NF-κB, TNF-α and IL-6 levels in Liver and Kidney of High-Fructose-Fed Rats

Short title: High Fructose Diet

Abstract

Background: Fructose constituting an important part of human diet, was reported to facilitate fat depositing in the abdominal region in case of excessive consumption, therefore increasing the risk of chronic illness more rapidly than expected, and inducing development of various diseases such as diabetes, metabolic syndrome, hypertension and atherosclerosis. The aim of this study was to investigate nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels in liver and kidney tissues of high-fructose-fed rats and to determine the role of dietary addition of fructose on inflammation.

Methods: The rats were randomly divided into two groups of 7 rats as control (C) and fructose (F). The fructose group received 30% (v/w) fructose in drinking water for 8 weeks. Serum samples were used for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and uric acid measurements. The liver and kidney tissues of the rats were washed with 0.9% NaCl for TNF-α, IL-6 and NF-κB measurements.

Results: TNF-α, IL-6 and NF-κB levels in liver tissues were found significantly higher in the fructose group than the control group ($p<0.001$, $p<0.05$, $p<0.001$, respectively). TNF-α, IL-6 and NF-κB levels in the kidney tissue of the fructose group were statistically significantly higher than the control group ($p<0.001$, $p<0.001$, $p<0.001$, respectively).

Conclusion: Fructose fed diet increased liver and kidney damage through augmenting NF-κB, TNF-α and IL-6 levels.

Key words: fructose, interleukin-6, nuclear factor-kappa B, tumor necrosis factor-alpha

1.INTRODUCTION

As a matter of fact, eating habits today are rapidly changing worldwide, which cause an increased consumption of sugar and sugar-rich nutrients in the daily diet. Significant changes occurred in carbohydrate content of foods, and intake of complex carbohydrates rich in fiber content decreased while sugary products with high glycemic index increased. Fructose is the major sweetener of sugar-sweetened beverages as part of sucrose molecule or high fructose corn syrup (HFCS) besides glucose [1]. HFCS contains 42-90% fructose and is the main source of fructose in the diet. Fructose is widely used in fruit juices, canned fruits, jams, jellies, breakfast cereals and pastries. One of the reasons of high preference of fructose in food industry is its lower cost in comparison to saccharose. Another reason is that fructose-containing food and beverages delay the feeling of satiety while inducing the feeling of a new hunger sooner [2]. Given that fructose is rapidly metabolized in the body and converted directly into fatty acids unlike glucose, it was reported to contribute to lactic acidosis, liver steatosis, obesity, insulin resistance, diabetes and lipid metabolism disorders and also to play an important role in hypertension and cardiovascular diseases [3,4].

A wide variety of substances are secreted from the adipose tissue, including inflammatory cytokines to begin with. Cytokines are chemical signaling molecules existing in peptide or glycoprotein forms and mediate the development and regulation of inflammatory and immune responses in membranes [5]. These molecules are mainly produced by T cells and macrophages and secreted from cells with different characteristics. Cytokines are classified in 2 groups, namely pro-inflammatory and anti-inflammatory cytokines, and interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are involved in the proinflammatory group [6]. IL-6 is an important cytokine playing a role in many physiological and pathological events, such as immune response, acute phase response of the liver, hemopoiesis, regulation of neuronal functions and osteoclast formation. The synthesis and circulating level of IL-6 increase in parallel to the adipose tissue. Approximately one-third of the IL-6 in the systemic circulation originates from the adipose tissue [7]. IL-6 is distinctive with its endocrine activity and as a circulating cytokine, where most other cytokines function via autocrine or paracrine mechanisms. TNF-α is produced by monocytes and macrophages, in response to a variety of inflammatory and immunomodulatory stimuli. TNF-α has a considerably wide range of bioactivities, and most cells are sensitive to TNF-α. Under normal physiological conditions, TNF-α is involved in the formation of immune response, cellular homeostasis as well as cell survival, proliferation, migration and differentiation. TNF-α enhances lipolysis and apoptotic adipocyte death by inhibiting lipogenesis [8].

The liver is crucial for fructose metabolism, and for this very reason has a role in important events such as metabolic dysfunction of glucose, dyslipidemia and inflammation. Lipid accumulation leads to the development of oxidative stress in the liver and increased activation of nuclear factor kappa B (NF-κB) [9]. NF-κB is a transcription factor and a significant regulator of many genes responsible for inflammation, immune response, lymphocyte activation, cell growth and apoptosis [10]. NF-κB exists in all cell types and is involved in the production of cellular responses to stimuli such as stress, inflammatory cytokines, free radicals, ultraviolet radiation, carcinogens, tumor-forming agents, chemotherapeutics, bacterial and viral agents. NF-κB plays a central role in the pathophysiology of clinically important diseases of many organ systems and inflammatory cell damage [11,12].

This study was conducted to investigate NF-κB, TNF-α and IL-6 levels in liver and kidney tissues of high-fructose-fed rats and to determine the role of dietary addition of fructose on inflammation.

2. MATERIALS AND METHODS

2.1. Experimental Animals and Experimental Groups

Atatürk University Experimental Animals Ethics Committee approval was obtained before starting the study. As experimental animals, we used 14 male Wistar rats weighing about 200-250 g and 8 weeks old. The rats were randomly divided into two groups of 7 rats as control (C) and fructose (F). Experimental animals were kept under appropriate temperature and ventilation conditions as well as in hygienic conditions. There was no restrictions for feed and water. The control group was fed with the standard diet. The fructose group received 30% (v/w) fructose in drinking water for 8 weeks [13].

At the end of eight-week experiment, all the rats in both groups were decapitated under anesthesia with ketamine (60 mg/kg, Ketalar, Eczacibasi, Istanbul, Turkey) and xylazine (12 mg/kg; Rompun, Bayer, Istanbul, Turkey). Blood samples of the rats were rapidly collected in biochemistry tubes and centrifuged at 4000xg for 10 minutes to separate the sera. Serum samples were used for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and uric acid measurements. The liver and kidney tissues of the rats were washed with 0.9% NaCl for TNF-α, IL-6 and NF-κB measurements and packaged and stored at -80°C in the deep freezer until the day of measurement.

2.2. Biochemical Analysis

Serum AST, ALT, ALP, BUN and uric acid levels were determined by autoanalyzer. Liver and kidney tissues were homogenized with 1/10 cold 0.1 M phosphate buffer (pH: 7.4) by using homogenizer. Tissue homogenates were centrifuged at 4000xg and at 4°C for 20 minutes and supernatants were obtained. In the kidney and liver tissue samples, TNF-α , IL-6 and NF-κB levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method using commercial kits (SunRed Biological Technology, Co., Ltd. Shanghai). Absorbances were read at 450 nm in the ELISA reader. The TNF-α, IL-6 and NF-κB results were stated as ng/ml.

2.3. Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) 24.0 program and Student's T-Test. All results were expressed as mean±standard deviation and $p < 0.05$ was considered statistically significant.

3. RESULTS

Serum AST, ALT and ALP enzyme activities with BUN and uric acid levels of the study groups are presented in Table 1. Serum AST, ALT, ALP enzyme activities and BUN and uric acid levels of the fructose group were statistically significantly higher than the control group ($p<0.001$). TNF-α, IL-6 and NF-κB levels in liver and kidney tissues are presented in Table 2. TNF-α, IL-6 and NF-κB levels in liver tissues were found significantly higher in the fructose group than the control group ($p<0.001$, $p<0.05$, $p<0.001$, respectively). TNF-α, IL-6 and NF-κB levels in the kidney tissue of the fructose group were statistically significantly higher than the control group ($p<0.001$, $p<0.001$, $p<0.001$, respectively).

4. DISCUSSION

Changes in the living conditions and eating habits lead to more fructose intake due to the preference of ready-to-eat foods, hence increased calories through daily diet. Different carbohydrate types such as fructose, fructose-corn syrup and sucrose abounding in processed and packaged, ready-to-eat foods and beverages, can cause metabolic dysfunctions associated with chronic diseases due to high-energy contents and differences in their metabolisms [14,15]. In this study, we investigated the effect of 30% fructose added to drinking water on both liver and kidney tissues as well as on the liver enzyme profile.

Due to its roles in protein synthesis, carbohydrate and lipid metabolisms and being the main organ responsible for fructose metabolism, liver has an important place in xenobiotic metabolism and it is the organ affected at most by the metabolic changes and damaged first. For this reason, loss of liver function is vital for the human beings [16]. Both clinical and experimental studies in the literature reported that liver function markers such as serum AST, ALT and ALP had the tendency to increase in obesity and non-alcoholic liver steatosis [17,18,19]. In this study too, we observed a statistically significant increase in the serum levels of AST, ALT and ALP of the fructose-fed group compared to the control group. Masterjohn et al. [20] reported plasma ALT and AST activities were elevated in comparison to the control group in high fructose-fed mice. In another study, high fructose-fed rats displayed higher levels of plasma ALT and AST activities than the control group [21]. There are studies in the literature reporting similar results as ours as well as those with opposite results. Ackerman et al. [22] showed that there was no change in ALT and AST levels of rats fed a diet containing 60% fructose for 5 weeks. Various mechanisms and different mediators are mentioned in fructose-induced renal damage. BUN and uric acid are metabolic waste products that are excreted from the kidneys. In this study, we observed a statistically significant increase in the serum levels of BUN and uric acid of the fructose-fed group compared to the control group. When designing this study, we knew that fructose caused liver and kidney diseases. But the primary factor initiating and developing disease process was the real question that shoud be answered. Oxidative stress and inflammation are the issues discussed at most. In our study, by means of both kidney and liver tissues, we tried to determine how strong was the effect of fructose on inflammation. Cytokines are molecules having roles in various biological processes such as inflammation, apoptosis, necrosis, and fibrosis [23]. They are usually released from lymphocytes and monocytes and are effective in intercellular communication and modulation of the immune response. By inhibiting apoptosis during the inflammatory process, IL-6 triggers events that develop chronic disease progression [24].

The release of IL-6 is induced by interleukin-1 (IL-1) and TNF-α, and IL-6 displays synergistic effects with these cytokines. The best defined effects of IL-6 are on hepatocytes and B lymphocytes and cause hepatocytes to synthesize many plasma proteins that contribute to the acute phase response. IL-6 was also reported to be associated with signal transduction pathways and expected to increase in hepatic cirrhosis and liver cancer due to regeneration [25]. Yang et al. [26] examined renal tissues of high fructose-fed rats and reported that fructose reduced kidney weight, led to renal tubular damage, and increased TNF-α and IL-6

levels significantly. In our study too, there was a statistically significant increase in TNF-α and IL-6 levels in both liver and kidney tissues compared to the control group. Gersch et al. [27] reported that a high-fructose diet might induce inflammation, because fructose accelerated renal disease in association with an increase in monocyte-macrophage infiltration. The increase in adipose tissue due to high fructose consumption is one of the most important causes of the secretion of inflammatory cytokines in the systemic circulation. Adipose tissue dysfunction can impair energy consumption, primarily inflammatory cytokines and insulin signaling [28,29]. Both human and rat studies in the literature stated that high fructose diet caused increase in visceral adiposity, and as a response to that, inflammatory cytokines increased and organ-related morphological and functional changes were observed [30,31].

In the literature, TNF-α was reported to induce NF-κB activation, which leads to an increase in proinflammatory cytokines through various mechanisms, resulting in an increase in TNF-α release and consequently development of hepatosteatosis and insulin resistance [32,33]. In our study, levels of NF-κB, a transcription factor involved in the expression of cytokines that cause proinflammatory cytokine elevation, were measured in both liver and kidney tissues. The liver and kidney NF-κB levels of the group with fructose added to drinking water were significantly higher than the control group. In the study by Veličković et al., [34] high fructose diet was reported to increase NF-κB and TNFα levels in the liver. In another study by Zheng et al., [35] excessive fructose consumption caused inflammation by increasing fatty acid biosynthesis and fat accumulation in the liver. Vasiljević et al. [36] reported increased levels of hepatic TNF-α and NF-κB in high fructose-fed rats for 9 weeks and stated that high fructose contributed to the development of NF-κB-mediated inflammation. NF-κB is one of the key regulators in inflammatory processes and stimulates the sythesis of TNF-α, IL-6 and adipokine, which increase in inflammation [37,38]. We can say that fructose-induced chronic inflammation leads to the activation of NF-κB, which stimulates the release of proinflammatory cytokines as a result of cell damage in liver and kidney tissues due to excessive fructose consumption [39].

5. CONCLUSION

In conclusion, in this study investigating the effects of high fructose administration on the liver and kidney, we determined that fructose increased inflammation by regulating the TNF-α and IL-6 signaling pathway as a result of NF-κB activation.

REFERENCES

1. Rebollo A, Roglans N, Alegret M. Way back for fructose and liver metabolism: Bench side to molecular insights. World J Gastroenterol 2012;18(45):6552-6559.

2. Bellamkonda R, Karuna R, Sasi Bhusana Rao B, Haritha K, Manjunatha B, Silpa S, Saralakumari D. Beneficiary effect of Commiphora mukul ethanolic extract against high fructose diet induced abnormalities in carbohydrate and lipid metabolism in wistar rats. J Tradit Complement Med 2017;8(1):203-211.

3. Nomura K, Yamanouchi T. The role of fructose-enriched diets in mechanisms of nonalcoholic fatty liver disease. J Nutr Biochem 2012;23:203-208.

4. Park JH, Ku HJ, Kim JK, Park JW, Lee JH. Amelioration of High Fructose-Induced Cardiac Hypertrophy by Naringin. Sci. Rep 2018;8(1): 9464.

5. Abbas AK, Lichtman AH. Basic immunology: functions and disorders of the immune system. 2nd ed. Phiadelphia, PA: W.B Saunders Co, Updated edition 2006-2007.

6. Avci B, Alacam H, Dilek A, Kozan A. Effects of asymmetric dimethylarginine on inflammatory cytokines (TNF-α, IL-6 and IL-10) in rats. Toxicol. Ind. Health 2015;31(3):268-73.

7. Chan DC, Watts GF, Ng TW, Uchida Y, Sakai N, Yamashita S, Barrett PH. Adiponectin and other adipocytokines as predictors of markers of triglyceride-rich lipoprotein metabolism. Clin Chem 2005;51(3):578-85.

8. Tsatsanis C, Zacharioudaki V, Androulidaki A, Dermitzaki E, Charalampopoulos I, Minas V, Gravanis A, Margioris AN. Adiponectin induces TNF-alpha and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli. Biochem Biophys Res Commun 2005;335(4):1254-63.

9. Francini F, Castro MC, Schinella G, García ME, Maiztegui B, Raschia MA, Gagliardino JJ, Massa ML. Changes induced by a fructose-rich diet on hepatic metabolism and the antioxidant system. Life Sci 2010;86:965–971.

10. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. Oncogene 2006;25:6680-4.

11. Baker RG, Hayden MS, Ghosh S. NF-κB, inflammation and metabolic disease. Cell Metab. 2011;13(1):11–22.

12. Sanz AB, Sanchez-Niño MD, Ramos AM, Moreno JA, Santamaria B, Ruiz-Ortega M, Egido J, Ortiz A. NF-kappaB in renal inflammation. J Am Soc Nephrol 2010;21:1254-1262.

13. Volynets V, Spruss A, Kanuri G, Wagnerberger S, Bischoff SC, Bergheim I. Protective effect of bile acids on the onset of fructose-induced hepatic steatosis in mice. J Lipid Res 2010;51:3414-3424.

14. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev 2005;63(5):133-57.

15. 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(4):537-43.

16. Friedman SL. Seminars in medicine of the Beth Israel Hospital, Boston. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. N Engl J Med 1993;328:1828-1835.

17. Nieto N, Friedman SL, Cederbaum AI. Stimulation and proliferation of primary rat hepatic stellate cells by cytochrome P450 2E1-derived reactive oxygen species. Hepatology 2002;35:62-73.

18. Malaguarnera L, Di Rosa M, Zambito AM, dell'Ombra N, Nicoletti F, Malaguarnera M. Chitotriosidase gene expression in Kupffer cells from patients with non-alcoholic fatty liver disease. Gut 2006;55:1313-1320.

19. Malaguarnera L, Di Rosa M, Zambito AM, dell'Ombra N, Di Marco R, Malaguarnera M. Potential role of chitotriosidase gene in nonalcoholic fatty liver disease evolution. Am J Gastroenterol 2006;101:2060-2069.

20. Masterjohn C, Park Y, Lee, J. Dietary fructose feding ıncreases adipose methylglyoxal accumulation in rats in association with low expression and activity of glyoxalase-2. Nutrients 2013;5:3311-3328.

21. Giriş M, Abbasoğlu SD, Kumral A. Effect of carnosine alone or combined with α-tocopherol on hepatic steatosis and oxidative stres in fructose-induced insulin-resistant rats. J Physiol Biochem 2014;70:385-395.

22. Ackerman Z, Herman MO, Rosenthal MGT, Pappo O, Link G, Sela BA. Fructose-induced fatty liver disease hepatic effects of blood pressure and plasma triglyceride reduction. Hypertension 2005;45(5):1012-8.

23. Falasca K, Ucciferri C, Dalessandro M, Zingariello P, Mancino P, Petrarca C, Pizzigallo E, Conti P, Vecchiet J. Cytokine patterns correlate with liver damage in patients with chronic hepatitis B and C. Ann Clin Lab Sci 2006;36(2):144-150

24. Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. J Immunol 2005;175(6):3463-3468.

25. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: From physiopathology to therapy. J Hepatol. 2016;64(6):1403-15.

26. Yang M, Jiang R, Lin X. Ginger extracts improve renal injury of high fructose-fed SD rats by inhibiting the expression of proinflammatory cytokines. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2014;30(9):929-32.

27. Gersch MS, Mu W, Cirillo P, Reungjui S, Zhang L, Roncal C, Sautin YY, Johnson RJ, Nakagawa T. Fructose, but not dextrose, accelerates the progression of chronic kidney disease. Am J Physiol Renal Physiol 2007;293:1256-1261.

28. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Investig 2009;119:1322-1334.

29. Pektas MB, Koca HB, Sadi G, Akar F. Dietary fructose activates insulin signaling and inflammation in adipose tissue: Modulatory role of resveratrol. BioMed Res Int 2016;2016:8014252.

30. Zhang DM, Jiao RQ, Kong LD. High Dietary Fructose: Direct or Indirect Dangerous Factors Disturbing Tissue and Organ Functions. Nutrients 2017; 9:1-25.

31. Jin YH, Park YJ, Ham M, Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. Mol Cells 2014;37:365-371.

32. Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterol 1996;111:1645-1653.

33. George DK, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, Jazwinska EC, Powell LW. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterol 1998;114:311-318.

34. Veličković N, Djordjevic A, Vasiljević A, Bursać B, Milutinović DV, Matić G. Tissue-specific regulation of inflammation by macrophage migration inhibitory factor and glucocorticoids in fructose-fed Wistar rats. Br J Nutr 2013;110(3):456–465.

35. Zheng J, Peng C, Ai Y, Wang H, Xiao X, Li J. Docosahexaenoic Acid Ameliorates Fructose-Induced Hepatic Steatosis Involving ER Stress Response in Primary Mouse Hepatocytes. Nutrients 2016;8(1):55.

36. Vasiljević A, Bursać B, Djordjevic A, Milutinović DV, Nikolić M, Matić G, Veličković N. Hepatic inflammation induced by high-fructose diet is associated with altered 11βHSD1 expression in the liver of Wistar rats. Eur J Nutr 2014;53(6):1393-402.

37. Basaranoglu M, Basaranoglu G, Sabuncu T, Sentürk H. Fructose as a key player in the development of fatty liver disease. World J Gastroenterol 2013;19(8):1166-72.

38. Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, Nicol CJ, Vinson C, Gonzalez FJ, Reitman ML. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. J Biol Chem 2003;278(36):34268-76.

39. Sunami Y, Leithäuser F, Gul S, Fiedler K, Güldiken N, Espenlaub S. Hepatic activation of IKK/NF-kappa B signaling induces liver fibrosis via macrophage-mediated chronic inflammation. Hepatology 2012;56(3):1117-28.

Table 1. Serum biochemical parameters of the groups

	Control	Fructose
AST (U/L)	45,00±15,15[a]	150,00±20,50[b]
ALT (U/L)	34,00±12,50[a]	172,00±23,34[b]
ALP (U/L)	120,45±22,35[a]	269,35±40,85[b]
BUN (mg/dL)	16,45±3,12[a]	58,35±5,22[b]
Uric acid (mg/dL)	4.20±0,80[a]	17,34±4,62[b]

Data were given as mean ±SD.

AST : Aspartate aminotransferase, ALT : Alanine aminotransferase, ALP: Alkaline phosphatase, BUN: Blood Urea Nitrogen

[*]Different superscripts within the same columns differ, ($p<0.001$)

Table 2. Tissue biochemical parameters of the groups

	Groups	TNF-α	IL-6	NF-κB
Liver	Control	1160,75±476,33[a]	1,03±0,26[a]	0,89±0,28[a]
	Fructose	3510,64±400,37[b]	1,75±0,32[c]	2,62±0,57[b]
Kidney	Control	203,08±26,54[a]	0,16±0,06[a]	0,36±0,08[a]
	Fructose	528,59±101,43[b]	0,37±0,10[c]	0,81±0,20[b]

Data were given as mean ±SD.

TNF-α: Tumor necrosis factor alpha, IL-6: Interleukin-6, NF-κB**:** Nuclear factor kappa B

[a]Significantly different when compared with control group, ($p<0.001$)

[b]Significantly different when compared with control group, ($p<0.05$)

[c]Significantly different when compared with control group, ($p<0.01$)

Highlights

- Fructose fed diet causes a potentially fatal hepatotoxicity and nephrotoxicity
- Levels of NF-κB, a transcription factor involved in the expression of cytokines that cause proinflammatory cytokine elevation, were measured in both liver and kidney tissues.
- Fructose increased inflammation by regulating the TNF-α and IL-6 signaling pathway as a result of NF-κB activation.