Chapter 10
Insulin Resistance in the Metabolic Syndrome

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Introduction

In 1988, Gerald Reaven coined the term "Syndrome X" to describe a complex of metabolic abnormalities, including glucose intolerance, hypertriglyceridemia and reduced levels of HDL-cholesterol, present in individuals at increased risk for cardiovascular disease [1]. Since then, attempts to quantify cardiovascular disease risk have led to the development of clinical criteria for the diagnosis of this syndrome, now known as the "metabolic syndrome" or "insulin resistance syndrome". Although these criteria continue to evolve, those put forth by the National Cholesterol Education Program (NCEP), World Health Organization (WHO), European Group for the Study of Insulin Resistance (EGIR), International Diabetes Federation (IDF) and American Association of Clinical Endocrinologists (AACE), all include hyperglycemia, hypertriglyceridemia, low HDL-cholesterol and hypertension (reviewed in [2)] (Table 1). It is clear now that the metabolic syndrome is associated with many diseases in addition to cardiovascular disease. These include cholesterol gallstones, non-alcoholic fatty liver disease, which ranges from benign steatosis to non-alcholic steatohepatitis (NASH), polycystic ovary disease (PCOS) and neurodegenerative disease.

The prevalence of the metabolic syndrome has risen at an alarming rate; more than one in three adults and increasing numbers of children now carry the diagnosis of the metabolic syndrome [3]. Despite the prevalence of metabolic syndrome, and the serious morbidity and mortality associated with it, the underlying pathophysiology of this disorder remains unclear. Dr. Reaven postulated in 1988 that insulin resistance plays a central role in the metabolic syndrome [1]. Since then, a great deal of data has shown a strong association between insulin resistance and the different components of the metabolic syndrome, but proving a causal role has been difficult [4].

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Table 1 Criteria for the diagnosis of the metabolic syndrome (NCEP:ATPIII, 2001).

Metabolic syndrome (three or more of the following)
Abdominal obesity
Men: waist circumference >40 in.
Women: waist circumference >35 in.
Fasting plasma glucose ≥110 mg/dl
Blood pressure ≥130/80 mmHg
Triglycerides ≥140 mg/dl
High-density lipoprotein cholesterol
Men <40 mg/dl
Women <35 mg/dl

Insulin resistance is only one of the multiple derangements in the hormonal and metabolic milieu which occur in the metabolic syndrome. Some of these derangements could be synergistic with insulin resistance whereas others could be antagonistic. Some changes are so intricately linked to insulin resistance—e.g., hyperglycemia is not only secondary to insulin resistance, but it also appears to exacerbate insulin resistance, resulting in a feed-forward cycle—that it is difficult to study one in isolation of the other. Finally, some of the genetic and dietary factors that induce the metabolic syndrome, like excess dietary fat, could not only act by promoting insulin resistance, but could also act independent of insulin resistance to alter metabolism.

Nonetheless, defining the role of insulin resistance is a fundamental problem with important clinical implications. If insulin resistance does not play a pathogenic role in the metabolic syndrome, one should identify and treat the individual components of the metabolic syndrome, as has been advocated by some experts [4]. For example, dyslipidemia and hypertension should be identified and treated before they progress to cardiovascular disease. If insulin resistance is the central driver of this disorder, we should identify and treat insulin resistance itself, potentially even before the development of dyslipidemia and hypertension, let alone cardiovascular disease.

Over the past 20 years, we have learned a great deal about the mechanisms of insulin signaling. To comprehend how these findings shape our understanding of insulin resistance in the metabolic syndrome, we will review the better known components of the insulin signaling pathway, how defects in the insulin signaling pathway could contribute to the metabolic syndrome phenotype and how such defects arise.

Clinical Versus molecular definitions of insulin resistance

Clinically, insulin resistance is defined as the failure of insulin to maintain normal serum glucose levels. Thus, the hyperinsulinemic euglycemic clamp is the gold standard for the measurement of insulin resistance, and surrogate measurements involving serum insulin and glucose levels, like the homeostatic model assessment

(HOMA), are also based on glucose metabolism. Such definitions of insulin resistance, however, fail to acknowledge the underlying complexities of insulin signaling in two respects. First, insulin regulates many processes within the cell. It increases glucose uptake, promotes glycogen synthesis and suppresses hepatic glucose production. However, it also stimulates lipogenesis and triglyceride secretion, increases salt and water retention, and regulates many other processes including bile acid metabolism, growth and differentiation. The clinical definitions of insulin resistance imply that all of these processes become resistant in parallel with glucose metabolism, though this is unlikely to be true.

In fact, Dr. Reaven postulated that some pathways remain sensitive to insulin in the metabolic syndrome, while the pathways by which insulin stimulates glucose uptake, particularly by the muscle and fat, become resistant [1]. The resulting hyperglycemia stimulates insulin secretion from the pancreatic β-cell, leading to hyperinsulinemia, which then over-stimulates those pathways that are still sensitive to insulin. For example, hyperinsulinemia triggers excessive lipogenesis and triglyceride secretion, resulting in hypertriglyceridemia and hepatic steatosis, salt and water retention which produces hypertension, and excessive androgen synthesis, resulting in PCOS [5].

The definitions of insulin resistance based on disturbances in glucose homeostasis fail to recognize the intrinsic complexities of the insulin signaling cascade. The insulin signaling pathway (described below) consists of multiple nodes, with many nodes represented by multiple isoforms with seemingly redundant capabilities. Clinical definitions of glucose intolerance suggest that insulin resistance is a homogenous phenomenon, which can only vary quantitatively. In contrast, the presence of so many signaling components suggests that insulin resistance could be a heterogeneous phenomenon, i.e., the phenotype produced by insulin resistance could vary depending on the components affected.

Therefore, it is also useful to consider the concept of molecular insulin resistance, defined as specific defects in one or more components of the insulin signaling pathway. Molecular insulin resistance could be present even in the absence of abnormalities in glucose homeostasis, and conversely, it is possible that multiple forms of molecular insulin resistance could produce abnormal glucose homeostasis. Molecular insulin resistance is not a concept that can, at present, be used in the clinical setting, but our hope is that it will clarify our understanding of the metabolic syndrome, and provide insights into its therapy.

Insulin Signaling Pathway

Insulin elicits a complex cascade of signaling events, involving multiple nodes. Although we shall present the insulin signaling pathway as a linear chain, it is important to acknowledge the great deal of complexity underlying each node. At most nodes, there are numerous isoforms, which are theoretically capable of responding to and generating subtly different signals. In addition, there is crosstalk

among the different components of the cascade. Here, we will review some of the better studied nodes of the insulin signaling pathway (Fig. 1).

Insulin Receptor. The insulin receptor binds insulin and triggers a complex cascade of signaling events. The insulin receptor gene encodes a single chain precursor protein which is post-translationally processed into an α- subunit and a β-subunit, which are covalently linked by disulfide bonds. The α-subunit is extracellular and binds insulin, whereas the β-subunit is intracellular and contains a tyrosine kinase domain. The α/β subunit complexes dimerize with one another to form the insulin receptor. Upon binding insulin, the β-subunits phosphorylate one another and their intracellular substrates.

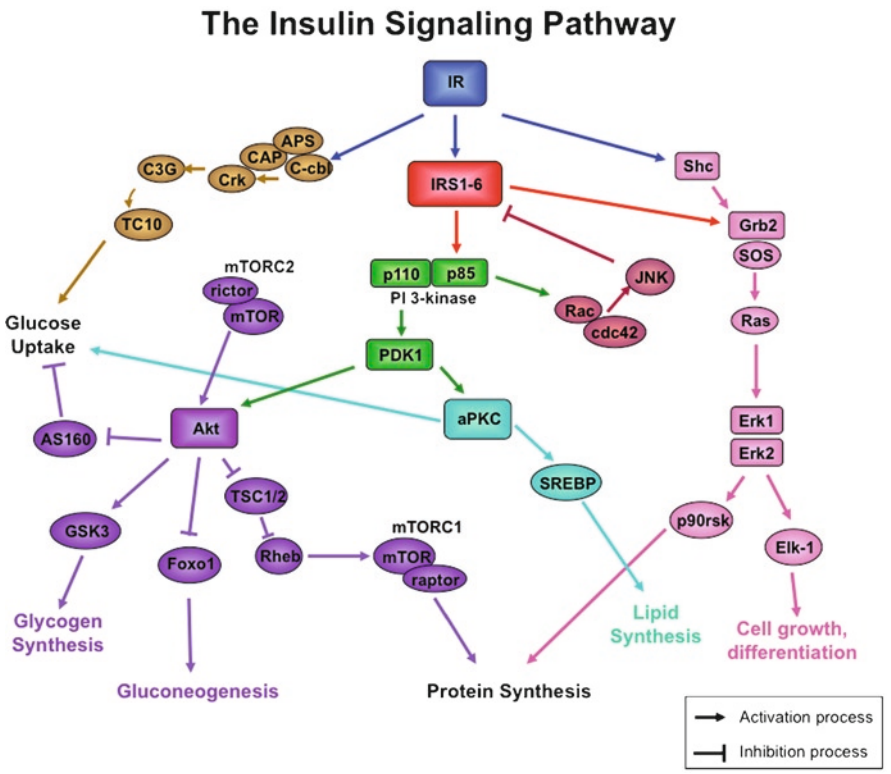

Fig. 1 The insulin signaling network. Upon binding insulin, the insulin receptor (IR) activates the IRS proteins and initiates a complex cascade of signaling events. Here, we show the major branches of this signaling network. Many of the effects of insulin are mediated by PI 3-kinase, which activates Akt, the atypical PKCs (aPKCs) and the stress kinase JNK. Akt in particular mediates many of insulin's metabolic effects by regulating gluconeogenesis, glycogen synthesis and protein synthesis, whereas the aPKCs activate lipid synthesis. In contrast, the proliferative effects of insulin are largely mediated by ERK1 and ERK2, independent of PI 3-kinase. Finally, insulin promotes glucose uptake through Akt, the aPKCs, and the CAP/Cbl complex. *Plain arrows* indicate stimulation and *blocked arrows* indicate inhibition

At this signaling node, heterogeneity is generated by alternative splicing and cross-talk with the insulin-like growth factor (IGF)-1 signaling pathway. Alternative splicing of exon 11 yields two isoforms, IR_A which lacks the 12 amino acids encoded by this exon and IR_B which contains them. The 12 amino acids encoded by exon 11 are contained in the α-subunit and increase the affinity of the receptor for the related growth factor, IGF-2 [6]. In addition, the two isoforms activate different downstream events under certain conditions [7].

The insulin receptor is a member of a subfamily of receptor tyrosine kinases which also includes the IGF-1 receptor and the insulin receptor related receptor (IRR). The α/β subunit complex encoded by the IGF-1 receptor gene not only dimerizes with itself to form the IGF receptor, but also dimerizes with the α/β subunit complex of the insulin receptor to generate hybrid receptors. The insulin receptor, IGF receptor and hybrid receptor all bind insulin and IGF-1 with varying affinities (Fig. 2). Although there is a great deal of overlap between insulin and IGF signaling, insulin tends to regulate metabolism whereas IGF tends to regulate growth and proliferation. Hyperinsulinemia in the metabolic syndrome could

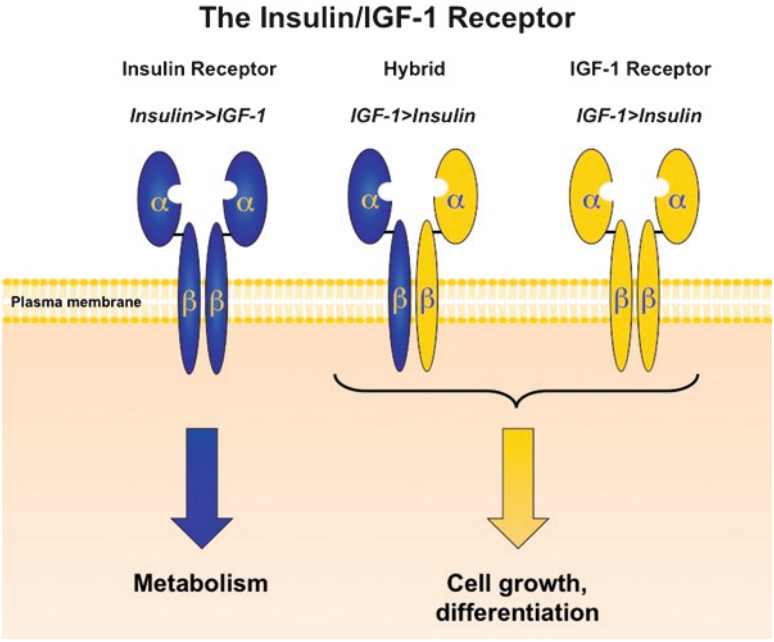

Fig. 2 The insulin /IGF-1 receptor. Both the insulin receptor and the IGF receptor are encoded by single genes which are processed into an α-chain and β-chain that remain linked by disulfide bonds. These α/β complexes can either homodimerize to form insulin receptors or IGF receptors, or heterodimerize to form hybrid receptors. Insulin binds preferentially to the insulin receptor whereas IGF-1 binds preferentially to the IGF-1 and hybrid receptors. Although there is a great deal of overlap in their function, the insulin receptor is more closely linked with metabolic effects whereas the hybrid receptor and IGF receptor are more closely linked with proliferation

potentially lead to the activation of the hybrid receptor or IGF receptor, driving cell growth and proliferation. This has been implicated in the pathogenesis of acanthosis nigricans, the thickening of the skin, particularly in the neck and axilla, present in insulin resistant individuals, the increased risk of cancer in patients with the metabolic syndrome, pseudoacromegaly and PCOS. However, other factors, including changes in the IGF binding proteins which alter IGF activity, could also play a role in these processes.

Insulin Receptor Substrate Proteins. Upon binding insulin, the insulin receptor phosphorylates and activates its numerous substrates. At least 11 substrates of the insulin receptor kinase have been identified, with the most prominent being the 6 members of the Insulin Receptor Substrate (IRS 1-6) family of proteins [8]. The IRS proteins share a similar structure: the N-terminal region contains a pleckstrin homology (PH) domain which mediates protein–lipid and protein–protein interactions and a phosphotyrosine binding (PTB) domain; the remainder of the molecule contains numerous tyrosine, serine and threonine residues which could potentially undergo phosphorylation. Phosphorylation of the IRS proteins on tyrosine residues activates these proteins, enabling them to recruit and activate their downstream targets [8]. In contrast, phosphorylation of the IRS proteins on serine residues appears to impair insulin signaling as discussed below.

The reason for the existence of so many IRS proteins is still unclear. The fact that IRS-4 is expressed primarily in embryonic tissues or cell lines, suggests that it may be important in producing tissue-specific responses to insulin [8]. IRS-1 and IRS-2, on the other hand, are widely distributed and though largely redundant, may have subtle distinctions in their functions. For example, IRS-1 may play the more important role in mediating insulin-stimulated glucose uptake in muscle [8] whereas IRS-2 may be more important in mediating glucose transport in brown adipocytes [9] and maintaining β-cell mass; [8] in the liver, IRS-1 and IRS-2 may play different roles in the regulation of glucose versus lipid metabolism [8].

The subtle differences in IRS-1 and IRS-2 function could be due to structural differences between the proteins. For example, IRS-2, but not IRS-1, possesses a Kinase Regulatory Loop Binding (KRLB) domain that appears to impair its ability to be tyrosine phosphorylated in response to insulin; [8, 10] the absence of this domain could contribute to preferential signaling through IRS-1 versus IRS-2. Alternatively, the fact that IRS-1 is associated with the low-density microsome (LDM) fraction, whereas IRS-2 is found in both the cytosol and LDM fraction, suggests that the two proteins differ in their subcellular distribution [8]. IRS-1 and IRS-2 expression levels could vary independently of one another, as they appear to be regulated by different mechanisms. For example, prolonged exposure of hepatocytes to insulin leads to a decrease in IRS-2 but not IRS-1 [11]. Consistent with this, hepatic IRS-2 expression is highest in the fasted state [12]. Ultimately, these differences in structure, subcellular localization and expression could yield differences in the downstream signals produced. For example, the Abl tyrosine kinase and the phosphatase SHP2 binds to IRS1, but not IRS-2, while the proteins Grb2, Crk and phospholipase Cg bind to IRS1 with a greater affinity than IRS2 [8]. Similarly, IRS-1 binds to 14-3-3ε protein and PKCα, leading to the formation of a complex that modulates insulin signaling in fibroblasts [13].

Additional targets of the insulin receptor tyrosine kinase include Src-homology-2 containing protein (Shc) which ultimately promotes proliferation; Cas-Br-M (murine) ectopic retroviral transforming sequence homologue (Cbl) which initiates glucose uptake; β-arrestin-2, a member of the β-arrestin family of adaptor proteins originally discovered as desensitizers of G-protein coupled receptors, which complexes Akt to the insulin receptor; [14] and Grb-2 associated binder 1 (Gab1) [8]. Gab1, in contrast to the others, appears to be a negative regulator of insulin signaling since knockout of Gab1 in the liver improves insulin sensitivity and signaling through IRS-1 and IRS-2 [15].

PI 3-Kinase. The class Ia phosphatidylinositol 3-kinase (PI 3-kinase) is a lipid kinase which plays a central role in insulin signaling (reviewed in [16)]. Active PI 3-kinase phosphorylates phosphotidylinositol-4,5-bisphosphate (PIP_2) to generate phosphotidylinositol-3,4,5-triphosphate (PIP_3). The PI 3-phosphates bind the pleckstrin homology domains of other signaling molecules, activating them or altering their subcellular location. PI 3-kinase thereby activates PDK1 (PI dependent kinase 1), which is in turn able to activate Akt and the atypical forms of protein kinase C, which are critical mediators of insulin action.

PI 3-kinase consists of a catalytic subunit, of which there are three isoforms, and a regulatory subunit, of which there are eight isoforms. As in the case of the IRS proteins, the roles of these different isoforms are not clear. Knockout of the catalytic subunits produces glucose intolerance [17]. However, partial reductions in the regulatory subunits, for example by deleting a single isoform such as p85α alone, surprisingly improve glucose tolerance [18]. Thus, either the stoichiometry of the regulatory to catalytic subunits is important or the regulatory subunits have some negative role in insulin signaling.

The importance of PI 3-kinase in insulin signaling is highlighted by studies showing that virtually all of insulin's metabolic effects, including glucose transport, lipogenesis and glycogenesis, are abolished by either inhibitors or dominant negative mutants of PI 3-kinase [19, 20].

MAP Kinases. The main members of the mitogen activated protein (MAP) kinase family involved in insulin signaling are the extracellular signal-regulated kinases, ERK1 and ERK2, and the stress kinase c-Jun NH2-terminal kinase (JNK). These proteins have different roles in both propagating and terminating the insulin signal. Insulin stimulates the binding of a complex containing the Src homology 2 (SH2)-containing adaptor protein Grb2 and the guanyl nucleotide exchange factor SOS to phosphotyrosines on the IRS proteins, Shc and Gab-1. This binding triggers the sequential activation of the small GTPase Ras, the kinase Raf, the dual specificity kinases MAPK/Erk Kinase (MEK)-1 and -2, and, ultimately ERK1 and ERK2. Activated ERK1 and ERK2 phosphorylate p90 ribosomal protein S6 kinase (p90RSK), transcription factors such as Elk1 and other targets [8].

ERK1 and ERK2 are mainly involved in mediating cell growth, survival and differentiation. Thus, pharmacological inhibitors and dominant negative mutants of these proteins inhibit the stimulation of cell growth by insulin, but do not alter insulin's metabolic or anabolic effects [16]. Although ERK1 and ERK2 have similar functions, ERK1, but not ERK2, has been shown to be required for adipogenesis both in vitro and in vivo [8].

Three JNK-coding genes (JNK1-3) have been described in mammals, each with multiple splice variants. JNK1 and JNK2 are expressed ubiquitously, whereas JNK3 expression is restricted to neuronal tissues [8]. Insulin has been shown to activate JNK1 and -2 in various cellular systems by a mechanism that may involve Rac and cdc42 [9, 21]. JNK takes part in a negative feedback loop of insulin action by phosphorylating IRS1 on serine residues, impairing the ability of IRS-1 to be activated by insulin. The fact that genetic deletion or downregulation by siRNA, expression of dominant negative mutant or endogenous inhibitory proteins of JNK, especially JNK1, improves insulin sensitivity and many metabolic functions in obese mice suggests that this may be the primary role of JNK in insulin signaling [22]. ERK1 and ERK2 may also have negative roles in insulin signaling [8].

The CAP/Cbl Pathway. Insulin also appears to be able to induce glucose transport independent of PI 3-kinase, by assembling signaling platforms that emanate from lipid rafts. This process is initiated by phosphorylation of the proto-oncogene c-cbl and the formation of a multiprotein complex at the plasma membrane, composed of c-cbl, c-Cbl associated protein (CAP) and the adaptor protein APS. CAP contains a sorbin homology domain which appears to bind the scaffolding protein flotillin, and localize the complex to the lipid raft. There, tyrosine-phoshorylated c-cbl is able to recruit CrkII, via its SH2 domain, and activate the guanyl nucleotide exchange protein C3G, which in turn activates the G-protein TC10. Once activated, TC10 promotes the formation of new signaling complexes that inhibit the rab31 GTPase [8]. Together, these events appear to facilitate the translocation, docking and fusion of the glucose transporter Glut4 at the plasma membrane.

Akt. In response to insulin, PI 3-kinase activates the serine/threonine kinase Akt (also known as protein kinase B, or PKB). There are three isoforms of Akt: Akt1 is ubiquitously expressed; Akt 2 is expressed predominantly in insulin-sensitive tissues, such as liver, fat and muscle; and Akt3 is expressed primarily in the brain. PI 3-kinase activates Akt through several mechanisms [20, 23]. First, the generation of PI 3-phosphates, particularly PIP_3, activates PDK1. PIP_2 and PIP_3 also recruit Akt to the plasma membrane through the pleckstrin homology domain of Akt, thus bringing it into proximity with its kinase PDK1. Additionally, binding of PIP_3 to the pleckstrin homology domain of Akt induces a conformational change that allows Thr 308 to be phosphorylated by PDK1. Akt activation also requires phosphorylation on Ser473 by mTORC2, a protein complex which includes the protein kinase mTOR (mammalian target of rapmycin) and the regulatory protein, rictor (rapamycin-insensitive companion of mTOR) [24]. Phosphorylation of Thr308 and Ser473 results in Akt activation. Akt plays a key role in mediating the effects of insulin on glucose transport, protein synthesis, glycogen synthesis and gene expression as described below.

Atypical PKCs. PI 3-kinase also activates the atypical PKCs, PKCζ (zeta) and PKCλ (lambda)/ι (iota) PKCλ is the mouse ortholog of PKCι, which is present in humans. The atypical PKCs (aPKCs) differ from the conventional PKCs [α (alpha), β (beta)I, β (beta)II, γ (gamma)] and the novel PKCs [δ (delta), ε (epsilon), η (eta), θ (theta), μ (mu)] in that they do not require diacylglycerol (DAG) for activation. More importantly, conventional and novel PKCs appear to be negative regulators of

insulin signaling, whereas the atypical PKCs are important mediators of insulin action. In muscle and fat, the aPKCs stimulate glucose transport in response to insulin. Overexpression of PKCζ or PKCλ results in increased translocation of the insulin-sensitive glucose transporter GLUT4 to the plasma membrane [25, 26]. Conversely, dominant negative mutants of PKCλ inhibit insulin-stimulated glucose uptake [27]. In the liver, however, the aPKCs appear to stimulate lipogenesis.

mTOR. Another important downstream target of insulin is the mTORC1 complex [8]. mTORC1, like mTORC2, contains the protein kinase mTOR. However, the two complexes are functionally distinct, and mTORC1 complex contains the regulatory protein raptor (regulatory associated protein of TOR), instead of rictor. Akt activates mTORC1 by phosphorylating and inhibiting tuberin, or tuberous sclerosis complex-2 (TSC2), which is in a complex with hamartin, or TSC1 [28]. The TSC1/TSC2 complex inhibits the GTPase Ras homologue enriched in brain (Rheb) [29]. Rheb is an activator of mTORC1. Thus, activation of Akt by insulin results in the dis-inhibition of Rheb and the activation of mTORC1. mTORC1 promotes protein synthesis by phosphorylating eukaryotic translation initiation factor 4E binding protein 1 (4EBP1). 4EBP1 inhibits translation by binding eukaryotic translation initiation factor 4E (eIF4E), a limiting component of the translation inititation complex. Phosphorylation of 4EBP1 allows eIF4E to dissociate, and thereby increases translation. In addition, mTORC1 phosphorylates and activates p70 ribosomal S6 kinase (S6K), which increases ribosome biosynthesis.

GSK3. In the liver, insulin is a key signal to promote glycogen synthesis. There are two isoforms of glycogen synthase kinase 3 (GSK3), GSK3α and GSK3β, encoded by two different genes. GSK3α and GSK3β phosphorylate and inhibit glycogen synthase, the enzyme catalyzing the final step in glycogen synthesis. Akt inactivates GSK3α and GSK3β by phosphorylating them on Ser 21 and Ser9, respectively [30]. Thus, insulin activates Akt, which inactivates GSK3, and derepresses glycogen synthase, leading to a stimulation of glycogen synthesis. Mutation of the Akt phosphorylation sites of GSK3—i.e., mutation of serine 21 to alanine in GSK3α (S21A) and mutation of serine 9 to alanine in GSK3β (S9A)—renders it insensitive to insulin. Studies of mice with knockin of the S21A mutation in GSK3α or the S9A mutation in GSK3β show that GSK3β is more important in the regulation of muscle glycogen synthase by insulin [31]. Consistent with this, mice with muscle-specific knockout of GSK3β show improved glucose tolerance, due to enhanced stimulation of glycogen synthase by insulin [32]. GSK3α may have a more important role in the liver, as mice with whole body knockout of GSK3α have improved whole-body glucose tolerance and hepatic insulin sensitivity, but mice with liver-specific knockout of GSK3β show no change in glucose or insulin tolerance or glycogen content [32, 33].

AS160. Akt substrate of 160 kDa (AS160) is phosphorylated by Akt. AS160 contains an intrinsic rab GTPase activating domain. In its GDP bound form, AS160 is inactive; phosphorylation by Akt inhibits its GTPase activity, allowing a switch to the GTP bound form, which promotes translocation of GLUT 4-containing vescicles to the cell surface, thereby increasing glucose uptake [34].

FoxO1. Insulin exerts many of its effects at the transcriptional level. Of the many transcription factors and coactivators involved, FoxO1 and SREBP-1c are among the most well studied. Akt inactivates FoxO1 by phosphorylating it on residues Thr-24, Ser-256 and Ser-319 though other kinases have been implicated [35]. Phosphorylated FoxO1 is excluded from the nucleus and targeted for degradation. Insulin may also regulate FoxO1 by acetylation [35] and modulation of its transcriptional co-activator, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC)-1α.[36] In the absence of insulin, FoxO1 activates transcription both directly, by binding to insulin response elements (IREs) in the promoters of its target genes [37], and indirectly by co-activating other transcription factors [38]. FoxO1 induces the gluconeogenic enzymes, glucose-6-phosphatase (*G6pc*) and phosphoenolpyruvate carboxykinase (*Pck1*). Therefore, increased expression of FoxO1 leads to increased fasting glucose and impaired glucose tolerance [39] whereas knockdown of FoxO1 decreases gluconeogenic gene expression and decreases serum glucose levels[40].

Although the effects of FoxO1 on glucose metabolism are its most prominent effects in insulin signaling, FoxO1 has many other important effects in the cell. For example, FoxO1 regulates triglyceride metabolism by inducing microsomal triglyceride transfer protein (*Mttp*), which promotes the lipidation of apolipoprotein B (ApoB), a rate-determining step in VLDL secretion; [41] FoxO1 induces ApoCIII, an apolipoprotein which inhibits lipoprotein lipase activity and promotes hypertriglyceridemia; [42] FoxO1 inhibits lipogenic gene expression [39]. In addition, FoxO1 promotes the expression of the cholesterol efflux transporters, *Abcg5* and *Abcg8* [43], which stimulates cholesterol efflux into the bile, [44] and plays an important role in the regulation of bile acid metabolism, [45] cell growth and differentiation, protection from reactive oxygen species and may even modulate insulin sensitivity (reviewed in [46)].

Insulin also regulates the related protein FoxA2. Akt phosphorylates Foxa2 on Thr156, preventing its nuclear localization and thereby inactivating FoxA2. FoxA2 promotes the transcription of the enzymes of fatty acid oxidation, Mttp, and gluconeogenic genes [47, 48]. FoxA2 also regulates the bile acid transporters, and the knockout of FoxA2 in the liver leads to intrahepatic cholestasis [49].

SREBP-1c. The sterol regulatory element-binding proteins (SREBPs) are a family of three nuclear transcription factors encoded by two genes [50]. SREBP-1a and SREBP-1c are derived from the same gene, and both appear to regulate lipogenic gene transcription. SREBP-1c, however, is the dominant isoform in liver and adipose. SREBP-1c is capable of activating the entire program of monounsaturated fatty acid synthesis. Mice expressing a constitutively active isoform of SREBP-1c have an increase in lipogenic gene expression and hepatic triglyceride content [51]. In addition, SREBP-1c inhibits transcription of IRS-2 [52] and the gluconeogenic genes [53], potentially contributing to changes in glucose metabolism as well.

The SREBPs are subject to complex regulation at the transcriptional and post-translational levels [50]. Their transcripts encode membrane bound precursors, which are retained in the endoplasmic reticulum by Insig proteins. Sterol depletion causes dissociation of the Insig proteins, allowing the SREBPs to proceed to the

golgi, where the Site 1 and Site 2 proteases reside. These proteases release a soluble fragment of SREBP that can translocate into the nucleus to activate transcription.

Several lines of evidence suggest that SREBP-1c is regulated by insulin. First, SREBP-1c transcript and nuclear protein are increased by insulin treatment in hepatocytes [54]. Second, streptozotocin treatment, which renders mice insulin deficient, results in a decrease in SREBP-1c [54]. Similarly, fasting, which also lowers insulin levels, decreases SREBP-1c [55]. Conversely, refeeding induces an exaggerated insulin response which is accompanied by an increase in SREBP-1c [55]. Knockout of SREBP-1c impairs the lipogenic response to insulin in the context of re-feeding [56].

The mechanisms by which insulin induces SREBP-1c are not clear. Insulin appears to induce transcription of SREBP-1c via the nuclear hormone receptor, Liver X Receptor (LXR), as knockout of LXR prevents insulin from inducing SREBP-1c and its targets [57]. Insulin has been reported to increase the stability of LXR mRNA, [58] and has been suggested to induce the oxysterol ligand of LXR. Insulin also acts post-transcriptionally to induce the processing of SREBP-1c to its active nuclear form by suppressing expression of Insig2a [59]. As Insig levels fall, SREBP-1c is no longer retained in the endoplasmic reticulum. In addition, SREBP-1c may undergo other modifications which regulate its activity, including phosphorylation and ubiquitination. The insulin signaling components which mediate insulin's effects on SREBP-1c are not clear, and PI 3-kinase, PKC-λ, GSK-3β and MAPK have been implicated.

It should also be noted that SREBP-1c is under complex control, and insulin is not the only, or even the dominant, regulator of SREBP-1c. Thus, dietary factors, such as carbohydrates and polyunsaturated fatty acids and hormonal factors, such as leptin, also regulate SREBP-1c [60]. It is therefore possible that these other pathways activate SREBP-1c independent of insulin signaling in the metabolic syndrome.

Metabolic Effects of Insulin Resistance: Lessons from Knockout Mice

The existence of so many redundant components and branches of the insulin signaling cascade suggests that, (a) a lesion in a given node in the insulin signaling cascade will only have phenotypic consequences if the other isoforms in that node are unable to compensate for it, and (b) the phenotype produced by a given lesion in the insulin signaling cascade will depend on the location of the lesion. These concepts have been validated by studies using mice with targeted mutations of different components of the insulin signaling cascade (reviewed in [16)]. In particular, mice with liver-specific mutations in the insulin receptor, IRS-1 and -2, PI 3-kinase and PKC-λ have been generated. Here, we will discuss how defects in the different insulin signaling components, even within the same tissue, vary in their contribution to the metabolic syndrome.

Insulin Receptor Knockout (LIRKO) Mice. LIRKO mice show greater than 95% deletion of the insulin receptor in liver. This results in complete insulin resistance, as the insulin receptor is unable to activate any of its downstream targets. Consistent with the role of insulin in suppressing hepatic gluconeogenesis, LIRKO mice are hyperglycemic, with increased expression of the gluconeogenic genes, increased hepatic glucose output, marked glucose intolerance and hyperglycemia [61]. LIRKO mice are also markedly hyperinsulinemic, and this is due both to β cell compensation, because the β cells secrete excess insulin in response to the hyperglycemic stimulus, and to decreased insulin clearance, because insulin receptors in the liver play an important role in the clearance of insulin from the serum. However, unlike diet-induced obese mice and humans with the metabolic syndrome, LIRKO mouse livers do not to respond at all to hyperinsulinemia.

LIRKO mice also show a decrease in SREBP-1c and lipogenic gene expression, particularly in the re-fed state [62]. Although the triglyceride content of the liver is similar to wild type controls, VLDL secretion is markedly abnormal in LIRKO mice. VLDL contains triglycerides, cholesterol and phospholipids in complex with ApoB, the principal apolipoprotein component of VLDL. As expected from the decrease in SREBP-1c and its targets, VLDL-triglyceride secretion is decreased in LIRKO mice. However, ApoB secretion is increased. This discrepancy could be due to the fact that insulin inhibits ApoB lipidation, by inhibiting transcription of *Mttp* transcription by FoxO1,[41] and that insulin targets ApoB protein for degradation [63]. Consequently, LIRKO livers secrete VLDL particles that are relatively poor in triglycerides and rich in cholesterol.

In addition to abnormal VLDL particles, LIRKO mice show reduced levels of HDL-cholesterol [62]. When stressed with an atherogenic diet, LIRKO mice develop marked hypercholesterolemia, which is associated with decreased expression of the low density lipoprotein (LDL) receptor, and decreased LDL clearance. Consequently, all of the LIRKO mice but none of the controls develop atherosclerosis after being fed the atherogenic diet for less than 4 months [62].

In addition, the cholesterol transporters *Abcg5* and *Abcg8* are increased threefold at the mRNA levels in LIRKO livers. These transporters reside on the cannilicular membrane of the hepatocyte and regulate the efflux of cholesterol into bile. Consequently, biliary cholesterol secretion is increased threefold in LIRKO mice [43]. This finding is important because increased biliary cholesterol secretion contributes to gallstone formation in obese humans [64]. Not surprisingly, when fed a lithogenic diet, 36% of LIRKO mice, but none of the control mice, develop cholesterol gallstones within 1 week [43].

Knockout of the Insulin Receptor Substrates (IRS). Unlike LIRKO mice, liver-specific knockout of either IRS-1 or IRS-2 showed very subtle phenotypes [8]. Consistent with the fact that IRS-1 is expressed at higher levels in the fed state, mice with liver-specific knockout of IRS-1 alone showed increased gluconeogenic gene expression and hepatic glucose production, glucose intolerance and decreased lipogenic gene expression in the fed state [12]. However, these abnormalities were absent in the fasted state. In contrast, mice with liver-specific knockout of IRS-2 alone showed increased gluconeogenic gene expression, increased hepatic glucose

production, glucose intolerance and decreased lipogenic gene expression in the fasted, but not fed state [12]. Mice with hepatic knockout of either IRS-1 or IRS-2 did not show changes in serum triglyceride or cholesterol levels [12].

On the other hand, mice with knockout of both IRS-1 and IRS-2 in the liver show marked metabolic changes including increased hepatic glucose production and glucose intolerance in both the fed and fasted states, decreased serum triglyceride secretion, decreased serum triglycerides and decreased HDL cholesterol [12, 65]. In addition, genetic ablation of FoxO1 in the livers of these mice restores gluconeogenic gene expression, fasting glucose, insulin levels, serum triglyceride and HDL levels towards normal. This underscores the importance of FoxO1 in this phenotype [12, 65].

Knockout of PI 3-Kinase. The two major PI 3-kinase regulatory subunits expressed in the liver are p85α and p85β. By mating mice with whole-body knockout of the p85β (βKO) to mice with liver-specific knockout of the *Pik3r1* gene, which encodes p85α, as well as the less abundant isoforms, p55α and p50α (αLKO), mice harboring both these deletions (p85α/β–DKO) were generated [66]. Mice with knockout of either p85α in the liver or p85β in the whole body show no changes in the activation of the downstream targets of PI 3-kinase, glucose or triglyceride metabolism. However, p85α/β–DKO mice fail to activate PI 3-kinase [66]. Consequently, p85α/β–DKO mice show blunted responses of Akt, PKCλ, FoxO1, GSK3β, TSC2 and p70S6 kinase to insulin. They show increased gluconeogenic gene expression, hyperglycemia and hyperinsulinemia. In addition, SREBP-1c, its downstream target, fatty acid synthase, and serum triglycerides are decreased. Mice with acute knockdown of PI 3-kinase in the liver show a similar phenotype [67]. These mice were generated by injecting wild type mice with adenovirus encoding a dominant negative mutant of p85α, which abolishes basal and insulin stimulated PI 3-kinase. Acute knockdown of PI 3-kinase also produces hyperglycemia, hyperinsulinemia and a marked reduction in serum triglyceride and cholesterol levels.

PKC-λ knockout. Matsumoto and colleagues [68] have characterized mice with knockout of PKC-λ in the liver (L-λKO mice). Although knockout of PKC-λ induced hyperinsulinemia, it did not impair the ability of insulin to suppress gluconeogenic gene expression in vitro or in vivo. Consequently, L-λKO mice showed normal serum glucose levels and liver glycogen content after being challenged with a glucose load. However, they showed reduced levels of SREBP-1c mRNA and protein, decreased expression of the SREBP-1c target gene, fatty acid synthase and reduced hepatic triglyceride levels. Serum triglyceride levels were normal. In addition, treatment with an LXR agonist was able to fully restore SREBP-1c levels in L-λKO mice.

Similarly, knockout of liver PKC-λ impairs the ability of constitutively active PI 3-kinase, delivered by adenovirus, to increase expression of SREBP-1c but not its ability to decrease serum glucose levels. Interestingly, L-λKO mice show normal levels of serum and hepatic cholesterol. Taken together, these data indicate that PKC-λ is an important driver of lipogenesis, but not glucose metabolism. Consistent with this, restoration of PKC-λ in L-λKO livers was able to increase expression of SREBP-1c and liver triglyceride content, but did not alter serum glucose levels.

Taken together, these data illustrate several points about insulin resistance. First, studies in LIRKO mice show that insulin resistance can produce several components of the metabolic syndrome: hyperglycemia, low HDL cholesterol and increased susceptibility to atherosclerosis and cholesterol gallstones. This is a very important finding because it indicates that, at least in mice, insulin can play a causative role in the metabolic syndrome phenotype. Whether defects in the IRS proteins or other downstream insulin signaling molecules also produce this phenotype is yet to be determined. Second, the insulin resistance phenotype varies with the particular node involved. For example, LIRKO mice and p85α/β–DKO show hyperglycemia and decreased levels of SREBP-1c. In contrast, mice with knockout of PKC-λ only show decreased levels of SREBP-1c. L-λKO mice also show that molecular insulin resistance can exist even in the absence of hyperglycemia. Third, studies in mice with knockout of the IRS proteins or the PI 3-kinase regulatory subunits show that the severity of the phenotype produced by an insulin signaling defect depends on the extent to which the node itself is compromised. Thus, knockout of IRS-1, IRS-2, p85α or p85β alone produces rather subtle phenotypes. However, when both IRS isoforms or both p85 isoforms are knocked out in the liver, a phenotype very similar to the LIRKO results.

Molecular Mechanisms of Insulin Resistance

The metabolic syndrome is caused by a combination of genetic and environmental factors. One of the most important environmental factors is overnutrition. Here, we will review some of the mechanisms which have been suggested to underlie the associations between obesity and insulin resistance, including inflammation, lipotoxicity, ER stress and hyperglycemia (Figs. 3, 4).

Inflammation. Overnutrition and obesity appear to trigger an inflammatory response. Hence, macrophage activation and infiltration are commonly observed in the adipose tissue of obese humans and mice [22]. This is associated with increased secretion of chemokines and pro-inflammatory cytokines such as TNF-α, interleukin (IL)-1 and IL-6, resulting in a generalized state of inflammation [69]. Inflammation can produce insulin resistance through several mechanisms. Inflammation increases phosphorylation of IRS-1 on Ser307. TNF-α, for example, initiates the formation of a multiprotein signaling complex that triggers the activation of a kinase cascade which activates JNK [22]. JNK phosphorylates IRS-1 on Ser 307. Though IRS-1 Ser-307 phosphorylation has been extensively described as a marker of insulin resistance in mice and humans, it is not clear how this modification impairs insulin signaling. Ser-307 phosphorylation may interfere with IRS-1 function by disrupting its interaction with the insulin receptor or promoting the interaction of IRS-1 with 14-3-3 proteins, impairing its ability to activate its downstream targets [8]. Serine phosphorylation has also been shown to alter the intracellular localization of IRS-1 and induce its degradation [8].

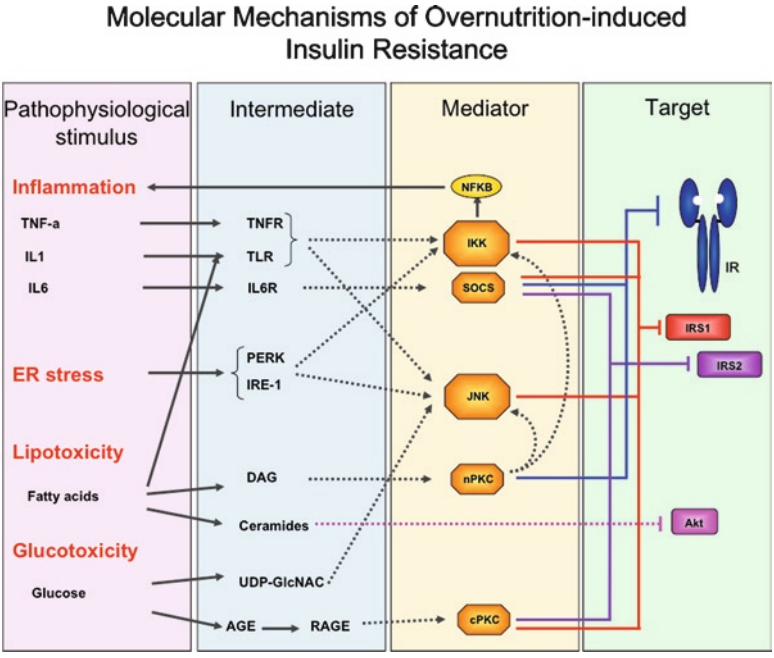

Fig. 3 Molecular mechanisms of overnutrition-induced insulin resistance. Overnutrition appears to induce insulin resistance through many pathways, including inflammation, ER stress, lipotoxicity and glucotoxicity. Together, these processes induce the SOCS proteins and activate JNK, IKK, the conventional PKCs and novel PKCs. This ultimately inhibits signaling through the insulin receptor, IRS-1, IRS-2 and Akt. However, it is not clear which of these pathways, if any, plays the major role in causing the insulin resistance associated with obesity

Inflammation also increases expression of the suppressors of cytokine signaling (SOCS) proteins. For example, IL-6 induces transcription of the SOCS proteins by activating the STAT family of transcription factors [70]. SOCS -1 and SOCS-3 in particular decrease insulin signaling by (a) direct interaction with the insulin receptor, which could prevent binding of the IRS proteins or decrease the kinase activity of the insulin receptor, and (b) by promoting degradation of the IRS proteins [8]. In addition, SOCS3 promotes leptin resistance in the hypothalamus [70] and may regulate β cell mass and proliferation [71]. Consistent with this, mice heterozygous for a deletion of SOCS3 are resistant to diet-induced obesity and insulin resistance [70].

Inflammation activates the NFκB pathway. Cytokines, such as IL-1, stimulate the formation of signaling platforms involving TNF-receptor associated factor (TRAF) proteins and transforming growth factor-β activated kinase (TAK) 1 (reviewed in [72)], which activate the inhibitor κB kinase (IKK). IKK is a central mediator of the inflammation response, as it activates the transcription factor, nuclear factor kappa B (NFκB). NFκB drives the production of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6, thereby further promoting inflammation and insulin resistance [73]. Forced expression of IKK is sufficient to induce insulin

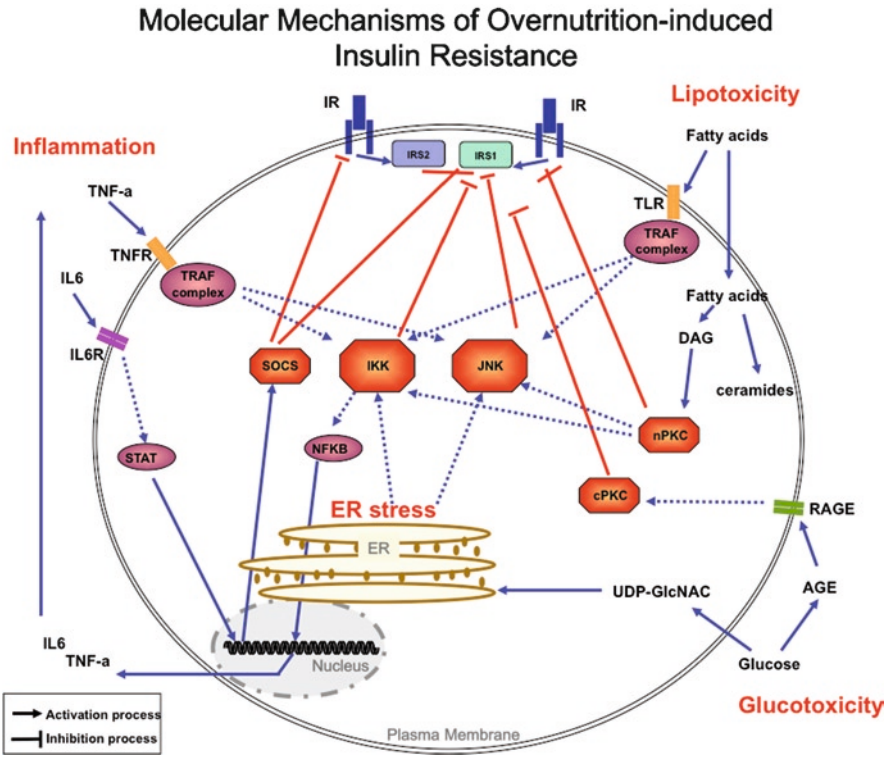

Fig. 4 Negative effects of glucotoxicity, lipotoxicity and inflammation on insulin signaling

resistance [22] whereas deletion of the gene protects against high fat diet-induced insulin resistance [22]. In addition, obesity increases IKK activity and salicylates, IKK-β inhibitors, have proven to be efficient insulin sensitizers in mice and humans [22].

Lipotoxicity. Free fatty acids themselves promote inflammation by activation of the toll-like receptor (TLR)-2 and -4 pathways [22]. TLRs signal via the formation of TRAF protein-containing complexes, and activate the JNK and the IKK/NFκB pathways [72]. Excess fatty acids can also become deposited into tissues such as the liver and muscle, where they drive the formation of diacylglycerol (DAG) and other potentially toxic lipid metabolites, such as GM3 ganglioside or ceramides [74]. DAG activates JNK and the novel PKCs, PKCθ and PKCε. PKCθ is present in muscle and stimulates activation of IKK-β and JNK [75]. PKCε is present in liver and has been shown to directly interact with the insulin receptor and decrease its activity [76]. GM3 ganglioside and ceramides, on the other hand, have been reported to induce insulin resistance by interfering with the activation of the insulin receptor and Akt, respectively (as reviewed in [74])].

Endoplasmic Reticulum (ER) Stress. The ER is an organelle dedicated to the synthesis, folding and maturation of all secreted and membrane proteins. When the

ER becomes overloaded with misfolded proteins, it triggers the unfolded protein response (UPR), a coordinate response involving inositol-requiring enzyme 1 (IRE-1), double stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6). Under normal physiological conditions, the protein chaperone, BiP, interacts with IRE-1, PERK and ATF6, and maintains them in an inactive conformation. Under conditions of stress, the misfolded proteins bind and sequester BiP. As a result of BiP being removed from IRE-1, PERK and ATF-6, these proteins become activated, producing the UPR [77].

The UPR leads to a general attenuation of translation, which decreases the influx of more unfolded proteins, and increases expression of the chaperone proteins necessary to bind the unfolded proteins present. The UPR therefore represents a homeostatic mechanism. However, it also leads to insulin resistance. IRE-1 directly binds to TRAF-2 and triggers the activation of JNK [22] and IKK [22]. Furthermore, PERK phosphorylates the alpha subunit of the translation initiation factor 2 (eIF2a), and this results in decreased expression of IκB-α, a negative regulator of NFκB [22]. Consequently, genetic manipulations or chemical treatment that reduce the UPR have been shown to improve insulin sensitivity [22].

Glucose Toxicity. Insulin resistance eventually leads to hyperglycemia. In a vicious cycle, hyperglycemia leads to glucose toxicity, which further impairs insulin signaling, as well as insulin secretion. The effects of hyperglycemia on insulin signaling are thought to be mediated by the hexosamine pathway. This starts with the production of glucosamine 6-phosphate from fructose 6-phosphate by the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). The major end product is UDP-*N*-acetylglucosamine (UDP-GlcNAC), which provides a substrate for the glycosylation of proteins and lipids [78]. Although the mechanism by which this pathway impairs insulin action is unclear, possible mechanisms include ER stress and JNK activation [79]. Alternatively, the fact that overexpression of *O*-GlcNAc transferase (OGT) decreases Akt Thr308 phosphorylation and increases IRS-1 Ser307, 632/635 phosphorylation suggests that glycosylation of key signaling molecules may also occur [79]. In addition, hyperglycemia leads to the formation of advanced glycation end (AGE) products through non-enzymatic modification of proteins by reducing sugars. AGEs bind and activate the receptor for AGE (RAGE). The RAGE receptor activates PKCα, which in turn induces serine phosphorylation of IRS-1 and IRS-2 [80].

Other Mechanisms of Insulin Resistance. Studies to understand how environmental factors interfere with insulin signaling have focused on IRS-1 Ser 307 phosphorylation by JNK. However, there are several important points to note. First, there are over 70 potential serine phosphorylation sites on IRS-1, and other IRS proteins may also undergo serine phosphorylation. The importance of IRS-1 Ser 307 relative to these other sites has not been adequately studied. Second, although many studies have demonstrated negative effects of serine phosphorylation, there is some evidence that serine phosphorylation of certain sites may serve a positive role. Finally, multiple other mechanisms could contribute to insulin resistance in the metabolic syndrome [8].

Insulin resistance could result from increases in the activity or amount of the enzymes which normally terminate the insulin signal, including the phosphotyrosine phosphatases, such as PTP1b, which de-phosphosphorylate and de-activate the insulin receptor. Increased tyrosine phosphatase activity has been described in insulin resistant states, and knockout of PTP1b improves insulin sensitivity in different models of insulin resistance [8]. Similarly, increased levels of the PIP_3 phosphatases, e.g., PTEN and SHIP, which dephosphorylate the PIP_3 produced by PI 3-kinase, would be expected to impair insulin signaling. Consistent with this, reduction of PTEN and SHIP2 expression improves insulin sensitivity in various models of insulin resistance through upregulation of PI3-K and Akt signaling [8]. In addition, the pseudo-kinase TRB3, a mammalian homolog of drosophila tribbles, has been proposed to contribute to obesity-induced insulin resistance by interacting with Akt and downregulating its activity, [8] and endogenous Akt inhibitors such as CTMP and PHLPP may also play a role [8].

Thus, it is likely that many mechanisms, independent of JNK and IRS-1 Ser 307 phosphorylation, play a role in mediating the effects of overnutrition on insulin signaling. In particular, it is likely that different genetic and environmental insults will produce their own signature of molecular insulin resistance, with a defined cluster of defects in the insulin signaling pathway.

Insulin Resistance in Humans

Identifying insulin signaling defects associated with the metabolic syndrome in humans is complicated by significant methodological challenges (reviewed in [81)]. Two approaches have been used. First, biopsies have been taken from insulin-resistant subjects and their controls both before and after being subject to a hyperinsulinemic euglycemic clamp, and insulin signaling has been examined. Alternatively, biopsies have been taken from individuals, and cultured in vitro in the presence or absence of insulin. Comparing the results of these different studies is difficult because of the numerous variables present, such as the patient population, the dose and duration of insulin, the site of biopsy and how the biopsy is processed for signaling studies. Therefore it is not surprising that some studies have identified defects in the insulin signaling pathway whereas others have not (reviewed in [16)]. Nonetheless, defects in insulin binding, insulin receptor tyrosine phosphorylation, IRS-1 tyrosine phosphorylation have been documented. Similarly defects in PI 3-kinase and its downstream targets, Akt and aPKC have been shown. The most consistent insulin signaling defect to be observed in muscle and adipose is in the aPKCs, and this occurs in the presence or absence of defects in Akt. In contrast, activities of ERK1/2 and JNK appear to be relatively intact.

In a complementary approach, humans with defined lesions in the insulin signaling cascade have been studied [82]. Patients with mutations in the insulin receptor are hyperglycemic and hyperinsulinemic. Consistent with the studies in LIRKO mice, they show low serum triglycerides, their VLDL is relatively poor in triglycerides

and they show no increase in either lipogenesis or hepatic triglyceride content. In contrast, humans with mutations in Akt2 develop hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and increased lipogenesis and hepatic triglyceride content. Taken together, these studies indicate that multiple defects in the insulin signaling pathway are possible in humans, but that not all pathways are equally affected. Furthermore, the phenotype of the individual will depend on the specific complement of lesions he or she harbors.

Conclusions

The role of insulin resistance in the metabolic syndrome is yet to be resolved. However, recent advances in our understanding of the insulin signaling cascade show that many of the ideas put forth by Dr. Reaven, over 20 years ago, are likely correct. First, studies, particularly in LIRKO mice, indicate that many features of the metabolic syndrome, in addition to hyperglycemia, can be produced by insulin resistance, namely, low HDL cholesterol, susceptibility to atherosclerosis and susceptibility to cholesterol gallstones. Further characterization of mice and humans with defined lesions in the insulin signaling pathway may even show other features to be related. Second, the existence of multiple branches and nodes in the insulin signaling pathway explain how some pathways of the metabolic syndrome can remain sensitive to insulin, while others become resistant. For example, a specific defect in Akt could allow continued signaling through the insulin receptor/IRS/PI 3-kinase/PKC-λ pathway leading to the activation of lipogenesis. Since Akt regulates FoxO1 and gluconeogenesis, defects in Akt could produce both hyperglycemia and increased serum and hepatic triglycerides, as observed in humans with mutations in Akt. Although the precise lesions that cause insulin resistance in humans, particularly in tissues such as the liver, are yet to be determined, studies to date suggest that defects do occur.

Finally, the unexpected finding of so many redundant components in the insulin signaling cascade suggests that the metabolic syndrome may be even more complex than we had initially thought. In other words, the number of defects possible in the insulin signaling cascade is staggering, and each defect is likely to produce a slightly different phenotype. The exact complement of lesions harbored by any individual will depend on both the genetic and environmental insults to which he or she is subjected. Ultimately, we hope to be able to diagnose and treat individuals with the metabolic syndrome based on their specific insulin signaling defects, leading to more effective therapy with fewer adverse effects.

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References

1. Reaven, G. M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes, 37*(12), 1595–1607.
2. Cornier, M. A., Dabelea, D., Hernandez, T. L., et al. (2008). The metabolic syndrome. *Endocrine Reviews, 29*(7), 777–822.
3. Ford, E. S., Giles, W. H., & Mokdad, A. H. (2004). Increasing prevalence of the metabolic syndrome among U.S. adults. *Diabetes Care, 27*(10), 2444–2449.
4. Kahn, R., Buse, J., Ferrannini, E., & Stern, M. (2005). The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care, 28*(9), 2289–2304.
5. Reaven, G. (2004). The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinology and Metabolism Clinics of North America, 33*(2), 283–303.
6. Yamaguchi, Y., Flier, J. S., Benecke, H., Ransil, B. J., & Moller, D. E. (1993). Ligand-binding properties of the two isoforms of the human insulin receptor. *Endocrinology, 132*(3), 1132–1138.
7. Leibiger, B., Leibiger, I. B., Moede, T., et al. (2001). Selective insulin signaling through A and B insulin receptors regulates transcription of insulin and glucokinase genes in pancreatic beta cells. *Molecular cell, 7*(3), 559–570.
8. Taniguchi, C. M., Emanuelli, B., & Kahn, C. R. (2006). Critical nodes in signalling pathways: insights into insulin action. *Nature Reviews. Molecular Cell Biology, 7*(2), 85–96.
9. Fasshauer, M., Klein, J., Ueki, K., et al. (2000). Essential role of insulin receptor substrate-2 in insulin stimulation of Glut4 translocation and glucose uptake in brown adipocytes. *The Journal of Biological Chemistry, 275*(33), 25494–25501.
10. Wu, J., Tseng, Y. D., Xu, C. F., Neubert, T. A., White, M. F., & Hubbard, S. R. (2008). Structural and biochemical characterization of the KRLB region in insulin receptor substrate-2. *Nature Structural & Molecular Biology, 15*(3), 251–258.
11. Hirashima, Y., Tsuruzoe, K., Kodama, S., et al. (2003). Insulin down-regulates insulin receptor substrate-2 expression through the phosphatidylinositol 3-kinase/Akt pathway. *The Journal of Endocrinology, 179*(2), 253–266.
12. Kubota, N., Kubota, T., Itoh, S., et al. (2008). Dynamic functional relay between insulin receptor substrate 1 and 2 in hepatic insulin signaling during fasting and feeding. *Cell Metabolism, 8*(1), 49–64.
13. Oriente, F., Andreozzi, F., Romano, C., et al. (2005). Protein kinase C-alpha regulates insulin action and degradation by interacting with insulin receptor substrate-1 and 14-3-3 epsilon. *The Journal of Biological Chemistry, 280*(49), 40642–40649.
14. Luan, B., Zhao, J., Wu, H., et al. (2009). Deficiency of a beta-arrestin-2 signal complex contributes to insulin resistance. *Nature, 457*(7233), 1146–1149.
15. Bard-Chapeau, E. A., Hevener, A. L., Long, S., Zhang, E. E., Olefsky, J. M., & Feng, G. S. (2005). Deletion of Gab1 in the liver leads to enhanced glucose tolerance and improved hepatic insulin action. *Nature Medicine, 11*(5), 567–571.
16. Biddinger, S. B., & Kahn, C. R. (2006). From mice to men: insights into the insulin resistance syndromes. *Annual Review of Physiology, 68*, 123–158.
17. Brachmann, S. M., Ueki, K., Engelman, J. A., Kahn, R. C., & Cantley, L. C. (2005). Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. *Molecular and Cellular Biology, 25*(5), 1596–1607.
18. Terauchi, Y., Tsuji, Y., Satoh, S., et al. (1999). Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. *Nature Genetics, 21*(2), 230–235.
19. Cheatham, B., Vlahos, C. J., Cheatham, L., Wang, L., Blenis, J., & Kahn, C. R. (1994). Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase,

DNA synthesis, and glucose transporter translocation. *Molecular and Cellular Biology, 14*(7), 4902–4911.
20. Shepherd, P. R., Withers, D. J., & Siddle, K. (1998). Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *The Biochemical Journal, 333*, (Pt 3):471–490.
21. Bishop, A. L., & Hall, A. (2000). Rho gtpases and their effector proteins. *The Biochemical Journal, 348*, Pt 2:241–255.
22. Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature, 444*(7121), 860–867.
23. Vanhaesebroeck, B., & Alessi, D. R. (2000). The PI3K-PDK1 connection: more than just a road to PKB. *The Biochemical Journal, 346*, Pt 3:561–576.
24. Sarbassov, D. D., Guertin, D. A., Ali, S. M., & Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mtor complex. *Science, 307*(5712), 1098–1101.
25. Bandyopadhyay, G., Standaert, M. L., Zhao, L., et al. (1997). Activation of protein kinase C (alpha, beta, and zeta) by insulin in 3T3/L1 cells. Transfection studies suggest a role for PKC-zeta in glucose transport. *The Journal of Biological Chemistry, 272*(4), 2551–2558.
26. Tremblay, F., Lavigne, C., Jacques, H., & Marette, A. (2001). Defective insulin-induced GLUT4 translocation in skeletal muscle of high fat-fed rats is associated with alterations in both Akt/protein kinase B and atypical protein kinase C (zeta/lambda) activities. *Diabetes, 50*(8), 1901–1910.
27. Kotani, K., Ogawa, W., Matsumoto, M., et al. (1998). Requirement of atypical protein kinase clambda for insulin stimulation of glucose uptake but not for Akt activation in 3T3-L1 adipocytes. *Molecular and Cellular Biology, 18*(12), 6971–6982.
28. Inoki, K., Li, Y., Zhu, T., Wu, J., Guan, K. L. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mtor signalling. *Nature Cell Biology, 4*(9), 648–657.
29. Inoki, K., Li, Y., Xu, T., & Guan, K. L. (2003). Rheb gtpase is a direct target of TSC2 GAP activity and regulates mtor signaling. *Genes & Development, 17*(15), 1829–1834.
30. Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., & Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature, 378*(6559), 785–789.
31. McManus, E. J., Sakamoto, K., Armit, L. J., et al. (2005). Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *The EMBO journal, 24*(8), 1571–1583.
32. Patel, S., Doble, B. W., MacAulay, K., Sinclair, E. M., Drucker, D. J., & Woodgett, J. R. (2008). Tissue-specific role of glycogen synthase kinase 3beta in glucose homeostasis and insulin action. *Molecular and Cellular Biology, 28*(20), 6314–6328.
33. MacAulay, K., Doble, B. W., Patel, S., et al. (2007). Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metabolism, 6*(4), 329–337.
34. Ishikura, S., Bilan, P. J., & Klip, A. (2007). Rabs 8A and 14 are targets of the insulin-regulated Rab-GAP AS160 regulating GLUT4 traffic in muscle cells. *Biochemical and Biophysical Research Communications, 353*(4), 1074–1079.
35. Accili, D., & Arden, K. C. (2004). Foxos at the crossroads of cellular metabolism, differentiation, and transformation. *Cell, 117*(4), 421–426.
36. Puigserver, P., Rhee, J., Donovan, J., et al. (2003). Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature, 423*(6939), 550–555.
37. Foufelle, F., & Ferre, P. (2002). New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *The Biochemical Journal, 366*(Pt 2), 377–391.
38. Kodama, S., Koike, C., Negishi, M., & Yamamoto, Y. (2004). Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Molecular and cellular biology, 24*(18), 7931–7940.
39. Zhang, W., Patil, S., Chauhan, B., et al. (2006). Foxo1 regulates multiple metabolic pathways in the liver: effects on gluconeogenic, glycolytic, and lipogenic gene expression. *The Journal of biological chemistry, 281*(15), 10105–10117.
40. Samuel, V. T., Choi, C. S., Phillips, T. G., et al. (2006). Targeting foxo1 in mice using antisense oligonucleotide improves hepatic and peripheral insulin action. *Diabetes, 55*(7), 2042–2050.

41. Kamagate, A., Qu, S., Perdomo, G., et al. (2008). Foxo1 mediates insulin-dependent regulation of hepatic VLDL production in mice. *The Journal of Clinical Investigation, 118*(6), 2347–2364.
42. Altomonte, J., Cong, L., Harbaran, S., et al. (2004). Foxo1 mediates insulin action on apoc-III and triglyceride metabolism. *The Journal of Clinical Investigation, 114*(10), 1493–1503.
43. Biddinger, S. B., Haas, J. T., Yu, B. B., et al. (2008). Hepatic insulin resistance directly promotes formation of cholesterol gallstones. *Nature Medicine, 14*(7), 778–782.
44. Graf, G. A., Yu, L., Li, W. P., et al. (2003). ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. *The Journal of Biological Chemistry, 278*(48), 48275–48282.
45. Li, T., Kong, X., Owsley, E., Ellis, E., Strom, S., & Chiang, J. Y. (2006). Insulin regulation of cholesterol 7alpha-hydroxylase expression in human hepatocytes: roles of forkhead box O1 and sterol regulatory element-binding protein 1c. *The Journal of Biological Chemistry, 281*(39), 28745–28754.
46. Gross, D. N., van den Heuvel, A. P., & Birnbaum, M. J. (2008). The role of foxo in the regulation of metabolism. *Oncogene, 27*(16), 2320–2336.
47. Wolfrum, C., Asilmaz, E., Luca, E., Friedman, J. M., & Stoffel, M. (2004). Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes. *Nature, 432*(7020), 1027–1032.
48. Zhang, L., Rubins, N. E., Ahima, R. S., Greenbaum, L. E., & Kaestner, K. H. (2005). Foxa2 integrates the transcriptional response of the hepatocyte to fasting. *Cell Metabolism, 2*(2), 141–148.
49. Bochkis, I. M., Rubins, N. E., White, P., Furth, E. E., Friedman, J. R., & Kaestner, K. H. (2008). Hepatocyte-specific ablation of Foxa2 alters bile acid homeostasis and results in endoplasmic reticulum stress. *Nature Medicine, 14*(8), 828–836.
50. Horton, J. D., Goldstein, J. L., Brown, M. S. (2002). Srebps: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *The Journal of Clinical Investigation, 109*(9), 1125–1131.
51. Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., & Goldstein, J. L. (1997). Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *The Journal of Clinical Investigation, 99*(5), 846–854.
52. Ide, T., Shimano, H., Yahagi, N., et al. (2004). Srebps suppress IRS-2-mediated insulin signalling in the liver. *Nature Cell Biology, 6*(4), 351–357.
53. Yamamoto, T., Shimano, H., Nakagawa, Y., et al. (2004). SREBP-1 interacts with hepatocyte nuclear factor-4 alpha and interferes with PGC-1 recruitment to suppress hepatic gluconeogenic genes. *The Journal of Biological Chemistry, 279*(13), 12027–12035.
54. Shimomura, I., Bashmakov, Y., & Horton, J. D. (1999). Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *The Journal of Biological Chemistry, 274*(42), 30028–30032.
55. Horton, J. D., Bashmakov, Y., Shimomura, I., & Shimano, H. (1998). Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proceedings of the National Academy of Sciences of the United States of America, 95*(11), 5987–5992.
56. Liang, G., Yang, J., Horton, J. D., Hammer, R. E., Goldstein, J. L., & Brown, M. S. (2002). Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *The Journal of Biological Chemistry, 277*(11), 9520–9528.
57. Repa, J. J., Liang, G., Ou, J., et al. (2000). Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, lxralpha and lxrbeta. *Genes & Development, 14*(22), 2819–2830.
58. Tobin, K. A., Ulven, S. M., Schuster, G. U., et al. (2002). Liver X receptors as insulin-mediating factors in fatty acid and cholesterol biosynthesis. *The Journal of Biological Chemistry, 277*(12), 10691–10697.

59. Yabe, D., Komuro, R., Liang, G., Goldstein, J. L., & Brown, M. S. (2003). Liver-specific mrna for Insig-2 down-regulated by insulin: implications for fatty acid synthesis. *Proceedings of the National Academy of Sciences of the United States of America, 100*(6), 3155–3160.
60. Kakuma, T., Lee, Y., Higa, M., et al. (2000). Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. *Proceedings of the National Academy of Sciences of the United States of America, 97*(15), 8536–8541.
61. Michael, M. D., Kulkarni, R. N., Postic, C., et al. (2000). Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Molecular Cell, 6*(1), 87–97.
62. Biddinger, S. B., Hernandez-Ono, A., Rask-Madsen, C., et al. (2008). Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metabolism, 7*(2), 125–134.
63. Sparks, J. D., & Sparks, C. E. (1994). Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochimica et Biophysica Acta, 1215*(1–2), 9–32.
64. Shaffer, E. A., & Small, D. M. (1977). Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. *The Journal of Clinical Investigation, 59*(5), 828–840.
65. Dong, X. C., Copps, K. D., Guo, S., et al. (2008). Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. *Cell Metabolism, 8*(1), 65–76.
66. Taniguchi, C. M., Kondo, T., Sajan, M., et al. (2006). Divergent regulation of hepatic glucose and lipid metabolism by phosphoinositide 3-kinase via Akt and pkclambda/zeta. *Cell Metabolism, 3*(5), 343–353.
67. Miyake, K., Ogawa, W., Matsumoto, M., Nakamura, T., Sakaue, H., & Kasuga, M. (2002). Hyperinsulinemia, glucose intolerance, and dyslipidemia induced by acute inhibition of phosphoinositide 3-kinase signaling in the liver. *The Journal of Clinical Investigation, 110*(10), 1483–1491.
68. Matsumoto, M., Ogawa, W., Akimoto, K., et al. (2003). Pkclambda in liver mediates insulin-induced SREBP-1c expression and determines both hepatic lipid content and overall insulin sensitivity. *The Journal of Clinical Investigation, 112*(6), 935–944.
69. Tilg, H., & Moschen, A. R. (2008). Inflammatory mechanisms in the regulation of insulin resistance. *Molecular Medicine (Cambridge, Mass.), 14*(3–4), 222–231.
70. Howard, J. K., Cave, B. J., Oksanen, L. J., Tzameli, I., Bjorbaek, C., & Flier, J. S. (2004). Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nature Medicine, 10*(7), 734–738.
71. Nielsen, J. H., Galsgaard, E. D., Moldrup, A., et al. (2001). Regulation of beta-cell mass by hormones and growth factors. *Diabetes, 50*, Suppl 1:S25–29.
72. Verstrepen, L., Bekaert, T., Chau, T. L., Tavernier, J., Chariot, A., & Beyaert, R. (2008). TLR-4, IL-1R and TNF-R signaling to NF-kappab: variations on a common theme. *Cellular and Molecular Life Sciences: CMLS, 65*(19), 2964–2978.
73. Shoelson, S. E., Lee, J., & Yuan, M. (2003). Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 27*, Suppl 3:S49–52.
74. Holland, W. L., Knotts, T. A., Chavez, J. A., Wang, L. P., Hoehn, K. L., & Summers, S. A. (2007). Lipid mediators of insulin resistance. *Nutrition reviews, 65*(6 Pt 2), S39–46.
75. Gao, Z., Zhang, X., Zuberi, A., et al. (2004). Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. *Molecular Endocrinology (Baltimore, Md.), 18*(8), 2024–2034.
76. Savage, D. B., Petersen, K. F., & Shulman, G. I. (2007). Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiological Reviews, 87*(2), 507–520.
77. Malhotra, J. D., & Kaufman, R. J. (2007). The endoplasmic reticulum and the unfolded protein response. *Seminars in Cell & Developmental Biology, 18*(6), 716–731.

78. Buse, M. G. (2006). Hexosamines, insulin resistance, and the complications of diabetes: current status. *American Journal of Physiology. Endocrinology and Metabolism, 290*(1), E1-E8.
79. Srinivasan, V., Tatu, U., Mohan, V., & Balasubramanyam, M. (2009). Molecular convergence of hexosamine biosynthetic pathway and ER stress leading to insulin resistance in L6 skeletal muscle cells. *Molecular and Cellular Biochemistry, 328*(1–2), 217–224.
80. Miele, C., Riboulet, A., Maitan, M. A., et al. (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. *The Journal of Biological Chemistry, 278*(48), 47376–47387.
81. Frojdo, S., Vidal, H., & Pirola, L. (2009). Alterations of insulin signaling in type 2 diabetes: a review of the current evidence from humans. *Biochimica et Biophysica Acta, 1792*(2), 83–92.
82. Semple, R. K., Sleigh, A., Murgatroyd, P. R., et al. (2009). Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. *The Journal of Clinical Investigation, 119*(2), 315–322.