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Original Research Article

Effect of high fructose consumption on plasma levels of leptin, adropin and insulin, and serum biochemical parameters in mature adult rats

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Abstract

Purpose: *To determine the effect of high-fructose consumption on plasma levels of insulin, leptin, and adropin, as well as serum biochemical parameters in mature adult rats.*
Methods: *Sixteen (16) mature adult rats were assigned to control group (CG) and fructose group (FG). The CG was fed ad libitum with standard rat feed and drinking water, while FG was fed standard rat feed and 20 % fructose in drinking water for 8 weeks. Plasma hormonal analyses for insulin, leptin and adropin were performed by enzyme-linked immunosorbent assay (ELISA), while serum biochemical analysis was carried out with an autoanalyzer.*
Results: *In FG, plasma levels of insulin and leptin significantly increased, while adropin levels decreased, when compared to CG ($p < 0.05$). On the other hand, while serum glucose and triglycerides were significantly increased by fructose feeding, total cholesterol, HDL-C and ALT levels were decreased ($p < 0.05$).*
Conclusion: *High fructose consumption triggers metabolic syndrome in mature adult similar to young rats, resulting in hyperleptinemia and hyperinsulinemia. Moreover, high fructose consumption decreases plasma adropin levels.*

Keywords: *Adropin, Fructose, Insulin, Leptin*

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INTRODUCTION

In the last forty years, fructose consumption has increased by approximately 30 % [1]. At the same time, increased fructose consumption has led to rising cases of metabolic irregularities such as obesity and metabolic syndrome (MetS) [1]. It has been reported that chronic fructose consumption for 8 weeks increased serum insulin and leptin levels, and caused leptin resistance in juvenile rat brain [2]. Thus, hyperinsulinemia and hypertriglyceridemia may mediate leptin resistance in juvenile rat brain [2]. Furthermore, fructose does not acutely stimulate the production of leptin and insulin [3]. Therefore, insulin and leptin resistance develop in long-term fructose consumption.

Adropin is a newly-discovered peptide hormone encoded by the energy homeostasis-associated

(ENHO) gene. This gene, which is mostly expressed in the liver, is regulated together with other genes involved in glucose and lipid metabolism in the liver [4]. Indeed, a study has demonstrated a link between adropin deficiency and insulin resistance in the liver [5]. In that study, it was observed that there was a defect in the suppression of hepatic glucose production in AdrKO mice, resulting in insulin resistance in the liver [5]. Since production of glucose in liver is an important factor that influences fasting blood glucose level, and since it has a core role in the effects of insulin, these results suggest that adropin affects insulin function and hepatic glucose metabolism. Indeed, hyperinsulinemia and hyperglycemia have been reported in AdrKO mice [5].

In humans, studies have shown that fructose consumption increased plasma adropin, irrespective of duration, sex or age [6]. However, in adult rats aged 5 - 6 months, not much is known on the effect of long-term high fructose diet on plasma levels of adropin, insülin, leptin and some biochemical parameters. This research was done to investigate the effects of high fructose consumption on plasma insulin, leptin and adropin levels, as well as serum levels of fasting glucose, total cholesterol (C), HDL-C, LDL-C, triglycerides and uric acid, in mature adult rats. In addition, the effects of high fructose feeding on serum activities of AST, ALT, GGT and LDH in the mature adult rats, were studied.

EXPERIMENTAL

This study was carried out at the Experimental Animal Breeding and Experimental Research Center of Burdur Mehmet Akif Ersoy University. The research received approval from the ethical Authority of same institution (approval no. 725/86), and it was carried out according to the guidelines of Principles of Laboratory Animal Care [7].

Experimental animals

Sixteen (16) adult male Sprague Dawley rats used in the study were housed in two plastic rat cages (8 rats per cage) during the study. The rats were fed *ad libitum* with standard feed at mean room temperature of 22 ± 2 ^{o}C and relative humidity of 55 ± 10 %, in an environment with 12-h day/12-h dark photoperiod. After one-week adaptation, the rats were assigned to two equal groups: CG and FG. The animals were fed with a standard rat diet composed of 6.6 % raw ash, 24.3 % protein, 3 % fat, 5.1% cellulose, and standard vitamin mixture. Rats in CG received regular rat feed and water during the experiment, while rats in FG were fed regular rat feed and water containing 20 % fructose. Rats in both groups were fed their respective treatments *ad libitum* for 8 weeks.

Preparation of drinking water containing fructose

Fructose in drinking water was prepared fresh everyday by dissolving 20 g of fructose in 100 mL of water**.** The 20 % fructose solution was given every day for eight weeks *ad libitum* [8].

Collection of samples

At the end of the experiment, all rats were placed under general anesthesia using thiopental at a dose of 40 mg/kg. Blood samples obtained *via* cardiac puncture were collected in EDTA-treated tubes for plasma samples, while blood samples for sera were collected in plain red-topped tubes. After centrifugation at +4 °C and 3000 rpm for 15 min, the resultant plasma and serum samples were preserved at −20 °C prior to analysis. After collection of blood samples, the rats were sacrificed via cervical dislocation.

Biochemical analysis

Serum uric acid, glucose, total-C, HDL-C, LDL-C and triglycerides, and serum activities of AST, ALT, GGT and LDH were measured spectrophotometrically with Konelab 60 brand autoanalyzer.

Analysis of plasma hormone levels

Plasma insulin levels were determined using competitive enzyme-linked immunoassay method with USCN Life EIA kit (Cat No: E0448r). Plasma adropin levels were determined with competitive enzyme-linked immunosorbent assay (ELISA) using commercial kits (Phoenix EIA kits, Cat. No. EK-067-29). Plasma leptin levels were measured with sandwich ELISA using commercial kit (BioVendor kiti cat. no. RD291001200R). All ELISA tests were performed in line with the procedure outlined in the kit manual.

Statistical analysis

Measurement data are expressed as mean ± standard deviation (SD). First of all, normality test was used to determine whether the data were parametric or non-parametric. Since the sample size in each group was < 30, the results were interpreted according to the Shapiro-Wilk test in the normality test. Based on this test, the variables of total cholesterol, HDL-C, triglycerides, GGT, LDH, and adropin in CG and

the LDH variable in fructose group did not show normal distribution. Thus, Mann-Whitney U test, which is a non-parametric test, was used for analyzing total cholesterol, HDL-C, triglycerides, GGT, LDH and adropin, while independent two-group *t*-test, which is a parametric test, was applied for all other variables. At the end of the analysis, $p < 0.05$ was accepted as statistically significant.

RESULTS

The initial mean weight of the animals in each group was approximately 400 g ($p > 0.05$). At the end of the study, while the body weight of animals in the fructose group was 528.143 ± 18.099 g, the body weight of the animals in the control group was 466.375 ± 10.939 g ($p < 0.05$). These results are shown in Table 1.

Table 1: Effect of high fructose consumption on body weight

Body weight (g)	Control (n=8)	Fructose (n=7)
Initial	400.875 ± 8.774[a]	404.14 ± 6.819[a]
Final	466.375 ± 10.939[a]	528.143 ±18.099[b]

For each group, different letters in the columns indicate statistically significant differences (p<0.05)

At the end of high fructose diet, while glucose and triglyceride levels were increased, there were decreases in levels of HDL-C and ALT in rats that consumed high fructose for 8 weeks, when compared to CG ($p < 0.05$). Moreover, serum uric acid levels and serum activities of AST, LDH and GGT were raised in the fructose group, although the increases were not statistically significant. These data are presented in Table 2.

Table 2: Effect of high fructose consumption on serum biochemical parameters

Parameter	Control(n=8)	Fructose (n=7)
Glucose (mg/dL)	136.63±5.470[a]	201.71±4.699[b]
Uric acid (mg/dL)	1.788±0.359[a]	2.129±0.245[a]
Total Cholesterol (mg/dL)	53.13±3.148[a]	39.86±2.807[b]
HDL-C (mg/dL)	41.25±2.743[b]	23.86±2.595[a]
LDL-C (mg/dL)	10.71±2.112[a]	10.75±0.526[a]
Triglycerides (mg/dL)	30.13±2.255[b]	124.00±16.553[a]
AST (U/L)	99.50±2.922[a]	105.71±13.467[a]
ALT (U/L)	57.900±3.136[b]	33.214±3.271[a]
GGT (U/L)	5.63±0.324[a]	7.00±0.756[a]
LDH (U/L)	557.75±134.554[a]	1216.14±485.255[a]

Different letters in each of the rows indicate statistically significant differences ($p < 0.05$)

After 8 weeks of high fructose consumption, while plasma leptin and insulin levels increased, plasma adropin levels decreased in fructose group, when compared to the control group ($p < 0.05$; Table 3).

Table 3: Effect of high fructose consumption on plasma hormone levels

Parameter	Control (n=8)	Fructose (n=7)
İnsulin (ng/mL)	0.763±0.06[a]	0.926± 0.09[b]
Leptin (pg/mL)	37.493 ± 1.709[a]	295.096 ± 52.711[b]
Adropin (ng/mL)	5.966 ± 0.445[a]	4.711 ± 0.268[b]

Different letters in each of the rows indicate statistically significant differences ($p < 0.05$)

DISCUSSION

Studies on Sprague Dawley and Wistar Albino rats have demonstrated that high fructose diet induced hyperglycemia, impaired glucose tolerance, induced insulin resistance, and led to weight gain [9,10]. Thus, the results obtained in the current study on Sprague Dawley rats are in agreement with data from earlier investigations.

Some studies have reported increases in energy intake, body weight and amount of fat in animals fed high fructose diets [9,10]. In contrast, in a study conducted on 3-week-old Wistar albino rats, it was reported that after six weeks of fructose feeding, the rats were neither obese nor overweight [11].

However, in the current study, the body weights of animals given drinking water containing 20 % fructose for 8 weeks increased more than the body weights of rats in the control group. While the average final body weight of rats in the fructose diet group was 528 g, some researchers reported that the final body weight of rats after consumption of fructose for 24 weeks was 415 g [12]. The higher weight gain in the current study may be due to the difference in the ages of rats used: the current study used 6-month-old rats, while the previous study was carried in the out on 4-month-old rats [12]. This disparity may account for the higher weight gain in this study.

When carbohydrate (e.g. fructose) intake exceeds daily energy requirements, blood glucose level is elevated, and insulin is produced by the pancreas to allow cells take up glucose. Thus, prolonged excessive consumption of carbohydrates leads to persistently high blood glucose levels. Under this condition, insulin is constantly secreted to lower blood glucose. Therefore, high carbohydrate intake has been reported to cause insulin resistance [13]. It is reported that administration of high fructose diet for 8 weeks caused increases in fasting blood glucose levels and plasma triglyceride concentrations in 7-week-old rats [14]. A group of

researchers fed young rats 20 % fructose-containing water for 8 weeks to create metabolic syndrome model, and the end of the experiment, there were increases in plasma glucose levels in the fructose group [15]. In the current study, it was also observed that plasma glucose levels increased in rats given 20 % fructose-containing water for 8 weeks.

Hypertension, hypertriglyceridemia, and hyperglycemia have been reported in young rats after 8 weeks of administration of 20 % fructose diet [15]. In a previous study, fructose feeding in rodents was consistently associated with increased hepatic *de novo* lipogenesis and increased plasma triglycerides [14]. A study on fructose-fed hamsters showed increased plasma triglycerides [10]. It has been reported that high fructose consumption for 5 weeks decreased plasma HDL levels in rats [16]. In another study, while fructose administration to 3-week-old rats for 6 weeks increased the total cholesterol/triglyceride ratio and cardiovascular risk, this risk was absent after 3 months of study on the fructose-enriched diet [11]. In the current study, it was found that triglyceride levels were increased, while HDL levels were decreased in rats given 20 % fructose water for 8 weeks.

Excessive fructose consumption is associated with elevated plasma uric acid. Renal uric acid excretion is suppressed by chronic fructose consumption, thereby increasing serum uric acid levels. Several studies have shown that high fructose consumption caused increases in blood uric acid levels [17,18]. A study reported hyperuricemia in adult Sprague–Dawley rats due to administration of 10 % fructose in drinking water and high-fructose diet [18]. In addition, uric acid level was increased in fructose group, although the increase was not significant. Furthermore, a study on obese subjects showed decreases in ALT activity in individuals who consumed fructose-sweetened beverages for 10 weeks [17]. Indeed, in the present study, there was a decrease in plasma ALT activity in rats in the fructose group, and the rats were overweight. Fructose-induced hypertriglyceridemia is associated with significant hyperinsulinemia [19]. Fructose does not stimulate secretions of insulin and leptin secretion after acute ingestion, and reduce postprandial ghrelin suppression. Therefore, chronic intake of high fructose diets may contribute to increased calorie intake and obesity [20]. Studies have reported that chronic fructose intake is associated with elevated plasma leptin levels in rats [14, 20]. On the other hand, fructose consumption for 8 weeks increased plasma insulin and leptin levels and weight gain in rats. Moreover, intake of high fructose diet (60 %) for 8 weeks caused elevated plasma leptin levels and hyperinsulinemia [14]. In the present study, chronic fructose consumption also caused hyperinsulinemia and elevated plasma leptin levels. The hyperinsulinemia and increased adipose tissue mass seen in the fructose group may be responsible for the increased plasma leptin levels.

Adropin is another peptide hormone associated with energy metabolism and disorders. It is a novel hormone which regulates glucose and lipid metabolism. It has also been reported that serum adropin levels are affected by food, especially carbohydrate intake. For example, decreases in serum adropin and increases in levels of fasting glucose, have been reported in mice given carbohydrate-rich feed [5]. Furthermore, low circulating adropin levels were linked to weight gain in high-fructose-fed Rhesus macaques. While adropin levels dropped, leptin and fasting glucose increased in these animals [21]. It has also been reported that DIO mice treated with adropin exhibited improved glucose tolerance and insulin resistance [22]. In addition, adropin reduced hyperglycemia by increasing insulin sensitivity in type 2 diabetic animals. The regulation of metabolism by adropin appears to be due to its effect on hepatic glucose synthesis and increased hepatic glucose oxidation [23]. In this study, it was also found that high fructose diet lowered plasma adropin. Moreover, it was shown that the only independent factor for fatty liver disease was a decrease in adropin level while leptin level was not an independent pre-disposing factor for the disease [24]. In addition, a report showed that administration of adropin in diet-induced obese mice improved insulin signaling in skeletal muscle and liver [22]. Therefore, low adropin level should correlate with hyperglycemia [22]. However, the mechanism by which fructose or glucose modulates adropin levels remains to be investigated [23].

CONCLUSION

The findings of this study show that high fructose consumption triggers metabolic syndrome in mature adult similar to young rats due to hyperleptinemia and hyperinsulinemia. Moreover, high fructose consumption lowers plasma adropin levels in mature rats. These findings are valuable contributions to existing literature, especially in view of existing conflicting reports on the effects of fructose consumption on levels of plasma adropin.

DECLARATIONS

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Ethical approval

This study was approved by the ethical Authority of Burdur Mehmet Akif Ersoy University local Ethics Committee forAanimal Experiments (approval no. 725/86).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hale Ergin Egritag and Seyfullah Haliloglu conceived and designed the study, collected and analysed the data, and wrote the manuscript. The two authors read and approved the manuscript for publication.

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