Deleterious Effect of Fructose on the Heart Function of Hypertriglyceridemic Rats

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ABSTRACT

A high-fructose intake (HFI) in food, sweetened beverages, and soft drinks appears to be one of the risk factors that worsens human metabolic and cardiovascular health, although the more accurate mechanism remains unclear. Hypertriglyceridemic (HTG) rats represent a suitable animal model of metabolic syndrome where the consumption of an HFI could have an additional aggravating impact. We aimed to study the effect of fructose on the heart functions. Male HTG rats had HFI or a standard diet for five weeks. Heart function was tested *ex vivo* on the perfused heart using the Langendorff technique. Isolated hearts underwent 25 min ischemia (I) and 30 min reperfusion (R). Left ventricular developed pressure (LVDP), ventricular premature beats, and dysrhythmias were monitored during R. At the end of the R, ventricular fibrillation (VF) was evoked electrically. Systolic blood pressure, glucose level, serum total cholesterol (TC), triglycerides (TAG), and thiobarbituric acid reactive substances (TBARS) in the kidney were determined. The LVDP showed a reduced return to the input values, the duration of VF in R increased, and the threshold for VF induction decreased. Serum TC, TAG, and kidney TBARS were increased. The effect of HFI on heart ventricular impairment was associated with the reduced threshold for induction of VF and aggravated dyslipidemia. The results point to the adverse impact of dietary high-fructose intake in rats with hypertriglyceridemia.

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INTRODUCTION

Metabolic syndrome (MetS) is characterized by a cluster of several simultaneously present risk factors as abdominal obesity, hypertension, hyperlipidemia, hyperglycemia, and insulin resistance. These disorders consequently lead to the development of cardiovascular diseases, diabetes mellitus type 2, non-alcoholic fatty liver disease, etc. (10, 39). The increased prevalence of MetS is related to an unhealthy lifestyle, mainly sedentary work, low physical activity, and consumption of unhealthy food with high amounts of saturated fats, added sugar, and low-fiber food. The introduction of high-fructose corn syrup in the 1970s accelerated fructose consumption. Fructose is sweeter than sucrose, and its production is less expensive (1). Over the past few decades, epidemiological studies have demonstrated that a high-fructose intake is an etiological factor of MetS. Fructose is metabolized in the liver to lipids, and finally, it increases triacylglycerol concentration here thus a high-fructose intake is partly analogous to a high-fat diet and endangers health (25). A high-fructose intake evokes the expression of all main features of MetS (11). A changed serum lipid profile characterized by dyslipidemia was documented, especially the increased levels of low-density lipoprotein cholesterol linked to cardiovascular diseases (17, 38). Some evidence suggests that a high-fructose intake increases cardiovascular risk, although the mechanism remains unclear. It was reported that high-fructose feeding elicits insulin resistance, hyperinsulinism, and hypertension in normal mongrel dogs (22), in experimental mice and rats resulted in high triglyceride levels, glucose intolerance, insulin resistance, and obesity (18). Some differences in fructose overload effects were observed depending on the rat strain. Fructose in drinking water (10%) administered to Wistar and Sprague-Dawley (SD) rats for eight weeks resulted in hypertension and hypertriglyceridemia in SD rats. However, no metabolic changes were seen in the Wistar rats. These differences could be attributed to the active behavior of Wistar rats causing a higher metabolic rate (7). As hereditary hypertriglyceridemic (HTG) rats are considered a suitable animal model for the study of MetS-like conditions (14), we focused on the impact of a high-fructose intake as an additional stress on the organism, which is already burdened by the presence of several MetS risk factors in HTG rats (41). Our study aimed to find the effect of high-fructose intake on the heart function of HTG rats. In addition, some biometric, biochemical, and physiological parameters were determined.

MATERIAL AND METHODS

ANIMALS AND DESIGN OF EXPERIMENT

All experimental procedures involving animals conformed to Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Ethical Committee of the Centre of Experimental Medicine of the Slovak Academy of Sciences, and the Animal Health and Animal Welfare Division of the State Veterinary and Food Diet Administration of the Slovak Republic (the number of the permit 385/18-221/3). Adult male HTG rats, 15 weeks old at the onset of the experiment, weighed 293 ± 4 g, $n =$ 28 were used in a chronic 5-week lasting experiment. Rats were divided into two groups: a control group fed a standard diet (Control; $n =$14), and a group fed a high-fructose diet with 60% of fructose in the pellets (Fructose; $n = 14$). A standard rodent diet (1g/13.26 kJ) was produced by the certified pellet producer (Centrum of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dobrá Voda, Slovakia). A modified high-fructose diet (1g/14.57 kJ) was prepared by the same pellet producer with a 60% fructose addition and omitting an adequate amount of wheat and barley. Rats were kept on a 12h/12h light/dark cycle, placed in cages of five animals and they had food and water *ad libitum.* The amount of pellets and water was recorded *per* cage and recalculated to the consumption of one rat/day.

HEART FUNCTION

The heart function was tested *ex vivo* on the perfused heart under constant pressure (15) using the Langendorff technique. The hearts of 7 rats from each group were retrogradely perfused via cannulated aorta in Langendorff mode with oxygenated Krebs-Henseleit solution (mmol/l: 120.0 NaCl; 4.2 KCl; 1.75 $CaCl_2$; 1.25 $MgSO_4$. $4H_2O$; 12.5 glucose; 25.0 $NaHCO_3$; pH 7.4; temperature 37.0 °C). To assign basal diastolic pressure to the left ventricle, a water-filled latex balloon was inserted into the left ventricular cavity, and adjusted to the value of 80 mmHg (1 mmHg = 133.32 Pa). After 10 min of the heart equilibration (a stabilization period), ischemic conditions (I) were induced by stop-flow (25 min), and reperfusion (R) was applied for a further 30 min. Left ventricular developed pressure (LVDP), number of ventricular premature beats (VPB), and heart dysrhythmias (ventricular tachycardia; VT and ventricular fibrillation; VF) were monitored. At the end of the R period, myocardial susceptibility to persistent VF was determined by electrical stimulation via electrodes located at the epicardium of the right ventricle. The heart was subjected to programmed electrical stimulation: current strength 10 mA, train duration 2s, stimulation rate 100 pps, and stimulus duration 0.2 ms was used (Electrostimulator ST-3, Medicor, Hungary). In case of sustained VF lasting > 2 min was not induced, the stimulus intensity was increased in 5 mA steps until a maximum of 50 mA to detect the fibrillation threshold. The system BioLab F ver.1 (Institute of Measurement Science, Slovak Academy of Sciences, Bratislava, Slovakia) was used for data collection and offline analysis.

ENDOTHELIUM-DEPENDENT RELAXATION OF THE AORTA

The thoracic aorta was prepared and placed in a modified Krebs solution (mmol/l: NaCl 122.2; KCl 5.9; $NaHCO_3$ 15.0; D-glucose 11.0; $MgCl_2$ 1.25; $CaCl_2$ 1.25). Two millimeter-long rings were clamped between two hooks of a triangular shape and immersed into Krebs solution gassed with 95% O_2 and 5% CO_2, pH 7.4 at 37 °C. The holder with an aorta ring was connected to a tensiometry sensor. Rings were stabilized at an optimal tension of 10 mN for 60 min

(A static-dynamic apparatus M 1101, Czech Republic). During the stabilization period, the rings were washed out several times with Krebs solution. Rings were contracted by Krebs solution with 100 mM KCl, washed out, and stabilized. Phenylephrine (10^{-6} mol/l) was added to induce contraction. At the plateau of contraction, acetylcholine was administered cumulatively in concentrations of 10^{-8} to 10^{-5} mol/l, and the endothelium-dependent relaxation response was monitored (Kutesz 185, Hungary).

SERUM LIPID PROFILE

The blood was collected from the retro-orbital plexus after 14 hours of starvation. Diagnostics kits for total cholesterol and triacylglycerols (Erba Lachema Ltd, Czech Republic) were used to determine the lipid profile from rat blood serum. The absorbance of resulting colored compounds was measured spectrophotometrically (LabSystems 352 Multiscan MS Microplate Reader, ThermoFisher Scientific, USA).

THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) ASSAY

The thiobarbituric acid (TBA) test was used as an index for lipid peroxidation based on the reactivity of the final lipid peroxidation product malondialdehyde with TBA to form a red adduct (9). The double heating method, according to Draper and Hadley (8) was used to determine TBARS. The trichloroacetic acid solution was added to the kidney tissue, homogenated into centrifuge tubes, and placed in a hot water bath. After 15 min, the mixture was cooled with water and centrifuged for 10 min at 3000 × g and 4 °C. The supernatant was added to the TBA solution and placed in a hot water bath of 95 °C for 15 min. Subsequently, the solution was cooled in water and its absorbance was measured on microplates spectrophotometrically (Labsystem Multiscan RC, Canada).

FASTING GLUCOSE

The blood was drawn from the retro-orbital plexus after 14 hours of starvation (the same collection as for serum lipid profile determination), and the level of glucose was measured in a drop of blood by glucose meter (Contour, Bayer, Germany).

BLOOD PRESSURE

Blood pressure was measured by non-invasive tail-cuff plethysmography (PowerLab 4/30, AD Instruments, USA). Rat-friendly modification of blood pressure measurement according to Liptak et al. (2017) was used to reduce the stress of animals. Systolic blood pressure was calculated as the average of five consecutive measurements.

STATISTICAL EVALUATION

Data were expressed as means ± SEM. An unpaired t-test was used to compare the means of two unrelated groups and determine a significant difference between them. The level of p < 0.05 was considered a statistically significant difference.

RESULTS

The rats consumed the same amount of food per g/rat/day regardless of the type of diet (Tab. 1). The addition of 60% fructose to the pellets meant a subsequent reduction of saccharides, fats, and protein content to 40% in the fructose-fed group. However, the fructose group had a higher energy intake (Table 1).

Yet, this increased energy intake lasting five weeks in the Fructose group of rats resulted in a not quite significant increase in the body weight gain compared to Control rats. HTG rats with a high-fructose intake drank on average significantly less water compared to Controls. Concerning the impact of a high-fructose intake on cardiovascular damage, a marked deterioration of LVDP was found during the reperfusion of the heart represented by a significantly slower return to the input values compared to Controls (Fig. 1A).

Moreover, the duration of VF was several times longer than in Control group of rats (Table 1). At the end of the 30-minute reperfusion period, VF was evoked by electri-

Tab. 1 Impact of a high-fructose intake on basic parameters and heart function in hypertriglyceridemic rats.

Parameter (units)	CONTROL	FRUCTOSE	Significance
Food consumption (g/day/rat)	28.68 ± 0.73	28.06 ± 0.81	n.s.
Energy intake (kJ/day/rat)	380.32 ± 4.33	408.86 ± 11.02	$p \leq 0.05$
Water Drinking (ml/day/rat)	26.23 ± 0.53	21.16 ± 0.78	$p \leq 0.001$
Body weight gain (g/5 weeks/rat)	55.64 ± 3.83	66.93 ± 5.12	n.q.s.
VPB in R (number)	474.36 ± 121.79	394.44 ± 115.38	n.s.
VT duration in R (s)	160.26 ± 83.33	243.59 ± 153.85	n.s.
VF duration in R (s)	115.38 ± 89.74	673.08 ± 230.77	$p \leq 0.05$
VF stimulation threshold (mA)	29.10 ± 0.31	18.81 ± 2.24	$p \leq 0.05$

Values are expressed as means ± S.E.M. VPB – ventricular premature beats; VT – ventricular tachycardia; VF – ventricular fibrillation; TBARS – thiobarbituric acid reactive substances; R – reperfusion; n.s. not significant; n.q.s. not quite significant. Basic values, n = 14 rats/group; values obtained by Langendorff technique, n = 7 rats/group. Student t-test.
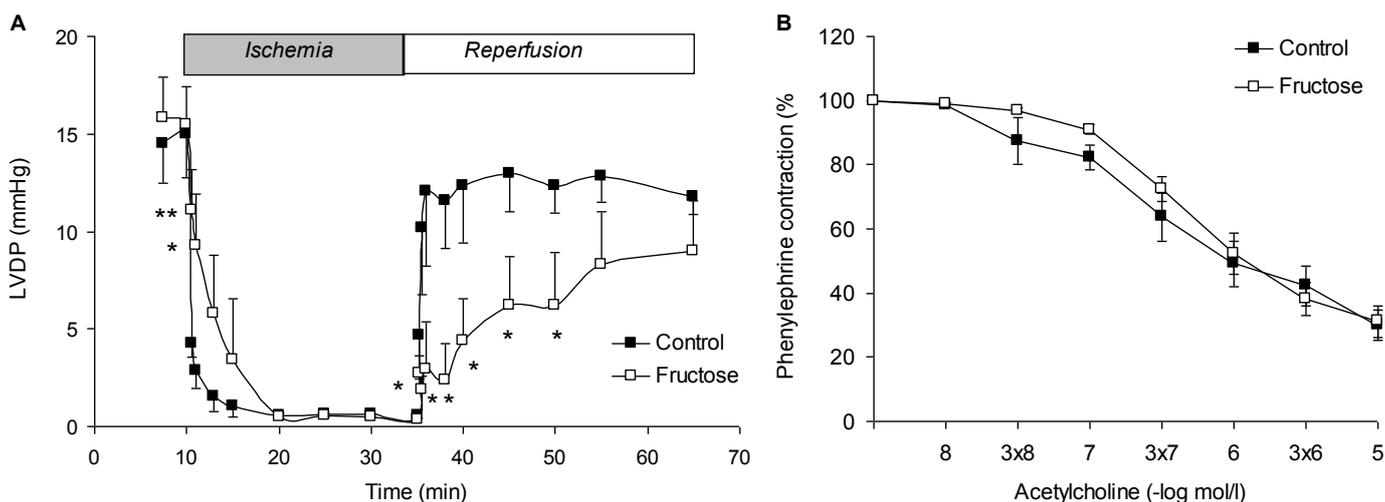
Fig. 1 Effect of a high-fructose intake on **(A)** the left ventricular developed pressure (LVDP). Isolated rat hearts according to the Langendorff technique underwent a stop-flow ischemia (25 min) followed by reperfusion (30 min). During the reperfusion period, a return of LVDP to the onset values was significantly slower in the Fructose group (n = 7) compared to the Control rats (n = 7; *p < 0.05). Effect of a high-fructose intake on **(B)** the thoracic aorta endothelial-dependent relaxation. Phenylephrine-precontracted vessels were relaxed by cumulatively adding acetylcholine (10^{-8} to 10^{-5} mol/l). No significant differences were observed between the responses of Control (n = 14) and Fructose group (n = 14). Values are means ± S.E.M. Student t-test.

cal stimulation. As a substantial finding of 5 weeks lasting high-fructose intake impact, we consider an increased sensitivity to the induction of life-threatening VF, as the electrical stimulation intensity needed to evoke VF was significantly lower comparing Control rats. A high-fructose intake did not deteriorate endothelium-dependent relaxation of the thoracic aorta (Fig. 1B). A significant increase was found in the total serum cholesterol (TC) level (1.52 ± 0.05 *vs*. 1.38 ± 0.04 mmol/l; $p ≤ 0.05$) and the triacylglycerol (TAG) level (3.41 ± 0.19 *vs*. 2.44 ± 0.17 mmol/l; $p ≤ 0.001$) due to a high-fructose intake compared controls at the end of the experiment. Similarly, significantly increased oxidative stress, measured as TBARS level, was observed in the kidneys of rats exposed to a high-fructose intake (6.30 ± 0.28 *vs*. 5.50 ± 0.20 mmol/mg protein; $p ≤ 0.05$). The systolic blood pressure and the blood fasting glucose level were not affected by the 5-week lasting high-fructose intake comparing controls.

DISCUSSION

The simultaneous presence of several risk factors of MetS elevates rates and severity of cardiovascular diseases including coronary atherosclerosis and calcification, microvascular dysfunction, cardiac dysfunction, myocardial infarction, and heart failure (10, 17, 32, 39). Hereditary HTG rats are characterized by hypertriglyceridemia, mild hypertension, and insulin resistance and they have some disturbances in glucose metabolism (41). The occurrence of the aforementioned metabolic disturbances allows their use as a model of MetS and related cardiovascular diseases, even though they are non-obese. We assumed that the consumption of high-fructose intake could have an aggravating impact on rats with metabolic disturbances. This would mimic the situation when people with MetS consume a lot of fructose in soft drinks and industrially processed food and sweets. There is the sug-

gestion that oxidative stress and inflammation may play a key role in high fructose-induced cardiac dysfunction. Cardiac inflammation due to high-fructose intake was induced via macrophage recruitment in cardiomyocyte (37). High-fructose intake increased the expression of TNF-α, IL-6, IL-1β, and NF-κB in rat hearts, resulting in cardiac inflammation in fructose-fed diabetic rats (16). Further, fructose exposure induced oxidative stress, mitogen-activated protein kinases (MAPK) signaling, mitochondrial signaling, and inflammatory signaling, increased NLRP3 inflammasome activation due to the induction of CD36 expression and heart tissue oxidative stress (13, 37), stimulated the expression of p38, ERK1/2 and JNK (40).

We focused on heart and vessel dysfunctions. In the present work, marked deterioration of LVDP represented by a slower return to the input values and many times increased durations of life-threatening VF during the reperfusion period was shown due to a high-fructose intake compared to the control group. Impairment of ventricular diastolic function in rats due to the fructose-rich diet was found by Xing and co-workers (2010). An increased susceptibility to myocardial I/R injury was shown by Maarman et al. (2017). Topçu and co-workers (2022) fed SD rats with high fructose for four weeks and their hearts underwent 30 min ischemia followed by 60 min reperfusion *ex vivo*. Similarly, they observed the left developed ventricular pressure was still depressed at the end of 60 min reperfusion compared to the control group. 60% fructose intake increased cardiac fibrosis, cardiomyocytes size, and relative wall thickness of the left ventricle in adult C57BJ/L6 mice (37).

Our original and most important finding is that the threshold for VF induction decreased, thus the sensitivity to the heart functional disorders induction increased. The lowering of the threshold for the VF induction was not accompanied by either an increase in the blood pressure or a change in glucose level, therefore it seems the only measurable indicator of approaching impairment of heart

function by fructose is presented by an increased level of TAG. Even though TAGs are highly atherogenic (24, 33), no detrimental effect on the endothelium-dependent relaxation of the thoracic aorta was observed here. The reason could be the short duration of the experimental diet to develop vessel impairment, even though the 60% fructose in pellets represents an intensive diet. It was suggested that an increase in plasma TAG levels is associated with an increase in cardiovascular risk, therefore plasma TAG levels may serve in the prognosis of the heart disease risk (12). The negative influence of increased TAG levels was reported in humans by Tikhonoff et al. (2024), who determined a prognostic cutoff value for increased cardiovascular risk events to 89 mg/dl of TAG. In the present work, a significant increase in the serum TAG levels was found with an additional TC level increase due to a high-fructose intake. The mechanism of the harmful effect of a high-fructose intake on heart function found here could be associated with observed dyslipidemia. Therefore therapies targeting dyslipidemia, and the TAG-lowering action may be useful in improving cardiovascular outcomes (26) even in high-fructose intake-induced heart dysfunctions.

The deleterious effect of high-fructose feeding may be related to renal damage (6), and can lead to oxidative stress in the kidneys (28). We also determined increased lipid peroxidation in the kidney tissue in the present work. Consistent with our observation, a negative effect of drinking 10% fructose, lasting 15 weeks, was documented in the kidneys in a recent study by Vrbjar et al. (34). These authors have found an increase in lipid peroxidation and a decrease in thiol-redox balance estimated by determining the GSH/GSSG ratio. In connection with this finding, in our previous work, we have demonstrated that the combination of antioxidants (Vitamin E, 100 mg/kg/day and the pyridoindole antioxidant coded SMe1EC2, 15 mg/kg/day, *p.o.*) suppressed the occurrence of serious dysrhythmias (VT+VF) within the group of HTG rats fed a high-fat-fructose diet (1% cholesterol, 7.5% pork lard, 10% fructose) in comparison to the control HTG animals fed standard diet (3). Thus an oxidative stress-reducing diet may have a positive effect on unhealthy high fat and sugar-rich intake sequale.

In addition to the main effect of the increased level of TAG in response to a high-fructose intake, it could lead to induction of insulin resistance by inhibiting the insulin signaling pathways (2), induction of non-alcoholic fatty liver diseases as increased fat is stored within the liver cells (27), and may exhibit proapoptotic effects *via* inhibition the PI3K/Akt axis, and thus contribute to the death of cardiomyocytes (4, 19). Modern concepts offer a new hypothesis regarding the direct myocardiotoxic effects of fructose (19, 23, 29). This might be the base for corresponding changes in molecular mechanisms that could lead to systemic consequences ending up in cardiovascular impairment. So far studies elucidating such backgrounds are infrequent (5, 29, 36).

CONCLUSIONS

Results from the present experimental study show the substantial impact of uncontrolled unhealthy fructose consumption which can trigger the progression of cardiovascular events and ventricular arrhythmogenesis associated with dyslipidemia. We proved that a high-fructose intake increased the duration of life-threatening VF and weakened LVDP during the rat heart reperfusion followed by ischemia. Most importantly, a decreased threshold for inducing VF was observed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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