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Effect of high- fructose diet on plasma leptin levels, morphological and biochemical parameters in ovariectomized rats

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Leptin, the obese gene product, is an adipocyte-derived hormone that regulates body weight and metabolism by influencing food intake and energy expenditure. Postmenopausal status and high consumption of fructose are associated with increased incidence of visceral obesity and risk of metabolic syndrome, type 2 diabetes and cardiovascular diseases. The main objective of this study was to determine alterations in body weight, abdominal fat mass, plasma leptin levels, serum glucose and lipid profile (total cholesterol, triacylglycerol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol) in ovariectomized rats subjected to a fructose rich diet. Twenty-four adult female Wistar rats were divided into four groups: control sham-operated (SHAM, n = 6), ovariectomized (OVX, n = 6), sham-operated plus fructose (SHAM-F, n = 6) and ovariectomized plus fructose (OVX-F, n = 6). Fructose was given to the rats as 10% solution in drinking water for 8 weeks. The results indicated that plasma leptin levels were increased significantly in OVX and OVX-F groups compared with SHAM and SHAM-F groups (4466,66 ± 179,23 pg/ml and 2758.33 ± 682.47 pg/ml vs. 1965.33 ± 179.23 pg/ml and 1706.50 ± 232.15 pg/ml, respectively). Although leptin levels were not significantly different in the sham-operated and sham-operated plus fructose groups, there is a tendency for lower leptin levels in the sham-operated plus fructose group. The body weight was significantly increased in the OVX, OVX-F and SHAM-F groups compared with the SHAM group. Abdominal adipose tissue and biochemical parameters were significantly higher in OVX and OVX-F compared to those of control animals. In conclusion, excessive consumption of fructose may contribute to metabolic disorders and expressed predisposition to chronic non-infectious diseases observed in the postmenopausal condition. Furthermore, the results showed that a high-fructose diet and ovariectomy affect leptin levels in different directions, which may be due to interference on the various signal-transduction mechanisms in leptin synthesis and secretion.

Key words: leptin, ovariectomy, obesity, metabolic syndrome, high-fructose diet

INTRODUCTION

The lack of ovarian function in the postmenopausal period is associated with a high incidence of visceral type obesity and several metabolic disorders [1]. Estrogen deficiency has been proposed as an important obesity-triggering factor [2]. Experimental studies in rodents and humans support the involvement of estrogen in the control of energy balance [3], food intake [4], and body fat distribution [5]. In ovariectomized rodents – an animal model used to study human menopause – higher body weight and visceral adipose tissue have been observed than in sham-operated rodents. These ovariectomy disorders have been reversed by estradiol administration [6]. Postmenopausal women who have low levels of estrogen showed a significant increase in waist-to-hip ratio compared with postmenopausal women receiving hormone replacement therapy [7].

Together with changes in fat distribution and metabolic disorders, the lack of estrogen is accompanied by abnormalities in the synthesis and signaling pathways of a number of peripheral peptides which participate in the regulation of energy homeostasis, such as leptin. Leptin is a 16-kDa peptide hormone that is secreted mainly by adipose cells [8]. It reduces feeding and increases energy expenditure by acting at sites primarily within the central nervous system [9], where it binds to leptin receptors (OB-Rs).There are multiple OB-Rs isoforms, all of which are products of a single OB-Rs gene, and it is thought that a long form of leptin receptor (OB-RL) is mainly responsible for regulating energy balance [10]. An intimate relationship between leptin and estrogen was described in many in vitro and in vivo studies, demonstrating a direct effect of estrogen on leptin expression [11] and leptin signaling in the brain [12].

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In recent decades, there is a tendency to increase energy intake due to the widespread use of high-fructose corn syrup in beverages. High fructose consumption has been linked to the development of insulin resistance, dyslipidemia and visceral obesity [13]. An animal study has observed that dietary fructose leads to a decrease in serum leptin levels, increased food intake and weight gain [14].
Accordingly, the objective of this study was to investigate the effect of a high-fructose diet on weight gain, abdominal fat mass, lipid profile, serum glucose level and plasma leptin concentration in ovariectomized and sham-operated rats.

MATERIALS AND METHODS

Experimental Animals.

Female Wistar rats weighing 190 – 220 g were studied. The rats were kept in the accredited vivarium of the Medical Faculty, Trakia University at 25±1 °C with a light/dark photoperiod of 12/12 hours and free access to chow diet and water for a week before the study.

Experimental design.

The twenty-four experimental animals were randomly divided into two groups: ovariectomized group (n=12) and sham-operated-group (n=12). The ovariectomy procedure was performed by a dorsal midline skin incision through which the ovaries were bilaterally clamped and removed. The uterine horns were tied and the uterus was left intact. In the sham-operated group, the ovaries were exteriorized to create similar stress, but were not clamped and removed. After surgery, the animals were allowed to recover for 4 weeks. After recovery, the ovariectomized and sham-operated groups were each subdivided into two new groups, based on the type of diet: control sham-operated (SHAM, n=6), sham-operated plus fructose (SHAM-F, n=6), ovariectomized (OVX, n=6) and ovariectomized plus fructose (OVX-F, n=6). During the 8-week period, all the rats were fed with a standard diet; in addition, the fructose groups received a 10% solution of fructose in drinking water. Body weight was measured at the end of the recovery period and at the end of every two weeks during the experiment. At the end of the experiment, the rats fasted overnight were anesthetized with a mixture of ketamine - xylazine (90 and 10 mg/kg, respectively, i.p.). Blood samples were collected for serum and plasma separation by abdominal aorta puncture. The mesenteric (MAT), retroperitoneal (RAT) and subcutaneous (SAT) adipose tissue were dissected and externalized. The fat was weighed immediately after dissection in order to avoid weight loss by evaporation.

The experiment was conducted in compliance with the requirements of national legislation and the European Directive 2010/63/EU of 22.09.2010 on the protection of animals used for scientific purposes.

Determination of glucose and lipid profile.

Glucose and lipid parameters (triglycerides, total cholesterol, HDL- and LDL-cholesterol) in serum were examined using an automatic analyzer Mindray BS300.

Determination of leptin levels.

All samples were centrifuged and the plasma was stored at -20 °C until analysis. Levels of plasma leptin were assessed using ELISA kit (BioVendor Mouse and Rat Leptin ELISA kit) according to the manufacturer's instructions.

Statistical analysis

The data were analyzed using the Student's t-test on Statsoft Statistica v.8. Results were expressed as mean ± standard deviation (SD) and values of $p > 0.05$ were considered statistically insignificant, while those of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Body weight and adipose tissue. Figure 1 shows the changes in body weight of the different groups for the duration of the study. As expected, after recovery for 4 weeks the OVX rats exhibited the highest body weight. This is consistent with other clinical [15] and experimental studies [6] which demonstrate that lower levels of estrogen after ovarian function failure are associated with weight gain (Fig. 1).

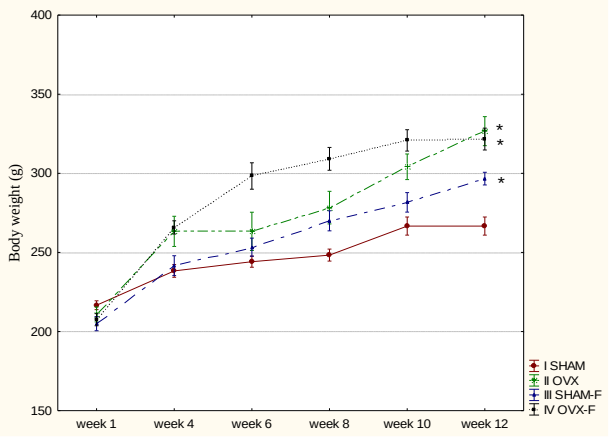

Fig. 1. Changes in body weight of different studied groups for the duration of the study, *p < 0.05.

After the 8-week feeding period with 10% fructose solution, a statistically significant difference ($p < 0.05$) was found between the mean body weight of the OVX, OVX-F and SHAM-F groups compared with the SHAM group. Mesenteric and retroperitoneal fat were significantly increased in the OVX and OVX-F groups compared with the SHAM group. Moreover, the OVX-F group showed a significant increase in MAT when compared with the SHAM-F group; while retroperitoneal fat depot weight was significantly higher in the OVX group that in the SHAM-F group (Table 1).

Bocarsly et al. [16] observed such changes in body weight and fat depot weight after 7 months of a high-fructose diet in female rats with intact ovarian function. Another study has demonstrated that the application of a 10% fructose solution in drinking water for 24 weeks in female rats increases visceral fat accumulation but does not alter body weight [17].

Our results demonstrate that ovariectomy leads to a significant increase in visceral fat depot. These results align with others previously reported [7]. It has been proposed that estrogen can modulate body fat distribution, adipocytes size and number [18] as well as adipocyte lipolisis [19]. This is supported by the presence of estrogen receptors in the abdominal and subcutaneous fat [20]. Moreover, estrogen receptors have been found to be expressed in hypothalamic nuclei which are linked to the regulation of energy homeostasis [21].

Plasma Leptin Concentrations. In the OVX group and OVX-F group, leptin levels were significantly elevated compared with the SHAM and SHAM-F groups (4466,66 ± 179,23 pg/ml and 2758.33 ± 682.47 pg/ml vs. 1965.33 ± 179.23 pg/ml and 1706.50 ± 232.15 pg/ml, respectively). No statistical difference was found in plasma leptin levels between SHAM and SHAM-F groups. However, there was a tendency for lower leptin levels in the SHAM-F group. Moreover, the OVX group showed significantly higher plasma leptin levels compared with the OVX-F group (Fig. 2).

Our results support other authors who have demonstrated that estrogen deficiency is accompanied with higher plasma leptin levels [22, 23] and this may be explained by the effect of estrogen on leptin synthesis in adipose tissue [24] and on leptin sensitivity in the hypothalamus [25, 26]. However, other researchers have found a positive correlation between serum estrogen and leptin concentration [27]. Interestingly, Chu et al. have demonstrated that ovariectomy in rats leads to an initial decrease in serum leptin levels, followed by an increased production of leptin [28].

Table 1. Changes in anthropometrical and biochemical parameters in SHAM, OVX, SHAM-F and OVX-F rats at the end of the experimental period. Values are presented as mean ± SD; *a* - p<0,05 significantly different from the SHAM group, *b* - p<0,05 significantly different from the OVX group, and *c* - p<0,05 significantly different from the SHAM-F group.

Parameters	SHAM	OVX	SHAM-F	OVX-F
Serum total cholesterol (mmol/l)	3,5 ± 0,6	4,9 ± 0,2 *a,c*	4,0 ± 0,3	5,5 ± 0,5 *a,c*
Serum HDL-cholesterol (mmol/l)	1,19 ± 0,1	1,3 ± 0,1	1,2 ± 0,2	1,2 ± 0,1
Serum LDL-cholesterol (mmol/l)	1,7 ± 0,6	3,2 ± 0,3 *a,c*	2,2 ± 0,4	3,7 ± 0,6 *a,c*
Serum TAG (mmol/l)	1,9 ± 0,1	2,0 ± 0,4	2,2 ± 0,1	2,6 ± 0,2
Serum glucose (mmol/l)	4,9 ± 0,8	8,8 ± 1,8 *a,c*	6,5 ± 1,2	9,8 ± 1,7 *a,c*
Retroperitoneal fat depot weight (g)	3,6 ± 0,4	6,1 ± 1,2 *a,c*	4,4 ± 0,8	5,5 ± 1,4 *a*
Mesenteric fat depot weight (g)	4,4 ± 0,7	6,4 ± 1,6 *a*	5,5 ± 1,0	7,1 ± 1,2 *a,c*
Subcutaneous fat depot weight (g)	2,9 ± 0,5	3,3 ± 0,7	3,2 ± 0,4	3,1 ± 0,2

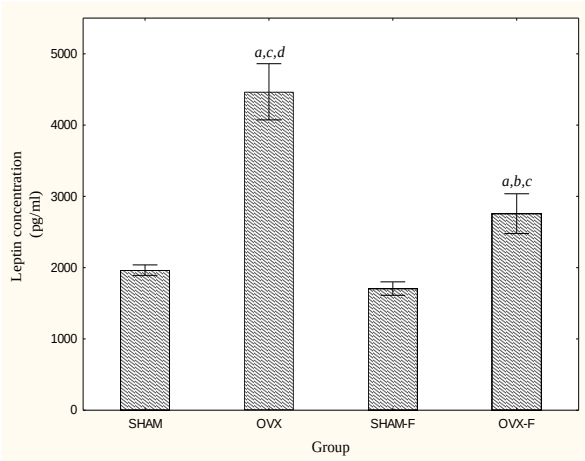

Fig. 2. Leptin concentration in plasma (pg/mL) of different studied groups at the end of the study. a: significantly different from the SHAM group, b: significantly different from the OVX group, and c: significantly different from the SHAM-F group, d: significantly different from the OVX-F group, $p< 0.05$.

In this study we have found that dietary fructose decreases plasma leptin levels in the OVX-F and SHAM-F groups compared with the OVX group. This supports the results obtained by Teff et al. [14]. Another study has found that fructose, unlike glucose, does not stimulate insulin secretion [29]. Low levels of insulin in a high-fructose diet could explain the increased food intake through the following mechanisms. First, in the central nervous system, insulin has a direct inhibitory effect on food intake [30] and second, insulin may modulate food intake by its effects on leptin secretion [31]. Another study has suggested that fructose consumption may induce central leptin resistance, while serum leptin levels, body weight and fat mass remain unchanged [32]; this may be due to the impairment of leptin transport across the blood-brain barrier, which includes leptin receptors and can be easily saturated [33]. An important role in the saturation of this transport mechanism is attributed to hypertriglyceridemia associated with a high-fructose diet [34].

Serum glucose and lipid profile. We have observed that in the OVX-F and OVX groups the levels of serum glucose, total cholesterol and LDL cholesterol were significantly increased when compared to the SHAM and SHAM-F groups (Table 1).

The present study shows that ovariectomy causes serious disturbances in the lipid profile, expressed in increased total cholesterol and LDL-cholesterol accompanied by impaired glucose homeostasis. We have found no significant difference in serum triglyceride levels in all of the studied groups. Regarding serum glucose, our results show that administration of a 10% fructose solution for 8 weeks in sham-operated as well as in ovariectomized animals is accompanied by high levels of fasting blood glucose. Insulin resistance has been proposed as a major cause of impaired glucose tolerance in a high-fructose diet [35]. Moreover, we have found that ovariectomy leads to impaired glucose tolerance, which may also be due to impaired insulin-mediated glucose utilization as a result of estrogen deficiency [36]. However, the exact mechanism of insulin resistance in the bsence of ovarian function remains unclear.

CONCLUSION

This study demonstrates that 8 weeks of administration of a high-fructose diet as a 10% solution in drinking water on ovariectomized Wistar rats leads to manifest metabolic and morphologic disturbances and an alteration in leptin metabolism. Low plasma levels of leptin as a result of a high-fructose diet suggest that fructose affects mostly leptin production and secretion. This indicates that fructose could contribute to decreased satiety and increased food intake. In contrast, estrogen deficiency is associated with higher plasma concentrations of leptin, which could be due to its influence on leptin signal transduction. In conclusion, disturbances in leptin metabolism determine a positive energy balance and weight gain. Further studies are needed to elucidate the role of a high-fructose diet on leptin action in the regulation of food intake and to provide detailed understanding of the mechanisms of postmenopausal obesity.

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ЕФЕКТ НА ВИСОКОФРУКТОЗНАТА ДИЕТА ВЪРХУ ПЛАЗМЕНИТЕ ЛЕПТИНОВИ НИВА, МОРФОЛОГИЧНИ И БИОХИМИЧНИ ПОКАЗАТЕЛИ ПРИ ОВАРИЕКТОМИРАНИ ПЛЪХОВЕ

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(Резюме)

Лептинът е продукт на ob-гена и адипокин, който регулира телесното тегло и метаболизма чрез повлияване приема на храна и разхода на енергия. Постменопаузата и високата консумация на фруктоза са свързани с повишена честота на висцерално затлъстяване и риск от метаболитен синдром, диабет тип 2 и сърдечно-съдови заболявания. Основната цел на това проучване е да се определят промените в телесното тегло, мастната тъкан, плазмените нива на лептина, серумната глюкоза и липидния профил (общ холестерол, триацилглицерол, HDL- и LDL-холестерол) в овариектомирани плъхове, подложени на богата на фруктоза диета. Двадесет и четири възрастни женски Wistar плъхове бяха разделени в четири групи: контролна фалшиво оперирани (Sham, 6 броя), овариектомирани (OVX, 6 броя), фалшиво оперирани плюс фруктоза (Sham-F, 6 броя) и овариектомирани плюс фруктоза (OVX-F, 6 броя). Фруктозата беше давана на плъховете като 10% разтвор в питейната вода за 8 седмици. Резултатите показват, че плазмените нива на лептина се увеличават значително в OVX и OVX-F групи в сравнение с Sham и Sham-F групи (4466,66 ± 179,23 pg/ml и 2758,33 ± 682.47 pg/ml срещу 1965,33 ± 179.23 pg/ml и 1706,50 ± 232.15 pg/ml, съответно). Въпреки че лептиновите нива не се различават сигнификантно във фалшиво оперираната и фалшиво оперираната плюс фруктоза групи, се наблюдава тенденция за намаляване на нивата на лептина във фалшиво оперираната плюс фруктоза група. Телесното тегло е значително повишено в OVX, OVX-F и Sham-F групи в сравнение с Sham-групата. Теглата на абдоминалната мастна тъкан и стойностите на биохимичните параметри са сигнификантно по-високи в OVX и OVX-F в сравнение с тези на контролните животни. В заключение, прекомерната консумация на фруктоза може да допринесе за метаболитни нарушения и предразположение към хронични неинфекциозни заболявания, наблюдавани в периода на постменопаузата. Освен това резултатите показват, че високофруктозната диета и овариектомията повлияват лептиновите нива в различни посоки, което може да се дължи на смущения на различни сигнално-трансдукционни механизми в лептиновия синтез и секреция.