Published in "The FASEB Journal, 2010
doi: 10.1096/fj.09-151340"
which should be cited to refer to this work.

JNK1 and IKKβ: molecular links between obesity and metabolic dysfunction

Giovanni Solinas*,[1] and Michael Karin†

*Laboratory of Metabolic Stress Biology, Department of Medicine, Physiology, University of Fribourg, Fribourg, Switzerland; and †Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology, School of Medicine, University of California–San Diego, San Diego, California, USA

ABSTRACT **Inflammation is thought to underlie the pathogenesis of many chronic diseases. It is now established that obesity results in a state of chronic low-grade inflammation thought to contribute to several metabolic disorders, including insulin resistance and pancreatic islet dysfunction. The protein kinases JNK1 and IKKβ have been found to serve as critical molecular links between obesity, metabolic inflammation, and disorders of glucose homeostasis. The precise mechanisms of these linkages are still being investigated. However, as we discuss here, JNK1 and IKKβ are activated by almost all forms of metabolic stress that have been implicated in insulin resistance or islet dysfunction. Furthermore, both JNK1 and IKKβ are critically involved in the promotion of diet-induced obesity, metabolic inflammation, insulin resistance, and β-cell dysfunction. Understanding the molecular mechanisms by which JNK1 and IKKβ mediate obesity-induced metabolic stress is likely to be of importance for the development of new treatments for a variety of obesity-associated diseases.—Solinas, G., Karin, M. JNK1 and IKKβ: molecular links between obesity and metabolic dysfunction. *FASEB J.* 24, 000–000 (2010). www.fasebj.org**

Key Words: type 2 diabetes · glucotoxicity · lipotoxicity · metabolic inflammation · metabolic stress

Chronic inflammation is believed to be a key pathogenic mechanism underlying diseases such as rheumatoid arthritis, Crohn's disease, and atherosclerosis, as well as cancers of the liver, stomach, and colon (1). It is now well established that obesity is associated with a state of chronic low-grade inflammation (metabolic inflammation) that is a major player in the pathogenesis of several metabolic disorders including type 2 diabetes and the so-called metabolic syndrome (2, 3). The stress-activated protein kinases JNK1 and IKKβ are central signal transducers in innate immunity and stress responses that control the expression of several proinflammatory genes. The JNK subgroup of stress-activated kinases, comprising the JNK1-3 isoforms and various splicing variants, belongs to the mitogen-activated protein kinases (MAPK) family, whose constituents are activated in response to growth factors, proinflammatory cytokines, microbial components such as lipopolysaccharide (LPS), and a variety of stresses (4, 5). Once activated, the JNKs phosphorylate and activate transcription factors including TCF/Elk-1, ATF2, and c-Jun, leading to increased *Jun* and *Fos* gene transcription and increased AP-1 transcription factor activity (4, 5). The JNKs have also been implicated in post-transcriptional control of gene expression by causing stabilization of inherently unstable mRNAs encoding cytokines and other inflammatory mediators (6, 7) (**Fig. 1*A***). IKKβ, on the other hand, is one of the 2 catalytic subunits (together with IKKα) of the IkB kinase (IKK) complex, whose activity is rapidly stimulated by proinflammatory cytokines, viral and microbial components, and numerous other inducers (8) (Fig. 1*B*). IKK is responsible for activation of the NF-kB/Rel family of transcription factors through its ability to phosphorylate their IkB inhibitors and target them to proteolitic degradation (8). Once freed from the IkB grasp, NF-kB dimers enter the nucleus and regulate transcription of genes involved in innate immunity and inflammation, as well as the maintenance of cell survival and tissue homeostasis (9) (Fig. 1*B*). Generally speaking the JNKs and the IKK complex are situated at the focal points of different signaling pathways involved in innate immunity, inflammation, and stress, whose main function is to activate host defense and maintain homeostasis (4, 8, 10).

Recently it has become evident that interference with either JNK1 or IKKβ activity improves insulin signaling in mouse models of obesity and lipid-induced glucose intolerance (11, 12). Because almost all metabolic stressors that cause insulin resistance or islet dysfunction activate JNK1 and/or IKKβ (see Fig. 3, 4), these protein kinases occupy key roles in linking obesity to insulin resistance, type 2 diabetes, and the metabolic syndrome and are therefore the focus of this review.

[1] Correspondence: Laboratory of Metabolic Stress Biology, Department of Medicine, Physiology, University of Fribourg, Chemin du Musée 5, CH-1700 Fribourg, Switzerland. E-mail: giovanni.solinas@unifr.ch

doi: 10.1096/fj.09-151340

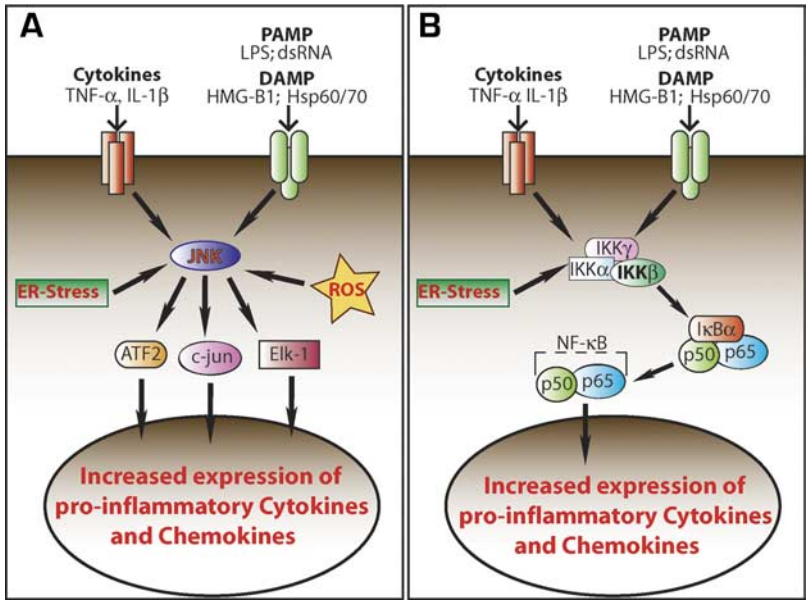

Figure 1. JNK and IKKβ are key signal transducers implicated in the innate immune response. During infection or tissue damage, pathogen-associated molecular pattern (PAMP), damage-associated molecular pattern (DAMP), and proinflammatory cytokines are sensed by specific receptors that activate the proinflammatory gene expression program. The kinases JNK (*A*) and IKKβ (*B*) are activated by several PAMP, DAMP, and cytokine receptors and play a central role in transmitting these proinflammatory signals from the cell surface to transcription factors involved in the control of the expression of proinflammatory cytokines and chemokines. TNF-α, tumor necrosis factor α; IL-1β, interleukin-1 β; LPS, lipopolysaccharide; HMG-B1, high mobility group box-1; Hsp60/70, heat-shock proteins 60/70; ROS, reactive oxygen species; ER stress, endoplasmic reticulum stress.

OBESITY AND TYPE-2 DIABETES ARE CHARACTERIZED BY CHRONIC LOW-GRADE METABOLIC INFLAMMATION

The first evidence for a pathogenic role of inflammation in type 2 diabetes is dated to more than a century ago when it was shown that the anti-inflammatory drug salicylate has beneficial effects on diabetic patients (13, 14). The "metabolic inflammation" hypothesis was revived and gained new popularity with the demonstration that interference with the proinflammatory cytokine TNF-α protects from obesity-induced insulin resistance in rodent models (15, 16). In addition to TNF-α, other proinflammatory cytokines, such as IL-6 and IL-1, are induced during obesity and were shown to cause insulin resistance in some models (17, 18). However, the role of IL-6 in the pathogenesis of insulin resistance in humans has been challenged (19, 20).

Consistent with a role for innate immunity and inflammation in obesity-induced insulin resistance, it was reported that mice bearing a mutation in the gene encoding Toll-like receptor 4 (TLR4), the sensor for bacterial LPS and other agonists, show improved insulin sensitivity in a model of free fatty acid (FFA)-induced insulin resistance (21). A hallmark of obesity-induced inflammation is macrophage infiltration into adipose tissue (22, 23). In obesity, adipose tissue macrophages (ATMs) are polarized toward the inflammatory M1 phenotype and express the CD11c marker, and most of them are localized around necrotic adipocytes or next to the residual lipid droplet left behind a dead adipocyte and form typical crown-like structures (CLSs) (24–26) (**Fig. 2*A***). Macrophages are believed to play an important role in obesity-induced insulin resistance by sensing lipid accumulation or cell damage and producing cytokines and other mediators that interfere with insulin signaling (2). The importance of macrophages and hematopoietic cells in general is supported by several studies in mice bearing specific genetic mutations in hematopoietic cells (generated by bone marrow transplantation) or in myeloid cells (obtained by conditional Cre-Lox recombination). Such studies have shown that disruption of the *Ikk*β gene in myeloid cells or the *Jnk1* locus in hematopoietic cells reduces metabolic inflammation and improves insulin sensitivity in models of diet-induced obesity (27–29). Genetic deletion of fatty acid binding protein *FABP* (30) or Cbl-associated protein *CAP* (31) within hematopoietic cells also reduces metabolic inflammation and improves insulin sensitivity, whereas mice with a conditional ablation of the *Ppar*γ gene in macrophages show increased susceptibility to obesity-induced inflammation and insulin resistance (32, 33). The latter is due to loss of the anti-inflammatory action of PPARγ (32, 33). It was also shown that recruiting macrophages to adipose tissue by overexpression of the chemokine MCP-1 in adipose tissue of lean mice is sufficient to induce insulin resistance (34). Conversely, depletion of CD11c-positive myeloid cells by injection of diphtheria toxin in mice overexpressing the diphtheria toxin receptor under the control of the CD11c promoter restores insulin sensitivity in obese insulin-resistant mice (35).

How hematopoietic cells induce insulin resistance and diabetes is a matter of intensive investigation. Nonetheless, it is well established that macrophages are a major source of proinflammatory cytokines proposed to contribute to the development of insulin resistance as well as β-cell destruction. In addition to macrophages, other types of hematopoietic cells may be important. It was recently shown that mast cells accumulate in adipose tissue during obesity and that genetic deficiency or pharmacological stabilization of these cells protects mice from diet-induced obesity and glucose intolerance (36). Obesity also modifies the population of adipose tissue resident T cells by decreasing the number of T_H2 and T_{reg} cells and increasing the number of $CD8^+$ T cells and T_H1 T

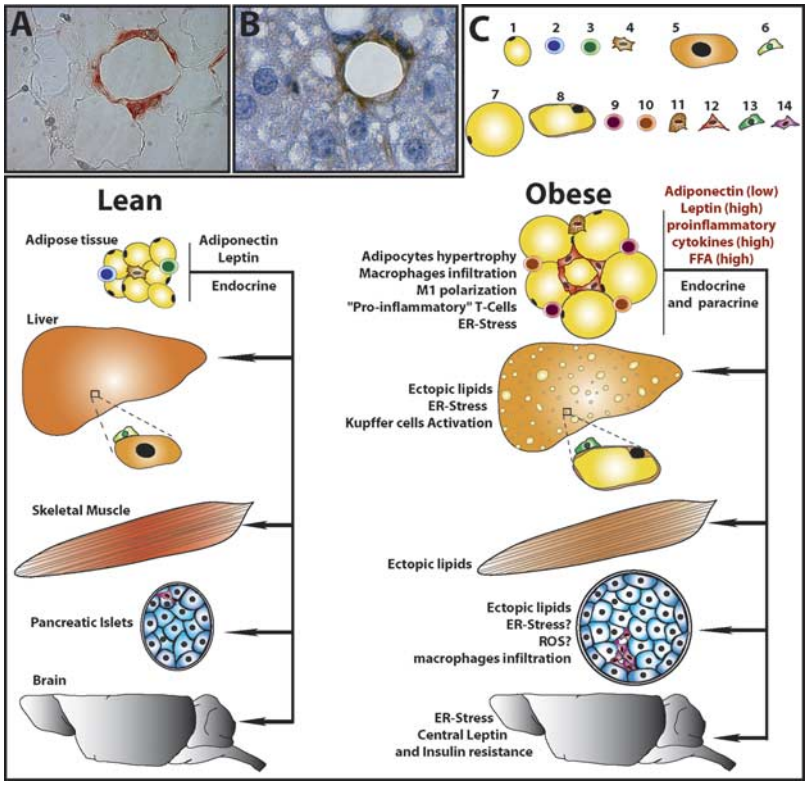

Figure 2. Obesity is characterized by adipocyte hypertrophy, deposition of ectopic lipids, and metabolic inflammation. *A*) MAC-2 staining of adipose tissue from an obese mouse showing a crown-like structure (CLS) comprising a dead adipocyte surrounded by adipose tissue macrophages (ATM). *B*) F4/80 staining of a fatty liver from an obese mouse showing Kupffer cells (brown F4/80 positive cells) surrounding a large lipid vacuole from a fatty hepatocyte. *C*) Obese adipose tissue is characterized by production of proinflammatory cytokines, hypertrophic adipocytes, alteration of the resident T-cell population, mast cell recruitment, and infiltration of M1 proinflammatory macrophages to form CLSs. Obese adipose tissue infiltrates other organs, possibly changing the exposure of some nonadipose tissues to adipose tissue released factors from endocrine to paracrine. Lipid deposition in nonadipose tissue further contributes to metabolic stress, leading to loss of glucose homeostasis and tissue damage. Fatty liver also displays increased expression of proinflammatory cytokines, and activated Kupffer cells are probably a major source. During obesity, macrophages infiltrate into pancreatic islets and are probably contributing to the local production of proinflammatory cytokines. It was reported that obesity is associated with endoplasmic reticulum stress in neurons leading to central leptin and insulin resistance, thereby further promoting obesity and loss of glucose homeostasis. *1*) Adipocyte. *2*) T_H2 T cell. *3*) T_{reg} ($CD4^+$) T cell. *4*) M2-polarized anti-inflammatory adipose tissue macrophage. *5*) Hepatocyte. *6*) Kupffer cell. *7*) Hypertrophyc adipocyte. *8*) Fatty hepatocyte. *9*) Effector ($CD8^+$) T cell. *10*) T_H1 ($CD4^+$) T cell. *11*) Mast cell. *12*) M1-polarized proinflammatory adipose tissue macrophage. *13*) Activated Kupffer cell. *14*) Pancreatic islet-associated macrophage.

cells, which cause macrophage recruitment and insulin resistance (37–40).

Because in the obese state ATMs are localized around necrotic adipocytes or their residual lipid droplets (24), it appears likely that adipocyte necrosis is a key factor in adipose tissue inflammation. The frequency of CLSs and necrotic adipocytes correlates better with adipocyte size than adiposity *per se*. Both necrotic adipocytes and CLSs are elevated in hormone-sensitive lipase-knockout mice, a model of adipocyte hypertrophy without increased adiposity (24). Increased ATM numbers were also observed in mice bearing a targeted mutation at the *Pparγ2* gene crossed with *ob/ob* mice (POKO mice), a model of limited adipose tissue expansion characterized by adipocyte hypertrophy without overt obesity (41). POKO mice display severe derangement in glucose homeostasis caused by both insulin resistance and loss of β-cell function, which correlates with ectopic deposition of toxic lipids and increased numbers of CLSs in adipose tissue (41).

Morbidly obese patients that had undergone bariatric surgery, which decreases adipocyte size, exhibited decreased CLS numbers within 3 mo, and the remaining macrophages expressed the anti-inflammatory cytokine IL-10 (42). Moreover, weight-loss surgery decreases the expression of the proinflammatory genes *MCP-1*, *CSF-3*, *HIF-1α*, and *PLAUR* in the stromal-vascular fraction of white adipose tissue (42). This observation is consistent with data from another laboratory showing that bariatric surgery decreases the circulating levels of the inflammatory markers MCP-1, IL-6, CRP, and IL-18 (43). Therefore, the chronic inflammatory process induced by obesity can be resolved by weight loss, supporting the idea that the presence of hypertrophic adipocytes is required to sustain a state of chronic metabolic inflammation. Interestingly, structures similar to CLSs were observed in fatty liver, where dead fatty hepatocytes are enclosed by Kupffer cells (44) (Fig. 2*B*), suggesting that "lipid-intoxicated" hepatocytes could play a role in steatohepatitis and Kupffer cell activation. CLS morphology suggests an explanation for the chronic nature of metabolic inflammation: whereas acute inflammation is resolved as soon as the causative factor disappears, in obesity new CLSs will keep forming as soon as adipocytes become hypertrophic and then necrotic, finally leaving behind a lipid droplet. Furthermore, when adipose tissue expansion approaches its limits, toxic lipids start to accumulate in other tissues, such as the liver and pancreatic islet (45–47), and circulating FFA concentrations increase. Finally, exposure to FFA leads to activation of inflammatory signaling in several cell types, including adipocytes (21, 48), hepatocytes (49, 50), myocytes (51), pancreatic islets (50), and macrophages (21, 27, 28). Therefore, the critical factor

in metabolic inflammation is not adiposity *per se* but an exhaustion of the lipid storage capacity of adipocytes. This occurs in obesity and lipodistrophy, 2 conditions that are characterized by hypertrophic adipocytes, adipocyte necrosis, ATM infiltration, CLS formation, and ectopic accumulation of toxic proinflammatory lipids. Hence, hypertrophic dysfunctional adipocytes, ectopic deposition of toxic lipids, and high circulating levels of FFA are the likely causes of metabolic inflammation. Both of our suspects, JNK1 and IKKβ, play a key role in transducing signals generated at cytokine receptors, such as TNF-α and IL-1 receptors, to transcription factors that control the expression of several proinflammatory genes, including *TNF*-α and *IL-1* themselves. Furthermore, JNK and IKKβ are also downstream of pathways activated by toxic lipids and excessive glucose levels.

METABOLIC STRESS PATHWAYS CONVERGE ON JNK1 AND IKKβ

Obesity is characterized by alterations of the metabolic milieu leading to insulin resistance and to a cluster of obesity-associated diseases referred to as the metabolic syndrome. We use the term "metabolic stress" to indicate stress caused by perturbations in the metabolic milieu. Metabolic stress can be described as intracellular or extracellular depending on whether the source of metabolic stress is exclusively intracellular, such as intracellular accumulation of toxic lipids, or extracellular, such as high levels of circulating FFA or exceedingly high blood glucose. Almost all the stressors and pathways proposed to induce insulin resistance or β-cell dysfunction result in the activation of JNK or IKKβ or both, independently of whether the signal is initiated at membrane receptors that sense the extracellular milieu, or whether it originates in response to intracellular stress.

Circulating FFAs

Elevated plasma concentration of FFAs is a major derangement of the extracellular milieu associated with obesity. Administration of FFAs has been shown to induce insulin resistance (52). Consistent with a role of the innate immune system in insulin resistance, several laboratories have shown that long-chain saturated fatty acids (LCSFAs), and in particular palmitic acid (PA), can activate TLR2 and TLR4, pattern recognition receptors that sense lipopetides and lipopolysaccarides of bacterial walls (21, 51, 53). Engagement of either TLR4 or TLR2 leads to recruitment of the MyD88 adapter protein and formation of signaling complexes containing the interleukin-1 receptor associated kinases (IRAKs) and the TNF receptor associated factor (TRAF) family members TRAF3 and TRAF6. The latter leads to recruitment and eventual activation of the MAP kinase kinase kinase (MAP3K) TAK1, which is required for JNK and IKKβ activation (54) (**Fig. 3*A***). Peritoneal macrophages bearing a targeted disruption at the *Tlr4* gene ($Tlr4^{-/-}$) show partially reduced JNK and IKKβ activation in response to PA (21), suggesting the existence of additional receptors. Another study showed that isolated soleous muscles from mice with a loss of function mutation at the *Tlr4* locus (*CH/HeJ* mutants) are protected from the negative effects of PA on insulin signaling and insulin-induced glucose uptake (55). A role for TLR4 signaling in FFA-induced insulin resistance is supported by *in vivo* experiments showing that $Tlr4^{-/-}$ mice compared to wild-type controls are protected from insulin resistance induced by lipid infusion and, to a lesser extent, from high-fat diet-induced insulin resistance (21, 55, 56). Adoptive transfer experiments suggest that the hematopoietic compartment is an important site of the diabetogenic action of TLR4 signaling (57).

IL-1

IL-1 is induced during obesity, and it has been reported that humans with detectable circulating IL-1β and increased concentrations of blood IL-6 are more likely to develop type 2 diabetes than individuals with high IL-6 but undetectable IL-1β (58). Also, 3T3-L1 adipocytes exposed to either IL-1α or IL-1β display defective insulin signaling and insulin-stimulated glucose uptake (17, 59). Most notably, interference with IL-1 receptor (IL-1R) signaling using recombinant IL-1 receptor antagonist (IL-1Ra) ameliorates glucose homeostasis in type 2 diabetes patients by improving pancreatic β-cell function (60). Similarly to TLR4, IL-1R activates JNK1 and IKKβ *via* the MyD88 signaling pathway (Fig. 3*B*).

TNF-α

TNF-α expression is elevated in obese rodents and humans, and treatment with TNF-α-neutralizing antibodies improves insulin sensitivity in obese rats (16). Similarly, mice bearing a targeted disruption of the *Tnf*α gene or of the genes encoding TNF-α receptors 1 and 2 (TNFR1, 2) are protected from obesity-induced insulin resistance (15). TNF-α transiently activates both IKKβ and JNK1 through a pathway different from the one used by IL-1R or TLR2/4 (61). After binding of trimeric TNF-α, TNFR1 undergoes trimerization, and its death domain (DD), located on its intracellular portion, forms a complex with the scaffolding kinase RIP1 and the adaptor protein TRADD, which bind TRAF2 and TRAF5 leading to IKKβ and JNK1 activation (Fig. 3*C*). RIP1 and TRAF2 recruit the IKK complex to activated TNFR1, where the IKKβ subunit undergoes phosphorylation and activation. JNK activation is also TRAF2 dependent but RIP independent and requires

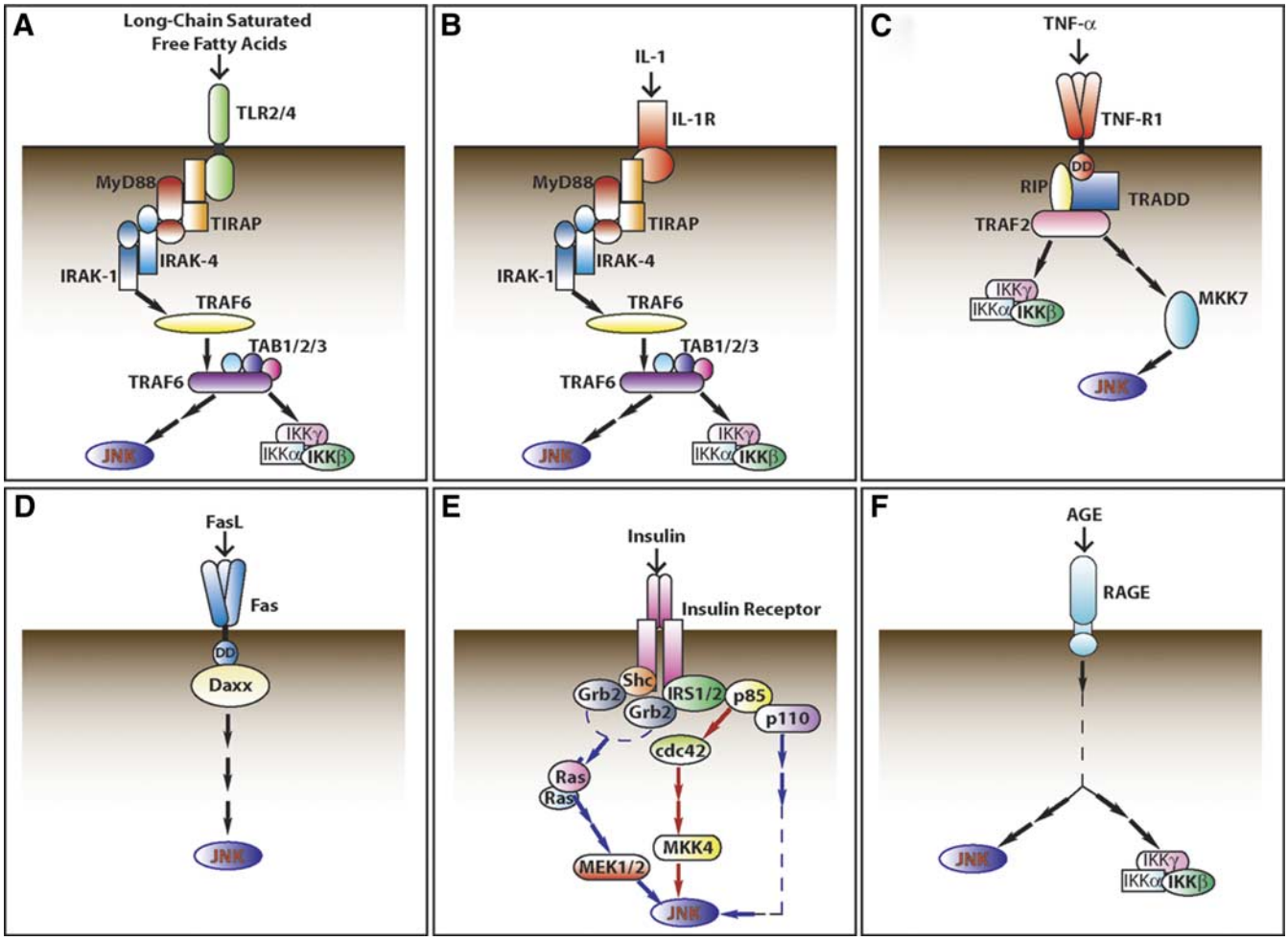

Figure 3. Extracellular metabolic stress signaling leads to JNK and IKKβ activation. Obesity-caused derangements of the extracellular milieu include hyperglycemia, increased levels of circulating FFAs, hyperinsulinemia, and increased levels of proinflammatory cytokines. *A*) Extracellular FFAs activate JNK and IKKβ *via* Toll-like receptor (TLR) signaling pathway. *B*) Obesity is characterized by elevation of the proinflammatory cytokine IL-1, whose receptor activates JNK and IKKβ using signaling modules common to the TLR pathway. *C*) The proinflammatory cytokine TNF-α is elevated in obesity and activates JNK and IKKβ by a specific signaling pathway. *D*) Fas ligand (FasL), which was proposed to play a role in β-cell dysfunction and death, activates JNK by a pathway involving the adaptor protein Daxx. *E*) Chronic hyperinsulinemia was also implicated in JNK activation and different signaling pathways were proposed. *F*) Hyperglycemia leads to increased levels of advanced-glycation end products (AGE), which activate JNK and IKKβ by binding their receptor RAGE.

the MAP3K MEKK1, leading to activation of the JNK kinases MKK4 and MKK7 (61, 62).

Fas ligand (*FasL*)

FasL and its receptor Fas were proposed to play a role in β-cell death and dysfunction (63, 64). Fas is a member of the TNFR superfamily, whose expression is controlled by the IKK/NF-KB and JNK/AP-1 signaling, and it activates JNK by a pathway dependent on the adapter protein DAXX (65) (Fig. 3*D*).

Hyperglycemia and advanced glycated end products (AGEs)

Hyperglycemia and AGEs have been proposed to contribute to insulin resistance (66–69) and β-cell dysfunction (70, 71). One mechanism by which hyperglycemia could trigger insulin resistance is through consequent hyperinsulinemia (see below). Hyperglycemia also leads to nonenzymatic reactions between glucose and amino groups of proteins and other molecules, leading to the formation of glycated proteins and AGEs, such as methylglyoxal (72). AGEs are considered to play an important role in diabetic complications (72); moreover, glycated albumin was shown to induce defective insulin signaling in L6 myotubes (69). Although the role of JNK1 and IKKβ in AGE-induced insulin resitance is not established, AGEs activate both IKKβ and JNK1 *via* a specific AGE receptor (RAGE) by a mechanism that remains to be identified (73–75). RAGE is a pattern-recognition receptor that recognizes a large family of compounds whose activation promotes inflammation (76, 77). Mice bearing a targeted deletion at the *Rage* gene are resistant to septic shock, and the AGE-RAGE pathway was proposed to

play a role in innate immunity by sensing molecules generated at the site of tissue injury and promoting inflammation (78, 79).

Insulin

Insulin has been reported to activate JNK in different cell culture models by a phosphatidyl-inositide 3 kinase (PI3K)-dependent pathway, and it was proposed that insulin-mediated JNK activation constitutes a negative feedback loop in insulin signaling (80). Two different mechanisms were proposed for insulin-dependent JNK activation: one requires PI3K activity and the Grb2→Ras→MEK1/2 pathway (80), whereas the second mechanism is independent of PI3K activity but requires its adapter subunit p85 for activation of JNK *via* Cdc42 and MKK4 (81) (Fig. 3*E*).

Intracellular glucotoxicity

The contribution of hyperglycemia to intracellular metabolic stress depends on the cell type. This view is supported by the fact that in type 1 diabetes, hyperglycemia-induced tissue damage is limited to a few cell types, most notably neurons and endothelial cells (82). A possible explanation could be that some cells, such as smooth muscle cells, down-regulate glucose uptake in the hyperglycemic environment to avoid large fluctuations in intracellular glucose concentrations, whereas other cells, such as endothelial cells, which are particularly sensitive to glucotoxicity, are unable to efficiently down-regulate glucose uptake and therefore develop intracellular hyperglycemia (82, 83). A rise in intracellular glucose can activate metabolic stress pathways, some of which have been proposed to play a role in insulin resistance and islet dysfunction. Consistent with this view, it was shown that exposure to a hyperglycemic environment alone is sufficient to activate JNK in endothelial cells (84) but not in hepatocytes (50).

Hexosamine pathway

The hexosamine pathway was also proposed as a mechanism by which excessive intracellular glucose concentrations could cause insulin resistance and β-cell dysfunction (85). Although the majority of intracellular glucose is channeled into glycolysis to form pyruvate, a small portion of fructose-6-phosphate is directed to the hexosamine pathway, which involves conversion to glucosamine-6-phosphate by the rate-limiting enzyme glutamine fructose-6-phosphate amidotransferase (GFAT), to subsequently generate uridindiphosphoglucose-*n*-acetylglucosamine (UDP-Gl-N), a basic substrate for most protein glycosylation pathways (85). Exposure to glucosamine has been shown to trigger insulin resistance in adipocytes (86), muscle cells (87), and *in vivo* (88). Furthermore, GFAT overexpression impairs glucose-stimulated insulin secretion in rat islets (89). Although the mechanism of glucosamine-induced insulin resistance remains unclear, it was shown that glucosamine induces endoplasmic reticulum stress (ER stress) (90), NF-kB activity (90), and JNK activation (91) (**Fig. 4*A***).

Intracellular AGEs

AGEs accumulate intracellularly, but to date it is not known whether intracellular AGEs play a direct role in insulin resistance. However, it was proposed that mitochondrial reactive oxygen species (ROS) production promotes the accumulation of intracellular AGEs and glucosamine by inactivation of the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and consequent reduction of glucose catabolism by glycolysis (92). Therefore, according to this model, mitochondrial ROS production, the hexosamine pathway, and intracellular AGEs might act as a cluster of stressors brought about by high intracellular glucose concentrations (82, 92, 93) (Fig. 4*A*).

De novo lipogenesis (DNL)

DNL is the pathway by which glucose is converted into lipids, a process occurring mostly in adipose tissue and liver under physiological conditions. It was proposed that tissues with a low lipogenic activity under normal conditions, such as muscle and pancreatic islets, might undergo substantial DNL in obese subjects, in response to hyperglycemia and hyperinsulinemia. Therefore, hyperglycemia and hyperinsulinemia might also contribute to ectopic lipid deposition and lipotoxicity during obesity (94) (Fig. 4*A*).

Intracellular lipids

Intracellular lipids were proposed to be a major factor in the pathogenesis of insulin resistance and pancreatic β-cell dysfunction (45–47, 95, 96). Lipids are normally stored in adipocytes, but chronic excessive energy intake in the context of low-energy expenditure leads to exceeding adipose tissue expansion and ectopic lipid deposition. In cell culture models, LCSFA exposure causes oxidative stress, ER stress, insulin resistance, β-cell dysfunction, and adipocyte hypertrophy and induces cell death in several cell types, including hepatocytes, muscle cells, adipocytes, and β cells (48–51, 97–99). The mechanisms for the deleterious effects of LCSFAs are only partially understood. Exposure of cultured cells to LCSFAs, and in particular PA, leads to changes in the composition of cellular membranes, resulting in decreased membrane fluidity and morphological abnormalities of mitochondria and ER (98–100). In addition to membrane structural derangements, lipid metabolites such as ceramides, diacylglycerol, lysophosphatidic acid, and lysophosphatidylcholine may also be involved in the toxic effects of high intracellular lipids

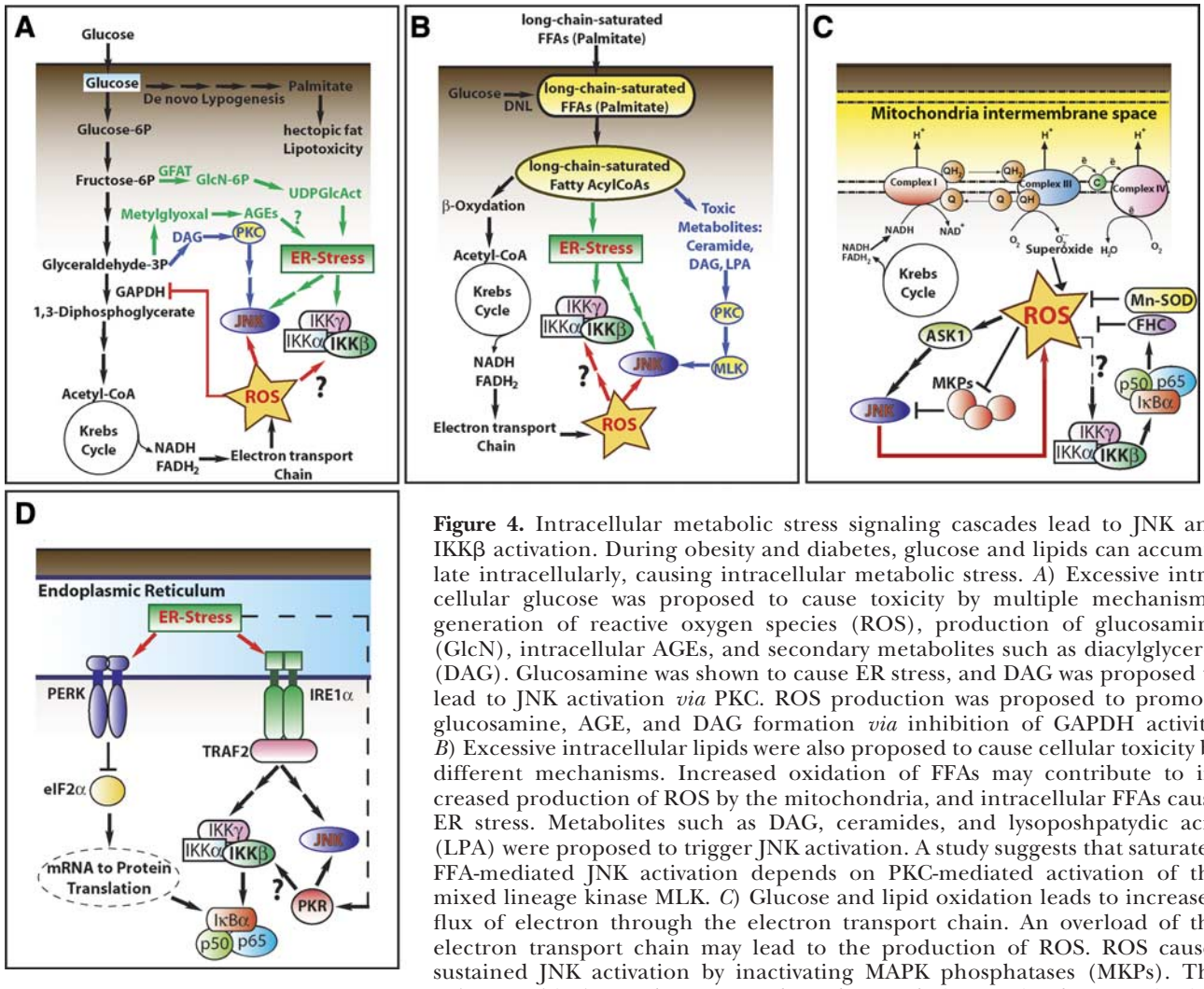

Figure 4. Intracellular metabolic stress signaling cascades lead to JNK and IKKβ activation. During obesity and diabetes, glucose and lipids can accumulate intracellularly, causing intracellular metabolic stress. *A*) Excessive intracellular glucose was proposed to cause toxicity by multiple mechanisms: generation of reactive oxygen species (ROS), production of glucosamine (GlcN), intracellular AGEs, and secondary metabolites such as diacylglycerol (DAG). Glucosamine was shown to cause ER stress, and DAG was proposed to lead to JNK activation *via* PKC. ROS production was proposed to promote glucosamine, AGE, and DAG formation *via* inhibition of GAPDH activity. *B*) Excessive intracellular lipids were also proposed to cause cellular toxicity by different mechanisms. Increased oxidation of FFAs may contribute to increased production of ROS by the mitochondria, and intracellular FFAs cause ER stress. Metabolites such as DAG, ceramides, and lysoposhpatydic acid (LPA) were proposed to trigger JNK activation. A study suggests that saturated FFA-mediated JNK activation depends on PKC-mediated activation of the mixed lineage kinase MLK. *C*) Glucose and lipid oxidation leads to increased flux of electron through the electron transport chain. An overload of the electron transport chain may lead to the production of ROS. ROS causes sustained JNK activation by inactivating MAPK phosphatases (MKPs). The MAPKKK ASK1 was also proposed to play a role in sustained JNK activation induced by ROS. The role of ROS on IKKβ activation is not clear, and IKKβ was shown to increase the expression of the antioxidant Mn-superoxyde dismutase (Mn-SOD) and ferritin heavy chain (FHC). *D*) Obesity causes ER stress in different cell types. ER stress leads to JNK and IKKβ activation *via* IRE1α-TRAF2 signaling. ER stress also leads to decreased protein translation *via* PERK-dependent phosphorylation of eIF2α, which may synergize with IKKβ-mediated NF-kB activation by reducing IkBα translation rate. Efficient ER stress-mediated JNK activation was reported to be dependent on the kinase PKR. However, the mechanism of ER stress-mediated PKR activation is not completely understood.

(2, 101). JNK activation is believed to play an important role in lipotoxicity. Long-chain saturated FFAs trigger sustained JNK activation in different cell types, and it was proposed that prolonged JNK activation causes insulin resistance, β-cell dysfunction, and cell death in these models (48–51). TLR4 and TLR2 have been proposed to contribute to JNK and IKKβ activation in response to circulating lipids by sensing the extracellular meillieu (21, 51), but it is unlikely that these membrane receptors are also the major sensors of lipids accumulating intracellularly. Thus, the major signaling pathway from intracellular lipids to JNK activation remains to be identified. It was suggested that, at least in some cells, PA activates JNK by increasing the intracellular concentrations of ceramides and diacylglycerol (DAG) causing sequential activation of protein kinase C (PKC), mixed lineage kinase 4 (MLK4), and MAPK kinases MKK4 and MKK7, culminating in JNK activation (102). Oxidative stress can also contribute to persistent JNK activation through the inhibition of MAPK phosphatases (MPKs) (103). ER stress, which is induced by LCSFAs but not by unsaturated FFAs (98), also can cause JNK and IKKβ activation (104). Overall, several signaling pathways are stimulated by intracellular toxic lipids, including ER stress, ROS, and PKC, which can all lead to JNK activation (Fig. 4*B*). Importantly, interference with JNK activation or activity is protective in different models of lipotoxicity (28, 48–50, 101).

Oxidative stress

Oxidative stress was proposed as an important mechanism through which excessive intracellular glucose

and lipids may impair glucose homeostasis. The exaggerated flux of glucose through glycolysis and the Krebs cycle results in NADH and FADH production that, when excessive, may overload the mitochondrial respiratory chain. Likewise, increased flux of lipids through β-oxidation may also contribute to an excessive electron flow. In such scenarios oxygen molecules could be converted to form superoxide anion, which can then be converted to hydrogen peroxide by superoxide dismutase (105–108). Superoxide and hydrogen peroxide react with other molecules in the cell to form different ROS (Fig. 4*C*). Another mechanism by which intracellular hyperglycemia could induce ROS production is by increased production of DAG, which can cause activation of NADPH oxidase *via* PKC (109). Oxidative stress was proposed to induce insulin resistance and pancreatic β-cell dysfunction, including reduced insulin production, defective insulin secretion, and β-cell death (110, 111). In addition to JNK activation (103), oxidative stress was proposed to activate NF-kB (112–114). However, the link between NF-kB and ROS has become complex because NF-kB activation has antioxidant functions (103, 115, 116). JNK activation may, however, promote ROS accumulation (117) and link ROS production to insulin resistance and loss of β-cell function (118–120).

ER stress

Nutrient deprivation, changes in calcium concentration, accumulation of unfolded proteins, abnormal post-translational modifications, and excessive elevation of intracellular lipids cause ER stress and trigger signaling cascades that are initiated at the ER and lead to activation of an adaptive response called the unfolded protein response (UPR), whose goal is to restore homeostasis (98, 99, 121–125). ER stress was proposed to be a major factor in the pathogenesis of obesity-induced insulin resistance (126), and it was shown that synthetic chaperones, which dissolve protein aggregates, restore normal glycemia in obese mice (127). ER stress leads to activation of JNK1 and IKKβ signaling (104). The pathways from ER stress to JNK1 and IKKβ are still being investigated; however, inositol-requiring enzyme 1α (IRE1α), a major signaling molecule in UPR, was shown to link ER stress to JNK and IKKβ activation (104). IRE1α forms a complex with the adapter protein TRAF2, leading to activation of apoptosis signal-regulating kinase 1 (ASK1) and consequent JNK1 activation (128) (Fig. 4*D*). It was reported that IKKβ forms a complex with IRE1α *via* association with TRAF2 and that ER stress activation of NF-kB is impaired in mouse embrionic fibroblasts lacking a functional *IRE1*α gene (129) (Fig. 4*D*). Furthermore, it was shown that ER stress and PA-mediated JNK activation are, at least in part, dependent on the proinflammatory kinase PKR, although the pathway linking PA and ER-stress to PKR is not completely understood (130) (Fig. 4*D*).

Islet amyloid polypeptide (IAPP)

IAPP forms amyloid aggregates in islets of type 2 diabetes patients and was proposed to play a role in β-cell death and dysfunction. IAPP activates JNK1 in β cells, and IAPP-induced cell death is inhibited by a JNK inhibitor and JNK1 antisense olygonucleotides (131).

JNK1 AND IKKβ LINK METABOLIC STRESS TO INSULIN RESISTANCE

JNK1 and IKKβ contribute to insulin resistance and type 2 diabetes through multiple mechanisms. Serine/threonine phosphorylation of insulin receptor substrates 1 and 2 (IRS1-2) is believed to be an important mediator of insulin resistance because it results in uncoupling of insulin receptor activation from downstream signaling (132–134). This hypothesis is supported by a study showing that mice expressing an IRS1 variant in which the serine phosphorylation sites Ser-302, Ser-307, and Ser-612 were replaced by alanines (IRS1 *Ser→Ala*) are protected from high-fat diet-induced insulin resistance (135). By contrast, mice expressing an IRS1 variant with only Ser-307 mutated to alanine are more susceptible to developing insulin resistance when fed a high-fat diet (136). Therefore, the inhibitory action of IRS1 serine/threonine phosphorylation on insulin signaling could be independent from Ser-307 phosphorylation. IKKβ was reported to phosphorylate IRS1 on multiple serine residues (137–139), but the contribution of direct IRS1 phosphorylation by IKKβ to induction of insulin resistance is not clear because IKKβ seems to exert its diabetogenic effects *via* NF-kB activation in a cell type-specific manner (29, 140). For JNK1, however, there is a good correlation between its activation and IRS1, IRS2 serine/threonie phosphorylation (11, 50, 141–143), a correlation that so far has not been observed for IKKβ.

Another mechanism by which JNK1 and IKKβ promote insulin resistance is by induction of proinflammatory cytokines in different cell types, especially within macrophages (28, 29). IKKβ overexpression in hepatocytes causes local and systemic induction of proinflammatory genes and systemic insulin resistance in the absence of obesity (140). Interestingly, IKKβ-induced insulin resistance is nearly fully reversed with neutralizing antibodies against IL-6 (140). Mice with a targeted disruption of the *Ikk*β gene in hepatocytes display decreased liver expression of proinflammatory cytokines and improved insulin-mediated suppression of hepatic glucose production, but glucose uptake and metabolism in other tissues remain unaffected (29). By contrast, disruption of IKKβ in myeloid cells results in a systemic improvement of insulin sensitivity in a model of diet-induced insulin resistance (29). These results strongly suggest that a major mechanism of IKKβ action in obesity-induced insulin resistance depends on

production of proinflammatory cytokines by myeloid cells, such as ATMs.

Induction of proinflammatory gene expression is also an important mechanism in JNK1-promoted insulin resistance. *Jnk1*$^{-/-}$ mice show decreased expression of proinflammatory cytokines in models of diet-induced insulin resistance (144), and a conditional deletion of *Jnk1* in adipocytes decreases IL-6 serum levels and improves hepatic insulin sensitivity (145). However, the role of adipocytes as critical sources for IL-6 remains to be established because it was reported that the majority of IL-6 released by human adipose tissue comes from cells other than adipocytes (146). It was also reported that LCSFA activate JNK in macrophages and induce IL-6, IL-1β, and TNF-α expression in a JNK1-dependent manner (27, 28). Furthermore, macrophages from *Jnk1*$^{-/-}$ mice exposed to PA release less IL-6 and TNF-α than macrophages from WT mice (145). In addition, conditioned medium of PA-exposed WT macrophages can induce insulin resistance in cultured muscle cells, whereas conditioned medium from *Jnk1*$^{-/-}$ macrophages exposed to PA does not (28). Nonetheless, it was reported that knockout of *Jnk1* in myeloid cells mediated by expession of *Cre* recombinase under control of the Lysozyme-M (LysM) promoter did not improve glucose homeostasis in obese mice (145). However, it is well established that the LysM promoter is not active in either Kupffer cells or in lymphocytes (147). As Kupffer cells (resident liver macrophages) are a major source for IL-6 in the liver during obesity (140) and PA-mediated induction of IL-6 gene expression in cultured Kupffer cells is JNK1 dependent (28), it is possible that JNK1 promotes insulin resistance by inducing expression of proinflammatory cytokines within Kupffer cells and perhaps other hematopoietic cells in which the LysM promoter is inactive. Consistent with this view, mice lacking *Jnk1* in hematopoietic cells (including lymphoid and Kupffer cells), generated by adoptive transfer, display decreased expression of proinflammatory cytokines during diet-induced obesity, and deletion of JNK1 within hematopoietic cells is protective toward obesity-induced insulin resistance (27, 28). Furthermore, JNK1 activity in hematopoietic cells is required for the transition from steatosis to steatohepatitis in the choline deficiency model of steatohepatitis (148). Altogether these studies show that JNK1 activity in hematopoietic cells and in adipocytes promotes metabolic inflammation during obesity and steatosis at least in part through the induction of inflammatory cytokines (**Fig. 5*A***).

A ROLE FOR JNK1 AND IKKβ IN THE DEVELOPMENT OF OBESITY

Both JNK1 and IKKβ exert substantial effects on the development of adiposity, and in the case of IKKβ, these effects were shown to depend on its activation within the hypothalamus. Increased NF-kB activity was observed in the hypothalamus of mice fed a high-fat diet (HFD) (149). Lentivirus-mediated overexpression of a dominant negative IKKβ mutant in the mediobasal hypothalamus (MBH) protected mice from HFD-induced obesity by decreasing food intake, whereas overexpression of a constitutively active IKKβ mutant in the MBH promoted food intake and weight gain (149). Congruently, mice bearing a conditional *Ikk*β gene disruption in the brain are resistant to HFD-induced obesity and display decreased food intake compared to controls (149). It was proposed that activation of the IKKβ→NF-kB axis in the hypothalamus promotes food intake and obesity through induction of SOCS3 gene expression, leading to inhibition of leptin signaling *via* the JAK-STAT axis (149) (Fig. 5*B*). It was also proposed that central activation of the IKKβ→NF-kB axis in mice fed an HFD is in part due to ER stress (149) and that ER stress is involved in central leptin and insulin resistance (149, 150). To date it is not known how a high-fat diet causes ER stress in the hypothalamus and whether ER stress operates in synergy with other pathways. Indeed, mice with a targeted disruption of the *MyD88* gene in the central nervous system are protected from diet-induced obesity and leptin resistance and display reduced central IKKβ activation by HFD (151). Because ER stress is also known to activate JNK, it was proposed that hypothalamic activation of JNK1 could also favor positive energy balance (152) (Fig. 5*B*). *Jnk1*-knockout mice were found to be obesity resistant in both dietary and genetic models (11). Total adiposity is decreased by ~50% in *Jnk1*$^{-/-}$ mice fed an HFD, with subcutaneous and intraperitoneal adipose tissue depots affected to the same extent (28).

The mechanism by which JNK1 affects energy balance and/or lipid metabolism is still under investigation. This function has been mapped to a tissue of nonhematopoietic origin involved in the control of energy expenditure (28). Conditional *Jnk1* gene mutation studies suggest that JNK1 activity in neurons but not in adipocytes, myocytes, hepatocytes, or myeloid cells is required for efficient HFD-induced obesity (143, 145, 153, 154). It was also shown that conditional *Jnk1* gene deletion in neurons leads to activation of the hypothalamic-pituitary-thyroid axis. Indeed, mice lacking JNK1 in the nervous system display increased expression of hypothalamic thyrotropin-releasing hormone (TRH) and pituitary thyroid-stimulating hormone (TSH). This enhanced TRH and TSH expression was associated with increased circulating levels of thyroid hormones (T3 and T4). Another study showed that JNK1 in neurons is not required for HFD-induced leptin resistance (151). Whether JNK1 plays a role in HFD-induced neuronal insulin resistance is not known (Fig. 5*B*).

Comparison of mice bearing *Jnk1* deletion in the nonhematopoietic compartment to weight-matched controls indicates that the improved glucose homeostasis in *Jnk1*$^{-/-}$ mice is partially due to their leaner phenotype (28). Conditional gene deletion studies

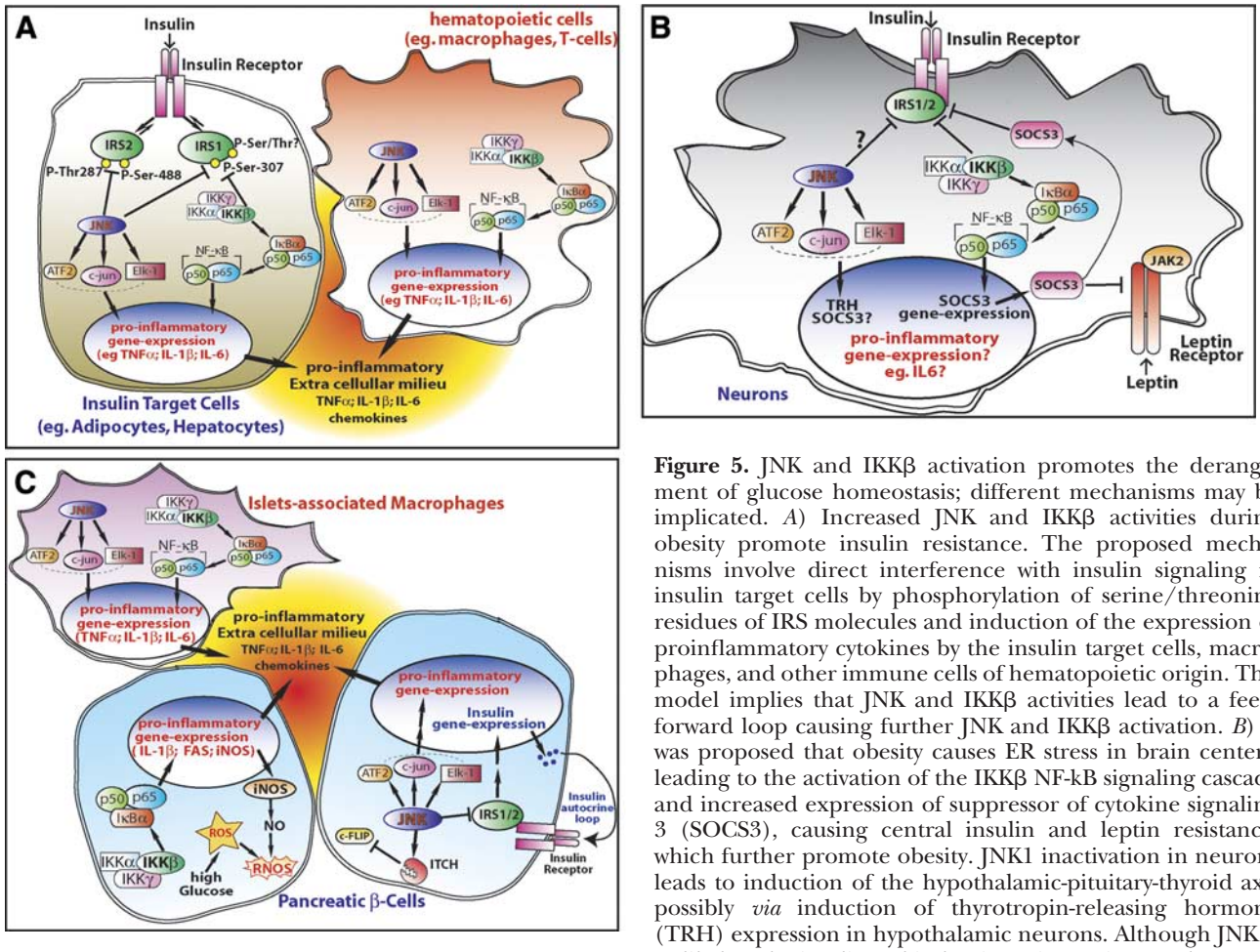

Figure 5. JNK and IKKβ activation promotes the derangement of glucose homeostasis; different mechanisms may be implicated. *A*) Increased JNK and IKKβ activities during obesity promote insulin resistance. The proposed mechanisms involve direct interference with insulin signaling in insulin target cells by phosphorylation of serine/threonine residues of IRS molecules and induction of the expression of proinflammatory cytokines by the insulin target cells, macrophages, and other immune cells of hematopoietic origin. This model implies that JNK and IKKβ activities lead to a feed-forward loop causing further JNK and IKKβ activation. *B*) It was proposed that obesity causes ER stress in brain centers, leading to the activation of the IKKβ NF-kB signaling cascade and increased expression of suppressor of cytokine signaling 3 (SOCS3), causing central insulin and leptin resistance, which further promote obesity. JNK1 inactivation in neurons leads to induction of the hypothalamic-pituitary-thyroid axis possibly *via* induction of thyrotropin-releasing hormone (TRH) expression in hypothalamic neurons. Although JNK is unlikely to be implicated in leptin resitance it may play a role in central insulin resistance. Furthermore, SOCS3 transcription is induced by IL-6, whose gene expression is controlled by JNK and IKKβ in different systems. *C*) JNK and IKKβ were proposed to be involved in β-cell dysfunction. Possible mechanisms may involve induction of proinflammatory cytokines gene expression such as IL-1β in pancreatic β-cells and in islet-associated macrophages, and interference with insulin autocrine signal. IKKβ activation also leads to increased iNOS gene expression and excessive NO production might be toxic for the β cell. We propose here that sustained JNK activation may also sensitize β cells to apoptosis by decreasing the intracellular concentration of the antiapoptotic protein c-FLIP$_L$. RNOS, reactive nitrogen oxide species.

consistently show that JNK1 inactivation in neurons, which causes a leaner phenotype, is sufficient to improve insulin and glucose tolerance in the HFD model, whereas JNK1 inactivation in myeloid cells, adipocytes, hepatocytes, or myocytes at best improved insulin tolerance but not glucose tolerance (143, 145, 153, 154). Overall, these results suggest that the improved glucose tolerance observed in JNK1 knockout mice is partly due to their leaner phenotype.

JNK1 AND IKKβ IN OBESITY-INDUCED β-CELL FAILURE

Glucose homeostasis depends on the equilibrium between insulin sensitivity and insulin production by pancreatic β cells, and type 2 diabetes develops in insulin-resistant subjects with insufficient compensatory insulin secretion (155). The β cells are particularly sensitive to metabolic stress because they have low antioxidant defenses (156) and are susceptible to cytokine-induced cell death (157). Recent data strongly suggest that targeting inflammation might be an effective approach to improve the β-cell compensatory response in type 2 diabetes. Indeed, a placebo-controlled double-blind clinical trial involving 70 type 2 diabetes patients showed that treatment with IL-1 receptor antagonist (IL-1Ra) improves glucose homeostasis by enhancing β-cell function with no observable side effects (60). This observation was recapitulated in mice fed an HFD in which IL-1Ra administration improved glucose homeostasis and β-cell survival and function (158). The fact that JNK1 and IKKβ are activated by IL-1R (Fig. 3*B*) makes them candidate effectors for the deleterious effects of IL-1 signaling on pancreatic islets. Indeed, it was shown that transgenic mice expressing in β cells a nondegradable form of the IKKβ substrate IkBα, known as IkBα super repressor (IkBα-SR), are protected from induction of diabetes by multiple low-

dose streptozotocin injections, a model characterized by loss of β-cell mass and function (159). Moreover mouse islets expressing the IkBα-SR transgene are protected from the deleterious effects of IL-1β + INF-γ (159). Human islets infected with an adenovirus expressing IkBα-SR are also protected from the effects of IL-1β on glucose-stimulated insulin secretion and were more resistant to Fas-induced apoptosis (160). The mechanism by which the IKKβ-NF-kB signaling pathway promotes β-cell death and dysfunction, while promoting survival in most other cell types, is not established. However, NF-kB also mediates the induction of proinflammatory cytokines, Fas, and inducible nitric oxyde synthase, all of which can contribute to β-cell cytotoxicity (Fig. 5*C*).

There is ample evidence that JNK is a major mediator of islet dysfunction and death. *Jnk1*$^{-/-}$ mice are protected from multiple low-dose STZ administration (161), and JNK inhibition improves glycemic control in mice after pancreatic islet transplantation (162). Moreover, obese mice treated with a specific JNK peptide inhibitor (DJNKi) showed improved glucose tolerance and increased pancreatic insulin mRNA and protein levels (163). We reported that pancreatic islets from *Jnk1*$^{-/-}$ mice or wild-type islets treated with DJNKi are protected from inhibition of glucose-induced insulin gene expression by PA (50). Others have observed that mouse islets infected with an adenovirus expressing a dominant negative JNK1 mutant are also protected from inhibition of insulin gene expression by oxidative stress (162). It was also shown that exposure of human pancreatic islets to high glucose and leptin leads to decreased insulin secretion and causes β-cell death, by a mechanism involving an autocrine IL-1β production and JNK activity (164). The mechanism by which JNK contributes to β-cell dysfunction might involve inhibition of autocrine insulin signaling (50) and nuclear-cytoplasmic translocation of the transcription factor PDX-1 (165), which are both required for insulin gene expression. Although the downstream mediators of JNK action in β-cell death are not known, we found that sustained JNK activation induces degradation of the antiapoptotic protein c-FLIP$_L$ in different cell types (166). c-FLIP$_L$ is a major survival signal in β cells, required for protection from TNF-α-induced β-cell death (167). c-FLIP$_L$ also switches high-glucose-induced Fas signaling from apoptosis to cell proliferation (168). We therefore speculate that during obesity sustained JNK activation within β cells could promote cell death at least in part by inducing c-FLIP$_L$ degradation.

In obesity, macrophages infiltrate into pancreatic islets, and because macrophages are a major source of proinflammatory cytokines, it is plausible that islet-associated macrophages play an important role in β-cell dysfunction and death (169). The role of JNK1 and IKKβ in islet-associated macrophages was not investigated, but it is likely to be similar to their role in other macrophages where JNK1 and IKKβ are implicated in the production of cytokines and other inflammatory mediators (27–29).

CONCLUSIONS

Well before the discovery of insulin, it was known that high doses of salicylates have beneficial effects on diabetic patients (13). The discovery that interference with TNF-α signaling improves glucose homeostasis in obese rodents demonstrated that inflammation might be involved in obesity-induced glucose intolerance (15, 16). Today, a large body of evidence supports the view that metabolic inflammation is a major cause of obesity-induced insulin resistance and β-cell dysfunction. A recent clinical trial showed that the salicylate salsalate improves insulin sensitivity and decreases inflammatory markers in obese nondiabetic individuals (170), whereas another trial showed that IL-1Ra decreases markers of inflammation and ameliorates glycemic control by improving β-cell function in type 2 diabetes patients (60). These studies demonstrate that specific anti-inflammatory interventions in obese individuals and individuals with type 2 diabetes may improve glycemic control, insulin sensitivity, and β-cell function.

The proinflammatory kinases JNK1 and IKKβ are activated by almost all the signaling pathways proposed to cause insulin resistance or β-cell dysfunction, and their inhibition provides protection from obesity and glucose intolerance in rodents (11, 12, 27–29, 144, 149, 162, 163). Interestingly JNK1 and IKKβ are activated downstream from IL-1R (Fig. 3*B*), and salicylate inhibits IKKβ activity and JNK activation at doses effective for its antidiabetogenic activity (171, 172). Therefore, it is possible that reduced JNK1 and IKKβ signaling may account for at least part of the beneficial effects of salsalate and IL-1Ra observed in clinical trials. Furthermore, a mutation on the gene expressing the JNK interacting protein-1 (JIP-1), causing reduced negative modulation on JNK signaling, was shown to segregate with type 2 diabetics (173). Taken together, the evidence strongly suggests that JNK1 and IKKβ are candidate targets for a new class of oral antidiabetogenic drugs targeting metabolic stress.

This review is supported by a Swiss National Science Foundation grant SNF31003A-118172 to G.S. The authors thank Barbara Becattini and Clive M. Brown for their critical comments.

REFERENCES

1. Karin, M., Lawrence, T., and Nizet, V. (2006) Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell* **124,** 823–835
2. Schenk, S., Saberi, M., and Olefsky, J. M. (2008) Insulin sensitivity: modulation by nutrients and inflammation. *J. Clin. Invest.* **118,** 2992–3002

3. Hotamisligil, G. S. (2006) Inflammation and metabolic disorders. *Nature* **444,** 860–867
4. Weston, C. R., and Davis, R. J. (2007) The JNK signal transduction pathway. *Curr. Opin. Cell Biol.* **19,** 142–149
5. Chang, L., and Karin, M. (2001) Mammalian MAP kinase signalling cascades. *Nature* **410,** 37–40
6. Chen, C. Y., Del Gatto-Konczak, F., Wu, Z., and Karin, M. (1998) Stabilization of interleukin-2 mRNA by the c-Jun NH2-terminal kinase pathway. *Science* **280,** 1945–1949
7. Chen, C. Y., Gherzi, R., Andersen, J. S., Gaietta, G., Jurchott, K., Royer, H. D., Mann, M., and Karin, M. (2000) Nucleolin and YB-1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. *Genes Dev.* **14,** 1236–1248
8. Hacker, H., and Karin, M. (2006) Regulation and function of IKK and IKK-related kinases. *Sci. STKE* 2006, re13
9. Senftleben, U., and Karin, M. (2002) The IKK/NF-kappa B pathway. *Crit. Care Med.* **30,** S18–S26
10. Luo, J. L., Kamata, H., and Karin, M. (2005) IKK/NF-kappaB signaling: balancing life and death: a new approach to cancer therapy. *J. Clin. Invest.* **115,** 2625–2632
11. Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C. Z., Uysal, K. T., Maeda, K., Karin, M., and Hotamisligil, G. S. (2002) A central role for JNK in obesity and insulin resistance. *Nature* **420,** 333–336
12. Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z. W., Karin, M., and Shoelson, S. E. (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* **293,** 1673–1677
13. Williamson, R. (1901) On the treatment of glycosuria and diabetes mellitus with sodium salicylate. *Br. Med. J.* **1,** 760–762
14. Reid, J., Macdougall, A. I., and Andrews, M. M. (1957) Aspirin and diabetes mellitus. *Br. Med. J.* **2,** 1071–1074
15. Uysal, K. T., Wiesbrock, S. M., Marino, M. W., and Hotamisligil, G. S. (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* **389,** 610–614
16. Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **259,** 87–91
17. Jager, J., Gremeaux, T., Cormont, M., Le Marchand-Brustel, Y., and Tanti, J. F. (2007) Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* **148,** 241–251
18. Klover, P. J., Clementi, A. H., and Mooney, R. A. (2005) Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* **146,** 3417–3427
19. Pedersen, B. K., and Febbraio, M. A. (2007) Point: interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. *J. Appl. Physiol.* **102,** 814–816
20. Pedersen, B. K., and Febbraio, M. A. (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* **88,** 1379–1406
21. Shi, H., Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H., and Flier, J. S. (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. *J. Clin. Invest.* **116,** 3015–3025
22. Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, J., Nichols, A., Ross, J. S., Tartaglia, L. A., and Chen, H. (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* **112,** 1821–1830
23. Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W., Jr. (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* **112,** 1796–1808
24. Cinti, S., Mitchell, G., Barbatelli, G., Murano, I., Ceresi, E., Faloia, E., Wang, S., Fortier, M., Greenberg, A. S., and Obin, M. S. (2005) Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J. Lipid Res.* **46,** 2347–2355
25. Lumeng, C. N., Bodzin, J. L., and Saltiel, A. R. (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* **117,** 175–184
26. Lumeng, C. N., Deyoung, S. M., Bodzin, J. L., and Saltiel, A. R. (2007) Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes* **56,** 16–23
27. Vallerie, S. N., Furuhashi, M., Fucho, R., and Hotamisligil, G. S. (2008) A predominant role for parenchymal c-Jun amino terminal kinase (JNK) in the regulation of systemic insulin sensitivity. *PLoS ONE* **3,** e3151
28. Solinas, G., Vilcu, C., Neels, J. G., Bandyopadhyay, G. K., Luo, J. L., Naugler, W., Grivennikov, S., Wynshaw-Boris, A., Scadeng, M., Olefsky, J. M., and Karin, M. (2007) JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab.* **6,** 386–397
29. Arkan, M. C., Hevener, A. L., Greten, F. R., Maeda, S., Li, Z. W., Long, J. M., Wynshaw-Boris, A., Poli, G., Olefsky, J., and Karin, M. (2005) IKK-beta links inflammation to obesity-induced insulin resistance. *Nat. Med.* **11,** 191–198
30. Furuhashi, M., Fucho, R., Gorgun, C. Z., Tuncman, G., Cao, H., and Hotamisligil, G. S. (2008) Adipocyte/macrophage fatty acid-binding proteins contribute to metabolic deterioration through actions in both macrophages and adipocytes in mice. *J. Clin. Invest.* **118,** 2640–2650
31. Lesniewski, L. A., Hosch, S. E., Neels, J. G., de Luca, C., Pashmforoush, M., Lumeng, C. N., Chiang, S. H., Scadeng, M., Saltiel, A. R., and Olefsky, J. M. (2007) Bone marrow-specific Cap gene deletion protects against high-fat diet-induced insulin resistance. *Nat. Med.* **13,** 455–462
32. Odegaard, J. I., Ricardo-Gonzalez, R. R., Goforth, M. H., Morel, C. R., Subramanian, V., Mukundan, L., Eagle, A. R., Vats, D., Brombacher, F., Ferrante, A. W., and Chawla, A. (2007) Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* **447,** 1116–1120
33. Hevener, A. L., Olefsky, J. M., Reichart, D., Nguyen, M. T., Bandyopadyhay, G., Leung, H. Y., Watt, M. J., Benner, C., Febbraio, M. A., Nguyen, A. K., Folian, B., Subramaniam, S., Gonzalez, F. J., Glass, C. K., and Ricote, M. (2007) Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. *J. Clin. Invest.* **117,** 1658–1669
34. Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., Kitazawa, S., Miyachi, H., Maeda, S., Egashira, K., and Kasuga, M. (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* **116,** 1494–1505
35. Patsouris, D., Li, P. P., Thapar, D., Chapman, J., Olefsky, J. M., and Neels, J. G. (2008) Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metab.* **8,** 301–309
36. Liu, J., Divoux, A., Sun, J., Zhang, J., Clement, K., Glickman, J. N., Sukhova, G. K., Wolters, P. J., Du, J., Gorgun, C. Z., Doria, A., Libby, P., Blumberg, R. S., Kahn, B. B., Hotamisligil, G. S., and Shi, G. P. (2009) Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat. Med.* **15,** 940–945
37. Lumeng, C. N., Maillard, I., and Saltiel, A. R. (2009) T-ing up inflammation in fat. *Nat. Med.* **15,** 846–847
38. Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., Yoshimura, K., Kadowaki, T., and Nagai, R. (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.* **15,** 914–920
39. Winer, S., Chan, Y., Paltser, G., Truong, D., Tsui, H., Bahrami, J., Dorfman, R., Wang, Y., Zielenski, J., Mastronardi, F., Maezawa, Y., Drucker, D. J., Engleman, E., Winer, D., and Dosch, H. M. (2009) Normalization of obesity-associated insulin resistance through immunotherapy. *Nat. Med.* **15,** 921–929
40. Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A. B., Benoist, C., Shoelson, S., and Mathis, D. (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* **15,** 930–939
41. Medina-Gomez, G., Gray, S. L., Yetukuri, L., Shimomura, K., Virtue, S., Campbell, M., Curtis, R. K., Jimenez-Linan, M., Blount, M., Yeo, G. S., Lopez, M., Seppanen-Laakso, T., Ashcroft, F. M., Oresic, M., and Vidal-Puig, A. (2007) PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. *PLoS Genet.* **3,** e64
42. Cancello, R., Henegar, C., Viguerie, N., Taleb, S., Poitou, C., Rouault, C., Coupaye, M., Pelloux, V., Hugol, D., Bouillot, J. L., Bouloumie, A., Barbatelli, G., Cinti, S., Svensson, P. A., Barsh,

G. S., Zucker, J. D., Basdevant, A., Langin, D., and Clement, K. (2005) Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* **54,** 2277–2286
43. Schernthaner, G. H., Kopp, H. P., Kriwanek, S., Krzyzanowska, K., Satler, M., Koppensteiner, R., and Schernthaner, G. (2006) Effect of massive weight loss induced by bariatric surgery on serum levels of interleukin-18 and monocyte-chemoattractant-protein-1 in morbid obesity. *Obes. Surg.* **16,** 709–715
44. Lefkowitch, J. H., Haythe, J. H., and Regent, N. (2002) Kupffer cell aggregation and perivenular distribution in steatohepatitis. *Mod. Pathol.* **15,** 699–704
45. Unger, R. H. (2003) Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology* **144,** 5159–5165
46. Savage, D. B., Petersen, K. F., and Shulman, G. I. (2007) Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol. Rev.* **87,** 507–520
47. Virtue, S., and Vidal-Puig, A. (2008) It's not how fat you are, it's what you do with it that counts. *PLoS Biol.* **6,** e237
48. Nguyen, M. T., Satoh, H., Favelyukis, S., Babendure, J. L., Imamura, T., Sbodio, J. I., Zalevsky, J., Dahiyat, B. I., Chi, N. W., and Olefsky, J. M. (2005) JNK and tumor necrosis factor-alpha mediate free fatty acid-induced insulin resistance in 3T3–L1 adipocytes. *J. Biol. Chem.* **280,** 35361–35371
49. Malhi, H., Bronk, S. F., Werneburg, N. W., and Gores, G. J. (2006) Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. *J. Biol. Chem.* **281,** 12093–12101
50. Solinas, G., Naugler, W., Galimi, F., Lee, M. S., and Karin, M. (2006) Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 16454–16459
51. Senn, J. J. (2006) Toll-like receptor-2 is essential for the development of palmitate-induced insulin resistance in myotubes. *J. Biol. Chem.* **281,** 26865–26875
52. Zheng, C., Xiang, J., Hunter, T., and Lin, A. (1999) The JNKK2-JNK1 fusion protein acts as a constitutively active c-Jun kinase that stimulates c-Jun transcription activity. *J. Biol. Chem.* **274,** 28966–28971
53. Nguyen, M. T., Favelyukis, S., Nguyen, A. K., Reichart, D., Scott, P. A., Jenn, A., Liu-Bryan, R., Glass, C. K., Neels, J. G., and Olefsky, J. M. (2007) A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. *J. Biol. Chem.* **282,** 35279–35292
54. Kawai, T., and Akira, S. (2007) TLR signaling. *Semin. Immunol.* **19,** 24–32
55. Tsukumo, D. M., Carvalho-Filho, M. A., Carvalheira, J. B., Prada, P. O., Hirabara, S. M., Schenka, A. A., Araujo, E. P., Vassallo, J., Curi, R., Velloso, L. A., and Saad, M. J. (2007) Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes* **56,** 1986–1998
56. Poggi, M., Bastelica, D., Gual, P., Iglesias, M. A., Gremeaux, T., Knauf, C., Peiretti, F., Verdier, M., Juhan-Vague, I., Tanti, J. F., Burcelin, R., and Alessi, M. C. (2007) C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet. *Diabetologia* **50,** 1267–1276
57. Saberi, M., Woods, N. B., de Luca, C., Schenk, S., Lu, J. C., Bandyopadhyay, G., Verma, I. M., and Olefsky, J. M. (2009) Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab.* **10,** 419–429
58. Spranger, J., Kroke, A., Mohlig, M., Hoffmann, K., Bergmann, M. M., Ristow, M., Boeing, H., and Pfeiffer, A. F. (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* **52,** 812–817
59. He, J., Usui, I., Ishizuka, K., Kanatani, Y., Hiratani, K., Iwata, M., Bukhari, A., Haruta, T., Sasaoka, T., and Kobayashi, M. (2006) Interleukin-1alpha inhibits insulin signaling with phosphorylating insulin receptor substrate-1 on serine residues in 3T3–L1 adipocytes. *Mol. Endocrinol.* **20,** 114–124
60. Larsen, C. M., Faulenbach, M., Vaag, A., Volund, A., Ehses, J. A., Seifert, B., Mandrup-Poulsen, T., and Donath, M. Y. (2007) Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N. Engl. J. Med.* **356,** 1517–1526
61. Liu, Z. G., Hsu, H., Goeddel, D. V., and Karin, M. (1996) Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. *Cell* **87,** 565–576
62. Matsuzawa, A., Tseng, P. H., Vallabhapurapu, S., Luo, J. L., Zhang, W., Wang, H., Vignali, D. A., Gallagher, E., and Karin, M. (2008) Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science* **321,** 663–668
63. Schumann, D. M., Maedler, K., Franklin, I., Konrad, D., Storling, J., Boni-Schnetzler, M., Gjinovci, A., Kurrer, M. O., Gauthier, B. R., Bosco, D., Andres, A., Berney, T., Greter, M., Becher, B., Chervonsky, A. V., Halban, P. A., Mandrup-Poulsen, T., Wollheim, C. B., and Donath, M. Y. (2007) The Fas pathway is involved in pancreatic beta cell secretory function. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 2861–2866
64. Maedler, K., Spinas, G. A., Lehmann, R., Sergeev, P., Weber, M., Fontana, A., Kaiser, N., and Donath, M. Y. (2001) Glucose induces beta-cell apoptosis via upregulation of the Fas receptor in human islets. *Diabetes* **50,** 1683–1690
65. Chang, H. Y., Nishitoh, H., Yang, X., Ichijo, H., and Baltimore, D. (1998) Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx. *Science* **281,** 1860–1863
66. Oku, A., Nawano, M., Ueta, K., Fujita, T., Umebayashi, I., Arakawa, K., Kano-Ishihara, T., Saito, A., Anai, M., Funaki, M., Kikuchi, M., Oka, Y., and Asano, T. (2001) Inhibitory effect of hyperglycemia on insulin-induced Akt/protein kinase B activation in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **280,** E816–E824
67. Nawano, M., Oku, A., Ueta, K., Umebayashi, I., Ishirahara, T., Arakawa, K., Saito, A., Anai, M., Kikuchi, M., and Asano, T. (2000) Hyperglycemia contributes insulin resistance in hepatic and adipose tissue but not skeletal muscle of ZDF rats. *Am. J. Physiol. Endocrinol. Metab.* **278,** E535–E543
68. Nawano, M., Ueta, K., Oku, A., Arakawa, K., Saito, A., Funaki, M., Anai, M., Kikuchi, M., Oka, Y., and Asano, T. (1999) Hyperglycemia impairs the insulin signaling step between PI 3-kinase and Akt/PKB activations in ZDF rat liver. *Biochem. Biophys. Res. Commun.* **266,** 252–256
69. Miele, C., Riboulet, A., Maitan, M. A., Oriente, F., Romano, C., Formisano, P., Giudicelli, J., Beguinot, F., and Van Obberghen, E. (2003) Human glycated albumin affects glucose metabolism in L6 skeletal muscle cells by impairing insulin-induced insulin receptor substrate (IRS) signaling through a protein kinase C alpha-mediated mechanism. *J. Biol. Chem.* **278,** 47376–47387
70. Olson, L. K., Redmon, J. B., Towle, H. C., and Robertson, R. P. (1993) Chronic exposure of HIT cells to high glucose concentrations paradoxically decreases insulin gene transcription and alters binding of insulin gene regulatory protein. *J. Clin. Invest.* **92,** 514–519
71. Robertson, R. P., Olson, L. K., and Zhang, H. J. (1994) Differentiating glucose toxicity from glucose desensitization: a new message from the insulin gene. *Diabetes* **43,** 1085–1089
72. Goh, S. Y., and Cooper, M. E. (2008) Clinical review: the role of advanced glycation end products in progression and complications of diabetes. *J. Clin. Endocrinol. Metab.* **93,** 1143–1152
73. Taguchi, A., Blood, D. C., del Toro, G., Canet, A., Lee, D. C., Qu, W., Tanji, N., Lu, Y., Lalla, E., Fu, C., Hofmann, M. A., Kislinger, T., Ingram, M., Lu, A., Tanaka, H., Hori, O., Ogawa, S., Stern, D. M., and Schmidt, A. M. (2000) Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* **405,** 354–360
74. Lander, H. M., Tauras, J. M., Ogiste, J. S., Hori, O., Moss, R. A., and Schmidt, A. M. (1997) Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J. Biol. Chem.* **272,** 17810–17814
75. Huttunen, H. J., Fages, C., and Rauvala, H. (1999) Receptor for advanced glycation end products (RAGE)-mediated neurite outgrowth and activation of NF-kappaB require the cytoplasmic domain of the receptor but different downstream signaling pathways. *J. Biol. Chem.* **274,** 19919–19924

76. Chavakis, T., Bierhaus, A., and Nawroth, P. P. (2004) RAGE (receptor for advanced glycation end products): a central player in the inflammatory response. *Microbes Infect.* **6,** 1219–1225
77. Lin, L. (2006) RAGE on the Toll road? *Cell. Mol. Immunol.* **3,** 351–358
78. Liliensiek, B., Weigand, M. A., Bierhaus, A., Nicklas, W., Kasper, M., Hofer, S., Plachky, J., Grone, H. J., Kurschus, F. C., Schmidt, A. M., Yan, S. D., Martin, E., Schleicher, E., Stern, D. M., Hammerling, G. G., Nawroth, P. P., and Arnold, B. (2004) Receptor for advanced glycation end products (RAGE) regulates sepsis but not the adaptive immune response. *J. Clin. Invest.* **113,** 1641–1650
79. Bianchi, M. E. (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. *J. Leukoc. Biol.* **81,** 1–5
80. Lee, Y. H., Giraud, J., Davis, R. J., and White, M. F. (2003) c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. *J. Biol. Chem.* **278,** 2896–2902
81. Taniguchi, C. M., Aleman, J. O., Ueki, K., Luo, J., Asano, T., Kaneto, H., Stephanopoulos, G., Cantley, L. C., and Kahn, C. R. (2007) The p85alpha regulatory subunit of phosphoinositide 3-kinase potentiates c-Jun N-terminal kinase-mediated insulin resistance. *Mol. Cell. Biol.* **27,** 2830–2840
82. Brownlee, M. (2005) The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* **54,** 1615–1625
83. King, G. L., and Loeken, M. R. (2004) Hyperglycemia-induced oxidative stress in diabetic complications. *Histochem. Cell. Biol.* **122,** 333–338
84. Ho, F. M., Liu, S. H., Liau, C. S., Huang, P. J., and Lin-Shiau, S. Y. (2000) High glucose-induced apoptosis in human endothelial cells is mediated by sequential activations of c-Jun NH(2)-terminal kinase and caspase-3. *Circulation* **101,** 2618–2624
85. Rossetti, L. (2000) Perspective: hexosamines and nutrient sensing. *Endocrinology* **141,** 1922–1925
86. Marshall, S., Bacote, V., and Traxinger, R. R. (1991) Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system: role of hexosamine biosynthesis in the induction of insulin resistance. *J. Biol. Chem.* **266,** 4706–4712
87. Ciaraldi, T. P., Carter, L., Nikoulina, S., Mudaliar, S., McClain, D. A., and Henry, R. R. (1999) Glucosamine regulation of glucose metabolism in cultured human skeletal muscle cells: divergent effects on glucose transport/phosphorylation and glycogen synthase in non-diabetic and type 2 diabetic subjects. *Endocrinology* **140,** 3971–3980
88. Veerababu, G., Tang, J., Hoffman, R. T., Daniels, M. C., Hebert, L. F., Jr., Crook, E. D., Cooksey, R. C., and McClain, D. A. (2000) Overexpression of glutamine: fructose-6-phosphate amidotransferase in the liver of transgenic mice results in enhanced glycogen storage, hyperlipidemia, obesity, and impaired glucose tolerance. *Diabetes* **49,** 2070–2078
89. Kaneto, H., Xu, G., Song, K. H., Suzuma, K., Bonner-Weir, S., Sharma, A., and Weir, G. C. (2001) Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. *J. Biol. Chem.* **276,** 31099–31104
90. Werstuck, G. H., Khan, M. I., Femia, G., Kim, A. J., Tedesco, V., Trigatti, B., and Shi, Y. (2006) Glucosamine-induced endoplasmic reticulum dysfunction is associated with accelerated atherosclerosis in a hyperglycemic mouse model. *Diabetes* **55,** 93–101
91. Andreozzi, F., D'Alessandris, C., Federici, M., Laratta, E., Del Guerra, S., Del Prato, S., Marchetti, P., Lauro, R., Perticone, F., and Sesti, G. (2004) Activation of the hexosamine pathway leads to phosphorylation of insulin receptor substrate-1 on Ser307 and Ser612 and impairs the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin insulin biosynthetic pathway in RIN pancreatic beta-cells. *Endocrinology* **145,** 2845–2857
92. Brownlee, M. (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* **414,** 813–820
93. Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S., Matsumura, T., Kaneda, Y., Yorek, M. A., Beebe, D., Oates, P. J., Hammes, H. P., Giardino, I., and Brownlee, M. (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* **404,** 787–790
94. Dulloo, A. G., Gubler, M., Montani, J. P., Seydoux, J., and Solinas, G. (2004) Substrate cycling between de novo lipogenesis and lipid oxidation: a thermogenic mechanism against skeletal muscle lipotoxicity and glucolipotoxicity. *Int. J. Obes. Relat. Metab. Disord.* **28**(Suppl. 4), S29–S37
95. Unger, R. H., and Orci, L. (2001) Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J.* **15,** 312–321
96. Unger, R. H. (2002) Lipotoxic diseases. *Annu. Rev. Med.* **53,** 319–336
97. Takahashi, K., Yamaguchi, S., Shimoyama, T., Seki, H., Miyokawa, K., Katsuta, H., Tanaka, T., Yoshimoto, K., Ohno, H., Nagamatsu, S., and Ishida, H. (2008) JNK- and IkappaB-dependent pathways regulate MCP-1 but not adiponectin release from artificially hypertrophied 3T3–L1 adipocytes preloaded with palmitate in vitro. *Am. J. Physiol. Endocrinol. Metab.* **294,** E898–E909
98. Karaskov, E., Scott, C., Zhang, L., Teodoro, T., Ravazzola, M., and Volchuk, A. (2006) Chronic palmitate but not oleate exposure induces endoplasmic reticulum stress, which may contribute to INS-1 pancreatic beta-cell apoptosis. *Endocrinology* **147,** 3398–3407
99. Borradaile, N. M., Han, X., Harp, J. D., Gale, S. E., Ory, D. S., and Schaffer, J. E. (2006) Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J. Lipid Res.* **47,** 2726–2737
100. Listenberger, L. L., Han, X., Lewis, S. E., Cases, S., Farese, R. V., Jr., Ory, D. S., and Schaffer, J. E. (2003) Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 3077–3082
101. Han, M. S., Park, S. Y., Shinzawa, K., Kim, S., Chung, K. W., Lee, J. H., Kwon, C. H., Lee, K. W., Park, C. K., Chung, W. J., Hwang, J. S., Yan, J. J., Song, D. K., Tsujimoto, Y., and Lee, M. S. (2008) Lysophosphatidylcholine as a death effector in the lipoapoptosis of hepatocytes. *J. Lipid Res.* **49,** 84–97
102. Jaeschke, A., and Davis, R. J. (2007) Metabolic stress signaling mediated by mixed-lineage kinases. *Mol. Cell* **27,** 498–508
103. Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H., and Karin, M. (2005) Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* **120,** 649–661
104. Zhang, K., and Kaufman, R. J. (2008) From endoplasmic-reticulum stress to the inflammatory response. *Nature* **454,** 455–462
105. Korshunov, S. S., Skulachev, V. P., and Starkov, A. A. (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett.* **416,** 15–18
106. Loschen, G., Flohe, L., and Chance, B. (1971) Respiratory chain linked H(2)O(2) production in pigeon heart mitochondria. *FEBS Lett.* **18,** 261–264
107. Boveris, A., Oshino, N., and Chance, B. (1972) The cellular production of hydrogen peroxide. *Biochem. J.* **128,** 617–630
108. Boveris, A., and Chance, B. (1973) The mitochondrial generation of hydrogen peroxide: general properties and effect of hyperbaric oxygen. *Biochem. J.* **134,** 707–716
109. Inoguchi, T., Sonta, T., Tsubouchi, H., Etoh, T., Kakimoto, M., Sonoda, N., Sato, N., Sekiguchi, N., Kobayashi, K., Sumimoto, H., Utsumi, H., and Nawata, H. (2003) Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase. *J. Am. Soc. Nephrol.* **14,** S227–S232
110. Eriksson, J. W. (2007) Metabolic stress in insulin's target cells leads to ROS accumulation: a hypothetical common pathway causing insulin resistance. *FEBS Lett.* **581,** 3734–3742
111. Kaneto, H., Katakami, N., Kawamori, D., Miyatsuka, T., Sakamoto, K., Matsuoka, T. A., Matsuhisa, M., and Yamasaki, Y. (2007) Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxid. Redox Signal.* **9,** 355–366
112. Manna, S. K., Zhang, H. J., Yan, T., Oberley, L. W., and Aggarwal, B. B. (1998) Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *J. Biol. Chem.* **273,** 13245–13254
113. Sen, C. K., and Packer, L. (1996) Antioxidant and redox regulation of gene transcription. *FASEB J.* **10,** 709–720
114. Schreck, R., Rieber, P., and Baeuerle, P. A. (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J.* **10,** 2247–2258

115. Li, N., and Karin, M. (1999) Is NF-kappaB the sensor of oxidative stress? *FASEB J.* **13,** 1137–1143
116. Pham, C. G., Bubici, C., Zazzeroni, F., Papa, S., Jones, J., Alvarez, K., Jayawardena, S., De Smaele, E., Cong, R., Beaumont, C., Torti, F. M., Torti, S. V., and Franzoso, G. (2004) Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. *Cell* **119,** 529–542
117. Ventura, J. J., Cogswell, P., Flavell, R. A., Baldwin, A. S., Jr., and Davis, R. J. (2004) JNK potentiates TNF-stimulated necrosis by increasing the production of cytotoxic reactive oxygen species. *Genes Dev.* **18,** 2905–2915
118. Temkin, V., and Karin, M. (2007) From death receptor to reactive oxygen species and c-Jun N-terminal protein kinase: the receptor-interacting protein 1 odyssey. *Immunol. Rev.* **220,** 8–21
119. Matsuzawa, A., and Ichijo, H. (2008) Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signaling. *Biochim. Biophys. Acta* **1780,** 1325–1336
120. Kaneto, H., Matsuoka, T. A., Katakami, N., Kawamori, D., Miyatsuka, T., Yoshiuchi, K., Yasuda, T., Sakamoto, K., Yamasaki, Y., and Matsuhisa, M. (2007) Oxidative stress and the JNK pathway are involved in the development of type 1 and type 2 diabetes. *Curr. Mol. Med.* **7,** 674–686
121. Diakogiannaki, E., Welters, H. J., and Morgan, N. G. (2008) Differential regulation of the endoplasmic reticulum stress response in pancreatic beta-cells exposed to long-chain saturated and monounsaturated fatty acids. *J. Endocrinol.* **197,** 553–563
122. Diakogiannaki, E., and Morgan, N. G. (2008) Differential regulation of the ER stress response by long-chain fatty acids in the pancreatic beta-cell. *Biochem. Soc. Trans.* **36,** 959–962
123. Guo, W., Wong, S., Xie, W., Lei, T., and Luo, Z. (2007) Palmitate modulates intracellular signaling, induces endoplasmic reticulum stress, and causes apoptosis in mouse 3T3–L1 and rat primary preadipocytes. *Am. J. Physiol. Endocrinol. Metab.* **293,** E576–E586
124. Wei, Y., Wang, D., Topczewski, F., and Pagliassotti, M. J. (2006) Saturated fatty acids induce endoplasmic reticulum stress and apoptosis independently of ceramide in liver cells. *Am. J. Physiol. Endocrinol. Metab.* **291,** E275–E281
125. Wu, J., and Kaufman, R. J. (2006) From acute ER stress to physiological roles of the unfolded protein response. *Cell Death Differ.* **13,** 374–384
126. Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L. H., and Hotamisligil, G. S. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306,** 457–461
127. Ozcan, U., Yilmaz, E., Ozcan, L., Furuhashi, M., Vaillancourt, E., Smith, R. O., Gorgun, C. Z., and Hotamisligil, G. S. (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* **313,** 1137–1140
128. Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P., and Ron, D. (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* **287,** 664–666
129. Hu, P., Han, Z., Couvillon, A. D., Kaufman, R. J., and Exton, J. H. (2006) Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol. Cell. Biol.* **26,** 3071–3084
130. Nakamura, T., Furuhashi, M., Li, P., Cao, H., Tuncman, G., Sonenberg, N., Gorgun, C. Z., and Hotamisligil, G. S. (2010) Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. *Cell* **140,** 338–348
131. Zhang, S., Liu, J., Dragunow, M., and Cooper, G. J. (2003) Fibrillogenic amylin evokes islet beta-cell apoptosis through linked activation of a caspase cascade and JNK1. *J. Biol. Chem.* **278,** 52810–52819
132. Tanti, J. F., Gremeaux, T., van Obberghen, E., and Le Marchand-Brustel, Y. (1994) Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J. Biol. Chem.* **269,** 6051–6057
133. White, M. F. (2003) Insulin signaling in health and disease. *Science* **302,** 1710–1711
134. Aguirre, V., Werner, E. D., Giraud, J., Lee, Y. H., Shoelson, S. E., and White, M. F. (2002) Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J. Biol. Chem.* **277,** 1531–1537
135. Morino, K., Neschen, S., Bilz, S., Sono, S., Tsirigotis, D., Reznick, R. M., Moore, I., Nagai, Y., Samuel, V., Sebastian, D., White, M., Philbrick, W., and Shulman, G. I. (2008) Muscle-specific IRS-1 Ser->Ala transgenic mice are protected from fat-induced insulin resistance in skeletal muscle. *Diabetes* **57,** 2644–2651
136. Copps, K. D., Hancer, N. J., Opare-Ado, L., Qiu, W., Walsh, C., and White, M. F. (2010) Irs1 serine 307 promotes insulin sensitivity in mice. *Cell Metab.* **11,** 84–92
137. Gao, Z., Hwang, D., Bataille, F., Lefevre, M., York, D., Quon, M. J., and Ye, J. (2002) Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J. Biol. Chem.* **277,** 48115–48121
138. Gao, Z., Zuberi, A., Quon, M. J., Dong, Z., and Ye, J. (2003) Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. *J. Biol. Chem.* **278,** 24944–24950
139. Herschkovitz, A., Liu, Y. F., Ilan, E., Ronen, D., Boura-Halfon, S., and Zick, Y. (2007) Common inhibitory serine sites phosphorylated by IRS-1 kinases, triggered by insulin and inducers of insulin resistance. *J. Biol. Chem.* **282,** 18018–18027
140. Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., and Shoelson, S. E. (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat. Med.* **11,** 183–190
141. Aguirre, V., Uchida, T., Yenush, L., Davis, R., and White, M. F. (2000) The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J. Biol. Chem.* **275,** 9047–9054
142. Sharfi, H., and Eldar-Finkelman, H. (2008) Sequential phosphorylation of insulin receptor substrate-2 by glycogen synthase kinase-3 and c-Jun NH2-terminal kinase plays a role in hepatic insulin signaling. *Am. J. Physiol. Endocrinol. Metab.* **294,** E307–E315
143. Sabio, G., Kennedy, N. J., Cavanagh-Kyros, J., Jung, D. Y., Ko, H. J., Ong, H., Barrett, T., Kim, J. K., and Davis, R. J. (2010) Role of muscle JNK1 in obesity-induced insulin resistance. *Mol. Cell. Biol.* **30,** 106–115
144. Tuncman, G., Hirosumi, J., Solinas, G., Chang, L., Karin, M., and Hotamisligil, G. S. (2006) Functional in vivo interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 10741–10746
145. Sabio, G., Das, M., Mora, A., Zhang, Z., Jun, J. Y., Ko, H. J., Barrett, T., Kim, J. K., and Davis, R. J. (2008) A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* **322,** 1539–1543
146. Fain, J. N., Madan, A. K., Hiler, M. L., Cheema, P., and Bahouth, S. W. (2004) Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* **145,** 2273–2282
147. Maeda, S., Kamata, H., Luo, J. L., Leffert, H., and Karin, M. (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* **121,** 977–990
148. Kodama, Y., Kisseleva, T., Iwaisako, K., Miura, K., Taura, K., De Minicis, S., Osterreicher, C. H., Schnabl, B., Seki, E., and Brenner, D. A. (2009) c-Jun N-terminal kinase-1 from hematopoietic cells mediates progression from hepatic steatosis to steatohepatitis and fibrosis in mice. *Gastroenterology* **137,** 1467–1477
149. Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., and Cai, D. (2008) Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. *Cell* **135,** 61–73
150. Ozcan, L., Ergin, A. S., Lu, A., Chung, J., Sarkar, S., Nie, D., Myers, M. G., Jr., and Ozcan, U. (2009) Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metab.* **9,** 35–51

151. Kleinridders, A., Schenten, D., Konner, A. C., Belgardt, B. F., Mauer, J., Okamura, T., Wunderlich, F. T., Medzhitov, R., and Bruning, J. C. (2009) MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell Metab.* **10,** 249–259
152. Yang, L., and Hotamisligil, G. S. (2008) Stressing the brain, fattening the body. *Cell* **135,** 20–22
153. Sabio, G., Cavanagh-Kyros, J., Barrett, T., Jung, D. Y., Ko, H. J., Ong, H., Morel, C., Mora, A., Reilly, J., Kim, J. K., and Davis, R. J. (2010) Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1. *Genes Dev.* **24,** 256–264
154. Sabio, G., Cavanagh-Kyros, J., Ko, H. J., Jung, D. Y., Gray, S., Jun, J. Y., Barrett, T., Mora, A., Kim, J. K., and Davis, R. J. (2009) Prevention of steatosis by hepatic JNK1. *Cell Metab.* **10,** 491–498
155. Prentki, M., and Nolan, C. J. (2006) Islet beta cell failure in type 2 diabetes. *J. Clin. Invest.* **116,** 1802–1812
156. Tiedge, M., Lortz, S., Drinkgern, J., and Lenzen, S. (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* **46,** 1733–1742
157. Kim, K. A., and Lee, M. S. (2009) Recent progress in research on beta-cell apoptosis by cytokines. *Front. Biosci.* **14,** 657–664
158. Sauter, N. S., Schulthess, F. T., Galasso, R., Castellani, L. W., and Maedler, K. (2008) The antiinflammatory cytokine interleukin-1 receptor antagonist protects from high-fat diet-induced hyperglycemia. *Endocrinology* **149,** 2208–2218
159. Eldor, R., Yeffet, A., Baum, K., Doviner, V., Amar, D., Ben-Neriah, Y., Christofori, G., Peled, A., Carel, J. C., Boitard, C., Klein, T., Serup, P., Eizirik, D. L., and Melloul, D. (2006) Conditional and specific NF-kappaB blockade protects pancreatic beta cells from diabetogenic agents. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 5072–5077
160. Giannoukakis, N., Rudert, W. A., Trucco, M., and Robbins, P. D. (2000) Protection of human islets from the effects of interleukin-1beta by adenoviral gene transfer of an Ikappa B repressor. *J. Biol. Chem.* **275,** 36509–36513
161. Fukuda, K., Tesch, G. H., and Nikolic-Paterson, D. J. (2008) c-Jun amino terminal kinase 1 deficient mice are protected from streptozotocin-induced islet injury. *Biochem. Biophys. Res. Commun.* **366,** 710–716
162. Kaneto, H., Xu, G., Fujii, N., Kim, S., Bonner-Weir, S., and Weir, G. C. (2002) Involvement of c-Jun N-terminal kinase in oxidative stress-mediated suppression of insulin gene expression. *J. Biol. Chem.* **277,** 30010–30018
163. Kaneto, H., Nakatani, Y., Miyatsuka, T., Kawamori, D., Matsuoka, T. A., Matsuhisa, M., Kajimoto, Y., Ichijo, H., Yamasaki, Y., and Hori, M. (2004) Possible novel therapy for diabetes with cell-permeable JNK-inhibitory peptide. *Nat. Med.* **10,** 1128–1132
164. Maedler, K., Schulthess, F. T., Bielman, C., Berney, T., Bonny, C., Prentki, M., Donath, M. Y., and Roduit, R. (2008) Glucose and leptin induce apoptosis in human beta-cells and impair glucose-stimulated insulin secretion through activation of c-Jun N-terminal kinases. *FASEB J.* **22,** 1905–1913
165. Kawamori, D., Kajimoto, Y., Kaneto, H., Umayahara, Y., Fujitani, Y., Miyatsuka, T., Watada, H., Leibiger, I. B., Yamasaki, Y., and Hori, M. (2003) Oxidative stress induces nucleo-cytoplasmic translocation of pancreatic transcription factor PDX-1 through activation of c-Jun NH(2)-terminal kinase. *Diabetes* **52,** 2896–2904
166. Chang, L., Kamata, H., Solinas, G., Luo, J. L., Maeda, S., Venuprasad, K., Liu, Y. C., and Karin, M. (2006) The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover. *Cell* **124,** 601–613
167. Cottet, S., Dupraz, P., Hamburger, F., Dolci, W., Jaquet, M., and Thorens, B. (2002) cFLIP protein prevents tumor necrosis factor-alpha-mediated induction of caspase-8-dependent apoptosis in insulin-secreting betaTc-Tet cells. *Diabetes* **51,** 1805–1814
168. Maedler, K., Fontana, A., Ris, F., Sergeev, P., Toso, C., Oberholzer, J., Lehmann, R., Bachmann, F., Tasinato, A., Spinas, G. A., Halban, P. A., and Donath, M. Y. (2002) FLIP switches Fas-mediated glucose signaling in human pancreatic beta cells from apoptosis to cell replication. *Proc. Natl. Acad. Sci. U. S. A.* **99,** 8236–8241
169. Ehses, J. A., Perren, A., Eppler, E., Ribaux, P., Pospisilik, J. A., Maor-Cahn, R., Gueripel, X., Ellingsgaard, H., Schneider, M. K., Biollaz, G., Fontana, A., Reinecke, M., Homo-Delarche, F., and Donath, M. Y. (2007) Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* **56,** 2356–2370
170. Fleischman, A., Shoelson, S. E., Bernier, R., and Goldfine, A. B. (2008) Salsalate improves glycemia and inflammatory parameters in obese young adults. *Diabetes Care* **31,** 289–294
171. Jiang, G., Dallas-Yang, Q., Liu, F., Moller, D. E., and Zhang, B. B. (2003) Salicylic acid reverses phorbol 12-myristate-13-acetate (PMA)- and tumor necrosis factor alpha (TNFalpha)-induced insulin receptor substrate 1 (IRS1) serine 307 phosphorylation and insulin resistance in human embryonic kidney 293 (HEK293) cells. *J. Biol. Chem.* **278,** 180–186
172. Yin, M. J., Yamamoto, Y., and Gaynor, R. B. (1998) The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* **396,** 77–80
173. Waeber, G., Delplanque, J., Bonny, C., Mooser, V., Steinmann, M., Widmann, C., Maillard, A., Miklossy, J., Dina, C., Hani, E. H., Vionnet, N., Nicod, P., Boutin, P., and Froguel, P. (2000) The gene MAPK8IP1, encoding islet-brain-1, is a candidate for type 2 diabetes. *Nat. Genet.* **24,** 291–295