Endoplasmic Reticulum Stress and Inflammation in Obesity and Diabetes

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Abstract: Obesity is a major problem worldwide that increases risk for a wide range of diseases, including diabetes and heart disease. As such, it is increasingly important to understand how excess adiposity can perturb normal metabolic functions. It is now clear that this disruption involves not only pathways controlling lipid and glucose homeostasis but also integration of metabolic and immune response pathways. Under conditions of nutritional excess, this integration can result in a metabolically driven, low-grade, chronic inflammatory state, referred to as “metaflammation,” that targets metabolically critical organs and tissues to adversely affect systemic homeostasis. Endoplasmic reticulum dysfunction is another important feature of chronic metabolic disease that is also linked to both metabolic and immune regulation. A thorough understanding of how these pathways intersect to maintain metabolic homeostasis, as well as how this integration is altered under conditions of nutrient excess, is important to fully understand, and subsequently treat, chronic metabolic diseases. (Circ Res. 2010;107:579-591.)

Key Words: obesity ■ ER stress ■ inflammation

Current lifestyle trends in modern society, characterized by caloric abundance, reduced physical activity, and increased lifespan, have propelled the incidence of obesity to epidemic proportions, including its recent emergence in previously unaffected populations such as developing countries and children. This alarming increase in worldwide incidence of obesity is also associated with an array of metabolic pathologies, including type 2 diabetes and heart disease, spurring intense research efforts to understand the mechanisms underlying these disorders. Although the results of such efforts have led to the development of new treatment options, these conditions remain among the leading causes of global mortality and morbidity, emphasizing the need for more effective therapeutic and preventive measures. Existing trends indicate that the scope of the problem is only likely to grow, especially in rapidly developing parts of the world. Many contributing agents have now been identified, including genetic, dietary, and environmental factors. However, the mechanisms by which excess nutrients and adiposity can ultimately result in one or more of a large cluster of chronic diseases are still being elucidated. In this review, we highlight research detailing how such a shift in energy balance alters systemic metabolic regulation and the important role that chronic inflammation, endoplasmic reticulum (ER) dysfunc-
A primary event in response to nutritional abundance is sequestration of excess fuel in adipocytes. 5 Despite this built-in storage function, excessive accumulation of adipose tissue is a major risk factor for metabolic disease. If mechanisms are in place to convert surplus energy into fat, then why does excess buildup often ultimately lead to disease? A potential answer lies in the limitations of even this organ to deal with continual demand on its lipid storage and processing functions. Beyond a critical threshold, the adipocyte begins exhibiting signs of stress, including hypertrophy and associated mechanical stress, compositional changes of lipids and other nutrients, hypoxia, disruption of mitochondrial function, production of reactive oxygen species, apoptotic signaling, increased fatty acid (FA) release, altered adipokine signaling, inflammation, and ER stress. 6–8 Many of these processes are interconnected, influence the function of each other, and have now been linked to metabolic dysfunction and disease. For example, excess circulating free FAs are taken up and deposited in other tissues not equipped to deal with them. The resulting “lipotoxicity” 9 has been shown to influence glucose utilization and insulin action in liver and muscle tissues. 10,11 Lipid signaling has also recently been linked to increased inflammatory and stress signaling. 7 Likewise, in conditions of obesity, alterations in circulatory levels of adipokines and other adipocyte-derived signals have been linked to disruption of an array of pathways important for metabolic homeostasis. 12–14 This includes control of energy intake and expenditure by the central nervous system, insulin and glucose signaling in peripheral tissues, and systemic lipid metabolism. 12–14 Finally, aberrant immune signaling and ER dysfunction have been implicated as important and integrating factors influencing metabolic regulation and contributing to the emergence of metabolic disease. 7

The link among metabolism, inflammation, and ER stress is not surprising when one considers how the function of each intersects with the others. All 3 processes are tightly regulated adaptive responses that are fundamental to survival and dependent on the availability of sufficient energy for proper function. It is therefore logical that these pathways would be integrated to successfully manage the distribution of available resources to enhance overall organismal well-being under diverse environmental conditions. For example, during the course of infections the ability of the immune system to divert energy for itself in response to pathogens is essential for resolution of the threat. Thus, both the metabolic needs of an organism and the energy requirement of immune cells must be properly coordinated. Likewise, processing of proteins destined for membranes or secretion occurs in the ER and is closely coupled to energy availability and cellular energy needs. Moreover, both metabolic processes and immune response pathways must closely intersect with pathways mediating protein folding and quality control to ensure generation and proper processing of numerous secreted proteins that are required for their function. As a result, it is logical to have developed extensive integration and communication between these essential energy-requiring processes.

During the development of energy using systems in higher mammals, nutrient scarcity was more prevalent than surplus. Unfortunately, present lifestyle trends have occurred too rapidly for evolutionary mechanisms to adjust to them. As a result, an imbalance exists between how our body deals with energy-providing substrates and pathogens, the evolution of our adaptive responses, and our existing metabolic and immunologic needs. Although short-term nutritional surplus itself is not detrimental (or appropriate adaptive mechanisms are in place to mitigate acute stresses 15,16), mechanisms to manage caloric surplus are not equipped to handle chronic exposure to continual nutritional excess. In the presence of long-term abundance of energy-providing substrates, it is now clear that the pathways regulating excess fuel disposal or storage are disrupted, resulting in deregulation of glucose and lipid homeostasis, as well as other cellular signaling pathways. 5 This review focuses on existing knowledge of how excess nutrients modulate immune signaling, ER stress, and the UPR, and how these in turn influence metabolic homeostasis in the context of obesity. A detailed understanding of how these pathways intersect, and how this balance is disrupted by exposure to constant nutritional surplus, will enhance our understanding of why metabolic diseases de-

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The discovery of increased expression of the proinflammatory cytokine tumor necrosis factor (TNFα) in adipose tissue of obese mice almost 2 decades ago was the first indication that inflammatory mediators are associated with obesity. In the ensuing years, it has become well accepted that changes in inflammatory signaling by adipocytes and infiltration of adipose tissue by immune cells are key features of obesity-induced insulin resistance and associated metabolic disease in animal models and humans (Figure 1). In obese mice, both adipocytes and macrophages (and potentially other cell types) residing in adipose tissue secrete a number of cytokines including TNFα, interleukin (IL)-6, IL-1β, and migration inhibitory factor. Increased expression of inflammatory mediators has also been observed in visceral fat of obese humans. These cytokines have been shown to disrupt insulin signaling through several mechanisms, including induction of the suppressors of cytokine signaling (SOCS) family of proteins, which have been shown to inhibit insulin receptor kinase activity, interfere with binding of insulin receptor substrate (IRS)1 and IRS2 to the insulin receptor, and promote IRS degradation. Cytokines also activate inflammatory signaling via c-Jun N-terminal kinase (JNK) and inhibitor of kappa β kinase (IKKβ) pathways in both immune and neighboring nonimmune cells. This results in increased inflammation and direct inhibition of insulin action, as well as possible alterations in other metabolic targets, that in combination contribute to overall metabolic deterioration. Numerous genetic and chemical studies in mice have verified the negative impact of inflammatory pathway activation on systemic carbohydrate metabolism. For example, deletion of either JNK or IKKβ or neutralization of TNFα or IL-1β leads to decreased inflammatory signaling and improved insulin responsiveness and glucose tolerance in mouse models of obesity. Treatment with an IL-1 receptor antagonist has also been shown to improve insulin sensitivity in humans. Tissue-specific deletion studies have shown that both immune and neighboring nonimmune cells are important in the observed improvements in glucose homeostasis. Of note, manipulation of levels of these inflammatory mediators can impact insulin resistance and other metabolic parameters regardless of whether obesity or lipid accumulation in liver or muscle occur. This indicates that alterations in immune signaling triggered by excess adiposity are essential mediators of the metabolic dysfunction observed in obesity.

Although early studies focused on inflammatory signaling in adipose tissue, it has become increasingly evident that other metabolically important tissues are also involved in the intersection of immune and metabolic responses and contribute significantly to disease states. For example, macrophage-like Kupffer cells, which reside in the liver in close communication with hepatocytes, are involved in hepatic inflammation, liver lipid metabolism, and impaired insulin signaling in the liver. In addition, islet inflammation in pancreatic β cells has been proposed to contribute to impaired insulin secretion and reduced β-cell mass in diabetes. Macrophage infiltration has also been observed in skeletal muscle, suggesting that local inflammatory signaling could directly influence muscle insulin resistance, although further studies are needed to verify such an effect. It is important to note that in many experimental systems, muscle effects appear to emerge secondary to alterations in other organs, including adipose tissue and liver. However, muscle-specific expression of the antiinflammatory cytokine IL-10 in mice was shown to improve muscle insulin sensitivity and reduce inflammation in this tissue despite normal development of obesity when fed a high-fat diet (HFD). Finally, increased inflammatory signaling in the brain has been observed in response to overnutrition, or in the context of obesity, resulting in improper regulation of energy uptake and energy expenditure by peripheral tissues. Thus, it appears that increased inflammation is a systemic feature associated with energy surplus. Therapeutics aimed at reducing inflammatory signaling induced by metabolic stress would therefore be expected to improve systemic energy homeostasis at multiple levels.

**Inflammation and Metabolism**

**Inflammatory Signaling in Obesity**

The discovery of increased expression of the proinflammatory cytokine tumor necrosis factor (TNFα) in adipose tissue of obese mice almost 2 decades ago was the first indication that inflammatory mediators are associated with obesity. In the ensuing years, it has become well accepted that changes in inflammatory signaling by adipocytes and infiltration of adipose tissue by immune cells are key features of obesity-induced insulin resistance and associated metabolic disease in animal models and humans (Figure 1). In obese mice, both adipocytes and macrophages (and potentially other cell types) residing in adipose tissue secrete a number of cytokines including TNFα, interleukin (IL)-6, IL-1β, and migration inhibitory factor. Increased expression of inflammatory mediators has also been observed in visceral fat of obese humans. These cytokines have been shown to disrupt insulin signaling through several mechanisms, including induction of the suppressors of cytokine signaling (SOCS) family of proteins, which have been shown to inhibit insulin receptor kinase activity, interfere with binding of insulin receptor substrate (IRS)1 and IRS2 to the insulin receptor, and promote IRS degradation. Cytokines also activate inflammatory signaling via c-Jun N-terminal kinase (JNK) and inhibitor of kappa β kinase (IKKβ) pathways in both immune and neighboring nonimmune cells. This results in increased inflammation and direct inhibition of insulin action, as well as possible alterations in other metabolic targets, that in combination contribute to overall metabolic deterioration. Numerous genetic and chemical studies in mice have verified the negative impact of inflammatory pathway activation on systemic carbohydrate metabolism. For example, deletion of either JNK or IKKβ or neutralization of TNFα or IL-1β leads to decreased inflammatory signaling and improved insulin responsiveness and glucose tolerance in mouse models of obesity. Treatment with an IL-1 receptor antagonist has also been shown to improve insulin sensitivity in humans. Tissue-specific deletion studies have shown that both immune and neighboring nonimmune cells are important in the observed improvements in glucose homeostasis. Of note, manipulation of levels of these inflammatory mediators can impact insulin resistance and other metabolic parameters regardless of whether obesity or lipid accumulation in liver or muscle occur. This indicates that alterations in immune signaling triggered by excess adiposity are essential mediators of the metabolic dysfunction observed in obesity.

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to metabolic changes elicited by this diet, recruits macrophages to obese adipose tissue, or a development that occurs later in obesity once inflammation has already been established. However, several recent reports support a contributory role for other immune cells in metabolic dysregulation regardless of when they are recruited into metabolic tissues. Genetic depletion of either mast cells or natural killer T cells resulted in reduced adipose tissue inflammation and improved insulin responsiveness and glucose homeostasis in mice. Mast cell deficiency also reduced diet-induced weight gain, whereas depletion of natural killer T cells did not alter body weight, indicating that obesity itself may not be a sole contributing factor to the observed effects. Whether other immune mediators act locally in other tissues and to what extent these effectors contribute to systemic metabolism remains to be established.

Despite the correlation between increased adipose tissue inflammation and metabolic dysfunction, the presence of immune cells in adipose tissue is not uniformly detrimental. Macrophages may play a role in the extensive tissue remodeling that occurs during adipose tissue growth. Likewise, mast cells have been suggested to contribute to angiogenesis and vascularization involved in tissue expansion. Recent work has highlighted the importance of the specific activation state of immune cells in determining functional consequences. The classically activated M1 state is associated with proinflammatory signaling and antimicrobial activity, whereas alternatively activated M2 macrophages display antiparasitic activity but also play a role in wound healing, tissue repair, and resolution of acute inflammation via antiinflammatory signaling. It has recently been proposed that M2/M1 switching underlies the negative metabolic impact of macrophages recruited to adipose tissue (Figure 1). In lean mice, adipocyte- or hepatocyte-derived IL-4 and IL-13 signaling has been shown to induce the M2 activation state, suggesting this may be one level at which metabolic cells can influence immune cell function and activity. Whether adipocyte- and hepatocyte-mediated expression of these cytokines is disrupted in obesity remains to be seen. Like macrophages, T cells can exist in 2 alternative populations, T-helper cell type 1 (Th1) and type 2 (Th2), which produce distinct subsets of cytokines with different inflammatory potentials. The proinflammatory Th1 subset has been suggested to be involved in macrophage recruitment and activation of the M1 phenotype. Moreover, Th1 polarization has been implicated in obesity-induced insulin resistance. Conversely, Th2 T-cell populations, like adipocytes, secrete IL-4 and IL-13 and would thus be expected to favor an M2 profile in macrophages. Finally, a distinct population of lymphocytes, regulatory T cells (T-regs), regulate antiinflammatory signaling and have been reported to be protective against insulin resistance. Specifically, adipose tissue of lean, but not obese, mice was enriched in T-regs. Moreover, experimental increase or decrease in T-reg populations influenced inflammatory signaling in adipose tissue and consequently insulin resistance. Overall, these results suggest that the relative abundance of pro-versus antiinflammatory immune cells is critical in regulating immune signaling and determining metabolic outcomes in adipose tissue, liver, and possibly other metabolically important organs. Thus, the possibility of influencing the activation state of immune cells pharmacologically, or regulating metabolic signals that influence these states, may serve as potential sites for therapeutic intervention.

It is important to note that the altered inflammatory signaling observed in obesity has unique characteristics. In response to pathogens or acute injury, inflammation is robust but resolves quickly once the threat is removed. Conversely, obesity-associated inflammation is characterized by a low-level but chronic inflammatory state. In fact, some normal immune functions have been shown to be compromised in obesity, implying that the increased inflammation associated with excess adiposity is a result of disruption of immune signaling in a specific context, not a general overactive response. It is remarkable that this form of chronic inflammation is invariably associated with tissues predominantly involved in metabolic homeostasis. Hence, the possibility should be considered that this is a novel form of inflammation triggered by metabolic cues, such as nutrients themselves and metabolic changes triggered by caloric surplus, which could be referred to as “metaflammation.” A critical unanswered question is why chronic caloric surplus engages inflammatory pathways in this manner. Likely, such signaling developed as a protective response. For example, if pathogens are encountered with a meal, activation of immune pathways by nutrients could serve as a signal that sufficient energy is present to meet the metabolic demands of the organism and thus help to redirect available resources toward fighting infection. Alternatively, immune cells could be involved in scavenging and disposing of lipids, lipid metabolites, and other nutrients that might otherwise be harmful to the organism when present in excess. Regardless, these potentially beneficial aspects of immune signaling appear not to be designed to handle the constant and extreme nutrient surplus we encounter today. The work described above suggests that in the long-term, these processes become maladaptive and ultimately result in the emergence of metaflammation and disruption of metabolic function. A major remaining challenge is to understand both the initiating signals and downstream mechanisms involved in the establishment of this type of inflammation. In subsequent sections, we discuss existing knowledge on how metabolic cues, such as lipids and adipokines, modulate this obesity-associated inflammation.

**Lipid-Mediated Effects on Metabolism and Inflammation**

Increased exposure to FAs, whether because of the increased fat content of modern diets or aberrant lipolysis in adipocytes, has been proposed as one of the key activators of both altered metabolic and immune signaling in obesity (Figure 1). Although some advances have been made in understanding how specific substrates engage various signaling pathways (discussed below), in general, there is a large gap in our understanding of these processes. In one example of nutrient-mediated signaling, free FAs and intracellular lipids have been shown to elevate diacylglycerol levels and induce the classic and novel protein kinase (PK)C isoforms. Furthermore, the novel isoforms, PKCδ, PKCe, and PKCθ, have all been implicated in development of insulin resistance.
Of note, it was recently reported that the saturated FA palmitate, but not monounsaturated oleic acid, was involved in PKC\(\theta\) action in the hypothalamus.\(^{53}\) PKC signaling has also been shown to play a role in various aspects of immune function.\(^{56-59}\) For example, PKC\(\theta\) is involved in T-cell development and function. Although it is clear that lipid signals are involved in the emergence of inflammatory responses, a role for FA-mediated induction of PKCs in metatflammation has not been carefully examined.\(^{67,69}\) Indeed, the mechanisms by which FAs contribute to the emergence of inflammatory and stress responses remain an important avenue for future research.

One mechanism by which FAs have been proposed to directly influence immune cell signaling is through recognition by pathogen-sensing molecules.\(^{60,61}\) This includes signaling through Toll-like receptors (TLRs) and subsequent JNK activation and engagement of inflammatory signaling cascades.\(^{60,61}\) However, it remains unclear whether FAs are directly recognized by TLRs or whether other factors are involved. In recent studies, we have identified PKR as another pathogen-sensing molecule capable of responding to lipids as well as ER stress signals (see below).\(^{62}\) The ligand binding domain of PKR is required for its ability to sense and respond to nutrients and stress,\(^{62}\) supporting the concept that certain metabolic cues may directly engage components of pathogen-sensing systems in the cell. PKR is also capable of interacting with other stress signaling molecules, such as JNK and IKK, and metabolic regulators, such as IRS and eukaryotic translation initiation factor (eIF2)\(\alpha\). This interaction could be envisioned to lead to formation of a protein complex, or “mefatflammosome,” where major metabolic and immune roads cross. If such a hypothesis is accurate, identification of the components and regulatory status of such a node (or nodes) could provide valuable insights into how chronic inflammation and metabolic pathways intersect and how this crosstalk is disrupted in metabolic diseases.

Of note, not all FA-mediated signaling is detrimental in the context of metabolism or inflammation. For example, FA-mediated signaling through the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors has been shown to have beneficial effects on metabolic parameters.\(^{63}\) However, the exact nature of the FA(s) or FA metabolite(s) that act as physiological ligands for PPAR has not been definitively determined. Moreover, liver X receptor (LXR)\(\alpha\)-mediated de novo lipogenesis in macrophages was recently shown to be protective against atherosclerosis and lipid-mediated ER stress (discussed below).\(^{64}\) This was at least partly attributed to increased production of the monounsaturated FA palmitoleate, which has been reported to exert beneficial effects on systemic metabolic regulation. Both the PPAR and LXR families of nuclear receptors have been shown to negatively impact inflammatory signaling in addition to their roles in metabolic regulation.\(^{63,65}\) PPAR\(\gamma\) and PPAR\(\delta\) activity also favors development of the alternatively activated M2 state in macrophages instead of the classic proinflammatory M1 state.\(^{46,66}\) Conversely, inflammatory signals can inhibit signaling by these receptors, resulting in disruption of metabolic processes.\(^{63,65}\) Thus, lipids and lipid metabolites can either enhance or disrupt metabolic and immune signaling depending on their composition or the specific signaling pathway engaged. The exact timing of how these signals intersect is unclear. However, in the context of obesity a possible scenario is that inflammatory signaling through TLRs and PKR predominate over antiinflammatory effects of nuclear receptor mediated lipid signaling. As a result both nuclear receptor–mediated lipid metabolism and antiinflammatory signaling are disrupted, leading to metabolic dysfunction through multiple mechanisms.

Thus far, we have discussed how nutrient surplus relates to obesity and metabolic disease, with little consideration for the nature of the energy-providing substrates. However, not just overall caloric intake, but the composition of lipids and other metabolic substrates in the diet are also likely to be critical in obesity and metabolic disease. Specifically, diets high in saturated fats, or sucrose and fructose, have been implicated in the development of obesity and insulin resistance.\(^{67}\) Indeed, FA composition plays an important role on downstream metabolic outcomes. For example, aberrant lipolysis and release of free FAs from adipose tissue has been linked to muscle insulin resistance and liver steatosis, whereas increased de novo lipogenesis, and specifically generation of palmitoleate, was recently shown to improve muscle insulin sensitivity and suppress hepatosteatosis.\(^{12}\) Moreover, the nature of inflammatory responses depends on the specific FA involved, with saturated FAs generating a proinflammatory response but polyunsaturated FAs showing little or no inflammatory effect.\(^{60}\) In contrast, a number of metabolites of FAs, as well as \(\omega-3\) and \(\omega-7\) FAs, have been reported to potentiate antiinflammatory signaling and resolution of inflammation.\(^{68}\) The mechanism by which different species of lipids mediate distinct metabolic and inflammatory outcomes remains a critical area for future research.

Another proposed contributing factor to the detrimental outcomes associated with certain nutrients is the efficiency of a particular substrate as an energy source. Support for this hypothesis is highlighted by recent work implicating the gut microbial environment in the development of obesity and disruption of energy balance (reviewed\(^{69}\)). Microorganisms residing in the gut provide protection from pathogenic bacteria and aid in the digestion of food. In both mice and humans, changes in diet have been shown to alter the composition of this “beneficial” bacteria.\(^{69}\) Moreover, HFD specifically alters the ratio of \textit{Bacteroidetes} versus \textit{Firmicutes}, which has been suggested to improve energy extraction from the diet, thus contributing to obesity. Of note, genetic deletion of TLR5, a TLR family member that is highly expressed in the intestine, was recently shown to result in development of features of metabolic syndrome.\(^{31}\) These results were attributed to a role for TLR5 signaling on the composition of gut microbiota. Moreover, an altered microbiotic environment has been shown to increase levels of circulating bacterial extracts such as lipopolysaccharides, potentially impacting immune regulation as well.\(^{69}\) The intersections between the gut microbiota and host is emerging as a fascinating area of research in the metabolic disease field and may carry exciting potential for therapeutic interventions.
Adipokines and Inflammation

The signaling function of adipocytes is another important aspect of adipose tissue function that is disrupted in obesity with potential impact on immune signaling (Figure 1). This includes increased secretion of traditional inflammatory mediators such as TNFα that can influence immune cell activation state or target cell function. However, adipokines, with roles in metabolic signaling, can also modulate immune function (Figure 1). For example, adiponectin, which acts to enhance systemic insulin sensitivity, also exhibits antiinflammatory properties. Conversely, resistin interferes with insulin action and glucose homeostasis and also promotes inflammatory signaling. The number of identified adipokines secreted by adipocytes has continued to grow over the years, and these more recently discovered adipokines also appear to modulate both metabolic and immune pathways. For example, chemerin and vaspin are emerging as potential regulators of insulin sensitivity and adiponectin expression that also decrease inflammatory signaling, whereas Rbp4 (retinol-binding protein 4) and omentin have not yet been determined to have effects on immune signaling but have been implicated in metabolic regulation. Clearly, the diverse range of proteins secreted by adipocytes act in coordination to propagate specific signaling patterns that modulate inflammation, energy homeostasis, and metabolism of glucose and lipids. Thus, a shift in the profile of adipokines and cytokines released by adipocytes during obesity has the capability of directly influencing both metabolic and immune signaling systemically.

The adipokine leptin has an interesting profile with regard to integration of metabolism and inflammation. It is generally considered to exert beneficial effects in the context of metabolic disease because of its control of appetite suppression and energy expenditure through the central nervous system, as well as regulation of lipid and glucose metabolism in peripheral tissues. However, leptin has also been shown to enhance proinflammatory responses and play a role in modulation of T cell–mediated immune responses. Specifically, leptin has been reported to influence Th1 versus Th2 activation of T cells and reduce T-reg subpopulations, resulting in establishment of a proinflammatory population of T cells. As a central sensor of energy status, it is possible that the dual role of leptin in reducing metabolic energy overload and enhancing immune signaling is a function of sensing fuel status and energy availability for immune regulation and other important biological processes. Such a profile suggests that leptin may act to channel energy for the service of the immune response when needed. Indeed, leptin is also implicated in regulation of other energy-requiring pathways, including growth and reproduction.

ER Stress and Metabolism

The Unfolded Protein Response: An Overview

As described in the previous section, nutritional excess and related metabolic cues can trigger both metabolic and inflammatory changes that lead to metabolic dysfunction. The ER is also highly responsive to cellular nutrient and energy status. Recent studies have suggested that ER-mediated stress signaling and adaptation pathways (referred to as the unfolded protein response [UPR]) are also activated under obese conditions and can mediate both metabolic and immune responses (Figure 2). The UPR consists of 3 main branches controlled by the ER membrane proteins inositol-requiring enzyme (IRE)-1, protein kinase-like ER kinase (PERK), and activating transcription factor (ATF)α. In response to changes in the protein-folding status within the ER, these proteins activate distinct and sometimes overlapping pathways. The UPR has also been shown to induce autophagy, which is important in organelle maintenance and recycling and likely aids in the degradation of misfolded proteins. Activation of these adaptive responses serves to induce signaling and transcriptional events that reestablish ER homeostasis, or induce apoptosis if the stress cannot be resolved. In this manner, the UPR maintains protein integrity during fluctuations in various environmental and cellular conditions.

A thorough description of the UPR has been provided in several recent reviews and is, therefore, only briefly summarized here to aid in illustrating the link between ER stress, inflammatory signaling, and metabolism. The PERK arm of the UPR mediates inhibition of protein translation via phosphorylation of the α subunit of eIF2α. This results in...
decreased protein production in an attempt to reduce the protein-folding load in the ER but has also been linked to activation of nuclear factor (NF)-κB signaling.81,82 Specifically, the global decrease in protein translation results in reduced levels of IκB and subsequent removal of inhibition of NF-κB activity. Signaling through the PERK arm of the UPR is also responsible for activation of a subset of translational targets. This includes increased expression of ATF4 and subsequent induction of genes involved in antioxidant response and amino acid transport.83,84 Finally, PERK activation results in expression of the proapoptotic transcription factor CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) and induction of Gadd34 (growth arrest and DNA damage-inducible protein 34), which results in negative feedback of eIF2α phosphorylation and attenuation of PERK signaling.83,84

Activation of IRE-1 results in splicing of X-box binding protein–1 (XBP-1) mRNA and translation of the spliced form, which then translocates to the nucleus and regulates expression of ER chaperones and proteins involved in ER-associated degradation (ERAD).85 IRE-1 activity can also induce apoptosis and inflammatory signaling by forming complexes with other proteins. Specifically, the cytosolic domain of IRE-1α can associate with TRAF2 (TNF receptor–associated factor 2) to activate the JNK pathway.86,87 and this function of IRE-1α can be induced independent of XBP-1 splicing.88 Moreover, IRE-1 has been suggested to activate the p38, extracellular signal–regulated kinase (ERK), and NF-κB pathways through interaction with the adaptor protein Nck (noncatalytic region of tyrosine kinase) and a protein complex composed of IKK and TRAF2.89 The proapoptotic Bcl-2–associated proteins Bax and Bak can also bind to IRE-1α to prolong signaling through this arm of the UPR and promote apoptosis. In addition to the ubiquitously expressed IRE-1α, a second IRE-1 isoform (IRE-1β) also exists with selective expression in intestinal epithelial cells, suggesting a specialized role for IRE-1 signaling in the intestine, an organ equipped with one of the largest secretory repertoires.90

The third canonical branch of the UPR is composed of the ATF6 family of transcription factors, ATF6α and ATF6β. However, ATF6α is a much stronger transcriptional regulator in response to ER stress and has been characterized more extensively than ATF6β.91 ATF6 proteins translocate to the Golgi in response to ER stress, where they are cleaved into an active amino-terminal form.92 This N-terminal ATF6 then proceeds to the nucleus, where it regulates ER chaperone expression.93,94 In addition, ATF6α can induce expression of XBP-1 and has recently been shown to also enhance induction of ERAD proteins by forming a heterodimer with spliced XBP-1.91 Like IRE-1 and PERK, ATF6α can also engage inflammatory pathways through regulation of NF-κB activity.95

Finally, additional levels of control have been described for all 3 arms of the UPR (Figure 2). Specialized signaling through the ATF6 arm of the UPR involves a family of ATF6-like transmembrane bZIP transcription factors that exhibit differences in activating stimuli and tissue distribution, indicating unique roles for each of these factors in regulating stress responses and other cellular processes. Fine-tuning of IRE-1 signaling involves the interaction of a number of proteins that modulate the nature and duration of signaling by this arm of the UPR.89 In addition, the ribonuclease activity of IRE-1 was recently shown to cleave other double-stranded mRNAs besides XBP-1. This function is proposed to assist in degradation of mRNAs but could potentially result in the generation of short-lived signaling molecules, although such a function for RIDD (regulated IRE-dependent decay) and the resulting molecules has not been verified.96,97 Finally, the PERK arm of the UPR can be differentially activated depending on the stress encountered. This is mediated by a family of eIF2α kinases, including PERK, PKR, GCN2, and HRI (heme-regulated eIF2α kinase), that all induce eIF2α phosphorylation and subsequent reduction in global protein translation.98 However, each kinase is activated by different stressors and can differentially modulate signaling downstream of eIF2α.98 The existence of a number of proteins that modulate ER stress signaling with different tissue distribution and activation profiles suggests the evolution of a more fine-tuned ER stress response in mammals that allows for distinct responses depending on the cell or tissue involved, the specific stressor encountered, and the duration of the stress. This added complexity results in a more specialized response with possible implications for ER stress function in obesity and is a promising area of research with potential for additional translational possibilities.

ER Stress and Metabolic Disease

Studies in our laboratory have demonstrated that improper functioning of the UPR plays an important role in chronic metabolic diseases, including obesity, insulin resistance, and diabetes.87 Specifically, obesity results in increased ER stress, particularly in the liver and adipose tissue of mice.7 Mice with XBP-1 haploinsufficiency develop insulin resistance when placed on a HFD, despite being on a genetic background normally resistant to diet-induced obesity and diabetes. This was attributable, at least in part, to ER stress–mediated induction of JNK activity and inhibition of insulin action.87 Functional studies in other laboratories, using genetic manipulation of ER stress mediators in mice, have confirmed an important role for this pathway in regulation of systemic glucose and lipid homeostasis. Mice heterozygous for an nonphosphorylatable eIF2α allele develop normally but become obese and insulin resistant in response to HFD. These mice also exhibit defects in insulin secretion.99 In addition, ER stress has been shown to be increased in the hypothalamus of obese mice, leading to disruption of leptin signaling.36,100 Aberrant UPR signaling has also been implicated in human disease.101–103 Increased expression of UPR mediators and ER chaperones was significantly correlated with patient body mass index and modestly correlated with insulin sensitivity in adipose tissue of lean compared to obese individuals.103 Moreover, gastric bypass surgery–induced weight loss was shown to both improve insulin sensitivity and reduce ER stress in obese humans.102

Evidence also suggests that ER stress is important for β-cell development and survival. It has been reported that PERK+/− mice develop diabetes within the first few days of life because of defects in β-cell development, resulting in an inability to maintain ER integrity when stressed with the
postnatal requirement to secrete insulin. Likewise, mice homozygous for a nonphosphorylatable eIF2α undergo severe β-cell deficiency, resulting in perinatal death. Similar to observations in mice, PERK mutations in humans result in early-onset and severe diabetes caused by β-cell loss. These models of ER stress–related disease are likely more relevant to certain forms of juvenile diabetes, in which β-cell loss is an initiating event in disease manifestation. In the context of obesity and type 2 diabetes, decreased β-cell mass is likely an event preceded by insulin resistance and metabolic dysfunction. However, peripheral insulin resistance results in a tremendous strain on β cells caused by increased demand for insulin production. Thus, increased stress on the UPR during prolonged nutritional excess and insulin resistance may play a role in the eventual transition from insulin resistance to overt diabetes, which is characterized by β-cell loss and dysfunction.

In contrast to some of the other UPR markers, it was recently reported that ATF6α expression is reduced in the liver of obese mice and reconstitution with adenoviral ATF6α improves glucose homeostasis in these animals. This suggests a possible dissociation between chaperone production to enhance protein processing and other UPR-mediated signaling events that could underlie some of the detrimental metabolic effects seen in obesity. The observation that treatment with chemical ER chaperones reduces both obesity/insulin resistance and atherosclerosis in mice further supports this conclusion. Moreover, treatment of insulin-resistant humans with TUDCA, a conjugated bile acid derivative that inhibits ER stress–induced apoptosis, results in increased insulin sensitivity. Genetic overexpression of the ER chaperones ORP150 (oxygen-regulated protein 150) and GRP78 (glucose-regulated protein 78) also improves metabolic regulation in mice. Finally, several recent reports have implicated disruption of autophagy in metabolic dysfunction. Specifically, studies in our laboratory have shown that reduction in expression of autophagy related protein (ATG)7, a member of a group of ATG proteins that are essential mediators of the autophagic process, in liver cells, or in mouse liver tissue, results in defective insulin responsiveness and increased ER stress. Conversely, reconstitution of ATG7 in the liver of obese mice (which exhibits marked defects in autophagy) attenuated ER stress and improved insulin signaling and glucose tolerance. In combination, these findings support a model in which UPR-mediated pathways that aid in reestablishment of ER homeostasis are protective in the context of metabolic disease. On the other hand, prolonged UPR signaling, or engagement of other signaling pathways, such as those involved in immune signaling, might negatively impact metabolic homeostasis. It is important to note that additional research is required to verify this model. However, examination of traditional roles of the ER and established pathways altered by ER stress supports such a hypothesis (Figure 3, and discussed below).

**ER Stress and Inflammation**

One mechanism by which ER stress has been proposed to negatively impact metabolic homeostasis is through induction of inflammatory signaling networks. Indeed, all 3 arms of the UPR can activate JNK and/or NF-κB signaling, as well as potentially other pathways. This signaling has been proposed to result in subsequent inhibition of insulin action via IRS-1 phosphorylation, although other mechanisms are also possible. Moreover, increased JNK and NF-κB signaling would be expected to induce other inflammatory mediators that are associated with metabolic disease. In addition, ER stress–mediated regulation of autophagy could play a role in modulation of inflammatory pathways because autophagy has been implicated in immune cell function at multiple levels. Although this remains to be definitively proven, it supports the idea that ER stress may be at least a contributing factor to the low-grade inflammation observed in obesity.

Another important function of the UPR is to determine when ER stress reaches an insurmountable level and, subsequently, when to induce proapoptotic signaling. Likely, this is a mechanism to prevent release and accumulation of misfolded proteins from this quality-control checkpoint, which can have negative impacts on cellular function. Moreover, the energy requirements needed to maintain UPR signaling may eventually be deemed too costly. However, ER stress–induced apoptosis may also play a role in increased inflammatory signaling and other aspects of metabolic disease. For example, adipocyte death in obesity has been proposed as a potential trigger for the recruitment of macrophages and other inflammatory cells into adipose tissue. The causes of adipocyte demise in obesity remain unknown but may involve ER stress. In support of this, ER stress has been implicated in pancreatic β-cell death observed in later stages of hyperglycemia and insulin resistance, as well as in plaque rupture of atherosclerotic lesions. Genetic deletion of CHOP has been shown to improve β-cell survival in both Akita and db/db models of insulin resistance and protect against myocardial infarction in mouse models of atherosclerosis, supporting a role for ER stress–induced apoptosis in these processes. However, definitive and mechanistic links between UPR-mediated cell death and metabolic diseases will require additional studies with these or other models.
Another intriguing possibility is that demand on, or disruption of, the protein-processing function of the ER is involved in obesity-mediated induction of ER stress and subsequent adverse metabolic and/or inflammatory outcomes. Proper folding and secretion of proteins is a well-defined function of the ER that is modulated by the UPR. For example, IRE-1 signaling and XBP-1 splicing are important mediators of antibody secretion by plasma B cells. Moreover, lipoprotein metabolism and secretion has been shown to be altered by ER stress. Finally, a large body of research has been devoted to understanding how disruption or hyperactivation of the ER stress response modulates insulin processing and secretion. As mentioned earlier, adipose tissue and associated immune cells secrete a number of adipokines and cytokines that play important roles in systemic metabolism and inflammation. It would be reasonable to postulate that either the excess nutrients associated with obesity increase secretory demand for these signaling molecules, thereby inducing ER stress signaling, or the increased ER stress observed in obesity disturbs normal production or distribution of these adipokines. Consistent with this, upregulation of hepatic apolipoprotein B100 (apoB100), either through exposure to a HFD or by genetic overexpression strategies, induced ER stress and hepatic insulin resistance. Conversely, ER stress activation has been associated with impaired ApoB100 secretion and subsequent very-low-density lipoprotein (VLDL) assembly. However, a functional role for the UPR in mediating, or being mediated by, adipokine production and secretion has not been examined and remains an interesting postulate to test.

**The UPR and Regulation of Lipid and Glucose Metabolism**

Thus far, we have discussed how the UPR could in various ways modulate immune signaling and indirectly influence metabolic processes. Recent evidence suggests that the ER can also directly affect lipid and glucose metabolism (Figure 2). In fact, ER stress mediators can modulate some of the key pathways disrupted in obesity, including lipogenesis and gluconeogenesis, highlighting the importance of the UPR in metabolic regulation. In pancreatic β cells, PERK and eIF2α can influence expression of sterol-regulatory binding proteins (SREBPs), which are key regulators of cholesterol and FA synthesis. PERK has also been shown to increase SREBP activity and lipogenesis in mammary epithelial cells. In addition, XBP-1 has been implicated in induction of FA synthesis in the liver. In contrast, activation of ATF6α inhibits SREBP-mediated induction of lipogenic genes by recruiting the corepressor histone deacetylases to SREBP on target gene promoters in liver cells. In combination, these findings suggest different branches of the UPR can differentially modulate lipid metabolism. It was recently shown that the nature and severity of the stress encountered also impacts metabolic regulation by ER stress mediators. Liver-specific deletion of ATF6α, IRE-1α, or expression of a nonphosphorylatable eIF2α all enhanced the effects of TM treatment, suggesting a fully functioning ER stress response may be required to maintain lipid homeostasis in response to high levels of stress. Of note, the mouse model lacking hepatic ATF6α, but not IRE-1α or expressing mutant eIF2α, exhibited increased hepatic steatosis despite downregulation of lipogenesis, indicating other aspects of lipid metabolism such as lipoprotein packaging and secretion may be differentially regulated between UPR branches in response to TM.

UPR mediators also exhibit distinct functions in regulation of glucose homeostasis. Inhibition of PERK signaling by inducible expression of the eIF2α phosphatase Gadd34 results in defective gluconeogenesis in response to fasting, implicating this arm of the UPR in induction of gluconeogenesis. In contrast, ATF6α has been shown to inhibit gluconeogenesis in the mouse liver by modulating the activity of the key transcriptional regulator CREB (cAMP-response element binding protein) through competition for the transcriptional cofactor TORC2 (transducer of regulated CREB activity). In response to ER stress, TORC2 was shown to associate with ATF6α at the promoters of UPR targets, thereby reducing its availability to regulate CREB-mediated hepatic glucose output. Moreover, overexpression of ATF6α repressed the gluconeogenic program in a mouse model of diabetes, supporting a functional role for this activity in metabolic disease.

The ability of all 3 main UPR mediators to modulate lipid and glucose homeostasis highlights the presence of a direct link between ER stress and substrate metabolism. Moreover, the nature and severity of the stress encountered also impacts metabolic regulation by ER stress mediators.
the ability of nutrients such as glucose and FAs to activate the UPR, as well as the observed increase in expression of some UPR markers in obesity, suggests ER stress pathways play a role in metabolic regulation under conditions of nutritional excess. The differential roles of each arm on these processes suggest that a view of ER stress as simply being detrimental or beneficial in regards to proper metabolic function may not be accurate. Rather, the respective contribution of each branch, as well as factors such as the timing or nature of a specific stress and how it directs signaling through each arm, need to be considered when evaluating metabolic outcomes and translational possibilities. As a result, it is becoming increasingly important to understand how the 3 arms of the UPR (or other yet to be discovered branches) respond to distinct environmental situations, including dietary exposure, as well as how differential signaling through these pathways modulates other biological pathways including metabolism, apoptosis, and inflammation.

Conclusions

It is now clear that increased ER stress and aberrant inflammation are important factors associated with obesity that likely contribute to metabolic dysfunction and cardiovascular disease. Metabolic, inflammatory, and ER stress signaling are all tightly integrated and can each influence the other (Figure 4). As a result, we can envision that these fundamental cellular pathways are kept in balance to respond to specific environmental conditions in a manner consistent with the needs of the organism. Moreover, important mediators of signaling for each pathway, such as PKR, TLRs, and nuclear receptors, serve as complex sensors that intersect all 3 pathways in an attempt to maintain a healthy metabolic equilibrium (Figure 4). The complexity and interconnected nature of networks controlling nutrient and energy homeostasis is further highlighted when one considers numerous other pathways not discussed in this review, such as mTOR signaling, nitrogen or iron metabolism, calcium homeostasis, and mitochondrial function that also impact metabolic well-being.

Under metabolically challenging conditions such as obesity, disruption of any of these processes, and in particular at key nodes where multiple pathways intersect, could ultimately lead to a shift in metabolic equilibrium, resulting in disease (Figure 4). Indeed, both genetic and environmental factors play a role in individual susceptibility to metabolic disease, arguing against a single underlying cause. Thus, the fundamental question lies in addressing what changes triggered by diet, lifestyle, and/or aging result in a shifting of this balance at any given point. Moreover, most obesity-associated diseases are also diseases associated with aging. Thus, we should perhaps consider metabolic dysfunction on 2 levels: initial events that trigger underlying disruption of energy-sensing pathways and mechanisms by which these disturbances ultimately result in disease over time (Figure 5). A number of processes associated with aging, such as increased reactive oxygen species production and accumulation of protein, DNA and organelle damage likely contribute to the emergence of these advanced disease states. The ultimate progression to disease could thus be viewed as the result of continuous exposure to nutritional excess, in combination with other adverse consequences of aging, which eventually leads to a “tipping point” where adaptive responses fall short, resulting in advanced metabolic deterioration. When and if this point is reached, as well as the resulting disease presented, is likely determined by unique genetic susceptibility profiles of each individual. ER stress and inflammatory signaling also potentially play a role in determining when this tipping point is reached because of their ability to influence cellular responses and adaptation to changing environmental conditions. Thus, metaflammation and ER dysfunction are emerging as critical processes that, if successfully targeted therapeutically, have the potential to shift the tipping point, strengthen overall adaptive capacity, and improve various metabolic parameters. Indeed, encouraging results have been obtained in both preclinical and clinical studies for targeting these pathways to treat chronic metabolic diseases.

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References


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