HHS Public Access

Author manuscript

J Nutr Biochem. Author manuscript; available in PMC 2024 April 24.

Published in final edited form as:

J Nutr Biochem. 2023 April ; 114: 109224. doi:10.1016/j.jnutbio.2022.109224.

Fructose impairs fat oxidation: Implications for the mechanism of western diet-induced NAFLD

Mustafa Kutlu Inci[a], **Se-Hyung Park**[b,c], **Robert N. Helsley**[b,c,d], **Suzanna L. Attia**[b], **Samir Softic**[b,c,e,*]

[a] Sanko University, School of Medicine, Gaziantep, Turkey

[b] Department of Pediatrics, University of Kentucky College of Medicine, Lexington, KY, USA

[c] Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, USA

[d] Saha Cardiovascular Research Center, University of Kentucky, Lexington, KY, USA

[e] Joslin Diabetes Center and Department of Medicine, Harvard Medical School, Boston, MA, USA

Abstract

Increased fructose intake from sugar-sweetened beverages and highly processed sweets is a well-recognized risk factor for the development of obesity and its complications. Fructose strongly supports lipogenesis on a normal chow diet by providing both, a substrate for lipid synthesis and activation of lipogenic transcription factors. However, the negative health consequences of dietary sugar are best observed with the concomitant intake of a HFD. Indeed, the most commonly used obesogenic research diets, such as “Western diet”, contain both fructose and a high amount of fat. In spite of its common use, how the combined intake of fructose and fat synergistically supports development of metabolic complications is not fully elucidated. Here we present the preponderance of evidence that fructose consumption decreases oxidation of dietary fat in human and animal studies. We provide a detailed review of the mitochondrial β-oxidation pathway. Fructose affects hepatic activation of fatty acyl-CoAs, decreases acylcarnitine production and impairs the carnitine shuttle. Mechanistically, fructose suppresses transcriptional activity of PPARα and its target CPT1α, the rate limiting enzyme of acylcarnitine production. These effects of fructose may be, in part, mediated by protein acetylation. Acetylation of PGC1α, a co-activator of PPARα and acetylation of CPT1α, in part, account for fructose-impaired acylcarnitine production. Interestingly, metabolic effects of fructose in the liver can be largely overcome by carnitine supplementation. In summary, fructose decreases oxidation of dietary fat in the liver, in part, by impairing acylcarnitine production, offering one explanation for the synergistic effects of these nutrients on the development of metabolic complications, such as NAFLD.

* Corresponding author at: Samir Softic, Department of Pediatrics, University of Kentucky, 900 South Limestone, Lexington, KY 40536, USA. samir.softic@uky.edu (S. Softic).

Declaration of Competing Interests

The authors declare that there are no conflicts of interest.

Keywords

Sugar; Fructose; Fatty acid oxidation (FAO); Western diet; Non-alcoholic fatty liver disease (NAFLD)

1. Introduction

Consumption of a high-fat diet (HFD) was initially considered to be the primary driver of the worldwide epidemic of obesity and its associated metabolic complications [1]. Thus, over the last three decades a strong emphasis has been placed on reducing total fat intake. Despite successfully lowering the percentage of energy intake from dietary fat on a population level [2], the incidence of obesity, heart disease, and non-alcoholic fatty liver disease (NAFLD) has not been tamed. Recently, there has been a paradigm shift and more attention has been focused on the detrimental effects of sugar, primarily fructose, as a contributor to the obesity epidemic [3]. The most sugar in our diet is consumed as sucrose (glucose and fructose disaccharide) or high-fructose corn syrup (55% fructose and 45% glucose monosaccharide). Indeed, the combined intake of fructose and sucrose robustly supports weight gain, whereas consumption of fructose alone does not always result in increased weight gain [4]. However, even weight neutral fructose intake may worsen metabolic complications [5,6].

Several metabolic properties of fructose make it an ideal candidate to support the development of obesity and its complications. First, fructose consumption contributes to an increased caloric load. For example, desserts are consumed after a meal when the subject is no longer hungry; and sugar sweetened beverages are consumed to quench thirst, not hunger, resulting in excess caloric intake. On the other hand, even isocaloric fructose restriction may improve weight gain and metabolic dysfunction [7–9]. Next, fructose is considered a highly lipogenic nutrient and serves as both a substrate for lipogenesis and a key inducer of the lipogenic pathway. Additionally, fructose metabolism is exceedingly fast compared to glucose metabolism leading to depletion of adenosine triphosphate (ATP) and uric acid production. Uric acid can lead to lower AMP-activated protein kinase (AMPK) activity [10] and thus decreased fatty acid oxidation. The interaction between fructose and fat metabolism may be the most interesting aspect of fructose-induced effects since these nutrients are commonly consumed together in the obesogenic “Western diet.” In spite of the strong evidence showing that the most detrimental effects of fructose are observed in the setting of a HFD intake, the effects of fructose on fat oxidation are poorly defined. Therefore, the aim of this review is to summarize the available evidence and propose a mechanistic understanding of how fructose intake decreases fatty acid oxidation.

2. Is high fat diet or high sugar intake causing obesity? The truth lies in between

A positive energy balance is required for the development of obesity and its complications. Due to its high caloric density, diets high in fat have indisputably been found to strongly induce obesity. Some studies, in mice, even suggest that dietary fat is the necessary and

sufficient nutrient to induce obesity. A comparison of 29 different diets with varying amounts of protein, fat, and sugar found that only increasing the fat content up to 60% of the total calories results in greater obesity, whereas increasing sugar or protein content from 5 to 30% of the calories had no effect on weight gain [11]. This study did not examine the effects of sugar free diets and interestingly, increasing the percent of fat above 60% of the total calories, at an expense of reducing carbohydrate intake, resulted in lower weight gain [11]. This part of the study is in agreement with a well-known paradigm that high-fat, sugar-free keto genic diets promote decreased weight gain [12]. Indeed, high-fat, ketogenic diets reduce adiposity, as long as they are free of sugar, since even a small amount of sugar precludes ketosis. Thus, reducing sugar intake from an obesogenic high-fat diet, even at the expense of further increasing dietary fat, results in reduced weight gain [13], diminished liver lipid accumulation [14–16], decreased insulin resistance [17,18], and an improved lipid profile [19,20]. In addition to being sugar free, ketogenic diets are also low in total carbohydrate load. A proposed mechanism for metabolic improvements is that sugar-free ketogenic diets stimulate a fasting-like state [21] and rely on fat oxidation to sustain metabolic functions [22]. In agreement with this, we published that abrogating fructose metabolism in the livers of mice on a most commonly utilized obesogenic HFD (Research diets, D12492, 60% calories from fat), which also contains 6.7% of sugar, leads to increased mitochondrial fatty acid oxidation and improved metabolic health [23]. Thus, while HFDs strongly induce obesity and metabolic dysfunction, a relatively small amount of dietary sugar is a required co-ingredient to turn off fatty acid oxidation and allow for the full manifestation of metabolic derangements to become evident.

Sugar intake is clearly associated with weight gain and metabolic dysfunction in human [6,24–27] and animal studies [28–30]. Studies showing a positive correlation between sugar intake and obesity generally use a supra-physiologic amount of sugar, such as greater than 60% fructose in solid diet [31–35], or provide sugar-sweetened beverages (SSBs) on top of a standard diet [27–29,36], inducing a hypercaloric state. Therefore, the prevailing opinion is that sugar is a vehicle for increased caloric intake and that the caloric load principally drives the negative health consequences associated with sugar intake [37]. This hypothesis decreases the enthusiasm for avoiding sugar intake, as a greater decrease in caloric load can be achieved by reducing fat, which contains a higher caloric load. However, emerging data shows that isocaloric fructose intake [5,7,38] or fructose, as compared to equicaloric glucose intake [6,39,40], supports the development of metabolic complications independent of total caloric load.

In our studies, we find that 30% fructose-sweetened water consumption does result in increased weight gain in mice on standard chow diet (LabDiet, 9F 5020, 21.6% calories from fat), but this does not lead to severe metabolic complications after 10 weeks on the diet [41]. Interestingly, we observed that 30% fructose supplementation of mice on a low-fat diet (Research Diets, D12450K, 10% calories from fat) over the same time period does not result in weight gain (unpublished observation). Others have also shown that fructose consumption on a normal diet (*e.g.,* 13% calories from fat) does not induce weight gain [42], suggesting that a higher percent of fat in a diet is required to observe the obesogenic properties of fructose. This hypothesis is in line with the well-established fact that fructose intake from fruits and vegetables, as a part of well-balanced diet, does not lead to obesity

and metabolic complications [43,44]. On the other hand, we showed that 30% fructose supplementation of mice on a HFD worsens weight gain and metabolic dysfunction as compared to 30% glucose supplementation [41]. Others have also observed that the most severe metabolic derangements are induced by the combined intake of fructose and a HFD [45–50]. Therefore, a Western diet, which is high in both fat and sugar has become a diet of choice in animal studies to induce obesity [51]. Indeed, a recent systemic review of 3,920 rodent models of NAFLD found that the livers of rodents fed a high-fat, high-fructose diet most closely resemble the pattern of severe liver injury observed in human subjects with NAFLD [52]. Taken together, these studies suggest that the effects of fructose on metabolic dysfunction are dependent on higher dietary fat intake, and that a HFD must contain some sugar to fully support development of obesity-associated complications. While the combined intake of fructose and fat in our Western diet is necessary for the optimal obesogenic phenotype, the mechanism that governs these synergistic effects remains elusive.

3. Fructose supports *de novo* lipogenesis

Most studies in the literature, including our early work, report on a high lipogenic potential of fructose. Indeed, fructose stimulates hepatic *de novo* lipogenesis (DNL) to a greater extent than glucose [36,53,54], starch [5,55,56], or HFD [14,57,58]. Mechanistically (Fig. 1), fructose uptake across the plasma membrane is largely facilitated by glucose transporters (GLUT2, GLUT5), and the sodium glucose cotransporter 5 (SGLT5) [59,60]. Tissue-specific distribution of these transporters accounts for highly compartmentalized fructose metabolism in the selected organs, such as in the liver (GLUT2), intestine (GLUT5), and kidney (GLUT5/SGLT5) [60]. Phosphorylation and subsequent metabolism of fructose by the rate-limiting enzyme ketohexokinase (KHK) and downstream aldolase B (ALDOB) provides the triose-phosphate intermediates, dihydroxyacetone phosphate (DHAP) and glyceraldehyde (GA), while triose kinase/FMN Cyclase (TKFC) produces glyceraldehyde 3-phosphate (GA3P) from GA. These three carbon metabolites are also intermediates of glycolysis and serve as precursors for lipid synthesis. Aside from providing substrate for lipogenesis, fructose metabolism activates lipogenic transcription factors that govern the expression of lipogenic enzymes.

In human [61] and animal [62] studies, fructose robustly upregulates carbohydrate response element-binding protein (ChREBP) transcription factor, as well as its binding to DNA [63]. ChREBP then upregulates the transcription of enzymes involved in fatty acid synthesis including ATP citrate lyase (*Acly*), acetyl-CoA carboxylase (*Acc*), fatty acid synthase (*Fasn*), and stearoyl-CoA desaturase-1 (*Scd1*). This then enables lipid synthesis associated with fructose intake. At the same time, ChREBP also upregulates gluconeogenesis, as well as fructolytic enzymes *Khk, AldoB, Tkfc*, and *Glut5* in a feed forward cycle [64]. Moreover, ChREBP knockout in the liver leads to decreased expression of KHK and other fructolytic enzymes [65]. A bidirectional relationship between fructose metabolism and ChREBP is further highlighted by the studies utilizing a small molecule KHK inhibitor reporting that decreased fructose metabolism leads to ChREBP inactivation [66]. In spite of the strong evidence that ChREBP mediates some aspects of fructose metabolism in the liver, its role in fructose-induced metabolic dysfunction is complex. First, it has been reported that ChREBP dissociates hepatic lipid accumulation from the development of insulin

resistance [67]. Next, hepatic ChREBP is required to protect the liver from fructose-induced hepatotoxicity, as fructose-fed liver-specific ChREBP knockout mice exhibit dysregulated glycogen metabolism, cholesterol synthesis, and ATP homeostasis [33,35]. While ChREBP functions in the liver to mediate some fructose-induced metabolic effects, in the intestine ChREBP regulates sugar absorption and taste preference for sweets. Therefore, ChREBP knockout in the intestine leads to fructose malabsorption, bacterial proliferation, and symptoms consistent with irritable bowel syndrome [65,68,69]. In summary, fructose intake induces ChREBP, which in turn further increases fructolysis, but ChREBP upregulation appears to protect the liver from the toxic effects induced by this dietary sugar.

Fructose can also induce DNL via upregulation of sterol regulatory element-binding protein 1c (SREBP1c) transcription factor. SREBP1c upregulates the expression of largely overlapping DNL genes as does ChREBP. Unlike its direct action on ChREBP, fructose effects on SREBP1c are mainly thought to be mediated via its strong propensity to induce insulin resistance. We have recently reviewed the data and mechanistic details of how fructose induces hepatic insulin resistance [70]. Insulin resistance leads to hyperinsulinemia, and insulin is a strong mediator of the SREBP1c-dependent signaling machinery. Insulin stimulates all aspects of SREBP1c control including transcription, proteolytic cleavage into its active form, and nuclear translocation [71]. However, SREBP1c nuclear translocation, as well as concomitant increases in lipogenic genes, can be induced by fructose feedings even in mice with liver-specific knockout of insulin receptor, indicating that fructose can stimulate SREBP1c action in the setting of a complete lack of insulin signaling [72]. Peroxisome proliferator-activated receptor-gamma coactivator 1 beta (PGC1β), a transcriptional coactivator for SREBP1, may mediate these effects, since a knockout of PGC1β reduces SREBP1c expression and downstream lipogenic genes in the liver [73]. Therefore, upregulation of SREBP1c by fructose likely occurs via insulin dependent and independent mechanisms and contributes to transcriptional activation of hepatic lipogenesis.

Lastly, fructose metabolism leads to depletion of ATP, thereby resulting in uric acid production [62,74]. Elevated uric acid can promote lipogenesis by activating ChREBP [62] and SREBP1c [75]. In addition, uric acid inhibits aconitase, an enzyme in the tricarboxylic acid cycle (TCA) cycle *i.e.,* responsible for catabolizing citrate, further supporting the dual action of fructose to increase transcriptional DNL machinery and to increase the substrate availability for lipogenesis.

While fructose strongly supports lipogenesis in rodent models on a normal chow diet, we find that the effects of fructose on lipid synthesis are decreased in the presence of a HFD [41]. This observation is intuitive, as a HFD provides ample supply of free fatty acids (FFA) and obviates the need for ongoing DNL. Although lipogenesis is reduced with the combined intake of fructose and a HFD, the metabolic complications on a Western diet are more evident than on either a HFD or fructose diet alone. Therefore, fructose-induced lipogenesis does not fully account for the metabolic effects of fructose. Rather, the combined intake of fructose and a HFD synergistically supports the development of severe metabolic dysregulation even in the setting of reduced lipogenesis.

4. Fructose impairs fatty acid oxidation in human and animal studies

Based on the cumulative negative effects when fructose and fat are co-ingested, it is reasonable to assume that their concomitant metabolism acts synergistically. Indeed, numerous studies document that fructose decreases the oxidation of dietary fat (Table 1). This hypothesis is supported by clinical studies in human subjects. In the 1980's, Tappy and colleagues showed that 4h after ingesting a single 75g dose of fructose, seventeen healthy volunteers experienced a significantly greater decrease in lipid oxidation *vs.* subjects ingesting the same amount of glucose [76]. Similarly, Chong et al., gave fructose or glucose test meals labeled with ($^{2}H_2$)Palmitate and (^{13}C)D-fructose or (^{13}C)D-glucose to fourteen subjects after an overnight fast. The subjects who consumed fructose had a higher respiratory exchange ratio (RER) and more carbons from fructose recovered in breath CO_2 indicative of greater carbohydrate utilization. Conversely, net fat oxidation and beta-hydroxybutyrate (BHB) were significantly lower after fructose intake. The authors concluded that fructose contribution to DNL is small, but its effect on altering the partitioning of fatty acids toward esterification may be considerable when fructose and fat are co-ingested [77]. These single dose studies are in agreement with a short-term study of 6 d fructose overfeeding in eight male subjects showing decreased lipid oxidation (0.28±0.11 mg/kg/min) when compared to the basal reading (0.54±0.11 mg/kg/min) on a diet containing 35% of calories from fat [78]. Lastly, in a seminal paper, Cox et al., showed that long-term consumption of fructose- but not glucose-sweetened beverages for 10 weeks reduced net postprandial fat oxidation and energy expenditure in overweight/obese men and women [40].

Decreased fat oxidation with fructose feeding has also been reported in rodent studies since the 1970's [79–82], providing a model to investigate the underlying mechanism. Since then, several hypotheses have been proposed to mediate the fructose-induced decrease in oxidation of dietary fat. Given the high propensity of fructose to support *de novo* lipogenesis, the first mechanism explored the role of malonyl-CoA. Malonyl-CoA is produced from acetyl-CoA by the action of ACC1 and is the first committed step in fatty acid synthesis. Subsequently, FASN sequentially adds malonyl-CoA to extend the growing fatty acid chain by two carbons to form saturated fatty acids, such as palmitoyl-CoA. Besides supporting lipogenesis, malonyl-CoA also acts as a allosteric inhibitor of CPT1α [83], the rate limiting enzyme in mitochondrial fatty acid oxidation. By inhibiting CTP1α, malonyl-CoA prevents the two competing processes, fatty acid synthesis and fatty acid oxidation, to occur at the same time. However, regulation of liver CPT1α isoform is much less sensitive to malonyl-CoA inhibition than its counterpart CPT1β found in the muscle [84]. Moreover, malonyl-CoA levels are regulated, in part, by the nutrient sensor AMPK. When AMP and/or ADP levels increase relative to ATP, such as during fasting and fructose catabolism, AMPK is activated leading to phosphorylation and inactivation of its substrate ACC1 and decreases conversion of acetyl-CoA to malonyl-CoA. Consistent with this finding, we have found that hepatic levels of malonyl-CoA are not increased by fructose feeding on a HFD [23]. Therefore, despite the strong propensity of fructose to actively drive lipogenesis, there is no evidence that fructose metabolism on a HFD leads to accumulation of malonyl-CoA in the liver to explain the decrease in FAO observed with fructose feeding.

Another mode of regulation by which fructose can impair FAO is through disruption of a gene regulatory network. The nuclear receptor peroxisome proliferator-activated receptor α (PPARα) controls the transcriptional regulation of genes involved in FAO including *Cpt1a* [85]. While stimulation of PPARα drives fatty acid utilization, PPARα deficiency leads to hepatic lipid accumulation and inflammation in response to a HFD-feeding [86]. Numerous reports have demonstrated that fructose feeding lowers the expression and activity of PPARα [87–91] and leads to a reduction in target gene expression and ultimately lower FAO. For example, Nagai et al., demonstrated that eight weeks of high-fructose feeding in rats decreased PPARα mRNA and CPT1α protein levels, which can be partially restored by treatment with the PPARα agonist fenofibrate [87]. However, unraveling the mechanisms by which fructose alters PPARα signaling warrants further investigation.

In addition to transcriptional regulation, fructose may also impede FAO by altering the post-translational modification (PTM) of proteins involved in FAO and leading to their decreased enzymatic activity and/or protein stability. Acetylation is the most common nutrient dependent PTM of mitochondrial proteins. We have published that fructose or glucose supplementation uniquely alters acetylation of mitochondrial proteins in mice on either chow or HFD [92]. Further, it has been shown that fructose reduces Sirt1, a major protein deacetylase, and leads to acetylation and inactivation of peroxisome proliferator activated receptor gamma coactivator 1α (PGC1α), resulting in reduced FAO [93]. In our studies of the combined intake of fructose and fat, we find that fructose increases the acetylation of mitochondrial proteins, specifically ACADL and CPT1α, which mediate FAO. These effects are dependent on KHK, as a knockdown of KHK via siRNA lowers ACADL acetylation and increases CPT1α protein [23,92]. Thus, fructose-induced acetylation of mitochondrial proteins contributes to the decreased FAO observed with dietary fructose intake.

Here we present a large amount of data that fructose decreases FAO in human and rodent studies. The effects of fructose are mediated, in part, via faster fructose metabolism producing greater carbohydrate oxidation, high lipogenic potential of fructose stimulating hepatic malonyl-CoA, lower PPARα transcriptional activity and fructose-induced acetylation of mitochondrial proteins (Fig. 1). Based on these studies, we propose that the fructose component of dietary sugar decreases mitochondrial fatty acid oxidation and consequently augments HFD-induced lipotoxicity. This hypothesis explains why fructose intake on a HFD, but not on low-fat diet, leads to cumulative detrimental effects in terms of obesity and the development of metabolic complications.

5. A review of the mitochondrial β-oxidation pathway

Next, we will provide a detailed review of fatty acid oxidation pathway in the liver (Fig. 2). Adipose tissue lipolysis provides the major source of fatty acids delivered to the liver [94]. Fatty acids bound to albumin circulate in the blood and are taken up into the liver via plasma membrane-associated proteins. Several transporters mediate fatty acid uptake in the liver, such as fatty acid translocase (FAT, aka CD36), liver-fatty acid binding protein (L-FABP), caveolins, and fatty acid transport proteins (FATPs). CD36 plays a major role in fatty acid uptake in heart and skeletal muscle. Its expression is relatively low in the liver, but it is highly inducible by lipid overload [95]. Moreover, increased hepatic expression

of CD36 in mice contributes to the dyslipidemia associated with diet-induced obesity [96]. Expression of L-FABP is very high in the liver and it may represent 2–5% of cytosolic protein [97]. L-FABP levels are elevated in NAFLD patients [98], whereas silencing of L-FABP ameliorates hepatic steatosis in mice [99]. Caveolins (types 1–3) were initially characterized as cholesterol transporters and later implicated in fatty acid transport [100]. Mice deficient in caveolin-1 show reduced fat accumulation in the hepatocytes. Together, CD36, L-FABP Caveolin-1, and calcium-independent membrane phospholipase A2 are thought to form a heterotetrameric protein complex within the hepatocyte plasma membrane to promote fatty acid uptake [101]. Another pathway that facilitates fatty acid uptake across cellular plasma membrane is via FATPs. There are six members of this family, but only FATP2 and FATP5 contribute to lipid transport in the hepatocytes [102]. FATP5 is localized on the plasma membrane, while FATP2 is found in the endoplasmic reticulum [103]. These proteins possess translocase activity, but also esterify fatty acids into fatty acyl-CoAs [104]. Fatty acids activated into their acyl-CoA thioesters are unable to passively diffuse out of the cell and are committed to utilization within the cell. This step is of paramount importance to the subsequent partitioning of fatty acids into unique metabolic processes so that FATPs have been renamed for their acyl-CoA synthesis activity, such that FATP2 is also known as very long-chain acyl-CoA synthetase 1 (ACSVL1), and FATP5 as ACSVL6. There are 26 enzymes that possess acyl-CoA synthetase activity and this is highly regulated process dependent on the nutrient status. Once synthesized, acyl-CoAs support a variety of cellular processes such as the synthesis of complex lipids including triglycerides, phospholipids, and cholesterol esters. On the other hand, they can be converted to acyl-carnitines, which is a process that commits them to oxidation and energy production.

Indeed, conversion of acyl-CoAs into acyl-carnitines is the rate-limiting step of fatty acid oxidation. Mitochondrial β-oxidation is the primary site in the liver for oxidation of short, medium, and long-chain fatty acids. Carnitine palmitoyltransferase 1 (CPT1) catalyzes the conversion of long chain (12–18 carbons) acyl-CoAs into acyl-carnitines. CPT1 is localized on the outer mitochondrial membrane and is the rate-limiting enzyme of mitochondrial FAO. There are three CPT1 isoforms encoded by different genes. CPT1α is mainly found in the liver, CPT1β localizes to the muscle and adipose tissue, while CPT1c is present in the brain and testes. Due to its paramount importance, CPT1α is regulated at many levels. As previously mentioned, CPT1α is transcriptionally governed by PPARα and post-translationally regulated by malonyl-CoA and insulin. Its activity is also dependent on dimer and tetramer assemble [105], as well as acetylation of its lysine residues [23]. Long chain acyl-carnitines made by CPT1α are transported across the mitochondrial membrane by carnitine-acylcarnitine translocase (CACT). Inside the mitochondrial matrix acyl groups are transferred back to CoA, and free carnitine is generated by the enzyme CPT2. CPT2 in the mitochondrial matrix catalyzes the reverse reaction of CPT1 in the cytosol. Although CPT1 accounts for 80% of control over the pathway flux [106], recycling of carnitine via the carnitine shuttle is crucial for FAO to work properly. Additionally, carnitine is shuttled back to the mitochondrial inter-membrane space by CACT and then passively diffuses across the outer mitochondrial membrane into the cytosol, so that the cycle can begin anew. Cytoplasmic carnitine levels are also mediated by organic cation/carnitine transporter 2

(OCTN2), which transports carnitine from the plasma across the hepatocyte cytoplasmic membrane. Plasma carnitine levels are mainly a reflection of dietary L-carnitine intake.

Inside the mitochondrial matrix, newly transported acyl-CoAs undergo progressive shortening by oxidative removal of two carbon (acetyl) units via a four-step process. This process is termed mitochondrial beta oxidation. The first step is mediated by acyl-CoA dehydrogenases (ACAD). Dependent on acyl-CoA chain length, short, medium, long, and very long chain acyl-CoA dehydrogenases catalyze the production of 2-enoyl-CoA. ACAD9 has been more recently recognized as acyl-CoA dehydrogenase *i.e.,* active in brain tissue. This dehydrogenation step utilizes FAD + to make FADH2, which subsequently donates electrons to complex II of the mitochondrial electron transport chain. The second step is mediated by 2-enoyl-CoA hydratases (ECH) and produces 3-hydroxyacyl-CoA. There are two ECH enzymes: short-chain (ECHS1) and long-chain, which is a part of trifunctional protein (HADHA). This hydration step reduces a double bond between carbons two and three of the acyl-CoA chain. The third step is another dehydrogenation reaction mediated by 3-hydroxyacyl-CoA dehydrogenase (HADH) to produce 3-ketoacyl-CoA. It also reduces NAD + to NADH which donates electrons to complex I to support ATP synthesis. Similar to ECH, there are short- (SCHAD) and long-chain HADHs (HADHA). The fourth and final step of the beta cycle is mediated by acetyl-CoA acyltransferase 2 (ACAA2) to produce acetyl-CoA and shortened acyl-CoA chain. There are short-, medium- (ACAA2) and long-chain (HADHB) enzymes that possess thiolase function, although the short-chain may not play a role in beta oxidation. The last three reactions, which metabolize longer chain acyl-CoAs, are catalyzed by the multi-domain mitochondrial trifunctional protein (MTP). MTP is made of eight subunits. Four alpha subunits are encoded by the HADHA gene, and four beta subunits are produced from the HADHB gene. The alpha subunits harbor long-chain enoyl-CoA hydratase and long-chain 3-hydroxyacyl-CoA dehydrogenase enzymes. The beta subunits contain long-chain acetyl-CoA acyltransferase enzyme. In summary, once cycle of beta oxidation yields a fatty acyl-CoA shortened by two carbons, one acetyl-CoA molecule, and two electrons. The cycle is repeated until two acetyl-CoA molecules remain. Acetyl-CoA can be further reduced in the TCA cycle to generate citrate and additional electrons to support ATP production. In the liver, acetyl-CoA may be also be converted to ketone bodies that are used during fasting as energy source in other tissue.

6. The effects of fructose on import, activation, transport and oxidation of FFA

First, we will examine the effects of fructose on fat transporters. CD36 knockout mice show improved glucose uptake in the muscle on normal chow diet; however, as expected, they develop glucose intolerance and insulin resistance on a HFD. As previously stated, CD36 is not abundant in the liver as compared to the muscle, so whole body CD36 KO results in increased hepatic fatty acid uptake. Interestingly, CD36 KO mice develop worse glucose intolerance and insulin resistance on a high-fructose diet as compared to a HFD, suggesting that fructose decreases fatty acid oxidation in these mice [107]. On the other hand, L-FABP KO mice are protected from high-fat and high-fructose diet-induced NAFLD and liver fibrosis. This provides further evidence that fructose intake is insufficient to induce

NAFLD without adequate fat supply to the liver [108]. While there is no direct evidence that dietary fructose impairs hepatic fat transporters, the perturbations that result in increased or decreased fat transport in the liver dictate the metabolic outcomes of a high-fructose diet.

There is direct evidence that fructose downregulates activation of fatty acids to their corresponding acyl-CoAs in the liver. Dong et al., showed that a high-fructose diet markedly reduces the protein and mRNA expression of ACSL3 in hamster livers [109]. Pharmacologic activation of ACSL3 in these mice reduced hepatic triglyceride accumulation. Others have also show that the expression of several ACSL isoforms is differentially controlled by fasting followed by refeeding a 69% sucrose diet [110]. For example, fasting increased ACSL1 and ACSL4 mRNA abundance in the liver, while refeeding fructose decreased their expression. Conversely, ACSL3 and ACSL5 are reduced in fasted mice, and their mRNA is restored by feeding a high-fructose diet [110]. Based on these studies it can be concluded that fructose decreases activation of specific ACSL, which likely function to shunt fatty acyl-CoA towards mitochondrial oxidation.

We have already presented multiple studies documenting strong downregulation of CPT1α by dietary fructose (Table 1). We [23] and others [28] have shown that these effects are dependent on KHK mediated fructose metabolism [111]. We could not find evidence that fructose directly affects other enzymes in the carnitine shuttle, such as CACT and OCTN2. However, treating fructosefed rats with carnitine is sufficient to reverse fructose-induced metabolic derangements [112]. Similarly, L-Carnitine supplementation in mice attenuates fructose-mediated lipid accumulation, counteracts mitochondrial damage, and decrease the production of reactive oxygen species [113]. Together this evidence shows the strong propensity of fructose to lower fatty acid oxidation by decreasing acyl-carnitine production, an effect that can be reversed by carnitine supplementation.

The reports documenting the direct effects of fructose on enzymes mediating the four steps of mitochondrial β-oxidation are lacking. In summary, we can conclude that fructose inhibits FAO by decreasing CPT1α-mediated acylcarnitine production and activation of fatty acids into specific acyl-CoAs, while the data is lacking that fructose directly alters fatty acid uptake and the enzymes mediating mitochondrial β-oxidation. Thus, the profound effects of fructose to decrease FAO can be explained by decreased acylcarnitine production to account for the additive effects of fructose and a HFD on NAFLD pathogenesis.

7. Is FAO impaired in patients with NAFLD?

Obesity is a major risk factors for development of NAFLD [114,115]. We propose that a mechanism by which a Western diet induces NAFLD is mediated, in part, via a fructose-induced decrease in fat oxidation. Dietary fructose clearly increases the risk of developing a more severe form of NAFLD [116,117], but is there evidence that FAO is impaired in patients with NAFLD? This question is challenging to answer due to difficulty in measuring FAO, thus leading to conflicting reports. Naguib et al., reported that FAO of orally-delivered ^{13}C-labeled palmitate, as a part of mixed meal test, is decreased in NAFLD patients compared to healthy controls [118]. The authors suggest that this is likely due to decreased mitochondrial β-oxidation and propose the use of a ^{13}C-palmitate breath

test to noninvasively assess FAO. Another study also found reduced hepatic fat oxidation in patients with NAFLD [119]. Conversely, Kotronen et al., published that whole-body lipid oxidation is increased because of peripheral insulin resistance, but that hepatic lipid oxidation is unchanged in NAFLD patients [120]. They used labeled glucose, indirect calorimetry, and a euglycemic hyperinsulinemic clamp to measure metabolic flux in patients with NAFLD. However, some studies have even found that fatty acid oxidation is higher in patients with non-alcoholic steatohepatitis (NASH) [121–123]. In spite of the lack of clear evidence from tracer studies that FAO is impaired in NAFLD, it has been reported that PPARα is downregulated in patients with advanced fatty liver disease compared to those with simple steatosis [124], and that PPARα expression negatively correlates with severity of NAFLD. Similarly CPT1α has been documented to be decreased in patients with NAFLD [125]. Therefore, approaches to increase FAO are currently being investigated as treatment options for NAFLD [126,127].

Elafibranor, is a dual PPARα and δ agonist that was clinically tested for treatment of NAFLD. It showed promising results in a phase 2b clinical study [128]. However, in a phase 3 clinical trial in patients with NASH, elafibranor did not meet the predefined primary endpoint and has been discontinued. Next in line, the new generation pan-PPAR agonist, lanifibranor, has been subsequently shown to lower steatosis, inflammation, and fibrosis in humans and mice [129,130]. Lanifibranor is currently in stage 3 clinical trials studying its effectiveness in improving NASH and fibrosis. Similarly, gene therapy to increase CPT1α activity and hepatic fatty acid oxidation has proved effective to decrease hepatic steatosis in mice [131]. Together, these studies suggest that restoring fructose-induced decrease in hepatic fatty acid oxidation may be a potentially promising strategy to treat NAFLD.

8. Conclusions

Fructose is a highly lipogenic nutrient that has been implicated in the development of obesity and its complications, such as NAFLD. Here we present a large body of evidence demonstrating that fructose also decreases dietary fat oxidation. In spite of the strength and abundance of the evidence, this aspect of fructose metabolism has been largely understudied and often overlooked. However, the enormous relevance of these findings to those affected by consumption of a Western diet warrant further investigation. Future studies are needed to elucidate the mechanism of how dietary fructose decreases PPARα and CPT1α function. These effects are not likely to be mediated by malonyl-CoA in the liver, but could be secondary to protein acetylation leading to decreased PPARα transcriptional activity and lower CPT1α protein levels. A deeper understanding of this not so simple sugar metabolism may offer new treatment options for management of obesity-associated complications.

Funding

This work was also supported by NASPGHAN Foundation Young Investigator Award, Pediatric Scientist Development Program Award (HD0 0 0850) and COCVD Pilot and Feasibility Grant (GM127211) awarded to SS. National Institutes of Health grants K01DK128022 and NIH National Center for Advancing Translational Sciences through grant number UL1TR001998 awarded to RNH.

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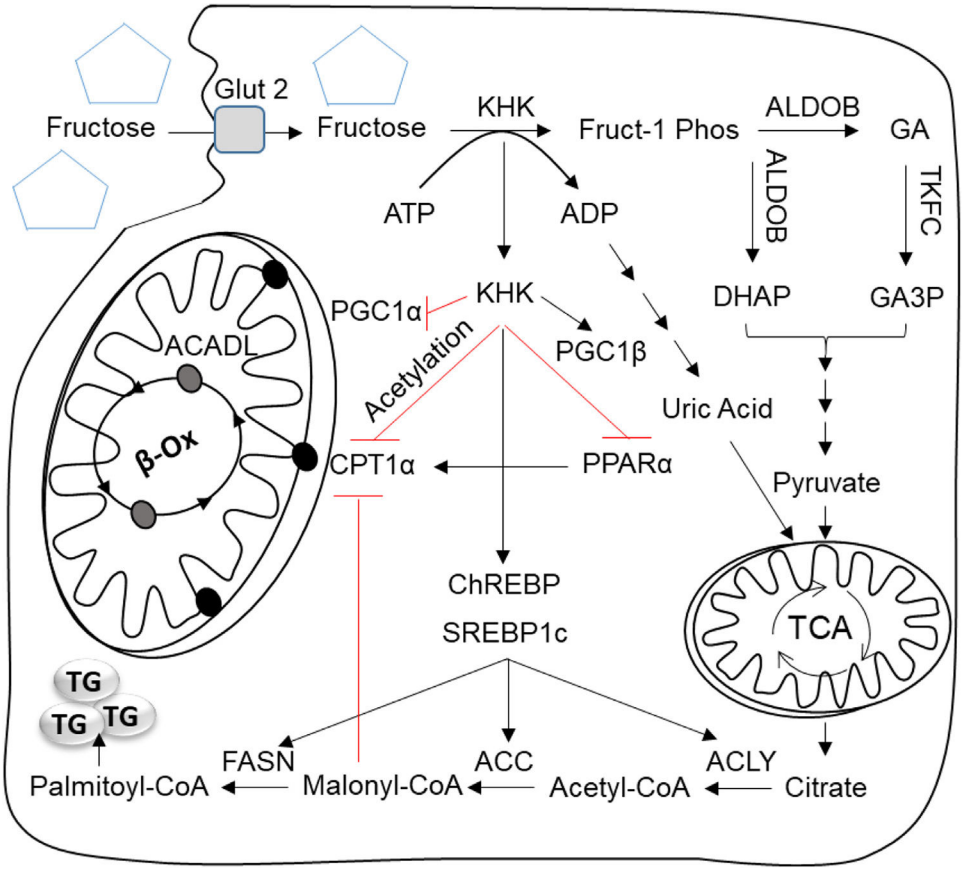

Fig. 1.

The effect of fructose on lipogenesis and mitochondrial oxidation. In the liver fructose is taken up by solute carrier family 2, facilitated glucose transporter, member 2 (SLC2A2 aka GLUT2) and phosphorylated by ketohexokinase (KHK) to fructose-1 phosphate (Fruct-1 Phos). KHK mediated fructose phosphorylation is rapid leading to depletion of adenosine triphosphate (ATP), accumulation of adenosine diphosphate (ADP) and eventually production of uric acid. Fruct-1 Phos is further metabolized by aldolase b (ALDOB) into dihydroxyacetone phosphate (DHAP) and glyceraldehyde (GA). GA is then phosphorylated by triokinase and FMN cyclase (TKFC) into glyceraldehyde-3 phosphate (GA3P). DHAP and GA3P are also intermediates of glycolysis pathway and they are metabolized into pyruvate. Pyruvate enters the tricarboxylic acid cycle (TCA) to produce electrons for energy production. When the cellular energy stores are plentiful citrate is transported out of mitochondria into the cytosol to serve as a substrate for de novo lipogenesis (DNL). Uric acid produced by fructose metabolism inhibits aconites, an enzyme in TCA cycle, to further increase citrate production. In the cytosol, citrate is converted to acetyl-CoA by the enzyme ATP citrate lyase (ACLY). Acetyl-CoA carboxylase (ACC1), then catalyzes the production of malonyl-CoA and fatty acid synthase (FASN) extends the growing fatty acid chain to synthesize palmitoyl-CoA, a major building block of triglyceride (TG) formation. Malonyl-CoA, an intermediate in DNL also inhibits carnitine palmitoyltransferase 1 alpha (CPT1α) the rate limiting enzyme of mitochondrial fatty acid oxidation (FAO). In addition to providing substrate for DNL fructose through KHK affects the function of

transcription factors mediating DNL and FAO. KHK and carbohydrate responsive element binding protein (ChREBP) regulate each other via a bidirectional loop, and upregulated ChREBP increases the expression of genes involved in DNL. Fructose through KHK also upregulates another lipogenic transcription factor, sterol regulatory element-binding protein 1c (SREBP1c), either directly via PPARG coactivator 1 beta (PGC1β) or indirectly by inducing selective insulin resistance and hyperinsulinemia. We present additional evidence that fructose through KHK decreases FAO. These effects are mediated, in part, via lower peroxisome proliferator activated receptor alpha (PPARα), a transcription factor that regulates expression of FAO enzymes, leading to decreased CTP1α protein. Additionally, fructose decreases PGC1α, a cofactor required for optimal PPARα activity via increasing PGC1α acetylation. Lastly, our research indicates that fructose through KHK increases CPT1α acetylation and decreases its protein stability.

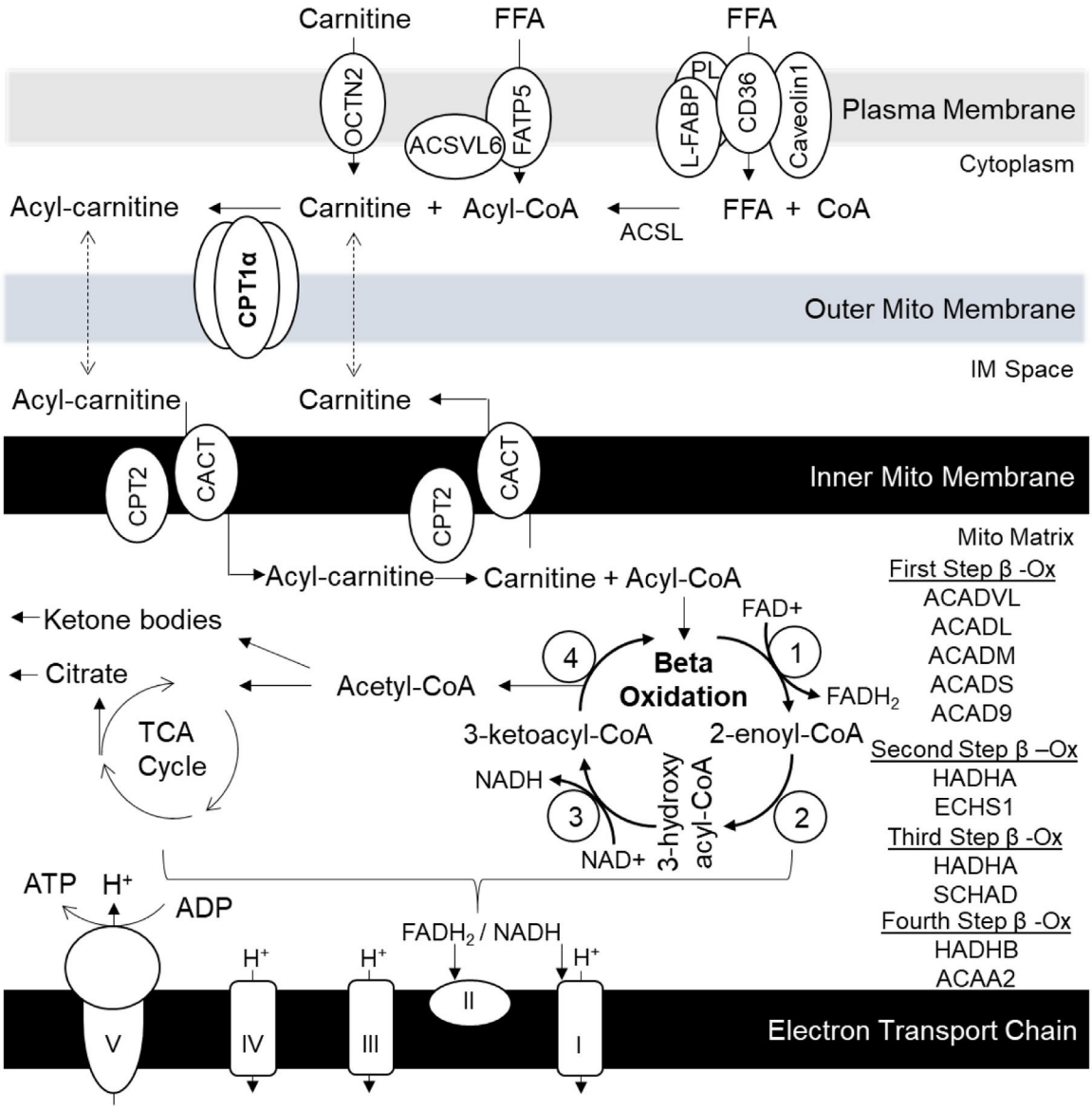

Fig. 2.

The Pathway of Mitochondrial Fatty Acid Oxidation in the Liver. Long-chain fatty acids are transported into the liver via a complex of proteins consisting of fatty acid translocase (FAT, aka CD36), liver-fatty acid binding protein (L-FABP), caveolins and phospholipase A2 (PL). In the cytosol fatty acids are activated into fatty acyl-CoA by the actions of long-chain acyl-CoA synthetases (ACSL). Acyl-CoAs are unable to diffuse out of the cell and are committed to different cellular processes such as lipid synthesis or oxidation. The metabolic fate of acyl-CoAs is likely mediated by unique ACSL isoforms. Another pathway that mediates fatty acid import into the hepatocytes is via fatty acid transport protein 5 (FATP5). This enzyme complex additionally contains very long-chain acyl-CoA synthetase 6 (ACSVL6) activity to generate Acyl-CoA. Acyl-CoAs designated for energy production are conjugated to carnitine by carnitine palmitoyltransferase 1 alpha (CPA1a). This is the rate limiting enzyme of fatty acid oxidation accounting for 80% of the

control over the pathway. Carnitine is transported into the hepatocytes by OCTN2. Acyl-carnitines can cross outer mitochondrial membrane and are transported across the inner mitochondrial membrane by carnitine-acylcarnitine translocase (CACT) in an exchange for free carnitine transported from mitochondrial matrix into inter membrane space. Inside the mitochondrial matrix carnitine palmitoyltransferase 2 (CPT2) converts acyl-carnitines into free carnitine and acyl-CoA. Beta oxidation involves a four-step process where long chain acyl-CoA are progressively shortened by two carbons to generate acetyl-CoA and two electrons. The first step dehydrogenates acyl-CoA into 2-enoly-CoA, and is mediated by acyl-CoA dehydrogenases (ACADs) of different chain lengths. This dehydrogenation step yields $FADH_2$, which donates electrons to complex II of the mitochondrial electron transport (ETC) chain for ATP production. The next three steps are mediated by mitochondrial trifunctional protein (MTP). The second step is hydration step and it produces 3-hydroxyacyl-CoA by the action of 2-enoyl-CoA hydratases (ECH). The third step is another dehydrogenation step mediated by 3-hydroxyacyl-CoA dehydrogenase (HADH) to produce 3-ketoacyl-CoA. This dehydrogenation step yields NADH, which donates electrons to complex I of the mitochondrial ETC chain for ATP production. The fourth step is mediated by acetyl-CoA acyltransferase 2 (ACAA2) to produce shortened acyl-CoA chain and acetyl-CoA. Acetyl-CoA can be further reduced in tricarboxylic acid (TCA) cycle and it yields citrate when energy stores are plentifully. During fasting, acetyl-CoA may be converted to ketone bodies that are used during fasting as energy source in other tissue.

J Nutr Biochem. Author manuscript; available in PMC 2024 April 24.

Table 1

Studies documenting fructose-induced decrease in fatty acid oxidation.

Model	Diet comparison	Dose & duration	Fatty acid oxidation	Mechanism	Refs
Humans	Fructose or Glucose, 75g	A single drink, 4h metabolic monitoring	-Higher RER	N/A	Tappy, 1986 [76]
Humans	Fructose or Glucose in a drink containing fat	A single drink containing 0.75 g sugar/kg body weight and 0.5g/kg of oil	-Higher RER -Higher $^{13}CO_2$ from labeled fructose -lower BHB	N/A	Chong, 2007 [77]
Humans	Fructose drink in addition to regular diet *vs.* regular diet alone	25% additional calories from fructose (3.5mg/kg dose) for 6 d	-Decreased lipid oxidation mg/kg/min and -BHB only in male subjects	N/A	Couchepin, 2008 [78]
Humans	Fructose or Glucose-drinks on *ad libitum* diet	25% of energy requirement on normal diet containing 30% fat for 10-wk	-Decreased postprandial fat oxidation and -increased carbohydrate oxidation	N/A	Cox, 2012 [40]
Perfused Rat Liver	Fructose	25 and 45 mg fructose/100 mL of blood	Decreased ^{14}C incorporation into CO_2 and lower ketone bodies.	N/A	Topping 1972 [81]
Rat Liver and serum	Fructose, Glucose, Glyceraldehyde, Sorbitol injection in fasted rats	1 mL of 30% fructose, glucose, glyceraldehyde, or sorbitol were injected intramuscular	Decreased in ketone bodies	N/A	Rawat 1975 [79]
Rat Liver	Fructose addition to mitochondria-supernatant system	5.56 mM fructose treatment along with ^{14}C labeled palmitate	Decreased conversion of ^{14}Cpalmitate to $^{14}CO_2$	N/A	Prager 1976 [80]
Rat Liver Isolated rat hepatocytes	High fructose diet 67% carbohydrate (98%fructose)	−8 wk of high fructose diet -Isolated hepatocytes in 25mM fructose	Decreased expression of FAO genes	-Reduced PPARα protein and activity -Decreased CPT1α	Nagai 2002 [87]
Rat Liver	Fructose drink on chow diet	10% fructose in water for 2 wk	Decreased β-oxidation activity nmol/min/mg	Decreased PPARα and target gene CPT1α	Roglans 2002 [91]
Rat Liver	Fructose or glucose drink on normal diet	10% fructose or glucose in water for 14 d	Decreased β-oxidation activity nmol/min/mg	Decreased PPARα and CPT1α protein & mRNA	Roglans 2007 [88]
Rat Liver & human hepatocytes	Sucrose in rats/fructose in hepatocytes	40% sucrose diet for 10 wk/ 5mM fructose in vitro	Decreased BHB	AMPD2 mediated decrease in AMPK activity	Lanaspa 2012 [132]
Rat Liver Rat hepatoma cells	Fructose drink on of regular diet	−10% fructose drink for 14 d;	Decreased β-oxidation activity	-Decreased PPARα and Sirt1	Rebollo 2014 [93]
Human hepatocytes	In vitro fructose, glucose and mannitol	−25 mM fructose for in vitro experiments	nmol/min/mg	-Increased acetylation of PGC1α	
Rat Liver	Fructose supplementation of regular diet	20% fructose solution for 14 wk	Decreased FAO gene expression	DNA methylation at PPARa and CPT1A promoter regions	Ohashi 2015 [90]

Model	Diet comparison	Dose & duration	Fatty acid oxidation	Mechanism	Refs
Mouse liver and Hepatocytes	30% Fructose or Glucose drinks on chow and HFD	In vitro 25 mM fructose vs. glucose for 24 hr	Decreased β-oxidation (OCR pmol/min) with 25 mM fructose	Acetylation of metabolic enzymes dependent on KHK	Softic 2019 [23]
Mouse liver	15% or 30% fructose in water on chow diet	Wild type and KHK A/C KO mice treated for 25 wk	Decreased BHB with fructose	KHK KO restored BHB	Ishimoto 2012 [28]