

LEPTIN AND GLUCOCORTICOID SIGNALING PATHWAYS IN THE HYPOTHALAMUS OF FEMALE AND MALE FRUCTOSE-FED RATS

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Abstract - Alterations in leptin and glucocorticoid signaling pathways in the hypothalamus of male and female rats subjected to a fructose-enriched diet were studied. The level of expression of the key components of the leptin signaling pathway (neuropeptide Y /NPY/ and suppressor of cytokine signaling 3 /SOCS3/), and the glucocorticoid signaling pathway (glucocorticoid receptor /GR/, 11 β -hydroxysteroid dehydrogenase type 1 /11 β HSD1/ and hexose-6-phosphate dehydrogenase /H6PDH/) did not differ between fructose-fed rats and control animals of both genders. However, in females, a fructose-enriched diet provoked increases in the adiposity index, plasma leptin and triglyceride concentrations, and displayed a tendency to decrease the leptin receptor (ObRb) protein and mRNA levels. In male rats, the fructose diet caused elevations in plasma non-esterified fatty acids and triglycerides, as well as in both plasma and hypothalamic leptin concentrations. Our results suggest that a fructose-enriched diet can induce hyperleptinemia in both female and male rats, but with a more pronounced effect on hypothalamic leptin sensitivity in females, probably contributing to the observed development of visceral adiposity.

Key words: Fructose diet, hypothalamus, leptin, leptin receptor, glucocorticoids, glucocorticoid receptor

INTRODUCTION

Over the past decades there was a general trend towards an increased total energy intake due to use of high-fructose corn syrup in soft-drinks, which was followed by the increased prevalence of obesity and metabolic syndrome worldwide (Bray, 2008). It has been shown that a long-term fructose-enriched diet may lead to metabolic and cardiovascular disturbances, including dyslipidemia, insulin resistance, hypertension, hyperuricemia and weight gain (Tappy and Le, 2010). Additionally, as a highly lipogenic sugar, fructose leads to increased plasma non-esterified fatty acid (NEFA), leptin and adiponectin levels, as well as to abdominal adiposity

and impaired insulin sensitivity (Melanson et al., 2008).

Previous studies have reported gender differences in the susceptibility and progression of metabolic disturbances (Denzer et al., 2009; Regitz-Zagrosek et al., 2006). Males and females differ in distribution of adipose tissue, which is located predominantly in the abdominal region in males and carries much greater metabolic risk than adipose tissue distributed subcutaneously, as in females (Wajchenberg, 2000). Animal studies have shown that males exhibit a greater resistance to increase in body mass than females. On the other hand, female rats seem to be less susceptible to carbohydrate- or lipid-induced insu-

lin resistance (Horton et al., 1997; Riant et al., 2009). Besides, male rats are more prone to diet-induced hypertension and hyperinsulinemia (Roberts et al., 2001), while female rats develop these symptoms only after ovariectomy (Galipeau et al., 2002).

The hypothalamus is the key site for regulation of food intake, body weight and energy expenditure (Morton et al., 2006). Leptin is a hormone secreted by adipose tissue, and is known to reduce food intake and stimulate energy expenditure (Friedman and Halaas, 1998). In the hypothalamus, leptin suppresses food intake by modulating the synthesis and secretion of both orexigenic and anorexigenic peptides. Leptin acts through the leptin receptor (OBR). Five alternatively spliced isoforms of the receptor differ in the length of the C-terminus and the long form (OBRb) is capable of activating intracellular signaling (Myers et al., 2008). By binding to OBRb, leptin stimulates tyrosine phosphorylation of Janus kinase 2 (JAK2), which then phosphorylates and activates the signal transducer and activator of transcription 3 (STAT3). After dimerization, activated STAT3 translocates to the nucleus and stimulates gene transcription. Leptin may also lead to the inhibition of its own signaling pathway through feedback inhibitors, such as the suppressor of cytokine signaling 3 (SOCS3) (Myers et al., 2008).

Obesity is frequently associated with elevated plasma leptin concentrations and leptin resistance (Oswal and Yeo, 2010). Leptin resistance is characterized by a decreased expression of OBRb, JAK2 and STAT3, as well as with increased expression of SOCS3 in the hypothalamus (Howard and Flier, 2006). Additionally, an impaired leptin transport across the blood-brain barrier may contribute to the development of leptin resistance. Available data from diet-induced obesity in rodents strongly suggest that central leptin resistance contributes to the development of obesity (Schwartz et al., 1996).

Glucocorticoids are important metabolic regulators of glucose availability and energy balance. Over the past decades, it has been shown that glucocorticoid excess is causatively linked to obesity and meta-

bolic syndrome (Vegiopoulos and Herzig, 2007). Glucocorticoids affect appetite indirectly, through influencing the levels of other hormones and neurotransmitters, such as orexigenic neuropeptide Y (NPY), Agouti-related protein (AgRP) and anorexigenic proopiomelanocortin (POMC) (Shimizu et al., 2008). In the hypothalamus, glucocorticoids counteract the anorexigenic actions of leptin and insulin, and interplay between these hormones is crucial for metabolic homeostasis (Vegiopoulos and Herzig, 2007). At the molecular level, glucocorticoids exert their physiological effects through binding to the glucocorticoid receptor (GR), which belongs to the nuclear receptor superfamily of transcription factors. The intracellular concentration of glucocorticoids is determined not only by their plasma levels, but also by the activity of 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1), the enzyme which catalyzes the intracellular conversion of inactive glucocorticoids to active forms (Seckl et al., 2004).

Considering the link between increased fructose intake and the growing rate of metabolic disturbances, including obesity and metabolic syndrome, as well as gender differences in the susceptibility and progression of these disturbances, the objective of this study was to examine alterations in leptin and glucocorticoid signaling in the hypothalamus of male and female rats subjected to a fructose-rich diet.

MATERIALS AND METHODS

Animals and treatment

Male and female 21-day-old Wistar rats were separated from their mothers and divided into two experimental groups according to diet regime: control group and fructose-fed group. Each group consisted of nine animals (n=9), which were housed three per cage, and kept at 22 \pm 2°C, under 12 h light-dark cycle, and constant humidity. All experimental groups had *ad libitum* access to commercial chow and either 10% (w/v) fructose solution (rats on fructose diet) or tap water (control group). During a 9-week treatment, food and liquid intake was measured daily, and body mass was measured weekly. Caloric intake was

calculated as the sum of calories ingested as food and liquid. All animal procedures complied with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes, and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

Blood plasma preparation, tissue collection and determination of plasma parameters

Animals were sacrificed by rapid decapitation after overnight fasting. Immediately after decapitation, visceral omental adipose tissue and hypothalamus were carefully isolated, weighed and frozen in liquid nitrogen for subsequent processing. Adiposity index (%) was calculated as [(adipose tissue mass/body mass) x 100].

Trunk blood was rapidly collected into EDTA containing tubes and the triglyceride concentration in the blood was measured on site by MultiCare strips (Biochemical Systems International, Italia). Plasma was obtained by centrifugation at 1600 x g for 10 min at room temperature and stored at -70°C. The plasma level of non-esterified fatty acids (NEFA) was determined using a modified version of Duncombe's method (Duncombe, 1964). Leptin concentrations in both plasma and hypothalamic tissue were determined by Rat Leptin ELISA Kit (EZRL-83K, Millipore), according to the manufacturer's instructions. Absorbance at 450 nm (reference 650 nm) was read using a plate reader (Multiskan Spectrum, Thermo Electron Corporation, Finland). The data were fitted by 4PL curve (RedaerFit Software, MiraiBio Group of Hitachi Solutions America, Ltd.) and leptin concentration is expressed in ng/ml for plasma samples, and in ng/mg of protein for tissue samples.

Preparation of hypothalamic whole cell extracts

Hypothalami from 3 animals were pooled and homogenized in 4 vol. (w/v) of RIPA buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet NP40, 0.1% SDS, 2 mM DTT, 1 mM EDTA-Na₂, protease and

phosphatase inhibitors) using a glass/teflon (Potter-Elvehjem) homogenizer. The homogenates were sonicated on ice (3 x 10 s at 10 MHz, Hielscher Ultrasound Processor) and incubated 60 min at 0°C prior to 20 min centrifugation at 14000 x g. The resulting supernatants were stored at -70°C. Protein content was determined according to Spector (1978).

SDS-PAGE and immunoblotting

Proteins were resolved on 7.5% SDS-polyacrylamide gels using Mini-Protean II Electrophoresis Cell (Bio-Rad Laboratories, Hercules, CA). Transfer of proteins from acrylamide gels to PVDF membranes (Immobilon-FL, Millipore) was performed in 25 mM Tris buffer, pH 8.3 containing 192 mM glycine and 20% (v/v) methanol, at 135 mA overnight in Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked by phosphate-buffered saline (PBS, 1.5 mM KH₂PO₄, 6.5 mM Na₂HPO₄, 2.7 mM KCl, 0.14 M NaCl, pH 7.2) containing 2% non-fat dry milk for 90 min at room temperature. After extensive washing (PBS containing 0.1% Tween20), membranes were incubated overnight at 4°C with respective primary antibodies: rabbit polyclonal anti-leptin receptor (ab5593, Abcam), rabbit polyclonal anti-GR (sc-1004, Santa Cruz Biotechnology), rabbit polyclonal anti-11β-HSD1 (ab109554, Abcam), rabbit polyclonal anti-H6PD (sc-67394, Santa Cruz Biotechnology) and mouse monoclonal anti-β-actin (AC-15, Sigma-Aldrich), which was used as an equal loading control. After extensive washing, all membranes were incubated with alkaline phosphatase conjugated secondary antibodies (Amersham Pharmacia Biotech, 1:20000). The immunoreactive proteins were visualized by an enhanced chemifluorescence method (ECF, Amersham Pharmacia Biotech, UK) and quantitative analysis was performed by Image-Quant software (GE Healthcare, USA).

RNA isolation and Reverse Transcription

Total hypothalamic RNA was isolated using TRIreagent® (AmBion). RNA was dissolved in RNase-DNase free water (Eppendorf) and its concentration and pu-

rity were tested spectrophotometrically (OD 260/280 > 1.8 was considered satisfactory). RNA integrity was confirmed by 1% agarose gel electrophoresis. RNase inhibitor (Applied Biosystems) was added and the samples were frozen at -80°C until use. Prior to cDNA synthesis, DNA contamination was removed by DNase I treatment (Fermentas). cDNA was synthesized from 2 µg of RNA. Reverse transcription was performed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's instructions, and cDNA was stored at -80°C until use.

Real-time PCR

Quantification of ObR, GR, NPY and SOCS3 mRNA levels in the hypothalamus was performed by TaqMan[®] Real Time PCR. Primers and probes for GR, ObRb, NPY and SOCS3 (Rn01433205_m1, Rn00561369_m1, Rn01410145_m1 and Rn00585674_s1, respectively) were obtained from Applied Biosystems Assay-on-Demand Gene Expression Products. TBP (Rn01455646_m1) was used as a previously validated endogenous control. Real-time PCR was performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems) in a total volume of 25 µl containing 1× TaqMan[®] Universal Master Mix with AmpErase UNG, 1× Assay Mix (Applied Biosystems) and the cDNA template (20 ng of RNA converted to cDNA) at cycle conditions: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 90 s. No template control was used in each run. All reactions were run in triplicate. Relative quantification of target genes was performed using the comparative $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). The obtained results were analyzed by Sequence Detection Software version 1.2.3 for 7000 System SDS Software RQ Study Application (Applied Biosystem) with a confidence level of 95 % ($p \leq 0.05$).

Statistical analyses

Statistical analyses were performed using Prism software 5.00 (GraphPad, San Diego, CA, USA) and SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). To analyze the effects of two factors, gender and fructose,

as well as their interaction, we performed two-way ANOVA, followed by the Bonferroni *post hoc* test. The results are expressed as mean \pm SD for biochemical and hormonal parameters, and as mean \pm SEM for data from Western blot analysis and qPCR. Analysis of covariance (ANCOVA) was used to evaluate between-group differences in plasma leptin levels after adjusting for body mass. Values were considered statistically significant when the P value was less than 0.05.

RESULTS

Body parameters of male and female fructose-fed rats

Daily food and liquid intake was measured during 9 weeks of treatment. Two-way ANOVA revealed the significant effects of gender and fructose on caloric intake (Table 1). Subsequent *post-hoc* analyses showed that the caloric intake of both female and male fructose-fed rats was significantly increased in comparison to the rats of the same gender on standard diet (Table 1, $P < 0.05$ for males and females, control *vs.* fructose).

Two-way ANOVA analysis showed a significant effect of gender on body mass and significant effects of both factors, gender and fructose on visceral adipose tissue mass and adiposity index (Table 1). *Post-hoc* analysis showed a significant difference in body mass between males and females in both control and fructose-fed groups (Table 1, * $P < 0.05$ control males *vs.* control females, ** $P < 0.01$ fructose-fed males *vs.* fructose-fed females). Visceral adipose tissue mass and adiposity index were significantly increased in fructose-fed females in comparison to the control female group (Table 1, $P < 0.05$). The adiposity index was also significantly higher in fructose-fed female rats compared to fructose-fed males (Table 1, # $P < 0.05$).

Plasma levels of triglycerides and NEFA in male and female fructose-fed rats

Two-way ANOVA showed a significant effect of the fructose diet on plasma triglyceride levels and the

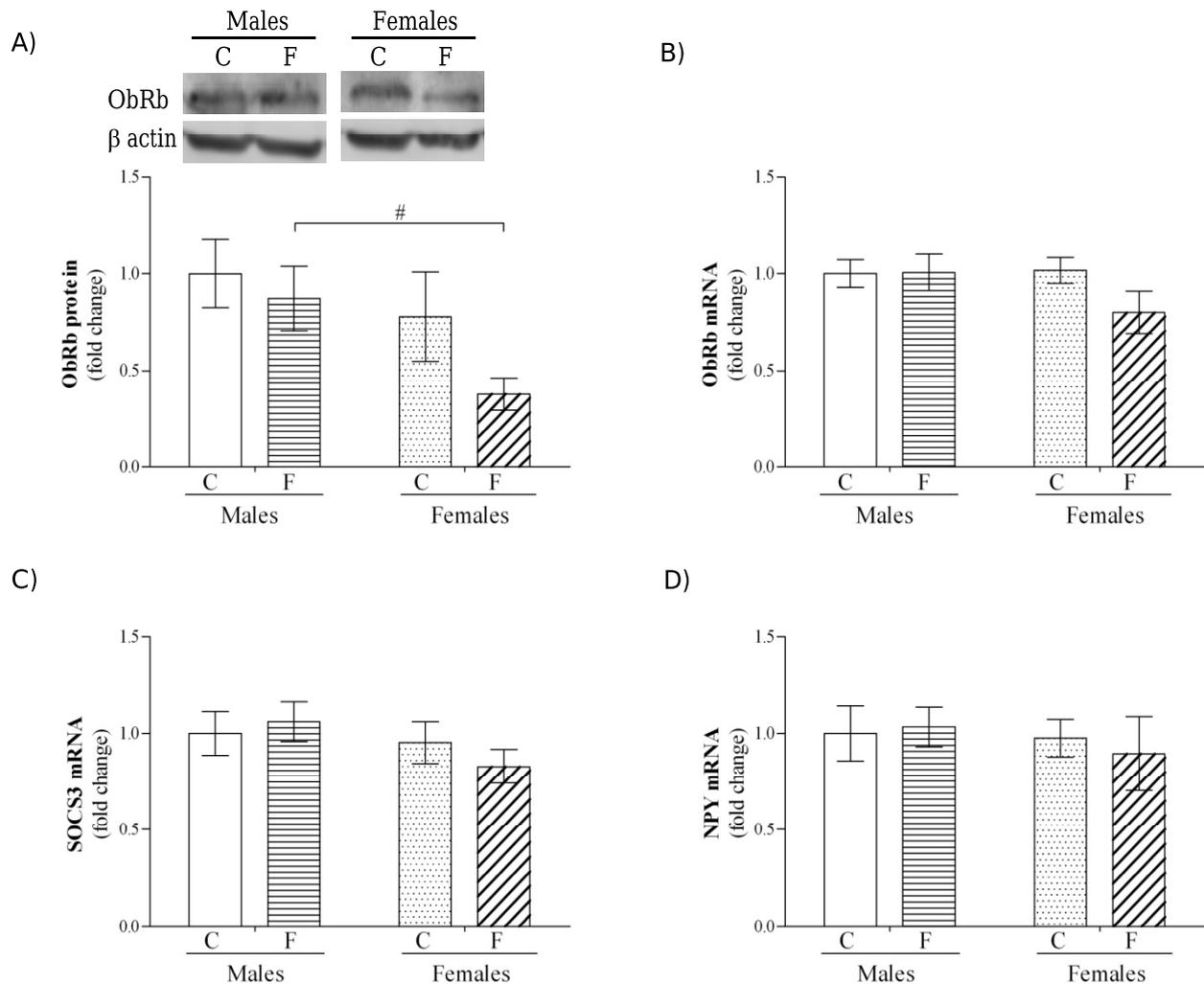


Fig. 1. Effects of high fructose diet on the OBRb protein level and OBRb, SOCS3 and NPY mRNA levels in the hypothalamus. Representative Western blots and relative quantification of OBRb protein (A) in the hypothalamic whole cell extracts of control male and female rats, as well as male and female rats on a fructose-rich diet. Lower parts of the blots were probed with β -actin antibody as a loading control. Relative quantification of OBRb (B), NPY (C) and SOCS3 (D) mRNA levels in the hypothalamus of control male and female rats, and male and female rats on a fructose-rich diet. C – control rats, F – fructose-fed rats. Data are presented as mean \pm SEM. A value of $P < 0.05$ was considered statistically significant. Significant between group difference is indicated as $^{\#}P < 0.05$ for fructose-fed males vs. fructose-fed females.

effect of gender on plasma NEFA concentrations (Table 1). Increased triglycerides were observed in both female and male fructose-fed rats in comparison to control ones (Table 1, $P < 0.01$, fructose-fed females vs. control females, $P < 0.05$, fructose-fed males vs. control males). A significant increase in plasma NEFA concentrations was observed in the male

fructose-fed rats in comparison to controls (Table 1, $P < 0.05$). *Post-hoc* analysis showed a significant difference in plasma NEFA concentrations between males and females in both control and fructose-fed groups (Table 1, $^*P < 0.05$ control males vs. control females, $^{\#}P < 0.05$ fructose-fed males vs. fructose-fed females).

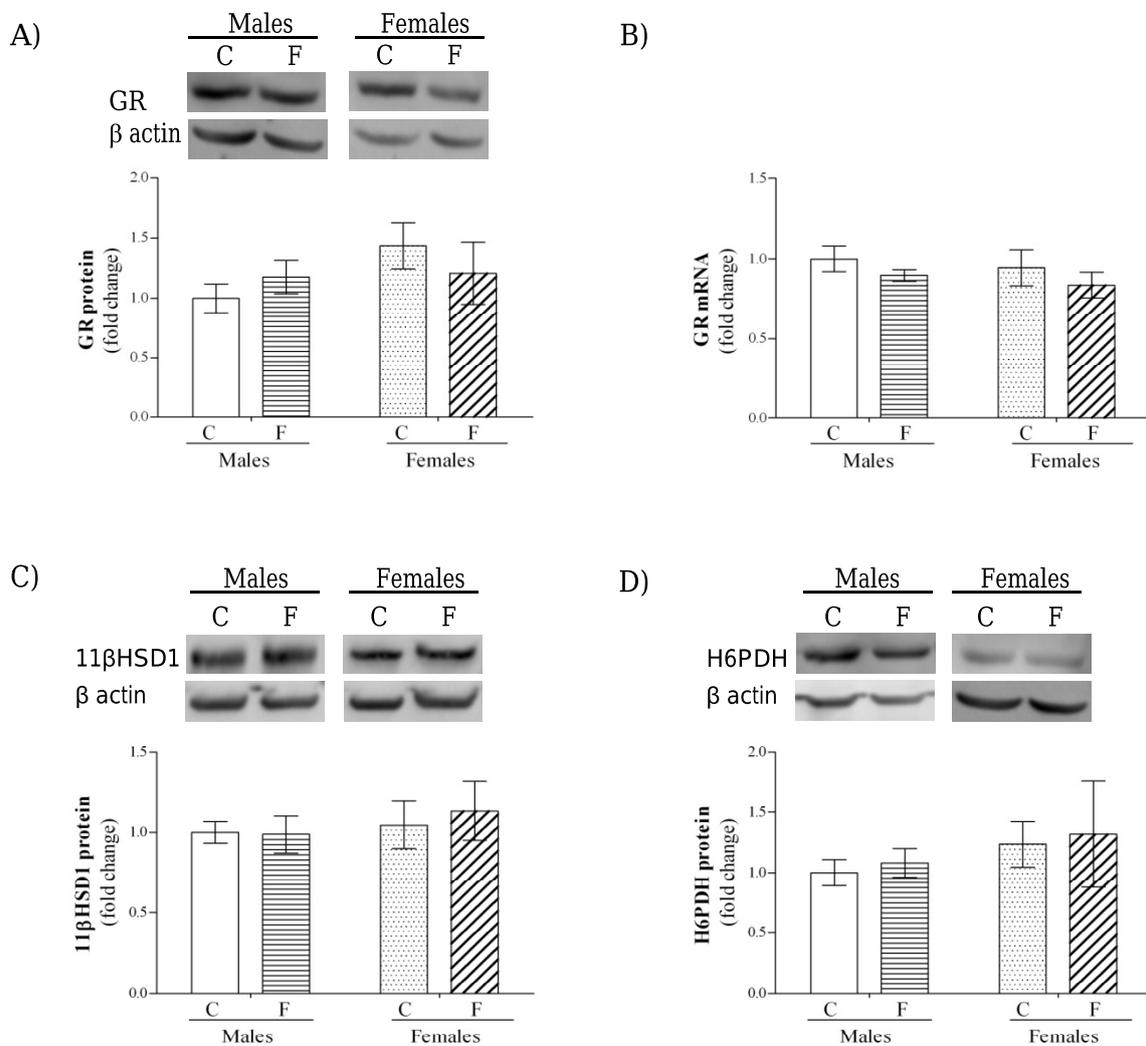


Fig. 2. Effects of high fructose diet on the GR, 11βHSD1 and H6PDH protein levels and GR mRNA level in the hypothalamus. Representative Western blots and relative quantification of GR protein (A) and GR mRNA (B) levels. Representative Western blots and relative quantification of 11βHSD1 (C) and H6PDH (D) proteins in the hypothalamic whole cell extracts of control male and female rats, and male and female rats on fructose-rich diet. Lower parts of the blots were probed with β-actin antibody as loading control. C – control rats, F – fructose-fed rats. Data are presented as mean ± SEM.

Leptin concentrations in the plasma and hypothalamus of male and female fructose-fed rats

Two-way ANOVA showed a significant effect of fructose diet on plasma leptin levels (Table 1, $P < 0.01$), while the effect of gender was not observed. Plasma leptin concentrations were increased in fructose-fed rats of both genders compared to the controls (Ta-

ble 1, $P < 0.05$). When the analysis of plasma leptin was repeated by ANCOVA, with body mass as a covariate, a between-group difference was observed ($F(3,32) = 2.92$, $P < 0.05$). After adjusting for body mass, estimated plasma leptin concentrations were 1.23 ± 0.41 ng/ml for control males, 2.39 ± 0.41 ng/ml for fructose-fed males, 1.47 ± 0.42 ng/ml for control females and 2.51 ± 0.42 ng/ml for fructose-fed

Table 1. Caloric intake, body weight, visceral adipose tissue mass, adiposity index, plasma parameters and concentration of leptin in the hypothalamus.

	Male			Female			Two-way ANOVA		
	Control	Fructose	P	Control	Fructose	P	Gender	Fructose	GxF
Caloric intake (kJ/day/animal)	226.23 ± 28.90	283.65 ± 24.51	<0.05	191.51 ± 32.89	236.06 ± 30.21	<0.05	<0.05	<0.01	ns
Body mass (g)	338.89 ± 40.45*	311.25 ± 37.58**	ns	259.00 ± 28.66	271.23 ± 25.44	ns	<0.001	ns	ns
Mass of VAT (g)	2.59 ± 1.14	3.16 ± 1.80	ns	3.20 ± 1.32	5.06 ± 2.48	<0.05	<0.05	<0.05	ns
Adiposity index (%)	0.75 ± 0.28	1.01 ± 0.48 [#]	ns	1.24 ± 0.52	1.83 ± 0.75	<0.05	<0.001	<0.05	ns
Triglycerides (mmol/l)	1.49 ± 0.36	1.90 ± 0.35	<0.05	1.39 ± 0.29	1.87 ± 0.38	<0.01	ns	<0.01	ns
NEFA (mmol/l)	0.61 ± 0.08*	0.70 ± 0.07 [#]	<0.05	0.84 ± 0.17	0.83 ± 0.18	ns	<0.001	ns	ns
Leptin (ng/ml) ¹	1.42 ± 1.17	2.62 ± 1.23	<0.05	1.24 ± 0.60	2.28 ± 1.42	<0.05	ns	<0.01	ns
Leptin x10 ¹² (ng/mg) ²	5.42 ± 0.98	11.98 ± 5.49	<0.05	12.1 ± 8.34	12.47 ± 3.65	ns	ns	ns	ns

The data represent the mean values ± SD of 9 animals per group. GxF indicates gender and fructose-diet interactive effect. ¹Plasma level; ²Level in the hypothalamus. A value of P<0.05 was considered statistically significant. *P<0.05 control males vs. control females; [#]P<0.05 fructose-fed males vs. fructose-fed females; **P<0.01 fructose-fed males vs. fructose-fed females; ns – stands for non-significant.

females, with no differences in plasma leptin concentrations between genders.

Leptin concentration in the hypothalamic whole-cell extract was higher in the fructose-fed males compared to the rats on control diet (Table 1, P<0.05), while in females the leptin concentration in the hypothalamus remained unaltered by fructose overconsumption (Table 1).

Leptin signaling in the hypothalamus of male and female fructose-fed rats

The effects of a high fructose diet on the expression of hypothalamic OBRb isoform, NPY and SOCS3 were examined by quantitative Real-time PCR and semi-quantitative Western blot. The results from two-way ANOVA showed a significant effect of gender on OBRb relative protein concentration in the hypothalamus (F(1,21)=4.40, P<0.05), while there were no effects of fructose or interaction of both factors. Subsequent *post-hoc* analysis showed that fructose-fed females had significantly decreased hypothalamic OBRb relative protein concentration compared to fructose-fed males (Fig. 1, [#]P<0.05). Besides, fructose-fed females showed a tendency toward a decrease of OBRb at the protein and mRNA level (P<0.1) in comparison to females on the nor-

mal diet. When the level of OBRb, NPY and SOCS3 mRNA in the hypothalamus were analyzed, two-way ANOVA did not reveal any significant effect of gender and fructose, or their interaction (Fig. 1).

Glucocorticoid signaling in the hypothalamus of male and female fructose-fed rats

The impact of a high fructose diet on glucocorticoid signaling was also assessed in the hypothalamus of male and female rats. The results of two-way ANOVA showed no significant effects of gender and fructose, or they interaction, on the GR mRNA and GR, 11βHSD1 and H6PDH protein levels. The expression of the key enzymes of pre-receptor glucocorticoid metabolism, 11βHSD1 and H6PDH, was not affected by the high-fructose diet in animals of both sexes (Fig. 2), and was paralleled by unaltered expression of both GR protein and mRNA (Fig. 2).

DISCUSSION

The results of the present study demonstrate that a fructose-rich diet induces an increase in plasma leptin concentrations in both female and male rats. However, the impact of fructose on hypothalamic leptin sensitivity differs in a gender-specific manner. Females display a tendency towards decrease in

ObRb protein and mRNA levels in parallel with the development of visceral adiposity. On the other hand, male rats have elevated plasma NEFA and hypothalamic leptin concentrations. Expression of the key components of the glucocorticoid signaling pathway (GR, 11 β HSD1 and H6PDH) and leptin signaling pathway (NPY and SOCS3) did not differ between fructose-fed and control animals of both genders.

Both male and female rats on the fructose-rich diet had elevated caloric intake originating from increased fructose consumption. Interestingly, increased caloric intake did not influence the rat body mass, regardless of sex. However, females on the fructose diet displayed increased visceral adipose tissue mass and significantly elevated adiposity index. Furthermore, in female rats the fructose diet led to hypoglycemia and triglyceridemia, which are important parameters of metabolic disturbance. It was previously shown that fructose-induced increase in rat abdominal fat is accompanied by hypertriglyceridemia (Bocarsly et al., 2010). In contrast to females, male rats on the fructose-rich diet did not present an increased mass of visceral adipose tissue and adiposity index, but had increased triglycerides and NEFA. Visceral adiposity is characterized not only by the different distribution of body fat, but also by changes in certain hormone levels, such as leptin and insulin, which may affect the hypothalamus (Melanson et al., 2008). A number of studies demonstrated a relationship between increased adiposity and increased plasma leptin and insulin levels (Clegg et al., 2006). It is known that leptin and insulin send afferent signals to the hypothalamus in an endocrine feedback loop that regulates body adiposity and that the receptors for these hormones share common signaling pathways in this brain structure (Gerozissis, 2008). Our results showed that a fructose-rich diet led to elevated plasma leptin levels in both genders. It should be noted that the existing literature data regarding plasma leptin concentrations in female and male rats are contradictory. While some authors reported unchanged plasma leptin level (Wu-Peng et al., 1999), others showed increased (Clegg et al., 2003) or even decreased leptin concentration in females compared to males (Mulet et al., 2003). In this

study, we did not observe differences in plasma leptin concentrations between the genders even after controlling for body mass. It should be emphasized that the main difference in leptin signaling was observed at the level of its hypothalamic intracellular concentration. Namely, the observed increase in plasma leptin in both male and female rats on the fructose-rich diet was accompanied by elevated hypothalamic leptin concentration only in the males. This result suggests that in females on a fructose-rich diet, leptin originating from enlarged adipose tissue did not reach the hypothalamic leptin receptor (ObRb), pointing to the presence of hypothalamic leptin resistance possibly due to a transport defect at the level of the blood-brain barrier. It is known that leptin is transported across the blood-brain barrier by a saturable transport mechanism, which may be affected by a number of circulating substances, including triglycerides (Banks et al., 2004). Banks et al. (2004) showed that serum triglycerides interfere with the ability of the blood-brain barrier to transport leptin and represent a likely cause of the leptin resistance observed in both starvation and obesity. However, it is unlikely that this is the sole reason for a lack of leptin transport into the hypothalamus of females, since this phenomenon was not observed in fructose-fed males, who also had elevated plasma triglycerides. It was previously shown that fructose effects on metabolism might be modified by sex hormones (Galipeau et al., 2002). Studies on intact adult rats confirmed that females were protected against the deleterious metabolic effects of fructose through the actions of estrogen (Galipeau et al., 2002). Additionally, there are data showing that estrogen-deficient rats are leptin insensitive (Ainslie et al., 2001). Accordingly, the lack of estrogen in the first weeks of fructose diet in our juvenile females could contribute to disturbed leptin sensitivity in the hypothalamus. It was previously demonstrated that disturbed leptin signaling is associated with an increase in visceral fat deposition and impaired lipolysis (Verploegen et al., 1997), which overall resulted in unchanged NEFA levels, as seen in females in our study. On the other hand, the preserved leptin sensitivity in the hypothalamus of fructose-fed male rats was associated with undisturbed leptin action

on the periphery, as evidenced by increased NEFA release from this tissue and depleted lipid deposition in the adipose tissue (Gallardo et al., 2007; Shimabukuro et al., 1997). Moreover, the elevation of plasma NEFA in male fructose-fed rats could be attributed to lipolysis that originates from enhanced glucocorticoid actions in the adipose tissue, as previously demonstrated in our laboratory (Bursać et al., 2012).

Other factors contributing to leptin resistance in obesity involve the downregulation of ObRb and disturbed downstream signaling at the level of STAT and SOCS proteins (Howard and Flier, 2006; Myers et al., 2008). As previously mentioned, fructose-fed females had an unchanged hypothalamic leptin level accompanied with a tendency towards decreased ObRb protein and mRNA levels. It has already been shown that leptin administration causes a reduction in ObRb expression in cell lines (Liu et al., 2004) and Liu et al. (2007) have reported a negative relationship between plasma leptin concentration and ObRb gene expression in the hypothalamus and liver. In our study, a decrement in ObRb protein level in fructose-fed females was significantly lower compared to the males, and this tendency towards leptin resistance in females could develop further if the animals were exposed to the diet for a longer period. In line with this assumption, Shapiro et al. (2008) have shown that chronic (6 months) fructose consumption induced leptin resistance through decreased hypothalamic STAT3 phosphorylation and elevated triglycerides, but with no changes in SOCS3 gene expression. Our results also did not confirm fructose-related changes in the hypothalamic SOCS3 mRNA level in both genders, and we assume that the impaired hypothalamic leptin sensitivity in the females could be caused by alterations downstream of the leptin receptor.

Apart from leptin effects on the regulation of food intake and energy metabolism, there is evidence suggesting that glucocorticoids also play an important role in the control of feeding behavior (la Fleur, 2006). For example, glucocorticoids may affect the nutritional state through the regulation of hypothalamic expression of orexigenic peptides, such

as NPY, AgRP and POMC (Shimizu et al., 2008). Tissue-specific glucocorticoid concentrations depend on the activity of 11 β HSD1, which may be regulated by diet regime (London and Castonguay, 2009; Seckl et al., 2004). Our results showed that a fructose-rich diet did not change the pre-receptor metabolism of glucocorticoids, GR protein and mRNA levels in the hypothalami of males and females. Additionally, unchanged GR levels coincided with an unchanged expression of its transcriptional target, NPY. Since NPY is not the only orexigenic peptide produced in the hypothalamus, it is possible that the effects of the fructose diet were mediated through other orexigenic signals in the brain.

In summary, the results of the present study show that long-term fructose consumption exerted more harmful effects on central leptin sensitivity in female than in male rats, probably shaping a gender-specific metabolic phenotype characterized by increased visceral adiposity only in females.

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