have shown decreased vasculature of adipose tissue in obese versus lean conditions (75). This "starvation" of the adipose in the face of plenty may be sufficient to induce stress. Support for this concept comes from recent work by Hosogai et al. (76), indicating that obese mouse adipose tissue shows signs of hypoxia compared with wild-type tissue. In cultured adipocytes, hypoxia induces the UPR, as shown by BiP and CHOP mRNA induction, eIF2α phosphorylation, and XBP-1 splicing.

ER stress may also be caused by the inflammatory state of obese adipose tissue. As described above, the UPR is able to induce inflammatory gene expression in a variety of cell types. Remarkably, the converse may also be true: that inflammation induces the UPR. Indeed, TNF-α was shown to activate the UPR in mouse fibrosarcoma cells (77). The mechanism of TNF-α UPR induction was shown to be dependent upon ROS. Therefore, oxidative stress is yet another possibility for the cause of obesity-related ER stress, as various studies have shown that ROS is able

to activate the UPR (77, 78). This is relevant given recent evidence of an increase in oxidative stress markers in the adipose tissue of obese/insulin-resistant mice and humans (79), and in cultured adipocytes ROS have been shown to cause insulin resistance (80). Many of these stress signals converge on common pathways and can regulate each other. It is likely that their coordinated regulation is a feature of obesity and may be the cause of feedback regulation and the perpetuation of inflammation.

THERAPEUTIC POTENTIAL FOR THE ADIPOCYTE ER

The cause-effect cycles in obesity with respect to stress and inflammation are complex. Vicious forward loops may occur because the causes of ER stress (i.e., ROS or inflammation) are also the consequences. Therefore, increased oxidative stress or inflammation will result in its own amplification, as it attacks various organelles and signaling pathways in the cell (**Fig. 3**). Given this complexity, one approach to therapy may be to focus on whole organelles as sources of multiple stressors, the relief of which may have multiple beneficial effects. Maintenance or enhancement of proper ER function may be one such method of organelle therapy. It has been demonstrated that ER folding is enhanced with the treatment of small molecules classified under the category of chemical chaperones (81). Although the mechanism(s) of action for chemical chaperones is unclear, they are classified as such for their ability to protect the cell from ER stress (82) and to facilitate protein folding and export. Two of these molecules, 4-phenyl butyric acid (PBA) and taurine-conjugated ursodeoxycholic acid (TUDCA), were recently tested in our laboratory in a mouse model of obesity (59). In obese and insulin-resistant ob/ob mice, treatment with either PBA or TUDCA dramatically improved glucose tolerance, decreased blood glucose and insulin levels, and increased systemic insulin sensitivity, without significantly affecting body weight. When liver and adipose tissues were investigated for UPR activation, tissues from chaperone-treated mice showed a marked decrease in UPR signaling compared with untreated mice, despite the presence of severe obesity. This was demonstrated by a significant reduction in the phosphorylation of PERK and IRE-1α and decreased JNK activity. Coupled with the decrease in UPR signaling was an increase in insulin sensitivity in the treated tissues, as indicated by increased tyrosine phosphorylation of IRβ, IRS-2, and insulin-responsive Akt activation. In view of this outcome, chemical chaperones may provide a highly promising avenue of therapy, targeting an organelle for the alleviation of stress and the enhancement of function.

Further support for the beneficial effects of enriching ER function comes from the study of the ORP150 transgenic mouse (61). Diabetic mice overexpressing the ER chaperone ORP150 displayed improved glucose tolerance compared with nontransgenic diabetic mice. Insulin signaling in liver and muscle was also enhanced in the trans-

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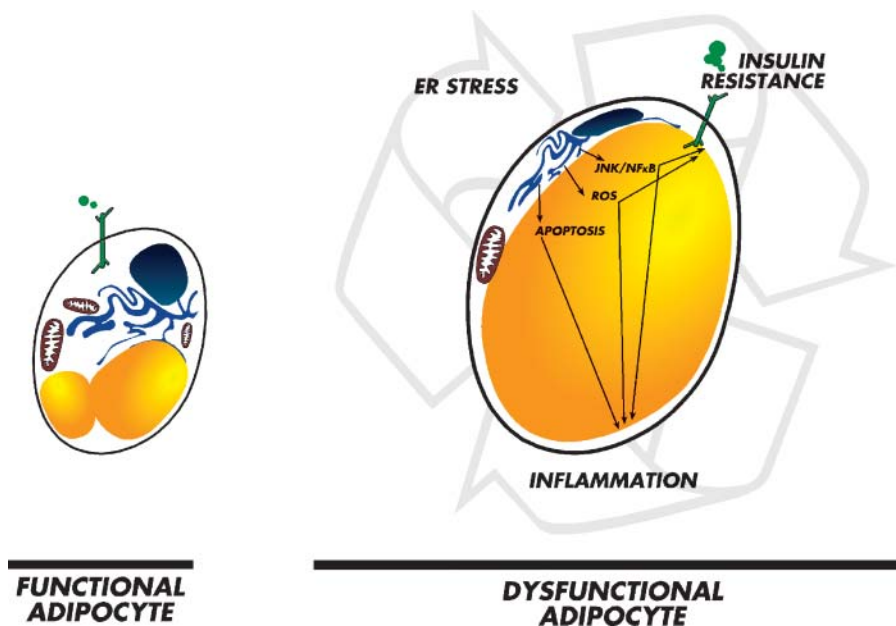
Fig. 3. Adipocyte stress in the obese and insulin-resistant state. In the transformation of a healthy functional adipocyte to a hypertrophic, dysfunctional adipocyte, many alterations take place. The adipocyte increases in size, coinciding with an increase in lipid storage in the TG droplet. Mitochondrial function is lost, and multiple stress-signaling cascades are initiated from the ER. These include the JNK and NFκB pathways, reactive oxygen species (ROS) generation, and apoptotic signaling. The ultimate consequences of these pathways are increased inflammation, increased ER stress, and the inhibition of insulin signaling pathways, which may each independently exacerbate the other, culminating in severe adipocyte dysfunction. Artistic design by Deniz Hotamisligil.

genic mouse. An alternative way to reduce ER stress may be through direct targeting of UPR-involved molecules. For example, the small molecule drug Salubrinal was recently shown to inhibit the dephosphorylation of eIF2α, leading to a sustained repression of protein synthesis and rescue from ER stress (83). Indeed, Salubrinal protected cells from ER stress-induced cell death via agents such as tunicamycin and brefeldin A. In one model of neuronal toxicity (caused by increased glutamate receptor activation), Salubrinal treatment protected against ER stress and cell death in vitro and, importantly, in vivo in the rat (84). However, given that the effects of Salubrinal may vary according to tissue (85), testing in animal models of ER stress diseases remains an important future step. Intriguingly, two chemicals in use or in clinical trials for the treatment of type 2 diabetes have also been shown to have effects on eIF2α. Thiazolidinediones, known agonists of PPARγ, were shown to phosphorylate eIF2α and inhibit protein synthesis independent of PPARγ (86). Second, the anti-inflammatory salicylates also lead to increased eIF2α phosphorylation via PERK activation (87). It is exciting to speculate that the beneficial effects of these therapies may be related to their ability to protect/maintain ER function through UPR effectors.

Although the preliminary results from ER therapy are promising, many questions remain to be answered. What are the effects of ER stress relief on other negative regulators of insulin signaling such as oxidative stress and inflam-mation? Will targeting one source alleviate many causal agents? Through what organs and by what mechanism do the chaperones exert their beneficial effects? Specifically, is ER recovery in adipose tissue critical to the restoration of insulin sensitivity and systemic metabolic homeostasis? Efforts to address these questions are ongoing and should yield important insights regarding both mechanisms and potential therapeutic applications.

To conclude, the adipocyte, and thus adipose tissue, has been and will continue to be central to the study of obesity and its related pathologies. It is imperative to understand the alterations that occur from the life of a healthy adipocyte to a hypertrophic insulin-resistant adipocyte (Fig. 3) to perhaps even a dead adipocyte. A deeper knowledge of adipose tissue expansion and the dysfunction that follows will be critical to our thinking and to therapy in the coming years.

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