World Applied Sciences Journal 28 (12): 2249-2253, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.28.12.2072

Effects of Dietary Fructose, Sucrose and Lactose in Induction of Nonalcoholic Fatty Liver in Rat

[1]Binesh Fariba, [2]Salmanroghani Hassan, [3]Ghasemalian Shahin and [4]Rajabzadeh Yavar

[1]Clinical and Surgical Pathology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2]Internal Medicine, Gastroenterologist, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3]Internalist, Shahid Sadoughi University of Medical Sciences,yazd, Iran
[4]General Practitioner, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abstract: *Background*: Non alcoholic fatty liver disease(NAFLD) has a very high prevalence. Some studies have shown that diets high in sugars have etiologic role. In this study the effects of different sugars on histology of rat's liver studied. *Methods*: Male Ratus Morvegicus Dvivius rats (n=40) weighing 250-270 gram were obtained from the animal house of shahid sadoughi university of medical science. Lactose, sucrose and fructoses were supplied as 10% drinking solutions. When the rats were 25% overweight, the animals were sacrificed and their liver was extracted and histologically was evaluated. *Results*: No evidence of macro or microvesicular steatosis were found in liver specimens of control and tests groups.According to PV=1,there was no meaningful difference regarding histological findings in tests and control groups. *Conclusion*: To explain these subjects we should say that 1-NAFLD is a multifactorial disorder and only added sugar is inadequate in development of this disease. 2-More complex diet is needed.3- Longer term exposure to sugars is necessary and 4- Genetic factor is important. Since neither control nor test groups show fatty liver, it is possible that our rats were not genetically susceptible to fatty liver. There is no objective ground to support that moderate intake of sugars is unsafe.

Key words: Rat · Nonalcoholic fatty liver · Sucrose · Lactose · Fructose

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) encompasses a disease spectrum ranging from simple hepatic steatosis to steatohepatitis (NASH), fibrosis and cirrhosis. It has a very high prevalence in worldwide. The major risk factors are obesity and insulin resistance [1]. Increase in affluence, with accompanying changes in lifestyle; have led to reduced physical activity and increased intake of easily available calorie-rich foods resulting in increased obesity [2]. Recent studies have shown that diets high in saturated fat, sugars and meat are associated with an increased risk of obesity and NAFLD/NASH [3-4]. The most common sugars in our nutrition are sucrose, fructose and glucose. It has been long recognized that feeding a high-fructose diet for more than 1 week increases plasma total- and VLDL-triglycerides in healthy volunteers and in patients with insulin resistance or type 2 diabetes. An increase in total cholesterol was also encountered in some of these studies [5]. The mechanisms underlying fructose-induced dyslipidemia have been partially elucidated. Altogether, epidemiological studies at this stage provide an incomplete, sometimes discordant appraisal of the relationship between fructose or sugar intake and metabolic/cardiovascular diseases. Previous studies in rodents have demonstrated various histological alterations of liver tissue after fructose consumption [6-8]. Several large-scale epidemiological studies have also suggested a positive relationship between fructose consumption and NAFLD [4, 9-10]. This study aimed to evaluate the effects of fructose, lactose and sucrose in development of NAFL in rat.

Corresponding Author: Dr. Fariba Binesh, Clinical and Surgical Pathology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Tel: +983518113698, Fax:+983518224100.

MATERIALS AND METHODS

This is an experimental (lab trial) study and aimed to evaluate the effects of fructose, lactose and sucrose in development of NAFL in rat.

Male Ratus Morvegicus Dvivius rats (n=40) weighing 250-270 gram were obtained from the animal house of shahid sadoughi university of medical science. During the experiment, the animals were kept in collective polyethylene cages (10 rats per cage) in a closed biotherium at 25°C under a light/dark photoperiod of 12/12 hours. The animals received commercial chow for rodents (containing 17.44% protein 1.95%fat 4.58% fiber, 0.48% salt, 0.62%p hosphorus, 0.56%calcium, 1% various vitamins and 73.37% starch). Body weight, as well as food and liquid intake per cage, was measured and recorded once a week. All experiments involving animals were reviewed and approved by the Ethics Committee. The animals were divided randomly into four groups (n= 10 per group) according to the liquid source utilized. Control Group (C): animals drank water as the only liquid source; fructose group (F): animals drank 10%fructose solution, sucrose group (S): the rats drank 10%sucrose solution and lactose group(L): animals drank 10% lactose solution.

When the rats were 25% overweight, the animals were sacrificed by decapitation for sample collection. We performed a subchondral incision and the liver was extracted. The specimens were send to pathology ward in 10% formalin solution. Tissues were fixed in 10%formalin solution, dehydrated in alcohols and embedded in paraffin (Sigma-Aldrich, St. Louis, MO, USA) and 4-µ sections were stained with H&E. The histological analysis was performed blinded regarding treatment groups.

The statistical analysis was performed using SPSS, version 16 (SPSS Inc., Chicago, IL, USA) using ANOVA test. Pearson correlation coefficients were determined between diagnostic groups. A P value <.05 was considered to be significant.

RESULTS

Liver Histology: The histological analysis was performed blinded regarding studied groups. Steatosis attributable to NAFLD is typically macro vesicular rather than micro vesicular [11]. In the previous studies, macro vesicular steatosis was defined as a single vacuole that is larger than the nucleus and usually displaces it to the periphery of the cell [12]. But in all of the studied groups the liver architecture was intact and there was no evidence of macro or even micro vesicular steatosis. There was not

Table 1: Shows frequency of fatty change among control and test groups

Groups	Number	Fatty change
Control	10	Negative
Fructose group	10	Negative
Sucrose group	10	Negative
Lactose group	10	Negative

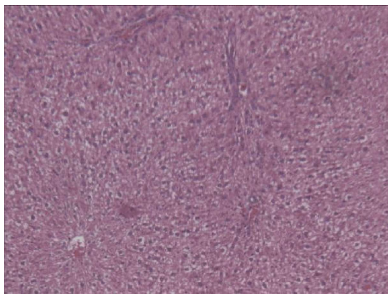

Fig. 1: Control group

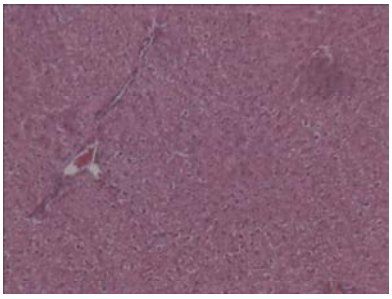

Fig. 2: Fructose group

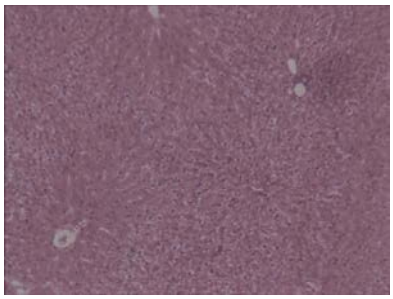

Fig. 3: Sucrose group

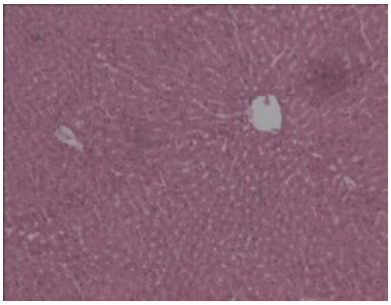

Fig. 4: Lactose group

inflammatory cells infiltration in the portal tracts (Fig. 1-4). Our research set up was based on Kazumi *et al.* studies in 1986 and 1997 [13-14]. According to these findings we reviewed our research and repeated our evaluation with 20 rats again, but the pathology records were identical to our previous results. According to PV=1, there was no meaningful difference regarding histological findings in tests and control groups (Table 1).

DISCUSSION

The pathogenesis of NAFLD is yet to be clearly defined. Obesity, hyperglycemia, type 2 diabetes and hypertriglyceridemia are most important etiologic factors. Genetic factors undoubtedly predispose to NAFLD, as supported by higher prevalence of steatosis in Hispanics than Caucasians and African-Americans [15]. On the other hand overconsumption of sugars promotes the development of overweight and obesity and is associated with metabolic disturbances, including intrahepatic fat accumulation and metabolic syndrome. In one study [13] the effects of different dietary sugars, with or without exogenously induced hyperinsulinemia, on rat plasma triglyceride kinetics have been studied. They concluded that dietary fructose not only increased triglyceride production, but also impaired triglyceride removal. Exogenously induced hyperinsulinemia further increased triglyceride production in those rats receiving dietary fructose, either as the monosaccharide or as sucrose, but not in those receiving only glucose. In another study [14] effects of dietary carbohydrates on triglyceride production and hepatic lipogenic enzyme activities have been examined in Wistar fatty rats, an animal model of noninsulin dependent diabetes mellitus, fed fructose or glucose and have been compared with those of Wistar lean rats. They found out that in genetically obese, diabetic rats feeding fructose and glucose is associated with an increase in hepatic lipogenic enzyme activities and triglyceride production and authors have suggested that fructose stimulates triglyceride production but impairs triglyceride removal, whereas glucose stimulates both of them. The question is that how does fructose play a role in the pathogenesis of these unfavorable effects? Although much is known about hepatic fructose metabolism, the detailed mechanism of the second hit associated with fructose consumption remains unclear [16]. Fructose has long been known to be metabolized differently than the other commonly consumed monosaccharide and recently this has caused it to be implicated in the development of the metabolic abnormalities of the metabolic syndrome [17]. Fructose, unlike glucose, does not stimulate insulin secretion from the pancreatic β-cells [18]. In one study, pure fructose, consumed with mixed meals, was shown to result in decreased circulating insulin and leptin and to attenuate postprandial suppression of Ghrelin (Ghrelin is counterpart of hormone leptin) in women, as compared with dietary glucose [19]. These authors concluded that the differential impact of fructose on energy regulatory systems as compared with glucose might contribute to increased caloric intake and ultimately contribute to weight gain and obesity during chronic consumption of a diet high in fructose. Fructose metabolism is known for its lipogenic potential. Thus, hepatic fructose metabolism (liver is the main organ capable of metabolizing this simple carbohydrate), can promote triglyceride synthesis. Experimental evidence has shown that fructose increases postprandial levels of triglyceride [19-20]. Another unique characteristic of fructose metabolism is the ability to raise uric acid level. Uric acid is a product of nucleotide metabolism, which is up-regulated by fructose. In addition, in an in vitro study, it was suggested that HFCS (high-fructose corn syrup) could be a significant source of reactive dicarbonyls that would by itself induce hyperuricemia [21]. Despite uric acid's early designation as an antioxidant [22], it is estimated to be responsible for as much as 60% of the antioxidant capacity of the plasma [23], it is now also known to have prooxidative properties [24]. Elevated levels are seen in a wide variety of metabolic disease states [25], which poses the question of whether hyperuricemia is secondary to the disease state or whether it plays a mechanistic role in the development of metabolic disease states [17]. Increasingly, the weight of evidence is in support of the latter. As mentioned previously we repeated our study for the second time and we didn't observe any difference between liver histology in control and tests group. To explain this difference we should be noted that: 1 –NAFL is a multifactorial disorder and insulin resistance, obesity-related inflammation, genetic, possible dietary and lifestyle factors are thought to play a key role. The rising incidence of obesity in today's generation is associated with many health complications in addition to NAFLD. These include cardiovascular diseases, diabetes, hyperlipidemia and hypertension. This constellation is recognized as metabolic syndrome. 70% of patients with fatty liver have metabolic syndrome and 30% of patients with metabolic syndrome have fatty liver. So it is probably that only

added sugar by itself is inadequate in development of this disease and metabolic syndrome has a critical role. 2- In comparison with some other studies[7]more complex diet (higher percent of fructose, lipid and other minerals especially iron) is needed. These complex diets are effective in development of metabolic syndrome and as noted the role of metabolic syndrome in NAFL is critical. 3-Since NAFL is a slow progressing disease it seems that probably long term exposure to sugars is an important factor. In our study the rats were exposure to various sugars for 60 days, whereas in other studies time was longer or the concentration of the sugars was higher. 4- Genetic factor is an important factor in this disease. Since in our study neither control nor test group showed fatty liver, it is possible that our rats were not genetically susceptible to fatty liver. Altogether epidemiological studies at this stage provide an incomplete, sometimes discordant appraisal of the relationship between sugar intake and metabolic diseases. Part of the discordances may be explained by the fact that intakes of sugars are often not recorded individually. In addition, fructose is essentially consumed as either sucrose or HFCS (high-fructose corn syrup), with the consequence that glucose intakes essentially varies with fructose intake. Confounding factors (i.e., interrelationship between sugar intake and intake of other nutrients, association with physical activity and life-style) are important and difficult to control for. Indeed, there appears to be strong evidence that consumption of sugars is associated with obesity. Presently, there is no single hint that the HFCS (high-fructose corn syrup) may have more deleterious effect than other sources of sugar.

CONCLUSION

There is, however, no objective ground to support that moderate intake of fructose, or of fructose consumed with fruits or honey, is unsafe. We recommended further studies with higher concentration of mono and disaccharides in longer period to elucidate the relationship between sugar consumption and NAFL.

ACKNOWLEDGMENTS

We thank shahid sadoughi university of medical sciences for their support.

Conflict of Interest: Not declared.

Funding Support: This work was supported by Shahid Sadoughi University of medical sciences and health services, Yazd, Iran.

REFERENCES

1. Schwimmer, J.B., R. Deutsch, J.B. Rauch, C. Behling, R. Newbury and J.E. Lavine, 2003. Obesity, Insulin Resistance and Other Clinicopathological Correlates of Pediatric Nonalcoholic Fatty Liver Disease. J. Pediatr, 143: 500-505.
2. Kerkar N., 2004. Non-alcoholic steatohepatitis in children. Pediatr Transplantation, 8: 613-618.
3. Musso, G., R. Gambino, F. De Michieli, M. Cassader, M. Rizzetto, E. Faga, *et al*., 2003. Dietary habits and their relations to insulinresistance and postprandial lipemia in non-alcoholic steatohepatitis. Hepatology, 37: 909-916.
4. Zelber-Sagi, S., D. Nitzan-Kaluski, R. Goldsmith, M. Webb, L. Blendis, Z. Halpern, *et al*., 2007. Long term nutritional intake and the risk ofnon-alcoholic fatty liver disease (NAFLD): a population basedstudy. J. Hepatol., 47: 711-717.
5. Bantle, J.P., D.C. Laine and J.W. Thomas, 1986. Metabolic effects of dietary fructose and sucrose in types I and II diabetic subjects. JAMAM, 256: 3241-3246.
6. Sanchez-Lozada, L.G., W. Mu, C. Roncal, Y.Y. Sautin, M. Abdelmalek, S. Reungjui *et al*., 2010. Comparison of free fructose and glucose to sucrose in the ability to cause fatty liver Eur. J. Nutr., 49: 1-9.
7. Ackerman, Z., M. Oron-Herman, M. Grozovski, T. Rosenthal, O. Pappo, G. Link *et al*., 2005. Fructose-induced fatty liver disease: hepatic effects of blood pressure and plasma triglyceride reduction Hypertension, 45: 1012-1018.
8. Armutcu, F., O. Coskun, A. Gurel, M. Kanter, M. Can, F. Ucar *et al*., 2005. Thymosin α 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats Clin Biochem., 38: 540-547.
9. Ouyang, X., P. Cirillo, Y. Sautin, S. McCall, J.L. Bruchette, A.M. Diehl *et al*., 2008. Fructose consumption as a risk factor for non-alcoholic fatty liver disease J. Hepatol., 48: 993-999.
10. Abdelmalek, M.F., A. Suzuki, C. Guy, A. Unalp-Arida, R. Colvin, R.J. Johnson, *et al*., 2010. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease Hepatology, 51: 1961-1971.

11. Brunt, E.M., 2001. Nonalcoholic steatohepatitis: definition andpathology. Semin Liver Dis., 21: 3-16.
12. Oleszczuk, A., M. Spannbauer, A. Tannapfel, M. Bluher, J. Hengstler, U.C. Pietsch, A. Schuhmacher, C. Wittekind, J.P. Hauss and M.R. Schon, 2007. Regenerative capacity differs between micro- and macrovesicular hepatic steatosis. Exp. Toxicol. Pathol., 59: 205-213.
13. Kazumi, T., M. Vranic and G. Steiner, 1986. Triglyceride kinetics: effects of dietary glucose, sucrose, or fructose alone or with hyperinsulinemia. Am. J. Physiol., 250(3 Pt 1): E325-30.
14. Tsutomu Kazumi, Hiroyuki Odaka, Toshiki Hozumi, Yoshihiko Ishida, Nobuyuki Amano and Gen Yoshino, 1997. Effects of Dietary Fructose or Glucose on TriglycerideProduction and Lipogenic Enzyme Activities in the LiverWistar Fatty Rats, an Animal Model of NIDDM, Endocrine Journal, 44(2): 239-245.
15. Browning, J.D., L.S. Szczepaniak, R. Dobbins, P. Nuremberg, J.D. Horton, J.C. Cohen, S.M. Grundy and H.H. Hobbs, 2004. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology, 40: 1387-1395.
16. Kyoko, Nomura and Toshikazu Yamanouchi, 2012. The role of fructose-enriched diets in mechanisms of nonalcoholic fatty liver diseaseThe Journal of Nutritional Biochemistry, 23(3): 203-208.
17. Johnson, R.J., M.S. Segal, Y. Sautin, T. Nakagawa, D.I. Feig, D.H. Kang, M.S. Gersch, S. Benner and L.G. Sánchez-Lozada, 2007. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease and cardiovascular diseased. Am. J. Clin Nutr., 86: 899-906.
18. Curry, D.L., 1989. Effects of mannose and fructose on the synthesis and secretion of insulin. Pancreas, 4: 2-9.
19. Teff, K.L., S.S. Elliott, M. Tschöp, T.J. Kieffer, D. Rader, M. Heiman, R.R. Townsend, N.L. Keim, D. D'Alessio and P.J. Havel, 2004. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin and increases triglycerides in women. J. Clin Endocrinol. Metab., 89: 2963-72.
20. Jeppesen, J., Y.D.I. Chen, M.Y. Zhou, T. Wang and G.M. Reaven, 1995. Effect of variations in oral fat and carbohydrate load on postprandial lipemia. Am. J. Clin Nutr., 62: 1201-5.
21. Lo, C.Y., S.M. Li, Y. Wang, D. Tan, M.H. Pan, S. Sang and C.T. Ho, 2008. Reactive dicarbonyl compounds and 5- (hydroxymethyl)-2-furfural in carbonated beverages containing high fructose corn syrup. Food Chem., 107: 1099-1105.
22. Glantzounis, G.K., E.C. Tsimoyiannis, A.M. Kappas and D.A. Galaris, 2005. Uric acid and oxidative stress. Curr. Pharm. Des., 11: 4145-51.
23. Nieto, F.J., C. Iribarren, M.D. Gross, G.W. Comstock and R.G. Cutler, 2000. Uric acid and serum antioxidant capacity: a reaction to atherosclerosis? Atherosclerosis, 148: 131-9.
24. Corry, D.B., P. Eslami, K. Yamamoto, M.D. Nyby, H. Makino and M.L. Tuck, 2008. Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. J. Hypertens., 26: 269-75.
25. Mellen, P.B., A.J. Bleyer, T.P. Erlinger, G.W. Evans, F.J. Nieto, L.E. Wagenknecht, M.R. Wofford and D.M. Herrington, 2006. Serum uric acid predicts incident hypertension in a biethnic cohort: the atherosclerosis risk in communities study. Hypertension, 48: 1037-42.