Fructose as a key player in the development of fatty liver disease

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Abstract

We aimed to investigate whether increased consumption of fructose is linked to the increased prevalence of fatty liver. The prevalence of nonalcoholic steatohepatitis (NASH) is 3% and 20% in nonobese and obese subjects, respectively. Obesity is a low-grade chronic inflammatory condition and obesity-related cytokines such as interleukin-6, adiponectin, leptin, and tumor necrosis factor-α may play important roles in the development of nonalcoholic fatty liver disease (NAFLD). Additionally, the prevalence of NASH associated with both cirrhosis and hepatocellular carcinoma was reported to be high among patients with type 2 diabetes with or without obesity. Our research group previously showed that consumption of fructose is associated with adverse alterations of plasma lipid profiles and metabolic changes in mice, the American Lifestyle-Induced Obesity Syndrome model, which included consumption of a high-fructose corn syrup in amounts relevant to that consumed by some Americans. The observation reinforces the concerns about the role of fructose in the obesity epidemic. Increased availability of fructose (e.g., high-fructose corn syrup) increases not only abnormal glucose flux but also fructose metabolism in the hepatocyte. Thus, the anatomic position of the liver places it in a strategic buffering position for absorbed carbohydrates and amino acids. Fructose was previously accepted as a beneficial dietary component because it does not stimulate insulin secretion. However, since insulin signaling plays an important role in central mechanisms of NAFLD, this property of fructose may be undesirable. Fructose has a selective hepatic metabolism, and provokes a hepatic stress response involving activation of c-Jun N-terminal kinases and subsequent reduced hepatic insulin signaling. As high fat diet alone produces obesity, insulin resistance, and some degree of fatty liver with minimal inflammation and no fibrosis, the fast food diet which includes fructose and fats produces a gene expression signature of increased hepatic fibrosis, inflammation, endoplasmic reticulum stress and lipopapoptosis. Hepatic de novo lipogenesis (fatty acid and triglyceride synthesis) is increased in patients with NAFLD. Stable-isotope studies showed that increased de novo lipogenesis (DNL) in patients with NAFLD contributed to fat accumulation in the liver and the development of NAFLD. Specifically, DNL was responsible for 26% of accumulated hepatic triglycerides and 15%-23% of secreted very low-density lipoprotein triglycerides in patients with NAFLD compared to an estimated less than 5% DNL in healthy subjects and 10% DNL in obese people with hyperinsulinemia. In conclusion, understanding the underlying causes of NAFLD forms the basis for rational preventive and treatment strategies of this major form of chronic liver disease.

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INTRODUCTION

Excessive accumulation of triglycerides in hepatocytes in the absence of significant alcohol consumption occurs in about 20%-30% of adults[1-5]. Excessive fat in the liver, called nonalcoholic fatty liver disease (NAFLD), predisposes to the development of nonalcoholic steatohepatitis (NASH). NASH constitutes the subset of NAFLD that is most worrisome because it is a significant risk factor for developing cirrhosis and its complications, including hepatocellular carcinoma (HCC)[6-9]. Because the accumulation of excess fat in the liver is a prerequisite for the development of NASH, understanding the underlying causes of NAFLD forms the basis for rational preventive and treatment strategies of this major form of chronic liver disease.

Obesity is a low-grade chronic inflammatory condition and obesity-related cytokines such as interleukin-6 (IL-6), adiponectin, leptin, and tumor necrosis factor (TNF) α may play important roles in the development of NAFLD. The prevalence of NASH is 3% and 20% in nonobese and obese subjects, respectively. Additionally, the prevalence of NASH associated with both cirrhosis and HCC was reported to be high among patients with type-2 diabetes with or without obesity.

OBESITY EPIDEMIC

A balance exists between energy demand and intake in the human body. Obesity is one of the major abnormalities of this well preserved equilibrium. Obesity, and its consequences such as insulin resistance and the metabolic syndrome, is a growing threat to the health of people in developed nations[10]. A diet based on high cholesterol, high saturated fat, and high fructose (cafeteria or fast food type) recapitulates features of the metabolic syndrome and nonalcoholic fatty liver disease and nonalcoholic steatohepatitis with progressive fibrosis in human and mice. NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

"FAST FOOD" OR "CAFETERIA" TYPE DIET COMPOSED OF HIGH SATURATED FATS, CHOLESTEROL, AND FRUCTOSE

The basis of the composition of “fast food” or “cafeteria” style food is high saturated fats, cholesterol, and fructose[11]. As the high fat diet produces obesity, insulin resistance, and some hepatic steatosis with minimal inflammation and no fibrosis, the fast food diet produces a gene expression signature of increased hepatic fibrosis, inflammation, endoplasmic reticulum stress and lipopoptosis (Figure 2). Our research group previously showed that consumption of fructose is associated with adverse alterations of plasma lipid profiles and metabolic changes in mice, the American Lifestyle-Induced Obesity Syndrome (ALIOS) model, which included consumption of a high-fructose corn syrup (HFCS) in amounts relevant to that consumed by some Americans[11]. The observation that the ALIOS mice indeed consumed a greater quantity of food beyond the additional calories consumed from the HFCS when fed HFCS compared with control water supports this observation and reinforces the concerns about the role of fructose in the obesity epidemic[12,13]. In adolescents, higher fructose consumption is associated with multiple markers of cardiometabolic risk, but it appears that these relationships are mediated by visceral obesity.

The most commonly used HFCS in soft drinks and other carbohydrate-sweetened beverages is a blend composed of 55% fructose, 41% glucose, and 4% complex polysaccharides. Fructose has increasingly been used as a sweetener since the introduction of high-fructose corn syrups in the 1960s[10,13,16] and is now an abundant source of dietary carbohydrate in the United States. The annual per capita consumption of extrinsic or added fructose was approximately 0.2 kg in 1970 to approximately 28 kg in 1997. This increased consumption has been linked to the increased prevalence of obesity, type 2 diabetes and fatty liver in the United States.

The liver is exquisitely sensitive to changes in nutrient delivery and is uniquely suited to metabolize ingested simple sugars, such as fructose and glucose[13,14]. Stress-activated protein kinases, principally the c-Jun N-terminal kinases (JNK), are activated by cell stress-inducing stimuli. Increased fructose supply provokes a hepatic stress response involving activation of JNK and subsequent reduced hepatic insulin signaling.

UNIQUE METABOLISM OF FRUCTOSE

Fructose, glucose, and galactose are the 3 major dietary...
monosaccharides. Sucrose (glucose-fructose), lactose (glucose-galactose), and maltose (glucose-glucose) are the major disaccharides. Dietary fructose occurs in 2 forms: mono- or disaccharide. The rate of fructose absorption appears to be between that of mannose and glucose\(^{12-15}\). Fructose is absorbed by carrier-mediated facilitated diffusion, an energy-dependent process. The fructose carrier is a member of the glucose transport family and is referred to as glucose transporter 5. Sucrose is cleaved to glucose and fructose by sucrase, an enzyme located in the brush border of small intestine enterocytes.

Fructose was previously accepted as a beneficial dietary component because it does not stimulate insulin secretion. However, since insulin signaling plays an important role in the central mechanisms of NAFLD, this property of fructose may be undesirable\(^{13-15}\). Additionally, fructose may prevent suppression of ghrelin secretion, resulting in impaired satiety mechanisms\(^{14}\). In large quantities, fructose can also stress the liver by depleting hepatic energy supplies. Normal subjects and patients with NASH exhibited a similar depletion of hepatic ATP levels after an injection of fructose, but recovery of ATP levels after depletion was slower in NASH patients compared with healthy controls. A mixture of fructose and glucose might induce metabolic abnormalities that differ from sucrose, a disaccharide cleaved to fructose and glucose in the small intestine.

Phosphorylation of glucose by glucokinase is a rate-de-

terminating step in hepatic glucose metabolism. In contrast to glucose, phosphorylation of fructose in the liver occurs via the enzyme fructokinase. In addition, the metabolism of fructose 1-phosphate in the liver occurs independently of phosphofructokinase, a second rate-determining step in glucose metabolism\(^{13-15}\). As a result, the liver is the primary site of fructose extraction and metabolism, with extraction approaching 50% to 70% of fructose delivery. Therefore, increased availability of fructose (e.g., high-fructose corn syrup) will increase not only abnormal glucose flux but also fructose metabolism in the hepatocyte. Thus, the anatomic position of the liver places it in a strategic buffering position for absorbed carbohydrates and amino acids.

Fructose extraction and metabolism by the liver are exceptionally high compared to glucose due both to the extensive amount of fructokinase that phosphorylates fructose to fructose 1-phosphate in the liver and to the subsequent metabolism of fructose 1-phosphate at the triose phosphate level, which bypasses flux control at phosphofructokinase\(^{13-16}\). Previous studies comparing the metabolism of fructose and glucose in postabsorptive humans over short intervals have shown that fructose is used faster than glucose and that more is converted to liver glycogen. Fructose oxidation represented a significant portion of fructose metabolism, accounting for 56% to 59% of the ingested fructose and approximately 33% of the infused fructose. It is likely that extrahepatic lactate oxidation subsequent to hepatic fructolysis contributed significantly to the estimated rate of fructose oxidation. Thus, increments in fructose after infusion produced immediate changes in hepatic and extrahepatic substrate metabolism, but did not induce changes in overall glucose production. An immediate fructose infusion in humans induced both hepatic and extrahepatic insulin resistance. These data are consistent with the notion that high concentrations of fructose elicit adaptations in the liver that include metabolic intermediates, gene expression, and insulin action.

**SYSTEMIC AND HEPATIC INSULIN RESISTANCE IN NAFLD**

While insulin receptor defects cause severe insulin resistance, most patients with insulin resistance have impaired post-receptor intracellular insulin signaling. Insulin binds \(\alpha\)-subunits of its receptor, which is a cell surface receptor on the major insulin sensitive cells such as skeletal muscle, adipocytes, and hepatocytes, leading to autophosphorylation of the cytoplasmic domains (\(\beta\)-subunits) of the receptor\(^{2-5,17}\). The insulin receptor has intrinsic tyrosine kinase activity activated by insulin binding and the autophosphorylated receptor activates its substrates that include insulin receptor substrate (IRS)-1, IRS-2, Src homology collagen, and adaptor protein with a pleckstrin homology and Src homology 2 domain by tyrosine phosphorylation. These phosphory-
lated docking proteins bind and activate several down-
stream components of the insulin signaling pathways. 
Activated IRS-1 associates with phosphatidyl inositol
3-kinase, which then activates Akt. These events and
insulin-dependent inhibition of hepatic glucose output
maintain glucose homeostasis. Insulin also affects glu-
cose homeostasis indirectly by its regulatory effect on 
lipid metabolism. Any interference in this insulin sig-
aling pathway causes glucotoxicity, insulin resistance and,
when islet beta cells are capable of responding, compen-
satory hyperinsulinemia.

Hepatic expression of insulin receptor protein in hu-
mans and the levels of both IRS-1 and IRS-2 in animals 
were decreased in chronic hyperinsulminemic states[11].
IRS-1 was more closely linked to glucose homeostasis 
with the regulation of glucokinase expression while IRS-2 
was more closely linked to lipogenesis with the regulation 
of lipogenic enzymes sterol regulatory element-binding 
protein-1c (SREBP-1c) and fatty acid synthase[18,19]. Addi-
tional physiological roles of insulin include regulating 
the metabolism of macronutrients and stimulating cellular 
growth. Insulin activates synthesis and inhibits catabolism
of lipids while shutting off the synthesis of glucose in 
the liver.

Adipose tissue is one of the major insulin sensitive 
organs in the human body and the process of differenti-
ation of preadipocytes to adipocytes is induced by insulin[17,18]. Within the adipose tissue, insulin stimulates 
triglyceride synthesis and inhibits lipolysis by upregulat-
ing lipoprotein lipase activity which is the most sensitive 
pathway in insulin action, facilitating free fatty acid up-
take and glucose transport, inhibiting hormone sensitive 
lipase, and increasing gene expression of lipogenic en-
zymes.

PROINFLAMMATORY SIGNALING IN 
INSULIN RESISTANCE

Protein kinase C theta (PKCθ) and inhibitor κB kinase
β (IKK-β) are two proinflammatory kinases involved in
insulin downstream signaling[17,18]. They are activated by 
lipid metabolites such as high plasma free fatty acid con-
centrations and there is a positive relationship between 
the activation of PKCθ and the concentration of inter-
mediate fatty acid products. PKCθ activates both IKK-β 
and JNK, leading to increased Ser 307 phosphorylation 
of IRS-1 and insulin resistance. Activation or overex-
pression of IKK-β diminishes insulin signaling and 
causes insulin resistance whereas inhibition of IKK-β 
improves insulin sensitivity. Inhibition of IKK-β activity 
prevented insulin resistance due to TNF-α in cultured 
cells. IKK-β phosphorylates the inhibitor of nuclear fac-
tor kappa B (NF-κB), leading to the activation of NF-
κB by the translocation of NF-κB to the nucleus. NF-
κB is an inducible transcription factor and promotes 
specific gene expression in the nucleus. For example,
NF-κB regulates the production of multiple inflamma-
tory mediators, such as TNF-α and IL-6. TNF-α and re-
active oxygen species could also activate NF-κB[19-22]. In 
contrast, antioxidants inhibit this activation. NF-κB has 
both apoptotic and anti-apoptotic effects. The finding 
that NF-κB deficient mice were protected from high-
fat diet-induced insulin resistance suggests that NF-κB 
directly participates in processes that impair insulin sig-
naling. High-dose salicylates also inhibit NF-κB and sub-
sequently improve insulin sensitivity. These subsequently 
promote hepatic and systemic insulin resistance. The 
study group also showed that these results were reversed 
by curcumin which inhibits NF-κB activity. Curcumin 
also has the ability to induce antioxidant enzymes and 
scavenge ROS.

Suppressors of cytokine signaling (SOCS) and induc-
ible nitric oxide synthase are two inflammatory mediators 
recently recognized to play a role in insulin signaling[23-25]. 
Induction of SOCS proteins (SOCS 1-7 and cytokine-
inducible src homology 2 domain-containing protein) 
by proinflammatory cytokines might contribute to the cyto-
kine-mediated insulin resistance in obese subjects[26-30]. In 
fact, the isoforms of SOCS are the members of a nega-
tive feedback loop of cytokine signaling, regulated by 
both phosphorylation and transcription events. SOCS-1, 
and particularly SOCS-3, are involved in the inhibition 
of insulin signaling either by interfering with IRS-1 and 
IRS-2 tyrosine phosphorylation or by the degradation 
of their substrates. SOCS-3 might also regulate central 
leptin action and play a role in the leptin resistance of 
obese human subjects. SOCS might be a link between 
leptin and insulin resistance because insulin levels are 
increased in leptin resistant conditions due to the dimin-
ished insulin suppression effect of leptin because of ins-
sufficient leptin levels. Moreover, SOCS proteins might 
influence insulin/insulin like growth factor-1 signaling. 
SOCS-1 knockout mice showed low glucose concentra-
tions and increased insulin sensitivity. SREBP-1c is one 
of the key mediators of lipid synthesis from glucose and 
other precursors (de novo lipogenesis) in the liver. Indeed, 
SOCS proteins markedly induce de novo fatty acid synth-
ase in the liver by both the up-regulation of SREBP-1c 
and persistent insulin resistance with hyperinsulinemia 
which stimulates SREBP-1c-mediated gene expression. 
Liver is the insulin clearance organ. Thus, decreased in-
sulin clearance in patients with NAFLD further elevates 
insulin levels in the circulation and de novo lipogenesis 
in the liver. SOCS-1 and SOCS-3 may exert these effects by 
inhibiting signal transduction and activator of transcription 
proteins (STAT), particularly STAT-3, via binding 
Janus tyrosine Kinase (JAK) tyrosine kinase because 
this binding diminishes the phosphorylation ability of 
JAK kinase to STAT-3. STAT-3 inhibits the activation of 
SREBP-1c. Specific STAT-3 knockout mice showed 
markedly increased expression of SREBP-1c and sub-
sequently increased fat content in the liver. Conversely, 
inhibition of SOCS proteins, particularly SOCS-3, im-
proved both insulin sensitivity and the activation of
SREBP-1c which eventually reduced liver steatosis and hypertriglyceridemia in db/db mice.

Nitric oxide synthase-2 (NOS2) or inducible nitric oxide synthase (iNOS) production are also induced by proinflammatory cytokines\[31\]. A high-fat diet in rats causes up-regulation of iNOS mRNA expression and increases iNOS protein activity. Increased production of NOS2 might reduce insulin action in both muscle and pancreas and decreased iNOS activity protects muscles from the high-fat diet induced insulin resistance. It was also shown that leptin deficient ob/ob mice without iNOS were more insulin sensitive than ob wild-type mice. Thus, the production of nitric oxide may be one link between inflammation and insulin resistance.

**SOURCES OF LIVER FAT**

Accumulation of triglycerides as fat droplets within the cytoplasm of hepatocytes is a prerequisite for subsequent events of NASH. Accumulation of excess triglyceride in hepatocytes is generally the result of increased delivery of non-esterified fatty acids (NEFAs), increased synthesis of NEFAs, impaired intracellular catabolism of NEFAs, impaired secretion as triglyceride, or a combination of these abnormalities\[32\]. Recent techniques, such as isotope methodologies, multiple-stable-isotope approach and gas chromatography/mass spectrometry, provided valuable information regarding the fate of fatty acids during both fasting and fed states\[33\] such as the relative contribution of three fatty acid sources to the accumulated fat in NAFLD: adipose tissue, de novo lipogenesis, and dietary fat. Additionally, these studies reported that the plasma NEFA pool is the main contributor of both hepatic triglycerides in the fasting state and very low-density lipoproteins (VLDL)-triglycerides in both fasting and fed states.

**DYSREGULATED PERIPHERAL LIPOLYSIS**

A study showed that adipose tissue makes a major contribution to the plasma NEFA pool, contributing 81.7% in the fasted state and 61.7% in the fed state\[34\]. Additionally, the contribution of dietary lipids to the plasma NEFA pool was found to be only 26.2% and 10.4% in fed and fasted states, respectively, in the same study. Finally, the contribution of newly made fatty acids (originating from the adipose tissue and liver) to the plasma NEFA pool was 7.0% and 9.4% for the fasted and fed states, respectively.

The liver takes up free fatty acids from the circulating NEFA pool and the rate of uptake depends only on the plasma free fatty acid concentrations. Hepatic NEFA uptake continues despite increased hepatic content of fatty acids and triglycerides\[35\]. The concentration of free fatty acids is increased in the portal circulation rapidly when feeding continues despite increased hepatic content of fatty acids in NAFLD. Cornstarch has increased fructose consumption in NAFLD\[35-39\]. Stable-isotope studies showed that increased de novo lipogenesis (DNL) in patients with NAFLD contributed to fat accumulation in the liver and the development of NAFLD\[33\]. Specifically, DNL was responsible for 26% of accumulated hepatic triglycerides and 15%-23% of secreted VLDL triglycerides in patients with NAFLD compared to an estimated less than 5% DNL in healthy subjects and 10% DNL in obese people with hyperinsulinemia. Interestingly, Donnelly and colleagues demonstrated the similarity between VLDL-triglycerides and hepatic-triglycerides regarding contributions of fatty acid sources (62% vs 59% for NEFA contribution, respectively; 23% vs 26% for DNL, respectively; and 15% vs 15% for dietary fatty acids, respectively) in NAFLD patients. Substrates used for the synthesis of newly made fatty acids by DNL are primarily glucose, fructose, and amino acids; oleic acid (18:1, a ω-6 monounsaturated fatty acid, which is relatively resistant to peroxidation) is the major end product of de novo fatty acid synthesis\[40\].

Moreover, simple sugars have the ability to stimulate lipogenesis\[39\]. Ingested carbohydrates are a major stimulus for hepatic delayed neuronal loss and are thus more likely to directly contribute to NAFLD than dietary fat intake\[41-44\].

In conclusion, fructose has increasingly been used as a sweetener since the introduction of high-fructose corn syrups in the 1960s and is now an abundant source of dietary carbohydrate in the United States\[45-50\]. The most commonly used HFCS in soft drinks and other carbohydrate-sweetened beverages is a blend composed of 55% fructose, 41% glucose, and 4% complex polysaccharides\[51-58\]. This increased consumption has been linked to the increased prevalence of obesity and type 2 diabetes and fatty liver in the United States by increased fructose supply, which provokes a hepatic stress response involving activation of JNK and subsequent reduced hepatic insulin signaling\[56-59\]. Understanding the underlying causes of NAFLD forms the basis for rational preventive and treatment strategies of this major form of chronic liver disease.

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