Excessive Dietary Fructose is Responsible for Lipid Peroxidation and Steatosis in the Rat Liver Tissues

AŞIRI BESİNSEL FRUKTOZ SIÇAN KARACİĞER DOKULARINDA YAĞLANMA VE LİPİD PEROKSİDASYONUNDAN SORUMLUDUR

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Abstract

Objective: Drinking large amounts of carbonated beverage is associated with increased risk for obesity and nonalcoholic steatohepatitis. Fructose is found in many fruits, vegetables, honey and in soft drinks, such as high fructose corn syrup. Excessive fructose consumption can adversely affect liver metabolism and increase lipid peroxidation, and this plays a role in the pathology associated with fructose feeding.

Material and Methods: The present study reports the effects of high-fructose diet (10% and 20% in the drinking water) on the liver lipid peroxidation of rats. At the end of the experiment, biochemical evaluation of plasma and histopathological evaluation of liver tissue were performed.

Results: Enhanced thiobarbituric acid-reactant substance levels and abnormal lipid changes were observed in high-fructose-fed rats. In 10% fructose-fed group, the most consistent findings in the histologic sections of liver tissues were the hepatocellular degenerative changes, minimal macrovesicular and microvesicular steatosis in zone 1. In the 20% fructose-fed group, the trabecular liver structure was more seriously affected than the 10% fructose-fed group. High fructose-induced degenerative changes were evident in numerous hepatocytes of zone 1; the cells were enlarged and had a light and foamy cytoplasm filled with vacuoles.

Conclusion: Our study suggests that fructose feeding is associated with lipid peroxidation and steatosis in liver and the dose of fructose is associated with the degree of tissue damage.

Key Words: Fructose; fatty liver; lipid peroxidation; rats

Özet

Amaç: Aşırı miktarda tatlandırılmış soğuk içecekler şişmanlık ve alkolik olmayan karaciğer yağlanması ile ilişkilidir. Fruktoz pek çok meyve, sebze bal ve yüksek fruktozlu mısır şurubu gibi, hafif içeceklerde bulunmaktadır. Aşırı fruktoz tüketimi karaciğer metabolizmasını kötü yönde etkileyebilir, lipit peroksidasyonunu arttırabilir ve bu, fruktoz ile beslenmeye bağlı patolojide rol oynayabilir.

Gereç ve Yöntemler: Bu çalışma yüksek fruktoz diyetinin (içme sularında %10 ve %20 oranında) sıçanlarda karaciğer lipit peroksidasyonuna etkilerini bildirmektedir. Deney sonunda, karaciğer dokularının histopatolojik ve plazma örneklerinin biyokimyasal değerlendirmesi yapıldı.

Bulgular: Yüksek fruktoz ile beslenen sıçanlarda artmış tiyobarbiturik asit reaktan madde düzeyleri ve anormal lipit değişiklikleri gözlendi. %10 fruktoz ile beslenen grupta karaciğer dokularının histolojik kesitlerinde en belirgin bulgular olarak hepatosellüler dejeneratif değişiklikler ve zon 1'de, minimal makroveziküler ve mikroveziküler yağlanma vardı. %20 fruktoz ile beslenen grupta trabeküler karaciğer yapısı %10 fruktoz ile beslenen gruptan daha ciddi olarak etkilenmiş idi. Yüksek fruktozla indüklenen dejeneratif değişiklikler zon 1'in çoğu karaciğer hücresinde görüldü; bu hücrelerin büyümüş, parlak ve vakuoller ile dolu köpüklü sitoplazmaları vardı.

Sonuç: Bizim çalışmamız fruktoz ile beslenmenin karaciğerde lipit peroksidasyonu ve yağlanmayla, fruktoz dozunun da doku hasarının derecesi ile ilişkili olduğunu göstermektedir.

Anahtar Kelimeler: Fruktoz; yağlı karaciğer; lipid peroksidasyonu; sıçanlar

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Drinking large amounts of carbonated beverage is associated with increased risk for obesity and nonalcoholic steatohepatitis (NASH). Excessive fructose consumption can adversely affect liver metabolism and increase lipid peroxidation, and this plays a role in the pathology associated with fructose feeding.[1]

Loading of the liver with fructose may potentiate hypertriglyceridemia, hypercholesterolemia, and hyperuricemia. Fructose rich diets were shown to have detrimental metabolic effects, including glucose intolerance, insulin resistance, dyslipidemia, and liver dysfunction. Some of these metabolic effects of fructose are attributed to its rapid hepatic uptake and the fact that it bypasses the phosphofructokinase regulatory step in glycolysis.[2,3]

NASH is an increasingly recognized form of chronic liver condition affecting both children and adults within the wide spectrum of fatty liver diseases. A 'two-hit' concept of disease pathogenesis has been proposed. The first hit is steatosis, and this is postulated to sensitize the liver to the second hit, which may be oxidative stress or abnormal cytokine production. Oxidative stress and lipid peroxidation are candidates for the second hit in the pathogenesis of NASH. When adult Wistar rats are given 10% fructose in the drinking water for 48 hours, hepatic fatty acid synthase is induced and *de novo* fatty acid synthesis and esterification are increased significantly.[4,5] The underlying mechanisms for the detrimental consequences of a high-fructose diet in animal models are not clear. However, increased lipid peroxidation plays a role in the pathology associated with fructose feeding.[6,7] The aim of this study was to evaluate, in tissue and blood samples, the effects of 10% and 20% fructose on lipid peroxidation, and to observe the resultant histopathological changes in liver.

Material and Methods

Animals and experimental design: Eighteen male Wistar albino rats (280-300 g) were randomly assigned to one of the 3 groups; the control group (I) which received a purified diet, 10% fructose-fed group (II) and 20% fructose-fed group (III). Each group contained six animals and all of the rats were preserved with 12 hours light, 12 hours dark cycle housing supplied with standard rat chow and freely available water. Ten percent and twenty percent (w/v) fructose dissolved in tap water was added to their drinking water ad libitum for ten days. At the end of the experiment period the rats were sacrificed. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Sample preparation: The animals were starved overnight for 12 h before the blood collection process. At the time of sacrification, rats were anesthetized with ketamin 0.5 mg/kg (Ketalar®, Eczacıbaşı) intraperitoneally. Blood samples were collected after death by entering the abdominal and thoracic cavities into tubes containing potassium EDTA using disposable syringes. Blood samples were centrifuged at 1000 x g for 10 min at 4°C to remove plasma. Plasma samples were transferred into polyethylene tubes and were stored at -40°C until the analysis of biochemical parameters.

Liver tissues were homogenized in four volumes of ice-cold Tris-HCl buffer using a glass teflon homogenizer (Ultra Turrax IKA T18) after cutting of the tissues into small pieces with scissors. The homogenate was then centrifuged at 5000 x g for 10 min to remove debris. The clear supernatant fluid was analyzed for thiobarbituric acid reactant substances (TBARS) and protein concentration. A separate amount of supernatant solution was extracted with an equal volume of ethanol/chloroform mixture (5/3; v/v). After centrifugation at 5000 × g for 30 min, the clear upper layer (the ethanol phase) was obtained and used in the tissue triglyceride assays. All preparation procedures were performed at +4°C.

Biochemical evaluation: TBARS content in plasma was measured by the double heating method of Draper and Hadley, protein analysis was performed by the Lowry method. [8,9] Triglyceride levels in liver extracs were determined by the method of Folch et al. and Grattagliano et al.[10,11] Plasma glucose, uric acid and triglyceride levels were determined by using colorimetric kits on an autoanalyzer (Cobas Integra 800).

Histopathologic examination: Liver tissues were harvested from the sacrificed animals, and the fragments from tissues were fixed in 10% neutral formaline solution, embedded in paraffin and then, stained with haematoxylin and eosin. Preparations

Table 1. Plasma glucose, triglyceride, uric acid and TBARS levels, and the tissue triglyceride and TBARS levels according to groups.

Parameters	Group I	Group II	Group III
Glucose (mg/dL)	88 ± 9.3	117 ± 12.8[a]	136 ± 15.2[c]
Triglycerides (mg/dL)	48 ± 5.1	67 ± 7.9[a]	95 ± 10.1[c]
Uric acid (mg/dL)	0.34 ± 0.07	0.45 ± 0.06[b]	0.51 ± 0.12[cd]
TBARS (µmol/L)	0.33 ± 0.04	0.42 ± 0.06[a]	0.57 ± 0.08[c]
Triglycerides mg/g wet tissue	296 ± 36.8	378 ± 65.8[a]	751 ± 103.4[c]
Tissue TBARS nmol/g protein	26.8 ± 2.15	44.1 ± 4.13[b]	58.3 ± 6.01[c]

[a]p< 0.001, [b] p< 0.01 when group II is compared with group I.
[c]p< 0.001, [d]p< 0.05 when group III is compared with group I and II.

were evaluated by a bright field microscope (Olympus B x 51).

Statistical analysis

Statistical analyses were performed by ANOVA test and post-hoc multiple comparison tests. Data were expressed as mean ± standard deviation (SD) and probability value of less than 0.05 was considered to be statistically significant.

Results

All the biochemical data were summarized in table 1. As shown in Table 1, the high fructose diet induced remarkable hypertriglyceridemia in both the liver and the plasma in our experimental model. Fructose-induced steatosis was also confirmed histopathologically (Figures 1-4). Enhanced lipid changes were observed in high-fructose-fed rats. Triglyceride, glucose and uric acid levels were increased in groups II and III. The increase in triglyceride was higher in group III than in group II. Fructose administration resulted in the generation of reactive oxygen species in the blood of rats by increasing the levels of TBARS, an indication of lipid peroxidation. Enhanced TBARS reactivity was higher in groups II and III than in group I. TBARS reactivity higher in group III than in group II.

In 10% fructose-fed group, the most consistent findings in the histological sections of liver tissues were the hepatocellular degenerative changes and minimal macrovesicular and microvesicular steatosis in zone 1. The trabecular liver structure was more seriously affected in group III than in group II. High fructose-induced degenerative changes were evident in numerous hepatocytes of zone 1; the cells were enlarged and had a light and foamy cytoplasm filled with vacuoles. In a few zone 1 hepatocytes, necrotic changes were evident; a small, pycnotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm were observed.

Discussion

The general increases in consumption of calories, and specifically of refined carbohydrates and fructose correlates positively with an alarming increase in metabolic syndrome. The increasing use of high fructose sweeteners over the past few decades has resulted in a considerable rise in the

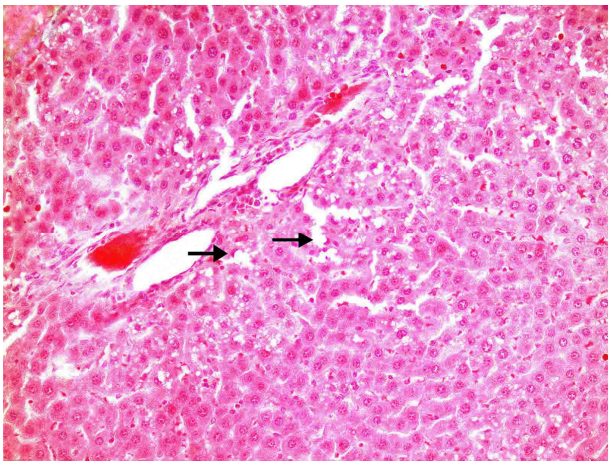

Figure 1. 10% fructose-fed group: Hepatocellular degenerative changes, minimal microvesicular and macrovesicular steatosis (arrows) are seen in zone 1 (H&E x 260).

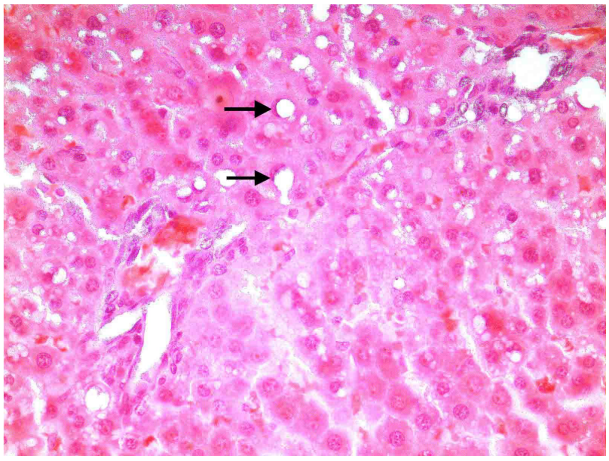

Figure 2. High magnification of Figure 1. Microvesicular and macrovesicular steatosis (arrows) are seen in zone 1 (H&E x 420).

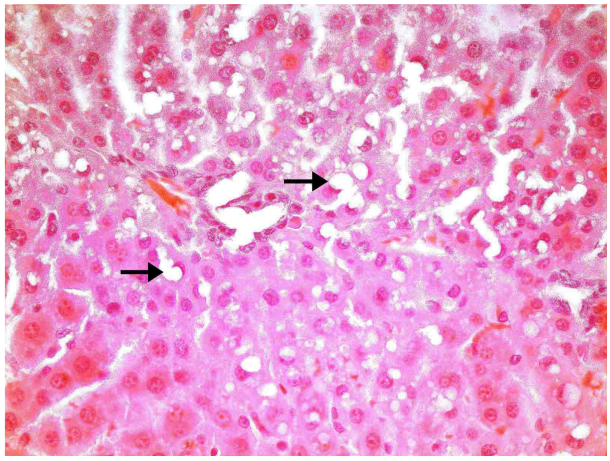

Figure 3. 20% fructose-fed group: Hepatocellular cells were enlarged and had a light and foamy cytoplasm filled with vacuoles in zone 1 (H&E x 260).

dietary intake of fructose. Westernization of diets has resulted in significant increases in added fructose, leading to typical daily consumptions amounting to 85-100 grams of fructose per day. Fructose is found as a free monosaccharide in many fruits and vegetables and in honey. In addition high fructose corn syrups are quite commonly found in soft drinks and juice beverages, and are incorporated into many convenient pre-packaged foods, such as breakfast cereals and baked goods. Fructose consumption has thus largely increased over the past few decades most likely as a result of this increased use of high fructose corn syrups, which contain 55-90% of fructose.[3,12] Exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance. Due to these negative effects of fructose, fructose metabolism has gained recent research attention.[1,13]

NASH is one of the most common liver diseases encountered in the United States and Europe. It is now established that fructose-induced oxidative damage is present in several animal models of steatohepatitis. The underlying mechanisms for the detrimental consequences of a high-fructose diet in animal models are not clear. However, the possibility exists that fructose feeding facilitates oxidative

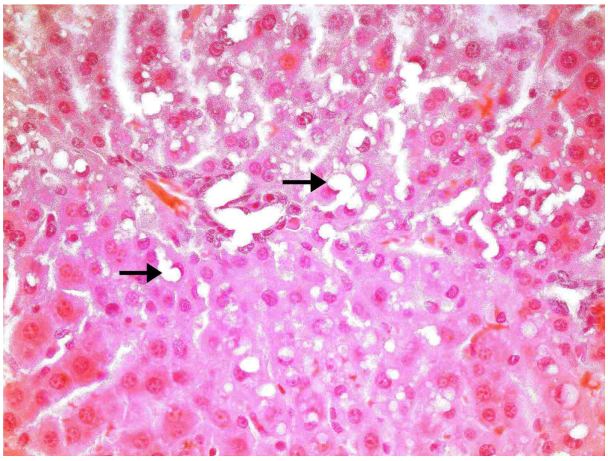

Figure 4. High magnification of Figure 3. In a zone 1 hepatocyte, necrotic changes were evident; a small, pycnotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm and microvesicular and macrovesicular steatosis (arrows) were observed (H&E x 420).

damage. It is known that hepatic fatty acid synthase is induced and *de novo* fatty acid synthesis is increased in Wistar rats that were fed fructose in drinking water.[6,7,14] In the present study, both liver and fasting plasma triacylglycerol levels were significantly elevated by dietary fructose. Fructose-induced increase in triacylglycerols may be due to the stimulation of triacylglycerol synthesis. In addition, steatosis and hepatocellular degenerative changes that were also observed in the 10% fructose-fed group were more diffuse in the 20% fructose-fed group.

Fructose consumption results in an excess production of uric acid due to an increased degradation of nucleotides. The enzyme xanthine oxidase catalyzes the oxidation of both hypoxanthine and xanthine. During the oxidation process free radicals are generated, which in turn, induce lipid peroxidation and cell membrane damage.[15] Following fructose consumption, excess amounts of NADH and NADPH are known to occur during the metabolism of fructose via the pentose shunt and sorbitol pathway. In addition, fructose-feeding results in increased xanthine oxidase activity, and to glyceraldehyde production, which may induce free radicals. During the metabolism of hypoxanthine by xanthine oxidase enzyme, both superoxide radical and hydrogen peroxide may be generated.[16-18] In our study, we observed a significant increase in plasma uric acid levels in the fructose-fed group when compared to the control group.

This study suggested that fructose-fed rats were more susceptible to peroxidative damage, as measured by TBARS. Which one of the possible pathways of free radical generation occurs in response to a high fructose diet is still unclear. Recent studies strongly suggest that oxidative stress occurs in rats fed a high fructose diet. Detrimental effects of fructose are enhanced when antioxidant defenses are decreased or when free radical production is increased.[19,20] Delbosc et al. showed that high fructose feeding was associated with an early (1-week) increase in ROS production by the aorta, the heart and circulatory polymorphonuclear cells, in association with enhanced markers of oxidative stress.[21] Studies involving commonly consumed fruit juices showed that natural fructose carbohydrates can alter lipid and protein oxidation biomarkers in the blood, and mediate oxidative stress responses *in vivo*.[22] Fructose-induced hypertriglyceridemia is a result of enhanced lipogenesis, overproduction of VLDL triglycerides and decreased peripheral catabolism.[2] Kelley et al. hypothesized that pro-oxidant stress response pathways might mediate hepatic increases in VLDL secretion and delayed clearance upon fructose feeding.[23] Another contributing factor to VLDL overproduction includes fructose effects on lipid peroxidation. High fructose diets may have a hypertriglyceridemic and prooxidant effect, and fructose fed rats have shown less protection from lipid peroxidation.[24] Fructose-fed rats were also less protected against lipid peroxidation as shown by TBARS in liver tissue homogenates, and fructose-fed rats were characterized by a higher plasma nitric oxide level, suggesting greater nitric oxide production.[14] Moreover, the susceptibility of tissues to oxidative stress may depend on alterations in lipid composition. Another possibility is that fructose induces the accumulation of advanced glycation end-products and that oxidative degradation of fructose adducts leads to production of free radicals.[25] An important but not well-appreciated dietary change has been the substantial increase in the amount of dietary fructose consumption from high intake of sucrose and high fructose corn syrup, a common sweetener used in the food industry. The alarming increase in fructose consumption may be an important contributor to the epidemic of obesity and insulin resistant diabetes, in both pediatric and adult populations. Thus, emerging evidence from recent epidemiological and biochemical studies clearly suggests that high dietary intake of fructose has rapidly become an important causative factor in the development of metabolic syndrome.[1,12]

In conclusion, lipid peroxidation may play a role in fructose-induced hepatocellular injury. ROS may be involved in the mechanism of non-alcoholic steatohepatitis; the links between impaired hepatic fatty acid oxidation and hypertriglyceridemia and the specific effects of diets with low and high amounts of sucrose or fructose should be studied further in humans.

REFERENCES

1. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab (Lond). 2005 21;2:5.
2. Mayes PA. Intermediary metabolism of fructose. Am J Clin Nutr 1993;58(5 Suppl):754S-765S.
3. Bantle JP, Raatz SK, Thomas W, Georgopoulos A. Effects of dietary fructose on plasma lipids in healthy subjects. Am J Clin Nutr 2000;72:1128-34.

4. Poulsom R. Morphological changes of organs after sucrose or fructose feeding. Prog Biochem Pharmacol 1986;21:104-34.
5. Koteish A, Diehl AM. Animal models of steatosis. Semin Liver Dis 2001;21:89-104.
6. Faure P, Rossini E, Lafond JL, Richard MJ, Favier A, Halimi S. Vitamin E improves the free radical defense system potential and insulin sensitivity of rats fed high fructose diets. J Nutr 1997;127:103-7.
7. Anurag P, Anuradha CV. Metformin improves lipid metabolism and attenuates lipid peroxidation in high fructose-fed rats. Diabetes Obes Metab 2002;4:36-42.
8. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421-31.
9. Lowry Oh, Rosebrough Nj, Farr AL, Randall Rj. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
10. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497-509.
11. Grattagliano I, Vendemiale G, Caraceni P, et al. Starvation impairs antioxidant defense in fatty livers of rats fed a choline-deficient diet. J Nutr 2000;130:2131-6.
12. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr 2004;79:537-43.
13. Moore MC, Cherrington AD, Mann SL, Davis SN. Acute fructose administration decreases the glycemic response to an oral glucose tolerance test in normal adults. J Clin Endocrinol Metab 2000;85:4515-9.
14. Armutcu F, Coskun O, Gurel A, et al. Thymosin alpha 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats. Clin Biochem 2005;38:540-7.
15. Fields M, Lewis CG, Lure MD. Allopurinol, an inhibitor of xanthine oxidase, reduces uric acid levels and modifies the signs associated with copper deficiency in rats fed fructose. Free Radic Biol Med 1996;20:595-600.
16. Van den Berghe G. Metabolic effects of fructose in the liver. Curr Top Cell Regul 1978;13:97-135.
17. Thornalley P, Wolff S, Crabbe J, Stern A. The autoxidation of glyceraldehyde and other simple monosaccharides under physiological conditions catalysed by buffer ions. Biochim Biophys Acta 1984,14;797:276-87.
18. Granot E, Kohen R. Oxidative stress in childhood--in health and disease states. Clin Nutr 2004;23:3-11.
19. Fields M, Ferretti RJ, Reiser S, Smith JC Jr. The severity of copper deficiency in rats is determined by the type of dietary carbohydrate. Proc Soc Exp Biol Med 1984;175:530-7.
20. Busserolles J, Gueux E, Rock E, Mazur A, Rayssiguier Y. High fructose feeding of magnesium deficient rats is associated with increased plasma triglyceride concentration and increased oxidative stress. Magnes Res 2003;16:7-12.
21. Delbosc S, Paizanis E, Magous R, et al. Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat. Atherosclerosis 2005;179:43-9.
22. Breinholt VM, Nielsen SE, Knuthsen P, Lauridsen ST, Daneshvar B, Sorensen A. Effects of commonly consumed fruit juices and carbohydrates on redox status and anticancer biomarkers in female rats. Nutr Cancer 2003;45:46-52.
23. Kelley GL, Allan G, Azhar S. High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. Endocrinology 2004;145:548-55.
24. Busserolles J, Gueux E, Rock E, Mazur A, Rayssiguier Y. Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. J Nutr 2002;132:3379-82.
25. Levi B, Werman MJ. Long-term fructose consumption accelerates glycation and several age-related variables in male rats. J Nutr 1998;128:1442-9.