

Appetite–regulating hormones in rats with fructose-induced metabolic changes

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Abstract

Objectives: The aim of this research is to examine the effects of fructose-drinking on the plasma levels of appetite-regulating hormones insulin, leptin and ghrelin in male and female rats.

Methods: Mature Wistar rats were divided as follows: two control groups - male (CM) and female (CF); two fructose-drinking groups - male (FDM) and female (FDF), received 15% fructose solution. The experiment lasted 11 weeks. At the end, insulin, leptin and ghrelin levels as well as lipid and glucose profile were assessed.

Results: Plasma concentrations of the examined hormones were elevated in fructose-drinking groups. However, in the FDM group only the leptin levels were significantly increased compared to the control. In the FDF group, all three appetite-regulating hormones showed the highest concentrations in comparison to the other groups.

Conclusion: Sex hormones may affect the appetite-regulation signals and could be a factor contributing to degree of metabolic changes caused by long-term fructose overconsumption.

Keywords

Appetite, Fructose, Ghrelin, Insulin, Leptin

Introduction

Widespread overconsumption of sugars and of fructose, in particular, has significantly increased in present times. This higher dietary intake of fructose has been suggested to contribute to the world epidemic of metabolic syndrome (MetS), which includes the following clinical symptoms and biochemical changes: impaired glucose tolerance, hypertension, dyslipidemia, and central obesity

(Tranchida et al. 2012). The exact mechanisms by which fructose overconsumption is involved in the development of MetS are not fully understood. It has been reported that regular consumption of fructose promotes obesity and lipid metabolism disorders by appetite stimulation (Sadowska and Rygielska 2019). It is well known that the control of food intake and energy balance is achieved by different signals, with central or peripheral origin, acting on specialized brain areas, located predominantly in the

hypothalamus (Lindqvist et al. 2008; Nabil et al. 2020). Among the most important factors, responsible for appetite regulation and the maintenance of body weight are the peptide hormones insulin, leptin and ghrelin (Melanson et al. 2007).

Leptin, recognized as anorexigenic hormone, is produced mainly by the adipose tissue and the gastric mucosa. It regulates energy balance by producing satiety and stimulating energy expenditure, thus having a role in weight control (Melanson et al. 2007; Mendoza-Herrera et al. 2021). Ghrelin is an orexigenic hormone, secreted by the enteroendocrine cells of the stomach, and is involved in the feeling of hunger, increased food intake and weight gain (Melanson et al. 2007). Besides its other functions, insulin suppresses the appetite by direct action on the brain or through its stimulation on leptin secretion (Melanson et al. 2007) and its indirect inhibitory effect on ghrelin activity (Colquitt 2011). Usually, the levels of blood glucose, insulin, and leptin are inversely related to circulating ghrelin levels, depending on the feeding or fasting state (Melanson et al. 2007).

A better understanding of the role of fructose overconsumption on obesity can be achieved by studying the effects that fructose elicits on appetite-regulating hormones (Colquitt 2011). High-fructose diet has been shown to induce leptin resistance, insulin resistance and hepatic steatosis (Aijälä et al. 2013; Mendoza-Herrera et al. 2021). Additionally, chronic high fructose feeding can result in an elevation of the pre-meal serum ghrelin concentrations. The study by Lindqvist et al (2008) reports that rats fed a fructose solution for two weeks have a significant increase in fasting serum ghrelin concentrations compared to those offered a water solution.

Based on the reported findings, we attempted to establish an experimental model of the features of MetS in rats, using dietary modification with fructose solution. The aim of the present study is to examine whether drinking 15% fructose solution for 11 weeks will affect the appetite-regulating hormones levels (insulin, leptin and ghrelin) in mature male and female rats and additionally, will there be any sex differences.

Materials and methods

Laboratory animals and procedures

All of the 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 Local Commission of Ethics (Medical Faculty of Trakia University, Stara Zagora). The animals were maintained according to the national rules and regulations and the experimental protocol was approved by the BFSa Ethics Committee (Statement of the National Ethic Commission №53/ 23.06.2016 and Ethical approval number 137 – A/ 23.03.2016).

Ten-week-old male and female Wistar rats, weighing 190–260 g, were housed two per cage in polycarbonate

wire floor cages in controlled conditions (12 h light/dark cycle, a temperature of 18–23 °C and humidity of 40–60%). After one-week acclimatization period, the animals were randomly divided into four weight-matched groups (n=6/group): two control groups - male (CM) and female (CF) rats, received tap water to drink, and two fructose-drinking (FD) groups - male (FDM) and female (FDF) rats, received 15% fructose solution. Additionally, the rats from the FD groups were injected after 14 days of the beginning of the experiment with a single streptozotocin dose of 20 mg/kg via intra-peritoneal administration. The controls received injection of saline solution. The design of the experiment was, according to Sadeghi et al. (2017) and Wilson and Islam (2012), with some modifications. The duration of the experiment was 11 weeks. During the experimental period body weight, naso-anal length and waist circumference of the animals were monitored weekly.

At the end of the experimental period, the overnight fasted animals were anesthetized with pentobarbital sodium (Nembutal) 50 mg/kg i.p. and exsanguinated. Fresh blood (8–10 ml) was collected directly from the heart in EDTA-containers. 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, glucose, insulin, leptin and ghrelin.

Biochemical laboratory analyses

Glucose, TG, CHOL, and HDL cholesterol concentrations were determined by using standard methods on a Mindray BS-300 analyser (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China). Plasma insulin, acylated ghrelin and leptin levels were determined using enzyme-linked immunosorbent assay kits (ELISA) from BioVendor R&D (BioVendor Laboratory Medicine, Inc., Czech Republic) 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:

$$\text{Fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mmol/l)} / 22.5$$

(Chao et al. 2018)

For verification of the metabolic disturbances, the following comprehensive lipid indexes (Cai et al. 2017) were calculated: CHOL/HDL ratio, TG/HDL ratio and lipoprotein combined index (LCI) = CHOL × TG × LDL/ HDL

Morphometric analysis

During the experimental period body weight, naso-anal length and waist circumference of the animals were monitored weekly. At the end of the experimental period the following morphometric indexes were calculated:

Lee index = the cubic root of body weight (in grams) divided by the naso-anal length (in mm) × 10⁴, according to Li et al. (1997).

Waist-to-length ratio = waist circumference (in mm) divided by naso-anal length (in mm), according to Egbunu and Ezeanyika (2012).

Statistical analysis

Obtained data were processed by the statistical program Statistica Version 8 (StatSoft, 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

Figs 1-3 represent the weekly monitored body parameters (once in a week during the whole experimental period). There were no significant differences in body weight between the fructose-drinking groups and the relevant control groups, although there was a tendency to gain weight after the 6th week in the FDM and FDF animals (Fig. 1). Regarding the linear growth (represented by the naso-anal length), there were not significant differences as well (Fig. 2). The waist circumference of the animals from CM and FDM groups also did not differ significantly. In the female groups, after week 6 of the experimental period, waist circumference of the FDF rats was increased, and in week 7 and 8 displayed a significant difference compared to the controls (Fig. 3).

Although being higher in the fructose-drinking animals, the calculated morphometric indices did not show statistical differences compared to the controls (*p* > 0.05), with the exception of the Lee index of the FDF group (Table 1).

Table 1. Morphometric analysis at the end of the experiment.

Parameter	CM	FDM	CF	FDF
Body weight (g)	327.87±7.85	338.89±15.11	249.17±7.12	258.33±4.59
Naso-anal length (mm)	225.56±2.56	225.00±2.04	214.17±2.00	212.50±1.12
Waist circumference (mm)	198.89±3.31	203.89±3.89	177.50±4.23	180.00±1.83
Waist-to-length ratio	0.88±0.01	0.91±0.02	0.83±0.01	0.85±0.01
Lee Index	308.04±1.55	309.33±3.49	293.69±1.15	299.67±1.37*

*vs. control females, *p* < 0.05; CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females

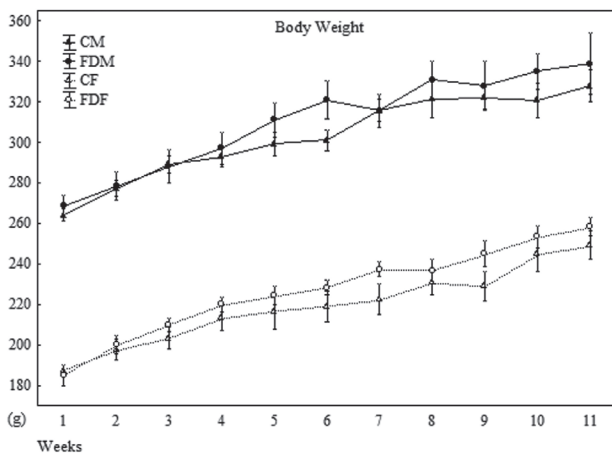


Figure 1. Body weight of the experimental animals, monitored weekly during the experimental period. **Note:** CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

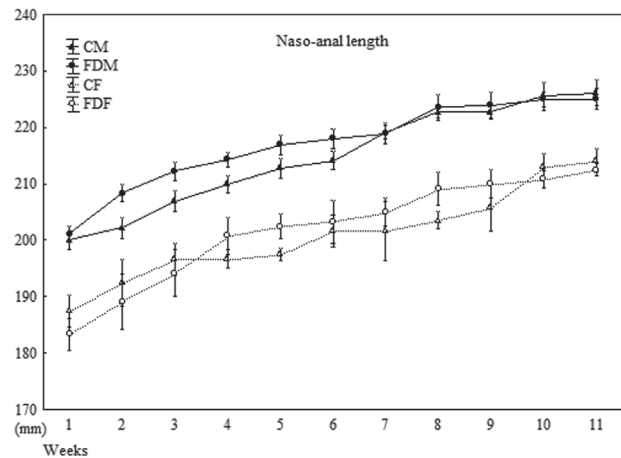


Figure 2. Naso-anal length of the experimental animals, monitored weekly during the experimental period. **Note:** CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

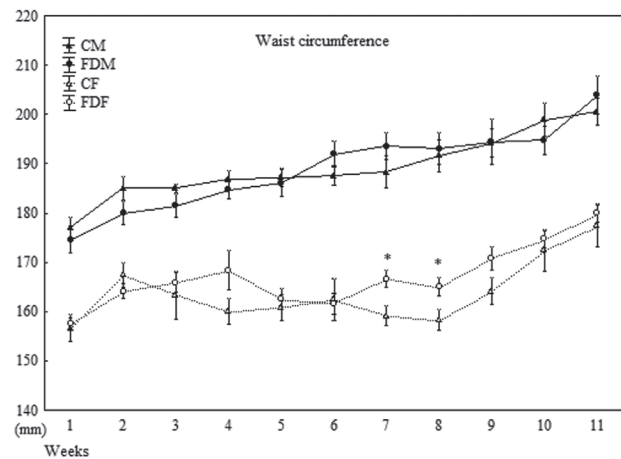


Figure 3. Waist circumference of the experimental animals, monitored weekly during the experimental period. **Note:** *vs. control females, *p* < 0.05. **Note:** CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

Regarding the lipid profile of the experimental animals (Table 2), no statistical differences were observed in the CHOL levels of the groups. The levels of HDL cholesterol of the animals from the FDM group were significantly reduced (*p* < 0.05). Plasma TG levels were elevated in both fructose-drinking groups, compared to the relevant controls (*p* < 0.05, Table 2).

A statistically significant increase in the TG/HDL index was registered in the FDM and FDF groups, compared to

Table 2. Lipid profile at the end of the experiment.

Group	CHOL	HDL	TG	CHOL/HDL	TG/HDL	LCI
CM	1.33 ± 0.04	0.54 ± 0.03	0.81 ± 0.07	2.51 ± 0.08	1.54 ± 0.13	0.86 ± 0.25
FDM	1.24 ± 0.08	0.43 ± 0.04*	1.73 ± 0.36*	2.92 ± 0.14*	3.74 ± 0.60*	1.15 ± 0.06
CF	1.30 ± 0.10	0.59 ± 0.02	0.86 ± 0.11	2.20 ± 0.11	1.49 ± 0.21	0.75 ± 0.15
FDF	1.30 ± 0.10	0.59 ± 0.04	2.75 ± 0.58*	2.21 ± 0.07	4.68 ± 0.92*	2.19 ± 0.07*

*vs. control males, *p* < 0.05, # vs. control females, *p* < 0.05; TG: triglycerides; CHOL: total cholesterol; HDL: high-density lipoprotein cholesterol; LCI - lipoprotein combined index; CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

the control ones. Additionally, the LCI index was elevated in FDF animals and the Chol/HDL index was elevated in FDM rats, compared to the controls (Table 2).

The two fructose-drinking groups showed significant hyperglycemia at the end of the study ($p < 0.05$, Fig. 4), but insulin levels did not statistically differ among the different groups ($p > 0.05$, Fig. 5).

The calculated HOMA-IR index was significantly elevated in both FDM and FDF groups (Fig. 6).

Ghrelin levels were higher in both fructose-drinking groups (FDM group 902.36 ± 20.67 ng/ml and FDF group 992.35 ± 20.88 ng/ml) when compared to the controls (842.32 ± 23.83 ng/ml and 759.82 ± 43.98 ng/ml for CM and CF groups, respectively). A statistically significant increase was detected only in the FDF group ($p < 0.05$, Fig. 7). Both the FDM and FDF groups had significantly elevated leptin concentrations (636.22 ± 94.67 and 1291.21 ± 210.17 ng/ml, respectively), and the FDF group had a more than twofold increase in this parameter, compared to the female control (485.66 ± 64.89 ng/ml), (Fig. 8).

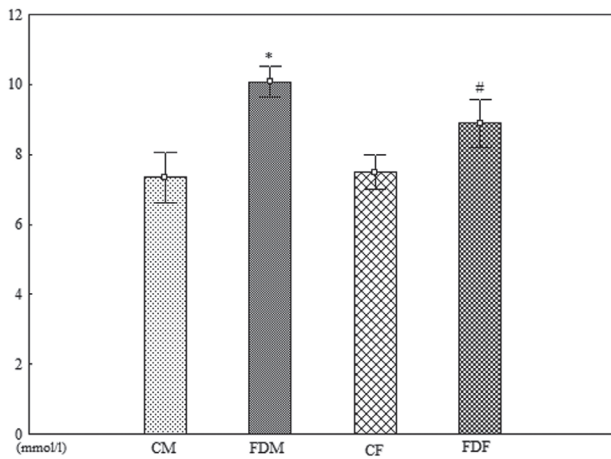


Figure 4. Glucose plasma levels at the end of the experiment. **Note:** *vs. control males, $p < 0.05$, # vs. control females, $p < 0.05$. **Note:** CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

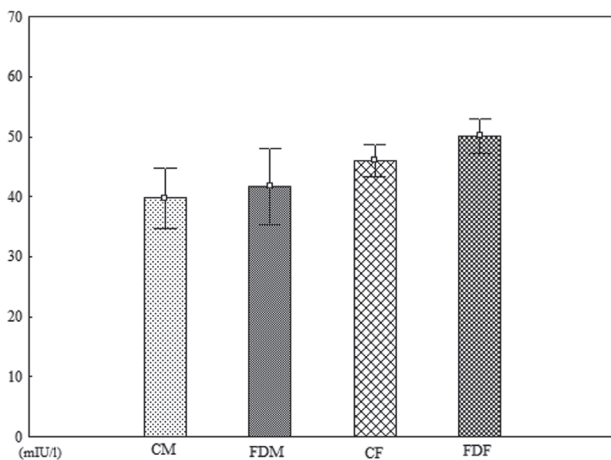


Figure 5. Plasma insulin concentration at the end of the experiment. **Note:** CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

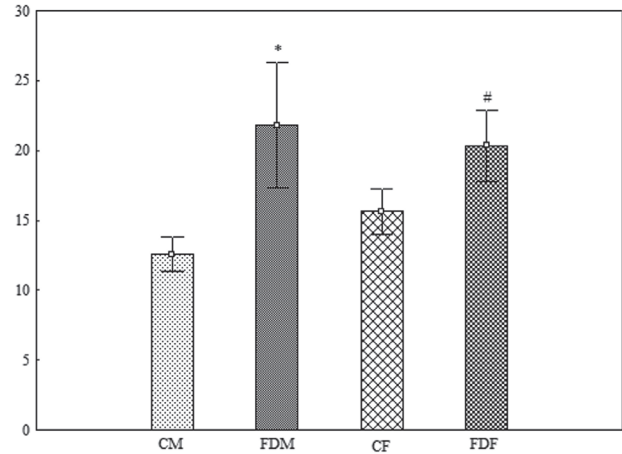


Figure 6. Insulin resistance index (homeostasis model assessment [HOMA]-IR) at the end of the experiment. **Note:** *vs. control males, $p < 0.05$, # vs. control females, $p < 0.05$. CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females

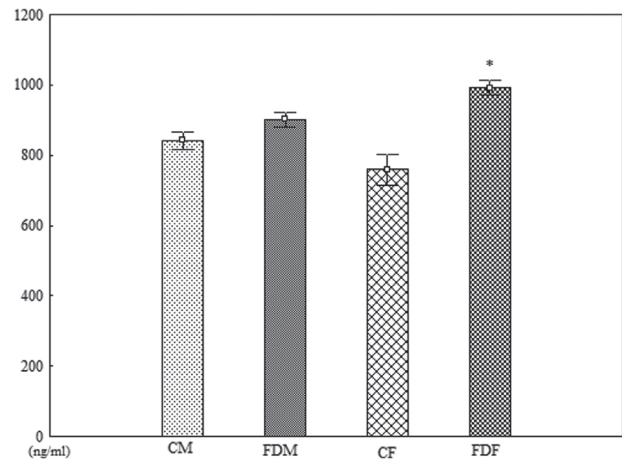


Figure 7. Ghrelin plasma levels at the end of the experiment. **Note:** *vs. control females, $p < 0.05$. **Note:** CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

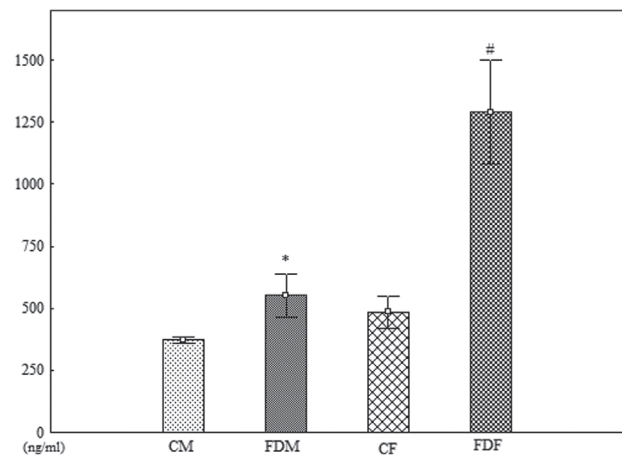


Figure 8. Leptin plasma levels at the end of the experiment. **Note:** *vs. control males, $p < 0.05$, # vs. control females, $p < 0.01$. CM-control males; CF-control females; FDM-fructose-drinking males; FDF-fructose drinking females.

Discussion

In the present study we demonstrate that rats drinking fructose solution for an extended period of time (almost 3 months) in a combination with a single low dose of streptozotocin develop significant metabolic changes such as hyperglycemia, dyslipidemia and insulin resistance. This is in accordance with research by other authors (Sharma et al. 2013; Sadeghi et al. 2017), showing that fructose-rich diet in combination with injection of low doses streptozotocin evokes desirable acceleration of the metabolic changes and leads to type 2 diabetes mellitus. The primary goal of the experiment was to examine whether and how plasma levels of the appetite-regulating hormones (insulin, leptin, ghrelin) would be affected in this experimental model, and additionally, if there would be any sex differences.

Our results showed that the animals belonging to both fructose-drinking groups did not become obese, as we did not observe significant differences in weight and most other morphometric characteristics between these groups and their relevant controls. This is consistent with findings of other researchers, who also reported that fructose diet did not induce a significant increase in body weight in rats (Alzamendi et al. 2012; Moreno-Fernández et al. 2018; Miranda et al. 2019). The impact of fructose on weight and fat gain is still debatable (Miranda et al. 2019). Generally, in fructose-manipulated rodent models different features of the MetS could be observed, depending on the animals used, the mode of fructose administration (fructose solution for drinking or fructose in the diet) and the duration of the experiment (Wong et al. 2016; Sadeghi et al. 2017). In our study, the fructose-drinking rats developed metabolic disorders, rather than obesity. We could speculate that perhaps in order to induce visible obesity, it is necessary to use fructose in combination with high fats/glucose in the diet and to prolong the experimental duration. However, our results revealed a tendency for weight gaining for both fructose-drinking groups, after the sixth week of the experiment. This was specifically expressed by the increase of in the waist circumference and Lee index of the FDF group, revealing sex-specific dynamics of the studied morphological parameters. Since these parameters correlate with visceral fat accumulation, their elevation in the FDF rats (although not significant) could be accepted as markers, predicting a possible future development of visible overweight and obesity.

Additionally, our results show that the changes resulting from long-term fructose intake affect the lipid profile and blood glucose levels first, and could cause obesity at a much later stage. Food intake, glucose and lipid homeostasis, and ultimately body adiposity, depend on the brain nerve centers that are targets of the circulating insulin, leptin and ghrelin (Nabil et al. 2020). The interactions among these peripheral signals may be a link to the control of eating behavior and energy expenditure (Melanson et al. 2007). There are accumulating data that fructose in diet causes hyperinsulinemia (Alzamendi et al. 2012; Nabil et al. 2020), elevated leptin levels (Lindqvist

et al. 2008; Alzamendi et al. 2012; Nabil et al. 2020) and alterations in ghrelin activity (Teff et al. 2004; Lindqvist et al. 2008; Colquitt 2011; Alzamendi et al. 2012). A study by Aijälä et al. (2013) summarizes that long-term consumption of fructose leads to elevated triglyceride levels, insulin and leptin resistance, thus ultimately causing impaired leptin signaling in the brain. In a study of Nabil et al. (2020) male fructose-drinking rats gained weight and exhibited hyperinsulinemia and hyperleptinemia, but at the same time their energy consumption was similar to that of the controls. The same authors suggest a disrupted connection between the peripheral and central appetite regulatory mechanisms. Moreover, fructose-enriched diet has been shown to induce brain oxidative stress, including hypothalamic inflammation (Nabil et al. 2020; Kovačević et al. 2021), which could further contribute to the impaired signaling.

The elevated plasma levels of insulin, leptin and ghrelin in the fructose-drinking groups of our experiment, are also a sign of a possible affected secretion and disturbed interaction between these appetite-regulating hormones. The FDF group showed higher ghrelin and leptin levels, suggesting that fructose consumption may affect the secretion of these hormones in a sex-dependent manner. It is known that there are sex differences in the prevalence of obesity-related metabolic disorders, and sex hormones have been proposed as regulators of fat distribution, energy homeostasis and plasma insulin and leptin levels (Shi et al. 2009). A study of Natelson et al. (2020) on leptin receptor deficient mice reports that only female ones developed diabetes and displayed diminished vertebral bone quality and alteration in intervertebral discs, suggesting a sex-dependent role of leptin in the pathological changes of the spine in diabetes and obesity. Additionally, it has been hypothesized that the different appetite regulation between males and females is in part due to different postprandial ghrelin levels (Leone et al. 2022). The oral intake of fructose does not suppress orexigenic hormones, which could serve as a possible explanation of the high ghrelin levels detected in the FD groups of our study. Moreover, the insulin resistance may contribute to the potential metabolic effects of fructose consumption. This puts the role of many additional factors (such as body composition, age, sex and insulin resistance), which may be of a significance for the peripheral hormonal responses in the energy homeostasis, appetite regulation and development of obesity (Yunker et al. 2021). In a study of Kovačević et al. (2021), fructose diet caused a greater extent of adipose tissue inflammation and insulin signaling impairment in female than in male rats. Interestingly, fructose overconsumption was not accompanied by obesity, which puts adipose tissue dysfunction at an earlier stage in the development of metabolic disorders and weight gain. In this regard, information on the protective effect of estrogens against the obesogenic effect of fructose still remains debatable. Further studies are required to reveal the possible role of sex hormones for the development of fructose-induced metabolic disorders and obesity.

Conclusion

In conclusion, drinking of 15% fructose solution for 11 weeks combined with a single low dose streptozotocin injection induced metabolic changes (hyperglycemia and disrupted lipid profile) and affected the peripheral appetite-regulating hormonal levels. These alterations precede the development of visible obesity and overweight in rats. The elevation of plasma insulin, leptin and ghrelin concentrations in fructose-drinking rats is likely to be sex-dependent. Probably sex hormones are involved in the shift of appetite-regulation signals and may be of importance for the degree of development of

the metabolic disorders observed in long-term fructose overconsumption.

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Competing interests

The authors have declared that no competing interests exist.

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