

The Role of High-Fructose Diet in Liver Function of Rodent Models: A Systematic Review of Molecular Analysis

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ABSTRACT

The present systematic review of animal studies on long-term fructose intake in rodents revealed a significant decrease in the activities of antioxidant enzymes due to a fructose-rich diet. The reduced activity of these enzymes led to an increase in oxidative stress, which can cause liver damage in rodents. Of eight studies analyzed, 5 (62.5%) and 1 (12.5%) used male and female rats, respectively, while 2 studies (25%) used female mice. Moreover, half of the studies used HFCS, but the other half employed fructose in the diet. Hence, it is essential to monitor dietary habits to ensure public health and nutrition research outcomes. **DOI: 10.52547/ibj.3965**

Keywords: Animal model, Fructose, Liver, Liver function tests, Oxidative stress

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INTRODUCTION

Fructose is naturally found in honey and sweet fruits, although for commercial purposes, it is typically produced in crystalline form or as syrup. Corn syrup is a common commercial product of fructose that has increasingly become popular in recent years and contains around 55% of free fructose. Over the past two decades, the use of HFCS as a sweetener in beverages and processed foods has raised by 20-30%^[1,2].

Hyperglycemia and insulin resistance are two risk factors for T2DM^[3]. This disorder can cause various hepatic injuries and abnormalities, including hepatitis, NAFLD, nonalcoholic steatohepatitis, cirrhosis, and hepatocellular carcinoma^[4,5]. The increased use of artificial sweeteners such as fructose and HFCS in processed foods might be related to the occurrence of these disorders.

Multiple preclinical and clinical studies have suggested a potential link between high fructose consumption and metabolic syndrome^[6]. It has also been demonstrated that excessive consumption of fructose by healthy individuals over a short period of time can predispose the individuals to be overweight. The harmful effects of fructose on health are attributed to how it is metabolized when consumed in food^[1,6,7]. High fructose intake has been associated with fatty liver disease, although the underlying mechanisms are not yet fully understood^[8]. Interestingly, a study has found that high-fructose diets can lead to significant effects and changes in various liver metabolism pathways^[1]. Apart from alterations in the transfer function of fatty acids, VLDL-TG assembly, cholesterol metabolism, and multiple molecular changes have been identified due to high-fructose diets. These changes include modifications in molecular chaperones involved in

List of Abbreviations:

Acc: acetyl-CoA carboxylase; **Alox3:** arachidonate lipoxygenase-3; **Alox8:** arachidonate 8-lipoxygenase; **CAT:** catalase; **ER:** endoplasmic reticulum; **Fas:** fatty acid synthase; **GRP78:** glucose-regulated protein; **GSH:** glutathione or L-γ-glutamyl-L-cysteinyl-glycine; **HFCS:** high-fructose corn syrup; **IL:** interleukin; **Insig:** insulin-induced gene; **MDA:** malondialdehyde; **MESH:** Medical Subject Headings; **mtDNA:** mitochondrial DNA; **MUFA:** monounsaturated fatty acid; **NAFLD:** nonalcoholic fatty liver disease; **PDI:** protein disulfide isomerase; **PPARα:** peroxisome proliferator activated receptor alpha; **PRISMA:** Preference Reporting Guidelines for Systematic Review and Meta-Analysis; **SOD:** superoxide dismutase; **TBARS:** thiobarbituric acid-reactive substance; **TG:** triacylglycerol; **T2DM:** type 2 diabetes; **UPR:** unfolded protein response; **VLDL-TG:** very low-density lipoprotein triglyceride

protein folding in the ER, and alteration in the protective activity of antioxidant. It has been observed that the high intake of fructose can have an adverse effect on liver metabolism, especially in the context of liver enzyme activity^[1,9]. The present study suggests a molecular-based approach that explains metabolic changes resulting from high-fructose diet.

Recent epidemiological studies have demonstrated a strong link between intrahepatic fat accumulation and saturated fats, carbohydrates, simple sugars, and trans fatty acids^[10]. However, further investigation is needed to fully understand relationship between specific nutrients containing sugars and specific nutrients containing fructose. From hepatology perspective, understanding the molecular effects of long-term fructose consumption on liver injury is of high importance. While rodent models have extensively been used in research and contributed immensely to the understanding of human diseases, there are still some uncertainties and limitations that need consideration. The authors of this study aimed to provide a comprehensive review of the outcomes of liver damage caused by high-fructose consumption. This survey could help to better understand the impact of high-fructose consumption on liver health and inform future research and potential strategies to prevent or treat liver damage. By better understanding the underlying molecular mechanisms, researchers can develop more effective treatments for patients suffering from liver-related illnesses.

METHODOLOGY

The present systematic review was conducted based on the PRISMA. Literature searches were carried out by two independent reviewers (from 1985 to June 2023) in four international databases, comprising Web of Science, PubMed, Embase, and Scopus. In searches, we used the MESH terms and other related keywords shown in [Supplementary Table 1](#).

Inclusion and exclusion criteria

Two independent reviewers were responsible for the selection of all animal studies. In case of disagreement, a third reviewer was consulted. By a refined search strategy, we removed duplicate studies, and by further screening, we eliminated irrelevant studies after assessing their titles, abstracts, and full texts. It is important to know that mice share many anatomical, physiological and genetic similarities with humans. The use of rodent models in preclinical research has played a vital role in advancing our comprehension of human diseases, allowing researchers to explore underlying mechanisms and potential therapies that may not be feasible to directly study on humans. By conducting genetic research on mice, scientists gain insights into the

biology of human disease and develop safe and effective treatments. Development of molecular biology has enabled researchers to extrapolate insights from rodent research and understand the molecular changes that cause liver injuries, paving the way for a better understanding of liver metabolism pathways and how they respond to different diets, including high-fructose diets. Following the investigation of underlying molecular mechanisms, they also can gain a better understanding of the pathogenesis of these diseases, which assists them to develop new treatments targeting specific molecular pathways, thereby revolutionizing the treatment of liver diseases. The inclusion criteria for this study were quite specific and limited to rodents that were exposed to either HFCS or a high-fructose diet, without any mixture. This review excluded studies in which species other than rodents were used. We also excluded a variety of research types, such as case studies, case series, reviews, editorials, conference reports, letters, human and in vitro studies, and epidemiological studies, which assessed the effects of on toxicity rather than liver toxicity.

Data extraction

Data were extracted based on a pre-defined checklist. The extracted data used for qualitative evaluation included the date and topics of study, author's name, sample size of the groups, information about the animals (species and genus), amount and routes of administration, age and gender of the rodents, duration of the study, methods of assessment, molecular findings, and the main findings of each study. A summary of the data extraction checklist is shown in the Table 1.

Quality assessment

The Joanna Briggs Institute Critical Appraisal tools was employed to evaluate the quality of the selected articles. Only studies answered yes to at least 60% of the questions on the specific checklist were included. This assessment ensured that we selected only high-quality articles for our systematic review. The authors carefully evaluated the quality of all articles and resolved discrepancies or disagreements by discussion.

RESULTS

Study selection

Following our search strategy, 127 published articles were identified. After removing 20 duplicates, 107 abstracts were selected for a more detailed review; 34 papers were excluded after screening for title and abstracts and 31 articles, including letters and review articles, were also removed. Eight articles were finally selected from the remaining 42 relevant papers for the current systematic review (Figs. 1 and 2).

Table 1. Summary of data extraction checklist

No.	Topics	Number of test group	Control group	Species /gender of animals	Amount of fructose/ HFCS	Administration routes	Duration of study	Molecular analysis/gene expression	Evaluation of exposure and outcome	Country	Authors/Date
1	Fructose-rich diet affects mtDNA damage and repair in rats	Two groups (five per group)	Yes	Male rats (Sprague Dawley)	HFCS: 20% solution	Oral- drinking water	8 weeks	Yes	Mitochondrial DNA damage A fructose-rich diet leads to mitochondrial damage (mtDNA), which may play a role in liver dysfunction and metabolic disease.	Italy	Cioffi 2017 ^[11]
2	<i>Globularia alypum</i> aqueous extract decreases hypertriglyceridemia and ameliorates the oxidative status of the muscle, kidney, and heart in rats fed a high-fructose diet.	Four groups (six per group)	Yes	Male rats (Wistar)	61(g/100 g)	Oral	14 weeks	Yes	Hypertriglyceridemia High-fructose intake in the diet causes hypertriglyceridemia.	Algeria	Taleb-Dida 2011 ^[12]
3	Preventive effect of <i>Catharanthus roseus</i> (Linn.) against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats	Four groups (eight per group)	Yes	Male rats (Albino Wistar)	High-fructose (66%) diet	Oral-fed with fructose diet	60 days	Yes	Oxidative stress and insulin resistance In fructose-fed rats, hepatic antioxidants and oxidative stress were observed.	India	Karuna 2011 ^[13]
4	Effects of sucrose and HFCS consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats	38 dolescents + 38 adults (12-13 per group)	Yes	Male rats (Sprague-Dawley)	HFCS-55 solution, prepared to contain equal carbohydrate weight/volume (11%)	Oral	30 days	Yes	Neurological and memory disorders Consumption of extra sugars in the diet, especially HFCS-55, has a negative effect on hippocampal function, metabolism reactions and neuro-inflammation in case of high-dose intake of fructose during puberty and growth	USA	Hsu 2015 ^[14]

No.	Topics	Number of test group	Control group	Species /gender of animals	Amount of fructose/ HFCS	Administration routes	Duration of study	Molecular analysis/gene expression	Evaluation of exposure and outcome	Country	Authors/Date
5	The severity of rat liver injury by fructose and high fat depends on the degree of respiratory dysfunction and oxidative stress induced in mitochondria	Four groups (four per group)	Yes	Male rats (Wistar)	with 25% fructose in the drinking water	Oral	6 weeks	Yes	Effect on the liver Fructose-induced liver damage was related to the extent of dysfunction and oxidative damage in mitochondria.	Mexico	García-Berumen 2019 ^[15]
6	HFCS-55 consumption alters hepatic lipid metabolism and promotes triglyceride accumulation	Four groups (seven per group)	Yes	Female rats	Water sweetened with 13% (w/v) HFCS-55	Oral	8 weeks	Yes	Effect on hepatic lipid metabolism Excessive consumption of sweetened beverages and the results of gene expression and analysis of fatty acid compounds showed that some sugars were more harmful to the liver in the hypercaloric state. Consumption of HFCS-55 should be limited.	USA	Mock 2017 ^[16]
7	Effect of Dietary Monosodium Glutamate on HFCS-Induced Hepatic Steatosis: Expression Profiles in the Liver and Visceral Fat	Four groups (20 per group)	Yes	Female mice	20% HFCS	Oral drinking water	Between 6 weeks of age until 32 weeks of age	Yes	Effect on liver This study suggests that HFCS may affect hepatic steatosis.	Saudi Arabia	Collison 2010 ^[17]
8	Endoplasmic reticulum stress is involved in hepatic SREBP-1 c activation and lipid accumulation in fructose-fed mice	22 mice (12 per group)	Yes	Female mice (CD-1)	Water containing 30% fructose	Oral	8 weeks	Yes	Effect on the liver Chronic fructose intake can trigger liver stress, which may be important in fructose-induced hepatitis.	China	Zhang 2012 ^[18]

Study characteristics

Eight studies, including experimental studies, were included in the systematic review. The selected articles were published between 2010 and 2019. In all the studies, rodents, including rats (male and female) and female mice, were examined.

Molecular analyses of high-fructose consumption in the liver

Male rats

Based on our search strategy, we found five studies in which molecular analyses were conducted on the liver of male rats. A study by Cioffi *et al.*^[11] showed that a fructose-rich diet significantly changed the levels of plasma 8-OHdG and CAT protein in the mitochondria of rat liver compared to the control rats; 8-OHdG was used as an indicator of oxidative DNA damage *in vivo* and *in vitro*. According to the above study, rats fed a high-fructose diet had a higher number of mtDNA lesions and an increasing mtDNA damage in their liver. Also, researchers observed a reduction in mtDNA copy number, indicating impaired mitochondrial biogenesis. Their findings revealed that a diet rich in fructose can cause mtDNA damage and affect mitochondria, which leads to liver dysfunction and metabolic diseases^[11]. According to a study performed by Taleb-Dida *et al.*^[12], CAT activity was not a sensitive test in the studied tissues, except for the liver, after consumption of a high-fructose diet for 14 weeks. However, high levels of TBARS and decreased SOD and CAT activities were found in the liver, which indicate an elevated oxidative stress in rats fed a high-fructose diet. Our findings suggest that fructose overfeeding can induce pro-oxidant effects by increasing TBARS levels and decreasing SOD and CAT activity in the liver. In Karuna and Saralakumari's^[13] study, reduced antioxidant enzymes activities decreased hepatic GSH levels and increased lipid peroxidation intermediates in the group fed fructose, which result in the development of oxidative stress in high-fructose diet-fed rats for 60 days. Moreover, rats in the fructose group showed significantly higher levels of TBARS and protein carbonyl groups and lower levels of hepatic GSH compared to the control rats. They also exhibited that the activities of antioxidants enzymes, particularly GR, GPx, GST, SOD, and CAT, were significantly lower in the fructose group rats than in the control group. Hsu *et al.*^[14] found that the exposure of adolescent rats to HFCS-55 within 30 days increased circulating insulin levels and hepatic proinflammatory cytokine expression. Overall, the data of their study showed significant side effects for excessive consumption of sugars, particularly HFCS-55, during critical periods of development, as well as highlighted

that metabolic disruptions could occur in adolescent who consume HFCS-55 and sucrose^[14]. In a study performed by García-Berumen *et al.*^[15], mitochondrial lipid peroxidation was exacerbated in the fructose-fed group. The group showed a low level of steatosis and no changes in ROS production, but high levels of mitochondrial lipid peroxidation. This observation shows that mitochondrial lipid peroxidation alone may not cause severe liver damage in the absence of other factors such as significant accumulation of fat or increased mitochondrial ROS production. The study also highlighted that induction of lipid peroxidation in the liver by fructose is linked to the depletion of antioxidant defenses^[15]. Despite producing high levels of lipid peroxidation, fructose had less deleterious effects on mitochondrial function and development of NAFLD. This reaction is because fructose partially decreases oxidative phosphorylation and induces a lower percentage of microvesicular steatosis, which can help protect it against the development of NAFLD. It is worth noting that the combination of a high-fat and high-fructose diet has more deleterious effects on mitochondria, including lipid peroxidation, increased ROS production, decreased complex I activity, and the full inhibition of oxidative phosphorylation. These findings are consistent with the severe liver damage induced by this diet^[15].

Female rats

Following our search, we found that only one study has examined the effect of high-fructose consumption on liver in female rats. According to Mock *et al.*^[16], rats that drank HFCS-55 had a significantly higher liver content of MUFA palmitoleic acid compared to those drinking sucrose and fructose solution. Additionally, rats drinking HFCS-55 solution had the highest hepatic content of MUFA palmitoleic acid among all the groups studied, which highlights the unique metabolic effects of HFCS-55 on the liver. PPAR α gene expression was downregulated in rats drinking HFCS-55 solution, as compared to the rats drinking water. The desaturation indices indicates a higher SCD-1 activity in rats consumed the HFCS-55 solution than the control group. Rats consumed HFCS-55 solution exhibited a pronounced hepatic *de novo* lipogenesis, as indicated by the upregulation of stearoyl-CoA desaturase-1 and increased oleic acid content. Such increased lipogenesis was accompanied by a decrease in β -oxidation, which is evident from the downregulation of hepatic peroxisome proliferator-activated receptor α . Researchers attributed the observed lipogenic effects in rats to the slightly higher fructose content of the HFCS-55 solution, despite that there is no difference in macronutrient and total caloric intake between the rats consumed HFCS-55 and

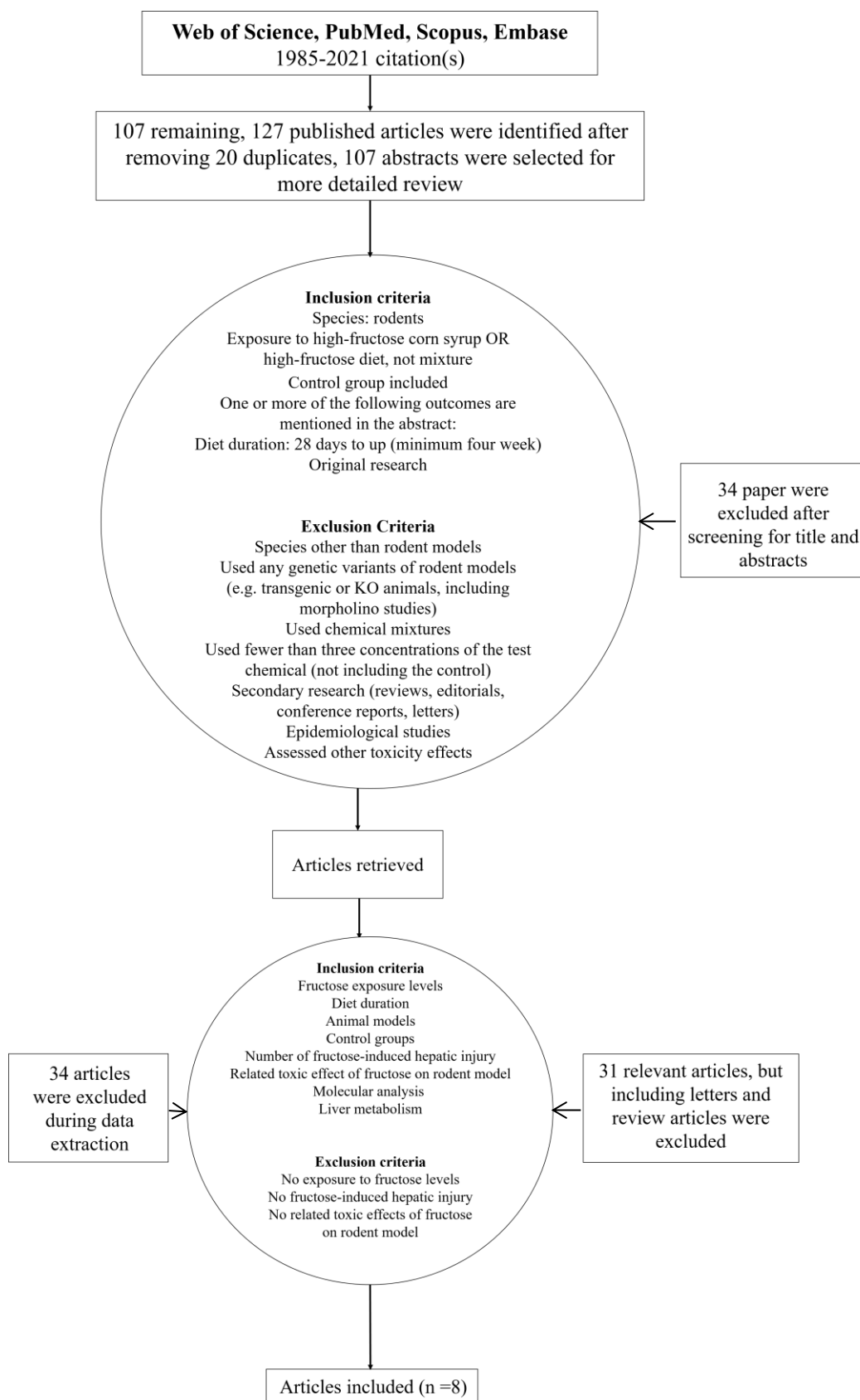


Fig. 1. Flow Diagram of study selection.

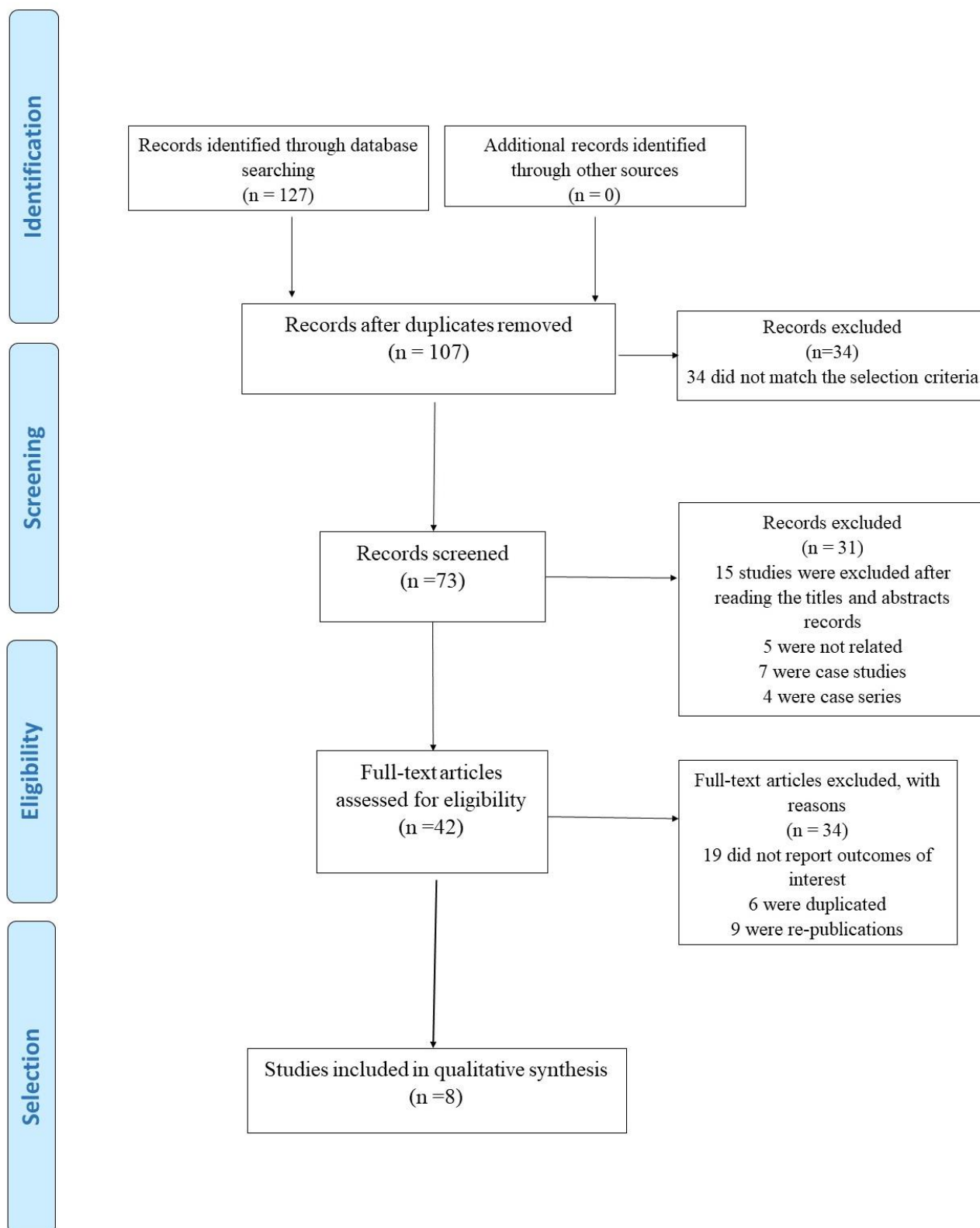


Fig. 2. PRISMA flowchart for included studies.

those consumed sucrose solution. Results from gene expression and fatty acid composition analysis showed that in a hypercaloric state, certain types of sugars are more detrimental to the liver. Results of this preclinical study showed that the excess consumption of caloric sweetened beverages, particularly those sweetened with HFCS, should be limited^[16].

Female mice

Based on our study strategy, we identified two studies investigating the hepatic effects of high fructose consumption on female mice. Collison *et al.*^[17] compared the gene expression profile in the liver and abdominal fat of animals fed with isocaloric standard chow under identical conditions with that of control animals. The consumption of HFCS resulted in hepatic steatosis and a significant increase in gene expression related to carbohydrate and lipid metabolism. The most significant impact on hepatic gene expression profile was observed in the mice fed with the HFCS diet. Enzymes involved in steroid and fatty acid metabolism from the cyp450 family, including oxidoreductases Cyp2b9 and Cyp2b10, were upregulated by HFCS. Levels of other oxidoreductases, Alox3 and Alox8, involving in inflammatory lipid metabolism, were also found to be increased by HFCS. Additionally, the HFCS diet induced the expression of several inflammatory mediators, including tumor necrosis factor (TNF- α) and IL-1. Rats that consumed the HFCS solution also showed an increase in endoplasmic reticulum stress-associated PDIA4. Levels of the genes involved in the apoptotic pathway, including G0s2 (the G0/G1 switch gene) and Tnfrsf10b (death receptor), were also upregulated in the HFCS-diet group. These data suggest that dietary HFCS causes an increase in the TG content of the liver by a mechanism that upregulates genes contributing to carbohydrate and lipid metabolism^[17]. According to Zhang *et al.*^[18], consumption of fructose solution resulted in the activation of hepatic SREBP-1c in mice, in which two target genes of SREBP-1c, *fas* and *acc*, were upregulated. The activation of hepatic SREBP-1c by fructose was associated with the depletion of Insig-1^[18]. An ER stress and UPR, as determined by an increased GRP78 expression and eIF2 α and PERK phosphorylation, were observed in the livers of mice fed with a fructose solution. Altogether, ER stress contributes, at least in part, to hepatic SREBP-1c activation and lipid accumulation in fructose-evoked NAFLD^[18]. The data collected from these studies are represented in Tables 2 and 3.

DISCUSSION AND CONCLUSION

In this systematic review, we investigated different studies evaluating the effects of fructose consumption on liver damage. Finally, we selected eight animal-based (rodent) studies, five (62.5%) used a male rat model, whereas one (12.5%) and two (25%) used female rats and mice, respectively. This study was conducted to provide better understanding of the molecular mechanism and expression profile of genes involving in the liver damage and caused by fructose in rodent diets.

Although mitochondrial damage and metabolic disorders are predominantly observed in males, females have been found to exhibit an increased hepatic lipogenesis. However, it is notable that both males and females indicate augmented expression of pro-inflammatory cytokines and hepatic inflammatory mediators^[14,17]. Inflammatory cytokines are signaling molecules secreted by helper T lymphocytes and macrophages, intensifying inflammation and increasing inflammatory reactions. These molecules, including IL-1, IL-12, IL-18, TNF- α , interferon gamma, and granulocyte-macrophage colony-stimulating factor, play a crucial role in the innate immune system^[19,20,21]. Studies have shown that feeding rodents with fructose can increase these factors. Han *et al.*^[22] reported the elevated levels of IL1, IL-6, and TNF- α in the high fructose-fed male mice. In another study, Al-Qahtani *et al.*^[23] found potential gender differences in lipid kinetics. Their study suggested that disparities in energy sensing between males and females could be a reason for the less noticeable metabolic changes in the livers of female rats caused by fructose.

As represented in Table 3, the levels of TBARS in rat liver increased with a high-fructose diet, whereas activities of antioxidants enzymes (GR, GPx, GST, SOD, and CAT) decreased^[12,13]. According to a study by Busserolles *et al.*^[24], rats fed with a fructose diet showed higher levels of TBARS in plasma and urine samples than those fed with a starch diet^[24]. Also, lipid peroxidation was found to be increased in samples obtained from patients with metabolic syndrome. Male rats fed with HFCS-55 showed metabolic disruptions, while female rats exhibited lipogenic effects on the liver^[14,16]. Also, in mice models, lipogenic gene expression increased when fed with HFCS^[17,25].

One of the major outcomes of a high-fructose diet is a raise in lipid peroxidation^[13,15]. Lipid accumulation in fructose-evoked NAFLD was observed in the mice fed a fructose solution^[18]. The rats that were fed with HFCS-55 solution showed a variety of outcomes and

Table 2. Effects of fructose consumption in molecular analyses of fructose-fed animals

Duration of study/treatment	The most important outcomes of molecular analyses	Species/gender of animals	Authors/date
8 weeks	Rats fed a fructose-rich diet had significantly higher levels of plasma 8-OHdG compared to the control group. CAT protein levels in rat liver mitochondria decreased, while liver mtDNA contained significantly more mtDNA lesions.	Male rats	Cioffi 2017 ^[11]
14 weeks	In the fructose diet group, compared to the control group, SOD activity decreased in the liver, and heart and inversely increased in adipose tissue. GPx activity reduced in the heart and increased in adipose tissue, whereas the values remained unchanged in the liver, muscle, and kidney. Reduced CAT activity was noted.	Male rats	Taleb-Dida 2011 ^[12]
60 days	Concerning liver antioxidant status in fructose-fed rats, oxidative stress was observed.	Male rats	Karuna 2011 ^[13]
30 days	Protein expression of pro-inflammatory cytokines (IL-6 and IL-1 β) increased in the dorsal hippocampus for the adolescent HFCS-55 group. Liver IL-1 β and plasma insulin levels elevated in adolescent-exposed HFCS-55 groups.	Male rats	Hsu 2015 ^[14]
6 weeks	In the fructose group, the exacerbation of mitochondrial lipid peroxidation was observed.	Male rats	García-Berumen 2019 ^[15]
8 weeks	REBP-1c was upregulated in the fructose group. Gene expression of PPAR α was downregulated in the HFCS-55 group compared to control.	Female rats	Mock 2017 ^[16]
26 weeks (between 6 weeks of age until 32 weeks of age)	Expression of carbohydrate and lipid metabolism genes was induced.	Female mice	Collison 2010 ^[17]
8 weeks	Fructose stimulation led to the activation of hepatic SREBP-1c, which is linked with the depletion of Insig-1. Additionally, noticeable ER stress and an UPR were observed in the liver.	Female mice	Zhang 2012 ^[18]

alterations in function, such as changes in the levels of plasma 8-OHdG and CAT protein, mitochondrial biogenesis, liver dysfunction, metabolic diseases, and fat oxidation.^[11,14,17] Oxidative stress was observed as a consequence of chronic fructose feeding in rats^[11,12,26].

It has also been observed that metabolic diseases can be triggered by chronic inflammation and oxidative stress, which are closely associated with a high-fat or high-carbohydrate diet. Such diets can lead to lipid peroxidation and protein carbonylation, as well as a decline in the body's antioxidant content and glutathione levels^[27]. Studies have shown that a fructose-rich diet can damage the liver, reduce antioxidant capacity and cause oxidative damage^[28,29]. Researchers have also suggested that a high-fructose diet may contribute to the development of metabolic syndrome, including hepatic steatosis^[30,31]. Hepatocytes have a complex network of nonenzymatic and enzymatic antioxidant defenses designed to eliminate or counteract ROS. This sophisticated system protects the liver from oxidative stress and maintains its proper function. However, in the presence of the excessive levels of ROS, it can overwhelm the hepatocellular antioxidant defenses,

which results in hepatocyte injury, ROS production, and cell death^[32].

The 8-OHdG is a great base modification in mammalian DNA, and its level is directly related to oxidative stress. Therefore, it can be used as a biomarker for systemic oxidative stress in *in vivo* conditions^[11,33-35]. The results of studies included in our review showed reduced CAT expression in rats fed with a high-fructose diet, which is consistent with previous studies showing an increase in oxidative stress in the hepatic mitochondria of rats fed with a fructose-rich diet^[11,28]. A review included the results of the studies relating to the effects of a fructose-rich diet on mitochondria, in which they have shown significant mtDNA, indicating that the long-term consumption of high fructose diet affects mitochondria and possibly causes metabolic disorders such as insulin resistance and hepatic steatosis^[11,36,37]. Based on the above-mentioned studies, lipid peroxidation can be considered as an extractor of nonalcoholic steatohepatitis by triggering signaling cascades, which mediate inflammation via increasing in the level of malondialdehyde and 4-hydroxynonenal, the end-

Table 3. Main molecular analysis outcomes of fructose in the liver of rat and mice

No.	Alteration/dysfunction	Decrease/reduction	Increase/enhancing/ inducing	Target organ	Type of fructose	Author/date
1	Changed levels of plasma 8-OHdG Changed CAT protein levels Mitochondrial biogenesis Liver dysfunction and metabolic diseases	mtDNA copy number	mtDNA damage Plasma 8-OHdG Oxidative stress	Rat liver mitochondria	HFCS-55	Cioffi 2017 ^[11]
2	CAT activity	SOD and CAT activities	Levels of TBARS Oxidative stress	Rat liver	High-fructose diet	Taleb-Dida 2011 ^[12]
3	-----	Activities of enzymatic antioxidants GR, GPx, GST, SOD, and CAT Levels of hepatic GSH	Levels of TBARS Protein carbonyl groups Lipid peroxidation intermediates	Rat liver	High fructose diet fed	Karuna 2011 ^[13]
4	Metabolic disruptions	-----	Circulating insulin levels Hepatic proinflammatory cytokine expression	(Liver) adolescent rats	HFCS-55	Hsu 2015 ^[14]
5	-----	Level of steatosis Oxidative phosphorylation	Mitochondrial lipid peroxidation	Male Rat liver	Fructose-fed group	García-Berumen 2019 ^[15]

No.	Alteration/dysfunction	Decrease/reduction	Increase/enhancing/ inducing	Target organ	Type of fructose	Author/date
6	Lipogenic effects	PPAR α gene expression (downregulated)	Liver content of the MUFA, palmitoleic acid Hepatic content of oleic acid SCD-1 (stearoyl-CoA desaturase-1) activity Hepatic de novo lipogenesis	Female rat liver	drinking HFCS-55	Mock 2017 ^[16]
7	Fat oxidation	The expression of key transcription coactivator PPARGC1A	Hepatic steatosis Expression of genes involved in carbohydrate and lipid metabolism Oxidoreductases Cyp2b9 and Cyp2b10 (upregulated) Oxidoreductases Alox3 and Alox8 The expression of TNF- α and IL-1 Endoplasmic reticulum stress-associated Pdia4 Levels of genes involved in the apoptotic pathway: G0s2, G0/G1, Tnfrsf10b (upregulated) The TG content of the liver The expression of lipogenic genes	Mice liver	HFCS	Collison 2010 ^[17]
8	ER stress and UPR lipid accumulation in fructose-evoked NAFLD	Insulin-induced gene (Insig)-1	Hepatic SREBP-1c fas and acc GRP78 expression eIF2 α and PERK phosphorylation	Mice liver	fructose solution	Zhang 2012 ^[18]

products of lipid peroxidation, suggesting that lipid peroxidation induced by fructose may trigger inflammation^[15,38,39]. An earlier study conducted on mice has found that fructose induces hepatic lipid accumulation, activates hepatic SREBP-1c, causes hepatic ER stress and decreases hepatic Insig-1 protein^[18]. A fructose-rich diet negatively affects liver function and metabolism^[32] because almost all the absorbed monosaccharides are primarily sent to the liver via portal blood, and approximately all ingested fructose is metabolized in the liver^[11,40,41]. Accordingly, the massive flow of ingested fructose and its handling in the liver cause a metabolic injury to the tissue, which is well-established^[11,42-44].

Based on the information provided, it seems that the research on the chronic and long-term effects of fructose consumption on rodent models is somewhat limited due to the small number of high-quality studies eligible for our criteria. This limitation do not allow to fully explain the relationships and molecular signalings associated with the consumption of both dietary high fructose and HFCS-55 solution on the liver of rodent models. Hence, high-quality studies are needed to better understand these relationships and molecular signalings. Overall, the results of this systematic review suggest that long-term fructose consumption may cause molecular damage to the liver in animal models.

Limitations

The present study investigated the effects of fructose consumption on the liver function of rodents, which is a limitation of our study. Examining the effects of a high-fructose diet on humans may provide better insight into the cause-and-effect relationships between high fructose intake and development of liver injury. However, further research is needed to fully understand the implications of fructose for human health. Hence, this issue can serve as a basis for future studies.

DECLARATIONS

Ethical statement

Not applicable.

Data availability

The data supporting the article are also included within the article and supplementary file. Additionally, the analyzed data sets generated during the study can be made available from the corresponding author upon reasonable request.

Author contributions

RM: data curation, formal analysis, investigation, review and editing, writing original draft, methodology, and conceptualization. R.K: data curation, formal

analysis, investigation, review and editing, and methodology. SAB: data curation, formal analysis, Investigation, review and editing, methodology, conceptualization, supervision, and project administration.

Conflict of interest

None declared.

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REFERENCES

1. Zhang L, Perdomo G, Kim DH, Qu S, Ringquist S, Trucco M, Dong HH. Proteomic analysis of fructose-induced fatty liver in hamsters. *Metabolism* 2008; **57**(8): 1115-1124.
2. Harada N, Nomura M, Yoda Y, Matsumura S, Inui H, Yamaji R. Food texture affects glucose tolerance by altering pancreatic β -cell function in mice consuming high-fructose corn syrup. *PloS one* 2020; **15**(5): e0233797.
3. Dongiovanni P, M Anstee Q, Valenti L. Genetic predisposition in NAFLD and NASH: impact on the severity of liver disease and response to treatment. *Current pharmaceutical design* 2013; **19**(29): 5219-5238.
4. Gupta M, Kaur A, Singh TG, Bedi O. Pathobiological and molecular connections involved in the high fructose and high fat diet induced diabetes associated nonalcoholic fatty liver disease. *Inflammation research* 2020; **69**(9): 851-867.
5. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of nonalcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Annals of medicine* 2011; **43**(8): 617-649.
6. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutrition and metabolism* 2005; **2**(1): 5.
7. Taghibiglou C, Carpentier A, Van Iderstine SC, Chen B, Rudy D, Aiton A, Lewis GF, Adeli K. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance: evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. *The journal of biological chemistry* 2000; **275**(12): 8416-8125.
8. Hsieh FC, Lee CL, Chai CY, Chen WT, Lu YC, Wu CS. Oral administration of *Lactobacillus reuteri* GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. *Nutrition and metabolism* 2013; **10**(1): 35.
9. Taskinen MR, Packard CJ, Borén J. Dietary fructose and the metabolic syndrome. *Nutrients* 2019; **11**(9): 1987.
10. Lee JS, Jun DW, Kim EK, Jeon HJ, Nam HH, Saeed WK. Histologic and metabolic derangement in high-fat, high-

- fructose, and combination diet animal models. *The scientific world journal* 2015; **2015**: 306326.
11. Cioffi F, Senese R, Lasala P, Ziello A, Mazzoli A, Crescenzo R, Liverini G, Lanni A, Goglia F, Iossa S. Fructose-rich diet affects mitochondrial DNA damage and repair in rats. *Nutrients* 2017; **9**(4): 323.
 12. Taleb Dida N, Krouf D, Bouchenak M. Globularia alypum aqueous extract decreases hypertriglyceridemia and ameliorates oxidative status of the muscle, kidney, and heart in rats fed a high-fructose diet. *Nutrition research* 2011; **31**(6): 488-495.
 13. Karuna R, Saralakumari D. Preventive effect of Catharanthus roseus (Linn.) against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats. *Journal of diabetes Mellitus* 2011; **1**(3): 63-70.
 14. Hsu TM, Konanur VR, Taing L, Usui R, Kayser BD, Goran MI, Kanoski SE. Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. *Hippocampus* 2015; **25**(2): 227-239.
 15. García Berumen CI, Ortiz Avila O, Vargas Vargas MA, del Rosario Tamayo BA, Guajardo López C, Saavedra Molina A, Rodríguez Orozco AR, Cortés Rojo C. The severity of rat liver injury by fructose and high fat depends on the degree of respiratory dysfunction and oxidative stress induced in mitochondria. *Lipids in health and disease* 2019; **18**(1): 78.
 16. Mock K, Lateef S, Benedito VA, Tou JC. High-fructose corn syrup-55 consumption alters hepatic lipid metabolism and promotes triglyceride accumulation. *The journal of nutritional biochemistry* 2017; **39**: 32-39.
 17. Collison KS, Maqbool ZM, Inglis AL, Makhoul NJ, Saleh SM, Bakheet RH, Al-Johi MA, Al-Rabiah RK, Zaidi MZ, Al-Mohanna FA. Effect of dietary monosodium glutamate on HFCS-induced hepatic steatosis: expression profiles in the liver and visceral fat. *Obesity* 2010; **18**(6): 1122-1134.
 18. Zhang C, Chen X, Zhu R-M, Zhang Y, Yu T, Wang H, Zhao H, Zhao M, Ji YL, Chen YH, Meng XH, Wei W, Xu DX. Endoplasmic reticulum stress is involved in hepatic SREBP-1c activation and lipid accumulation in fructose-fed mice. *Toxicology letters* 2012; **212**(3): 229-240.
 19. Zhang JM, Jianxiong A. Cytokines, inflammation and pain. *International anesthesiology clinics* 2007; **45**(2): 27-37.
 20. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. *International journal of molecular sciences* 2019; **20**(23): 6008.
 21. Wang C, Wang X, Song G, Xing H, Yang L, Han K, Chang YZ. A high-fructose diet in rats induces systemic iron deficiency and hepatic iron overload by an inflammation mechanism. *Journal of food biochemistry* 2021; **45**(1): e13578.
 22. Han X, Li W, Huang D, Yang X. Polyphenols from hawthorn peels and fleshies differently mitigate dyslipidemia, inflammation and oxidative stress in association with modulation of liver injury in high fructose diet-fed mice. *Chemico biological interactions* 2016; **257**: 132-140.
 23. Al-Qahtani S, Bryzgalova G, Efendić S, Berggren P, Portwood N. Activation of hepatic AMPK by 17 β -estradiol suppresses both nuclear receptor Nr2c2/TR4 and its downstream lipogenic targets, reduces gluconeogenic genes and improves insulin signaling. *Disease and molecular medicine* 2016; **4**: 55-67.
 24. Busserolles Jrm, Gueux E, Rock E, Demigne C, Mazur A, Rayssiguier Y. Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. *The journal of nutrition* 2003; **133**(6): 1903-1908.
 25. Collison KS, Saleh SM, Bakheet RH, Al-Rabiah RK, Inglis AL, Makhoul NJ, Maqbool ZM, Zaidi MZ, MA Al Johi MA, Al Mohanna FA. Diabetes of the liver: the link between nonalcoholic fatty liver disease and HFCS-55. *Obesity* 2009; **17**(11): 2003-2013.
 26. Martins CC, Bagatini MD, Simões JLB, Cardoso AM, Baldissarelli J, Dalenogare DP, Dos Santos DL, Chitolina Schetinger MR, Morsch VM. Increased oxidative stress and inflammatory markers contrast with the activation of the cholinergic anti-inflammatory pathway in patients with metabolic syndrome. *Clinical biochemistry* 2021; **89**: 63-69.
 27. Tan BL, Norhaizan ME, Liew WPP. Nutrients and oxidative stress: friend or foe? *Oxidative medicine and cellular longevity* 2018; **2018**: 9719584.
 28. Crescenzo R, Bianco F, Falcone I, Coppola P, Liverini G, Iossa S. Increased hepatic de novo lipogenesis and mitochondrial efficiency in a model of obesity induced by diets rich in fructose. *European journal of nutrition* 2013; **52**(2): 537-545.
 29. Botezelli JD, Cambri LT, Ghezzi AC, Dalia RA, Voltarelli FA, de Mello MAR. Fructose-rich diet leads to reduced aerobic capacity and to liver injury in rats. *Lipids in health and disease* 2012; **11**: 78.
 30. Kostogrys RB, Pisulewski PM. Effect of conjugated linoleic acid (CLA) on lipid profile and liver histology in laboratory rats fed high-fructose diet. *Environmental toxicology and pharmacology* 2010; **30**(3): 245-250.
 31. Zhang HF, Shi LJ, Song GY, Cai ZG, Wang C, An RJ. Protective effects of matrine against progression of high-fructose diet-induced steatohepatitis by enhancing antioxidant and anti-inflammatory defences involving Nrf2 translocation. *Food and chemical toxicology* 2013; **55**: 70-77.
 32. Lozano I, Van der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, eandier N, Maillard E, E, Sigrist S, Dal S. High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. *Nutrition and metabolism* 2016; **13**: 15.
 33. Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, Di Minno MND. 8-Hydroxy-2-deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature. *Antioxidants and redox signaling* 2016; **24**(10): 548-555.
 34. Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. 8-Hydroxyguanine, an abundant form of oxidative DNA

- damage, causes GT and AC substitutions. *Journal of biological chemistry* 1992; **267**(1): 166-172.
35. Guo Z, Liu X, Liu Y, Wu G, Lu X. Constructing a novel 8-hydroxy-2'-deoxyguanosine electrochemical sensor and application in evaluating the oxidative damages of DNA and guanine. *Biosensors and bioelectronics* 2016; **86**: 671-676.
 36. Nagai Y, Yonemitsu S, Erion DM, Iwasaki T, Stark R, Weismann D, Dong J, Zhang D, Jurczak MJ, Löffler MG, Cresswell J, Yu XX, Murray SF, Bhanot S, Monia BP, Bogan JS, Samuel V, Shulman GI. The role of peroxisome proliferator-activated receptor γ coactivator-1 β in the pathogenesis of fructose-induced insulin resistance. *Cell metabolism* 2009; **9**(3): 252-264.
 37. Baena M, Sangüesa G, Dávalos A, Latasa MJ, Sala Vila A, Sánchez RM, Roglans N, Laguna JC, Alegret M. Fructose, but not glucose, impairs insulin signaling in the three major insulin-sensitive tissues. *Scientific reports* 2016; **6**: 26149.
 38. Alwahsh SM, Xu M, Seyhan HA, Ahmad S, Mihm S, Ramadori G, Schultze FC. Diet high in fructose leads to an overexpression of lipocalin-2 in rat fatty liver. *World journal of gastroenterology* 2014; **20**(7): 1807-1821.
 39. George J, Pera N, Phung N, Leclercq I, Hou JY, Farrell G. Lipid peroxidation, stellate cell activation and hepatic fibrogenesis in a rat model of chronic steatohepatitis. *Journal of hepatology* 2003; **39**(5): 756-764.
 40. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. *Physiological reviews* 2010; **90**(1): 23-46.
 41. Crescenzo R, Cigliano L, Mazzoli A, Cancelliere R, Carotenuto R, Tussellino M, Liverini G, Iossa S. Early effects of a low fat, fructose-rich diet on liver metabolism, insulin signaling, and oxidative stress in young and adult rats. *Frontiers in physiology* 2018; **9**: 411.
 42. Li W, Lu Y. Hepatoprotective effects of sophoricoside against fructose-induced liver injury via regulating lipid metabolism, oxidation, and inflammation in mice. *Journal of food science* 2018; **83**(2): 552-558.
 43. Ferder L, Ferder MD, Inserra F. The role of high-fructose corn syrup in metabolic syndrome and hypertension. *Current hypertension reports* 2010; **12**(2): 105-112.
 44. Le MT, Frye RF, Rivard CJ, Cheng J, McFann KK, Segal MS, RJ Johnson, Johnson JA. Effects of high-fructose corn syrup and sucrose on the pharmacokinetics of fructose and acute metabolic and hemodynamic responses in healthy subjects. *Metabolism* 2012; **61**(5): 641-651.