Role of Endoplasmic Reticulum Stress and c-Jun NH_2-Terminal Kinase Pathways in Inflammation and Origin of Obesity and Diabetes

Gökhan S. Hotamisligil

Metabolic and immune systems are the most fundamental requirements for survival, and many metabolic and immune response pathways or nutrient- and pathogen-sensing systems have been evolutionarily highly conserved. Consequently, metabolic and immune pathways are also highly integrated and interdependent. In the past decade, it became apparent that this interface plays a critical role in the pathogenesis of chronic metabolic diseases, particularly obesity and type 2 diabetes. Importantly, the inflammatory component in obesity and diabetes is now firmly established with the discovery of causal links between inflammatory mediators, such as tumor necrosis factor (TNF)-α and insulin receptor signaling and the elucidation of the underlying molecular mechanisms, such as c-Jun NH_2-terminal kinase (JNK)- and inhibitor of nuclear factor-κB kinase–mediated transcriptional and posttranslational modifications that inhibit insulin action. More recently, obesity-induced endoplasmic reticulum stress has been demonstrated to underlie the initiation of obesity-induced JNK activation, inflammatory responses, and generation of peripheral insulin resistance. This article will review the link between stress, inflammation, and metabolic disease, particularly type 2 diabetes, and discuss the mechanistic and therapeutic opportunities that emerge from this platform by focusing on JNK and endoplasmic reticulum stress responses. *Diabetes* 54 (Suppl. 2):S73–S78, 2005

INFLAMMATORY CYTOKINES, ADIPOKINES, AND INSULIN ACTION

Inflammation is the body's response to deal with a broad range of injuries; however, this response does not assure a satisfactory outcome, particularly in the long term. This certainly appears to be the case in glucose homeostasis in obesity and type 2 diabetes. In contrast to the classic paradigm, the inflammatory responses in obesity are low grade and occur over an exceedingly extended time frame, thus increasing the risk of harm to multiple systems including, but not limited to, those involved in glucose homeostasis. In the past decade, several key lines of evidence emerged to establish firmly that inflammation lies at the heart of obesity and type 2 diabetes. First, a molecular link between inflammation and obesity was identified by the discovery of tumor necrosis factor (TNF)-α as an inflammatory cytokine overexpressed in the adipose tissues from rodent models of obesity, and this link disrupts insulin action in cells and whole animals (1,2). TNF-α is also overproduced in adipose and other tissues, such as muscle in obese humans (3–5). Administration of recombinant TNF-α to cultured cells or to whole animals impairs insulin action, whereas biochemical, pharmacological, or genetic blockade of TNF-α action results in improved insulin sensitivity (3). Thus, particularly in rodents, it is clear that adipose tissue overproduction of TNF-α is an important feature of obesity and contributes significantly to insulin resistance. Subsequently, it became clear that, in addition to TNF-α, several other inflammatory mediators are also dysregulated in obesity and impair insulin action both in experimental models and in humans. A comprehensive list of these with compelling evidence linking them to metabolic homeostasis can be found in another recent review (6). Second, clinical and epidemiological studies have linked inflammatory mediators to the development of insulin resistance and diabetes (7,8). For example, high levels of TNF-α, interleukin-6, C-reactive protein, plasma activator inhibitor 1, and many other inflammatory mediators were observed in obese individuals, those with insulin resistance and type 2 diabetes, and their first-degree relatives (4,6,9–12). Third, it became clear that the presence of chronic inflammatory diseases, such as rheumatoid arthritis or hepatitis, significantly increases the risk of development of insulin resistance and/or type 2 diabetes, thus providing an additional and important link between chronic inflammatory states and impaired insulin action in humans (13–15).

Recently, it was also proposed that infiltration of macrophages into adipose tissue, a phenomenon reminiscent of classic inflammation, might contribute to the inflammatory responses of obesity (16,17). Because this macrophage infiltration occurs rather late in the disease process, whether or to what extent it also contributes to insulin resistance or other metabolic abnormalities in obesity is unclear. It is worth noting, however, that there is a strikingly high degree of overlapping features between adipocytes and macrophages, which illustrates another interesting aspect of the integration and coregulation of metabolic and immune responses. This paradigm is best exemplified by the impact of fatty acid binding proteins and peroxisome proliferator–activated receptor (PPAR)-γ–LXR (liver X receptor) pathways on insulin action, type 2 diabetes, and atherosclerosis through their related actions on metabolic and inflammatory responses in adipocyte and macrophages, respectively (18–20). Finally, in

From the Departments of Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts.

Address correspondence and reprint requests to Gökhan S. Hotamisligil, MD, PhD, 665 Huntington Ave., Bldg. I, Rm. 205, Boston, MA 02115. E-mail: ghotamis@hsph.harvard.edu.

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ER, endoplasmic reticulum; IKK, inhibitor of nuclear factor-κB kinase; IRS, insulin receptor substrate; JIP, JNK-interacting protein; JNK, c-Jun NH_2 terminal kinase; TNF, tumor necrosis factor.

addition to the inflammatory cytokines regulating insulin action and metabolic homeostasis, hormones that are highly specifically produced by adipocytes, such as adiponectin, resistin, and visfatin, also act at the interface of metabolic and immune systems (21–25). Taken together, there is now a general consensus regarding the inflammatory origin of obesity and diabetes; however, the exact role of each target cell that might be critical in the different pathological outcomes and the underlying mechanistic details, especially regarding the initiating signals, are still not completely understood.

In addition, because many inflammatory mediators are simultaneously regulated in obesity and type 2 diabetes, it is unlikely that a single inflammatory mediator alone could explain the whole of the disease pathogenesis. Hence, an important consideration in designing therapeutics is the continuing need to understand more central mechanistic events that give rise to obesity-related inflammatory responses. In the recent years, there have been groundbreaking discoveries to uncover these core mechanistic pathways.

MECHANISMS LINKING INFLAMMATORY PATHWAYS WITH INSULIN ACTION

In the overwhelming majority of the individuals suffering from obesity-induced insulin resistance or type 2 diabetes, the principal defect in insulin action lies distal to the insulin receptor. Because of space limitations, insulin receptor signaling pathways will not be covered in detail here and the readers are referred to excellent recent reviews on insulin action and receptor signaling (26–28). Interestingly, inhibition of downstream components of the insulin receptor signaling pathways is also a primary mechanism by which inflammatory cascades are mechanistically linked to insulin resistance. Numerous studies have described that inhibitory phosphorylation of insulin receptor substrate (IRS)-1 on serine residues is a key event in generation of impaired insulin action upon exposure of cells to TNF-α or elevated free fatty acids or in the context of obesity and systemic insulin resistance in whole animals and humans (29–33). Serine phosphorylation of IRS-1 reduces both tyrosine phosphorylation of IRS-1 in response to insulin and the ability of IRS-1 to associate with insulin resistance and thereby suppresses downstream signaling and insulin action (30,34,35). The search for enzymatic mediators of this key modification has resulted in uncovering critical insights into mechanistic bases of insulin resistance. These studies have yielded several serine/threonine kinases that are activated by inflammatory or stressful stimuli and contribute to inhibition of insulin signaling (6). These include c-Jun NH_2-terminal kinase (JNK), inhibitor of nuclear factor-κB kinase (IKK), and protein kinase C-θ. Again, the activation of these "inflammatory" kinases in obesity and their involvement in insulin action illustrates the close relationship between metabolic and immune pathways. In particular, IKK and JNK control major inflammatory response pathways, and they are activated by a variety of stress signals and mediate the innate immune responses by Toll-like receptor signaling upon exposure to pathogens and their products (36). These signaling pathways can also affect T-cell function and adaptive immunity. Hence, the detailed mechanistic studies have also reinforced the close integration of immune and metabolic response pathways and their critical importance in the pathogenesis of type 2 diabetes.

JNK

The three members of the JNK group of serine/threonine kinases, JNK-1, -2, and -3, belong to the mitogen-activated protein kinase family and regulate multiple activities in development and cell function, in large part through their ability to regulate transcription by phosphorylating activator protein-1 (AP-1) complex proteins, including c-Jun and JunB (37). JNK-1 and JNK-2 are expressed in a wide range of tissues, whereas JNK-3 is restricted to brain, pancreatic islets, heart, and testis. Recent studies have demonstrated that JNK is a central player in the modulation of insulin action and a critical component of the pathogenesis of obesity, fatty liver disease, and type 2 diabetes (32). In particular, JNK1 appears to be the predominant isoform present in muscle, liver, and adipose tissues, and its activity is strikingly elevated in obesity at these sites (32). JNK can also directly phosphorylate IRS-1 at several sites, including serine 307 (31). There is also increased IRS-1 serine phosphorylation in obesity, which depends on the elevated JNK activity, and this modification leads to reduced insulin-stimulated IRS-1 tyrosine phosphorylation. In obese animals, deletion of the JNK-1 gene results in reversal of the obesity-induced increase in JNK activity and IRS-1 serine phosphorylation, whereas JNK-2 deficiency alone does not alter these significantly. There is also dramatic protection against insulin resistance and defective insulin receptor signaling in JNK-1–deficient obese animals (32). Again, none of these changes could be observed in the JNK-2–deficient animals, suggesting that this isoform alone is not sufficient to mediate the metabolic effects and JNK-1 is the principal isoform mediating obesity-induced JNK activity and consequently insulin resistance. However, it is possible that in the setting of JNK1 haploinsufficiency, which has little or no impact on systemic insulin action, JNK2 deficiency could influence insulin sensitivity, since JNK-2 can modulate JNK-1 action (38). JNK-interacting proteins (JIPs) are scaffolding molecules for JNK isoforms and can play an important role in determining kinase activity of all JNK isoforms. For example, a JIP-1 loss of function model creates a phenotype very similar to JNK-1 deficiency with reduced JNK activity and increased insulin sensitivity in mice (39). Interestingly, a point mutation in the human homolog of JIP-1, which leads to increased JNK activity, has been identified in a rare Mendelian form of diabetes in humans, providing crucial genetic proof-of-principle supporting the role of JNK in the pathogenesis of type 2 diabetes in humans (40).

In addition to the genetic loss of function models, several recent studies confirmed the critical impact of JNK in insulin resistance even after the establishment of disease in rodents. For example, adenovirus-mediated modulation of JNK activity with the expression of a dominant-negative JNK isoform in the livers of obese animals results in increased systemic insulin sensitivity and improved glucose homeostasis (41). Conversely, exogenous expression of JNK in the adult liver results in severe insulin resistance in mice (41). In these animals where JNK activity is blocked, there is also reduced IRS-1 serine phosphorylation and increased insulin receptor signaling, in perfect agreement with the genetic results obtained in JNK-deficient models. Finally, administration of small molecule or peptide inhibitors of JNK activity has resulted in significant increases in systemic insulin sensitivity in obese insulin-resistant animals (42,43). Inhibition of JNK is also effective against short-term lipid infusion-induced

insulin resistance in rats (C. Gorgun, J. Kim, G.S.H., unpublished data). The importance of these results is twofold. In addition to illustrating the strong therapeutic potential of JNK inhibition against type 2 diabetes, these studies also demonstrate the fact that the impact of JNK activity on systemic insulin action is independent of its effects on body weight, which is also regulated in JNK-deficient genetic models where JNK activity is blocked throughout the life of the animal (32).

Finally, it is likely that JNK activity modulates islet function and/or survival in numerous ways. First, there is convincing evidence for the involvement of JNK in islet cell inflammation and death mediated by cytokines (44–46). Second, JNK activation might generate a state of β-cell dysfunction and defective insulin production, thereby contributing to the development of frank diabetes (47). Third, administration of SP600125, a synthetic inhibitor of JNK, results in improved glucose-stimulated insulin production in isolated islets in the *db*/*db* model of obesity and diabetes (42). Hence, there is a strong possibility that JNK might integrate defects in insulin secretion with peripheral insulin resistance in type 2 diabetes through its actions in pancreatic β-cells as well as peripheral sites of insulin action. If this is the case, it is also likely that JNK might be important in the pathogenesis of type 1 diabetes, and recent studies have provided evidence to support a role for the JNK-2 isoform in this disease (48). Taken together, there is very strong evidence that abnormal JNK activation is a critical event in the deterioration of glucose homeostasis and offers a very promising target for therapeutic strategies.

Two other inflammatory kinases that play important roles in counteracting insulin action are IKK and, particularly in response to lipid metabolites, protein kinase C. Because of space limitations, these will not be discussed in detail, but readers are referred to recent excellent reviews in these areas (49,50). It is highly likely that there are interactions between the signaling and action of these key mediators, which might involve regulation of the activity of each other. For example, it has already been suggested that protein kinase C-θ can act through IKKβ to phosphorylate IRS-1 (49). Lipid exposure can modulate the activity of all three enzymes (51). Both JNK and IKKβ are required for TNF-induced insulin resistance in cells, and suppression of either can increase insulin sensitivity (52). All three enzymes can increase IRS-1 serine phosphorylation and alter inflammatory gene expression profiles (51,53). At high doses of salicylate exposure, both JNK and IKKβ activity could be suppressed (52). The action of both JNK and IKKβ appears to play an important role in their impact on systemic insulin action at similar target sites (32,51,54–56). Hence, it will be extremely interesting to examine the potential integration or synergy between these pathways, particularly with JNK and IKK, regarding the relevant target sites of insulin action, underlying mechanisms, and systemic metabolic outcomes.

In addition to these serine/threonine kinase cascades, several other pathways also contribute to inflammation-induced insulin resistance, including activation of inducible nitric oxide synthase, production of reactive oxygen species in adipose tissue, regulation of suppressor of cytokine signaling (SOCS) proteins, and alterations in AMP-K and mTOR pathways in obesity (57–61). It is also highly likely that many of these mediators share common regulatory cues and signaling pathways; however, their integration mechanisms are yet to be fully uncovered. Regrettably, these highly promising areas and additional mechanisms involving these molecules are not discussed in detail here because of space limitations.

ENDOPLASMIC RETICULUM STRESS AND DIABETES

One of the major areas that has been quite difficult to address has been the mechanism(s) underlying the origin of the stress and inflammatory responses associated with obesity. How does a cell interpret the presence of metabolic overload and start transmitting stress signals such as activation of JNK and IKK pathways to trigger a prolonged inflammatory response? In our recent studies, we have produced some insights into this issue and demonstrated that the endoplasmic reticulum (ER) might be a key site where the metabolic signals are sensed, integrated, and transmitted in the form of stress signals, including activation of JNK, IKK, and possibly other pathways (62). Accordingly, this model offers a new paradigm, where inflammatory pathways are activated by metabolic stresses originating from inside of the cell and propagated by extracellular signaling molecules or inflammatory mediators as described above. In particular, obesity-induced activation of the JNK pathway appears to be controlled, at least in part, by this mechanism. As such, ER can offer mechanistic models to integrate the molecular pathways shown to take part in the development of insulin resistance and type 2 diabetes.

The ER is a critical organelle where all secreted and transmembrane proteins are synthesized, folded into their correct three-dimensional structures, modified, and transported to their final cellular destinations. Under conditions in which the client traffic is increased or mutant proteins arrive at the ER, the organelle experiences stress and activates an adaptive mechanism called “unfolded protein response” to reestablish equilibrium (63). The principal events during unfolded protein response include transcriptional activation of chaperones to aid protein folding, selective attenuation of new protein synthesis (which allows ER to cope with the existing proteins), and activation of proteosome-mediated protein degradation. In addition to increased client load, changes in nutrient, energy, and Ca^{2+} availability and exposure to pathogens and pathogen components and hypoxic signals can also induce ER stress (63–65). Thus, it is possible to envision ER as one of the earliest sites evolved as a nutrient- and pathogen-sensing device in the cells. In our very recent studies, we have postulated that obesity is associated with many conditions that can lead to ER stress (62). These include an increase in protein and lipid synthesis, lipid accumulation, perturbations in intracellular nutrient and energy fluxes, and severe changes in tissue architecture. The latter is particularly the case for expanding adipocytes and adipose tissue. In testing this postulate, we observed elevation of several biochemical indicators of ER stress in liver and adipose tissues of obese animals compared with their lean counterparts (62). These observations held true for both dietary (high-fat diet–induced) and genetic (leptin-deficient, *ob*/*ob* mice) models of obesity and included phosphorylation of PERK, activation of JNK, and, depending on the stage of the disease, increased chaperone expression (62). Subsequently, these observations were independently confirmed by Nakatani et al. (66), who observed increased (immunoglobulin heavy chain–binding protein) Bip expression in the liver tissues of a genetic model of obesity (leptin receptor–deficient *db*/*db* mice).

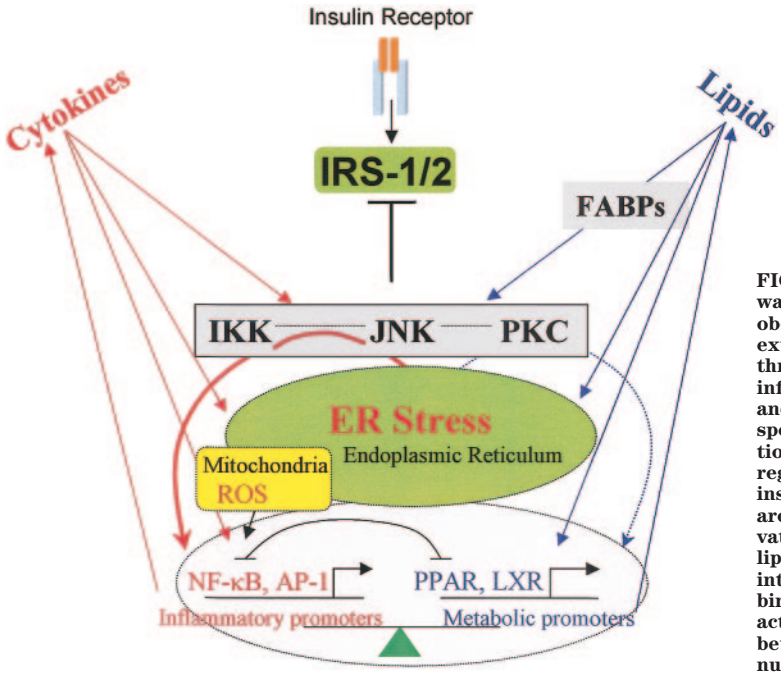

FIG. 1. Model of integration of inflammatory and metabolic pathways that interfere with insulin action. Inflammatory responses in obesity can be triggered in the adipocyte or macrophages by extracellular mediators such as cytokines or lipids, or initiated through ER stress. Signals from all of these mediators converge on inflammatory signaling pathways, including the kinases JNK, IKK, and protein kinase C (PKC) and production of reactive oxygen species (ROS). Once activated, JNK and IKK lead to the production of additional inflammatory mediators through transcriptional regulation and start a vicious cycle. They also directly inhibit insulin receptor signaling. Opposing the inflammatory pathways are transcription factors from the peroxisome proliferator–activated receptor (PPAR) and LXR families, which are regulated by lipids and promote nutrient transport and lipid metabolism. The integration of lipid signals is regulated by cytosolic fatty acid binding proteins (FABPs), which coordinate the distribution and action of these mediators. The cell thus must strike a balance between metabolism and inflammation for optimum function. NF, nuclear factor.

We next tested whether experimental manipulation of ER stress responses could lead to modification of insulin receptor signaling and insulin action. In cultured cells, induction of ER stress by tunicamycin or thapsigargin exposure or by deletion of XBP-1 (x-box–binding protein), a critical transcription factor regulating unfolded protein response, led to generation of ER stress, increased IRS-1 serine phosphorylation, and suppressed insulin-induced insulin receptor signal transduction (62). This ER stress–induced cellular insulin resistance was mediated by inositol-requiring enzyme-1 (IRE-1)–dependent activation of JNK and reversed by blocking the activity of either kinase (62). Conversely, reduction of ER stress by overexpression of a spliced and transcriptionally active form of XBP-1 rendered cells refractive to ER stress–induced insulin resistance. Existence of a similar causal relationship between ER stress and insulin action was also demonstrated in whole animals. Mice with XBP-1 haploinsufficiency in the Balb/C genetic background, which is normally resistant to insulin resistance and diabetes, were predisposed to high-fat diet–induced ER stress, insulin resistance, and hyperglycemia compared with wild-type controls. In liver and adipose tissues of these animals, an increase in ER stress markers including elevation in JNK activity and IRS-1 serine phosphorylation was evident. Consequently, $XBP^{+/-}$ animals exhibited significantly impaired insulin receptor signaling in adipose and liver tissues (62). In the absence of a high-fat diet or obesity, there was no indication of alterations in systemic insulin sensitivity or insulin receptor signaling capacity in liver or adipose tissues of lean $XBP\text{-}1^{+/-}$ animals (62). Taken together, these studies demonstrated that ER stress responses are closely linked to insulin receptor signaling and insulin action in both cultured cells and whole animals, and modification of these responses have a significant impact on systemic insulin sensitivity.

ER stress has been previously implicated in islet biology and function (63–65). The islet morphology of $XBP\text{-}1^{+/-}$ animals was similar to wild-type controls, both exhibiting comparable levels of hypertrophy and insulin content (62). There was also no apparent defect in glucose-stimulated insulin secretion in $XBP\text{-}1^{+/-}$ mice, even after exposure to a high-fat diet. However, because $XBP^{+/-}$ mice developed peripheral insulin resistance and hyperglycemia, it could be inferred that their islet responses were insufficient to compensate for the degree of insulin resistance present; therefore, a role for XBP-1 in islet biology cannot be ruled out at this time. Subsequently, two independent groups also demonstrated that adenovirus-mediated or genetic depletion of an ER chaperone leads to ER stress insulin resistance and diabetes, whereas in vivo overexpression renders animals refractory to insulin resistance and diabetes (66,67). In all of these models, increased ER stress was associated with impaired insulin receptor signaling at target tissues. Taken together, these results demonstrated that ER stress is a central feature of obesity and a key mechanism of insulin resistance and diabetes.

These findings stimulate consideration of new models in understanding the pathogenesis of type 2 diabetes and may have important therapeutic implications (Fig. 1). First, it is quite possible that ER is the site where metabolic stress is translated into inflammatory responses, particularly the activation of JNK and other inflammatory mediators. More experiments are needed to test these possibilities. For example, it is not yet clear whether modification of ER stress modulates local or systemic inflammatory cytokine levels in obesity. Another important question is whether ER stress–induced insulin resistance and type 2 diabetes could be rescued by blocking JNK activity in vivo, as is the case in vitro. This will firmly establish the ER stress–JNK activation axis as a key mechanism in this model. Interestingly, ER stress also stimulates the activity of the nuclear factor κB pathway, which might represent a further link between JNK- and IKK-regulated pathways, the two important signaling cascades leading to insulin resistance. Therefore, it will be highly informative to test the role of the IKK–NF-κB pathway and inflammation in general in ER stress–induced insulin resistance. Also, studies are critical that are aimed at understanding the components of obesity that lead to ER stress activation and the metabolic responses and the related pathologies that are regulated by ER stress. Of

particular interest are dyslipidemia and atherosclerosis, since cholesterol loading of macrophages has been shown to modify ER membrane properties and lead to an ER stress–mediated apoptotic response (68). The potential contribution of other lipid signals to triggering ER stress in obesity might open up new possibilities in understanding and preventing lipotoxicity in a variety of metabolically active tissues. Finally, there is need for development of physiologically relevant models of ER stress and highly sensitive reagents to monitor the biochemical indicators of ER stress and unfolded protein response and to study detailed mechanisms of metabolic regulation through this pathway during the course of obesity, insulin resistance, and type 2 diabetes.

Studies in the past decade clearly established that inflammation is an integral component of obesity and associated diseases. At the core of this mechanism lies the high degree of integration between nutrient- and pathogen-sensing pathways, the coordination of which is disrupted in obesity. Central to this obesity-induced inflammation is the activation of JNK and IKK and induction of ER stress. Along these lines, better decoding of the link between these response systems and modulation of inflammatory activity or ER stress pathways may offer multiple novel therapeutic and preventive strategies for metabolic diseases, particularly type 2 diabetes.

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REFERENCES

1. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
2. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389:610–614, 1997
3. Hotamisligil GS: Inflammation, TNFalpha, and insulin resistance. In *Diabetes Mellitus: A Fundamental and Clinical Text.* LeRoith D, Taylor SI, Olefsky JM, Eds. New York, Lippincott, 2004, p. 953–962
4. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB: The expression of tumor necrosis factor in human adipose tissue: regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 95:2111–2119, 1995
5. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA: The expression of TNF alpha by human muscle: relationship to insulin resistance. *J Clin Invest* 97:1111–1116, 1996
6. Wellen KE, Hotamisligil GS: Inflammation, stress, and diabetes. *J Clin Invest* 115:1111–1119, 2005
7. Pickup JC: Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* 27:813–823, 2004
8. Dandona PA, Aljada A, Bandyopadhyay A: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 25:4–7, 2004
9. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327–334, 2001
10. Nakanishi S, Yamane K, Kamei N, Okubo M, Kohno N: Elevated C-reactive protein is a risk factor for the development of type 2 diabetes in Japanese Americans. *Diabetes Care* 26:2754–2757, 2003
11. Alessi MC, Bastelica D, Morange P, Berthet B, Leduc I, Verdier M, Geel O, Juhan-Vague I: Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. *Diabetes* 49:1374–1380, 2000
12. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 95:2409–2415, 1995
13. Sattar N, McCarey DW, Capell H, McInnes IB: Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation* 108:2957–2963, 2003
14. Bahtiyar G, Shin JJ, Aytaman A, Sowers JR, McFarlane SI: Association of diabetes and hepatitis C infection: epidemiologic evidence and pathophysiologic insights. *Curr Diabetes Rep* 4:194–198, 2004
15. Knobler H, Zhornicky T, Sandler A, Haran N, Ashur Y, Schattner A: Tumor necrosis factor-alpha-induced insulin resistance may mediate the hepatitis C virus-diabetes association. *Am J Gastroenterol* 98:2751–2756, 2003
16. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr: Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808, 2003
17. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830, 2003
18. Makowski L, Hotamisligil GS: Fatty acid binding proteins: the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr* 134:2464S–2468S, 2004
19. Li AC, Glass CK: PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis. *J Lipid Res* 45:2161–2173, 2004
20. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA, Hotamisligil GS: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab* 1:107–119, 2005
21. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I: Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307:426–430, 2004
22. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I: Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol* 14:1431–1437, 1994
23. Steppan CM, Lazar MA: The current biology of resistin. *J Intern Med* 255:439–447, 2004
24. Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA: An inflammatory cascade leading to hyperresistinemia in humans. *Plos Med* 1:e45, 2004
25. Berg AH, Combs TP, Scherer PE: ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab* 13:84–89, 2002
26. Saltiel AR, Pessin JE: Insulin signaling pathways in time and space. *Trends Cell Biol* 12:65–71, 2002
27. White MF: The insulin signalling system and the IRS proteins. *Diabetologia* 40 (Suppl. 2):S2–S17, 1997
28. Saltiel AR, Kahn CR: Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414:799–806, 2001
29. Yin MJ, Yamamoto Y, Gaynor RB: The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* 396:77–80, 1998
30. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271:665–668, 1996
31. Aguirre V, Uchida T, Yenush L, Davis R, White MF: 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, 2000
32. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS: A central role for JNK in obesity and insulin resistance. *Nature* 420:333–336, 2002
33. Kanety H, Hemi R, Papa MZ, Karasik A: Sphingomyelinase and ceramide suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. *J Biol Chem* 271:9895–9897, 1996
34. Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF: Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* 277:1531–1537, 2002

35. Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y: A molecular basis for insulin resistance: elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. *J Biol Chem* 272:29911–29918, 1997
36. Medzhitov R: Toll-like receptors and innate immunity. *Nat Rev Immunol* 1:135–145, 2001
37. Davis RJ: Signal transduction by the JNK group of MAP kinases. *Cell* 103:239–252, 2000
38. Sabapathy K, Hochedlinger K, Nam SY, Bauer A, Karin M, Wagner EF: Distinct roles for JNK1 and JNK2 in regulating JNK activity and c-Jun-dependent cell proliferation. *Mol Cell* 15:713–725, 2004
39. Jaeschke A, Czech MP, Davis RJ: An essential role of the JIP1 scaffold protein for JNK activation in adipose tissue. *Genes Dev* 18:1976–1980, 2004
40. Waeber G, Delplanque J, Bonny C, Mooser V, Steinmann M, Widmann C, Maillard A, Miklossy J, Dina C, Hani EH, Vionnet N, Nicod P, Boutin P, Froguel P: The gene MAPK8IP1, encoding islet-brain-1, is a candidate for type 2 diabetes. *Nat Genet* 24:291–295, 2000
41. Nakatani Y, Kaneto H, Kawamori D, Hatazaki M, Miyatsuka T, Matsuoka TA, Kajimoto Y, Matsuhisa M, Yamasaki Y, Hori M: Modulation of the JNK pathway in liver affects insulin resistance status. *J Biol Chem* 279:45803–45809, 2004
42. Bennett BL, Satoh Y, Lewis AJ: JNK: a new therapeutic target for diabetes. *Curr Opin Pharmacol* 3:420–425, 2003
43. Kaneto H, Nakatani Y, Miyatsuka T, Kawamori D, Matsuoka TA, Matsuhisa M, Kajimoto Y, Ichijo H, Yamasaki Y, Hori M: Possible novel therapy for diabetes with cell-permeable JNK-inhibitory peptide. *Nat Med* 10:1128–1132, 2004
44. Ammendrup A, Maillard A, Nielsen K, Aabenhus Andersen N, Serup P, Dragsbaek Madsen O, Mandrup-Poulsen T, Bonny C: The c-Jun amino-terminal kinase pathway is preferentially activated by interleukin-1 and controls apoptosis in differentiating pancreatic beta-cells. *Diabetes* 49: 1468–1476, 2000
45. Bonny C, Oberson A, Negri S, Sauser C, Schorderet DF: Cell-permeable peptide inhibitors of JNK: novel blockers of beta-cell death. *Diabetes* 50:77–82, 2001
46. Abdelli S, Ansite J, Roduit R, Borsello T, Matsumoto I, Sawada T, Allaman-Pillet N, Henry H, Beckmann JS, Hering BJ, Bonny C: Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure. *Diabetes* 53:2815–2823, 2004
47. Kaneto H, Xu G, Fujii N, Kim S, Bonner-Weir S, Weir GC: Involvement of c-Jun N-terminal kinase in oxidative stress-mediated suppression of insulin gene expression. *J Biol Chem* 277:30010–30018, 2002
48. Jaeschke A, Rincon M, Doran B, Reilly J, Neuberg D, Greiner DL, Shultz LD, Rossini AA, Flavell RA, Davis RJ: Disruption of the Jnk2 (Mapk9) gene reduces destructive insulitis and diabetes in a mouse model of type I diabetes. *Proc Natl Acad Sci U S A* 102:6931–6935, 2005
49. Perseghin G, Petersen K, Shulman GI: Cellular mechanism of insulin resistance: potential links with inflammation. *Int J Obes Relat Metab Disord* 27 (Suppl. 3):S6–S11, 2003
50. Shoelson SE, Lee J, Yuan M: Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. *Int J Obes Relat Metab Disord* 27 (Suppl. 3):S49–S52, 2003
51. Gao Z, Zhang X, Zuberi A, Hwang D, Quon MJ, Lefevre M, Ye J: Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3–L1 adipocytes. *Mol Endocrinol* 18:2024–2034, 2004
52. Gao Z, Zuberi A, Quon MJ, Dong Z, Ye J: 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, 2003
53. Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, Ye J: Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J Biol Chem* 277:48115–48121, 2002
54. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE: Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKKbeta. *Science* 293:1673–1677, 2001
55. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M: IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 11:191–198, 2005
56. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE: Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11:183–190, 2005
57. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS, Van Obberghen E: SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *J Biol Chem* 276:47944–47949, 2001
58. Perreault M, Marette A: Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med* 7:1138–1143, 2001
59. Lin Y, Berg AH, Iyengar P, Lam TK, Giacca A, Combs TP, Rajala MW, Du X, Rollman B, Li W, Hawkins M, Barzilai N, Rhodes CJ, Fantus IG, Brownlee M, Scherer PE: The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J Biol Chem* 280:4617–4626, 2004
60. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I: Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 114:1752–1761, 2004
61. Khamzina L, Veilleux A, Bergeron S, Marette A: Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance. *Endocrinology* 146:1473–1481, 2005
62. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS: Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306:457–461, 2004
63. Kaufman RJ, Scheuner D, Schroder M, Shen X, Lee K, Liu CY, Arnold SM: The unfolded protein response in nutrient sensing and differentiation. *Nat Rev Mol Cell Biol* 3:411–421, 2002
64. Harding HP, Ron D: Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes* 51 (Suppl. 3):S455–S461, 2002
65. Shi Y, Taylor SI, Tan SL, Sonenberg N: When translation meets metabolism: multiple links to diabetes. *Endocr Rev* 24:91–101, 2003
66. Nakatani Y, Kaneto H, Kawamori D, Yoshiuchi K, Hatazaki M, Matsuoka TA, Ozawa K, Ogawa S, Hori M, Yamasaki Y, Matsuhisa M: Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. *J Biol Chem* 280:847–851, 2005
67. Ozawa K, Miyazaki M, Matsuhisa M, Takano K, Nakatani Y, Hatazaki M, Tamatani T, Yamagata K, Miyagawa J, Kitao Y, Hori O, Yamasaki Y, Ogawa S: The endoplasmic reticulum chaperone improves insulin resistance in type 2 diabetes. *Diabetes* 54:657–663, 2005
68. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA, Marks AR, Ron D, Tabas I: The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol* 5:781–792, 2003