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Fructose and the Metabolic Syndrome: Pathophysiology and Molecular Mechanisms

Angela C. Rutledge, BSc, PhD, and Khosrow Adeli, PhD, FCACB, DABCC

Emerging evidence suggests that increased dietary consumption of fructose in Western society may be a potentially important factor in the growing rates of obesity and the metabolic syndrome. This review will discuss fructose-induced perturbations in cell signaling and inflammatory cascades in insulin-sensitive tissues. In particular, the roles of cellular signaling molecules including nuclear factor kappa B (NFkB), tumor necrosis factor alpha (TNF-α), c-Jun amino terminal kinase 1 (JNK-1), protein tyrosine phosphatase 1B (PTP-1B), phosphatase and tensin homolog deleted on chromosome ten (PTEN), liver X receptor (LXR), farnesoid X receptor (FXR), and sterol regulatory element-binding protein-1c (SREBP-1c) will be addressed. Considering the prevalence and seriousness of the metabolic syndrome, further research on the underlying molecular mechanisms and preventative and curative strategies is warranted.

Key words: fructose, hepatic lipogenesis, insulin resistance, JNK, metabolic syndrome, obesity, NFkB, TNF-α

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INTRODUCTION

Technological advances and improved economic status in Western countries over the past century have resulted in a shift in the energy balance toward a more sedentary lifestyle accompanied by increased caloric intake. This has led to an increased incidence of the metabolic syndrome, an epidemic that now threatens developing countries.[1] An important but not well-appreciated dietary change over the past few decades has been a substantial increase in fructose consumption. Emerging evidence from recent epidemiological and biochemical studies clearly suggests that high fructose intake may be an important causative factor in the development of the metabolic syndrome. The present review will discuss potential molecular links between dietary fructose, insulin resistance, and metabolic dyslipidemia, as well as dietary animal models of the metabolic syndrome.

Drs. Rutledge and Adeli are with the Department of Clinical Biochemistry and Molecular Structure and Function, The Hospital for Sick Children, and the Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada.

Please address all correspondence to: Dr. Khosrow Adeli, Division of Clinical Biochemistry, DPLM, Hospital for Sick Children, University of Toronto, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8; Phone: 416-813-8682; Fax: 416-813-6257; E-mail: khosrow.adeli@sickkids.ca

INVOLVEMENT OF FRUCTOSE, A LIPOGENIC NUTRIENT, IN INITIATION OF THE METABOLIC SYNDROME

Fructose, found naturally in many fruits, is now consumed by humans in large quantities due to the popularity of convenient, prepackaged foods and the consumption of soft drinks and juice beverages containing sucrose (table sugar consisting of 50% fructose, 50% glucose) or high-fructose corn syrup (a single can of soft drink contains 25 g of fructose).[2] Interestingly, the approximate 25% increase in per capita fructose consumption over the past 30 years coincides closely with the increase in the prevalence of obesity and the metabolic syndrome.[2] In one longitudinal study involving about 500 schoolchildren, it was concluded that each serving of sugar-sweetened drinks increased body mass index (BMI) by 0.25 kg/m^2.[3] Similarly, mice allowed ad libitum access to water containing fructose or to a soft drink showed increased adiposity and specifically increased hepatic lipid storage.[4] High-fructose diets have been shown to induce insulin resistance, weight gain, hyperlipidemia, and hypertension in several animal models including rats, hamsters, dogs, and certain mice species.[2,5] In fact, fructose has been shown to induce insulin resistance in all animal models tested, except for certain mice strains such as C57BL/6. Polymorphisms within the sterol regulatory element-binding protein (SREBP)-1c gene appear to cause this differential sensitivity to fructose between mice strains.[6] In human studies, fructose consumption was associated with the development of

hepatic and adipose tissue insulin resistance and dyslipidemia due to its ability to induce hepatic de novo lipogenesis.[7]

Absorption of fructose from the intestine into the portal blood is aided by glucose transporter 5 (GLUT5) at the brush border and basolateral membranes of the jejunum. This route of absorption results in massive fructose uptake by the liver.[2] The hepatic metabolism of fructose differs greatly from that of glucose, as shown in Figure 1. Fructose is phosphorylated by fructokinase, forming fructose-1-phosphate, which can then be converted to several three-carbon molecules, including glyceraldehyde, dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate. Some of these three-carbon molecules could be converted to glucose through gluconeogenesis, or they could be used to generate other products such as triglyceride (TG). This aspect of fructose metabolism contrasts greatly with glucose metabolism. There are several processes inhibiting the formation of TG from glucose. These include the conversion of glucose to glycogen, the reformation of glucose from the three-carbon glycolysis products through gluconeogenesis, and, most importantly, the regulation of this process by the rate-limiting enzyme phosphofructokinase. The formation of fructose-1-phosphate from fructose bypasses phosphofructokinase, allowing the carbons from fructose to enter glycolysis downstream of this enzyme. The three-carbon molecules can eventually be used for the synthesis of glycerol and fatty acids, which through esterification can form TG. Thus, high fructose intake can result in large amounts of TG synthesis due to the relative lack of regulation of this pathway.[2] This TG can be packaged into very-low density lipoproteins (VLDL) by the liver. As VLDLs travel through the bloodstream, the TG can be hydrolyzed by lipoprotein lipase to form non-esterified fatty acids (NEFAs) and monoacylglycerol. Adipose tissue can take up these components and resynthesize TG. Therefore, excessive fructose consumption can lead to high levels of free fatty acids and obesity.

CONTRIBUTION OF FREE FATTY ACIDS TO INSULIN RESISTANCE

The main role of adipose tissue is to take up excess fatty acids provided by the diet and to store them in the form of TG to be used as an energy supply for the body in times of starvation, but there is a limited capacity of adipose tissue to store fat. This maximum capacity may be reached in states of obesity, resulting in an impaired ability of adipose tissue to acquire dietary fatty acids and, therefore, increased levels of fatty acids in the circulation. Signaling abnormalities in adipocytes can also trigger lipolysis of TG stores and efflux of fatty acids into the bloodstream, augmenting the problem. The presence of high levels of NEFAs in the bloodstream is proposed to function as a key mechanistic link between obesity and insulin resistance, type 2 diabetes, and metabolic dyslipidemia. Eventually, these NEFAs may be taken up ectopically by non-adipose tissues such as the liver and skeletal muscle, where they may be stored as TG or diacylglycerol and interfere with metabolic pathways

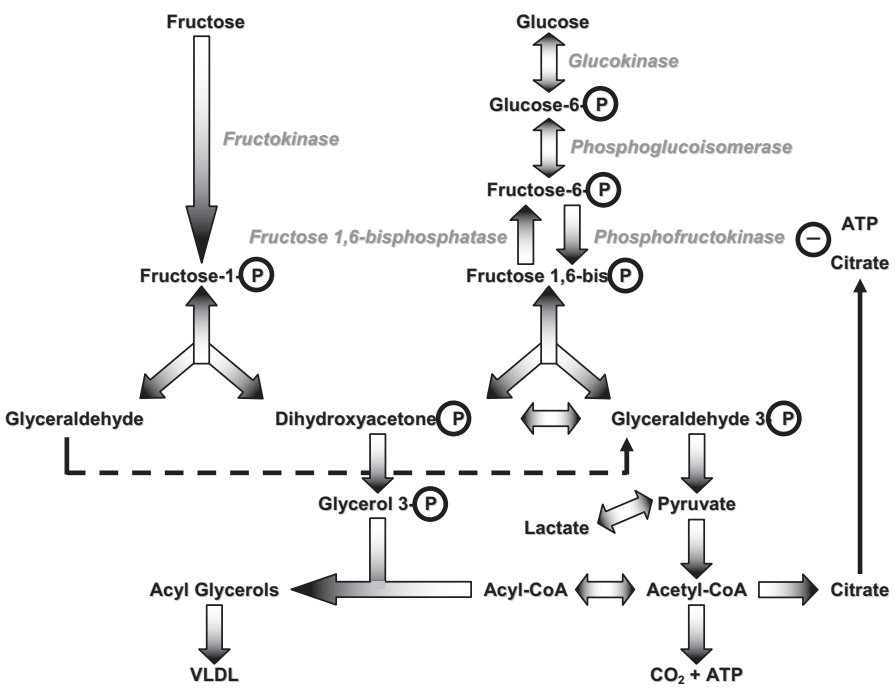

Figure 1. Comparison of pathways regulating triglyceride synthesis from fructose and glucose. (Adapted from Havel, 2005.[2])

such as the response to insulin, contributing to insulin resistance and the metabolic syndrome.[8]

Differences exist in the metabolic properties of the various sites of adipose tissue. Visceral or abdominal fat stores are believed to pose a greater risk for the development of insulin resistance and the metabolic syndrome than subcutaneous fat stores. Reasons for this include reduced responsiveness of visceral fat to the anti-lipolytic effects of insulin (due to lower expression and activity of hormone sensitive lipase [HSL], reduced tyrosine phosphorylation of the insulin receptor, decreased insulin receptor substrate [IRS]-1 expression, and increased protein tyrosine phosphatase [PTP]-1B activity); greater responsiveness of visceral fat to the lipolysis-inducing effects of catecholamines; and decreased uptake and acylation of fatty acids compared with subcutaneous fat, all of which result in amplification of NEFA levels in the blood.[9] Visceral fat is also located conveniently for these NEFAs to enter portal circulation for direct delivery to the liver, where they pose risks to hepatic insulin responsiveness.

ADIPOSE TISSUE INSULIN RESISTANCE

In addition to its role in fatty acid uptake, adipose tissue also takes up glucose via insulin-induced translocation of GLUT4 to the plasma membrane, a process that is impaired upon insulin resistance.[10] The reduction in GLUT4 translocation occurs due to inhibitory serine phosphorylation of IRS-1 or IRS-2 via a nuclear factor (NF)kB/c-Jun amino terminal kinase (JNK)-dependent pathway (discussed below in more detail).

Sterol regulatory element-binding protein (SREBP)-1c is found in the endoplasmic reticulum membrane, but upon its activation by insulin or low cholesterol levels, it travels to the Golgi, where it is cleaved to generate a transcription factor fragment that enters the nucleus. Genes regulated by SREBP-1c include acetyl-coenzyme (Co)A carboxylase (ACC) and fatty acid synthase (FAS). ACC is responsible for the synthesis of fatty acids from acetyl-CoA (derived from glycolysis products). FAS is responsible for combining fatty acid chains with glycerol, which can also be generated from glucose. Therefore, under insulin-sensitive conditions, insulin is able to stimulate glucose uptake via GLUT4 and to activate SREBP-1c to synthesize TG. While the cells are sensitive to insulin, the hydrolysis of TG stores by HSL is also inhibited by insulin. As adipocytes become overloaded with fat and inhibitory serine phosphorylation of IRS-1 and IRS-2 occurs, the cells become less able to respond to insulin. This impairs SREBP-1c activation, decreasing TG synthesis by adipocytes. The lack of glucose available due to decreased GLUT4 translocation also inhibits new TG synthesis. In addition, the inhibition of HSL by insulin is lost, resulting in hydrolysis of the adipocyte TG stores. Therefore, there is a net efflux of lipid from the adipose tissue in insulin resistance, which results in ectopic fat uptake by skeletal muscle and liver and insulin resistance in those tissues.

Adipose tissue also has a secretory role. Upon overload of adipose tissue with fat and the development of insulin resistance, there is increased secretion of inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6. The inflammation propagated by these cytokines has been proposed to contribute greatly to insulin resistance.[11,12] Therefore, a key contributor to insulin resistance and the metabolic syndrome appears to be the abundance of TG and fatty acids that occurs in obesity (perhaps in part due to high fructose intake), exceeding the storage capacity of adipose tissue and impairing adipocyte signaling. The end result is ectopic fat storage, accompanied by modified secretion of hormones and cytokines by adipose tissue and an inflammatory state, all of which cause damaging abnormalities in signaling within insulin-sensitive tissues.

KEY ROLE OF HEPATIC INSULIN RESISTANCE

Hepatic insulin resistance, an important pathophysiological feature of type 2 diabetes and the metabolic syndrome, can be defined as the failure of insulin to adequately suppress hepatic glucose production while lipogenic actions of insulin are not compromised.[13] Numerous studies suggest a critical role for at least three major contributors in the induction of hepatic insulin resistance: a) increased flux of NEFAs to the liver, b) induction of hepatic inflammation (e.g., by fructose), and c) specific defects in molecules of hepatic insulin signaling cascades. As numerous studies have already addressed the role of increased NEFA levels, we will focus on emerging new evidence detailing defects in insulin signaling cascades and hepatic inflammation.

Defects in insulin signaling cascades have been reported in the livers of both genetic (ob/ob mice and lipodystrophic mice)[14,15] and diet-induced (fructose-fed and fat-fed hamsters, rats, and mice)[16,17] models of insulin resistance. Insulin signaling involves a series of events initiated by insulin binding to its cell surface receptor. This is followed by receptor autophosphorylation and tyrosine phosphorylation of insulin receptor substrates, leading to the recruitment and activation of PI-3 kinase, which catalyzes the formation of the lipid second messenger phosphatidylinositol (3,4,5)–triphosphate (PIP-3) at the plasma membrane. This mediates activation of the Akt/protein kinase (PKB) pathway, which is necessary for insulin's modulation of glucose transport and glycogen synthesis.[18] Akt also phosphory-

lates and causes the nuclear exclusion and inactivation of Forkhead transcription factors (Foxa2, Foxo1), which mediate many actions of insulin.[19] Foxa2 stimulates hepatic production and secretion of VLDL, partly by upregulating microsomal TG transfer protein (MTP),[20] and activates genes involved in mitochondrial β-oxidation of fatty acids.[19] Deregulation of Foxa2-dependent mechanisms may thus be critically important in the development of diabetic dyslipidemia.

A common finding in obese and type 2 diabetic subjects is that insulin-stimulated IRS-1 tyrosine phosphorylation and PI-3 kinase activity are decreased in insulin-sensitive tissues.[21] A key phosphatase implicated in inactivation of the insulin receptor and IRS-1/IRS-2 is PTP-1B.[22] In the fructose-fed hamster model, we found that impaired hepatic insulin signaling was associated with elevated protein mass and activity of PTP-1B,[16] and PTP-1B overexpression in the liver has been found to directly stimulate VLDL-apolipoprotein (apo)B secretion.[23] In contrast, PTP-1B knockout mice exhibit improved insulin sensitivity on a high-fat diet[24] and were found to be resistant to fructose-induced dyslipidemia and VLDL-TG elevation,[23] strongly implicating PTP-1B in the development of fructose-induced VLDL overproduction.

Lipid phosphatases that dephosphorylate or inactivate PIP-3 can also potently inhibit insulin signaling. Such phosphatases include phosphatase and tensin homolog deleted on chromosome ten (PTEN), due to its 3′-phosphatase activity[25] and Src homology (SH2) domain-containing inositol phosphatase (SHIP2), due to its 5′-phosphatase activity.[26] Liver-specific deletion of PTEN causes insulin hypersensitivity and fatty liver via insulin-induced fatty acid synthesis.[27] We have also obtained evidence for increased protein mass of PTEN in livers of fructose-fed hamsters (unpublished data). Interestingly, overexpression of PTEN in HepG2 cells was found to attenuate PIP-3 levels and enhance apoB secretion, an effect mediated by MTP (unpublished observations). Targeted disruption of the SHIP2 gene in mice also protects against obesity from a high-fat diet,[28] and inhibition of SHIP2 through liver-specific expression of a dominant-negative SHIP2 improves insulin resistance in diabetic db/db mice.[29]

Many gene regulatory events induced by receptor-associated kinases such as the insulin receptor are mediated by mitogen-activated protein (MAP) kinases. Extracellular signal-regulated kinase (ERK)1/2 and JNK are members of the MAP kinase family of serine/threonine kinases. Negative regulation of ERK1/2 activity is mediated by p38, another serine/threonine kinase. There is increasing evidence that ERK1/2, JNK, and p38 may contribute to the development of insulin resistance. Increased basal levels of phosphorylation of ERK1/2, JNK, and p38 have been observed in adipocytes from type 2 diabetic subjects.[30] Studies from our laboratory[31] and by Huff et al.[32] also provide evidence for a potential link between the MAP kinase signaling cascade and hepatic assembly and secretion of apoB100-containing lipoproteins. In addition to their ability to regulate gene expression via phosphorylation of downstream signaling molecules, ERK/12 and JNK can perform inhibitory serine phosphorylation of IRS-1 and IRS-2, which may account for the insulin resistance attributed to these molecules.[33,34] Inflammation plays an important role in the activation of these MAP kinases, as will be described below.

FACTORS RESPONSIBLE FOR HEPATIC INFLAMMATION AND THEIR IMPACT ON INSULIN SENSITIVITY

There is increasing evidence that obesity and insulin-resistant states are associated with a low-grade inflammation resulting from chronic activation of the innate immune system.[35] Individuals with the metabolic syndrome produce a relative excess of proinflammatory mediators, such as TNF-α and IL-6, from both white adipose tissue and the liver.[36] A large number of macrophages infiltrate these tissues in obesity, and it may be these cells rather than the adipocytes and hepatocytes themselves that secrete these factors.[37] These cytokines appear to be critical to the pathogenesis of hepatic insulin resistance and the progression of fatty liver.[38] As fat accumulates in the liver, this induces sustained hepatic generation of proinflammatory cytokines, leading to a vicious cycle of worsening insulin resistance and steatosis.

Role of c-Jun Amino Terminal Kinases in Hepatic Inflammation

JNKs are serine/threonine kinases that, once activated by mitogen-activated protein kinase (MKK) 7, phosphorylate and thereby activate transcription factors such as c-Jun.[39] JNKs are known to be activated by TNF-α upon inflammation or the presence of high levels of NEFAs,[34] which are both very prominent in obesity. In agreement, JNK activity was found to be significantly increased in liver, skeletal muscle, and adipose tissue of obese mice,[34] and muscle samples from humans with insulin resistance also showed JNK activation.[40] The harm in activation of JNK is in its inhibitory phosphorylation of IRS-1 at Ser-307, which interferes with the tyrosine phosphorylation performed by the insulin receptor that is necessary to propagate insulin signaling (Figure 2). In a study in which mouse livers were treated with TNF-α to induce Ser-307 phosphorylation of IRS-1, a JNK inhibitor was able to completely prevent this phos-

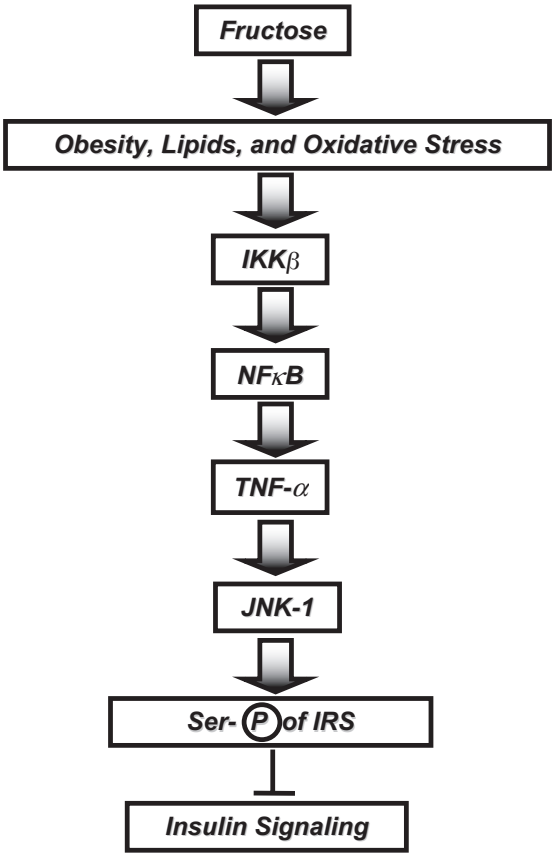

Figure 2. Overview of the nuclear factor kappa B (NFkB)/c-Jun amino terminal kinase 1 (JNK-1) pathway proposed to link fructose consumption and inflammation and lead to hepatic insulin resistance.

phorylation, demonstrating the importance of JNK.[34] Similarly, overexpression of wild-type JNK in livers of normal mice impaired insulin sensitivity, whereas overexpression of dominant-negative JNK in livers of obese mice reduced insulin resistance and lowered blood glucose.[41]

JNK activation is also induced by fructose feeding and oxidative stress.[17,42] Studies suggest that acute hepatic exposure to fructose can stimulate JNK. Exposure of primary rat hepatocytes to fructose for 2 to 4 hours caused activation of JNK, coinciding with increased phosphorylation of MKK7, increased inhibitory Ser-307 phosphorylation of IRS-1, and decreased tyrosine phosphorylation of IRS-1 and IRS-2. SP6000125, a JNK inhibitor, was able to normalize JNK activity and IRS-1 phosphorylation during the fructose treatment.[43] Similarly, single feedings of sucrose to rats for 6 hours resulted in hepatic JNK activation and increased Ser-307 phosphorylation of IRS-1.[17] However, our earlier findings that short-term (2-day) fructose feeding of hamsters or direct short-term exposure of cultured hepatocytes to fructose did not induce insulin resistance or VLDL overproduction suggest that some of the effects of fructose are indirect and require chronic activation of pathways such as hepatic inflammation.[44] For example, feeding male rats a high-fructose diet for a more chronic 2-week period induced JNK activation, along with oxidative stress, hypertriglyceridemia, VLDL overproduction, and insulin resistance. These outcomes were blocked by treatment with lipoxygenase inhibitors, suggesting the involvement of inflammatory pathways.[42] Therefore, acute exposure to fructose may play a role in JNK activation, but chronic fructose consumption may have more widespread effects on insulin-dependent pathways due to the development of obesity and increased NEFA levels and through the initiation of chronic inflammation, all of which result in JNK activation.

However, it is JNK-1 rather than JNK-2 that is involved in the regulation of insulin sensitivity in obesity. $Jnk1^{-/-}$ lean mice, but not $Jnk2^{-/-}$ lean mice, were protected against obesity and insulin resistance upon feeding of a high-fat diet.[34] Similarly, in the ob/ob obese background, $Jnk1^{-/-}$ mice gained less weight and had lower blood glucose levels than $Jnk1^{+/+}$ littermates.[34] These in vivo data support a role for JNK-1 in obesity-induced insulin resistance.

Involvement of Nuclear Factor Kappa B in Hepatic Inflammation and c-Jun Amino Terminal Kinase Activation

Another important mediator of fructose/obesity-induced insulin resistance is nuclear factor kappa B (NFkB). Under basal conditions NFkB is found in the cytosol bound to its inhibitor, IkB, but upon activation of IkB kinase (IKK)-β, which phosphorylates IkB and marks it for degradation, NFkB is allowed to enter the nucleus, where it induces transcription of specific genes.[45] The proteins encoded by these genes include pro-inflammatory cytokines such as plasminogen activator inhibitor (PAI)-1, TNF-α, IL-6, and IL-1β.[46] The mechanisms responsible for IKKβ and NFkB activation in obesity are unclear. One possibility is that the buildup of lipids in the liver and other tissues that occurs in obesity contributes to increased mitochondrial β-oxidation of fatty acids, generating peroxidation products that stimulate IKKβ and, therefore, NFkB activation.[47] Several studies have found a correlation between fatty acid or lipid treatment and NFkB activation.[48] Deficiency of CD36, a scavenger receptor for various forms of fatty acids and lipoproteins that is normally expressed on adipocytes, skeletal muscle, macrophages/monocytes, platelets, microvascular endothelial cells, and heart, also impairs NFkB activation, confirming the importance of lipids in NFkB activation.[49] Using liver-specific transgenic models of NFkB activation or inhibition, increased NFkB activity was shown to induce insulin resistance, whereas mice with reduced NFkB activation were resis-

tant to insulin resistance.[46,47] Hepatic insulin resistance induced by NFkB activation was associated with increased expression of TNF-α, IL-6, and IL-1.[35] Activation of IKKβ appears to be a key mediator of insulin resistance, which is supported by the finding that targeted gene disruption of IKKβ improves insulin sensitivity.[50]

Surprisingly, activation of NFkB does not appear to induce secretion of inflammatory cytokines or insulin resistance in skeletal muscle.[51] There is some evidence implicating the NFkB pathway, induced by conjugated linoleic acid, in adipocyte insulin resistance,[52] and the NFkB pathway also promotes insulin insensitivity in hepatocytes,[46] although myeloid cells (e.g., macrophages) may be more important than hepatocytes in terms of NFkB-induced insulin resistance. Arkan et al.[47] generated mice lacking IKKβ in hepatocytes or myeloid cells and fed them a high-fat diet. Loss of the NFkB pathway in liver maintained only hepatic insulin responsiveness, but could not protect against the development of insulin resistance in skeletal muscle or adipose tissue, whereas mice lacking the NFkB pathway in myeloid cells maintained global insulin sensitivity. Therefore, the inflammatory mediators produced by hepatocytes upon NFkB activation may act in a paracrine manner to induce hepatic insulin resistance, but may have little effect on adipose tissue and skeletal muscle. In contrast, the inflammatory mediators produced by myeloid cells appear to induce insulin resistance both locally and globally. Myeloid cells may undergo NFkB activation within the lipid-loaded tissues and propagate the inflammation via the production of pro-inflammatory cytokines and penetration of other tissues.[46] The production of inflammatory cytokines such as TNF-α by lipid-loaded cells upon NFkB activation may promote JNK-1 activation and insulin resistance via Ser-307 phosphorylation of IRS-1. Thus, NFkB and JNK-1 may cooperate to induce insulin resistance (Figure 2).

Furthermore, strong evidence for the critical role of inflammatory cascades involving NFκB and JNK in regulating insulin resistance comes from studies showing that inhibition of these pathways promotes insulin sensitivity. Nonsteroidal anti-inflammatories such as aspirin and other salicylates have long been known to have antidiabetic properties. One study found that phosphorylation of IRS-1 at Ser-307 in hepatic or adipocyte cell lines was accompanied by phosphorylation of JNK, c-Jun, and degradation of IκBα, whereas deficiency of JNK or IKK in mouse embryonic fibroblasts impaired Ser-307 phosphorylation of IRS-1. These results provide support for the involvement of NFκB, TNF-α, and JNK in insulin resistance. Aspirin was able to attenuate the inhibitory phosphorylation of IRS-1, the phosphorylation of JNK and c-Jun, and IκBα degradation. In addition, aspirin treatment of TNF-α-treated 3T3-L1 adipocytes improved insulin-induced glucose uptake.[53] Because myeloid cells appear to be an important contributor to insulin resistance, much of the insulin-sensitizing effect of salicylates may occur in myeloid cells through inhibition of IKKβ and JNK.[46] The insulin-sensitizing effects of JNK and IKK inhibition are not limited to salicylates. Co-culture of muscle cells with adipocyte-conditioned medium induced insulin resistance in muscle cells due to the presence of adipocyte-derived inhibitory factors. A highly specific IKK inhibitor, I229, completely restored insulin signaling in this model,[54] and significant improvements were also observed with a JNK inhibitor.[55]

MOLECULAR LINKS BETWEEN DIET-INDUCED HEPATIC INSULIN RESISTANCE AND DYSLIPIDEMIA

Nuclear Transcription Factors Acting as Intracellular Sensors

Hepatic lipid metabolism is now known to be under the control of a large family of ligand-activated nuclear transcription factors, including liver X receptor (LXR) and farnesoid X receptor (FXR). LXR and FXR act as intracellular sensors of cholesterol and bile acids, respectively, and induce transcriptional responses to maintain lipid, bile acid, and glucose metabolism.[56,57] LXRs promote the storage of carbohydrate- and fat-derived energy, while FXRs reduce TG levels and modulate glucose metabolism. Of particular relevance are the roles of LXR and FXR in the regulation of hepatic de novo lipogenesis and TG synthesis. Recent studies have shown that LXR activation leads to enhanced de novo lipogenesis via induction of SREBP-1c.[58] In a recent study,[5] we observed that in vivo activation of LXRα in the hamster perturbs hepatic insulin signaling and leads to hepatic overproduction of VLDL-apoB.

FXR also has substantial control over lipid and cholesterol metabolism. FXR knockout mice exhibit impaired glucose tolerance, insulin resistance, overt fatty liver, and elevated levels of plasma NEFAs, cholesterol, and TG.[59,60] In addition, FXR levels are reduced in rodent models of diabetes.[61] Interestingly, FXR represses expression of SREBP-1c by a mechanism that may involve interference with LXR activation, which is crucial for the expression of SREBP-1c and its downstream targets.[62] FXR has also been shown to repress expression of MTP.[63] In a recent study, Shulman et al.[64] employed the fructose-fed hamster model to demonstrate that activation of FXR decreases hepatic secretion of TG-rich lipoproteins, possibly by interfering with SREBP-1-mediated activation of stearoyl-CoA desaturase (SCD)-1 and attenuating de novo lipogenesis. Although they did

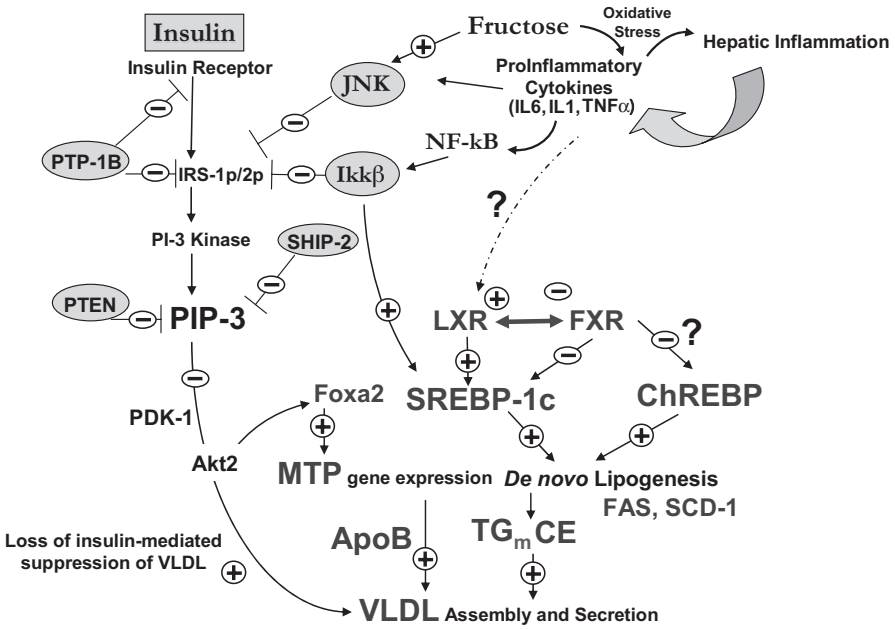

Figure 3. Mechanisms of fructose-induced hepatic insulin resistance and very low-density lipoprotein (VLDL) overproduction. Perturbations in insulin signaling—increased activity of protein tyrosine phosphatase 1B (PTP-1B), phosphatase and tensin homolog deleted on chromosome ten (PTEN), and Src homology (SH2) domain-containing inositol phosphatase (SHIP2)—lead to hepatic insulin resistance, increased expression of sterol regulatory element-binding protein-1c (SREBP-1c), induction of de novo lipogenesis, increased microsomal triglycerides and cholesteryl ester, and higher expression/activity of microsomal triglyceride transfer protein (MTP). Collectively, these changes massively stimulate the hepatic production of apolipoprotein B (apoB)-containing VLDL particles. Hepatic inflammation and intrahepatic production of cytokines can lead to activation of the nuclear factor kappa B (NFkB) and c-Jun amino terminal kinase 1 (JNK-1) systems, inducing both insulin resistance and hepatic steatosis.

not directly examine hepatic apoB secretion, this study clearly suggests that fructose-induced upregulation of VLDL production may be mediated by changes in the activation state of these nuclear orphan receptors.

Downstream Lipogenic Transcription Factors

LXR and FXR appear to exert their influence on hepatic lipogenesis via the well-characterized transcription factor SREBP-1c and the recently identified carbohydrate response element-binding protein, ChREBP.[65] SREBP-1c is the major regulator of de novo fatty acid synthesis from carbohydrate precursors, activating fatty acid biosynthesis in the liver via increased expression of FAS and SCD.[65] Increased hepatic SREBP-1c levels have been observed in numerous insulin-resistant animals.[66] We have also found increased hepatic and intestinal SREBP-1c in the fructose-fed hamster model of insulin resistance.[67] Interestingly, a genetic polymorphism of SREBP-1c appears to underlie differential sensitivity of mice to fructose-induced insulin resistance, clearly implicating this transcription factor in mediating the lipogenic effects of fructose.[6]

ChREBP induces de novo lipogenesis by binding to and activating carbohydrate response elements at promoters of lipogenic genes such as FAS and SCD-1.[68] Emerging evidence also suggests that ChREBP and SREBP-1c may act in concert to mediate insulin/glucose-induced stimulation of lipogenic gene expression.[68] Very recently, we have obtained preliminary evidence that not only glucose, but also fructose, can activate ChREBP (unpublished observations), suggesting that this carbohydrate sensor may play an important role in mediating the lipogenic effects of fructose.

INSIGHTS FROM DIET-INDUCED ANIMAL MODELS OF THE METABOLIC SYNDROME

Several genetic and diet-induced animal models of the metabolic syndrome have been employed to investigate the pathogenesis of this increasingly common disorder. Genetic models have been helpful in identifying key genetic factors involved in the development of this disease; however, they have the major drawback of not being relevant to the current etiology of the disease in humans, which is largely dietary in origin. Two rodent models of diet-induced insulin resistance have been particularly informative. These are the "sand rat" (*Psammomys obesus*), a gerbil that develops obesity and insulin resistance when fed standard rodent chow,[69] and the fructose-fed Syrian golden hamster (*Mesocricetus auratus*). The latter model was developed in our laboratory in

the mid-1990s to study the link between insulin resistance and VLDL overproduction, and is increasingly being used by others as a simple dietary model of insulin resistance. The Syrian golden hamster is an attractive model, as its lipoprotein metabolism resembles that of humans more closely than that of other rodents.[70] Hamster liver expresses only apoB100 (not apoB48) and secretes VLDL particles, which is similar to humans. Hamsters also develop hyperlipidemia and atherosclerosis in response to a modest increase in dietary cholesterol and saturated fat,[71] and can be made hypertriglyceridemic and insulin resistant by fructose feeding.[44]

Fructose feeding for a 2-week period induced hepatic and whole body insulin resistance and increased plasma TG, cholesterol, and FFA, but did not cause obesity or type 2 diabetes.[16,44] Fructose-induced insulin resistance was associated with decreased insulin receptor phosphorylation, decreased mass and tyrosine phosphorylation of IRS-1 and IRS-2, decreased PI-3 kinase and Akt activation, increased mass and activity of PTP-1B, increased hepatic MTP mass, increased hepatic intracellular stability of apoB100, heightened synthesis and secretion of TG by the liver, and VLDL overproduction.[16,44] Interestingly, we found that metabolic disturbances in the hamster model could be reversed by treatment with rosiglitazone, an insulin sensitizer.[72] We have also reported intestinal overproduction of apoB48-containing lipoproteins in this model.[67] Thus, the fructose-fed hamster is a model of relatively mild insulin resistance, hypertriglyceridemia, and increased hepatic and intestinal apoB-lipoprotein overproduction.

The relevance of fructose feeding as a model of the metabolic syndrome, insulin resistance, and metabolic dyslipidemia is further confirmed by studies in which humans were fed fructose-rich diets. Similar to hamsters, humans fed high-fructose diets developed hepatic and adipose tissue insulin resistance and dyslipidemia,[7] thus validating the fructose-fed hamster model as being applicable to the human disease.

CONCLUSION

It is clear that fructose is a potent inducer of hepatic lipogenesis. When consumed in excess, hepatic lipogenesis and overproduction of TG-rich VLDL particles can quickly lead to overload of adipocytes with fat, which then stimulates a cascade of events including the net efflux of fat from adipocytes and altered secretion of adipokines. Through these mechanisms, the adipocytes undergo crosstalk with other organs and modify their intracellular signaling pathways. TNF-α, upregulated by the NFkB pathway, appears to be a particularly important cytokine in the activation of JNK-1, a kinase that participates in inhibitory serine phosphorylation of IRS-1, especially in adipose tissue and the liver. Concurrently, fructose-induced hepatic lipogenesis and ectopic fat uptake by the liver can cause steatosis, hepatic inflammation involving inflammatory cytokine production by hepatic immune cells, and insulin resistance. Sustained hepatic generation of proinflammatory cytokines leads to a vicious cycle of worsening insulin resistance and steatosis. Figure 3 outlines our current hypothesis linking fructose consumption, hepatic inflammation, insulin resistance, and dyslipidemia.

Future research may uncover other metabolic pathways, transcription factors, or target genes, hormones, adipokines, tissues, or nutrients that contribute to the metabolic syndrome. Animal models such as the fructose-fed hamster will be especially useful tools in deciphering the nutritionally induced mechanisms underlying metabolic dyslipidemia and other phenotypic characteristics of the metabolic syndrome. Such models can be useful not only in further molecular and physiological characterization of the metabolic syndrome, but also in the evaluation of various strategies to prevent or mitigate the syndrome and its associated metabolic complications.

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