



Metabolic Disorders Induced by Fructose-drinking Water Affect Angiotensin II-mediated Intestinal Contractility in Male Wistar Rats

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Abstract

Introduction: The high-fructose diet in rats has been reported to cause metabolic disorders such as impaired fasting glucose levels, insulin resistance, dyslipidemia, and dysregulation of the renin-angiotensin system. This could lead to further complications, for instance, to the smooth muscle dysfunction.

Aim: The present study aimed at developing fructose-induced metabolic perturbations in rats and the investigation of their impact on angiotensin II-induced smooth muscle intestinal motility.

Materials and methods: Mature Wistar rats were randomly divided into two groups (9 rats per group): control group (drinking tap water) and fructose-drinking group (15% fructose, dissolved in tap water). At the end of the experimental period (11 weeks), the plasma levels of insulin, renin, angiotensin II and creatinine, as well as the lipid profile were assessed. Morphometric analysis and lipid index calculation were also performed. The contractile properties of ileum, colon and rectum were studied using stimulation with angiotensin II in the isolated tissue bath system.

Results: Our experiment showed that drinking 15% fructose solution induced dyslipidaemia accompanied by elevated lipid indexes as well as an increase in creatinine and renin plasma levels in the rats.

Conclusions: Fructose drinking and consequently the developed metabolic disorders modified the Ang II-induced intestinal activity causing a gradual alteration in the distal direction with the rectum being the most strongly affected organ.

Keywords

angiotensin II, fructose, intestinal dysfunction, metabolic perturbations

INTRODUCTION

Many studies reveal the fact that long-term fructose over-consumption produces metabolic alterations leading later to prediabetes and metabolic syndrome (MetS).^{1,2} Prediabetes is a condition that often precedes type 2 diabetes and represents an elevation of plasma glucose levels above the normal values but below that of clinical diabetes.³ The MetS is a combination of several clinical symptoms and biochemical changes that in combination increase the risk of developing type 2 diabetes, cardiovascular and other diseases. MetS is diagnosed by the presence of at least three of the following five pathological conditions: abdominal obesity, hypertension, hyperglycaemia, elevated triglycerides and reduced high-density lipoprotein (HDL) cholesterol.⁴ As elevated blood glucose is one of the components of the current definition of both MetS and prediabetes, they can be viewed as closely related to one another.³ The persistent hyperglycemia, with or without insulin resistance, causes an excess production of reactive oxygen species (ROS)^{5,6} and formation of advanced glycation end (AGE) products.^{2,7} Additionally, these factors could contribute to further complications such as the smooth muscle dysfunction. One of the consequences is an impaired gastrointestinal motility, leading to symptoms such as vomiting, abdominal pain, constipation, diarrhoea and faecal incontinence.⁸⁻¹⁰

The high-fructose diet in rats has been used for an induction of MetS or some of its elements as insulin resistance and dyslipidaemia.^{1,5,6} Several studies have established the involvement the overactive renin-angiotensin system (RAS) and the elevated levels of its main effector angiotensin II (Ang II) in insulin resistance and vascular dysfunction in rats on a fructose diet.^{6,11} Furthermore, the RAS blockade has been reported to improve some of the conditions, associated with these metabolic disorders.^{1,11} There is an evidence for a connection between elevated levels of Ang II and the insulin resistance.¹² Moreover, the increased RAS activity is associated with the impaired lipid metabolism, leading to the development of atherosclerosis.¹³ Comparatively, little is known about RAS influence on gastrointestinal tissues, although Ang II has a proven potent effect on the smooth muscles in the digestive tract.¹⁴ Despite the observed development of smooth muscle dysfunction and RAS dysregulation in prediabetes and MetS, the net effect of Ang II influence on the intestinal activity in these metabolic disorders is not clear.

AIM

The aim of this study was to investigate the impact of fructose consumption and the metabolic disorders developed as a consequence on the intestinal response to Ang II stimulation of Wistar rats.

MATERIALS AND METHODS

Laboratory animals and procedures

All experiments were carried out according to the guidelines of the Council Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. The animal experiments were approved by the Ethics Committee at the Medical Faculty of Trakia University, Stara Zagora.

Ten-week-old male Wistar rats, weighing around 250 g were housed in polycarbonate conventional cages 480×375×210 mm with floor area 1500 cm² (three animals per cage) in controlled conditions (12 h light/dark cycles), a temperature of 18–23°C and humidity of 40%–60%. After one-week acclimation period, two groups were formed (9 rats per group): a control group (animals drinking tap water) and experimental fructose-drinking group (animals drinking 15% fructose dissolved in tap water and injected after 14 days with a single streptozotocin dose of 20 mg/kg via intra-peritoneal injection). The duration of the experiment was 11 weeks. The design of the experiment was based on Sadeghi et al.¹⁵ and Wilson and Islam¹⁶ with some modifications. During the experimental period, body weight (BW), waist circumference (WC) and naso-anal length (NAL) of the animals were monitored weekly. The fasting levels of blood glucose were monitored weekly using a GlucoDr™ Plus Glucometer (Allmedicus, Anyang, Gyeonggi-do, Republic of Korea). Blood samples were collected from the tail vein in early morning hours.

Experimental protocols

At the end of the experimental period, the overnight fasted animals were anesthetized with 50 mg/kg of pentobarbital sodium (Nembutal[®]) intraperitoneally and then exsanguinated. Fresh blood (8–10 ml) was collected in EDTA-containers directly from the heart. The blood samples were centrifuged at 4000×g at 4°C for 10 minutes. The plasma samples were separated and used immediately for assays of: triglycerides (TG), total cholesterol (CHOL), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, creatinine, Ang II, renin, and insulin.

The abdominal cavity was opened, and segments from ileum, colon and rectum were dissected out. The ileal segment was taken from 1 cm proximal to the ileo-cecal valve; the colon segment - from the middle part; the rectum - from the most distal part. Residual contents in the lumen were gently cleared using Krebs solution. The isolated segments were promptly transferred to cold Krebs solution (3°C) with the following composition (in mM): NaCl, 118.0, KCl, 4.74, NaHCO₃, 25.0, MgSO₄, 1.2, CaCl₂, 2.0, KH₂PO₄, 1.2 and glucose, 11.0. The preparation of the intestinal strips and the recording of the mechanical activity were performed as previously described.¹⁷ In brief, longitudinal strips (approximately 10 mm long) were dissected

following the direction of the muscle bundles. Both ends of each strip were tied with ligatures. The distal end was connected to the organ holder; the proximal end was stretched and attached to a mechano-electrical transducer FSG-01 (Experimetria, Ltd., Hungary) via a hook. The preparations were mounted in organ baths TSZ-04/01 (Experimetria, Ltd., Hungary), containing Krebs solution, pH 7.4, continuously infused with Carbogen (95% O₂, 5% CO₂). The organ baths were mounted in parallel above an enclosed water bath, thus maintaining the solution temperature at 37°C constantly. Strips were placed under an initial tension (preload) of 1 g and allowed to equilibrate for at least 75 min (three periods: 15 min, 45 min, and 15 min and two washes with Krebs solution between them).

Biochemical laboratory analyses

TG, CHOL, HDL cholesterol, LDL cholesterol, and creatinine concentration were determined using standard methods on a Mindray BS-300 analyser (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China). Plasma insulin was measured with a rat enzyme immunoassay kit (Insulin Rat ELISA Wide range Kit, BioVendor, Czech Republic). Ang II and renin plasma levels were determined using Elabscience ELISA Kits (Elabscience Biotechnology Inc., USA) following the manufacturer's protocol. Plasma concentrations of both glucose and insulin were used for the calculation of the insulin resistance index (homeostasis model assessment [HOMA]-IR) according to the formula^{18,19}: fasting insulin (μU/mL) × fasting glucose (mmol/l)/22.5

Lipid indexes calculation

To verify the metabolic disturbances, we calculated the following comprehensive lipid indexes²⁰: the CHOL/HDL ratio, TG/HDL ratio, the lipoprotein combine index (LCI) = CHOL × TG × LDL/HDL, and the atherogenic index (AI) = non HDL/HDL ratio

Morphometric analysis

The following morphometric indexes were calculated:

Lee index²¹ = the cubic root of BW (in grams) divided by the NAL (in mm) × 104

Waist-to-stature ratio²² = WC (in mm) divided by NAL (in mm)

Analysis of the Ang II-induced intestinal activity

After the equilibration period, the intestinal preparations (n=9, per each segment, for each group) were treated with Ang II (1 μmol). The mechanical activity was digitized and recorded using ISOSYS-Advanced 1.0 Software (Experimetria, Ltd., Hungary). Data processing and storage for

subsequent analysis were performed with the specialized software KORELIA.^{23,24}

The duration of the interval for analysis of Ang II-induced intestinal contractions was measured from the beginning of the contraction until the moment at amplitude drop to 50% of its maximum. This definition was made in order to calculate uniformly the variations in the duration of the contractions. The recorded force versus time graphs allowed the determination of the amplitude and the integral force of the contraction (the latter represented by the area under the curve, AUC). The different phases of Ang II-induced intestinal contractions were analysed by the application of a time-parameter analysis²⁵, modified for the study of the smooth muscle contractions.²⁶ The following time-parameters were defined: half-contraction time (Thc): the time interval between the beginning of the contraction and half of its maximal force; contraction time (Tc): the time interval between the beginning of the contraction and its maximal force; half-relaxation time (Thr): the interval between reaching the maximal force until the amplitude fell by half; contraction plus half-relaxation time (Tchr): the interval from the beginning of contraction until the moment at which the amplitude drops to 50% of its maximum.

Chemicals and drugs

Ang II (Sigma-Aldrich, Germany) was dissolved in bidistilled water. Streptozotocin (Sigma-Aldrich, Germany) was dissolved in cold 0.1 M citrate buffer, pH 4.5. The fructose solution was prepared by using 150 g of fructose (Totem DD, Stara Zagora, Bulgaria) dissolved in one litre of drinking water. All reagents for the preparation of Krebs solution were purchased from Sigma-Aldrich, Germany.

Statistical analysis

The obtained data were analysed using Statistica software v. 8 (Stasoft, Inc., Tulsa, OK) and the results are presented as mean ± standard error (SE). The obtained values were compared by Student *t*-test. A *p* value less than or equal to 0.05 was considered to be statistically significant.

RESULTS

Plasma glucose and insulin levels

During the experimental period, the fasting blood glucose levels in the fructose-drinking group were significantly higher than those of the control group, but without hyperglycaemia (*p*<0.05) (Fig. 1).

There was no significant difference between the plasma insulin levels of the two groups and the calculated HOMA-IR, even though there was a tendency for elevation (*p*>0.05) (Fig. 2).

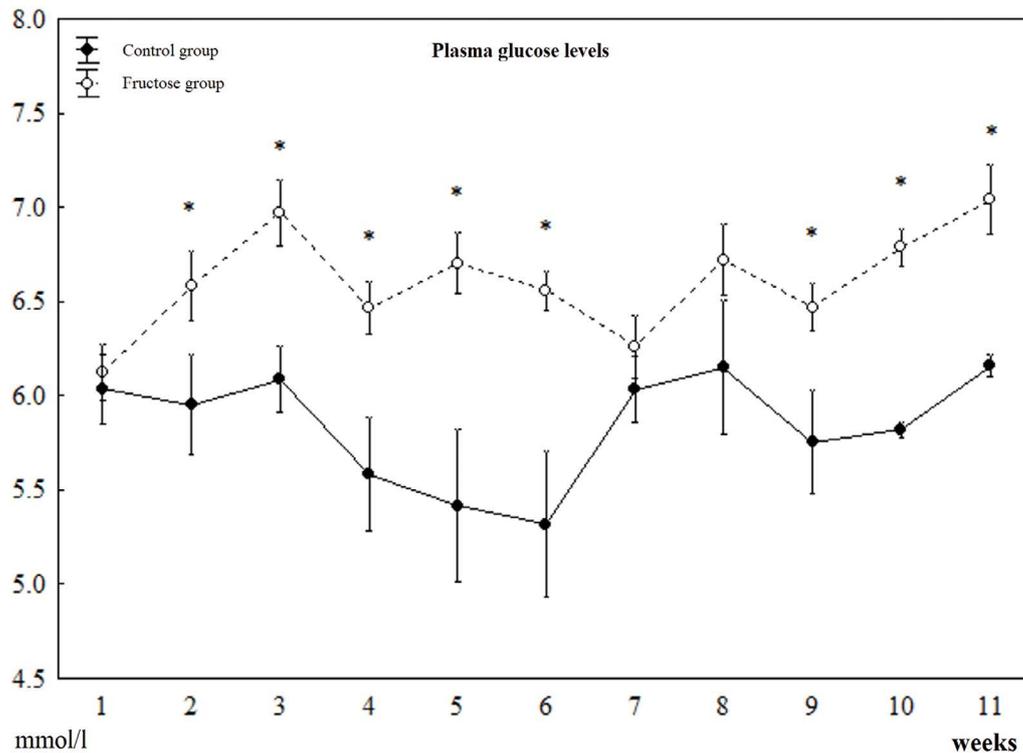


Figure 1. Fasting blood glucose levels monitoring weekly during the experimental period.

*vs. controls, $p < 0.05$

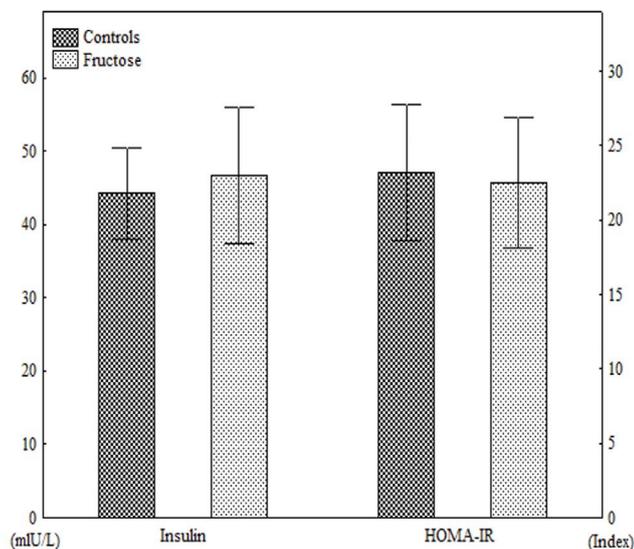


Figure 2. Plasma insulin concentration and HOMA-IR at the end of the experiment.

Lipid profile and creatinine levels

There were significant differences between the plasma lipids of the two groups, with the exception of the total

cholesterol. The fructose drinking induced a significant increase in TG, with concentration twice as high as that of the control group. Creatinine concentrations were significantly higher in the fructose-drinking group ($p < 0.05$) (Table 1).

Table 1. Lipid profile and plasma creatinine levels at the end of experiment

Parameter	Control group	Fructose-drinking group
CHOL (mmol/l)	1.33±0.04	1.24±0.08
LDL (mmol/l)	0.43±0.04	0.28±0.05*
HDL (mmol/l)	0.54±0.02	0.43±0.04*
TG (mmol/l)	0.81±0.06	1.73±0.36*
Creatinine (μmol/l)	51.22±1.85	66.25±2.20*

*vs. controls, $p < 0.05$, TG: triglycerides; CHOL: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol.

All calculated lipid indexes (Table 2) were higher in the fructose-drinking group, showing a statistically significant difference with the indexes of the control group ($p < 0.05$), with the exception of LCI which displayed only a tendency for an increase.

Table 2. Lipid indexes at the end of experiment

Parameter	Control group	Fructose-drinking group
Total cholesterol/HDL ratio	2.51±0.08	2.92±0.14*
Triglycerides/HDL ratio	1.54±0.13	3.74±0.6*
LCI	0.86±0.25	1.15±0.6
AI	1.70±0.06	2.18±0.15*

*vs. controls, $p < 0.05$, HDL: high-density lipoprotein cholesterol; LCI: lipoprotein combine index; AI: atherogenic index

Plasma renin and angiotensin II concentrations

There were significant differences between the plasma levels of renin of the two groups ($p > 0.05$) (Fig. 3).

Morphometric analysis

The morphometric analysis (Table 3) showed no statistically significant differences between the two study groups ($p > 0.05$) and all of the investigated parameters had similar values.

Table 3. Morphometric analysis at the end of experiment

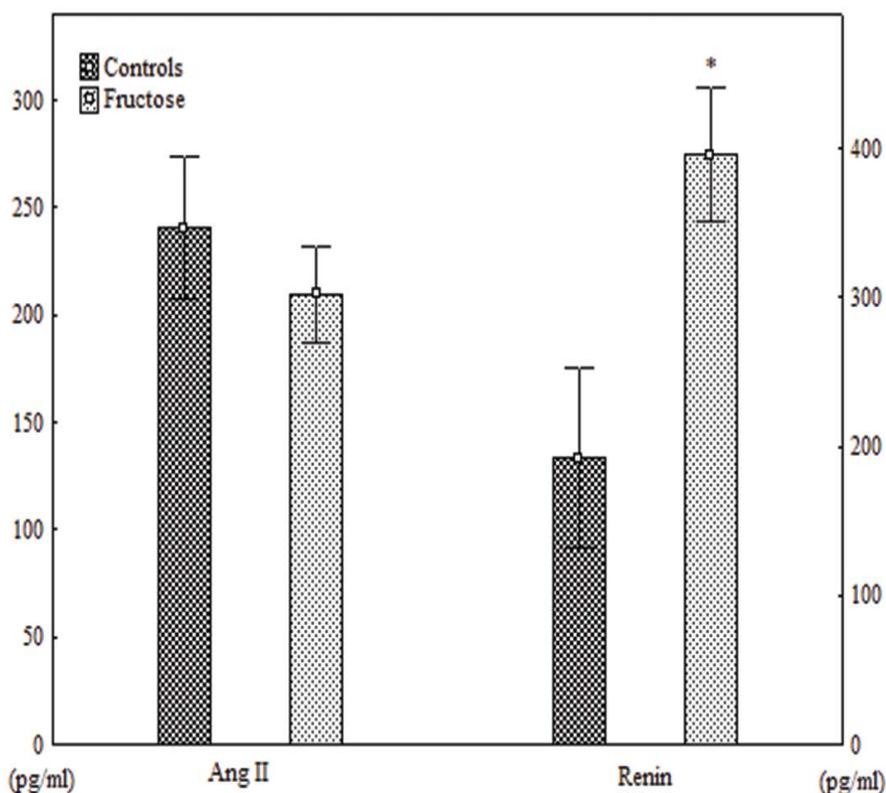
Parameter	Control group	Fructose-drinking group
BW (g)	336.67±12.86	338.89±15.11
WC (mm)	198.89±3.31	203.89±3.89
NAL (mm)	225.56±2.56	225.00±2.04
Waist-to-stature ratio	0.88±0.01	0.91±0.02
Lee Index	308.04±1.55	309.33±3.49

* vs. controls, $p < 0.05$, BW: body weight; WC: waist circumference; NAL: naso-anal length

Ang II-induced intestinal contractions

A graphical visualization of the average contractile process of Ang II-induced smooth muscle activity of the intestinal samples ($n=9$, per each segment, for each group) from both groups is presented in Figs 4-6. The amplitude of the contractions of the ileum from the fructose drinking group was similar to that of the controls (Fig. 4).

There was no significant difference in the AUC ($p > 0.05$) of both groups (232.1 ± 34.5 g*s controls vs. 271.5 ± 38.9 g*s experimental). The investigation of time parameters demonstrated a significantly faster initiation, followed by

**Figure 3.** Plasma renin and angiotensin II levels at the end of experiment.

*vs. controls, $p < 0.05$

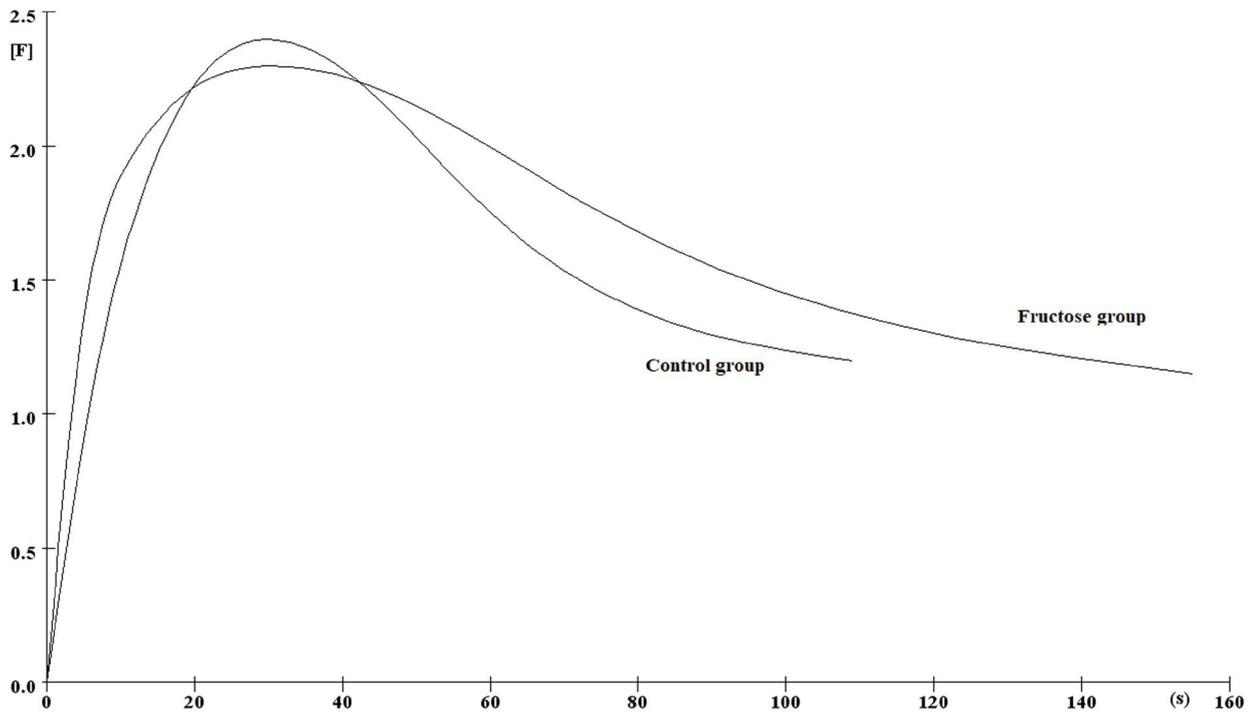


Figure 4. Graphic image of the average contractile pattern of angiotensin II-induced contractions of ileum strips ($n = 9$ for each group) from control and fructose-drinking group.

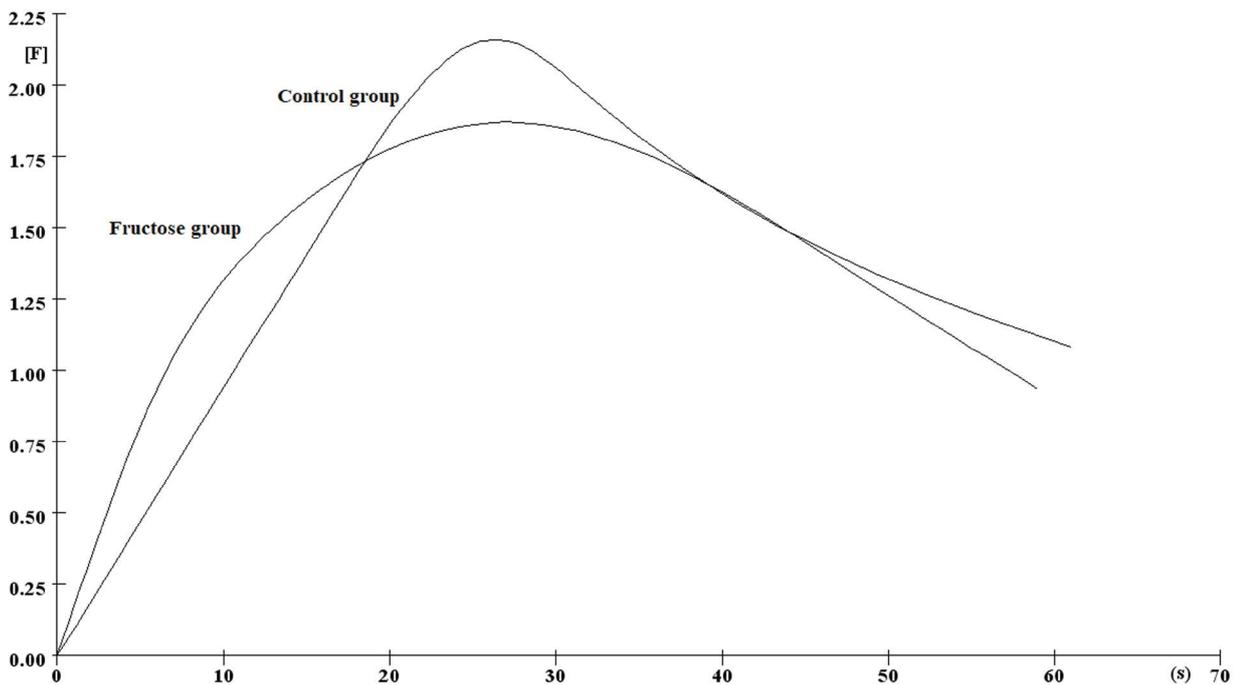


Figure 5. Graphic image of the average contractile pattern of angiotensin II-induced contractions of colon strips ($n = 9$ for each group) from control and fructose-drinking group.

a prolonged duration of the contraction of the ileum from the fructose-drinking group (**Table 4**).

The amplitude of the contractions of the colon (**Fig. 5**) from the fructose-drinking group (1.87 ± 0.22 g) was lower, but not statistically significant compared with the con-

trol one (2.16 ± 0.21 g), $p > 0.05$. There was no significant difference in the AUCs of the two groups as well (91.8 ± 19.3 g*s vs. 83.7 ± 17.9 g*s, $p > 0.05$). The time parameters of colon contractions (**Table 4**) were similar in both groups, with the exception of T_{hc} of the fructose-drinking group,

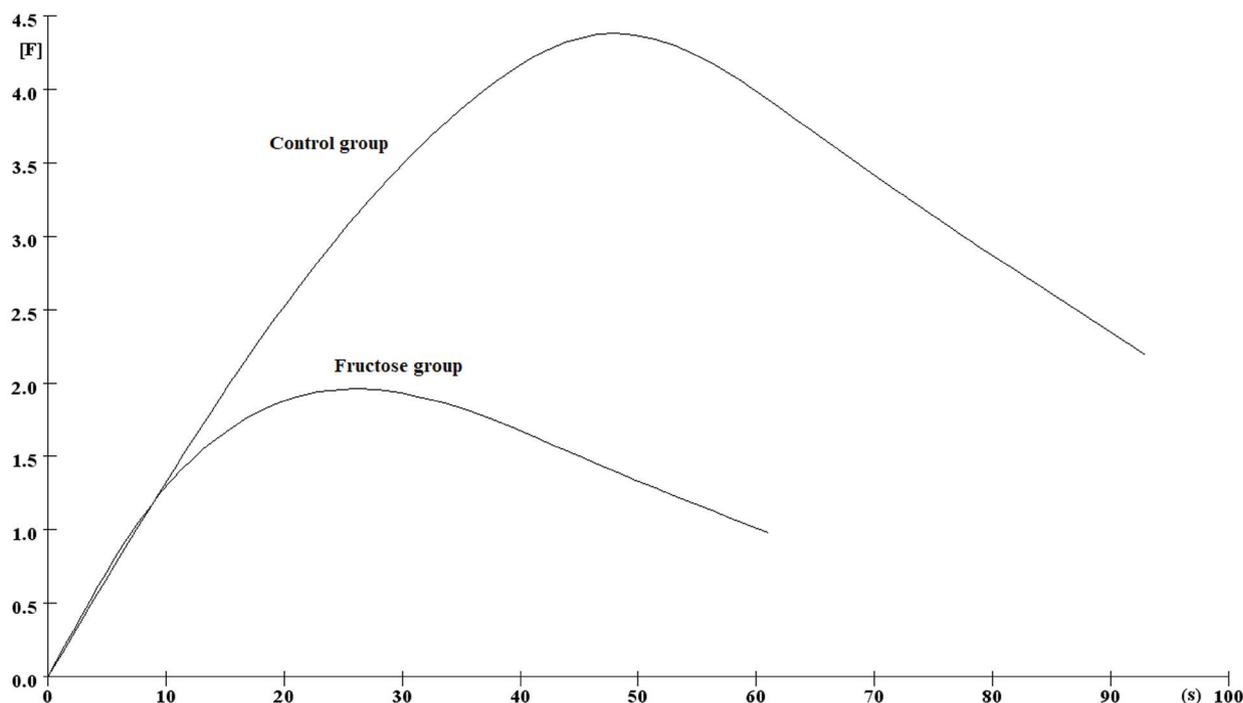


Figure 6. Graphic image of angiotensin II-induced contractions of rectal strips (n = 9 for each group) from control and fructose-drinking group.

Table 4. Time-parameters of angiotensin II -induced smooth muscle contractions

	Thc (s)	Tc (s)	Thr (s)	Tchr (s)
Ileum_C (n = 9)	7.3±1.25	30.25±5.3	78.8±7.3	109±8.3
Ileum_F (n = 9)	4.3±0.77*	29.6±4.1	126.1±19.2*	155.7±27.2*
Colon_C (n = 9)	11.5±1.5	26.5±5.5	34.5±4.8	61±8.8
Colon_F (n = 9)	5.75±0.48*	27.25±3.4	31.8±5.2	59±8.2
Rectum_C (n = 9)	17±2.8	45±6.5	48±5	93±11.1
Rectum_F (n = 9)	7.2±1.02*	26±4*	34.8±5.9*	60.8±7.9*

* vs. controls, $p < 0.05$, C: control group; F: fructose-drinking group; Thc: half-contraction time; Tc: contraction time; Thr: half-relaxation time; Tchr: contraction plus half-relaxation time.

demonstrating a faster initiation of the contraction.

Regarding rectum, both the amplitude and AUC of the contractions from the fructose-drinking group were significantly lower ($p < 0.05$) when compared with those of the controls (1.96±0.13 g vs. 4.38±0.56 g and 73.5±10.9 g*s vs. 274±22.7 g*s, respectively) (Fig. 6). There was a significant difference between all of the time-parameters of the two groups ($p < 0.05$) (Table 4), indicating both faster initiation and completion of the rectal contraction from the fructose-drinking animals.

DISCUSSION

The present study aimed at developing fructose-induced metabolic disorders in rats and the investigation of their impact on Ang II-induced smooth muscle intestinal motility.

Generally, the metabolic perturbations in fructose-drinking rodents are divergent depending on the used rats, the concentration of the fructose solution and the duration of the experiment.¹⁵ The results of our experiment showed that drinking of 15% fructose solution for 11 weeks in addition to a single treatment with low dose streptozotocin induced moderate metabolic perturbations, mainly in the lipid profile of the rats. These results are consistent with the findings of other researchers, about the elevated serum lipids as a consequence of the fructose in food diet^{1,5,6} or in drinking water.^{16,27} The lipid indexes, which were currently considered as more significant markers for increased metabolic risk²⁰, were especially indicative. In our experiment, however, the rats in fructose group did not become obese as we did not observe any differences in BW, WC and the other morphometric parameters between the two groups. Our results are consistent with the findings of many au-

thors^{1,18,28,29} who reported that the fructose diet did not induce an increase in body weight of the rats. The impact of fructose on weight and fat gain is still debatable.²⁹ Probably, to induce such weight gain, it was necessary to use a higher amount of fructose alone or in combination with high fats/glucose diet and to increase the experiment duration.

The mild changes in fasting glucose levels in fructose-drinking group did not affect significantly the plasma insulin levels and HOMA-IR indexes, suggesting the lack of insulin resistance. Similar findings have been reported by other authors.^{18,29} There is evidence that even the low fructose consumption affects the pancreatic islets without altering the serum insulin concentrations.²⁹

The significant increase of plasma creatinine concentration in the fructose-drinking group indicated a starting damage of the kidney functions. Similar results were described by Gersch et al.³⁰ who reported an acceleration of chronic kidney disease in rats on high fructose diet. The higher plasma renin levels after 11-week period of drinking fructose is in agreement with the hypothesis of the hyperactivity of RAS in both diabetes and MetS.^{1,11} It was further supported in the current study by the measured alterations of intestinal response to Ang II. This reveals a possible impact of fructose and the fructose drinking-induced metabolic perturbations on the smooth muscle properties. The three types of tested intestinal segments - ileum, colon and rectum, did not show an equal change in their response to Ang II when compared to the controls. From our results, we could conclude that the changes in the activity of ileum are still at an early stage of development as the only significant difference between the two groups is in the duration of their contraction. Regarding the large intestine, a significant worsening of the parameters of Ang II-induced contraction could be noted. Obviously the metabolic disorders gradually alter the intestinal motility in the distal direction of the gut with rectum being the most seriously affected organ. Our findings are consistent with Touw et al.³¹, who had reported a diminished motor activity of the large intestine in diabetic mice.

The role of oxidative stress in the pathogenesis of diabetes and in the development of consequent gastrointestinal complications is well recognized.^{9,32} The increased formation of ROS causes loss of enteric neurons, which in turn could lead to gastrointestinal dysfunction.¹⁰ It is the most likely cause for the reduced response to Ang II stimulation in the large intestine segments in the fructose-drinking group. The findings of a previous study of our research group³³ are in support of this hypothesis: we found elevated levels of ROS and other markers of oxidative stress in tissue homogenates from diabetic rats. Furthermore, it was found that the large intestine is more sensitive to the oxidative stress caused by diabetes when compared to the small intestine.³⁴ Another likely explanation for the reduced rectal contractile activity might be the accumulation of AGE products in the smooth muscle cells as a result of the increased fructose consumption.² The upregulated AGE products expression and the proliferation of the muscle layer in different

gastrointestinal segments of diabetic rats^{7,35} add support to this hypothesis. The accumulation of AGE products might be a factor for the structure remodeling and the change in the contractile properties of the intestinal smooth muscle.

CONCLUSIONS

Drinking 15% fructose solution for 11 weeks combined with a single 20 mg/kg streptozotocin injection induced moderate metabolic changes such as elevation of creatinine levels accompanied by dyslipidaemia and elevated renin levels. The fructose drinking and the subsequently developed metabolic disorders modified the Ang II-induced intestinal activity causing a gradual alteration in the distal direction, with the rectum being the most strongly affected organ. Further studies are needed in order to reveal the exact mechanism of the fructose diet on the RAS and Ang II-induced intestinal motility.

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Competing interests

The authors have declared that no competing interests exist.

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Нарушения обмена веществ, вызванные питьевой водой с фруктозой, влияют на опосредованную ангиотензином II сократимость кишечника у самцов крыс линии Wistar

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Резюме

Введение: Однозначно установлено, что диета с высоким содержанием фруктозы у крыс вызывает метаболические расстройства, такие как нарушение уровня глюкозы натощак, инсулинорезистентность, дислипидемия и нарушение регуляции ренин-ангиотензиновой системы. Это может привести к дополнительным осложнениям, таким как дисфункция гладких мышц.

Цель: Настоящее исследование направлено на провоцирование метаболических нарушений, вызванных фруктозой, у крыс и изучение их влияния на перистальтику гладких мышц кишечника, индуцированную ангиотензином-II.

Материалы и методы: Взрослых крыс линии Wistar случайным образом разделили на две группы (по 9 крыс в группе): контрольную группу (принимающие питьевую воду) и группу, получавшую фруктозу (15% фруктозы, растворенной в питьевой воде). В конце экспериментального периода (11 недель) оценивали уровни инсулина, ренина, ангиотензина-II и креатинина в плазме, а также липидный профиль. Также были выполнены морфометрический анализ и расчёт липидного индекса. Сократительные характеристики подвздошной, ободочной и прямой кишки исследовали с помощью стимуляции ангиотензином-II в ванне с изолированными тканями.

Результаты: Наш эксперимент показал, что употребление 15% раствора фруктозы вызывало дислипидемию, сопровождающуюся повышенными липидными индексами, а также повышением уровня креатинина и ренина в плазме у крыс.

Заключение: Потребление фруктозы и последующее развитие метаболических нарушений изменяют индуцированную Анг – II деятельность кишечника, вызывая постепенное изменение дистального направления, и прямая кишка является наиболее поражённым органом.

Ключевые слова

ангиотензин II, фруктоза, кишечная дисфункция, метаболические нарушения