Vol.67: e24230973, 2024
https://doi.org/10.1590/1678-4324-2024230973
ISSN 1678-4324 Online Edition

BABT
Brazilian Archives of Biology and Technology

Article - Biological and Applied Sciences

Vascular Dysfunction in Fructose-Treated Mice is Associated with Increased Sensitivity to Angiotensin ii and Decreased to Nitric Oxide

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 15-Sep-2023; Accepted: 25-Jan-2024.

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HIGHLIGHTS

- High-fructose intake has a profound influence in the vascular response of Ang II and Nitric Oxide.
- Fructose supplementation increased AT1 receptor in the mesenteric bed.
- Consumption of fructose reduced guanylate cyclase soluble in the mesenteric vessels.

Abstract: High fructose consumption has been recognized as a potential risk factor for increased incidence of cardiovascular disease, and diabetes. Some lines of evidence support endothelial dysfunction (ED) as a possible underlying mechanism linking insulin resistance and hypertension. However, there is little information on the vascular response to vasoactive mediators after the high-fructose intaking (HFI). In this study, swiss mice had access to fructose-water solution (at 30% w/v) ad libitum for ten consecutive weeks. After this period, the vascular reactivity was assessed by the analyses of the variations in mean arterial pressure induced by either angiotensin II (Ang II) or sodium nitroprusside (SNP). Results showed that HFI induced i) an increased response to the vasoconstrictive and hypertensive agent Ang II, and ii) a decreased response to the vasodilating and hypotensive agent SNP. Western blot analysis revealed that such events were paralleled by higher Ang II type 1 receptor (AT1) and lower guanylate cyclase (GC-β1) enzyme densities in the mesenteric arterial bed (MAB). The present study demonstrated that HFI leads to an impaired response to vasoactive substances and, consequently, to ED in MAB.

Keywords: Angiotensin II; Fructose; Arterial pressure; Nitric oxide; Mice.

INTRODUCTION

Consumption of sugar-sweetened beverages (SSBs) remains high, especially among children and adolescents [1]. A high intake of SSBs, which are composed of energy-containing sweeteners such as sucrose, high-fructose corn syrup (HFCS), or fruit juice concentrates, have been associated with the growing epidemics of obesity and type-2 diabetes in Western society [2]. A recent meta-analysis of rodent studies showed that chronic fructose consumption leads to increased body weight gain, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and elevated arterial pressure [3].

Hypertension remains a significant risk factor for global cardiovascular disease morbidity and mortality [4]. Janus and and coauthors (2016) [5], compiled a series of evidence that corroborates the fact that insulin resistance is related to endothelial dysfunction and hypertension. In addition, they demonstrated that pharmacological or non-pharmacological interventions improve insulin sensitivity and restore endothelial function. The endothelial balance is maintained by substances with vasodilatory (e.g., nitric oxide [NO] or prostaglandins [PGI2]) and vasoconstricting (e.g., Ang II or endothelin-1) actions [6]. Any perturbation affecting the capacity of the endothelium to metabolize, synthesize, and release these substances will cause endothelial dysfunction [7].

Some lines of evidence indicated that high-fructose intaking is related to cardiovascular dysfunction and endothelial damage in the aorta. The main underlying mechanisms include oxidative stress and inflammation that were associated with increased levels of angiogenin, C-reactive protein, myeloperoxidase, and TNF-α expression [8]. However, there is little information on the vascular response to vasoactive mediators after the high-fructose intaking.

Considering that the mesenteric bed, which receives 25% of cardiac output, contributes substantially to the total peripheral resistance and, therefore, to arterial pressure [9]; and that previous studies reported vascular dysfunction induced by high-fructose consumption in rodents [10], the present study investigated i) the vascular reactivity of high fructose-fed mice assessed by the analyses of the variation in MAP induced by either Ang II or SNP; and ii) the effects of chronic fructose intake on AT1 and GC-β1density in mice mesenteric bed. Moreover, given that gender is a crucial risk factor for various cardiovascular diseases and recognizing that the inclusion of both sexes enhances the validity of animal models, this study employed male and female mice.

MATERIAL AND METHODS

Animals

The experiments were conducted on 3-month-old male and female Swiss albino mice from the animal facility at Universidade Federal de Santa Catarina (UFSC, Florianópolis, Brazil). At the time of testing, the animals weighed from 48 to 63 g. The animals were randomly separated and kept in groups of 3 animals per cage in a controlled-temperature room (23 ± 1 °C) and subjected to a 12-h light cycle (lights on at 7 a.m.). Animals had free access to food and water. All animals were acclimated in the laboratory 24 h before the experiments. All procedures complied with the guidelines on animal care of the university Ethics Committee on Animal Use (Protocol 3026310817).

Experimental design

Adult male and female mice were equally distributed into two experimental groups (n = 12 per group) and fed daily during ten weeks with different solutions: (1) filtered potable water or (2) filtered potable water containing 30% fructose (w/v; LabSynth, Diadema, SP, Brazil). All solutions were replaced every two days. After ten weeks, half of the animals from each group were submitted to carotid arterial catheterization for the evaluation of vascular reactivity to Ang II and SNP. In the sequence, mice were euthanized by an overdose of chemical anesthetics, and mesenteric tissues were removed for the analysis of AT1 and guanylate cyclase (GC-β1) immunocontent (see below). On the other hand, after the ten weeks, the other half of the animals from each group were food-deprived for six hours, and the glucose tolerance test was performed. A day after, mice were again food-deprived for six hours, and the blood was collected from the heart to determine plasma cholesterol and triglyceride levels.

Biochemical Analysis

Glucose tolerance was assessed following 6 hours of fasting and i.p. injection of 2 g/kg D-(+)-glucose (Sigma Aldrich, St Louis, MO). Blood glucose was measured from the tail tip at baseline, 15, 30, 60, and 120 minutes using a glucometer (Accu-check, Roche Diagnostic). A day after, mice were again food-deprived for six hours, and whole blood was collected from the heart, immediately centrifuged at 1,000×g, and the plasma frozen at −80 °C. Total cholesterol and triglyceride levels were measured in plasma using the enzymatic kit according to the manufacturer's instructions (Gold Analisa Diagnóstica Ltda, Minas Gerais, Brazil).

Vascular reactivity

Mice were anesthetized with ketamine and xylazine (90 and 15 mg/kg i.p., respectively), and body temperature was maintained at 37°C using a heating pad. Animals breathed spontaneously. A heparinized PE-10 polyethylene catheter was inserted into the femoral vein for drug injections with a 300 µL syringe. After surgery, a catheter 24 GA (BD Angiocath, Bencton Dickinson, Juiz de Fora, MG, Brazil) was inserted into the right carotid artery for recording the MAP. This catheter was connected to a pressure transducer (Mikro-Tip, Millar Instruments, Inc., Houston, TX, USA) and coupled to a Powerlab 8/30 (AD Instruments Pty Ltd., Castle Hill, Australia). The signals were recorded by the data acquisition software LabChart 7 (AD Instruments Pty Ltd., Castle Hill, Australia). To assess vascular reactivity, the animals were prepared to MAP recording and, after stabilization, they received three consecutive doses of Ang II (25, 75 and 250 pmol/kg) followed by two doses of SNP (NO donor; 80 and 800 nmol/kg) diluted in 20 µL of sterile phosphate buffered saline. Each new dose was performed only after the return to the MAP baseline. The data were analyzed using the LabChart 8 Reader software (AD Instruments Pty Ltd., Castle Hill, Australia). Results were expressed as mean ± standard error of the mean (SEM) of the peak changes in MAP (mm Hg) after the administration of a given compound, relative to the baseline MAP (ΔMAP). Limitation: Only animals that completed the curve of vasoactive compounds were included in the study.

Western blot analysis

The mesenteric vascular bed was removed and quickly frozen in liquid nitrogen. The tissues were homogenized in ice-cold lysis buffer T-PER (78510, Thermo Fisher Scientific Inc., Rockford, IL, USA) containing protease inhibitor cocktail (P8340, Sigma-Aldrich, St. Louis, MO, USA), sodium orthovanadate (1 mM), and phenylmethanesulfonylfluoride (1 mM), centrifuged at 10.000 g at 4oC and the supernatant was collected. Equal amounts of protein extract (80 µg) were loaded per lane and electrophoretically separated using 10% denaturing polyacrylamide gel electrophoresis (SDS-PAGE). Afterward, the proteins were transferred to nitrocellulose membranes using a Mini Trans-Blot Cell apparatus connected to a PowerPac™ HC power supply (both from Bio-Rad, CA, USA) following the manufacturer's protocol. The membrane was blocked with 5% BSA in 0.05% TBS-T for 1 h at room temperature and then immunoblotted with primary antibodies anti-β-actin (A3854, 1:40000, Sigma-Aldrich, St. Louis, MO, USA), anti-AT1 (sc-1173, 1:250, Santa Cruz, CA, USA) and anti-GC-β1 (sc-20955, 1:1000, Santa Cruz, CA, USA) in blocking buffer at 4°C overnight. Following washing, the membranes were incubated with secondary antibodies conjugated to horseradish peroxidase (1:5000, Cell Signaling Technology, Danvers, MA, USA, for 1h at room temperature). The membranes were exposed to HPR substrate (Thermo Fisher Scientific Inc., Rockford, IL, USA), and immune complexes were visualized by chemiluminescence using Chemidoc MP System (Bio-Rad Laboratories). Bands were quantified by optical density using manufacturer's software and expressed as the ratio to β-actin by arbitrary units, or Ponceau Red staining was used as a loading control.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses were carried out using unpaired *Student t-test* or a one or two-way analysis of variance (ANOVA), with repeated measures when appropriate. Following significant ANOVA, multiple post hoc comparisons were performed using *Newman Keul's test*. The accepted level of significance for all tests was $P < 0.05$. All tests were performed using the STATISTICA® software package (StatSoft Inc, Tulsa, OK, USA).

RESULTS

Biochemical Analysis

One-way ANOVA with repeated measures indicated significant main effects for fructose treatment [$F(1, 10) = 6.91$, $p < 0.05$] and for repetition factor [$F(4, 40) = 33.27$, $p < 0.00005$] on blood glucose levels (mg/dL). Subsequent post hoc comparisons revealed a significant increase in blood glucose levels of fructose-treated

mice in comparison to control mice exposed to standard diet at 30 and 60 minutes post glucose load ($P < 0.05$) (Figure 1A). Moreover, the student's t-test indicated that the bodyweight of fructose-treated mice increased at a greater rate than in control mice ($t = 2.66$, $P < 0.05$). This becomes clearer when comparing bodyweight variation (Figure 1B). Finally, student's t-test indicated a significant increase in triglycerides levels ($t = 5.49$, $P < 0.05$; Figure 1C) and in total plasma cholesterol levels ($t = 2.23$, $P < 0.05$; Figure 1D) of fructose-treated mice in comparison to control mice.

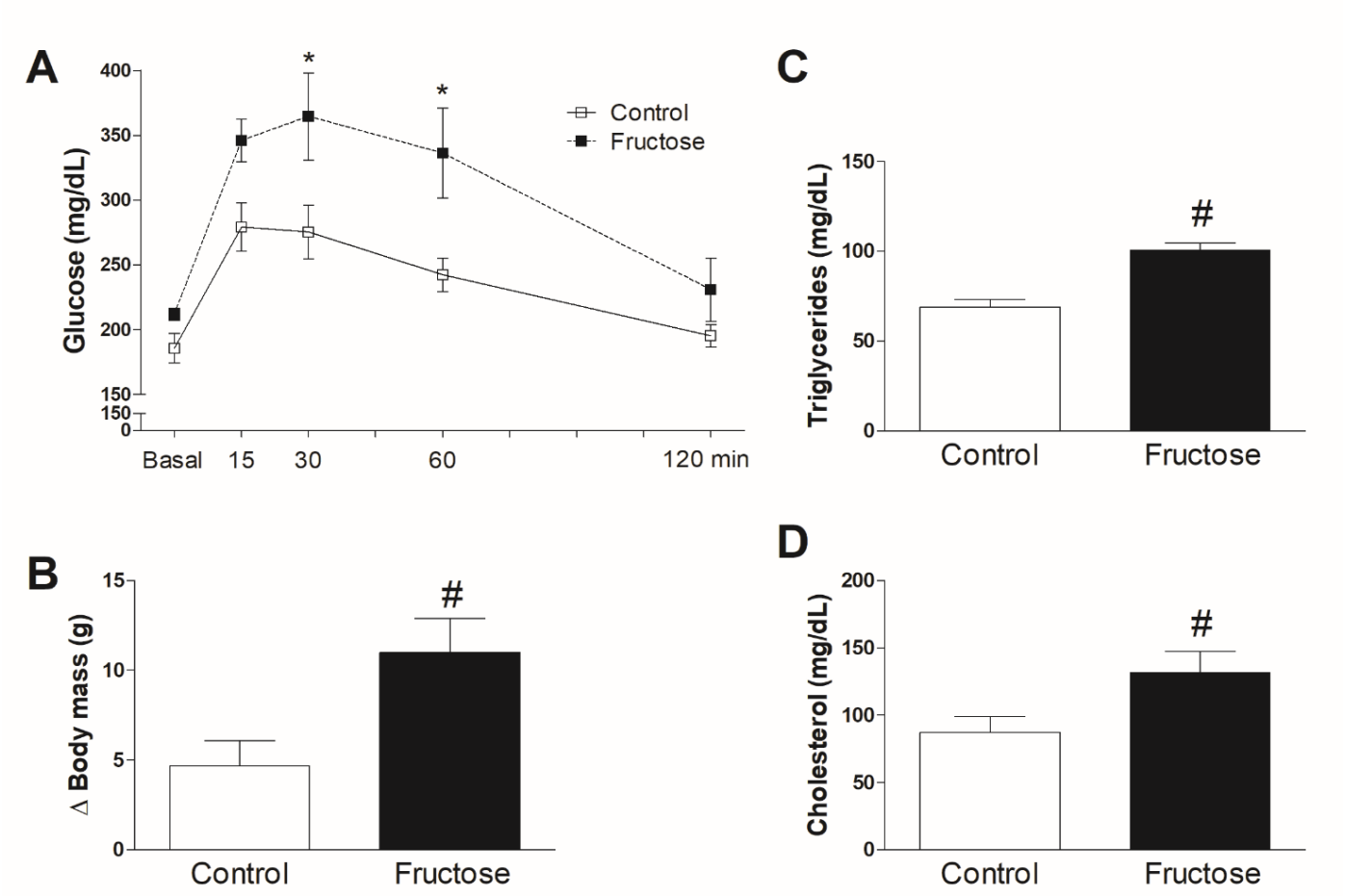

Figure 1. (A) Glucose tolerance test. (B) Body mass variation (g), (C) Plasma triglycerides levels, (D) Plasma triglycerides levels, were determined after 10 weeks of fructose supplementation. Data are expressed as mean + SEM (n = 6; animals per group). *P<0.05 compared to the respective control group (one-way ANOVA with repeated measures fallowed by the *Newman Keul's* post hoc test). #P<0.05 compared to the respective control group (*Student's "t" test*).

Vascular reactivity

Two-way ANOVA indicated main effects for Ang II concentration [$F = 41.63$, $p < 0.05$], for fructose treatment [$F = 44.44$, $p < 0.05$] and for the interaction of Ang II concentration by fructose treatment [$F = 3.47$, $p < 0.05$] on Δ MAP (mmHg). Subsequent post hoc comparisons revealed increase in Δ MAP of fructose-treated mice in comparison to control mice after angiotensin II treatment at 75 and 250 pmol/kg ($p < 0.05$) (Figure 2A). Additionally, two-way ANOVA indicated main effects for SNP concentration [$F = 10.36$, $p < 0.05$] and for fructose treatment [$F = 17.62$, $p < 0.05$] on Δ MAP (mmHg). Subsequent post hoc comparisons revealed decrease in Δ MAP of fructose-treated mice in comparison to control mice after sodium nitroprusside treatment at 80 and 800 nmol/kg ($p < 0.05$) (Figure 2B).

Western blot analysis

Student's t-test revealed that the AT1 receptor density in the mesenteric bed of fructose-treated mice was increased in comparison to control mice ($t = 2.97$, $P < 0.05$) (Figure 2C). Student's t-test also revealed that GC-β1 density in the mesenteric bed of fructose-treated mice was reduced in comparison to control mice ($t = 2.58$, $P < 0.05$) (Figure 2D).

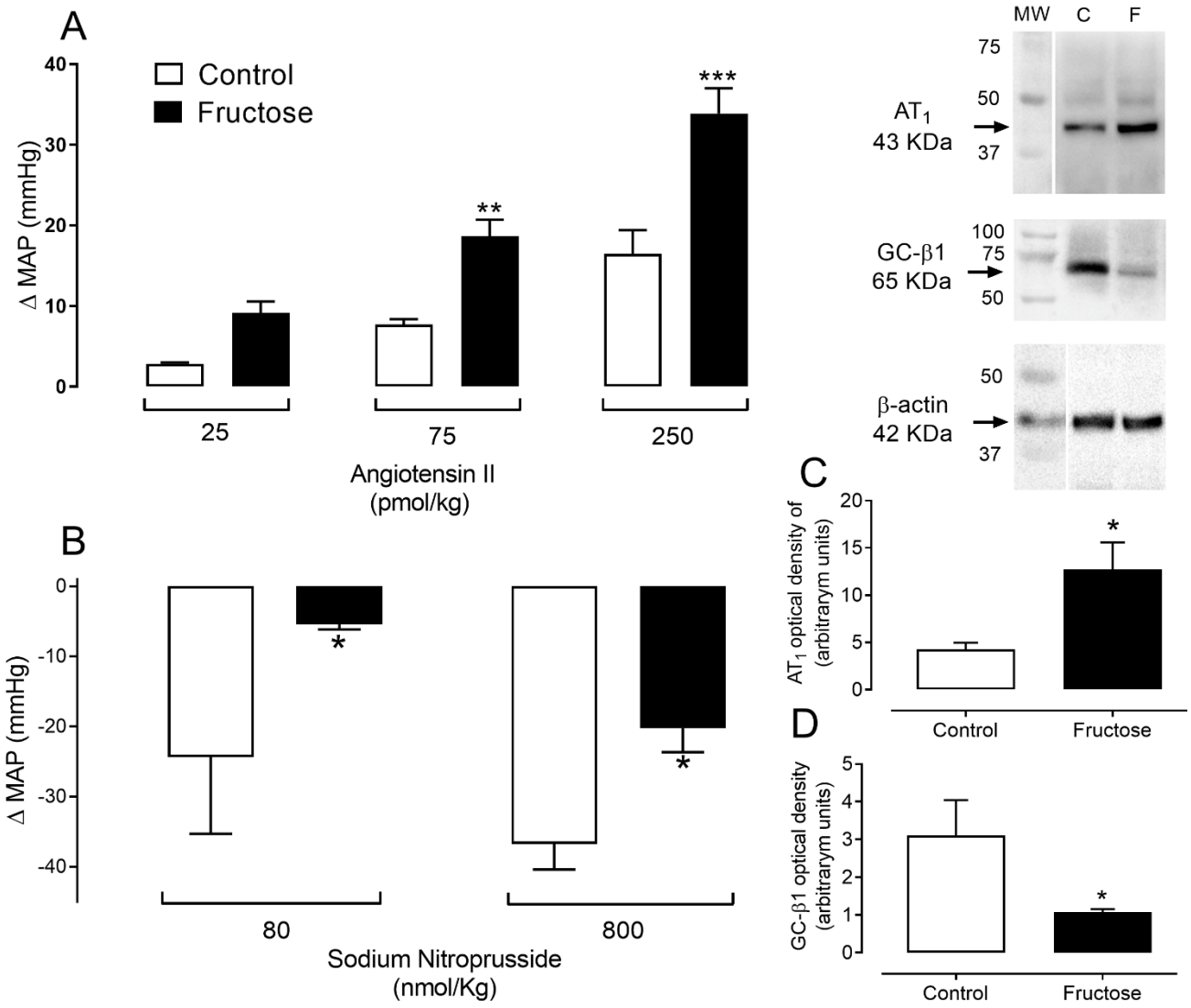

Figure 2. Mean arterial blood pressure (MAP) variation after (A) administration of 3 consecutive doses of angiotensin II (25; 75 and 250 pmol/kg) and (B) two doses of sodium nitroprusside (80 and 800 nmol/kg). (C) AT1 receptor immunocontent (n=6) and (D) guanylate cyclase (GC-β1) immunocontent (n=4) in the mesenteric bed. A and B: *P<0.05, **P<0.01 and ***P<0,001 when compared to the respective control group (two-way ANOVA fallowed by the *Newman Keul's* post hoc test). A and D: *P<0.05 compared to the respective control group (Student's "t" test). WM (Molecular Weight Marker); F (Fructose) and C (Control).

DISCUSSION

The main findings of the present study show that a high-fructose intake: i) Increased the effect of the vasoconstrictive and hypertensive agent Ang II; ii) reduced the effect of the vasodilating and hypotensive agent SNP; iii) increased the density of AT1 receptor; and i.v.) reduced the content of soluble guanylate cyclase β1 subunit in mice mesenteric arterial bed. As expected [11,12], fructose feeding increased body weight, raised blood cholesterol and triglycerides levels, and induced glucose intolerance.

There is a general agreement that a high-fructose diet induces cardiovascular changes, ranging from hypertension to morphological modifications in blood vessels and organs such as heart and kidneys [13-15]. Previous studies suggest that insulin resistance and hyperinsulinemia contribute to the development of hypertension since drugs that improve insulin sensitivity prevent or reverse the increase in blood pressure (BP) [16,17].

Ang II is the most important mediator of the renin-angiotensin system (RAS). This substance plays an essential role in the maintenance and control of BP. Most Ang II effects occur via the AT1 receptor, including vasoconstriction, cellular proliferation, and activation of the sympathetic nervous system [18].

Previous studies observed variations in the expression/density of AT1 receptors after chronic high-fructose intake. After three weeks in a high-fructose diet, the mRNA levels of AT1 receptors were increased

in the aorta and adipose tissue, but remained unchanged in the kidneys and other tissues [19-21]. On the other hand, the density of AT1 binding sites reduced in the aorta after four weeks of high-fructose diet [14], despite the high mRNA level in this tissue [21]. As expected, when the diet was maintained for 16 weeks, both protein content and mRNA in the aorta and kidneys were elevated [20]. Thus, one may assume that the expression of AT1 receptors depends on the duration of the diet and on the tissue evaluated. Despite the aforementioned differences across studies, a shared characteristic among them is that animals presented high BP. In our experiments, we did not find any differences on BP between the groups. However, our setup is set to register BP invasively in anesthetized mice. Nevertheless, the best way to record baseline BP in mice would be with non-anesthetized animals, which is a limitation of the study.

We demonstrated that the density of AT1 receptor is increased in the mesenteric bed after ten weeks of high-fructose intake. The mesenteric bed plays a more significant role in regulating BP than the conducting vessels such as the aorta. One may assume that, even with a reduction in AT1 receptors density in the aorta [14], BP would remain high due to the stimulation of AT1 receptors expressed in the mesenteric bed. It is noteworthy that these receptors seem to be active in the mesenteric bed, as the administration of three consecutive doses of Ang II promoted a substantial increase in the vasoconstrictor response compared to the control. Our findings suggest that an increased Ang II release could have a profound impact on the MAP, thus contributing to hypertension induced by high-fructose intake.

Endothelial cells play an important role in the regulation of vascular tone through synthesis and release of contracting and relaxing factors. One of the most important forms through which the endothelium promotes vasodilation is by activation of the NOS-3/NO/GC-β1/cGMP pathway. Nitric oxide is a powerful BP-lowering vasodilator that is generated by NOS-3 and activates GC-β1 to produce the second messenger cyclic guanosine monophosphate (cGMP). An increase in cytoplasmic cGMP, and the subsequent reduction of intracellular calcium concentration due to enhanced calcium extrusion and sequestration into intracellular stores, mediates the relaxation of vascular smooth muscle [22].

The role of fructose in this pathway is controversial; some authors have found a reduction of NOS-3 protein content both “in vitro”, in endothelial cell culture, and “in vivo”, in the mesentery and aorta of rats supplemented with high fructose (60%) [23-25]. Shaligram and coauthors (2018) [10] showed that the endothelium-dependent vasodilation induced by acetylcholine, as well as the relaxation responses to the NO donor SNP, are impaired in mesenteric arteries of fructose-supplemented rats although there are no changes in protein levels of NOS-3 in the mesentery. Souza and coauthors (2017) [26], have described similar results. Therefore, the effect of fructose on NOS-3 does not fully explain the vascular changes observed by the excessive consumption of this sugar.

In addition to confirming these results, our findings offer a new explanation as to why the response to SNP (and presumably, to endogenous NO) is reduced in fructose-fed mice. We found a substantial decrease in the β1 subunit of soluble guanylate cyclase. This enzyme is regarded as the physiological receptor of NO and responsible for several of its effects on vessels, platelets, and neurons [27]. The β1 subunit is essential for soluble guanylate cyclase catalytic activity [28], meaning that the degree of reduction in its content, like those we have found in the present study, will most likely prevent vessel relaxation. With an increased response to a vasoconstrictor such as Ang II, the profound reduction in the vasodilating capacity induced by fructose will likely induce a substantial endothelial dysfunction. These results also demonstrate that vascular changes induced by a high-fructose intake are not restricted to the endothelium; it may affect the smooth muscle of the vessels as the guanylate cyclase is abundantly expressed in this tissue [29]. Of note, decompensated diabetic animals have a 50% reduction in GC-β1 content, and insulin administration restores GC-β1 levels in the aortas [30].

CONCLUSION

Our study is limited in the context that it is valid for mice supplemented with fructose. Although preliminary, results indicated that a high-fructose intake has a profound influence in the vascular response to vasodilators and vasoconstrictors. These altered responses are caused, at least partially, by a reduced density of GC-β1 subunit in tandem with increased density of the AT1 receptor in mesentery. Considering the relevance of obesity in cardiovascular disease and the contribution of inadequate diet and supplements to this condition, it is worth investigating if our findings also hold true for humans.

Funding: Research supported by grants from the Brazilian funding agencies: CAPES, CNPq (Universal 424799/2019-9 and 401134/2016-4) and FAPESC (Jovem Pesquisador 10.029/2012-3).
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Bleich SN, Vercammen KA. The negative impact of sugar-sweetened beverages on children's health: an update of the literature. BMC Obes. 2018;5:6.
2. Pereira RM, Botezelli JD, da Cruz Rodrigues KC, Mekary RA, Cintra DE, Pauli JR, et al. Fructose Consumption in the Development of Obesity and the Effects of Different Protocols of Physical Exercise on the Hepatic Metabolism. Nutrients. 2017;9(4):405.
3. Toop CR, Gentili S. Fructose Beverage Consumption Induces a Metabolic Syndrome Phenotype in the Rat: A Systematic Review and Meta-Analysis. Nutrients. 2016;8(9):577.
4. Zhou D, Xi B, Zhao M, Wang L, Veeranki SP. Uncontrolled hypertension increases risk of all-cause and cardiovascular disease mortality in US adults: the NHANES III Linked Mortality Study. Sci. Rep. 2018;8(1):9418.
5. Janus A, Szahidewicz-Krupska E, Mazur G, Doroszko A. Insulin Resistance and Endothelial Dysfunction Constitute a Common Therapeutic Target in Cardiometabolic Disorders. Mediators. Inflamm. 2016;2016:3634948.
6. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler. Thromb. Vasc. Biol. 2003;23(2):168-75.
7. Su JB. Vascular endothelial dysfunction and pharmacological treatment. World. J. Cardiol. 2015;7(11):719-41.
8. Saygin M, Asci H, Cankara FN, Bayram D, Yesilot S, Candan IA, et al. The impact of high fructose on cardiovascular system: Role of α-lipoic acid. Hum. Exp. Toxicol. 2016;35(2):194-204.
9. Christensen KL, Mulvany MJ. Mesenteric arcade arteries contribute substantially to vascular resistance in conscious rats. J. Vasc. Res. 1993;30:73-9.
10. Shaligram S, Sangüesa G, Akther F, Alegret M, Laguna JC, Rahimian R. Differential effects of high consumption of fructose or glucose on mesenteric arterial function in female rats. J. Nutr. Biochem. 2018;57:136-44.
11. Ibitoye OB, Ajiboye TO. Dietary phenolic acids reverse insulin resistance, hyperglycaemia, dyslipidaemia, inflammation and oxidative stress in high-fructose diet-induced metabolic syndrome rats. Arch. Physiol. Biochem. 2018;124(5):410-7.
12. Benkhaled A, Réggami Y, Boudjelal A, Senator A, Bouriche H, Demirtaş I, et al. Chemical characterisation, hypoglycaemic and renoprotective effects of aqueous leaf extract of Limoniastrum guyonianum on fructose-induced metabolic syndrome in rats. Arch. Physiol. Biochem. 2022;128(4):914-23.
13. Kamide K, Rakugi H, Higaki J, Okamura A, Nagai M, Moriguchi K, et al. The renin-angiotensin and adrenergic nervous system in cardiac hypertrophy in fructose-fed rats. Am. J. Hypertens. 2002;15(1 Pt 1):66-71.
14. Iyer SN, Katovich MJ, Raizada MK. Changes in angiotensin AT1 receptor density during hypertension in fructose-fed rats. Adv. Exp. Med. Biol. 1996;396:49-58.
15. Dhar I, Dhar A, Wu L, Desai KM. Increased methylglyoxal formation with upregulation of renin angiotensin system in fructose fed Sprague Dawley rats. PLoS One. 2013;8(9):e74212.
16. Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. Hypertens. 1987;10(5):512-6.
17. Verma S, Bhanot S, McNeill JH. Antihypertensive effects of metformin in fructose-fed hyperinsulinemic, hypertensive rats. J. Pharmacol. Exp. Ther. 1994;271(3):1334-7.
18. Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol. Rev. 2000;52(4):639-72.
19. Giacchetti G, Sechi LA, Griffin CA, Don BR, Mantero F, Schambelan M. The tissue renin-angiotensin system in rats with fructose-induced hypertension: overexpression of type 1 angiotensin II receptor in adipose tissue. J Hypertens. 2000;18(6):695-702.
20. Fukui T, Hirano T, Shiraishi Y, Nagashima M, Adachi M. Chronic insulin infusion normalizes blood pressure and the gene expressions of angiotensin II type 1 receptor in fructose-fed rats. Hypertens. Res. 2008;31(1):127-33.
21. Nyby MD, Abedi K, Smutko V, Eslami P, Tuck ML. Vascular Angiotensin type 1 receptor expression is associated with vascular dysfunction, oxidative stress and inflammation in fructose-fed rats. Hypertens. Res. 2007; 30(5):451-7.
22. Schmidt HH, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biochim. Biophys. Acta. 1993;1178(2):153-75.
23. Glushakova O, Kosugi T, Roncal C, Mu W, Heinig M, Cirillo P, et al. Fructose induces the inflammatory molecule ICAM-1 in endothelial cells. J. Am. Soc. Nephrol. 2008;19(9):1712-20.
24. Palanisamy N, Venkataraman AC. Beneficial effect of genistein on lowering blood pressure and kidney toxicity in fructose-fed hypertensive rats. Br. J. Nutr. 2013;109(10):1806-12.
25. Okamura T, Tawa M, Geddawy A, Shimosato T, Iwasaki H, Shintaku H, et al. Effects of atorvastatin, amlodipine, and their combination on vascular dysfunction in insulin-resistant rats. J. Pharmacol. Sci. 2014;124(1):76-85.
26. Sousa GJ, Oliveira PWC, Nogueira BV, Melo AF, Junior Faria TO, Meira EF, et al. Fructose intake exacerbates the contractile response elicited by norepinephrine in mesenteric vascular bed of rats via increased endothelial prostanoids. J. Nutr. Biochem. 2017;48:21-8.
27. Murad F. Shattuck Lecture. Nitric oxide and cyclic GMP in cell signaling and drug development. N. Engl. J. Med. 2006;355(19):2003-11.
28. Shah RC, Sanker S, Wood KC, Durgin BG, Straub AC. Redox regulation of soluble guanylyl cyclase. Nitric Oxide. 2018;76:97-104.

29. Nardi GM, Scheschowitsch K, Ammar D, de Oliveira SK, Arruda TB, Assreuy J. Neuronal nitric oxide synthase and its interaction with soluble guanylate cyclase is a key factor for the vascular dysfunction of experimental sepsis. Crit. Care. Med. 2014;42(6):e391-e400.
30. Zanetti M, Barazzoni R, Stebel M, Roder E, Biolo G, Baralle FE, et al. Dysregulation of the endothelial nitric oxide synthase-soluble guanylate cyclase pathway is normalized by insulin in the aorta of diabetic rat. Atherosclerosis. 2005;181(1):69-73.