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REVIEWS: CURRENT TOPICS

The role of fructose-enriched diets in mechanisms of nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) currently affects 20%–30% of adults and 10% of children in industrialized countries, and its prevalence is increasing worldwide. Although NAFLD is a benign form of liver dysfunction, it can proceed to a more serious condition, nonalcoholic steatohepatitis (NASH), which may lead to liver cirrhosis and hepatocellular carcinoma. NAFLD is accompanied by obesity, metabolic syndrome and diabetes mellitus, and evidence suggests that fructose, a major caloric sweetener in the diet, plays a significant role in its pathogenesis. Inflammatory progression to NASH is proposed to occur by a two-hit process. The first “hit” is hepatic fat accumulation owing to increased hepatic *de novo* lipogenesis, inhibition of fatty acid beta oxidation, impaired triglyceride clearance and decreased very-low-density lipoprotein export. The mechanisms of the second “hit” are still largely unknown, but recent studies suggest several possibilities, including inflammation caused by oxidative stress associated with lipid peroxidation, cytokine activation, nitric oxide and reactive oxygen species, and endogenous toxins of fructose metabolites.
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Keywords: Fructose; Nonalcoholic fatty liver disease (NAFLD); Nonalcoholic steatohepatitis (NASH); Review

1. Introduction

High-fructose corn syrup (HFCS) is the major source of caloric sweeteners found in soft drinks, juice beverages, canned fruits, jams, jellies, breakfast cereals and baked goods. In 1970, HFCS represented <1% of all caloric sweeteners available for consumption in the United States. However, this percentage jumped rapidly in the 1980s, and HFCS represented 42% of all caloric sweeteners by 2000 [1]. HFCS contains 55%–90% fructose and thus constitutes a major source of dietary fructose. With the rise in HFCS consumption, obesity in the United States has increased to epidemic levels over the past three decades [2]. Many researchers have investigated the impact of fructose consumption on weight, lipid profiles and glucose metabolism in both rats and humans, and most of these researchers have agreed that a fructose-enriched diet contributes to the risks for obesity, metabolic syndrome and diabetes mellitus [3,4]. For example, we have previously shown that long-term consumption of sucrose (a disaccharide of glucose and fructose) causes weight gain, hyperglycemia, glucose intolerance and hyperinsulinemia in rats [5]. Stanhope and Havel reported similar findings in humans: fructose consumption at 25% of energy intake for 10 weeks resulted in increased visceral adiposity, lipid dysregulation and insulin resistance [6]. Some studies have observed these effects only with the consumption of fructose-sweetened beverages, and not glucose-sweetened ones [7–9], more often in men than in women [9] and more often in obese individuals than in nonobese individuals [7].

Nonalcoholic fatty liver disease (NAFLD) is the most common hepatic manifestation of obesity, affecting ~20%–30% of adults [10]. However, a study from Israel demonstrated that soft drink consumption was associated with NAFLD independent of metabolic syndrome [11] or in the absence of traditional risk factors, including obesity, diabetes or hyperlipidemia [12]. In fact, an increasing body of evidence indicates that fructose in the diet itself causes NAFLD. Previous studies in rodents have demonstrated various histological alterations of liver tissue after fructose consumption; these include focal inflammation in the periportal regions [13], macrovesicular steatosis in the periportal area [14], and macrovesicular and microvesicular steatosis [15,16]. We have previously reported the predominance of lobular inflammation over portal inflammation in rats fed a high-fructose diet (70%) for 5 weeks, which is consistent with findings in human nonalcoholic steatohepatitis (NASH) [17]. Several large-scale epidemiological studies have also suggested a positive relationship between fructose consumption and NAFLD [18–20]. Furthermore, among these, a study with a sample size of 427 conducted by Abdelmalek et al. demonstrated that daily fructose consumption was associated with higher fibrosis stage in biopsy-proven NAFLD, after controlling for age, gender, body mass index and total caloric intake [20].

Although NAFLD is a benign form of liver dysfunction, it can progress to the more serious disease NASH, which is a necroinflammatory condition affecting 2%–3% of adults [10]. NASH may progress to cirrhosis with subsequent liver failure and increases the risk for

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hepatocellular carcinoma. Given the evidence that a fructose-enriched diet may cause NAFLD, early intervention is needed to prevent the start of this disease progression. This review aims to clarify the role of fructose in mechanisms of NAFLD in light of recent findings in fructose-feeding studies in both human and animal models.

2. The "two-hit" theory of NAFLD: the first hit: hepatic lipid accumulation

According to the two-hit theory originally proposed by Day and James in 1998 [21], the inflammatory progression to NASH occurs with two sequential hits, with the first being hepatic steatosis and the second being hepatic inflammation. Hepatic steatosis is potentially reversible and does not necessarily lead to permanent hepatic injury. However, it is postulated to sensitize the liver to the second hit. Although less common, the second hit comprises more virulent inflammation associated with oxidative stress or endogenous toxins of fructose metabolites.

Fat accumulates within hepatocytes when the cellular input of fatty acids through either uptake or synthesis exceeds fatty acid output via degradation or export. Increased hepatocytic fatty acid input associated with fructose consumption is attributable to increased hepatic *de novo* lipogenesis, inhibition of fatty acid beta oxidation, impaired triglyceride (TG) clearance and reduced very-low-density lipoprotein (VLDL) export.

Fructose passes passively from the intestinal lumen to the blood via glucose transporter 5 (GLUT5), which is the sole transporter specific for fructose, with no ability to transport glucose or galactose [22]. From the portal blood, fructose is efficiently moved across hepatic plasma membranes by GLUT2 or 5. In the liver, fructose is converted to fructose-1-phosphate by fructokinase and further converted by aldolase B into the triose phosphates dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, which can enter the glycolytic pathway. In the case of glucose, its metabolism is regulated at the point where glucose carbon enters the glycolytic pathway: citrate and adenosine-5′-triphosphate (ATP) provide feedback inhibition of phosphofructokinase, reducing the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate (Fig. 1). However, because fructose bypasses this control step, it serves as an unregulated source of both glycerol 3-phosphate and acetyl-CoA, leading to enhanced lipogenesis [23]. This process is further enhanced by synergy with insulin resistance and/or obesity [24,25].

After fructose intake, triose phosphate is the main lipogenic precursor; it can be subsequently converted into pyruvate by pyruvate dehydrogenase and then further oxidized into CO_2 and H_2O in the hepatic mitochondrial tricarboxylic acid cycle [26]. When liver mitochondria cannot metabolize excess of acetyl-CoA substrate, the extra substrate exits the mitochondria and enters the cytosol in the form of citrate, leading to *de novo* lipogenesis [26]. *De novo* lipogenesis is mediated by two important proteins, carbohydrate response element binding protein (ChREBP) and sterol regulatory element binding protein 1c (SREBP-1c) [27,28]. Fructose administration induces the activation of ChREBP and acts in synergy with SREBP to increase the expression of lipogenic genes, including those encoding acetyl CoA carboxylase (ACC), fatty acid synthase (FAS) and stearoyl coenzyme-A desaturase-1 (SCD-1) [29]. ChREBP is localized in the cytosol, but it can be imported into the nucleus by protein phosphatase 2A (PP2A), which is promoted by xylulose 5-phosphate (Xu-5-P), an intermediate metabolite of the pentose phosphate pathway [30]. Thus, PP2A in nucleus activates ChREBP [29]. SREBP-1c is responsible for the insulin-mediated induction of lipogenic enzymes in the liver [27] or is expressed independently of insulin in fructose metabolism [31]. Recent studies have shown that peroxisome proliferator-activated receptor γ coactivator 1β (PCG-1β) [32] and a binding of scaffold attachment factor B1 (SAFB1) to an X-chromosome-linked RNA binding motif protein (RBMX) [33,34] may activate SREBP-1c.

Hepatic *de novo* lipogenesis is accelerated by the direct effect of fatty acid synthesis and indirect effect of increased levels of malonyl-CoA, which reduce the entry of fatty acids into the mitochondria by inhibiting liver carnitine palmitoyltransferase I (L-CPTI) [35]. This rate-limiting enzyme of beta oxidation regulates the transfer of long-chain acyl-CoA from the cytosol into the mitochondria, producing a shift from an oxidative to a reesterification pathway [36].

Another important molecule associated with fructose regulation of lipids in the liver is peroxisome proliferator-activated receptor alpha (PPARα), which belongs to the superfamily of ligand-activated nuclear hormone receptors. Fructose-1-phosphate reduces hepatic PPARα mRNA levels, and the decrease in PPARα reduces the expression of fatty acid oxidation enzymes [37–39]. A study group in Spain has suggested that the PPARα alteration may be induced by the impairment of hepatic transduction of the leptin signal [38–40]. Their studies have revealed that in fructose-fed rats, but not in glucose-fed rats, hyperleptinemia was induced and was caused by the impairment of leptin signal transduction mediated by Janus-activated kinase-2 (JAK-2) and the mitogen-activated protein kinase (MAPK) pathway [38–40]. PP2A expression, which is greatly increased by Xu-5-P, dephosphorylates Ser/Thr residues in JAK-2 and the MAPK pathway, and these in turn inhibit Akt and 5′ adenosine monophosphate-activated protein kinase (AMPK) activities, respectively. The subsequent increases in the activities of the unphosphorylated and active forms of the forkhead box O1 nuclear factor transrepresses PPARα activity, thus inducing hepatic lipogenesis [40]. The overstimulation of the only functional leptin signaling pathway in the liver finally induces enough suppressor of cytokine signaling-3 protein to further block leptin signaling mediated by the JAK-2 and MAPK pathways. This blockade establishes a self-perpetuating loop that maintains and enhances the metabolic disturbances produced by fructose [40].

After fatty acids are converted into TGs by esterification, TGs can be exported from the liver in VLDL particles, which are formed by the incorporation of TGs into apolipoprotein B (apoB). The degradation of apoB is dramatically reduced when the supply of fatty acids (and TG biosynthesis) is increased [41]; thus, the apoB level is increased in fructose metabolism [42]. Tsai et al. have suggested that hepatic synthesis and secretion of apoB are mediated by the inflammatory IκB kinase/nuclear factor-κB (NF-κB) signaling cascade [43]. The decrease in apoB degradation results in the accumulation of apoB in the hepatic endoplasmic reticulum (ER), which induces ER stress [44]. Su et al. have suggested that ER stress leads to the perturbed activation of glycogen synthase kinase 3 and glycogen synthase via the activation of c-Jun N-terminal kinase (JNK) and suppression of the insulin signaling cascade, to induce hepatic insulin resistance [44]. A study in ob/ob mice showed that ER stress also promotes SREBP-1c activation, thus contributing to *de novo* lipogenesis [45]. Indeed, the chaperone glucose-regulated protein 78, an ER stress reduction marker, alleviates hepatic TG levels and improves insulin sensitivity [45].

Insulin action in the liver exhibits many similarities to insulin action in muscle. In the liver, insulin activates insulin receptor kinase, which phosphorylates insulin receptor substrate-1 (IRS-1) and IRS-2, leading to the activation of phosphatidylinositol 3-kinase (PI3K) and ultimately Akt2 [46]. At this point, Akt2 activation promotes glycogen synthesis and inhibits gluconeogenesis. Thus, insulin resistance leads to increased hepatic glucose production, increased fasting glucose and insulin concentrations, and decreased glucose tolerance and apoB secretion [47,48]. Several mechanisms underlying fructose impairment of the insulin signaling pathway have been proposed [49–54]. For example, Wei et al. have demonstrated that fructose delivery increased JNK activity via the

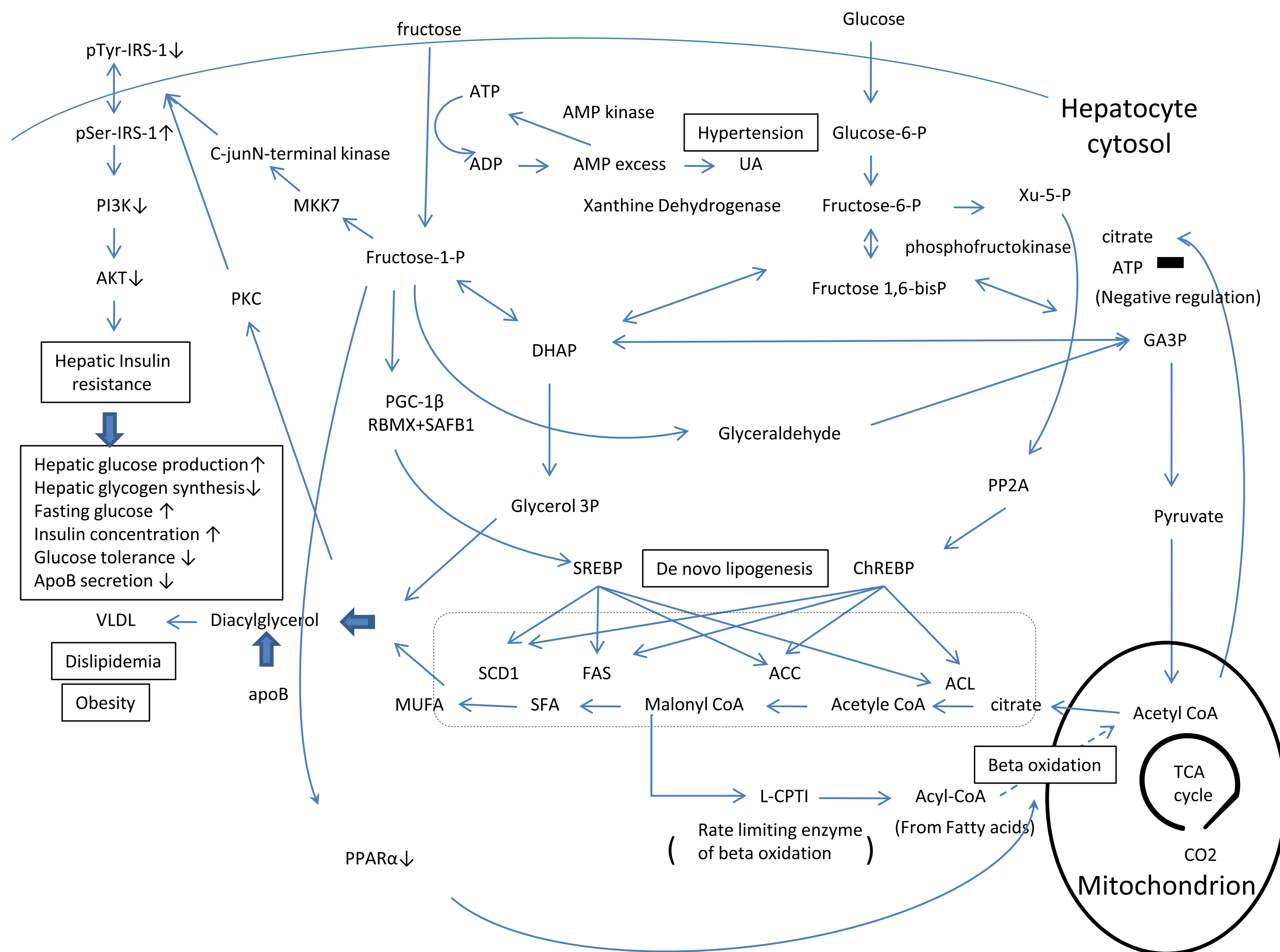

Fig. 1. Fructose metabolism in liver. Fructose is converted to fructose-1-phosphate by fructokinase and further metabolized into triose phosphate, entering the glycolytic pathway and serving as an unregulated source of glycerol-3-phosphate and acetyl-CoA, leading to enhanced *de novo* lipogenesis. *De novo* lipogenesis is activated through two gene expression proteins, ChREBP and SREBP-1c, which increase the expression of ACC, FAS and SCD-1. Finally, fatty acids are converted into TGs by esterification, and VLDL particles are formed by the incorporation of TGs into apoB. Fructose induces insulin resistance: fructose-1-phosphate increases serine phosphorylation of IRS-1 and reduces tyrosine phosphorylation of IRS-1, impairing the following PI3K/Akt signaling pathway. Insulin resistance leads to increased hepatic glucose production, increased fasting glucose and insulin concentrations, and decreased glucose tolerance and apoB secretion. Fructose also induces hyperuricemia and causes hypertension through the inhibition of endothelial NO synthesis. Abbreviation: ATP, adenosine-5′-triphosphate; ADP, 5′ adenosine diphosphate; fructose-1-p, fructose-1-phosphate; DHAP, dihydroxyacetone phosphate; GA3p, glyceraldehydes-3-phosphate; Xu-5-P, xylulose 5-P; PP2A, a protein phosphatase 2A; TCA, tricarboxylic acid; ChREBP, carbohydrate response element binding protein; SREBP-1c, sterol regulatory element binding protein 1c; PCG-1β, peroxisome proliferator-activated receptor γ coactivator 1β; SAFB1, scaffold attachment factor B1; RBMX, an X-chromosome-linked RNA binding motif protein; ACL, ATP-citrate lyase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD-1, stearoyl coenzyme-A desaturase-1; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; L-CPT1, liver carnitine palmitoyltransferase I; apo B, apolipoprotein B-100; PKC, novel protein kinase C; PPAR α, a peroxisome proliferator-activated receptor; MKK7, mitogen-activated protein kinase kinase 7; pSer-IRS-1, serine phosphorylation of IRS-1; pThy-IRS-1, tyrosine phosphorylation of IRS-1; IRS-1, insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase.

regulation of mitogen-activated protein kinase kinase 7 and scaffold proteins [50], increased serine phosphorylation of IRS-1 and reduced tyrosine phosphorylation of IRS-1, thereby impairing the subsequent PI3K/Akt signaling pathway [51]. In their review, Samuel et al. have suggested that an increased intrahepatic level of diacylglycerol may activate novel protein kinase C (PKC) [52], given the much higher affinity of novel PKC for diacylglycerol compared with other PKC family members [55]. The subsequent decrease in Tyr phosphorylation and/or increase in Ser phosphorylation of the insulin receptor and IRS-1 reduces the activity of PI3K and Akt, resulting in insulin resistance [52,53]. Indeed, the activation of novel PKC and Ser phosphorylation of IRS-1 were shown to improve insulin resistance in mice without PKC [56]. However, the kinases (i.e., JNK or IκB kinase β) that activate novel PKCs remain unknown [52].

Fructose may induce hypertension [57] as well as cardiovascular diseases [58] and metabolic syndrome [59,60]. These systemic conditions are associated with endothelial dysfunction involving uric acid inhibition of nitric oxide (NO) production by endothelial NO synthase (eNOS) [59]. The initial step in fructose metabolism, the phosphorylation of fructose to fructose-1-phosphate by fructokinase, requires ATP. The phosphorylation of fructose in the liver causes hepatic ATP depletion, and adenosine 5′-diphosphate is converted to AMP. The fate of AMP is determined by the relative activities of two competing enzymes, AMPK and xanthine dehydrogenase. When AMPK is more active than xanthine dehydrogenase, AMP is recycled to restore hepatic ATP content. When xanthine dehydrogenase is the more active enzyme, AMP is converted to uric acid [20]. The recovery from hepatic ATP depletion is severely impaired in patients with obesity-related NASH [61].

3. The second “hit”: inflammatory progression to NASH

The second hit is proposed to be inflammation caused by oxidative stress associated with lipid peroxidation, cytokine activation, NO,

reactive oxygen species (ROS) and endogenous toxins of fructose metabolites. The results of several studies in animals and humans have suggested that elevated dietary fructose intake induces bacterial overgrowth in the small intestine, accompanied by intestinal permeability; this increases endotoxin levels in the portal vein and contributes to the mechanism of NAFLD [62–64]. Although the evidence is limited to mice, hepatic lipid accumulation in fructose-fed mice was markedly reduced by antibiotics, suggesting that endotoxins play a critical role [65].

Endotoxins induce the expression of the endotoxin receptor toll-like receptor 4 (TLR-4) in the liver [62,66]. TLR-4 is a receptor for Gram-negative bacterial cell wall components, including lipopolysaccharides [67]. Upon pathogen recognition, TLR signaling promptly induces potent innate immune responses that signal through the adaptor molecule myeloid differentiation factor 88 to activate NF-κB [67–69], which in turn releases numerous proinflammatory mediators [70]. TLR facilitation of innate immune responses for the initial host defense against microorganisms is important in the liver because it is constantly exposed to microbial products from the enteric microflora that are carried through the portal circulation [71]. A normal liver can tolerate innate immune responses and does not usually induce inflammation in the TLR signaling pathway [72]. However, in NAFLD patients, a breakdown of this tolerance may allow the activation of an inappropriate immune response.

Although Kupffer cells are considered to be the primary cells in the liver that respond to TLR signaling, recent studies have revealed that TLR signaling occurs in hepatic nonimmune cell populations, including hepatocytes, biliary epithelial cells, endothelial cells and hepatic stellate cells [71]. The binding of lipopolysaccharides to TLR-4 in these cells induces lipid peroxidation and the production of proinflammatory cytokines and ROS [68]. Kupffer and hepatic stellate cells are involved in hepatic fibrosis, which is an important histological finding in NASH, through the TLR-4 signaling pathway [71,73]. The loss of TLR-4 and the destruction of Kupffer cells blunted hepatic expression of tumor necrosis factor alpha (TNF-α) and markers of fibrosis such as collagen α1 and transforming growth factor β in a methionine/choline-deficient diet [66] (i.e., an animal model for hepatosteatosis [74]).

Increased ROS formation has been repeatedly claimed as a major contributor to a proposed second-hit mechanism of NAFLD. Previous studies have investigated various markers of ROS formation, including 4-hydroxynonenal adducts [65,68,75–77], inducible NOS (iNOS) protein [68,77,78], malondialdehyde [79], thiobarbituric acid-reactive substances [16,80], NO [16] and xanthine oxidase activity [16]. These ROS markers were markedly elevated after fructose administration in both rodents [16,65,68,75–77,79,80] and humans (i.e., malondialdehyde) [12]. Among these studies, two studies in rodents investigated liver tissue histology; Bergheim et al. found a significantly increased number of infiltrating neutrophils [65], and Armutcu et al. reported a significant increase in macrovesicular steatosis [16]. Both of these findings are compatible with NAFLD, but not NASH. Another source of oxidative stress may be the overexpression of cytochrome P450 2EI (CYP2EI), a prooxidant enzyme [81]. ROS generated by CYP2EI or other sources may mediate the progression from steatosis to cell death through 4-hydroxynonenal by JNK/c-Jun overactivation [82].

Among ROS indicators, NO is a pluripotent, gaseous-free radical that possesses various physiological functions. It is produced from L-arginine by NOS, a three-member family comprising eNOS, iNOS and neuronal NOS [83]. In Section 2 of the present article, we discussed the impact of uric acid on eNOS in vascular diseases. However, compared with eNOS, iNOS produces much more NO [85], and iNOS has been found in hepatocytes, hepatic stellate cells and cholangiocytes [84]. NO may potentiate cytotoxicity by its reaction with superoxide anion to form peroxynitrite, which induces mitochondrial permeability, causing protein nitration and tissue injury leading to apoptosis [85]. The free radicals generated during the oxidation process by xanthine oxidase induce lipid peroxidation, resulting in premature cell death [86]. Recent studies conducted by Spruss et al. have reported that in livers of fructose-fed iNOS−/− mice, lipid peroxidation, phosphorylated IκB, NF-κB activity and TNF-α expression were not increased; these findings suggest that iNOS may be involved in mediating TLR-4-dependent effects on fructose-induced steatosis [68,78].

Several studies have proposed that fructose and fructose metabolites act as endogenous toxins [87–90]. A study group in Canada reported that the serum level of methylglyoxal, a glycolytic metabolite, is increased in fructose-fed rats [87,88]. Hyogo et al. found that serum levels of advanced glycation end products, which are formed from the fructose metabolite glyceraldehyde, were significantly elevated in NASH patients [89]. Hepatocytes are very resistant to H_2O_2, largely because of their high activities of catalase, glutathione (GSH), GSH peroxidase and GSH reductase. However, as little as 10 μM glyoxal was sufficient to increase hepatocyte susceptibility to noncytotoxic concentrations of H_2O_2. Cytotoxicities of fructose and glyoxal, a fructose metabolite, were increased about 100-fold and 200-fold, respectively, by noncytotoxic doses of H_2O_2 [87,88,90]. The major ROS sources in a cell are the mitochondria, because electrons passing through the electron transport chain can leak and reduce oxygen. Cytotoxicity was preceded by ROS and H_2O_2 formation, and the mitochondrial membrane potential was inhibited before cytotoxicity occurred; thus, the cytotoxic mechanism is likely oxidative stress, suggesting that mitochondrial toxicity can cause ROS formation [88]. On the other hand, cytotoxicity was prevented by deferoxamine, a ferric chelator, and by hydroxyl radical scavengers. Furthermore, fructose/ H_2O_2 cytotoxicity was further increased by adding trace amounts of ferric or cupric salts. These results suggest that hydroxyl radicals formed by the Fenton reaction [91] oxidized fructose to endogenous toxins, which contributed to the cytotoxic mechanism [92].

4. Conclusion

Fructose has been used as a major caloric sweetener over the past three decades, during which time the prevalence of obesity has increased. A growing body of evidence suggests that fructose in the diet induces NAFLD. Because NAFLD may progress to cirrhosis with subsequent liver failure and increased risk for hepatocellular carcinoma, early intervention is essential. In 2009, the American Heart Association recommended that dietary intake of added sugars be reduced by more than half [93]. Although much is known about hepatic fructose metabolism, the detailed mechanism of the second hit associated with fructose consumption remains unclear. Further research is warranted to determine the underlying mechanisms and possible therapeutic approaches for fructose-induced fatty liver disease.

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