Mechanism of ER Stress and Inflammation for Hepatic Insulin Resistance in Obesity

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Key Words

ER stress · Inflammation · Hepatic insulin resistance · Obesity

Abstract

Background: Obesity is a major risk factor in the development of hepatic insulin resistance, which is characterized by an impairment of insulin ability to inhibit glucose output. Although the underlying mechanism for the link between obesity and insulin resistance in the liver is unclear, it has been widely reported and suggested that hepatic endoplasmic reticulum (ER) stress and inflammation induced by obesity lead to the development of hepatic insulin resistance and gluconeogenesis. **Summary:** This review addresses the aspects of ER stress and inflammation currently understood to be involved in metabolic disease, including their role in obesity, hepatic insulin resistance, and hyperglycemia.

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Introduction

The liver plays a unique role in the regulation of glucose homeostasis by maintaining blood glucose concentration within a normal range [1]. However, impaired insulin action in the liver leads to insulin resistance characterized by impairment in the ability of insulin to inhibit glucose output [2]. Thus, hepatic insulin resistance, which

is the reduced sensitivity of the liver to insulin, causes gluconeogenesis and hyperglycemia, which are features of type 2 diabetes mellitus [1, 2].

Obesity is a major causal factor in the development of hepatic insulin resistance. Chronic excess energy intake leads to obesity, which can play a role in the development of hepatic insulin resistance by mechanisms that are not yet fully elucidated, and several questions remain unanswered regarding this process [3, 4]. Recent studies reported that inflammatory signals, including those produced by excess lipids, in an obese state can stimulate endoplasmic reticulum (ER) stress and inflammation in several cells and play key roles in insulin resistance [5–7].

This review addresses the aspects of ER stress and inflammation currently understood to be involved in metabolic disease, including their role in obesity, hepatic insulin resistance, and hyperglycemia.

The Role of the Liver in Glucose Homeostasis

The liver plays a unique role in the regulation of glucose homeostasis by maintaining blood glucose concentration within a normal range [1, 8]. Hepatic glucose metabolism mainly includes glycolysis, gluconeogenesis, glycogenesis, and glycogenolysis, and processes controlled by several hormones including insulin, glucagon, cortisol, growth hormones, and catecholamines. Moreover, many enzymes are involved in hepatic glucose me-

Fig. 1. Molecular mechanism for insulin in the liver. Insulin binds to the IR on the surface of hepatic cells, which initiates the tyrosine phosphorylation of the IRS proteins and the phosphorylation of PI3-K, which subsequently Akt/PKB. Akt/PKB causes the phosphorylation of the forkhead box protein O1, which results in the inhibition of the transcription of gluconeogenic genes and the phosphorylation of GSK3, resulting in the activation of glycogen synthesis.

tabolism and contribute to the regulation of glucose homeostasis [9, 10]. A key regulator hormone of glucose metabolism is insulin, the hormone that lowers blood glucose. Insulin directly and indirectly regulates glucose metabolism by binding to insulin receptors (IRs) in the liver, suppressing gluconeogenesis/glycogenolysis, and stimulating glycolysis/glycogenesis [2, 11].

During normal insulin action in the liver, especially after a carbohydrate-rich meal, insulin binds to IR, initiating tyrosine phosphorylation of the IR substrate (IRS) proteins, which lead to the activation of phosphatidylinositol-3-kinase (PI3-K) [12]. PI3-K subsequently activates protein kinase B (Akt/PKB) and causes the phosphorylation of the forkhead box protein O1, which results in the inhibition of the transcription of gluconeogenic genes such as phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase) [13, 14]. In addition, Akt/PKB leads to the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3). Inactivation of GSK3 by Akt/PKB offers protection from deactivating glycogen synthase; therefore, insulin increases glycogen synthesis (fig. 1) [15]. Conversely, during fasting periods, gluconeogenesis is strongly stimulated by a low level of insulin and a high level of glucagon, resulting in the production of

glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids [16–18]. Therefore, impaired insulin action in the liver (hepatic insulin resistance) plays a central role in the pathogenesis of metabolic disorders, such as metabolic syndrome, nonalcoholic fatty liver disease, and diabetes mellitus [2, 19, 20].

Obesity-Associated Hepatic Insulin Resistance

The liver is vital for maintaining normal glucose homeostasis, which is tightly regulated by insulin and glucagon during postabsorptive and fasting periods [1, 8, 16]. However, following the development of impaired hepatic insulin action, the liver manifests insulin resistance characterized by impairment in the ability of insulin to inhibit glucose output, finally resulting in gluconeogenesis [5, 6]. Thus, insulin resistance in the liver has a similar pathogenesis as type 2 diabetes. Insulin resistance occurs when the body produces sufficient insulin but the tissues respond abnormally to it [4]. A major factor in the development of insulin resistance is obesity, yet the molecular mechanisms underlying obesity and hepatic insulin resistance are controversial [3, 4, 21].

Fig. 2. Obesity-associated hepatic insulin resistance. High levels of glucose and insulin as well as the influx of pro-inflammatory cytokines, leptin and FFA from adipocyte into the blood, in-

duced by chronic excess energy intake, leads to inflammation and ER stress in the liver, which have negative effects on insulin signaling.

Obesity is defined as a medical condition with an abnormal accumulation of body fat, and carries a high risk of health problems, including metabolic syndrome, cardiovascular diseases, type 2 diabetes mellitus, and certain types of cancer. It has been reported that the risk of developing diabetes mellitus increases 10- to 40-fold at a BMI >30 compared with a normal BMI [22, 23]. Although the molecular mechanism linking obesity to hepatic insulin resistance is unclear, it has been widely reported and suggested that hepatic ER stress and inflammation induced by obesity lead to the development of hepatic insulin resistance and gluconeogenesis [24–26].

In most obese patients, white adipose tissue is characterized by an increased secretion of free fatty acids (FFA), leptin, and pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). The 'portal hypothesis' suggests that lipolysis of white adipose tissue expansion aggravates the influx of FFA into the blood, which leads to impaired IRS signaling and hepatic steatosis [27–30]. In addition, the expansion of white adipose tissue in an obese state leads to decreased secretion of adiponectin, which inhibits gluconeogenesis and improves insulin sensitivity in the liver [31]. Excess energy intake leads to an increase in the concentration of blood glucose and insulin produced by beta cells in the pancreas. The

blood environment that subsequently results from chronic increases in inflammation and ER stress has negative effects on insulin signaling in the liver (fig. 2) [24–26, 31, 32].

Unfolded Protein Response and ER Stress

The ER is one of the largest cytoplasmic organelles in eukaryotic cells and plays essential roles in calcium storage, lipid synthesis, and protein folding [33]. All secretory proteins enter the secretory pathway through the ER and must be properly folded and modified by asparagine links, glycosylation, and disulfide bonds. However, the physiological conditions that produce an imbalance between the cellular demand for protein folding and the capacity of the ER to promote protein maturation facilitate the accumulation of unfolded proteins in the ER lumen. This condition has been collectively defined as ER stress [34, 35].

Activation of the cellular response to ER stress, which has been termed the unfolded protein response (UPR), consists of 3 main signaling systems initiated by 3 ER transmembrane proteins: PKR-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring enzyme 1 (IRE1). Under normal conditions, the 3 ER transmembrane proteins are

maintained in an inactive state because they remain bound to the chaperone protein called binding immunoglobulin protein, also known as 78 kDa glucose-regulated protein (GRP78). However, chaperone protein dissociation from each ER transmembrane protein during the folding of unfolded proteins can trigger their activation and the induction of the UPR [34–36].

Phosphorylated and activated PERK phosphorylates the eukaryotic initiation factor 2 α subunit (eIF2 α), which results in translational attenuation. This process can reduce the workload of the ER by preventing the production of newly synthesized proteins and reducing the accumulation of proteins within the ER lumen. However, there are multiple apoptotic pathways related to the process [37, 38]. Phosphorylated eIF2 α also selectively enhances the translation of activating transcription factor 4, a member of the CCAAT/enhancer binding protein (C/EBP) family of transcription factors, which can lead to the activation of pro-apoptotic transcription factors, such as C/EBP homologous protein (CHOP). Growth-arrest and DNA-damage-inducible protein 34 is induced by CHOP, which acts as a negative feedback loop for the PERK pathway by dephosphorylating eIF2 α [39]. Activation of eIF2 α induces the translocation of ATF6 from the ER to the Golgi, which leads to the release of the N-terminal ATF6 fragment (ATF6-N). ATF6-N enters the nucleus and binds to the ER stress response element, which activates the expression of UPR target genes that encode ER chaperones and folding enzymes to help with protein folding and degradation (ER-associated degradation, ERAD) [40, 41]. Activation of IRE1 by ER stress leads to the splicing of X-box binding protein 1 (XBP-1), a transcription factor for regulating ER stress genes. Like ATF6-N, XBP-1 binds to ER stress response element and activates the expression of chaperones that can increase the folding capacity of the ER. However, when these corrective actions of the UPR are insufficient to attenuate ER stress and the ER stress response is prolonged, the UPR switches to a cell death mechanism. It has been reported that there are multiple apoptotic pathways, including the activation of CHOP mediated by activating transcription factor 4 and ATF6-N as well as c-Jun N-terminal kinase (JNK) activation mediated by the activation of IRE1 [42–44].

Mechanism of ER Stress to Hepatic Insulin Resistance in Obesity

The development of hepatic insulin resistance in an obese state is characterized by lipid accumulation and gluconeogenesis in the liver [5, 45]. Ectopic lipid accumu-

lation in the liver induced by high fat or excess energy intake leads to hepatic insulin resistance. The detailed molecular mechanisms underlying obesity-associated hepatic insulin resistance are not yet completely understood. However, there is evidence that hepatic ER stress and inflammation induced by obesity can lead to the development of hepatic insulin resistance and gluconeogenesis [24–26].

Several studies have indicated that severe ER stress leads to the activation of the JNK pathway, which is associated with the development of insulin resistance. Ozcan et al. [24] reported that obesity causes ER stress, which leads to the suppression of IR signaling through IRE1 α dependent activation of JNK and subsequent serine phosphorylation of IRS-1. They suggested that ER stress is a central feature of insulin resistance and type 2 diabetes.

A previous report by Achard et al. [32] demonstrated that exposure of human hepatoma HepG2 cells or mouse primary hepatocytes to saturated fatty acids enhanced ER stress in a dose-dependent manner, and that fatty acid-induced ER stress in HepG2 cells or hepatocytes resulted in reduced Akt phosphorylation and glycogen synthesis as well as in increased expression of G6Pase. In addition, a recent study revealed that GRP78 expression was decreased in the livers of diabetic *db/db* mice compared with diabetic *db/+* mice and that treatment of HepG2 cells with oleic acid reduced the expression of GRP78 [46]. A study by Min et al. [47] demonstrated that mice fed high fat diets enriched with lard had significantly higher PERK and CHOP protein expression and phosphorylation of eIF2 α concurrent with suppressed GRP78, while the serum insulin level and hepatic lipid deposition were increased. GRP78, a chaperone protein, binds newly synthesized proteins for subsequent folding and negatively regulates ER transmembrane proteins such as IRE1 α , PERK, and ATF6 α [46, 47]. These results indicate that the increased secretion of fatty acids from adipose tissue in the obese condition induces deleterious UPR signaling and contributes to insulin resistance in the liver through the activation of JNK, mediated by the activation of IRE1 and suppression of the expression of chaperone proteins and ERAD.

Continuous phosphorylation of eIF2 α in increased blood insulin conditions leads to the activation of C/EBPs. Birkenfeld et al. [48] demonstrated that the inhibition of the hepatic phosphorylation of eIF2 α signaling led to a decrease in hepatic glucose production, which could be attributed to reduced gluconeogenic gene expression. Oyadomari et al. [49] showed that eIF2 α phosphoryla-

Fig. 3. Activation of the UPR and ER stress signalling pathways in the liver. **a** Under normal conditions, the accumulation of unfolded proteins triggers the UPR for the ERAD or the expression of chaperone proteins to help with protein folding. **b** However, insufficient response to reduce the number of unfolded proteins or ag-

gravation of the accumulation of unfolded proteins in the chronic obesity-induced blood environment can lead to ER stress and cell death. In addition, ER stress promotes the translation of C/EBPs and the phosphorylation of JNK, which can cause gluconeogenesis and insulin resistance.

tion promoted C/EBPs translation in vitro. ER stress triggers an increase in the levels of C/EBPs mediated by eIF2 α phosphorylation, which leads to the expression of gluconeogenic genes, such as PEPCK or G6Pase, and induces hepatic glucose production. In addition, CHOP expression leads to the dysregulation of the C/EBPs [50, 51].

Based on the aforementioned, under normal conditions, the accumulation of unfolded proteins triggers the UPR pathway for the removal of unfolded proteins and the escape from ER stress through the expression of chaperone proteins to help with protein folding and ERAD. In chronic obesity-induced blood conditions, including high levels of glucose, insulin, FFA and pro-inflammatory cytokines, induction of ER stress and cell death mechanism can be activated in the liver by an insufficient response to the removal of unfolded proteins or aggravation of the accumulation of unfolded proteins. In addition, C/EBPs translation and activation of the JNK pathway induced by ER stress can cause gluconeogenesis and insulin resistance (fig. 3). Thus, hepatic ER stress in obesity may play a role in hepatic insulin resistance and metabolic dysregulation.

Mechanism of Inflammation to Hepatic Insulin Resistance in Obesity

Evidence of links between obesity and inflammation has existed for decades, and many reports have shown that obesity is associated with a chronic low-grade inflammation of white adipose tissue via chronic activation of the innate immune system [27, 52]. Moreover, obesity-associated inflammation can subsequently lead to insulin resistance in other peripheral tissues, including the liver and skeletal muscle [52–54]. The association between obesity, inflammation, and insulin resistance is well known, but the pathophysiological mechanism of this association is not completely understood.

Adipose tissue in obesity is characterized by progressive macrophage infiltration into adipose tissue and an increase in the production of pro-inflammatory cytokines. It has been reported that the number of macrophages in adipose tissue correlates with body mass index and adipose size. Adipocytes in obese individuals secrete low levels of TNF- α , which can lead to the production of monocyte chemoattractant protein-1 and subsequently induces macrophage infiltration into adipose tissue [52,

54–57]. Consequently, macrophage infiltration into adipose tissue triggers the release of pro-inflammatory cytokines, such as TNF- α , IL-6, and IL-1 β . Xu et al. [52] showed that adipocytes from obese mice produced chemokines and cytokines, including monocyte chemoattractant protein-1, MIPs, TNF- α , IL-6, and IL-1 β , which recruited monocytes into adipose tissue and activated their differentiation into macrophages. The specific trigger for the inflammatory response in adipose tissue in obesity is unknown. Some reports have hypothesized that metabolic stress, including malnutrition and overnutrition, can lead to an excess of metabolic building blocks in the ER or increased levels of FFA, which can subsequently activate inflammatory pathways in a variety of cell types [58–61]. In addition, production of pro-inflammatory cytokines as well as FFA from adipose tissue can activate inflammatory pathways in other tissues. Elevated levels of TNF- α have been observed in several experimental models of obesity and insulin resistance [62–64]. It was reported that anti-TNF- α therapy improved insulin sensitivity. Diehl et al. [65] showed that pretreatment with anti-TNF antibodies prevented regenerative induction of C/EBP expression, which is associated with mRNA levels of PEPCK. Gupta et al. [63] reported that TNF- α preincubation reduced insulin-stimulated tyrosine phosphorylation of the IR-beta and caused hyperphosphorylation of the IRS-1 serine residue. Overall, we can surmise from previous findings that obesity-induced adipocyte expansion leads to an increase in the levels of pro-inflammatory cytokines from the infiltrated macrophages and adipocytes as well as increased FFA in the blood. This blood environment promotes the inflammation response in other tissues including the liver and muscle, which can induce insulin resistance.

The mechanism for the progression from inflammation to insulin resistance in this blood environment can be explained by the activation of the nuclear factor- κ B (NF- κ B) pathway and JNK phosphorylation. Many recent reports have demonstrated the key roles of the NF- κ B signaling pathway and JNK phosphorylation in the development of inflammation-associated metabolic diseases in the liver [58–61]. Signaling from cytokine receptors on the cell surface can activate the NF- κ B pathway, which regulates the expression of target genes to activate the immune response such as the expression of pro-inflammatory cytokines. These intracellular pro-inflammatory cytokines, in addition to hematic pro-inflammatory cytokines, can induce JNK phosphorylation, which can cause gluconeogenesis and insulin resistance [60]. JNK is also activated upon exposure to FFA and ER stress, all

of which can be induced by obesity [60]. A previous study by Cai et al. [19] indicated that the production of pro-inflammatory cytokines in the liver was increased in high-fat-diet-induced mice and that lipid accumulation caused inflammation through NF- κ B activation and downstream cytokine production, leading to insulin resistance. Thus, pro-inflammatory cytokines-induced JNK phosphorylation directly promotes insulin resistance, and pro-inflammatory cytokines-induced activation of NF- κ B indirectly induces the local production of pro-inflammatory cytokines, which can inhibit insulin signaling via JNK phosphorylation (fig. 4).

Link between ER Stress and Inflammation in Obesity

In many recent studies on the pathological mechanisms of diseases, both ER stress and inflammation were observed in ER stress or inflammation-induced cells. A report by Ren et al. [66] documented that ER stress induced by tunicamycin increased pro-inflammatory cytokine expression and the activation of NF- κ B pathway in a murine acute liver failure model. Additionally, a study on cachexia progression by Narsale et al. [67] demonstrated that cancer caused inflammation as well as ER stress in the liver, mediated by the expression of binding immunoglobulin protein, IRE-1 α , inflammatory intermediate signal transducer and activator of transcription 3, and also increased mRNA expression of PEPCK. According to a study by Kim et al. [68], ER stress regulates the expression of different pro-inflammatory cytokine genes depending on the activation of GSK-3 β or XBP-1. These reports may indicate a correlation between ER stress and inflammation in several diseases, but the complete molecular mechanism is not yet understood. Hu et al. [69] found a link between ER stress and TNF- α through NF- κ B. They showed that blocking NF- κ B and TNF- α signaling inhibited ER stress-induced cell death; the ER stress-induced expression of TNF- α was IRE1 and NF- κ B dependent. Exposure to TNF- α induces ER stress, which itself can lead to the expression of TNF- α and inflammatory responses. In addition, ER stress-induced activation of CHOP plays a role in cytokine-induced pro-inflammatory responses and in the pathogenesis of inflammation [70, 71]. Overall, ER stress can activate NF- κ B signaling, which can subsequently induce inflammatory mediators, and these inflammatory mediators can induce ER stress. Therefore, the link between ER stress and inflammation is yet unknown.

This correlation between ER stress and inflammation can be found in previous studies on the mechanism

Fig. 4. Mechanism of inflammation by macrophage infiltration into adipose tissue to hepatic insulin resistance in the obese condition. Obesity-induced adipocyte expansion causes the infiltration of macrophages and the secretion of FFA and pro-inflammatory cytokines into the blood. This blood environment promotes the inflammation response in the liver, which can induce insulin resistance. Pro-inflammatory cytokines-induced JNK phosphorylation directly leads to hepatic insulin resistance, and pro-inflammatory cytokines-induced activation of NF- κ B indirectly induces the local production of pro-inflammatory cytokines, which can lead to hepatic insulin resistance via JNK phosphorylation.

Fig. 5. Obesity-associated ER stress, inflammation and hepatic insulin resistance. The blood environment under chronic excess energy intake can induce inflammation and ER stress in the liver. ER

stress and inflammatory pathways either directly or indirectly lead to hepatic insulin resistance by the inhibition of insulin signaling and the activation of gluconeogenic enzymes.

of obesity-induced metabolic dysregulation. Several recent reports have shown that obesity-induced excess lipids and other inflammatory signals can stimulate ER stress and an inflammatory response in several cells, and that ER stress correlates with inflammation [44, 47, 72]. Legry et al. [73] investigated the effect of treatment with the ER stress inducer tunicamycin, or conversely, with the ER protectant tauroursodeoxycholic acid in foz/foz mice, and found that the ER protectant failed to improve glucose intolerance, hepatic inflammation and apoptosis. It is still too early to conclude that ER stress and inflammation are the major mechanisms underlying obesity-induced hepatic insulin resistance because of a lack of sufficient evidence, although we can hypothesize that ER stress and inflammation play key roles in the mechanism based on the aforementioned.

Chronic excess energy intake-induced obesity leads to an increase in the levels of glucose, insulin, FFA and pro-inflammatory cytokines in the blood, which induce ER stress and inflammation in the liver. It is difficult to definitively conclude that the major role is played by either ER stress or inflammation because of the chicken or egg question. Thus, chronic excess energy intake-induced

obesity leads to simultaneous inflammation-induced ER stress and ER stress-induced inflammation, which can induce the aggravation of both ER stress and inflammation (fig. 5). Taken together, the effector of ER stress and inflammatory pathways in obesity either directly or indirectly disrupts metabolic function, including glucose and lipid metabolism, in several tissues.

Conclusions

In conclusion, the blood environment under chronic excess energy intake can induce inflammation and ER stress, which can increase the risk of developing insulin resistance through several mechanisms including the activation of gluconeogenic enzymes and can have negative effects on insulin signaling. Based on the aforementioned, ER stress can activate the inflammatory response and the inflammatory response can also activate ER stress, causing the development of hepatic insulin resistance in obesity. This research may provide insights into the mechanisms underlying the link between obesity and insulin resistance and lead to applications in therapeutic targets for hyperglycemia in obese patients.

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