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ORIGINAL ARTICLE

Time-Dependent Relationship Between Endothelial Dysfunction and High Blood Pressure in Fructose Drinking Rats

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ABSTRACT

Objectives: This study aims to investigate the duration required for endothelium dysfunction to develop in the fructose drinking-induced hypertension and examine the relative contributions of endothelium-dependent relaxing factors to changes in mesenteric arterial reactivity in male Wistar Albino rats.

Materials and Methods: Metabolic parameters (water intake and food consumption) and hemodynamic parameters systolic blood pressure (SBP) and diastolic blood pressure (DBP)-were monitored *in vivo*. Vascular reactivity was examined in the isolated organ bath. Endothelium-dependent relaxation (EDR) to acetylcholine was observed in the absence and presence of pharmacological inhibitors of endothelial nitric oxide (NO) synthase, cyclooxygenase, and KCa2.3 channels. Contractile responses to phenylephrine and relaxation of sodium nitroprusside (SNP) were also determined.

Results: A significant increase in daily water intake and decrease in food consumption were typically observed in rats treated with 10% fructose for 4 weeks ($p < 0.05$). SBP and DBP increased significantly as early as 2 weeks of induction and continued to rise gradually throughout the induction period ($p < 0.05$). Fructose consumption significantly impaired EDR at week 3 and worsened at week 4 ($p < 0.05$). Impairment of the KCa3.1 channel-mediated component of endothelium-dependent hyperpolarisation (EDH)-type relaxation contributed to worsening EDR, whereas the contribution of NO-mediated relaxation was not apparent compared with the controls. The reduction in EDH-type relaxation in fructose-fed rats appears to be partially compensated by increased NO sensitivity in the smooth muscle region, as fructose induction increased SNP relaxation compared with the control.

Conclusion: These data provide evidence of early endothelial dysfunction developing concurrently with increased blood pressure in 10% fructose-fed rats. Decreased KCa3.1-mediated part of EDH-type relaxation appears to contribute to the impairment of endothelium-dependent vasorelaxation over time in this model.

Keywords: Endothelial dysfunction, fructose, hypertension, mesenteric artery, rat

INTRODUCTION

In The 1970s, it has been seen the introduction of high fructose corn syrup as an affordable alternative for sweetening processed foods and soft drinks. Since then, there has been a progressive global increase in the consumption of packaged food containing fructose and corn syrup.[1-3] However, high fructose consumption in mice and rats has been associated with obesity, type 2 diabetes mellitus, insulin resistance in the liver and extrahepatic tissues, and elevated blood pressure (BP).[4-7] In 1987, Hwang et al.[4] first reported that male Sprague-Dawley rats fed pellets containing 66% fructose for 2 weeks developed systolic hypertension with increased plasma insulin levels and insulin resistance.

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Therefore, many investigations have been conducted to elucidate the underlying mechanism(s) of hypertension in this model. In summary, three main mechanisms through which high fructose consumption can raise BP have been reported: increased intestinal Na^+ absorption, central sympathetic outflow, and endothelial dysfunction.[8-10]

The vascular endothelium plays a vital role in regulating the vascular tone by releasing certain autocrine and paracrine factors.[11] In large-sized arteries, endothelium-dependent relaxation is achieved through diffusible factors like endothelium-derived nitric oxide (NO) and prostacyclin. In smaller arterioles and resistant arteries, the-opening of intermediate (KCa3.1)-and/or small (KCa2.3)-conductance calcium-activated potassium channels located on the endothelium triggers endothelial hyperpolarization that spreads toward smooth muscle and leads to vasorelaxation. This type of relaxation is called endothelium-dependent hyperpolarization (EDH)-type relaxation; in this way, the endothelium contributes to the regulation of regional blood flow and total peripheral vascular resistance in the microcirculation. Multiple studies have reported that fructose feeding diminishes NO production by reducing the activity and/or expression of endothelial NO synthase in the aorta, mesenteric artery, cardiac myocyte, and kidney homogenates.[12,13] Moreover, fructose utilization was reported to decrease acetylcholine-induced EDH-type relaxations.[9,14] Although these studies point to a possible link between endothelial dysfunction and the development of hypertension in the fructose-fed rat model, experiments need to be carried out, especially in the early period when fructose-induced endothelial changes begin to become evident and BP increases. Indeed, previous studies have been conducted to determine the inducing time required for endothelium dysfunction to occur in rats fed a 66% fructose diet.[15,16] Yet, these studies have been designed to deliver fructose in the rat's chow pellets,[14-16] and no such studies have been performed on the 10% *w/v* fructose in the drinking water design of the hypertensive rat model. Besides, most of these studies have been done on Sprague-Dawley rats, but not on the Wistar Albino rat strain. Whether Wistar rat strains respond to 10% fructose-water induction with endothelial dysfunction and to what extent they are sensitive to this model have not been previously studied in detail.

Consequently, in this study, it is aimed to determine the time required for the development of early endothelial dysfunction in 10% fructose drinking Wistar Albino rats. The second aim of this study is to examine its effect on the different components of endothelium-derived EDH-type relaxation in terms of increasing the duration of acute fructose consumption.

MATERIALS AND METHODS

Male Wistar Albino rats (8 weeks, 180-200 g) were obtained from Kobay Company (Ankara, Türkiye). Animals were housed for a week before the experiments to acclimatize to the laboratory settings and were maintained on a 12-hour light: 12-hour dark cycle. In compliance with institutional guidelines and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, the animals were handled and cared for as directed.

Animal procedures and protocols were approved by the Animal Ethics Committee of the University of Hacettepe (approval number: 2021/03-06, date: 30.03.2021).

Rats were divided randomly into four groups (n= 6 each). The fructose groups were given 10% fructose water (*w/v*) *ad libitum* for 3 and 4 weeks, whereas the control rats were given tap water for the same periods. All groups were fed standard rat chow. Water intake (mL/kg/day), food consumption (g/kg/day), and body weight (g/kg/day) were tracked twice weekly; systolic blood pressure [(SBP); mmHg], diastolic blood pressure [(DBP); mmHg], and heart rate [(HR); beats/minute] were measured weekly in all groups.

The non-invasive tail-cuff method was used for hemodynamic measurements (Biopac Systems, USA). Rats were habituated to the protocol 3 days before the experiments. Measurements were performed by the same researcher between 12:00 and 15:00. On the measurement day, the animals were kept in the procedure room (30 ± 2 °C) for 45 min and then sedated with isoflurane inhalation and oxygen mixture (5% induction and 1.5% maintenance) (Adeka, Türkiye).[17] Meanwhile, after the tails were warmed under a heat blanket, SBP, DBP, and HR were measured 5-6 times consecutively, and the average of the measurements was calculated.

For the *in vitro* organ bath experiments, rats in both the fructose (3 and 4 weeks) and control groups were euthanized by decapitation under CO_2 anesthesia. The superior mesenteric artery's main branch was cut and placed into a Petri dish with Krebs-Henseleit solution (KHS) at 4 °C. After removing the fat and connective tissues, the artery was divided into 2-mm-long rings. The rings were suspended at 37 °C in 5 mL organ baths filled with KHS gassed with 95% O_2-5% CO_2. One gram was determined as the optimum resting tension for the rings. The change in tension was measured by an isometric force transducer. Following an hour of rest, 80 mM KCl containing KHS was applied to the rings until consistent contractions were achieved.

In the main experiments, the assessment of vascular contractility required determining the total contractions to phenylephrine (10 nM-100 μM) for each group. After 1 h, the same rings were precontracted submaximally using 1 or 3 μM phenylephrine, and endothelium-dependent relaxations to acetylcholine (0.1 nM-10 μM) were then obtained. To examine the EDH-type component of acetylcholine relaxation, the same responses were repeated in the presence of N^w-nitro-L-arginine methyl ester (L-NAME; 100 μM), and indomethacin (10 μM), NO synthase inhibitors, and cyclooxygenase inhibitors, respectively. Moreover, a KCa3.1-mediated component of EDH-type vasorelaxation was also observed in rings incubated with L-NAME, indomethacin plus apamin (50 nM), a KCa2.3 channel blocker. In addition, to examine the NO-mediated component of acetylcholine-induced endothelial vasorelaxation, the same responses were repeated in the presence of indomethacin (10 μM), apamin (50 nM), and

TRAM-34 (1 μM), a KCa3.1 channel blocker. The incubation period was 45 minutes, and antagonist concentrations were selected using data from prior studies.[18-21] Finally, cumulative relaxation responses were obtained using sodium nitroprusside (SNP; 0.1 nM-10 μM), the NO donor.

Reagents

Fructose (Egepak Co., Türkiye) was dissolved in tap water. Acetylcholine chloride (Sigma-Aldrich, Germany, A6625), apamin (Sigma-Aldrich, A1289), L-NAME (Sigma, N-5751), L-phenylephrine hydrochloride (Sigma, P6126), and SNP (Sigma, S-0501) were dissolved in distilled water. Indomethacin (Sigma, I-7378) was dissolved in distilled water containing 0.7% Na_2CO_3 (*w/v*). KHS had the following composition (mM): NaCl 118.0, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.1. NaCl was replaced with equimolar KCl to obtain an 80 mM KCl solution.

Statistical analysis

All data was displayed as the mean ± standard error of the mean. The contractions induced by KCl (80 mM) were used to standardize cumulative phenylephrine contractions, whereas the percentage of precontraction generated by phenylephrine was used to standardize relaxation. pD_2 values [-log(EC_{50})] were expressed as the negative logarithm of the drug concentration, which constitutes the half-maximum response, and the area under the curve (AUC) was expressed as arbitrary units. All analyses were conducted using GraphPad Prism 9.4.1. Comparisons between the two groups were performed using the unpaired *t*-test. $p < 0.05$ was considered significant.

Results

Metabolic characteristics of fructose-fed rats

The addition of 10% fructose to drinking water in rats for 4 weeks resulted in a significant increase in daily water intake and a decrease in food consumption compared with rats fed tap water ($p < 0.05$; n= 6). These changes were significantly different from the control during all weeks of induction. Both groups gained weight during the 4 weeks, but no significant difference was detected between them (Figure 1).

Effects of fructose on hemodynamic parameters

High fructose intake caused a moderate increase in SBP levels starting from week 2 compared with the control [SBP values; control (2 weeks) 116.8 ± 0.98 mm Hg vs. Fructose (2 weeks) 129.4 ± 2.90 mmHg; $p < 0.05$, n= 6]. The increase in SBP continued gradually as the fructose induction was prolonged to week 4 [SBP values; fructose (2 weeks) 129.4 ± 2.90 mmHg vs. fructose (3 weeks) 137.3 ± 3.50 mmHg (n= 6), and vs. fructose (4 weeks) 145.3 ± 5.30 mmHg; $p < 0.05$, n= 6] (Figure 2).

DBP also increased in parallel with the increase in SBP in rats fed high fructose [DBP values; fructose (2 weeks) 88.9 ± 2.40 mmHg vs. fructose (3 weeks) 97.7 ± 1.50 mmHg ($p < 0.01$, n= 6), and vs. fructose (4 weeks) 97.7 ± 1.30 mmHg ($p < 0.01$, n= 6]. However, HR did not differ significantly between the groups (Figure 2).

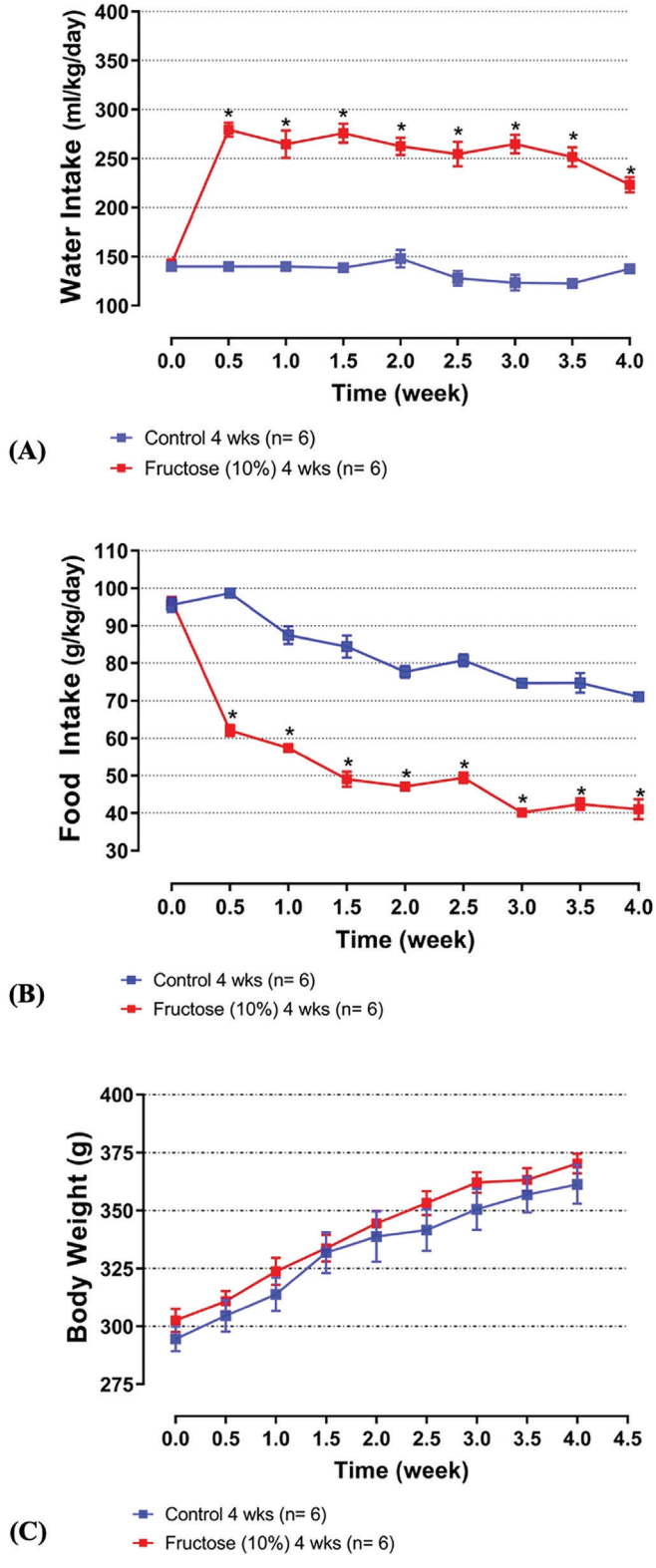

Figure 1. Change over time of daily water intake (A), food consumption (B), and body weight (C) in rats fed fructose (10%) or tap water (control) for 4 weeks (mean ± SEM, *fructose vs. control, unpaired t-test, *$p < 0.05$)

SEM: Standard error of the mean

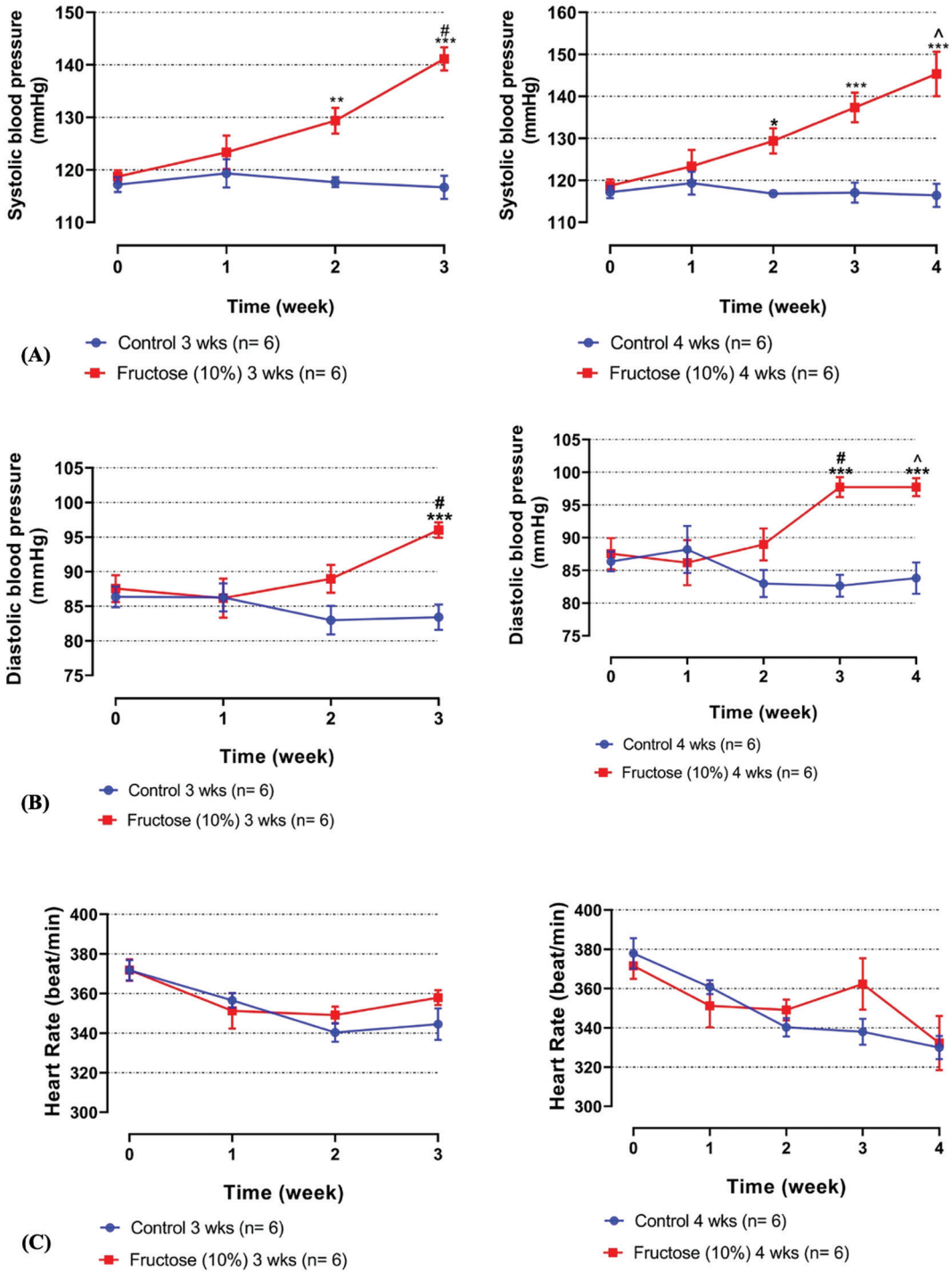

Figure 2. Change over time of SBP (A), DBP (B), and HR (C) in rats fed fructose (10%) or tap water (control) for 4 weeks (mean ± SEM, *fructose vs. control; #fructose 2nd vs. 3rd week; ^fructose 2nd vs. 4th week, unpaired *t*-test, *.#.^$p < 0.05$, ***$p < 0.001$)

SEM: Standard error of the mean, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HR: Heart rate

Effects of fructose on vascular reactivity

Cumulative contractions of phenylephrine (10 nM-100 µM) were examined in mesenteric arteries isolated from rats in the 3- and 4-week fructose groups. After 3 weeks of fructose induction, phenylephrine contractions were the same as those in the control (n= 6). When the induction period was extended to 4 weeks, a modest decrease in peak phenylephrine concentrations was observed, but the difference was not statistically significant (n= 6) (Figure 3). E_{max}, pD_2, and AUC values of contractile responses to phenylephrine were similar between the fructose groups (data not shown).

To assess endothelial function in fructose-fed rats, cumulative relaxation to acetylcholine (0.1 nM-10 µM) was observed in rings pre-contracted with phenylephrine (1 or 3 µM). Fructose

induction for 3 weeks led to a decrease in endothelium-dependent relaxation as opposed to contractions. The relaxation response curve of acetylcholine slightly but significantly shifted to the right without showing a change in E_{max} values in the 3-week fructose group ($p < 0.05$; n= 6) (Table 1). A similar but significantly higher rightward shift was detected in the rats at 4-week induction compared with that in the 3-week group ($p < 0.05$; n= 5) (Figure 4). The decrease in pD_2 values in the 4-week fructose group resulted in a significant decrease in AUC values compared with the control, which suggests a worsening of endothelium-dependent relaxation in correlation with the duration of fructose induction.

Acetylcholine-induced relaxation was repeated in the presence of L-NAME (100 µM) and indomethacin (10 µM). The remaining relaxation of acetylcholine were considered EDH-mediated and they were diminished in fructose-induced rats (Figure 5). The relaxation-response curves shifted to the right in both induction periods with decreasing pD_2 and AUC values. Moreover, the

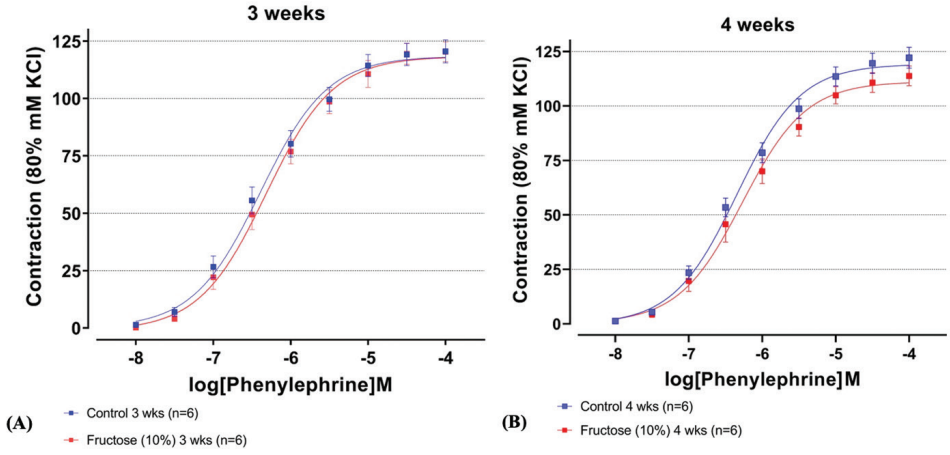

Figure 3. Phenylephrine-induced contractions in superior mesenteric arteries isolated from control and fructose groups at the 3rd (A) and 4th (B) weeks of fructose induction (mean ± SEM)

SEM: Standard error of the mean

Table 1. Comparison of the AUC, pD_2, and E_{max} values for the acetylcholine-induced relaxation response in the superior mesenteric arteries isolated from the control and fructose groups at the 3rd and 4th weeks of fructose induction

Acetylcholine	3rd week			4th week		
	Control (n= 5)	Fructose (n= 6)	*p* value	Control (n= 5)	Fructose (n= 5)	*p* value
AUC	302.30 ± 8.28	276.40 ± 7.15*	0.040	317.40 ± 17.77	255.70 ± 14.79*	0.030
pD_2	7.95 ± 0.05	7.69 ± 0.04*	0.003	8.13 ± 0.10	7.37 ± 0.09*, #	0.001
E_{max}	100 ± 0.00	99.26 ± 0.54	0.247	99.44 ± 0.56	97.24 ± 1.92	0.304
Acetylcholine in the presence of L-NAME + indomethacin	**Control (n= 4)**	**Fructose (n= 6)**	***p* value**	**Control (n= 4)**	**Fructose (n= 6)**	***p* value**
AUC	233.60 ± 15.00	175.20 ± 10.73*	0.012	238.90 ± 14.67	175.40 ± 10.39*	0.007
pD_2	7.22 ± 0.10	6.68 ± 0.07*	0.001	7.46 ± 0.10	6.84 ± 0.07*	0.001
E_{max}	96.40 ± 3.60	93.14 ± 3.97	0.584	93.35 ± 2.89	81.45 ± 2.57*, #	0.012
Acetylcholine in the presence of L-NAME + indomethacin + apamin	**Control (n= 5)**	**Fructose (n= 6)**	***p* value**	**Control (n= 6)**	**Fruktoz (n= 6)**	***p* value**
AUC	132.50 ± 12.41	115.10 ± 11.86	0.354	145.50 ± 16.14	115.20 ± 7.26	0.118
pD_2	6.50 ± 0.10	6.47 ± 0.09	0.827	6.70 ± 0.12	6.56 ± 0.08	0.331
E_{max}	75.42 ± 2.38	68.25 ± 5.16	0.320	77.93 ± 4.52	59.53 ± 3.28*	0.008
Acetylcholine in the presence of indomethacin + TRAM-34 + apamin	**Control**	**Fructose**	***p* value**	**Control (n= 3)**	**Fruktoz (n= 2)**	***p* value**
AUC	-	-	-	234.30 ± 21.42	214.30 ± 17.33	0.540
pD_2	-	-	-	7.45 ± 0.18	7.15 ± 0.14	0.324
E_{max}	-	-	-	92.97 ± 4.62	96.1 ± 2.8*	0.654

*Fructose vs. control, #3 week vs. 4 week fructose, unpaired t-test, *,#$p < 0.05$. AUC: Area under the curve, E_{max}: Maximum response, pD_2: Negative logarithm of drug concentration which constitutes half-maximum response, TRAM-34: Triarylmethane-34, NO: Nitric oxide, EDH: Endothelium-dependent hyperpolarization

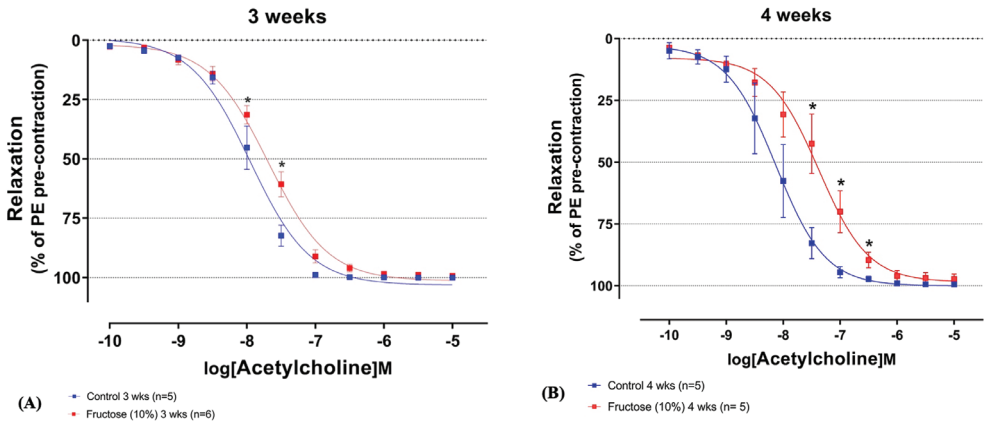

Figure 4. Acetylcholine-induced relaxations in superior mesenteric arteries isolated from control and fructose groups at the 3rd (A) and 4th (B) week of fructose induction (mean ± SEM, *fructose vs. control, unpaired *t*-test, *$p < 0.05$)

SEM: Standard error of the mean

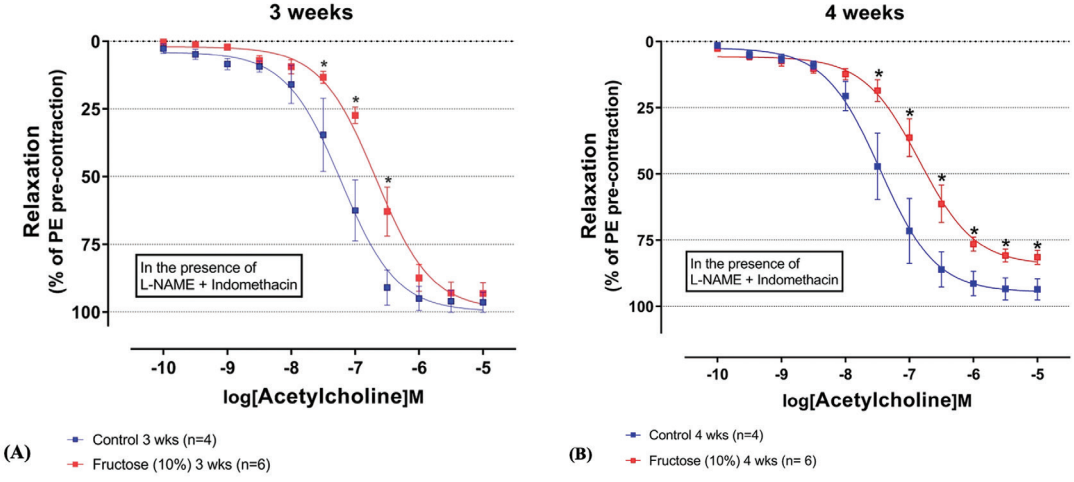

Figure 5. EDH-type relaxations obtained with acetylcholine in the presence of L-NAME and indomethacin in superior mesenteric arteries isolated from control and fructose groups at the 3rd (A) and 4th (B) week of fructose induction (mean ± SEM, *fructose vs. control, unpaired *t*-test, *$p < 0.05$)

EDH: Endothelium-dependent hyperpolarization, SEM: Standard error of the mean

amplitude of EDH-type relaxations decreased significantly only in the 4-week fructose group ($p < 0.05$; n= 6) (Table 1), suggesting that decreasing EDH-type vasorelaxation in time might partly contribute to the development of hypertensive responses in fructose-fed rats.

Because EDH-type relaxations are mediated by the activation of endothelial KCa3.1 and/or KCa2.3 channels, we examined acetylcholine relaxations in the presence of L-NAME (100 µM), indomethacin (10 µM), and apamin (10 nM), which are KCa2.3 channel blockers. The remaining relaxations in response to acetylcholine were considered to be solely mediated by KCa3.1 channels. Acetylcholine relaxations triggered by KCa3.1 activity did not change in the 3-week fructose group; however, as expected, a decrease in the amplitude of these relaxations was detected in the 4-week fructose group compared with the control or 3-week fructose group (Figure 6 and Table 1). These data suggest that impairment of endothelial KCa3.1 channel function contributes to the time-dependent reduction of EDH-type relaxation in fructose-fed rats.

There was significant potentiation in SNP-induced relaxation (0.1 nM-10 µM) in both fructose groups. Both relaxation response curves shifted significantly to the left compared to their controls (Figure 7). [pD_2 values; control (3 weeks) 8.70 ± 0.03 vs. fructose (3 weeks) 8.91 ± 0.04 ($p < 0.001$, n= 6); control (4 weeks) 8.07 ± 0.04 vs. fructose (4 weeks) 8.43 ± 0.06, $p < 0.001$, n= 6). These results indicate that smooth muscle sensitivity to NO and/or other downstream pathways increases under acute fructose induction.

DISCUSSION

The aim of this study was to determine the duration required for early endothelial dysfunction to develop in fructose drinking-induced hypertension in male Wistar Albino rats. BP and HR were measured simultaneously to detect changes in hemodynamic parameters associated with endothelial dysfunction. The second aim of this study is to detect changes in different components of endothelium-dependent relaxation in isolated mesenteric arteries of rats induced by high fructose.

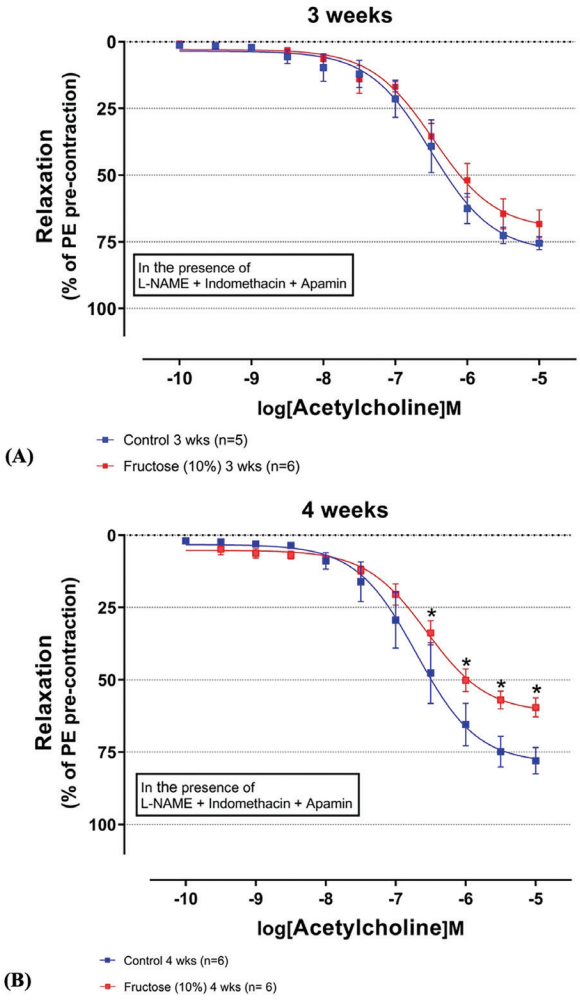

Figure 6. KCa3.1 channel-mediated component of EDH-type relaxations obtained with acetylcholine in the presence of L-NAME, indomethacin, and apamin in superior mesenteric arteries isolated from control and fructose groups at the 3rd (A) and 4th (B) week of fructose induction (mean ± SEM, *fructose vs. control, unpaired *t*-test, *$p < 0.05$)

EDH: Endothelium-dependent hyperpolarization, SEM: Standard error of the mean

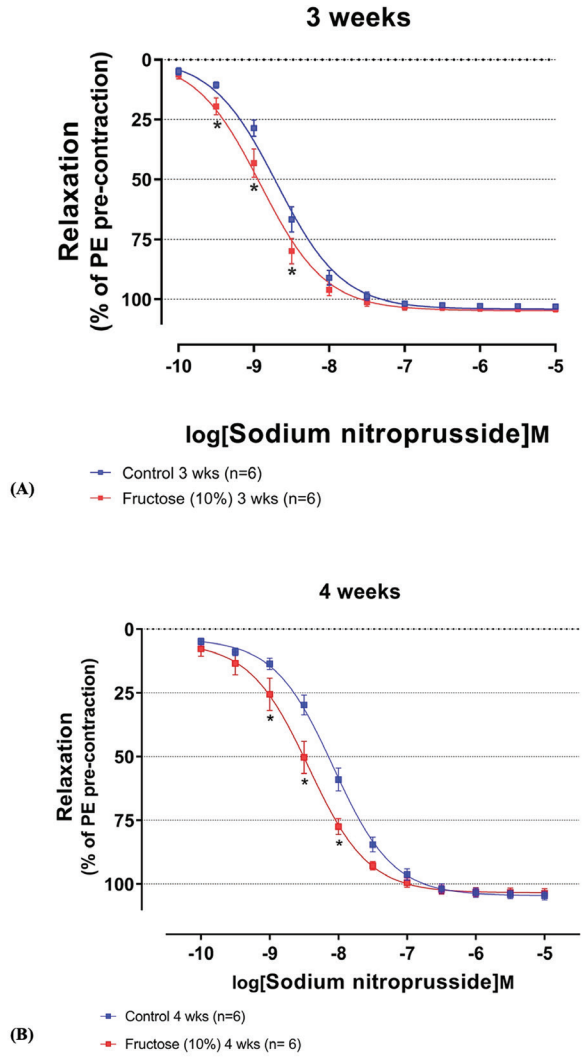

Figure 7. SNP-induced relaxation in superior mesenteric arteries isolated from control and fructose groups at the 3rd (A) and 4th (B) week of fructose induction (mean ± SEM, *fructose vs. control, unpaired *t*-test, *$p < 0.05$)

SNP: Sodium nitroprusside, SEM: Standard error of the mean

The duration of acute fructose exposure was determined as 3 and 4 weeks to evaluate the development phase of high BP and endothelial dysfunction in rats fed 10% fructose water. An increase in water intake and a corresponding decrease in food consumption observed in fructose induction were metabolic characteristics of this model since rats require less calorie intake if they have free access to fructose.[22] Consistent with the literature, these metabolic changes were used as model validation in this study.

Altered hemodynamic parameters during fructose induction are important symptoms of this experimental model and indicate metabolic syndrome development.[23] *In vivo* BP monitoring results showed that SBP began to increase with fructose induction as early as 2 weeks, and this increase continued gradually as the induction period was extended up to 4 weeks. DBP showed a similar increasing trend as SBP, but the rate of increase in DBP was more stable over the induction period. Early development of the increase in SBP has previously been reported in both fructose water and pellet design.[4,22,24] However, the pathophysiological mechanism(s) by which hypertension occurs in the fructose induction model has not been completely clarified, yet many studies have referred to endothelial dysfunction as a common underlying mechanism.[15,25] In a way that supports this, we were able to detect endothelial dysfunction in isolated mesenteric arteries of rats that consumed fructose (10%) for only 3 weeks, and it became more severe as the fructose consumption prolonged to 4 weeks. We also showed that endothelial dysfunction in fructose-fed rats was accompanied by a concomitant increase in SBP and DBP.

In our *ex vivo* organ bath experiments, contractile responses to phenylephrine were comparable between groups provided with or without 10% fructose water. In addition, fructose-related vascular impairment was characterized by decreased

endothelium-dependent relaxation in response to acetylcholine, which refers to dysfunctional endothelium, and increased smooth muscle sensitivity to SNP-induced relaxation. The maximal acetylcholine relaxations in both 3- and 4-week fructose-fed rats were not different from those in the control rats, despite being reported to lessen in other studies performed using the pellet design.[15,16] Nevertheless, the sensitivity of acetylcholine relaxation in our study design declined significantly in week 3 and worsened in week 4. Although not clear, this discrepancy might be due to differences in experimental protocols that might affect the sensitivity of mesenteric arteries to endothelium-dependent relaxant agents.

When the acetylcholine relaxation components were examined separately, it can be said that this inhibition was due to decreased EDH-type relaxation, which is compatible with the studies performed using the pellets.[14] Based on the obtained data, it was suggested that the KCa3.1-mediated component of EDH-type relaxation was decreased. The decrease in EDH-type relaxation in the short-term (3 or 4 weeks) fructose-induced hypertensive rats seems to be partially compensated by the increased NO sensitivity at the site of smooth muscle cells. This deduction was indirectly supported by the increase in sensitivity to SNP relaxations, although no functional increase in NO-mediated vasorelaxation in response to acetylcholine was detected in fructose-fed rats (Table 1). Other studies have reported no change in SNP relaxation with pellet design.[16,25] Furthermore, a long period of fructose consumption has been reported to decrease SNP relaxation,[26] suggesting that long-term exposure to fructose leads to the failure of this compensation mechanism.

Whether endothelial dysfunction is a cause or a consequence of hypertension remains a matter of debate. This paradox can be explained by simultaneously examining whether antihypertensive treatments improve endothelial dysfunction in fructose-induced hypertensive rats. According to our findings, the preservation of phenylephrine contractions in the mesenteric arteries after fructose induction indicates that smooth muscle sensitivity to contractile agents does not change and that the tonic inhibitory effect of endothelial factors against contractions continues. In this case, it can be argued that the two pathophysiologies may operate independently of each other. It has previously been suggested that fructose-mediated hypertension is associated with a central neuro-inflammatory response that triggers increased sympathetic outflow.[27] Blocking this mechanism with centrally acting anti-inflammatory agents can ameliorate hypertension by reducing only the sympathetic outflow without directly targeting endothelial dysfunction or hyperinsulinemia, which usually accompanies hypertension in this model.[24] On the other hand, in the pellet design of the fructose-induced hypertensive rat model, endothelial dysfunction has been reported to precede hypertension.[15] This means that further experiments are needed to conclude that hypertension and dysfunctional endothelium trigger each other.

CONCLUSION

In conclusion, rats subjected to 10% fructose water for only 3 weeks showed decreased relaxation responses to acetylcholine in isolated superior mesenteric arterial rings, supporting increases in SBP and DBP. Hypertensive response and corresponding endothelial dysfunction become more severe as the induction period with fructose increases. Decreased KCa3.1-mediated EDH-type relaxation appears to contribute to impaired mesenteric arterial relaxation in this model.

Ethics

Ethics Committee Approval: Animal procedures and protocols were approved by the Animal Ethics Committee of the University of Hacettepe (approval number: 2021/03-06, date: 30.03.2021).

Informed Consent: Not required.

Authorship Contributions

Concept: A.H., M.H.Ö., Design: A.H., M.H.Ö., Data Collection or Processing: A.H., Analysis or Interpretation: A.H., M.H.Ö., Literature Search: A.H., Writing: A.H., M.H.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Forshee RA, Storey ML, Allison DB, Glinsmann WH, Hein GL, Lineback DR, Miller SA, Nicklas TA, Weaver GA, White JS. A critical examination of the evidence relating high fructose corn syrup and weight gain. Crit Rev Food Sci Nutr. 2007;47:561-582.
2. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, Gersch MS, Benner S, Sánchez-Lozada LG. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr. 2007;86:899-906.
3. Marriott BP, Cole N, Lee E. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. J Nutr. 2009;139:1228S-1235S.
4. Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. Hypertension. 1987;10:512-516.
5. Klein AV, Kiat H. The mechanisms underlying fructose-induced hypertension: a review. J Hypertens. 2015;33:912-920.
6. Komnenov D, Levanovich PE, Rossi NF. Hypertension Associated with Fructose and High Salt: Renal and Sympathetic Mechanisms. Nutrients. 2019;11:569.
7. Sukiasyan L. Fructose-Induced Alteration of the Heart and Vessels Homeostasis. Curr Probl Cardiol. 2023;48:101013.
8. Soleimani M, Alborzi P. The role of salt in the pathogenesis of fructose-induced hypertension. Int J Nephrol. 2011;2011:392708.
9. El-Bassossy HM, Dsokey N, Fahmy A. Characterization of vascular complications in experimental model of fructose-induced metabolic syndrome. Toxicol Mech Methods. 2014;24:536-543.
10. Tran LT, MacLeod KM, McNeill JH. Selective alpha(1)-adrenoceptor blockade prevents fructose-induced hypertension. Mol Cell Biochem. 2014;392:205-211.

11. Krüger-Genge A, Blocki A, Franke RP, Jung F. Vascular endothelial cell biology: an update. Int J Mol Sci. 2019;20:4411.

12. Palanisamy N, Venkataraman AC. Beneficial effect of genistein on lowering blood pressure and kidney toxicity in fructose-fed hypertensive rats. Br J Nutr. 2013;109:1806-1812.

13. Miatello R, Risler N, Gonzalez S, Castro C, Rüttler M, Cruzado M. Effects of enalapril on the vascular wall in an experimental model of syndrome X. Am J Hypertens. 2002;15:872-878.

14. Katakam PV, Ujhelyi MR, Miller AW. EDHF-mediated relaxation is impaired in fructose-fed rats. J Cardiovasc Pharmacol. 1999;34:461-467.

15. Katakam PV, Ujhelyi MR, Hoenig ME, Miller AW. Endothelial dysfunction precedes hypertension in diet-induced insulin resistance. Am J Physiol. 1998;275:788-792.

16. Verma S, Bhanot S, Yao L, McNeill JH. Defective endothelium-dependent relaxation in fructose-hypertensive rats. Am J Hypertens. 1996;9:370-376.

17. Hem NA, Phie J, Chilton L, Kinobe R. A volume-pressure tail cuff method for hemodynamic parameters: Comparison of restraint and light isoflurane anesthesia in normotensive male Lewis rats. J Pharmacol Toxicol Methods. 2019;100:106601.

18. Hamad A, Ozkan MH, Uma S. Trimethylamine-N-oxide (TMAO) selectively disrupts endothelium-dependent hyperpolarization-type relaxations in a time-dependent manner in rat superior mesenteric artery. Biol Pharm Bull. 2021;44:1220-1229.

19. Gerber R, Anwar M, Poston L. Enhanced acetylcholine induced relaxation in small mesenteric arteries from pregnant rats: an important role for endothelium-derived hyperpolarizing factor (EDHF). Br J Pharmacol. 1998;125:455-460.

20. Crane GJ, Gallagher N, Dora KA, Garland CJ. Small- and intermediate-conductance calcium-activated K+ channels provide different facets of endothelium-dependent hyperpolarization in rat mesenteric artery. J Physiol. 2003;553:183-189.

21. Ozkan MH, Uma S. β-adrenergic receptor blocker ICI 118,551 selectively increases intermediate-conductance calcium-activated potassium channel (IK_{Ca})-mediated relaxations in rat main mesenteric artery. Basic Clin Pharmacol Toxicol. 2018;122:570-576.

22. Dai S, McNeill JH. Fructose-induced hypertension in rats is concentration- and duration-dependent. J Pharmacol Toxicol Methods. 1995;33:101-107.

23. Tran LT, Yuen VG, McNeill JH. The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Mol Cell Biochem. 2009;332:145-159.

24. Ho CY, Lin YT, Chen HH, Ho WY, Sun GC, Hsiao M, Lu PJ, Cheng PW, Tseng CJ. CX3CR1-microglia mediates neuroinflammation and blood pressure regulation in the nucleus tractus solitarii of fructose-induced hypertensive rats. J Neuroinflammation. 2020;17:185.

25. Miller AW, Hoenig ME, Ujhelyi MR. Mechanisms of impaired endothelial function associated with insulin resistance. J Cardiovasc Pharmacol Ther. 1998;3:125-134.

26. 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;57:136-144.

27. Wu CJ, Cheng PW, Kung MH, Ho CY, Pan JY, Tseng CJ, Chen HH. Glut5 Knockdown in the nucleus tractus solitarii alleviates fructose-induced hypertension in rats. J Nutr. 2022;152:448-457.