



## Fructose and sugar: A major mediator of non-alcoholic fatty liver disease

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### Summary

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome; its rising prevalence parallels the rise in obesity and diabetes. Historically thought to result from overnutrition and a sedentary lifestyle, recent evidence suggests that diets high in sugar (from sucrose and/or high-fructose corn syrup [HFCS]) not only increase the risk of NAFLD, but also non-alcoholic steatohepatitis (NASH). Herein, we review the experimental and clinical evidence that fructose precipitates fat accumulation in the liver, due to both increased lipogenesis and impaired fat oxidation. Recent evidence suggests that the predisposition to fatty liver is linked to the metabolism of fructose by fructokinase C, which results in ATP consumption, nucleotide turnover and uric acid generation that mediate fat accumulation. Alterations to gut permeability, the microbiome, and associated endotoxemia contribute to the risk of NAFLD and NASH. Early clinical studies suggest that reducing sugary beverages and total fructose intake, especially from added sugars, may have a significant benefit on reducing hepatic fat accumulation. We suggest larger, more definitive trials to determine if lowering sugar/HFCS intake, and/or blocking uric acid generation, may help reduce NAFLD and its downstream complications of cirrhosis and chronic liver disease.

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### Introduction

*“Apicius made the discovery, that we may employ the same artificial method of increasing the size of the liver of the sow, as of that of the goose; it consists in cramming them with dried figs, and when they are fat enough, they are drenched with wine mixed with honey, and immediately killed.”*

Pliny the Elder (The Natural History 1<sup>ST</sup> Century AD, eds John Bostock, Thomas Henry Riley)

Fructose is a simple sugar that is present in fruit and honey, but is also a major component of the two most commonly used sweeteners, sucrose (table sugar, a disaccharide of fructose and glucose), and high-fructose corn syrup (HFCS, a mixture of fructose and glucose monosaccharides). Fructose intake has increased markedly over the last several hundred years in parallel with the rise in intake of sucrose and HFCS. Currently the intake of added sugars approaches 15% of overall energy intake in the average western diet, with higher intakes among younger individuals (adolescents and adults in their twenties) and among ethnic minorities (African American, Hispanic, Native American, and Pacific Islanders).<sup>1–4</sup>

The association between fructose and fatty liver dates back to Pliny the Elder who noted that the famous Roman chef, Marcus Apicius, would make fatty liver (*foie gras*) by feeding geese dates (a rich source of fructose). Later the German chemist, Justus von Liebig, made the observation that simple

carbohydrates stimulated fat accumulation in the liver. Indeed, by the 1960s numerous scientists reported that fructose was distinct from glucose in its unique ability to increase both plasma triglycerides and liver fat.<sup>5–7</sup> Metabolic studies in which fructose was labelled showed a two- to threefold greater labelling of plasma and liver triglycerides than that observed with glucose.<sup>8</sup> However, the overall amount of fructose being converted to triglycerides was relatively small (1 to 3% of the fructose), and did not account for the lipogenic response observed.<sup>9,10</sup> This led some scientists to question the importance of fructose as a means of stimulating lipid synthesis and accumulation.

However, the importance of fructose re-emerged with a report in this journal linking intake of sugar-sweetened beverages, and in particular fructose, with non-alcoholic fatty liver disease (NAFLD),<sup>11</sup> an association that has been confirmed in numerous other studies and is now a major area of research.<sup>12–15</sup> Herein, we provide an update on the association and potential mechanisms by which fructose causes fatty liver. A key finding is that the fructose molecule itself is not primarily responsible for making triglycerides, but rather fat accumulates in the liver by the general activation of lipogenesis and blocking of fatty acid oxidation.<sup>16,17</sup> Indeed, the weight of studies strongly suggest that sucrose and HFCS are major risk factors for NAFLD.

Keywords: Hepatic steatosis; Hepatic inflammation; Insulin resistance; Sugar consumption; Uric acid.

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### Key point

Fructose causes fatty liver through the general activation of lipogenesis and blocking of fatty acid oxidation.

### The discovery of NAFLD and its association with metabolic syndrome

The association of diabetes with liver disease and gout has been known for over 120 years<sup>18</sup> and is strongly associated with insulin resistance. Type 2 diabetes mellitus is the strongest predictor of NAFLD-related hepatic fibrosis and cirrhosis.<sup>19</sup> However, the recognition that people with obesity and prediabetes could develop NAFLD only emerged in the last several decades.<sup>20–22</sup> Many patients with NAFLD show characteristics observed in individuals with metabolic syndrome, including elevated plasma triglycerides, low HDL cholesterol, impaired fasting glucose levels, an increased waist circumference, and elevated blood pressure.<sup>23</sup> Indeed, NAFLD can be viewed as another clinical manifestation of the metabolic syndrome, like hyperuricemia, systemic inflammation (elevated C reactive protein), and microalbuminuria.

NAFLD was not recognised as a clinical entity until the 1980s,<sup>20–22</sup> but it has since been increasing in prevalence. It may progress to non-alcoholic steatohepatitis (NASH) or cirrhosis, with some patients eventually requiring liver transplantation.<sup>24,25</sup> NAFLD is also the most common chronic liver disease in children and adolescents, especially in obese patients, and has even been detected in infants of mothers with gestational diabetes, making this disorder relevant across a wide spectrum of ages.<sup>26–28</sup> Thus, identifying the aetiologies of NAFLD represents a major goal.

### Soft drinks and added sugar are associated with fatty liver

While fructose is present in honey and fruits, the major source of fructose is from sucrose and HFCS, especially in sugar-sweetened beverages. While sucrose-containing drinks have equal amounts of glucose and fructose, HFCS-containing beverages have varying ratios, usually varying from a 55/45 or 65/35 fructose:glucose ratio.<sup>29</sup>

### Experimental studies

Dietary fructose, sucrose, or HFCS have been shown to have a particular tendency to induce fatty liver<sup>6,17,30–34</sup> and inflammation<sup>35</sup> in experimental animals. To develop the fatty liver, it usually takes at least 8–24 weeks on a high-fructose diet, with more progressive disease requiring longer exposure.<sup>36</sup> Often the administration of fructose also induces other features of metabolic syndrome as well, including elevated blood pressure, elevated serum triglycerides, and insulin resistance.<sup>37</sup> In part, the fatty liver may be caused by increased energy intake, as high fructose intake induces leptin resistance in rats.<sup>38,39</sup> However, if diet is controlled so that the control group ingests the same amount of total energy, the fructose-fed rats will still develop features of metabolic syndrome, although weight gain will not be different

between groups.<sup>37,40</sup> Indeed, one can even induce fatty liver with a calorically restricted diet if the diet is high (40%) in sugar.<sup>41</sup> Others have also reported that a high-fructose diet can induce fatty liver in the absence of weight gain.<sup>35</sup>

Fructose has also been administered to primates. In one study in cynomolgus monkeys (*M. fascicularis*), the administration of fructose was shown to result in both an increase in liver fat and hepatic fibrosis after seven years, with the degree of fibrosis correlating with the length of fructose exposure.<sup>42</sup> Fructose-induced metabolic syndrome can also be induced in rhesus monkeys.<sup>43</sup>

Based on comparative studies in which isocaloric diets were administered using sucrose (glucose-fructose disaccharide) or a 50:50 mixture of glucose and fructose monosaccharides, the monosaccharide mixture appears to induce more fatty liver, although the differences are slight.<sup>34</sup> This may relate to differences in absorption or other pharmacokinetics.

Endogenously generated fructose may also have a role in fatty liver and NAFLD.<sup>44</sup> For example, the administration of high concentrations of glucose in drinking water will lead to obesity, insulin resistance and fatty liver in mice over time.<sup>44</sup> Our group reported that high levels of glucose in the portal vein can induce the expression of aldose reductase in the liver, which can convert the glucose to sorbitol, which is then further metabolised to fructose by sorbitol dehydrogenase (the polyol pathway). Indeed, glucose fed mice show increased fructose levels in their liver, and when fructose metabolism is blocked (by giving glucose to fructokinase knockout mice) the animals are almost completely protected from fatty liver and insulin resistance, and are partially protected from obesity.<sup>44</sup>

The NAFLD so commonly observed in patients with diabetes may also represent the effects of endogenous fructose accumulation. Indeed, either knocking down aldose reductase mRNA in the liver, or treatment with aldose reductase inhibitors, can attenuate hepatic steatosis in the type 2 diabetic (*db/db*) mouse.<sup>45</sup>

### Clinical studies

Sugar-sweetened beverage drink intake is also strongly associated with NAFLD in humans. Ouyang *et al.*<sup>11</sup> compared individuals with NAFLD, without cirrhosis, to controls that were matched for age, sex and BMI. Individuals with NAFLD had a two- to threefold higher intake of fructose from sugar-sweetened beverages than controls, and this was associated with an increased expression of fructokinase in the liver.<sup>11</sup> Subsequently, fructose from soft drinks has been associated with NAFLD in children, adolescents and adults, where it correlates with the severity of hepatic fibrosis in a dose-dependent manner.<sup>11,13,46–52</sup> Fructose intake has also been shown to predict the development of NAFLD.<sup>53</sup>

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Clinical studies also suggest a role for fructose in NAFLD. For example, administering sugary beverages to humans for six months resulted in increases in liver fat that were confirmed by magnetic resonance spectroscopy.<sup>54</sup> Conversely, the restriction of fructose for nine days in children with a high baseline fructose intake resulted in both a reduction in liver fat and *de novo* lipogenesis compared to controls fed an isocaloric diet.<sup>55</sup> In a subset of the same study, there was also an improvement in other features of metabolic syndrome, including diastolic blood pressure, serum triglycerides and insulin resistance.<sup>56</sup>

While experimental and clinical studies suggest an association between fructose intake and NAFLD, there is one epidemiological study from Finland that found an inverse relationship between fructose intake and NAFLD, but less than 10% of this population consumed soft drinks, and fruit intake was much more prevalent.<sup>57</sup> While fruits contain fructose, they are less likely to induce metabolic syndrome because of the lower fructose content per fruit (compared to a soft drink) and because they also contain constituents (flavonols, epicatechin, ascorbate, and other antioxidants) that may combat the effects of fructose.<sup>58</sup>

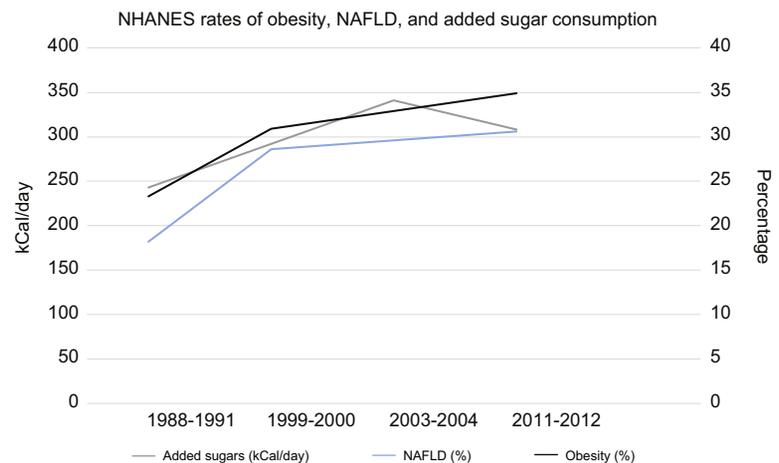
In addition to the aforementioned clinical studies, the rise in documented NAFLD prevalence in the National Health and Nutrition Examination Survey (NHANES) database (using a validated, noninvasive measurement,<sup>59</sup> the US Fatty Liver Index), relative to the rise in obesity and added sugar consumption (refined beet, and sugar cane sucrose and HFCS) is shown for the periods from 1988–1991, 1999–2000, 2003–2004 and 2011–2012 (Fig. 1).<sup>60–62</sup> There is a clear association between fructose intake from added sugars and the rise in obesity and NAFLD.

### Fructose effect on lipogenesis and fat oxidation

Fructose intake has been shown to stimulate *de novo* lipogenesis in animals, as well as to block hepatic  $\beta$ -fatty acid oxidation.<sup>16,17,63</sup> Similarly, studies in humans have also shown that fructose stimulates *de novo* lipogenesis and blocks fatty acid oxidation in the liver.<sup>13,63–66</sup> Fructose acutely (hours) stimulates thermogenesis and metabolic rate,<sup>67,68</sup> but it has been shown to chronically (days to weeks) reduce resting energy expenditure.<sup>66</sup> The mechanisms for these effects are discussed later, but it is evident how these processes could lead to fat accumulation in the liver and elsewhere.

### Differences in fructose and glucose metabolism

In order to understand how fructose intake might predispose an individual to the development of fatty liver, one must know how fructose metabolism is distinct from glucose metabolism. Glucose



**Fig. 1. Association of added sugar consumption with rates of NAFLD, obesity in NHANES data.** NAFLD, non-alcoholic fatty liver disease; NHANES, National Health and Nutrition Examination Survey.

is metabolised primarily by glucokinase or hexokinase, whereas fructose is principally metabolised by fructokinase. Fructokinase utilises ATP to phosphorylate fructose to fructose-1-phosphate, followed by the metabolism by aldolase B to generate D-glyceraldehyde and dihydroxyacetone phosphate. From this stage on, fructose metabolism is similar to glucose metabolism, and results in the generation of glucose, glycogen, and triglycerides.<sup>69</sup> Thus, the unique aspect of fructose metabolism lies in its first two enzymatic steps (Fig. 2).

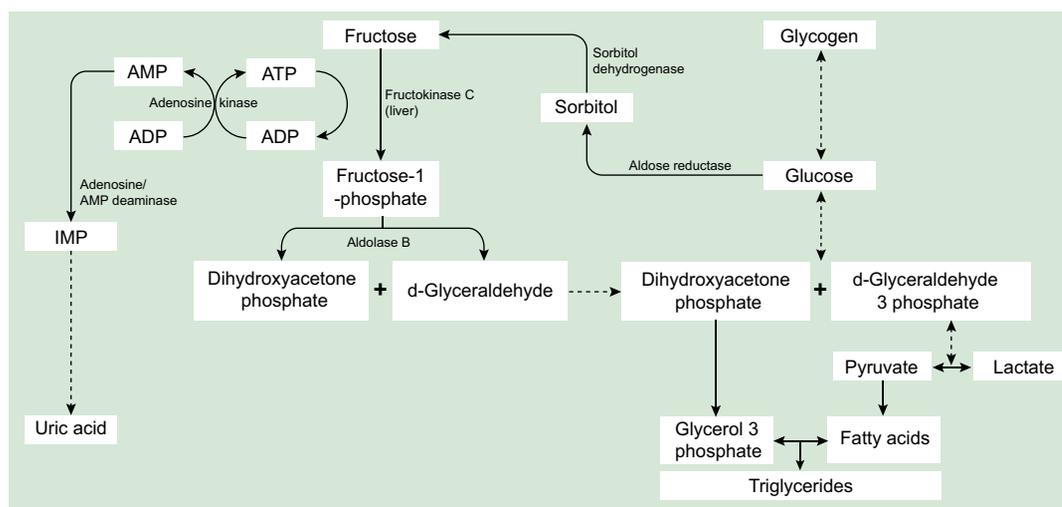
The principal isoform of fructokinase in the liver is fructokinase C, which phosphorylates fructose rapidly and without any negative feedback control, resulting in a drop in ATP and intracellular phosphate.<sup>70–73</sup> The fall in intracellular phosphate activates the enzyme, adenosine monophosphate (AMP) deaminase, that converts AMP to inosine monophosphate (IMP), resulting in purine nucleotide turnover that culminates in the formation of uric acid.<sup>74</sup> Fructose also stimulates the synthesis of uric acid from amino acid precursors.<sup>75,76</sup> The ability of fructose to induce ATP depletion was shown in humans with both intravenous<sup>49,70,77,78</sup> and orally<sup>79</sup> administered fructose. Likewise, an acute rise in uric acid also occurs following fructose ingestion.<sup>80–82</sup> Thus, a unique aspect of fructose metabolism is the transient decrease in intracellular phosphate and ATP levels, associated with nucleotide turnover and uric acid generation, which does not occur during glucose metabolism. This fall in ATP level induces a series of reactions, including a transient block in protein synthesis, induction of oxidative stress, and mitochondrial dysfunction that turn out to have a key role in fructose-mediated effects.<sup>17,70,83</sup>

### Fructokinase, the principal enzyme driving fructose-induced fatty liver

As mentioned, the hepatic metabolism of fructose by fructokinase C results in the breakdown of AMP

#### Key point

The unique aspect of fructose metabolism lies in the first two enzymatic steps, which lead to a fall in ATP and phosphate levels, and culminate in the formation of uric acid.



**Fig. 2.** Interaction of fructose, glucose and polyol pathway with uric acid and triglycerides.

to IMP and the generation of uric acid (Fig. 2), which is primarily due to a fall in intracellular phosphate that occurs following the rapid phosphorylation of fructose by fructokinase C in the liver.<sup>70,71</sup> In contrast, fructokinase A is a second isoform of fructokinase and is more ubiquitously expressed, differing from fructokinase C in that it phosphorylates fructose less efficiently and does not cause significant ATP depletion.<sup>84,85</sup> We have observed that mice lacking both fructokinase C and A are protected from fructose-induced fatty liver, while fructokinase A-knockout mice develop worse fatty liver than wild-type animals despite ingesting similar amounts of fructose.<sup>85</sup> These animals also show evidence of increased metabolism of fructose through the fructokinase C pathway and have higher intrahepatic uric acid levels.<sup>85</sup> In addition, another study by Softic, *et al.*<sup>85</sup> found that fructose, but not glucose drove lipogenic enzymes and insulin resistance through fructokinase, and that fructokinase levels are elevated in fructose-fed mice as well as obese humans with NASH.<sup>86</sup> Thus, these studies suggest that there is a unique property of the fructokinase C pathway that leads to hepatic steatosis, raising the possibility that hepatic steatosis may relate to transient ATP depletion, intracellular phosphate depletion or uric acid generation.<sup>16</sup>

#### Gut permeability and the microbiome

One potential mechanism by which fructokinase may drive fatty liver is via actions on the gut. While fructokinase C is the main enzyme that metabolises fructose in the liver, it is also highly expressed in the small intestine. We have found that the metabolism of fructose in the intestine results in disruption of the tight junctions and that this is not observed in fructokinase knockout mice.<sup>87</sup> This is likely to be responsible for the increased gut permeability that has been observed with fructose ingestion.<sup>88,89</sup> Indeed, studies by

Bergheim's group have shown that the increase in gut permeability results in endotoxin entering the portal vein, which is an important trigger for fatty liver formation.<sup>90</sup> Indeed, the administration of antibiotics to reduce endotoxemia can improve fatty liver.<sup>88,89</sup> Endotoxemia has also been shown to be elevated in children with NAFLD.<sup>91</sup> Thus, the microbiome may have a role in fructose-induced fatty liver through an interaction with fructose metabolism in the intestinal wall. Finally, fructose has been found to alter the gut microbiome, favouring NAFLD development, which along with increased gut permeability through loss of tight junctions, leads to more progressive disease.<sup>92–94</sup>

#### Role of the immune system

As noted, endotoxemia has been identified as a mechanism by which fructose may exacerbate NAFLD.<sup>88</sup> Endotoxemia acts in part by activating the innate immune system, and inflammation is known to have a role in NAFLD, especially in the transition from steatosis to steatohepatitis and cirrhosis.<sup>95</sup> In this regard, a role for T cells and NK cells (but not B lymphocytes) in fructose-induced NAFLD has been shown experimentally, using genetically modified mice that lack T cell or NK cell function.<sup>96</sup>

#### Uric acid and its role in fructose-mediated NAFLD

##### Fructose generates uric acid

As discussed earlier, fructose is the only common carbohydrate that generates uric acid during its metabolism (Fig. 2), with circulatory uric acid levels rising within minutes of fructose ingestion, and noted postprandially in individuals eating fructose-rich meals.<sup>80,82,97</sup> Fructose also increases uric acid levels in the liver.<sup>17</sup> Fructose also stimulates the synthesis of uric acid from amino acid precursors,<sup>75,76</sup> and diets high in fructose are asso-

ciated with increases in fasting serum uric acid levels.<sup>98</sup> Some, but not all epidemiological studies, have also linked high fructose intake with increases in fasting serum uric acid.<sup>99,100</sup>

### Experimental studies

One striking finding was that fructose-induced metabolic syndrome could be partially inhibited by treatment with allopurinol, a xanthine oxidase inhibitor that blocks uric acid generation.<sup>37</sup> Subsequent studies showed that xanthine oxidase inhibitors such as allopurinol or febuxostat could reduce fatty liver caused by fructose,<sup>33</sup> as well as in a genetic model of NAFLD,<sup>101</sup> diabetes-induced fatty liver,<sup>102</sup> high-fat diet associated NAFLD,<sup>103</sup> and alcohol-induced fatty liver.<sup>104</sup> This effect appears to be mediated by reducing uric acid levels and/or the effects of blocking xanthine oxidase-induced oxidative stress. Furthermore, acutely raising uric acid levels by administering a uricase inhibitor resulted in an acute increase in liver triglycerides and hepatic expression of fatty acid synthase (FAS),<sup>105</sup> and incubation of liver cells (HepG2 cells) with uric acid also resulted in an increase in intracellular triglycerides.<sup>17,83</sup>

Additional studies identified potential mechanisms by which a fructose-induced rise in uric acid can stimulate hepatic lipogenesis. Firstly, fructose was found to induce mitochondrial oxidative stress that was mediated by uric acid-induced activation of NADPH oxidase, which translocated to mitochondria.<sup>17</sup> Mitochondria contain a large number of enzymes, but two in particular are known to be sensitive to oxidative stress, aconitase-2 (in the Krebs cycle) and enoyl CoA hydratase (involved in  $\beta$ -fatty acid oxidation).<sup>106,107</sup> Fructose and uric acid have been shown to reduce aconitase-2 activity, leading to an accumulation of citrate that moves into the cytoplasm and activates lipogenesis by stimulating ATP citrate lyase.<sup>17</sup> Choi *et al.*<sup>82</sup> showed that the initial oxidative stress in the mitochondria is caused by NADPH oxidase, but later mitochondrial oxidative stress is stimulated via the electron transport chain, leading to endoplasmic reticulum (ER) stress, the activation of sterol regulatory element binding transcription factor 1 (SREBP-1c), and further stimulation of lipogenesis via activation of acetyl CoA carboxylase-1 and FAS.<sup>83</sup> Others have also shown fructose-induced induction of the transcription factor, SREBP-1c,<sup>83,108–110</sup> as well as the carbohydrate responsive-element binding protein (ChREBP).<sup>33,111</sup> The stimulation of ChREBP results in the stimulation of glucose-6-phosphatase that may mediate some of the gluconeogenic effects of fructose.<sup>112</sup>

We also documented that fructose-induced uric acid can impair fatty acid oxidation. While mitochondrial oxidative stress may be partially responsible for lowering enoyl CoA hydratase-1 activity, we also found that the activity of this enzyme is regulated by AMP-activated protein

kinase (AMPK) and AMP Deaminase-2 (AMPD).<sup>16</sup> As mentioned, the rapid metabolism of fructose leads to intracellular phosphate, GTP and ATP depletion, with the stimulation of both AMPK and AMPD activity. However, the stimulation of AMPD tends to dominate, possibly by removing AMP substrate, but also by generating uric acid which feeds back to inhibit AMPK.<sup>16,113</sup> The combined effects of inhibition of AMPK, coupled with AMPD overactivity, result in an inhibition of enoyl A CoA hydratase and the accumulation of lipid,<sup>16</sup> as well as the stimulation of gluconeogenesis.<sup>113</sup>

This process of reducing AMPK and stimulating AMPD can also result in a reduction in hepatic intracellular ATP levels. Baseline resting ATP levels are low in diabetic individuals with NAFLD and fall further following fructose challenge.<sup>77</sup> Individuals with NAFLD who have a higher serum uric acid level show a greater fall in ATP levels following the same fructose challenge.<sup>49</sup> Thus, uric acid may have a role not only in stimulating lipogenesis and gluconeogenesis, but also in blocking fatty acid oxidation, leading to a relatively low hepatic ATP state.

Thus, these studies show that the lipogenic response to fructose is not a result of the metabolism of the fructose molecule itself, but rather from the general stimulation of lipogenesis and blocking of fatty acid oxidation. Thus, studies using labelled acetate document lipogenesis better<sup>65</sup> than those using labelled fructose.<sup>9,10</sup>

Soluble uric acid has also been shown to have other proinflammatory effects that could play a role in NAFLD, including the activation of the transcription factor NF- $\kappa$ B, stimulation of chemokines such as monocyte chemoattractant protein-1, and the stimulation of NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasomes.<sup>114,115</sup>

### Clinical studies

Epidemiological studies have also linked hyperuricemia with NAFLD in both adults<sup>11,116–122</sup> and children,<sup>117–119</sup> in both cross-sectional and longitudinal studies<sup>123,124</sup> (Table 1). A liver biopsy study also found that in individuals with NAFLD, the higher the serum uric acid the greater the NAFLD score, lobular inflammation and steatosis grade.<sup>122</sup> In addition, a meta-analysis found a dose-dependent rise in the incidence of NAFLD by 3% for every 1 mg/dl increase in serum uric acid, even after accounting for metabolic syndrome and other lifestyle factors.<sup>125</sup> Finally, a larger meta-analysis of 55,573 patients found an OR of 1.92 (1.59–2.31) for NAFLD occurrence when comparing the highest to lowest serum uric acid.<sup>126</sup> While most subjects with NAFLD are obese, NAFLD can also occur in subjects with normal and low BMI, and elevated uric acid is common in these individuals.<sup>17,127</sup>

Hyperuricemia is also associated with NASH, the intermediate stage and progressive form of

### Key point

A striking finding of experimental studies, is that xanthine oxidase inhibitors that block uric acid generation were able to reduce fatty liver caused by fructose.

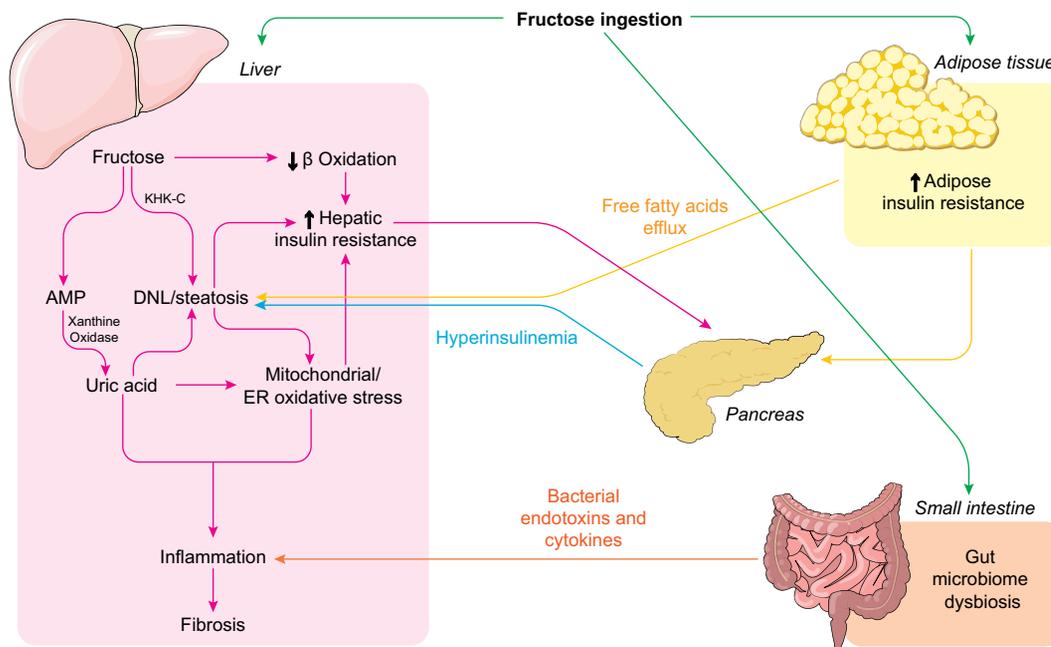
**Table 1. Studies with reported uric acid in NAFLD patients compared to non-NAFLD patients.**

Study	Uric acid (mg/dl) and NAFLD	p values
Ouyang X, et al. (2008), <i>Journal of Hepatology</i>	NAFLD 6.8 ± 1.6 vs. non-NAFLD 4.8 ± 0.9	p = 0.03
Sirota JC, et al. (2013), <i>Metabolism</i>	Non-NAFLD 5.09 ± 0.02 vs. mild NAFLD 5.19 ± 0.06 vs. moderate NAFLD 5.89 ± 0.06 vs. severe NAFLD 6.35 ± 0.10	p <0.0001
Liu J, et al. (2016), <i>Hepatol Res</i>	Obese hyperuricemia (>7.0 mg/dl males and >6.0 mg/dl females) OR 1.692 (1.371–2.087). Non-obese hyperuricemia 2.559 (1.870–3.503)	Not calculated
Liang J, et al. (2015), <i>Eur Rev Med Pharmacol Sci</i>	OR for NAFLD <sup>†</sup> : uric acid <3.7 OR 1; 3.7–<4.49 OR 1.53 (1.17–1.99); 4.49–<5.24 OR 2.22 (1.71–2.89); 5.24–<6.11 OR 2.64 (2.02–3.44); ≥6.11 OR 3.71 (2.83–4.88)	p <0.001
Ryu S, et al. (2011), <i>Metabolism</i>	Hyperuricemia (>7.0 mg/dl males only) OR 1.21 (1.07–1.38) for NAFLD <sup>‡</sup>	p = 0.004
Sartorio A, et al. (2007), <i>European Journal of Clinical Nutrition</i>	NAFLD 6.6 ± 1.7 vs. non-NAFLD 5.9 ± 1.6	p <0.0001
Sullivan JS, et al. (2015), <i>Pediatr Obes</i>	NAFLD 7.5 ± 1.4 vs. obese non-NAFLD 6.1 ± 1.6* vs. lean non-NAFLD 4.5 ± 1.6 <sup>#</sup>	*p = 0.04 #p = 0.0007
Li Y, Xu C, Yu C, Xu L, & Miao M (2009), <i>Journal of Hepatology</i>	NAFLD 6.2 ± 1.5 vs. non-NAFLD 5.4 ± 1.4	p <0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease.

<sup>†</sup>Model adjusted for sex, age, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL, LDL, log of triglycerides, Log AST, Log ALT.

<sup>‡</sup>Model adjusted for age, BMI, smoking, alcohol intake, exercise, total cholesterol, HDL, triglycerides, glucose, systolic blood pressure, insulin, hsCRP, and presence of metabolic syndrome.



**Fig. 3. Fructose mechanism mediating development and progression of NAFLD.** DNL, *de novo* lipogenesis; ER, endoplasmic reticulum; KHK-C, ketohexokinase-C; NAFLD, non-alcoholic fatty liver disease.

NAFLD. In a study of adolescents, hyperuricemia independently predicted the presence of NASH (OR 2.5 [1.87–2.83]) after adjusting for age, sex and other components of metabolic syndrome.<sup>51</sup> Hyperuricemia in males has also been found to associate more strongly with NASH than simple steatosis and was significantly associated with hepatocyte ballooning, BMI, and younger age in a multivariate analysis.<sup>128</sup>

One small randomised controlled study evaluated patients with ultrasound-diagnosed NAFLD to determine whether allopurinol treatment (n = 17) was superior to placebo (n = 14). They reported significant reductions in cytokeratin 18 (a marker of hepatic apoptosis and NASH<sup>129</sup>) (p = 0.006), lower alanine aminotransferase and aspartate

aminotransferase levels (p <0.001 and p = 0.013), and improved total cholesterol and triglycerides levels (p = 0.01 and p = 0.038) at three months.<sup>130</sup>

A diagram showing how fructose and its metabolite, uric acid, may play a role in NAFLD is shown (Fig. 3). This does not include the larger role uric acid likely plays in metabolic syndrome, including its effects on adipose tissue and islet cells.<sup>131</sup>

### Modulating factors

#### Factors that may exacerbate fructose-induced NAFLD

High-fat diets may also contribute to fatty liver. Indeed, when fructose is combined with a high-

fat diet, much more severe fatty liver occurs in mice.<sup>132</sup> One potential explanation is that, like fructose, high-fat diets induce mitochondrial oxidative stress.<sup>133</sup> Nevertheless, mice lacking fructokinase show marked protection from fatty liver and insulin resistance, highlighting the key role of fructose in western diet-induced NAFLD.<sup>132</sup>

Alcohol ingestion is well known to induce fatty liver and chronic liver disease that can be histologically similar to NAFLD. Alcohol combined with fructose was associated with worsening metabolic features (hyperlipidaemia), although interestingly they did not appear to have a synergistic effect on inducing liver injury.<sup>134</sup>

As mentioned, high glycaemic diets can induce endogenous fructose production.<sup>44</sup> However, we have found that high-salt diets can also induce hepatic aldose reductase expression (because of effects on osmolarity) leading to endogenous fructose production and NAFLD in mice, and mice lacking fructokinase are protected (Lanaspa MA, manuscript under review). High-salt diets are independently associated with metabolic syndrome/diabetes<sup>135,136</sup> and NAFLD.<sup>137</sup> Thus, it seems likely that both high glycaemic diets and/or high-salt diets might exacerbate fructose-induced NAFLD.

In addition, genetic factors likely play a role in fructose-induced NAFLD. For instance, in Hispanic children who were homozygous for the patatin-like phospholipase domain containing protein 3 (*PNPLA3*) gene variant rs738409 a positive correlation was found between liver fat content and carbohydrate ( $r = 0.38$ ,  $p = 0.02$ ) and total sugar ( $r = 0.33$ ,  $p = 0.04$ ) intake.<sup>138</sup> A similar correlation between liver fat content and sugar-sweetened beverage intake was made in Italian adolescents with the same variant.<sup>139</sup> Notably, in 18 adult patients with NAFLD (matched for liver fat content), a six-day low-calorie, low-carbohydrate diet revealed reductions in liver fat content, but was 2.5-fold greater in those who were *PNPLA3* GG homozygotes ( $n = 8$ ) vs. CC homozygotes ( $n = 10$ ).<sup>140</sup> Less is known about interactions with other genes such as transmembrane 6 superfamily member 2 (*TM6SF2*) which regulates very low-density lipoprotein secretion and glucokinase regulatory gene (*GCKR*) that regulates the glycolytic pathway. Overall, further investigations are warranted in this area, but initial data suggest a role for genetic polymorphisms interacting with fructose in the pathogenesis of NAFLD.

#### Protective factors for fructose-induced NAFLD

Omega 3 fatty acids, such as those found in fish oils and in Mediterranean diets, may also protect against NAFLD.<sup>141</sup> For example, mice on a western diet (high-fat, high-fructose) were partially protected from developing NAFLD if their diet was supplemented with combined omega-3 fatty acids and flavanols.<sup>142</sup> Likewise, fish oil treatment was

found to improve hypertriglyceridemia and insulin resistance in fructose-fed macaques, although liver fat was not assessed in that study.<sup>143</sup> A short-term fructose feeding study in humans also suggested fish oil might blunt the development of hypertriglyceridemia and *de novo* lipogenesis.<sup>64</sup> Further studies investigating this pathway are needed.

Likewise, there is evidence that many substances found in natural fruits, such as flavanols, epicatechin, vitamin C and other antioxidants may also protect against fructose-induced metabolic syndrome.<sup>58,144,145</sup> This may explain why intake of natural fruits is not associated with NAFLD. Fruit juices, which are associated with metabolic syndrome, contain higher amounts of fructose and are often ingested rapidly, leading to higher fructose concentrations that cause greater ATP consumption and depletion.

#### Other amplifying mechanisms

##### *High sugar exposure may enhance the metabolic effects of fructose*

Repeated exposure to sugar is known to upregulate the transport of fructose through the GLUT5 transporter,<sup>41,146</sup> and also increase fructokinase levels in the liver.<sup>41</sup> Uptake of fructose is also enhanced by glucose.<sup>147</sup> Interestingly, this may increase the risk of fatty liver. A study by Sullivan *et al.* investigated the absorption of fructose in lean children, obese children, and obese children with biopsy proven NAFLD. While fructose malabsorption was common in lean children, it was less in obese children and children with NAFLD absorbed almost all of the oral fructose challenge.<sup>119</sup> In addition, blood fructose levels were lower in the obese children with NAFLD, suggesting they also metabolised the fructose more rapidly. Furthermore, Jin *et al.* reported that children with NAFLD experience a greater rise in serum triglycerides in response to fructose than lean controls.<sup>148</sup>

##### *Uric acid as an amplification mechanism*

Elevated serum uric acid levels may also function to amplify the effects of fructose by creating a positive feedback system. For example, uric acid may feedback to increase endogenous fructose production by stimulating AR<sup>149,150</sup> and may also stimulate fructose metabolism by increasing expression and activity of fructokinase.<sup>33</sup> In contrast, high concentrations of uric acid can block xanthine oxidase.<sup>151,152</sup> Thus, high concentrations of uric acid may act to stimulate the upstream metabolism of fructose, which will further promote purine metabolism end-products, including uric acid.

#### Limitations

While the evidence for fructose as a risk factor for NAFLD seems strong, large clinical trials are still

**Table 2. Studies comparing fructose compared to glucose or other isocaloric intake on NAFLD, insulin resistance and lipids.**

Study	Cohort	Intervention	NAFLD measurement	Liver enzymes	Insulin resistance	Lipids
Maersk M, <i>et al.</i> , 2012 <sup>54</sup>	47 overweight non-diabetic patients (30 female)	1 L/d SSB (10), isocaloric milk (12), diet cola (12), and water (13) for 6 months	132–143% increase in liver fat over other beverages	n.a.	n.a.	n.a.
Johnson RD, <i>et al.</i> , 2013 <sup>161</sup>	32 centrally overweight males age 18–50.	Overfeeding fructose (25%) (n = 15) vs. glucose (25%) (n = 17) 2 weeks	No difference	No difference	No difference HOMA-IR	No difference in triglycerides
Aeberli I, <i>et al.</i> , 2013 <sup>162</sup>	9 healthy normal weight males age 21–25.	3 weeks crossover of medium fructose (40 g/d) (MF), and high fructose (HF), high glucose (HG), and high sucrose (HS) beverage at (80 g/d)	n.a.	n.a.	Decreased hepatic insulin sensitivity in HF compared to HG	Increased total cholesterol and LDL in MF, HF, and HS, but not HG. Free fatty acids only elevated in MF
LeCoultrre V, <i>et al.</i> , 2013 <sup>163</sup>	55 normal weight males (mean age 22.5)	6–7 days on weight maintenance diet followed by 6–7 day 1.5 g/kg/d (n = 7), 3 g/kg/d (n = 17), or 4 g/kg/d fructose, 3 g/kg/d glucose (n = 11) or 30% saturated fats overfeed	Higher intrahepatic fat in 3 g/kg/d and 4 g/kg/d fructose, 3 g/kg/day glucose, and saturated fat compared to baseline, with tendency for higher levels in fructose diets	n.a.	4 g/kg/d fructose and 3 g/kg/d of glucose increased hepatic glucose production. 4 g/kg/d and 3 g/kg/d fructose decreased hepatic insulin sensitivity	n.a.
Silbernagel G, <i>et al.</i> , 2011 <sup>164</sup>	20 healthy normal weight (mean age 30.5) (12 males, 8 females)	4 weeks over feeding with 150 g fructose or glucose	No difference	No difference	No difference	Increased triglycerides in fructose compared to glucose
Stanhope K, <i>et al.</i> , 2009 <sup>65</sup>	32 patients (age 42–71) overweight or obese (25–35 kg/m <sup>2</sup> ) (16 female)	10-week trial with 25% glucose vs. 25% fructose added to diet; 2 weeks inpatient energy balanced diet, followed by 8-week <i>ad libitum</i> diet	Not assessed though visceral adipose tissue, a marker for liver fat, was significantly higher in fructose, but not glucose diet	n.a.	Fasting insulin, glucose, and decreased insulin sensitivity in fructose diet, but not glucose diet	Increased fasting triglycerides in glucose, not fructose, but higher post prandial triglycerides as well as fasting apoB, LDL, and oxidized LDL in fructose diet only
Schwarz JM, <i>et al.</i> , 2015 <sup>165</sup>	8 healthy males (age 18–65) with BMI <30 kg/m <sup>2</sup>	Cross over 9-day study on isocaloric weight maintaining diet of either 25% fructose or fructose portion substituted with complex carbohydrates	Significant increase in liver fat by 137% in fructose diet compared to complex carbohydrate diet	n.a.	Higher endogenous glucose production during hyperinsulinemia in high fructose vs. complex carbohydrate diet	Increased <i>de novo</i> lipogenesis in high fructose as compared to complex carbohydrate diet

NAFLD, non-alcoholic fatty liver disease.

### Key point

While there remains a lack of large clinical trials, the majority of evidence suggests that fructose worsens lipid profiles and insulin sensitivity, likely contributing to the development of NAFLD.

lacking. Nevertheless, there are some groups that have argued that fructose intake may not increase the risk of NAFLD, especially in short-term (four weeks or less) trials that compare fructose to isocaloric diets,<sup>153</sup> as well as in studies of fructose-associated hypercaloric diets.<sup>154</sup> However, the development of fatty liver with fructose takes months in animals<sup>85,132</sup> and most of the trials were probably too short to note this effect. Some of these trials are listed (Table 2), with the longest performed by Stanhope *et al.* and Maersk *et al.* at ten weeks and six months, respectively, showing differences in either visceral fat, or liver and visceral fat were higher in patients receiving a fructose diet than a glucose or other isocaloric beverage, despite similar changes in weight. Other studies noted are shorter, although the majority of them support fructose worsening lipid profiles or insulin sensitivity compared to glucose, providing

likely mechanisms for the development of NAFLD. Of note, studies funded by the food industry, and/or in which the authors are funded by the food industry, often fail to show a relationship between sugar intake and metabolic disease.<sup>155,156</sup>

Experimentally, the data that uric acid may have a role in NAFLD is countered by a report in which exogenously administered uric acid reversed hepatic steatosis since it can function as an antioxidant.<sup>157</sup> However, there may be differences between exogenous uric acid and intracellular uric acid in terms of its effects on oxidative stress.<sup>17,158</sup> Finally, there is still much to learn about fructose metabolism that is not well understood. For example, fructose is now known to stimulate FGF21, which may counter some of the negative effects of fructose on craving, metabolic syndrome and liver disease.<sup>159,160</sup> Identifying how this factor modulates fructose responses is clearly of major interest.

## Conclusions

In summary, there has been a marked rise in sugar and HFCS intake that has paralleled the rise of NAFLD. Experimentally, the fructose component of sugar and HFCS appears to have a major role in inducing fatty liver by both stimulating *de novo* lipogenesis and blocking  $\beta$ -fatty acid oxidation. Evidence suggests these effects are caused by the unique metabolism of fructose by fructokinase that leads to a fall in ATP with nucleotide turnover and uric acid generation. The prooxidative and proinflammatory effects of uric acid lead to increases in gut permeability and endotoxemia that exacerbate the lipogenic process in the liver, which coupled with mitochondrial dysfunction, result in NAFLD. Clinically the intake of sugar-sweetened beverages is strongly linked with NAFLD. Reducing sugar or HFCS intake may have major benefits for patients with NAFLD. Clinical studies to investigate the potential benefit of lowering uric acid should also be performed. While there are many causes of NAFLD, the intake of fructose-containing sugars is likely to play a major role.

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## Conflict of interest

RJJ and ML disclose they are inventors on patents related to blocking fructose metabolism as a means to reduce sugar craving and metabolic syndrome. RJJ, LGL, DRT, and ML also have equity in Colorado Research Partners LLC, which is a startup company interested in developing novel fructokinase inhibitors. Dr Johnson has also received honoraria from Danone, Astra Zeneca and is on the Scientific Board of Kibow, Inc. RJJ, ML and TJ have submitted a patent for V1b antagonists for the treatment of IR and fatty liver disease. LGS-L receives research support from Danone Research and Kibow Biotech, Inc. MK, MG, CR, YS, KJN, DHK SS, KJN, MFA, AMD, HRR do not have any relevant disclosures.

Please refer to the accompanying [ICMJE disclosure](#) forms for further details.

## Authors' contributions

TJ and RJJ provided the main work of reviewing literature, formatting, the paper, and creation of table/figures along with editing the paper. MFA, SS, KJN, MG, AMD, CR, DHK, TN, LGS, MK, YS, HRR, and LGS-L all provided editorial feedback on the paper for additions and grammatical corrections.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jhep.2018.01.019>.

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