

High-fructose Intake in Obesity-related Nonalcoholic Fatty Liver Disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. NAFLD involves a spectrum of conditions that include pure steatosis without inflammation, nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Insulin resistance (IR) plays a main role in the pathophysiology of NAFLD by causing lipid accumulation in the hepatocytes, which may be followed by lipid peroxidation, production of reactive oxygen species and consequent inflammation. The consumption of carbohydrates, particularly sugars additives high in fructose increased dramatically in the past decades and appears to be at least one very important contributing factor in NAFLD pathogenesis. Recent studies suggest that the excessive consumption of fructose from sugar additives (mainly those found in sweetened beverages and processed foods) is linked to development and severity of NAFLD by induction of IR, postprandial hypertriglyceridemia and lipid accumulation in the liver, especially in individuals with overweight. We discuss the possible mechanisms involving fructose consumption, lipid accumulation and development of NASH. This review also presents the chief findings from all the studies that evaluated fructose consumption in human NAFLD.

Keywords: Fatty liver; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Fructose/High fructose corn syrup

Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive accumulation of triglycerides in hepatocytes in the absence of significant alcohol consumption. It encompasses a spectrum of clinicopathological conditions that ranges from simple hepatic steatosis (fatty liver) to hepatic steatosis associated with necroinflammatory lesions (nonalcoholic steatohepatitis [NASH]) with or without hepatic fibrosis that may progress to cirrhosis and hepatocellular carcinoma [1]. Currently, NAFLD is considered the most prevalent chronic liver disease in the Western and NASH is one of the most important emerging health issues [1,2]. NAFLD is usually associated with the metabolic syndrome (MS) [1,3,4]. The prevalence of obesity is rising worldwide and it parallels the increase in the frequency of NAFLD, cirrhosis, hepatocellular carcinoma and complications of the MS [5-7].

The pathogenesis of NAFLD is not fully elucidated, but insulin resistance (IR), and accumulation of triglycerides and free fatty acids in the liver play an essential role. Fat accumulation may be followed by subsequent inflammation and liver damage [2]. Many factors may influence the pathogenesis and progression of the liver disease including host factors (age, gender, presence of diabetes, genetic polymorphisms, and according to recent evidence, the gut microbiome) and environmental factors [1]. Increasing evidence suggests that the rise in consumption of carbohydrates, particularly refined sugars with high fructose content, appears to be a very important contributing factor in NAFLD pathogenesis. Although convince data demonstrate association between high-fructose intake and NAFLD progression in animals, data from human studies are less convincing. Searching for evidence of the role of fructose in the development and progression of NAFLD by addressing clinical human

studies is the focus of the present comprehensive review. The clarification of this issue is of crucial importance to the development of new strategies to prevent and/or to treat NAFLD.

NAFLD and Fructose Intake: Epidemiological Evidence

The role of environmental factors in the pathogenesis of NAFLD is supported by the increased in the prevalence of this condition in recent years. Currently, estimates of the worldwide prevalence of NAFLD, based on a variety of diagnostic methods, ranges from 6.3% to 33% with a median of 20% in the general population [8]. Thus, this condition represents the most common chronic liver disease in the general population and is expected to rise as a result of aging population, improving control of other major causes of chronic liver disease, and the epidemics of obesity and diabetes [1,7]. NASH, the progressive form of the disease, is the most common cause of cryptogenic cirrhosis and an increasingly common indication for liver transplantation [9].

Obesity is a common and well-documented risk factor for NAFLD. It is a known low-grade chronic inflammatory condition and obesity-related cytokines such as interleukin (IL)-6, adiponectin, leptin, and tumor necrosis factor (TNF)- α may play an important role in the development of NAFLD [5,10]. Both excessive body mass index (BMI) and visceral obesity are recognized risk factors for NAFLD. In patients with severe obesity undergoing bariatric surgery, the prevalence of NAFLD can exceed 90% and up to 5% of patients may have unsuspected cirrhosis [2,5,7]. A very high prevalence (up to 67%) of NAFLD, diagnosed by ultrasound (US), has been reported in individuals with type 2 diabetes mellitus (T2DM) [7,11]. In a recent study, 127 of 204 diabetic patients displayed fatty infiltration on US, and 87% of the patients with fatty infiltration who underwent liver biopsy had histologic confirmation of NAFLD [12]. 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 [1].

The general increase in calorie consumption, particularly of refined carbohydrates and fructose, correlates positively with an alarming increase in the prevalence of the MS and NAFLD [13]. Analysis of data from U.S. Government suggests an 18% and 41% increase in daily energy and carbohydrate intake between the 1977-1978 and 1999-2004, respectively [13]. Mean total fructose intake as a percentage of carbohydrate followed a similar trend pattern. More recently, data from the National Health and Nutrition Examination Survey suggest a decreasing trend in the average per capita and population mean energy intake of added sugar [14].

Relationship between fructose and risk of NAFLD

There has been much concern regarding the role of dietary fructose in the development of metabolic diseases. This concern arises from the increase in fructose consumption (and total added caloric sweeteners consumption) over the past few decades, and from the increased use of high-fructose corn syrup (HFCS) as a sweetener. Besides the large increase in the consumption of soft drinks and 'tasty' artificial juices, a significant decrease in milk intake has also been reported [15].

In this context, some studies suggest that the over-consumption of HFCS, primarily in the form of soft-drink, is linked to weight gain, particularly in children and adolescents, and increased risk of NAFLD [16,17]. A recent study showed a positive correlation between increase in the prevalence of type 2 diabetes and increasing intake of refined carbohydrate (corn syrup) concomitantly with decreasing intake of fiber [18]. Consumption of sweetened beverages is considered clearly associated with excess calorie intake, and increased risk of diabetes and cardiovascular diseases (CVD) due to increase in the body weight. The Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2015, advises restriction of refined carbohydrates for the most common diet-related chronic diseases such as CVD, hypertension, diabetes and diet-related cancers. Findings from a recent meta-analysis indicate a positive association between sugar-sweetened soft drink intake and type 2 diabetes risk, attenuated by adjustment for BMI [19].

Fructose is especially found in industrialized food and beverages. It is mainly consumed as added sugar. Soft drinks are the leading source of artificially added sugar in the world. Table sugar (sucrose) and HFCS are the two major dietary sources of fructose [20] while smaller consumed amounts come from fruits and honey. HFCS are quite commonly found in soft drinks and juice beverages, and are incorporated into many convenient pre-packaged foods, such as breakfast cereals and baked goods. HFCS contains variable amounts of fructose, between 55% and 90%. HFCS was first introduced into the human diet in 1967 [20,21]. Since then, the use of HFCS has increased an alarming 1000% between 1970 and 1990 [16]. Data show that individual consumption of fructose was 0.5 lb/year and 62.4 lb/year, in 1970 and in 1997, respectively [16]. Between 1909 and 1997, general sweetener use increased by 86%; and specifically, corn syrup sweeteners, nowadays, represent over 20% of total daily carbohydrate intake, at an increase of 2100% [15,16,18].

The role of fructose in the etiology and pathogenesis of NAFLD

The cause and the pathogenesis of NAFLD are not completely understood, but IR, accumulation of triglycerides and free fatty acids

in the liver play an essential role. Fat accumulation may be followed by inflammation and liver damage [2]. According to the most accepted theory, IR plays a major role initiating hepatic fat accumulation and, potentially, NASH [22,23]. IR affects lipid metabolism as it increases peripheral lipolysis, triglyceride synthesis, and hepatic uptake of free fatty acids (FFA) contributing to the accumulation of triglyceride in the hepatocytes [23]. This excessive deposition of triglyceride in the liver leads to a shift from carbohydrates to FFA mitochondrial β -oxidation, and may promote lipid peroxidation and accumulation of reactive oxygen species (ROS) in the hepatocytes. These compounds produce a variety of cellular stimuli with subsequent inflammatory response, hepatocellular injury and fibrosis [22,24].

Host factors, including age, gender, presence of diabetes, genetic polymorphisms and according to more recent evidence, the gut microbiome as well as environmental factors, especially the diet and physical activities are co-factors that influence NAFLD pathogenesis [1,25,26]. Intake of nutrients (including fructose found in sweetened beverages) may affect IR, carbohydrate levels, lipid metabolism, and hepatic steatosis [27]. A study including healthy volunteers showed that moderate amounts of fructose and sucrose significantly alter hepatic insulin sensitivity and lipid metabolism compared with similar amounts of glucose [28].

Obesity characterized by increased BMI or visceral obesity is a well-documented risk factor for NAFLD [6,8]. Gut microbiota is linked to both: obesity and NAFLD. The microbiota is related to obesity because it can increase energy harvesting from the diet and enhances energy storage [29]. Small intestinal bacterial overgrowth (SIBO) and increased intestinal permeability are related to NAFLD as they cause endotoxemia with subsequent cytokines release, systemic inflammation and IR [30]. It has been suggested that there is a causative relation between the increased prevalence of NAFLD and related disorders (i.e. obesity, diabetes, CVD, hypertension, cancer and MS) and intake of sweeteners, particularly fructose [14].

The "fructose hypothesis", fructose plays a unique and causative role in the increasing rates of cardiovascular disease, hypertension, diabetes, cancer, and nonalcoholic fatty liver disease, in part has been driven by data from animal studies and in part by historical trends [15,16]. Fructose is essentially metabolized in splanchnic tissues, where it is converted into glucose, glycogen, lactate, and, to a minor extent, fatty acids. In animal models, high fructose diets clearly stimulate hepatic de novo lipogenesis and cause hepatic steatosis. Specifically, animal studies have shown that high-fructose, compared with glucose, diets result in increased hepatic triglyceride content [17-21]. Unlike glucose, high-fructose diets cause development of features of MS and fatty liver in animals, in association with the development of leptin resistance [31], microvascular disease, and vascular inflammation [15,16,32,33]. In addition, some observations suggest that fructose may trigger hepatic inflammation and stimulate the development of hepatic fibrosis. These observations lead to consider the possibility that fructose may promote the progression of NAFLD to its more severe forms, i.e. NAHS and cirrhosis. Interestingly, it has been demonstrated in animal models that a four-week high-fructose diet increases lipopolysaccharide [LPS] contained in the gut microbiota and plasma LPS concentrations two to three times, which is considered metabolic endotoxemia. The induction of metabolic endotoxemia in mice, by continuous subcutaneous infusion of LPS during four weeks, was followed by a rise in the following parameters: fasting glycemia, insulinemia, markers of inflammation, liver triglyceride content, liver IR, and whole body, liver and adipose

tissue weight gain in a similar amount as occurred in high-fat diet fed mice [10]. Large amount of fructose consumption is also related to increase in endotoxin serum levels, proinflammatory response and steatosis. It was demonstrated in an elegant study conducted by Bergheim et al., that mice fed with fructose showed increased endotoxin levels in the portal blood, and higher intrahepatic lipid accumulation, lipid peroxidation and TNF- α expression [34].

The increasing use of high fructose sweeteners over the past few decades has resulted in a considerable rise in the dietary intake of fructose. A high flux of fructose to the liver, the major organ capable of metabolizing this simple carbohydrate, disturbs normal hepatic carbohydrate metabolism leading to two major consequences: perturbations in glucose metabolism and glucose uptake pathways, and a significantly enhanced rate of *de novo* lipogenesis and triglycerides (TG) synthesis, driven by the high flux of glycerol and acyl portions of the TG molecules coming from fructose catabolism. These metabolic disturbances appear to underlie the induction of IR commonly observed with high fructose feeding in both humans and animal models. Fructose induced insulin resistant states are commonly characterized by a profound metabolic dyslipidemia, which appears to result from hepatic and intestinal overproduction of atherogenic lipoprotein particles [15].

Fructose metabolism is different from glucose metabolism in many aspects. First of all, fructose ingestion cause less satiety sensation comparing to glucose, as insulin and leptin secretion is lower after glucose ingestion. This may lead to increase in food ingestion and inhibition in ghrelin secretion. Ghrelin is the hormone related to satiety. Leptin usually promotes reduction of food ingestion while low levels in plasma induce hyperphagia. Ghrelin acts in opposition to leptin inducing hungry. Second, after ingestion, most part of fructose is transported to the liver via GLUT5. Third, the fructose is converted in two triose phosphates (dihydroxyacetone and glyceraldehyde-phosphate) and uric acid by the enzymes fructokinase and aldolase B. Finally, part of triose phosphate excess can be used for lipogenesis and exportation of very low density lipoprotein (VLDL) to the hepatocytes, causing steatosis [35].

Fructose metabolism is associated with increase in lipogenesis, reduction in lipids oxidation, increase in free radicals production and in gut permeability, SIBO, and elevated levels of lipopolysaccharides [36-38].

Hepatic *de novo* lipogenesis (DNL) - fatty acid and triglyceride synthesis - is markedly increased in patients with NAFLD [39]. According to a stable-isotope study, the increase in DNL in patients with NAFLD contributed to fat accumulation in the liver and development of NAFLD. Interestingly, DNL was responsible for 26% of accumulated hepatic triglycerides and 15-23% of secreted very low-density lipoprotein triglycerides (VLDL-TG) in patients with NAFLD compared to an estimate of <5% in healthy subjects and of 10% in obese people with hyperinsulinemia [40].

In humans, there is strong evidence, based on several intervention trials, that fructose overfeeding increases fasting and postprandial plasma triglyceride concentrations, which are related to stimulation of hepatic DNL and VLDL-TG secretion, in association with decreased VLDL-TG clearance [41-43]. Recent data suggest that increased fructose consumption enhances fat mass, DNL and inflammation, and also induces IR and postprandial hypertriglyceridemia, particularly in overweight individuals [32,33,44-46]. However, in contrast to animal models, fructose intake by humans as high as 200 g/day decrease only

modestly hepatic insulin sensitivity, and has no effect on whole body (muscle) insulin sensitivity. A possible explanation may be that IR and dysglycemia develop mostly in the presence of sustained fructose exposures associated with changes in body composition. Such effects are observed with highly daily fructose intake, and there is no solid evidence that fructose, when consumed in moderate amounts, has deleterious effects [38,47,48].

Lecoultre et al., assessed the association between intrahepatic fat content and IR with daily fructose and energy intake during short-term overfeeding (6-7 days) in healthy male subjects [38]. The authors concluded that short-term overfeeding with fructose (up to 4 g fructose/kg/day) or glucose decreases hepatic insulin sensitivity and increases hepatic fat content. These observations suggest the possibility of short-term regulation of hepatic glucose metabolism by simple carbohydrates [38]. Considering that cardiovascular complications are a leading cause of death in NAFLD patients, a recent study showed a significant improvement in adipose insulin sensitivity, levels of high sensitivity C-reactive protein (hs-CRP) and low-density lipoprotein (LDL) oxidation in adolescents with NAFLD after reduction of fructose intake, which suggest that reduction in fructose consumption improves several important factors related to CVD despite a lack of measurable improvement in hepatic steatosis [49].

Although animal models are useful for elucidating the potential mechanisms for the development and progression of NAFLD, they cannot confirm the cause and pathophysiology in humans. Difficulties also occur in making extrapolations to humans from results obtained in experimental animals because results vary for different species, strains and gender, and with the specific source of dietary protein. Furthermore, a high-intake of fructose was used in many studies. An effect of fructose intake varies with the dose and with time of exposure. As for many macronutrients, extremely high levels of fructose intake can be toxic. Thus, the results of animals experiments using high dietary amounts of fructose administration over short periods of time can be misleading and should not be extrapolated to infer adverse healthy effects associated with chronic, low-dose consumption by humans [21,48,50].

Clinical evidence of fructose consumption in humans with NAFLD

In humans, a short-term fructose overfeeding stimulates DNL and significantly increases intrahepatic fat concentration, without however reaching the proportion encountered in NAFLD. Whether consumption of lower amounts of fructose over prolonged periods may contribute to the pathogenesis of NAFLD has not been convincingly documented in epidemiological studies and remains to be further assessed [21,35].

High fructose intake and the increase in diabetes and obesity seem to be the major factors for NAFLD development [51]. Human studies suggest an association between fructose intake and NAFLD [41,52,53]. This association was particularly high in the individuals with more severe liver disease (NASH) who ingested more amounts of carbohydrates (mainly fructose) when compared to healthy subjects [43,54]. Nevertheless, a recent meta-analysis [50] fails to show association between high-intake of fructose and NAFLD; furthermore, a recent cross-sectional study showed an inverse relation between the amount of fructose intake and the frequency of NAFLD development [55]. Table 1 shows the main findings of human studies evaluating fructose intake in NAFLD patients.

Study	Hepatic Disorder and Sample Size	Variables	Results
Kanerva et al. [55]	N=1611, 663 diagnosed with NAFLD by the algorithm Fatty Liver Index (FLI) and 511 by the NAFLD Fat Liver Score	Algorithm for definition of NAFLD, food frequency questionnaire, anthropometry, factors of MS	Subjects in the highest quartile of 28-44% fructose intake (range: 29.2-88.0 g/d) had smaller waist circumference values and lower risk of NAFLD assessed by using the FLI and NAFLD liver fat score. Association persisted after model adjusted for lifestyle and variables related to food consumption.
Volynets et al. [53]	20 NAFLD by ultrasonography (3 NASH) vs. 10 healthy controls	Food frequency questionnaire	NAFLD group had increased consumption of protein carbohydrates, sucrose, fructose and glucose. PAI-1, endotoxin, and ALT plasma levels were positively related to total protein and carbohydrate intake.
Abdelmalek et al. [43]	427 with biopsy proven NAFLD	Food frequency questionnaire, liver biopsy, biochemical tests	Group with daily consumption of industrialized beverages had higher triglyceride levels, fasting plasma glucose, total calories, proteins, carbohydrates and lipids compared to those who consumed moderately. Model adjusted for sex, age, BMI and total caloric intake showed that daily consumption of industrialized beverages was associated with a decrease in the degree of steatosis (OR = 0.4, CI 0.4-0.9; p = 0.02) and an increase in the degree of fibrosis (OR = 2.6, CI 1.4- 5; p = 0.004).
Abid et al. [36]	31 NAFLD with risk factors for metabolic syndrome (DM, obesity and hypertriglyceridemia), 29 NAFLD without risk factors for metabolic syndrome and 30 controls	Food record, HFCS consumption, ultrasound, HOMA, triglycerides	NAFLD with MS: increased HOMA (8.3 ± 8 vs. 3.7 ± 3.7 vs. 1.7 ± 0.5; p = 0.001) and triglycerides (208 ± 69 vs. 142 ± 64 vs. 108 ± 34; p = 0.001). Logistic regression demonstrated a strong association between consumption of industrialized beverages and hepatic fat (OR = 2; CI 95% = 1-5 p = 0.03).
Ouyang et al. [41]	49 NAFLD vs. 24 controls	Consumption on HFCS, triglycerides, cholesterol, uric acid, fructokinase, fattyacidsynthase	NAFLD group: increased consumption of HFCS (365 kcal / day vs. 190 kcal / day, p<0.05) and higher triglycerides, cholesterol and uric acid. Expression of fructokinase enzymes and fatty acid synthase were increased in NAFLD compared to samples from controls without steatosis.
Assy et al. [52]	31 NAFLD without classic risk factors of the disease (DM, obesity, dyslipidemia and hypertension) and 30 controls	Food record, ultrasound	NAFLD group: increased consumption of industrialized beverages compared to controls (80% vs. 20%, p = 0.001); higher consumption of added sugar in g/day (75.6 ± 8.4 vs. 33.6 ± 12.6; p = 0.001). Correlation between consumption of processed drinks and fatty liver infiltrate (r=0.63; p<0.01).
Toshimitsu et al. [54]	28 NASH vs. 18 steatosis	Three diet recalls	NASH group: increased consumption of carbohydrates (20-39 ys and 40-59 ys) compared to simple steatosis (56 ± 9 vs. 47 ± 13, p<0.05) (58 ± 9 vs. 49 ± 12, p<0.05).
Thuy et al. [57]	12 nondiabetic NAFLD and 6 healthy controls	Diet, endotoxin, TLR4, PAI-1 plasma and liver	NAFLD group: higher consumption of fructose (p<0.05; plasma levels of endotoxin (p<0.05), PAI-1 (p<0.05), hepatic TLR4 (p<0.05) and PAI-1 mRNA expression (p<0.05). PAI-1 concentrations correlated with endotoxin levels and with hepatic TLR4 mRNA expression. Hepatic mRNA expression of PAI-1 correlated with dietary intakes of carbohydrates, fructose, glucose and sucrose
Vos et al. [63]	149 children (110 boys, 79 girls) biopsy proven NAFLD	Sugar sweetened beverage per week, food questionnaire, demographic, anthropometric, clinical, laboratory and histology data	Sugar sweetened beverage consumption was low without correlation with histology hepatic. Uric acid was increased in NASH group (p=0.008). Median consumption of vitamin E was lower in children with higher grade of steatosis.
Jin et al. [61]	9 NAFLD (7 NASH, 1 steatosis, 2 NASH with fibrosis) and 10 controls without NAFLD	Intervention: balanced meal added to 1) a sweetened beverage with fructose (FB);	NAFLD group: increase in triglycerides after FB compared to GB both NAFLD and control groups. For all subjects, high-density lipoprotein cholesterol declined in the postprandial and overnight hours with FB, but not with GB (p=0.0006). Non sterified fatty acids were significantly higher in NAFLD.

		or 2) a drink sweetened with glucose (GB).	
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Table 1: Summary of clinical trials evaluating fructose consumption in humans with NAFLD. NAFLD: Nonalcoholic Fat Liver Disease; NASH: Nonalcoholic Steatohepatitis; DM: diabetes mellitus; HFCS: High Fructose Corn Syrup, PAI-1: Plasminogen Activator Inhibitor 1; TLR4: Toll-Like Receptor 4.

Toshimitsu et al. evaluated dietary habits and intake of nutrients in 46 patients with histological diagnosis of NAFLD. The authors found an excess intake of carbohydrates and energy in patients with NASH compared with patients with steatosis. Among carbohydrates, intake of simple carbohydrates was higher in those with NASH. Study results suggest that imbalanced diets play a role in development and progression of NASH and correction of these diets may be necessary in patients with NASH [54].

Assy, et al. evaluated the association between soft drink intake and the presence of fat liver disease diagnosed by ultrasonography in 31 patients with NAFLD not associated with classic risk factors (diabetes, obesity, hypercholesterolemia e hypertension) compared to 30 healthy controls [52]. Data on daily dietary intake of food and soft drink, and the source of added sugar were collected during two seven-day periods, at the beginning of the study, and within two weeks after the metabolic tests by using a validated food questionnaire given by a trained dietician. Eighty per cent of patients (25 of 31) consumed an excessive amount of soft drink beverages (more than 50 g/day of added sugar) for 36 months, compared with 20% in healthy controls ($p < 0.001$). Furthermore, patients with DHGNA presented higher levels of aspartate aminotransferase (AST), gamma-glutamyl transferase, glucoses, insulin e homeostatic model assessment (HOMA-IR) when compared to control group. Besides, soft drink consumption was higher in NAFLD patients [52].

Ouyang et al., (2008) investigated, by dietary history, 49 patients with evidence of biopsy-proven NAFLD without cirrhosis and 24 controls matched for gender, age, and body mass index (± 3 points). Consumption of fructose in patients with NAFLD was nearly 2-to 3-fold higher than controls [365 kcal vs 170 kcal ($p < 0.05$)]. In patients with NAFLD ($n=6$), hepatic mRNA expression of fructokinase, an important enzyme for fructose metabolism, and fatty acid synthase, an important enzyme for lipogenesis were increased ($p=0.04$ and $p=0.02$, respectively) [41].

The association of soft drink consumption with NAFLD with ($n=29$) and without MS ($n=31$) were assessed by Abid et al., and compared to 30 gender- and age-matched individuals without NAFLD [36]. The degree of fatty infiltration was measured by ultrasound. Data on physical activity and intake of food and soft drinks were collected during two 7-day periods over 6 months using a food questionnaire. IR, inflammation, and oxidant-antioxidant markers were measured. Excessive intake of soft drink beverages was found in 80% of patients with NAFLD compared to 17% of healthy controls. The NAFLD group consumed five times more carbohydrates from soft drinks compared to healthy controls (40% vs. 8%, $p < 0.001$). Patients with NAFLD with metabolic syndrome had similar malonyldialdehyde, paraoxonase, and C-reactive protein (CRP) levels but higher HOMA- IR and higher ferritin than NAFLD patients without MS. Logistic regression analysis showed that soft drink consumption is a strong predictor of fatty liver (odds ratio: 2.0; $p < 0.04$) independent of MS and CRP level. The authors conclude that NAFLD patients display higher soft drink consumption independent of MS diagnosis.

Results of animal experiments suggest that consumption of refined carbohydrates (e.g. fructose) can result in small intestinal bacterial overgrowth, increased intestinal permeability and endotoxin thereby contributing to the development of nonalcoholic fatty liver disease (NAFLD) disorder [10,41,56-58]. Furthermore, increased plasminogen activator inhibitor (PAI)-1 has been linked to liver damage of various etiologies including endotoxin. Thuy at al., developed a pilot study comparing dietary factors, endotoxin, and PAI-1 concentrations between NAFLD patients ($n=12$) and controls ($n=6$). Total energy, fat, protein, and carbohydrate intakes were similar between groups but patients with NAFLD consumed significantly more fructose than controls. Endotoxin and PAI-1 plasma concentrations as well as hepatic TLR4 and PAI-1 mRNA expression of NAFLD patients were significantly higher than in controls. Results suggested a contribution of dietary fructose intake; increased intestinal translocation of bacterial endotoxin, and PAI-1 to the development of NAFLD in humans [57].

A role of an altered dietary pattern (e.g., a diet rich in sugar) and also alterations at the level of the intestinal barrier in the development and progression of NAFLD were investigated by Volynets et al. [53]. Ten controls and 20 patients with NAFLD ranging from simple steatosis to steatohepatitis were included in the study. Bacterial overgrowth, orocecal transit time, and intestinal permeability were assessed. Alcohol, endotoxin, and plasminogen activator inhibitor (PAI-) 1 concentration were determined in plasma. Nutritional intake was assessed using a dietary history. The authors found a significant higher intake of protein, total carbohydrates, and mono- as well as disaccharides in patients with NAFLD than controls. PAI-1, endotoxin, and alanine aminotransferase (ALT) plasma levels were positively related to total protein and carbohydrate intake. The results also indicate that intestinal permeability, endogenous alcohol synthesis, and nutritional intake are markedly altered in patients with NAFLD [53]. One year later, the same group of authors observed that the reduction of fructose intake (reduction of 50% in comparison with baseline) during six months was associated with a decrease in hepatic lipid content, BMI, fasting plasma insulin concentrations, and serum levels of the aminotransferases, endotoxin and PAI-1 [59]. Corroborating the association between fructose and steatosis, Walker et al., investigated in a group of 37 obese young adults the presence of fructose malabsorption, assessed by hydrogen breath test, and correlated it with the grade of steatosis measured by magnetic resonance imaging [60]. The patients exhibited high consumption of fructose-containing beverages. The authors observed a negative correlation between fructose malabsorption and grade of steatosis, suggesting that fructose malabsorption might be protective against fatty liver disease [60].

Jim et al. performed a 2-d, crossover feeding study including 9 children with NAFLD and 10 matched controls without NAFLD [61]. They assessed plasma lipid levels over two nonconsecutive, randomly assigned, 24-h periods under isocaloric, isonitrogenous conditions with three macronutrient-balanced, consecutive meals and either: 1) a fructose-sweetened beverage (FB); or 2) a glucose beverage (GB) being

consumed with each meal. Differences in plasma glucose, insulin, TG, apolipoprotein B, high-density lipoprotein cholesterol, and nonesterified free fatty acid levels were assessed using mixed models and 24-h incremental areas under the time-concentration curve. After FB, triglyceride incremental area under the curve was higher vs. after GB both in children with NAFLD ($p=0.011$) and those without NAFLD ($p=0.027$); however, incremental response to FB was greater in children with NAFLD than those without NAFLD ($p=0.019$). For all subjects, high-density lipoprotein cholesterol declined in the postprandial and overnight hours with FB, but not with GB ($p=0.0006$). Nonesterified fatty acids were not impacted by sugar but were significantly higher in NAFLD. The dyslipidemic effect of dietary fructose occurred in both healthy children and those with NAFLD; however, children with NAFLD demonstrated increased sensitivity to the impact of dietary fructose [61].

Vos et al., conducted a cross-sectional registry based study evaluating demographic, anthropometric, clinical, laboratory and histology data, including the Block Brief Food Questionnaire from 149 children enrolled in the multi-center prospective NASH Clinical Research Network. They found no significant differences between children with steatosis compared to steatohepatitis for fraction of calories from fat, carbohydrates, and protein. Sugar sweetened beverage consumption was low and did not correlate with histologic features, although uric acid, a surrogate marker for fructose intake, was significantly increased in those with definite NASH ($p=.008$). Vitamin E consumption was insufficient compared to the recommended daily allowance for all groups and median consumption of vitamin E was lower in children with higher grade of steatosis [62,63].

A recent systematic review and meta-analysis evaluate fructose, high-fructose corn syrup, sucrose, and nonalcoholic fatty liver disease or indexes of liver health included 6 observational studies and 21 intervention studies (19 in healthy subjects). On the basis of indirect comparisons across study findings, the apparent association between indexes of liver health (ie, liver fat, hepatic de novo lipogenesis, alanine aminotransferase, AST, and g-glutamyltranspeptase) and fructose or sucrose intake appear to be confounded by excessive energy intake. The authors concluded that the evidence evaluated was not sufficiently robust to draw conclusions regarding effects of fructose, HFCS, or sucrose consumption on NAFLD [50]. Even though the authors had evaluated all study designs and all sources and forms of fructose intake in relation to liver health, limitations of conclusions are primarily inherited from poor-quality and heterogeneous studies included in the systematic review. In addition, most of the data were only available in healthy men, which limited the generalizability of the findings.

Abdelmalek et al. studied 427 adults with NAFLD enrolled in the NASH Clinical Research Network for whom Block food questionnaire data were collected within 3 months of a liver biopsy. Study was conducted to investigate the relationship between fructose consumption and disease severity in NAFLD. Fructose consumption was estimated based on reporting (frequency x amount) of Kool-aid, fruit juices, and nondietary soda intake, expressed as servings per week, and classified into none, minimum to moderate (<7 servings/week), and daily ($>$ or $=7$ servings/week). Increased fructose consumption was univariately associated with decreased age, male sex, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, decreased serum glucose, increased calorie intake, and hyperuricemia. The association of fructose intake with metabolic and histological features of NAFLD was analyzed using multiple linear and ordinal

logistic regression analyses with and without controlling for other confounding factors. Interestingly, daily fructose ingestion ($>$ or $=7$ servings/week) was associated with reduced hepatic steatosis but increased fibrosis [43].

Recent data from a large cross-sectional study from Finland shows conflicting results regarding the role of fructose in NAFLD. The authors examined the association between fructose intake and NAFLD by using the Fatty Liver Index (FLI) and the NAFLD liver fat score [62]. Nutritional, clinical and laboratorial parameters were also evaluated. Using validated and standardized 131-item food-frequency questionnaires habitual fructose and other dietary intake were assessed. In a model adjusted for age, sex, and energy intake, participants in the highest fructose intake quartile had lower risk of NAFLD assessed by using the FLI and NAFLD liver fat score than that of the lowest intake quartile. Study results, in disagreement with earlier studies, did not support the current hypothesis that high intake of fructose is associated with a higher prevalence of NAFLD as assessed by using the FLI and NAFLD liver fat score [55].

Conclusions

The data described here support that the notion that the consumption of carbohydrates, particularly sugars additives high in fructose, appears to be at least one very important contributing factor in NAFLD pathogenesis.

Even though growing evidence suggests that fructose contributes to the development and severity of NAFLD, available evidence is not sufficiently robust to draw conclusions regarding effects of fructose, HFCS, or sucrose consumption on NAFLD. Further studies to assess the impact of high-fructose intake as well as other sugars in humans with NAFLD and NASH are warranted.

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