Fructose drives de novo lipogenesis affecting metabolic health

Geidl-Flueck, Bettina ; Gerber, Philipp A

Abstract: Despite the existence of numerous studies supporting a pathological link between fructose consumption and the development of the metabolic syndrome and its sequelae, such as non-alcoholic fatty liver disease (NAFLD), this link remains a contentious issue. With this article, we shed a light on the impact of sugar/fructose intake on hepatic de novo lipogenesis (DNL), an outcome parameter known to be dysregulated in subjects with type 2 diabetes and/or NAFLD. In this review, we present findings from human intervention studies using physiological doses of sugar as well as mechanistic animal studies. There is evidence from both human and animal studies that fructose is a more potent inducer of hepatic lipogenesis than glucose. This is most likely due to the liver's prominent physiological role in fructose metabolism, which may be disrupted under pathological conditions by increased hepatic expression of fructolytic and lipogenic enzymes. Increased DNL may not only contribute to ectopic fat deposition (i.e., in the liver), but it may also impair several metabolic processes through DNL-related fatty acids (e.g., beta-cell function, insulin secretion, or insulin sensitivity).

DOI: https://doi.org/10.1530/JOE-22-0270

Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

Fructose drives de novo lipogenesis affecting metabolic health

Bettina Geidl-Flueck and Philipp A. Gerber

Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital Zurich (USZ) and University of Zurich (UZH), Switzerland.

Corresponding author:
Philipp A. Gerber
Department of Endocrinology, Diabetology, and Clinical Nutrition
University Hospital Zurich
Ramistrasse 100
CH - 8091 Zurich
Switzerland
Email: philipp.gerber@usz.ch

Short title: Fructose and de novo lipogenesis

Key words: sugar, glucose, fructose, de novo lipogenesis, fatty acids

Word count: Abstract: 176, Main text: 4392

Abstract

Despite the existence of numerous studies supporting a pathological link between fructose consumption and the development of the metabolic syndrome and its sequelae, such as non-alcoholic fatty liver disease (NAFLD), this link remains a contentious issue. With this article, we shed a light on the impact of sugar/fructose intake on hepatic de novo lipogenesis (DNL), an outcome parameter known to be dysregulated in subjects with type 2 diabetes and/or NAFLD. In this review, we present findings from human intervention studies using physiological doses of sugar as well as mechanistic animal studies. There is evidence from both human and animal studies that fructose is a more potent inducer of hepatic lipogenesis than glucose. This is most likely due to the liver's prominent physiological role in fructose metabolism, which may be disrupted under pathological conditions by increased hepatic expression of fructolytic and lipogenic enzymes. Increased DNL may not only contribute to ectopic fat deposition (i.e., in the liver), but it may also impair several metabolic processes through DNL-related fatty acids (e.g., beta-cell function, insulin secretion, or insulin sensitivity).

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

Introduction

Metabolic health is at risk in societies with an excess supply of energy-dense palatable food and drinks, and an everyday life with low physical activity. There is a global epidemic of metabolic syndrome (Saklayen 2018), which includes obesity (particularly visceral adipose tissue accumulation), dyslipidemia, impaired glucose tolerance, and hypertension. Importantly, this syndrome not only affects adults but also children and adolescents, in particular in developing countries (Noubiap, et al. 2022). Similarly, the prevalence of non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome, is increasing (Moore 2010; Riazi, et al. 2022; Sahota, et al. 2020). The metabolic syndrome, with all of its associated comorbidities, not only burdens the affected individual, but also the public health care system (Boudreau, et al. 2009).

It is commonly acknowledged that an increased body weight, associated with a positive energy balance, is a major trigger for the development of metabolic diseases. It is assumed, however, that factors other than an imbalanced energy intake and expenditure can influence metabolic health. A well-balanced macronutrient intake, characterized by a moderate fat and carbohydrate intake, with a focus on sugar restriction, is regarded as an important component of a healthy diet. A high intake of added sugars, and in particular of fructose – which is often present in a typical western diet – is considered to be a principal factor promoting metabolic derangements (Jensen, et al. 2018; Lim, et al. 2010). Despite numerous studies, it is still debated whether the metabolic effects of added sugars are mediated by excess energy intake/weight gain or whether fructose and glucose affect metabolism differently and independently of excess caloric intake. This review aims to shed a light on the current literature regarding this question.

Sugar consumption and its effects

Current recommendations

To reduce the risk of developing obesity and metabolic diseases, the World Health Organization recommends that adults and children consume less than 10% (preferably less than 5%) of their energy needs from free sugar (Organization 2015). Importantly, free sugars include monosaccharides, and disaccharides added to food and beverages as well as sugars naturally present in honey, syrups, fruit juices, and fruit juice concentrates. Recent studies on sugar intake in Europe, Latin America, and the United States found that mean sugar intakes in most countries were higher than the recommended intake (DiFrancesco, et al. 2022; Fisberg, et al. 2018; Löwik 2021). As a consequence, measures to reduce sugar intakes such as better food labeling or taxes on sweetened food are discussed or already implemented in many countries.

Dietary Glucose and Fructose

Glucose and fructose are stereoisomers. Fructose displays a higher sweetening power compared to glucose (Moskowitz 1970). Fructose and glucose occur naturally as monosaccharides in fruits and honey but also as sucrose (a disaccharide consisting of glucose and fructose). Other sugar sources include table sugar (sucrose) or high-fructose corn syrup (a mixture of fructose and glucose), concentrated fruit juices, agave or maple syrup, and so on. Sugar added to food and beverages as sweeteners are termed ”added sugars.” Importantly, the digestion/absorption of sugar from fruits is much slower than that of beverages and thus is unlikely to be associated with any negative effects. Unfavorable metabolic effects are particularly induced by beverages containing high amounts of free sugar that are rapidly absorbed, as detailed below. High fructose corn syrup (HFCS) is manufactured industrially

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

from corn starch through the isomerization of glucose to fructose. The proportion of fructose varies between 42 and 90% in HFCS (Serna-Saldivar 2016). HFCS with 42% fructose is widely used as a sweetener in processed foods, whereas HFCS with 55% fructose is commonly used in beverage production (Kay Parker 2010). HFCS was first introduced to the market in the US in the 1970s, and it is now a significant US export product, particularly to developing countries. The average fructose intake increased since the 70s in the US (Tappy and Lê 2010). High fructose corn syrup is a cheap sweetener used in the food and beverage industries, and its consumption is linked to the occurrence of type 2 diabetes (Kmietowicz 2012) and other metabolic diseases, as described below.

Sugar-sweetened beverage consumption is a risk factor for cardiometabolic diseases

A major source of added sugars are sugar-sweetened beverages (SSB) (Johnson, et al. 2009; Malik and Hu 2022). Their consumption has been linked not only to the development of obesity but also to its complications such as type 2 diabetes, NAFLD, and cardiovascular disease (Malik and Hu 2022). Prospective cohort studies from the United States and the United Kingdom found an association between high SSB consumption and an increased risk of type 2 diabetes, independently of obesity (Imamura, et al. 2015). Similarly, studies confirmed that habitual SSB consumption is associated with a dose-dependent increase in the risk of dyslipidemia and coronary heart disease (Te Morenga, et al. 2014; Yin, et al. 2021). Importantly studies showed that habitual SSB consumption has a dose-dependent effect on the risk of NAFLD (Chen, et al. 2019; Ouyang, et al. 2008) and that SSB intake in early childhood is associated with the later development of hepatic steatosis in adulthood (Sekkarie, et al. 2021). In addition to metabolic abnormalities, there is evidence of a link between SSB consumption and breast cancer, pancreatic and prostate cancer, and colorectal cancer (Malik and Hu 2022).

Worldwide, SSB intake is still rising (Malik and Hu 2022; Singh, et al. 2015). However, regional differences regarding SSB consumption are striking. Overall, SSB intake is highest in men and women in Latin America and the Caribbean (average SSB intake about 325g/day), where it has been rising for decades. In contrast, SSB intake in western high-income countries has stabilized since the 1990s at around 150-200g/day (Malik and Hu 2022). In Asian countries, SSB consumption is remarkably low (the average intake of SSB is about 30g/day). Given these data on global SSB consumption, the global burden of obesity and chronic diseases for societies is likely to rise further, particularly in developing countries.

Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

A specific role for fructose in the etiology of cardiometabolic diseases?

Differences between fructose and glucose metabolism

Although high sugar consumption is recognized as a risk factor for cardiometabolic diseases, the debate over whether the fructose component of consumed sugar plays a specific role in the etiology of such diseases is still ongoing. This question cannot be easily assessed by epidemiologic studies as fructose is rarely ingested in a pure form, but mostly co-ingested with glucose.

There are important differences regarding the cellular absorption and distribution of glucose and fructose (Maruhama and Macdonald 1973). Fructose is primarily absorbed via facilitated diffusion via glucose transporter 5 (GLUT5) (Burant, et al. 1992), which is expressed on epithelial intestinal cells, whereas glucose is absorbed via sodium-glucose-cotransporter 1 (SGLT1), an active transporter (Gorboulev, et al. 2012). A proportion of fructose is directly metabolized into glucose in enterocytes. However, when large amounts of fructose are consumed (for example, when consuming SSB), fructose spills over to the liver and large intestine (Jang, et al. 2018) (Figure 1). Fructose and glucose enter the circulation via GLUT5 and GLUT2, respectively (Koepsell 2020). Following that, the liver, which is the primary site of fructose metabolism, extracts a large portion of it (Mendeloff and Weichselbaum 1953). However, it can also be metabolized by the kidney, skeletal muscle, and adipose tissue. Hesley et al. provided a thorough review of tissue-specific fructose metabolism (Helsley, et al. 2020). In contrast, glucose is taken up and metabolized by most mammalian tissues (Thorens and Mueckler 2010). The majority of glucose is taken up by the liver and muscle and stored as glycogen, processes that require insulin. Further amounts of glucose are metabolized by the brain, adipose tissue, and the kidney (Gerich 2000). Following cellular uptake, fructose, and glucose are phosphorylated at different rates by specific kinases.

Fructokinase is expressed as the two isoforms ketohexokinase-A and C. KHK-C is primarily expressed in the liver, but it is also found in the kidney and intestines, whereas KHK-A is more widely expressed (Diggle, et al. 2009). KHK-C drives hepatic fructose uptake by phosphorylating fructose at a very high rate without feedback inhibition, resulting in a flux of fructose toward the liver (Ishimoto, et al. 2012) (Figure 1). Glucose is phosphorylated by glucokinase (GK). Importantly, the phosphorylation rate by KHK is 10 times higher than by GK. Phosphorylated fructose is cleaved into trioses and enters the glycolytic pathway. Fructose is mainly metabolized into lactic acid, converted to glucose or hepatic glycogen and lipids (Chong, et al. 2007; Parks, et al. 2008). Notably, fructose absorption is increased when it is co-ingested with glucose (Rumessen and Gudmand-Høyer 1986). Furthermore, animal studies have shown that consuming high amounts of fructose increases the expression of fructolytic and gluconeogenic enzymes and expands the intestinal cell surface, which improves nutrient absorption (Patel, et al. 2015a; Taylor, et al. 2021).

Metabolic effects of regular sugar/fructose intake

Traditionally, easily measurable outcome parameters of known clinical significance (cardiovascular risk markers) such as fasting glucose, insulin, c-peptide, insulin sensitivity/resistance, or serum lipids are measured for the risk assessment of dietary products regarding metabolic health. However, when metabolic health is defined just as the presence of ideal levels of these markers, fine metabolic changes may be missed. As a result, studies used more subtle outcome parameters to investigate how moderate sugar intake affects the metabolism of healthy men. Indeed, they provide evidence that consumption of SSB containing fructose in moderate amounts leads to metabolic derangements such as decreased hepatic insulin sensitivity (reflected by impaired suppression of glucose production during euglycemic-hyperinsulinemic clamps) (Aeberli, et al. 2013), induces a shift toward a more

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

atherogenic LDL subclass distribution (Aeberli, et al. 2011) in healthy men, or increases hepatic lipogenic activity (Geidl-Flueck, et al. 2021).

The latter, an increased de novo lipogenesis, is supposed to be linked to various metabolic complications/perturbations. As a result, the following section focuses on metabolic interactions between dietary sugars, specifically fructose, and DNL.

De novo lipogenesis (DNL) in health and disease

De novo lipogenesis (DNL) converts excess dietary CHO into fatty acids (FA). FA are formed during this process from acetyl-CoA molecules generated directly from CHO catabolism (i. e. glycolysis or fructolysis) or acetate generated by microbiota fructose fermentation (Zhao, et al. 2020). DNL necessitates the expression of lipogenic pathway enzymes by various cell types, particularly white adipocytes, and hepatocytes. DNL is contributing to the maintenance of glucose homeostasis. A healthy balance of hepatocyte and adipocyte DNL is essential for maintaining systemic insulin sensitivity (Song, et al. 2018). The master transcription factors sterol-responsive element binding protein (SREBP) induced by CHO intake/insulin signaling and carbohydrate responsive element binding protein (ChREBP) stimulated by CHO intake regulate the expression of lipogenic enzymes. DNL provides FA for the structural maintenance of the cells, allows storage of energy from CHO beyond the glycogen store (thus contributing to glucose homeostasis), and regulates FA oxidation.

The process of fatty acid synthesis in the liver has been identified as being of particular interest in the etiology of the metabolic syndrome, as well as a specific feature of NAFLD (Donnelly, et al. 2005; Imamura, et al. 2020; Lambert, et al. 2014). Clinical studies showed that DNL is increased in subjects with increased hepatic fat content (isotope approaches) (Diraison, et al. 2003; Lambert et al. 2014). Furthermore, DNL was found to be positively

199 related to intrahepatic TAG levels (Diraison et al. 2003; Lambert et al. 2014), and negatively
200 related to hepatic and whole-body insulin sensitivity (Smith, et al. 2020). DNL is supposed to
201 increase intrahepatic fat both by providing FA for TAG synthesis and inhibiting FA oxidation
202 promoting the re-esterification process. Importantly, accumulating intermediates (i. e.,
203 malonyl-CoA) inhibit FA import into the mitochondria and thus FA oxidation (Cox, et al.
204 2012; McGarry, et al. 1977). Furthermore, a clinical study (cross-over) showed that an
205 increase in DNL induced by a diet high in simple sugars correlates with triglyceridemia both
206 in lean and obese subjects (Hudgins, et al. 2000). In addition, increased concentrations of
207 DNL-related fatty acids (i. e. palmitate 16:0) have been linked to the metabolic syndrome in
208 observational and interventional studies (Vessby 2003). Mechanistic *in vitro* studies suggest
209 that palmitate impairs beta-cell function via ceramide formation, causing endoplasmic
210 reticulum stress, and induces the apoptotic mitochondrial pathway (Cunha, et al. 2008;
211 Maedler, et al. 2001; Maedler, et al. 2003). Other studies revealed that palmitate stimulates
212 interleukin-6 expression, a mechanism involved in the pathogenesis of insulin resistance and
213 vascular inflammation (Korbecki and Bajdak-Rusinek 2019; Rotter, et al. 2003; Staiger, et al.
214 2004; Testa, et al. 2006; Weigert, et al. 2004). Therefore, from a clinical perspective, DNL
215 may serve as a valuable marker for the development of cardiometabolic disease beyond
216 hepatic lipid accumulation / NAFLD.
217
218 ***The impact of macronutrients on DNL - insights from human intervention studies***
219 Regarding the question of how different macronutrients impact metabolic health, early human
220 studies compared the effects of diets with different carbohydrate and fat intake on metabolic
221 outcomes. Later, the effects of different forms of carbohydrates were compared (e.g., simple
222 sugars vs. complex carbohydrates or different types of sugar) in studies with children or
223 adults, with or without obesity / metabolic disease. Interventions aimed at increasing

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

sugar/fructose consumption, e.g. by SSB intake or decreasing sugar /fructose intake by prescription of sugar/fructose restriction (Donnelly et al. 2005; Lambert et al. 2014). Finally, they all contribute to the understanding of the relationship between CHO intake and metabolic complications in general, as well as the relative importance of fructose and glucose. Importantly, studies on the effects of sugar consumption on DNL are rarely comparable due to significant differences in the study populations, interventions, and/or methods used. (Studies discussed below are summarized in Table 1).

Of note, the process of hepatic DNL is assessed by applying different methods that all analyze FA bound to very low-density lipoproteins (VLDL). They range from calculating fatty acid desaturation indices to calculating the percentage of surrogate FA for newly formed FA (i. e., palmitate) in total FA to labeling newly formed FA with isotopes to calculate fractional DNL or fractional secretion rates of de novo synthesized FA (Hellerstein, et al. 1991). Measurement of DNL by isotope labeling methodology is considered the gold standard. However, it is costly and thus only appropriate for studies with small sample sizes. Initially, it was assessed by Hudgins et al. how the fat and CHO content of a diet impacts hepatic DNL in healthy men. Subjects were randomly assigned to either an eucaloric liquid high-fat diet (40% of calories as fat and 45% as glucose polymers, n=3) or a high-CHO diet (10% of calories as fat and 75% as glucose polymers, n=7) for 25 days. DNL was increased in men on a high CHO diet after 10 days, reflected as palmitate-enriched, linoleate-deficient VLDL triglycerides, and palmitate synthesis (mass isotopomer distribution analysis (MIDA) of palmitate labeled with 13C-acetate) was increased after 25 days compared to the high-fat diet (Hudgins, et al. 1996).

In a later study, Schwarz et al. compared the effects of a high-fructose (25% energy content), weight-maintenance diet to those of an isocaloric diet with the same macronutrient distribution but complex carbohydrates (CCHO) substituted for fructose (cross-over design,

n=8). Importantly, fructose was provided as beverages, whereas complex carbohydrates were provided as solid food. After 9 days of intervention, high fructose intake was associated with higher fractional hepatic DNL (MIDA of palmitate labeled with 13C-acetate) compared to the diet in which fructose was replaced by CCHO (Schwarz, et al. 2015). Stanhope et al. investigated the effects of glucose and fructose consumption on hepatic DNL in obese subjects after 10 weeks of consumption of glucose- or fructose-sweetened beverages providing 25% of energy requirements. Postprandial DNL was increased after fructose consumption (mass isotopomer distribution analysis (MIDA) of palmitate labeled with 13C-acetate) (Stanhope, et al. 2009).

The effects of different hexoses on hepatic DNL were investigated by Parks et al. Healthy subjects (n=6) were challenged with sweetened beverages (85g sugar) containing pure glucose (100:0), or mixtures of fructose and glucose (50:50 or 75:25) on three separate occasions in a random and blinded order. The beverages containing fructose stimulated DNL more potently compared with the beverages containing pure glucose (MIDA of palmitate labeled with 13C-acetate) (Parks et al. 2008).

Aside from the postprandial effect of fructose consumption on DNL which has been studied extensively, the effect of regular fructose consumption on basal hepatic lipogenic activity is of interest. Formation of new FA requires both the expression of lipogenic enzymes and the availability of substrate (acetyl-CoA). Fatty acid synthesis, as measured by a constant infusion of glucose (as a substrate for FA synthesis) and 13C- acetate, reflects hepatic lipogenic activity, which is determined by lipogenic enzyme expression. Thus, in such a setting differences regarding absorption rates of different sugar types do not influence the measurement. The effect of daily sugar-sweetened beverage consumption on liver lipogenic activity was studied in 94 healthy men by providing daily glucose, fructose, or sucrose-containing drinks (3x 2dl / 80g sugar intake per day) in a randomized way during 6 weeks.

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

The study with sugar sweetened beverage consumption in a close to real life setting showed that fructose and sucrose, but not glucose, increased the basal lipogenic activity of the liver (MIDA of palmitate labeled with 13C-acetate) (n=94, RCT) as compared to a control group. This is most likely due to fructose-containing beverages causing an increase in the expression of lipogenic genes in the liver (Geidl-Flueck et al. 2021).

Further studies assessed and clarified the role of DNL in fructose-induced hypertriglyceridemia and whether physical activity prevents hypertriglyceridemia. Egli et al examined healthy subjects (n=8) after 4 days of either a weight-maintaining low fructose diet (control), a high fructose diet with low physical activity, or a high fructose diet with high physical activity. Fasting and postprandial TAG as well as 13C-palmitate in triglyceride-rich lipoproteins were increased after a high fructose diet compared to control after an oral challenge with 13C-fructose. Those parameters remained unchanged after the high fructose/high physical activity intervention indicating that sport protects against fructose-induced triglyceridemia. The underlying mechanism induced by physical activity (i. e., reduced DNL from fructose or improved TAG clearance) was not resolved by this study. The same authors also tested the hypothesis that exercise prevents a fructose-induced rise in VLDL triglycerides (VLDL-TGs) by decreasing fructose conversion into glucose and VLDL-TGs and fructose carbon storage into hepatic glycogen and lipids (Egli, et al. 2015). Eight healthy men were placed on a weight-maintenance high-fructose diet (SSB) for 4 days before the metabolic fate of 13C-labeled fructose with or without physical activity was investigated. Exercise increased fructose oxidation. However, it did not abolish fructose conversion into glucose or did not prevent DNL (AUC of VLDL-13C palmitate). These findings imply that fructose-induced DNL occurs regardless of the degree of saturation of other fructose metabolism pathways.

So far studies were discussed that assessed the effect of increased CHO/sugar/fructose consumption on DNL. Overall, findings from various clinical studies indicate that carbohydrates, particularly when consumed as simple sugars and in liquid form, promote hepatic lipogenesis even when maintenance dietary interventions are used. Furthermore, studies using fructose, and glucose interventions revealed that fructose is a more potent inducer of hepatic lipogenesis than glucose.

In addition to these findings, some studies deal with the question of how a reduction/restriction of sugar/fructose consumption impacts de novo lipogenesis.

There is evidence that a general dietary sugar restriction (which also leads to a reduction in fructose intake) results in lower DNL. A link between free sugar consumption and DNL was confirmed by Cohen et al. who conducted a trial with adolescent boys suffering from NAFLD. A low-sugar diet for eight weeks reduced DNL (and hepatic fat content) compared to their usual diet, as measured by a lower percentage of newly synthesized palmitate in plasma TAG (labeled with deuterated 2H20) (Cohen, et al. 2021) (n=29, RCT). Similarly, Schwarz et al. demonstrated in a study with obese children (Schwarz, et al. 2017) that restricting sugar/fructose intake for 9 days reduced hepatic DNL (fractional DNL after a test meal containing 13C-acetate) (n=41). In this study, dietary sugars were substituted by complex carbohydrates.

Both intervention studies that increased sugar/fructose intake and those that reduced fructose intake provide evidence that sugar/fructose intake influences hepatic DNL. Importantly, the few studies that specifically assessed the effects of different hexoses (i.e., glucose and fructose) support the hypothesis that fructose is a more potent inducer of lipogenesis than glucose (Geidl-Flueck et al. 2021; Parks et al. 2008).

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

Fructose vs glucose metabolism – mechanistic insights from animal studies

Insights into mechanisms underlying the differences in glucose and fructose metabolism were gained from animal studies (Geidl-Flueck and Gerber 2017; Maruhama and Macdonald 1973). Several important transcription factors control carbohydrate metabolism. We focus on the role of ChREBP (Yamashita, et al. 2001) and SREBP (Wang, et al. 1994) in the regulation of CHO flux. They regulate glycolytic and fructolytic gene expression, as well as the expression of lipogenic genes. Glucose and fructose, to varying degrees, stimulate their expression, and activity. Importantly, the expression of both transcription factors is increased in the livers of NAFLD patients (Benhamed, et al. 2012; Kohjima, et al. 2007).

ChREBP is most strongly expressed in the liver, white and brown adipose tissue, and also the small intestine and muscle (Iizuka, et al. 2004). Lipogenic enzyme expression is reduced in mice with a genetic deletion of the ChREBP transcription factor (Iizuka et al. 2004). They display an impaired glucose tolerance as a consequence of reduced glucose disposal. ChREBP deletion shifts the flux from excess CHO to glycogen storage. It increases glycogen content in the liver and reduces the hepatic fat content. ChREBP-knock-out animals are fructose intolerant due to decreased expression of fructolytic and lipogenic enzymes, resulting in death when fed high sugar diets. Liver-specific knock-out of ChREBP in mice (L-ChREBP-/-) results in reduced SREBP1c at RNA and protein levels suggesting that both transcription factors co-ordinately regulate lipogenic gene expression (Linden, et al. 2018). Feeding studies revealed that fructose induces hepatic ChREBP and its targets more potently than glucose (Kim, et al. 2016; Koo, et al. 2009; Softic, et al. 2016; Softic, et al. 2017). Further, it is also activated by glycerol that is generated during fructolysis. As a result, ChREBP activation is thought to be related to hexose- and triose-phosphate levels (Kim et al. 2016).

SREBP is expressed in different isoforms. SREBP-1c induces lipogenic gene expression in response to carbohydrate feeding. SREBP1c mRNA expression is regulated by the TOR signaling pathway and the insulin signaling pathway. For full induction of SREBP-1c expression as well as for its translocation to the nucleus, hepatic insulin signaling is required. (Haas, et al. 2012). In mice, a high fructose diet induces SREBP-1c expression more potently than a standard chow diet.

Furthermore, mechanistic studies provided evidence that fructose reduces hepatic fatty acid oxidation by different mechanisms. One early *in vitro* study found that fructose, as a competing substrate for oxidation, inhibits long-chain fatty acid oxidation (Prager and Ontko 1976). A further study showed that fructose feeding reduces the expression of peroxisome proliferator-activated receptor and fatty acid oxidation enzymes (Nagai, et al. 2002). Furthermore, fructose feeding raises malonyl-CoA levels (which inhibits transport of FA by CPT1a into the mitochondria), causes mitochondrial dysfunction (reduced mitochondrial size and protein mass, specifically fatty acid oxidation pathway proteins and CPT1a levels), and increases acetylation of mitochondrial proteins in mice (Softic, et al. 2019).

The levels of expression of fructolytic pathway enzymes determine the relative contribution of tissues to fructose metabolism. Ketohexose-C (KHK-C) is considered to be a key enzyme in fructose metabolism phosphorylating fructose at a high rate as described above. KHK-C is highly expressed in hepatocytes (Diggle et al. 2009), but it is also found in the intestine, adipose tissue, kidney, and pancreas (Ishimoto et al. 2012). KHK-C knock-out mice fail to metabolize fructose leading to high fructose concentrations in the blood and urine (Patel, et al. 2015b). Both KHK-C deletion and KHK-C blockade protect against fructose-induced metabolic perturbations (Lanaspa, et al. 2018; Patel et al. 2015b; Softic et al. 2019). Deletion of the KHK-A isoform exacerbates fructose-induced metabolic syndrome probably due to an increased fructose supply to the liver (Ishimoto et al. 2012).

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

371 Clinical studies show that patients with NAFLD have increased expression of KHK-C in the
372 liver (Ouyang et al. 2008) and that inhibiting KHK-C reduces liver fat in NAFLD (Kazierad,
373 et al. 2021).

Possible mechanisms by which sugar/fructose consumption impacts fat distribution/deposition

Ectopic fat deposition is linked to metabolic syndrome and NAFLD and is thought to be exacerbated by a high sugar intake (Ma, et al. 2016). It is suggested that lipid deposition is promoted by CHO-induced DNL that reduces FA oxidation and by alterations of FA flux. A meta-analysis of randomized controlled trials demonstrated that high sugar (fructose or sucrose) hypercaloric diets increased liver and muscle fat in comparison to eucaloric control diets (Ma et al. 2016). Of course, data from studies that used "close to real life interventions" with high but not excessive sugar intake would provide the most relevant information about the effects of sugar consumption on fat distribution in individuals. A study by Maerks et al. compared the effects of SSB (1l) containing sucrose to those of isocaloric milk and a non-caloric soft drink on ectopic fat deposition. Consumption of sucrose-containing SSB for 6 months increases not only hepatic fat content but also muscle and visceral fat in obese subjects, whereas no such effects were observed in the other groups (Maersk, et al. 2012). However, studies that specifically compare the impact of different types of sugars on fat distribution are scarce (Lecoultre, et al. 2013). Stanhope et al. compared the effects of fructose and glucose-sweetened beverages on body fat distribution in subjects with obesity by quantification of subcutaneous, visceral, and abdominal fat. Consumption of fructose, but not glucose-sweetened beverages (providing 25% of energy requirements) for 10 weeks significantly increased visceral abdominal fat (Stanhope et al. 2009). In contrast, glucose consumption increased subcutaneous fat. Data about a fat deposition in the liver and muscle were not collected. In a later study, Schwarz et al. used magnetic resonance spectroscopy to investigate the effects of a high-fructose weight-maintenance diet on liver fat. They discovered that 9 days of a high fructose diet (25% energy content) increased both liver fat

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

and DNL (Schwarz et al. 2015). Different mechanisms underlying fat deposition have been suggested that implicate fructose. It is hypothesized that fructose consumption reduces fatty acid oxidation more than glucose consumption and that fructose consumption raises cortisol levels, promoting visceral adiposity, and/or lipid deposition in the liver. Cox et al. investigated the effects of SSB consumption on substrate utilization and energy expenditure in subjects with obesity. They found that the intake of fructose, but not glucose, reduced resting energy expenditure and postprandial fat oxidation while increasing postprandial carbohydrate oxidation. This finding suggests that lipid deposition may result from sparing FA from oxidation. DiNicolantonio et al. proposed that fructose plays a specific role in visceral fat deposition via glucocorticoid-mediated mechanisms (DiNicolantonio, et al. 2018). Visceral fat is known to accumulate under pathological conditions where cortisol levels are increased, such as Cushing's syndrome. Fructose consumption is thought to raise cortisol levels by promoting inflammatory processes in adipose tissue and stimulating the hypothalamus, resulting in the release of corticotropin-releasing factor. Cortisol increases the flux of FA from subcutaneous adipose tissue to visceral fat depots, impairing organ function (DiNicolantonio et al. 2018) and leading to an unfavorable fat distribution in lean individuals, i.e. a body shape described as thin outside, fat inside (TOFI), which is associated with an increased risk for the metabolic syndrome (DiNicolantonio et al. 2018). Taken together, studies provide evidence that fructose and sucrose consumption promote ectopic fat deposition associated with an increased risk for metabolic disease and cardiovascular events (Gruzdeva, et al. 2018). This is most likely due to a simultaneous increase in DNL and decrease in FA oxidation, but it could also be due to increased FA flux from subcutaneous adipose tissue to other tissues (visceral fat and the liver).

Conclusions

A high intake of free sugar as SSB increases the risk of obesity, cardiometabolic diseases, and NAFLD. A central role must be attributed to fructose in the development of these diseases. It is not only a strong inducer of DNL, but it is also a known cause of ectopic fat deposition by reducing fat oxidation and increasing FA flux to visceral fat and the liver. Most importantly, fructose-specific effects occur independently from overfeeding in healthy subjects. There are several mechanisms by which high fructose consumers increase fructose absorption and catabolism in the liver, exacerbating the metabolic effects. Sugar/fructose consumption should be reduced to avoid these unfavorable metabolic adaptations.

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

Declaration of interest: The authors declare no conflict of interests regarding this work.

Funding: This work was supported by the Heuberg foundation.

Author contributions statements: Bettina Geidl-Flueck and Philipp Gerber wrote and revised the manuscript.

References

Aeberli I, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, Berthold HK, Spinas GA & Berneis K 2011 Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. Am J Clin Nutr 94 479-485.

Aeberli I, Hochuli M, Gerber PA, Sze L, Murer SB, Tappy L, Spinas GA & Berneis K 2013 Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: a randomized controlled trial. Diabetes Care 36 150-156.

Benhamed F, Denechaud PD, Lemoine M, Robichon C, Moldes M, Bertrand-Michel J, Ratziu V, Serfaty L, Housset C, Capeau J, Girard J, Guillou H & Postic C 2012 The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. The Journal of clinical investigation 122 2176-2194.

Boudreau DM, Malone DC, Raebel MA, Fishman PA, Nichols GA, Feldstein AC, Boscoe AN, Ben-Joseph RH, Magid DJ & Okamoto LJ 2009 Health care utilization and costs by metabolic syndrome risk factors. Metab Syndr Relat Disord 7 305-314.

Burant CF, Takeda J, Brot-Laroche E, Bell GI & Davidson NO 1992 Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem 267 14523-14526.

Chen H, Wang J, Li Z, Lam CWK, Xiao Y, Wu Q & Zhang W 2019 Consumption of Sugar-Sweetened Beverages Has a Dose-Dependent Effect on the Risk of Non-Alcoholic Fatty Liver Disease: An Updated Systematic Review and Dose-Response Meta-Analysis. Int J Environ Res Public Health 16.

Chong MF, Fielding BA & Frayn KN 2007 Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr 85 1511-1520.

Cohen CC, Li KW, Alazraki AL, Beysen C, Carrier CA, Cleeton RL, Dandan M, Figueroa J, Knight-Scott J, Knott CJ, Newton KP, Nyangau EM, Sirlin CB, Ugalde-Nicalo PA, Welsh JA, Hellerstein MK, Schwimmer JB & Vos MB 2021 Dietary sugar restriction reduces hepatic de novo lipogenesis in adolescent boys with fatty liver disease. The Journal of clinical investigation 131.

Cox CL, Stanhope KL, Schwarz JM, Graham JL, Hatcher B, Griffen SC, Bremer AA, Berglund L, McGahan JP, Havel PJ & Keim NL 2012 Consumption of fructose-sweetened beverages for 10 weeks reduces net fat oxidation and energy expenditure in overweight/obese men and women. Eur J Clin Nutr 66 201-208.

Cunha DA, Hekerman P, Ladrière L, Bazarra-Castro A, Ortis F, Wakeham MC, Moore F, Rasschaert J, Cardozo AK, Bellomo E, Overbergh L, Mathieu C, Lupi R, Hai T, Herchuelz A, Marchetti P, Rutter GA, Eizirik DL & Cnop M 2008 Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. J Cell Sci 121 2308-2318.

DiFrancesco L, Fulgoni VL, Gaine PC, Scott MO & Ricciuto L 2022 Trends in added sugars intake and sources among U.S. adults using the National Health and Nutrition Examination Survey (NHANES) 2001–2018. Frontiers in Nutrition 9.

Diggle CP, Shires M, Leitch D, Brooke D, Carr IM, Markham AF, Hayward BE, Asipu A & Bonthron DT 2009 Ketohexokinase: expression and localization of the principal fructose-metabolizing enzyme. J Histochem Cytochem 57 763-774.

DiNicolantonio JJ, Mehta V, Onkaramurthy N & O'Keefe JH 2018 Fructose-induced inflammation and increased cortisol: A new mechanism for how sugar induces visceral adiposity. Prog Cardiovasc Dis 61 3-9.

Diraison F, Moulin P & Beylot M 2003 Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. Diabetes Metab 29 478-485.

Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD & Parks EJ 2005 Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. The Journal of clinical investigation 115 1343-1351.

Egli L, Lecoultre V, Cros J, Rosset R, Marques A-S, Schneiter P, Hodson L, Gabert L, Laville M & Tappy L 2015 Exercise performed immediately after fructose ingestion enhances fructose oxidation and suppresses fructose storage1. The American Journal of Clinical Nutrition 103 348-355.

Fisberg M, Kovalskys I, Gómez G, Rigotti A, Sanabria LYC, García MCY, Torres RGP, Herrera-Cuenca M, Zimberg IZ, Koletzko B, Pratt M, Aznar LAM, Guajardo V, Fisberg RM, Sales CH & Previdelli Á N 2018 Total and Added Sugar Intake: Assessment in Eight Latin American Countries. Nutrients 10.

Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

Geidl-Flueck B & Gerber PA 2017 Insights into the Hexose Liver Metabolism-Glucose versus Fructose. Nutrients 9.
Geidl-Flueck B, Hochuli M, Németh Á, Eberl A, Derron N, Köfeler HC, Tappy L, Berneis K, Spinas GA & Gerber PA 2021 Fructose- and sucrose- but not glucose-sweetened beverages promote hepatic de novo lipogenesis: A randomized controlled trial. J Hepatol 75 46-54.
Gerich JE 2000 Physiology of glucose homeostasis. Diabetes Obes Metab 2 345-350.
Gorboulev V, Schürmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, Veyhl-Wichmann M, Srinivasan A, Balen D, Breljak D, Rexhepaj R, Parker HE, Gribble FM, Reimann F, Lang F, Wiese S, Sabolic I, Sendtner M & Koepsell H 2012 Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes 61 187-196.
Gruzdeva O, Borodkina D, Uchasova E, Dyleva Y & Barbarash O 2018 Localization of fat depots and cardiovascular risk. Lipids Health Dis 17 218.
Haas JT, Miao J, Chanda D, Wang Y, Zhao E, Haas ME, Hirschey M, Vaitheesvaran B, Farese RV, Jr., Kurland IJ, Graham M, Crooke R, Foufelle F & Biddinger SB 2012 Hepatic insulin signaling is required for obesity-dependent expression of SREBP-1c mRNA but not for feeding-dependent expression. Cell Metab 15 873-884.
Hellerstein MK, Christiansen M, Kaempfer S, Kletke C, Wu K, Reid JS, Mulligan K, Hellerstein NS & Shackleton CH 1991 Measurement of de novo hepatic lipogenesis in humans using stable isotopes. The Journal of clinical investigation 87 1841-1852.
Helsley RN, Moreau F, Gupta MK, Radulescu A, DeBosch B & Softic S 2020 Tissue-Specific Fructose Metabolism in Obesity and Diabetes. Curr Diab Rep 20 64.
Hudgins LC, Hellerstein M, Seidman C, Neese R, Diakun J & Hirsch J 1996 Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. The Journal of clinical investigation 97 2081-2091.
Hudgins LC, Hellerstein MK, Seidman CE, Neese RA, Tremaroli JD & Hirsch J 2000 Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. Journal of Lipid Research 41 595-604.
Iizuka K, Bruick RK, Liang G, Horton JD & Uyeda K 2004 Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. Proc Natl Acad Sci U S A 101 7281-7286.
Imamura F, O'Connor L, Ye Z, Mursu J, Hayashino Y, Bhupathiraju SN & Forouhi NG 2015 Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. Bmj 351 h3576.
Imamura F, Fretts AM, Marklund M, Ardisson Korat AV, Yang WS, Lankinen M, Qureshi W, Helmer C, Chen TA, Virtanen JK, Wong K, Bassett JK, Murphy R, Tintle N, Yu CI, Brouwer IA, Chien KL, Chen YY, Wood AC, Del Gobbo LC, Djousse L, Geleijnse JM, Giles GG, de Goede J, Gudnason V, Harris WS, Hodge A, Hu F, Koulman A, Laakso M, Lind L, Lin HJ, McKnight B, Rajaobelina K, Riserus U, Robinson JG, Samieri C, Senn M, Siscovick DS, Soedamah-Muthu SS, Sotoodehnia N, Sun Q, Tsai MY, Tuomainen TP, Uusitupa M, Wagenknecht LE, Wareham NJ, Wu JHY, Micha R, Lemaitre RN, Mozaffarian D & Forouhi NG 2020 Fatty acids in the de novo lipogenesis pathway and incidence of type 2 diabetes: A pooled analysis of prospective cohort studies. PLoS Med 17 e1003102.
Ishimoto T, Lanaspa MA, Le MT, Garcia GE, Diggle CP, Maclean PS, Jackman MR, Asipu A, Roncal-Jimenez CA, Kosugi T, Rivard CJ, Maruyama S, Rodriguez-Iturbe B, Sánchez-Lozada LG, Bonthron DT, Sautin YY & Johnson RJ 2012 Opposing effects of fructokinase C and A isoforms on fructose-induced metabolic syndrome in mice. Proc Natl Acad Sci U S A 109 4320-4325.
Jang C, Hui S, Lu W, Cowan AJ, Morscher RJ, Lee G, Liu W, Tesz GJ, Birnbaum MJ & Rabinowitz JD 2018 The Small Intestine Converts Dietary Fructose into Glucose and Organic Acids. Cell Metabolism 27 351-361.e353.
Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C, Nakagawa T, Kuwabara M, Sato Y, Kang DH, Tolan DR, Sanchez-Lozada LG, Rosen HR, Lanaspa MA, Diehl AM & Johnson RJ 2018 Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. J Hepatol 68 1063-1075.
Johnson RK, Appel LJ, Brands M, Howard BV, Lefevre M, Lustig RH, Sacks F, Steffen LM & Wylie-Rosett J 2009 Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. Circulation 120 1011-1020.
Kay Parker MSaVCN 2010 High fructose corn syrup: Production, uses and public health concerns. Biotechnol Mol Biol Rev. 5 71–78.

554 Kazierad DJ, Chidsey K, Somayaji VR, Bergman AJ, Birnbaum MJ & Calle RA 2021 Inhibition of
555 ketohexokinase in adults with NAFLD reduces liver fat and inflammatory markers: A randomized
556 phase 2 trial. Med 2 800-813.e803.
557 Kim M-S, Krawczyk SA, Doridot L, Fowler AJ, Wang JX, Trauger SA, Noh H-L, Kang HJ, Meissen JK,
558 Blatnik M, Kim JK, Lai M & Herman MA 2016 ChREBP regulates fructose-induced glucose production
559 independently of insulin signaling. The Journal of clinical investigation 126 4372-4386.
560 Kmietowicz Z 2012 Countries that use large amounts of high fructose corn syrup have higher rates of
561 type 2 diabetes. BMJ : British Medical Journal 345 e7994.
562 Koepsell H 2020 Glucose transporters in the small intestine in health and disease. Pflügers Archiv -
563 European Journal of Physiology 472 1207-1248.
564 Kohjima M, Enjoji M, Higuchi N, Kato M, Kotoh K, Yoshimoto T, Fujino T, Yada M, Yada R, Harada N,
565 Takayanagi R & Nakamuta M 2007 Re-evaluation of fatty acid metabolism-related gene expression in
566 nonalcoholic fatty liver disease. Int J Mol Med 20 351-358.
567 Koo H-Y, Miyashita M, Simon Cho BH & Nakamura MT 2009 Replacing dietary glucose with fructose
568 increases ChREBP activity and SREBP-1 protein in rat liver nucleus. Biochemical and Biophysical
569 Research Communications 390 285-289.
570 Korbecki J & Bajdak-Rusinek K 2019 The effect of palmitic acid on inflammatory response in
571 macrophages: an overview of molecular mechanisms. Inflammation Research 68 915-932.
572 Lambert JE, Ramos-Roman MA, Browning JD & Parks EJ 2014 Increased de novo lipogenesis is a
573 distinct characteristic of individuals with nonalcoholic fatty liver disease. Gastroenterology 146 726-
574 735.
575 Lanaspa MA, Andres-Hernando A, Orlicky DJ, Cicerchi C, Jang C, Li N, Milagres T, Kuwabara M,
576 Wempe MF, Rabinowitz JD, Johnson RJ & Tolan DR 2018 Ketohexokinase C blockade ameliorates
577 fructose-induced metabolic dysfunction in fructose-sensitive mice. The Journal of clinical investigation
578 128 2226-2238.
579 Lecoultre V, Egli L, Carrel G, Theytaz F, Kreis R, Schneiter P, Boss A, Zwygart K, Lê KA, Bortolotti M,
580 Boesch C & Tappy L 2013 Effects of fructose and glucose overfeeding on hepatic insulin sensitivity
581 and intrahepatic lipids in healthy humans. Obesity (Silver Spring) 21 782-785.
582 Lim JS, Mietus-Snyder M, Valente A, Schwarz JM & Lustig RH 2010 The role of fructose in the
583 pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol 7 251-264.
584 Linden AG, Li S, Choi HY, Fang F, Fukasawa M, Uyeda K, Hammer RE, Horton JD, Engelking LJ &
585 Liang G 2018 Interplay between ChREBP and SREBP-1c coordinates postprandial glycolysis and
586 lipogenesis in livers of mice[S]. Journal of Lipid Research 59 475-487.
587 Löwik MRH 2021 Assessment and Evaluation of the Intake of Sugars in European Countries. Applied
588 Sciences 11 11983.
589 Ma J, Karlsen MC, Chung M, Jacques PF, Saltzman E, Smith CE, Fox CS & McKeown NM 2016
590 Potential link between excess added sugar intake and ectopic fat: a systematic review of randomized
591 controlled trials. Nutr Rev 74 18-32.
592 Maedler K, Spinas GA, Dyntar D, Moritz W, Kaiser N & Donath MY 2001 Distinct effects of saturated
593 and monounsaturated fatty acids on beta-cell turnover and function. Diabetes 50 69-76.
594 Maedler K, Oberholzer J, Bucher P, Spinas GA & Donath MY 2003 Monounsaturated Fatty Acids
595 Prevent the Deleterious Effects of Palmitate and High Glucose on Human Pancreatic β-Cell Turnover
596 and Function. Diabetes 52 726-733.
597 Maersk M, Belza A, Stødkilde-Jørgensen H, Ringgaard S, Chabanova E, Thomsen H, Pedersen SB,
598 Astrup A & Richelsen B 2012 Sucrose-sweetened beverages increase fat storage in the liver, muscle,
599 and visceral fat depot: a 6-mo randomized intervention study. Am J Clin Nutr 95 283-289.
600 Malik VS & Hu FB 2022 The role of sugar-sweetened beverages in the global epidemics of obesity
601 and chronic diseases. Nature Reviews Endocrinology 18 205-218.
602 Maruhama Y & Macdonald I 1973 Incorporation of orally administered glucose-U-14C and fructose-U-
603 14C into the triglyceride of liver, plasma, and adipose tissue of rats. Metabolism 22 1205-1215.
604 McGarry JD, Mannaerts GP & Foster DW 1977 A possible role for malonyl-CoA in the regulation of
605 hepatic fatty acid oxidation and ketogenesis. The Journal of clinical investigation 60 265-270.
606 Mendeloff AI & Weichselbaum TE 1953 Role of the human liver in the assimilation of intravenously
607 administered fructose. Metabolism 2 450-458.
608 Moore JB 2010 Non-alcoholic fatty liver disease: the hepatic consequence of obesity and the
609 metabolic syndrome. Proc Nutr Soc 69 211-220.
610 Moskowitz HR 1970 Ratio scales of sugar sweetness. Perception & Psychophysics 7 315-320.
611 Nagai Y, Nishio Y, Nakamura T, Maegawa H, Kikkawa R & Kashiwagi A 2002 Amelioration of high
612 fructose-induced metabolic derangements by activation of PPARalpha. Am J Physiol Endocrinol
613 Metab 282 E1180-1190.

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

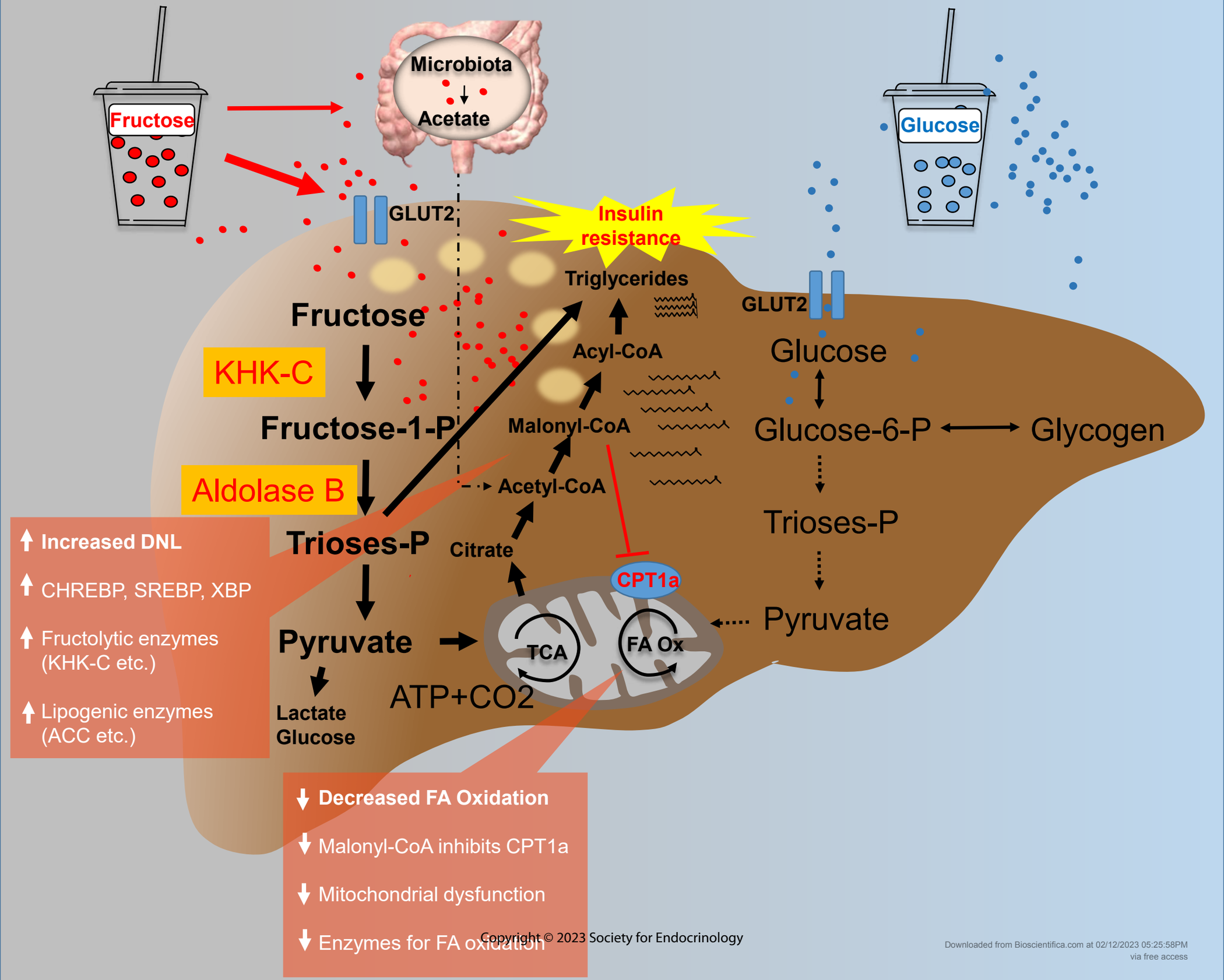
614 Noubiap JJ, Nansseu JR, Lontchi-Yimagou E, Nkeck JR, Nyaga UF, Ngouo AT, Tounouga DN, Tianyi
615 FL, Foka AJ, Ndoadoumgue AL & Bigna JJ 2022 Global, regional, and country estimates of metabolic
616 syndrome burden in children and adolescents in 2020: a systematic review and modelling analysis.
617 Lancet Child Adolesc Health 6 158-170.
618 Organization GWH 2015 Guideline: Sugars intake for adults and children. .
619 Ouyang X, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, Johnson RJ & Abdelmalek MF 2008
620 Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol 48 993-999.
621 Parks EJ, Skokan LE, Timlin MT & Dingfelder CS 2008 Dietary sugars stimulate fatty acid synthesis in
622 adults. J Nutr 138 1039-1046.
623 Patel C, Douard V, Yu S, Tharabenjasin P, Gao N & Ferraris RP 2015a Fructose-induced increases in
624 expression of intestinal fructolytic and gluconeogenic genes are regulated by GLUT5 and KHK. Am J
625 Physiol Regul Integr Comp Physiol 309 R499-509.
626 Patel C, Sugimoto K, Douard V, Shah A, Inui H, Yamanouchi T & Ferraris RP 2015b Effect of dietary
627 fructose on portal and systemic serum fructose levels in rats and in KHK-/- and GLUT5-/- mice. Am J
628 Physiol Gastrointest Liver Physiol 309 G779-790.
629 Prager GN & Ontko JA 1976 Direct effects of fructose metabolism on fatty acid oxidation in a
630 recombined rat liver mitochondria-high speed supernatant system. Biochimica et Biophysica Acta
631 (BBA) - Lipids and Lipid Metabolism 424 386-395.
632 Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, Swain MG, Congly SE, Kaplan
633 GG & Shaheen AA 2022 The prevalence and incidence of NAFLD worldwide: a systematic review
634 and meta-analysis. Lancet Gastroenterol Hepatol 7 851-861.
635 Rotter V, Nagaev I & Smith U 2003 Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1
636 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from
637 insulin-resistant subjects. J Biol Chem 278 45777-45784.
638 Rumessen JJ & Gudmand-Høyer E 1986 Absorption capacity of fructose in healthy adults.
639 Comparison with sucrose and its constituent monosaccharides. Gut 27 1161-1168.
640 Sahota AK, Shapiro WL, Newton KP, Kim ST, Chung J & Schwimmer JB 2020 Incidence of
641 Nonalcoholic Fatty Liver Disease in Children: 2009-2018. Pediatrics 146.
642 Saklayen MG 2018 The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep 20 12.
643 Schwarz J-M, Noworolski SM, Wen MJ, Dyachenko A, Prior JL, Weinberg ME, Herraiz LA, Tai VW,
644 Bergeron N, Bersot TP, Rao MN, Schambelan M & Mulligan K 2015 Effect of a High-Fructose Weight-
645 Maintaining Diet on Lipogenesis and Liver Fat. The Journal of Clinical Endocrinology & Metabolism
646 100 2434-2442.
647 Schwarz JM, Noworolski SM, Erkin-Cakmak A, Korn NJ, Wen MJ, Tai VW, Jones GM, Palii SP,
648 Velasco-Alin M, Pan K, Patterson BW, Gugliucci A, Lustig RH & Mulligan K 2017 Effects of Dietary
649 Fructose Restriction on Liver Fat, De Novo Lipogenesis, and Insulin Kinetics in Children With Obesity.
650 Gastroenterology 153 743-752.
651 Sekkarie A, Welsh JA, Northstone K, Stein AD, Ramakrishnan U & Vos MB 2021 Associations
652 between Free Sugar and Sugary Beverage Intake in Early Childhood and Adult NAFLD in a
653 Population-Based UK Cohort. Children (Basel) 8.
654 Serna-Saldivar SO 2016 Maize: Foods from Maize. In Reference Module in Food Science: Elsevier.
655 Singh GM, Micha R, Khatibzadeh S, Shi P, Lim S, Andrews KG, Engell RE, Ezzati M & Mozaffarian D
656 2015 Global, Regional, and National Consumption of Sugar-Sweetened Beverages, Fruit Juices, and
657 Milk: A Systematic Assessment of Beverage Intake in 187 Countries. PLoS One 10 e0124845.
658 Smith GI, Shankaran M, Yoshino M, Schweitzer GG, Chondronikola M, Beals JW, Okunade AL,
659 Patterson BW, Nyangau E, Field T, Sirlin CB, Talukdar S, Hellerstein MK & Klein S 2020 Insulin
660 resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. The Journal of
661 clinical investigation 130 1453-1460.
662 Softic S, Cohen DE & Kahn CR 2016 Role of Dietary Fructose and Hepatic De Novo Lipogenesis in
663 Fatty Liver Disease. Dig Dis Sci 61 1282-1293.
664 Softic S, Gupta MK, Wang GX, Fujisaka S, O'Neill BT, Rao TN, Willoughby J, Harbison C, Fitzgerald
665 K, Ilkayeva O, Newgard CB, Cohen DE & Kahn CR 2017 Divergent effects of glucose and fructose on
666 hepatic lipogenesis and insulin signaling. The Journal of clinical investigation 127 4059-4074.
667 Softic S, Meyer JG, Wang GX, Gupta MK, Batista TM, Lauritzen H, Fujisaka S, Serra D, Herrero L,
668 Willoughby J, Fitzgerald K, Ilkayeva O, Newgard CB, Gibson BW, Schilling B, Cohen DE & Kahn CR
669 2019 Dietary Sugars Alter Hepatic Fatty Acid Oxidation via Transcriptional and Post-translational
670 Modifications of Mitochondrial Proteins. Cell Metab 30 735-753.e734.
671 Song Z, Xiaoli AM & Yang F 2018 Regulation and Metabolic Significance of De Novo Lipogenesis in
672 Adipose Tissues. Nutrients 10.

673 Staiger H, Staiger K, Stefan N, Wahl HG, Machicao F, Kellerer M & Häring HU 2004 Palmitate-
674 induced interleukin-6 expression in human coronary artery endothelial cells. Diabetes 53 3209-3216.
675 Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL,
676 Dyachenko A, Zhang W, McGahan JP, Seibert A, Krauss RM, Chiu S, Schaefer EJ, Ai M, Otokozawa
677 S, Nakajima K, Nakano T, Beysen C, Hellerstein MK, Berglund L & Havel PJ 2009 Consuming
678 fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and
679 decreases insulin sensitivity in overweight/obese humans. The Journal of clinical investigation 119
680 1322-1334.
681 Tappy L & Lê KA 2010 Metabolic effects of fructose and the worldwide increase in obesity. Physiol
682 Rev 90 23-46.
683 Taylor SR, Ramsamooj S, Liang RJ, Katti A, Pozovskiy R, Vasan N, Hwang S-K, Nahiyaan N,
684 Francoeur NJ, Schatoff EM, Johnson JL, Shah MA, Dannenberg AJ, Sebra RP, Dow LE, Cantley LC,
685 Rhee KY & Goncalves MD 2021 Dietary fructose improves intestinal cell survival and nutrient
686 absorption. Nature 597 263-267.
687 Te Morenga LA, Howatson AJ, Jones RM & Mann J 2014 Dietary sugars and cardiometabolic risk:
688 systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure
689 and lipids. Am J Clin Nutr 100 65-79.
690 Testa R, Olivieri F, Bonfigli AR, Sirolla C, Boemi M, Marchegiani F, Marra M, Cenerelli S, Antonicelli
691 R, Dolci A, Paolisso G & Franceschi C 2006 Interleukin-6-174 G > C polymorphism affects the
692 association between IL-6 plasma levels and insulin resistance in type 2 diabetic patients. Diabetes
693 Res Clin Pract 71 299-305.
694 Thorens B & Mueckler M 2010 Glucose transporters in the 21st Century. Am J Physiol Endocrinol
695 Metab 298 E141-145.
696 Vessby B 2003 Dietary fat, fatty acid composition in plasma and the metabolic syndrome. Curr Opin
697 Lipidol 14 15-19.
698 Wang X, Sato R, Brown MS, Hua X & Goldstein JL 1994 SREBP-1, a membrane-bound transcription
699 factor released by sterol-regulated proteolysis. Cell 77 53-62.
700 Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F, Häring HU & Schleicher ED 2004
701 Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes
702 through proteasome-dependent activation of nuclear factor-kappaB. J Biol Chem 279 23942-23952.
703 Yamashita H, Takenoshita M, Sakurai M, Bruick RK, Henzel WJ, Shillinglaw W, Arnot D & Uyeda K
704 2001 A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver.
705 Proc Natl Acad Sci U S A 98 9116-9121.
706 Yin J, Zhu Y, Malik V, Li X, Peng X, Zhang FF, Shan Z & Liu L 2021 Intake of Sugar-Sweetened and
707 Low-Calorie Sweetened Beverages and Risk of Cardiovascular Disease: A Meta-Analysis and
708 Systematic Review. Adv Nutr 12 89-101.
709 Zhao S, Jang C, Liu J, Uehara K, Gilbert M, Izzo L, Zeng X, Trefely S, Fernandez S, Carrer A, Miller
710 KD, Schug ZT, Snyder NW, Gade TP, Titchenell PM, Rabinowitz JD & Wellen KE 2020 Dietary
711 fructose feeds hepatic lipogenesis via microbiota-derived acetate. Nature 579 586-591.
712
713

 Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

Figure legend

Figure 1: A comparison of the hepatic fructose (left) and glucose (right) metabolism after consumption of high loads of sugar in the form of SSB. It is hypothesized that an increased de novo lipogenesis after fructose intake in parallel with a decreased fatty acid oxidation leads to hepatic fat deposition. ACC: Acetyl-CoA-Carboxylase; ATP: adenosine triphosphate; CPT1a: carnitine palmitoyltransferase 1A; FA: fatty acid; GLUT: Glucose transporter; KHK-C: ketohexokinase-C; Ox: oxidation; P: phosphate; SSB: sugar-sweetened beverage¸ TCA: tricarboxylic acid cycle.



Accepted Manuscript published as JOE-22-0270.R1. Accepted for publication: 08-Feb-2023

Table 1: Overview of studies measuring effects of dietary interventions on hepatic DNL by tracer methodology

Intervention	Duration	Subjects	N	DNL measurement	Result	Reference
Eucaloric liquid formula diets - Low-fat diet (10% of calories as fat and 75% as glucose polymers) - High-fat diet (40% of calories as fat and 45% as glucose polymers)	25d	Healthy men and women Younger adults Normal weight	10	Postprandial DNL Labeling of palmitate with 13C-acetate, MIDA; linoleate dilution method	Dietary substitution of carbohydrate (CHO) for fat stimulates the hepatic fatty acid synthesis	Hudgins, L.C., et al., 1996 [66]
Isocaloric diets with the same macronutrient composition - High-fructose diet (25% caloric intake; beverage) - Complex CHO (solid) diet (replaced fructose)	9d	Healthy men All age groups Normal weight	8	Postprandial DNL Labeling of palmitate with 13C-acetate, MIDA	High-fructose diet is associated with higher hepatic DNL	Schwarz, J.-M., et al., 2015 [67]
Daily SSB consumption (25% of required caloric intake provided as SSB; 8 wks outpatient intervention with ad libitum diet, 2 wks energy-balanced inpatient intervention) - Glucose-SSB - Fructose-SSB	10wks	Men and women Middle-aged Overweight/obese	32	Postprandial DNL Labeling of palmitate with 13C-acetate, MIDA	High fructose increases hepatic DNL	Stanhope, K, et al., 2009 [68]
Beverage consumption containing glucose and/or fructose - 100:0 GLC: FRC - 50:50 GLC: FRC - 25:75 GLC: FRC	Single exposure	Healthy men and women Younger adults Normal weight	6	Postprandial DNL Labeling of palmitate with 13C-acetate, MIDA	Acute intake of fructose stimulates hepatic lipogenesis	Parks, E.J., et al., 2008 [38]
Daily SSB (80g sugar intake/day, 3x 2dl) consumption or SSB abstinence - Glucose-SSB - Fructose-SSB - Sucrose-SSB	6wks	Healthy men Younger adults Normal weight	94	Basal DNL Labeling of palmitate with 13C-acetate, MIDA	Fructose and sucrose increase basal hepatic lipogenic activity	Geidl-Flueck, B., et al., 2021 [45]
Dietary sugar restriction - Low free sugar diet - “Usual” diet	8wks	Obese boys with NAFLD	29	Labeling of palmitate with 2H2O, MIDA	Dietary sugar restriction reduces hepatic DNL	Cohen, C.C., et al., 2021 [70]
Isocaloric fructose restriction - Starch substituted for sugar (reduced caloric intake from fructose from 12% to 4% of total energy intake).	9d	Children (male and female) with obesity and metabolic syndrome and habitual highsugar consumption (fructose intake >50 g/d)	41	Postprandial DNL Labeling of palmitate with 13C-acetate, MIDA	Isocaloric fructose restriction decreases hepatic DNL	Schwarz, J. M., et al., 2017 [71]