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## Markers of low-grade inflammation in female and male rats, treated with combined high-fat high-carbohydrate diet

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### ABSTRACT

Low-grade inflammation is characterized by elevated serum levels of interleukin-6, C-reactive protein and other cytokines. Studies show that the application of combined high-fat high-carbohydrate diet in experimental models of diet-induced metabolic syndrome increases these cytokines in male rats, but its effect is not well studied on female laboratory animals. The aim of this work was to assess the effect of the combined high-fat high-carbohydrate diet on the serum concentrations of interleukin-6 and C-reactive protein in female and male rats. An equal number of female and male Wistar rats (n=12) were used. Half of them were exposed to combined high-fat high-carbohydrate diet and the other half (controls) received standard rat chew for 16 weeks. At the end of the experiment, the serum concentrations of cytokines were measured. Results were analyzed by two-way ANOVA. The enclosed diet had a significant main effect on the cytokines' levels. Dietary-manipulated animals had increased levels of interleukin-6 compared to controls ( $63.02 \pm 12.26$  pg ml<sup>-1</sup> vs  $20.98 \pm 12.26$  pg ml<sup>-1</sup>,  $P < 0.05$ ). The serum concentration of C-reactive protein in the dietary group was higher than that in controls ( $6.75 \pm 0.19$  mg l<sup>-1</sup> vs  $5.94 \pm 0.19$  mg l<sup>-1</sup>,  $P < 0.05$ ). The gender had a significant main effect only on the C-reactive protein, the female rats had higher levels than males ( $6.69 \pm 0.19$  mg l<sup>-1</sup> vs  $6.00 \pm 0.19$  mg l<sup>-1</sup>,  $P < 0.05$ ). The obtained results show that the 16-week application of the combined high-fat high-carbohydrate diet increases the levels of the tested cytokines and leads to development of low-grade inflammation in both sexes. In females the changes in C-reactive protein levels are expressed more than in male rats.

**Key words:** high-fat-carbohydrate diet, Wistar rats, metabolic syndrome, interleukin-6, C-reactive protein

## Introduction

Increased intake of lipids and carbohydrates and reduced physical activity lead to the development of obesity with low-grade inflammation. It is characterized by increased serum levels of interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor alpha (TNF- $\alpha$ ), and others. This state is a risk factor for the development of insulin resistance, diabetes, hypertension and metabolic syndrome. A number of studies on induced metabolic syndrome (MetS) through various dietary regimens have been done in order to clarify the diets' effects on the serum levels of cytokines. The obtained data is primarily concerned with male spontaneously hypertensive rats (SHR) and Sprague Dawley rats. SHR with diet-induced MetS by monosodium glutamate and high-carb

diet have increased levels of CRP and IL-6 (Leguisamo *et al.*, 2012; Škop *et al.*, 2016). In male Sprague Dawley rats, there have been found increased IL-6 after the administration of high-fat diet with and without added salt (Bao *et al.*, 2015) while a short-term, 6-week, administration of high-fat diet leads to low-grade inflammation with a slight increase of IL-6 (Crinigan *et al.*, 2015). A small number of studies are carried out to investigate the level of expression of IL-6 and CRP in Wistar rats with MetS induced by different diets. Metabolic syndrome induced by high-fat diet and a low dose of streptozotocin is manifested by increased CRP (Suman *et al.*, 2016) in male rats. Obviously the data on the effects of dietary regimens on the serum levels of interleukin-6 and C-reactive protein are incomplete, contradictory and are conducted primarily on male laboratory animals and its effects on intact female rats are scarce. The possible gender

differences in serum concentrations of CRP and IL-6 in male and intact female rats with dietary-induced MetS are not well defined. In order to follow the recommendations of the National Institute of Health (NIH, 1993) to conduct parallel investigations in both sexes, in this experiment male and female animals were used.

## Materials and Methods

An equal number of male (n=12) and female (n=12) Wistar rats were used, with an initial body mass of 160-180 g. They were kept in individual metabolic cages at  $20 \pm 2$  °C, controlled humidity and 12:12 h light-dark period. The experiment was approved by the Commission on Ethical Treatment of Animals of the Bulgarian Food Safety Agency (license № 119, 18.06.2015). In the preparatory period all rats received a standard rat chew for two weeks while adapting to the conditions in metabolic cages. Then half of the male (MD) and female (FD) rats were treated with combined high-fat-carbohydrate diet (HFCD) for 16 weeks and the other half received standard rat chew (controls, MC and FC). The energy content of the food of MC and FC groups was 2908 kcal.kg<sup>-1</sup> and of groups MD and FD - 4298 kcal.kg<sup>-1</sup> (Table. 1). In order to avoid possible gastrointestinal problems, the dietary-manipulated animals were subjected to transient diets with different percentages of high-fat-carbohydrate food, during the preparatory period. The combined HFCD, as well as transitional diets were prepared by adding lard and sucrose to standard pellets.

**Table 1.** Composition and energy value of food intake

	Control groups		Dietary-manipulated groups	
	g/100 g	En %	g/100 g	En %
Proteins	13.45	18.50	13.45	12.51
Lipids	3.40	10.50	14.40	30.15
Carbohydrates	51.60	71.00	61.60	57.34

The body weight of each animal was measured once a week. An hour before the food was temporarily deprived and returned to the metabolic cages after determining the body mass.

At the end of the trial, the experimental animals were decapitated under anesthesia, mixed blood was collected and centrifuged in order to measure the serum levels of interleukin-6 and C-reactive protein. The serum concentration of CRP was assayed using reagent of Thermosience (Finland) by analyzer Konelab 60 i (Thermophisher

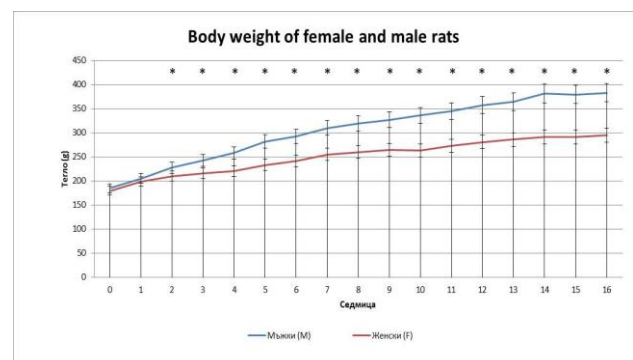
Scientific, USA), and those of IL-6 were measured by the sandwich ELISA method with IL-6 ELISA RAT Kit (№ BMS625, EBIOSCIENCE). Doubled samples were elaborated on Sirio microplate reader (SEAC, Italy) and the average of the two measurements was used as a representative for each rat.

The results are expressed as means  $\pm$  SEMs. The serum concentration of interleukin-6 and C-reactive protein were analyzed by two-way ANOVA, and those of the body weight - two-way ANOVA for repeated measures. The level of significance was set at  $P < 0.05$ .

## Results

At the beginning of the experiment there were no statistically significant differences between the body weights of the used groups - male control (MC -  $182.13 \pm 9.64$  g), female control (FC -  $172.00 \pm 16.49$  g), male dietary-manipulated (MD -  $184.75 \pm 13.66$  g) and female dietary-manipulated (FD -  $176.25 \pm 14.39$  g) rats ( $P > 0.05$ ).

During the experiment we found the existence of gender-dependent differences in animal weight. From the second week until the end of the experiment, the gender had a significant main effect on the body weight, as the males were with higher body mass than the females ( $P < 0.05$ ) (Fig.1).



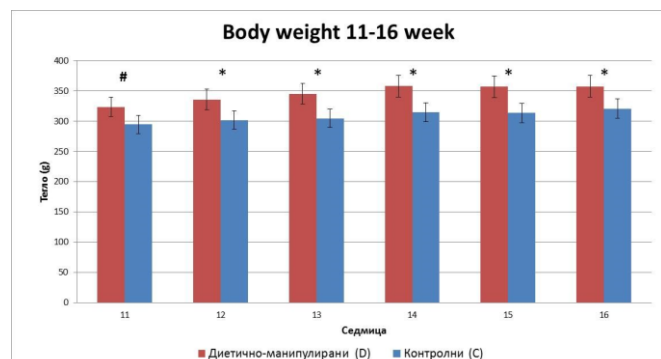
**Fig.1** Body weight of female and male rats (g) in the course of the experiment (\* $P < 0.05$ ).

By the tenth week the HFCD did not have any effects of on the weight of the experimental animals ( $P > 0.05$ ).

On the 11th week of application of the combined diet, a tendency for weight gain of test animals from dietary-manipulated groups compared with the controls was found ( $323.33 \pm 11.06$  g vs  $294.33 \pm 11.06$  g,  $P = 0.078$ ).

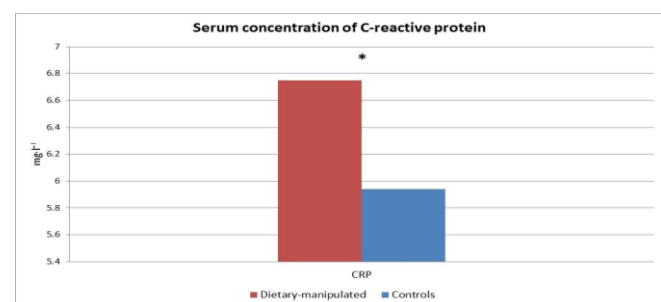
From the 12th week until the end of the experiment the HFCD had a significant main effect on body weight as dietary-manipulated animals had higher weight than controls ( $P < 0.05$ ). At the end of the trial, the dietary-manipulated animals had higher weight compared with the controls ( $357.33 \pm 12.24$  g vs  $320.42 \pm 12.24$  g,  $P < 0.05$ ), i.e. obesity

has been developed (Fig. 2). Analyzing the data of weight's changes during the experiment we did not find a significant interaction effect of the gender and the high-fat-carbohydrate diet.



**Fig. 2** Body weight of the experimental groups (g) from the 11th week until the end of the experiment # $P = 0.078$ , \* $P < 0.05$ , dietary-manipulated vs control groups.

Our data showed that gender as well as combined HFCD had significant main effects on the concentration of C-reactive protein. At the end of the experiment, female rats had higher serum CRP concentration compared with males ( $6.69 \pm 0.19 \text{ mg}\cdot\text{l}^{-1}$  vs  $6.00 \pm 0.19 \text{ mg}\cdot\text{l}^{-1}$ ,  $P < 0.05$ ) and dietary-manipulated rats had higher levels of CRP compared with controls ( $6.75 \pm 0.19 \text{ mg}\cdot\text{l}^{-1}$  vs  $5.94 \pm 0.19 \text{ mg}\cdot\text{l}^{-1}$ ,  $P < 0.05$ ) (Fig. 3). A significant interaction effect of the gender and the high-fat-carbohydrate diet was not found. Combined HFCD had a significant main effect on the IL-6 levels. The rats from the dietary-manipulated groups had increased concentration of interleukin-6 compared with the controls ( $63.02 \pm 12.26 \text{ pg}\cdot\text{l}^{-1}$  vs  $20.98 \pm 12.26 \text{ pg}\cdot\text{l}^{-1}$ ,  $P < 0.05$ ) (Fig.4). We did not find significant main effect of gender and a significant interaction effect of the gender and the high-fat-carbohydrate diet on the serum concentrations of IL-6 at the



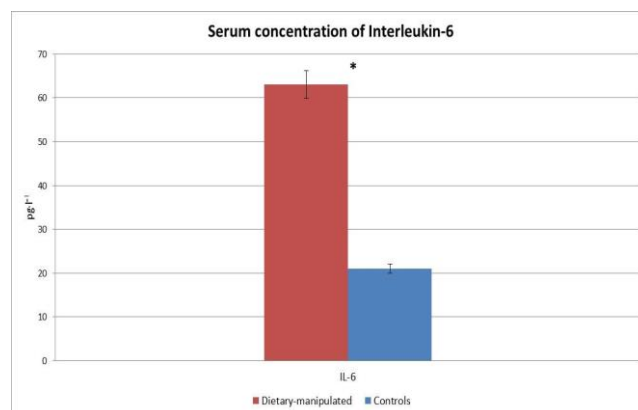
**Figure 3.** Serum concentration of C-reactive protein at the end of the experiment, \* $P < 0.05$ , dietary-manipulated vs control groups.

end of the experiment ( $P > 0.05$ ).

## Discussion

The obtained results show that 16-week application of combined HFCD leads to simultaneous increase in the serum levels of C-reactive protein and interleukin-6, which are markers of low-grade chronic inflammation in male as well as in female rats. Depending on the gender, the used diet had a different effect on the measured parameters – while the increase of IL-6 was similar in both sexes, the negative effect of the diet on CRP levels in female rats was greater. Basing on this we can suggest that low-grade inflammation is more obviously demonstrated in female rats. Obtained data show that the effect of the administered diet on the body weight depends on the duration of its application, and it is more demonstrative in male rats.

To our knowledge this is the first study to show increased serum concentration of CRP in intact female rats, treated with combined high-fat-carbohydrate diet. Similar higher levels of C-reactive protein have been measured in ovariectomized female rats compared with dietary-manipulated male rats with MetS (Balog *et al.*, 2015). Our results for male rats correspond with data from studies establishing elevated levels of C-reactive protein after 6 weeks administration of high-fat-high-fructose diet in male Wistar rats (Rodriguez Lanziet *al.*, 2016) and in male spontaneous hypertensive rats (Pravenec *al.*, 2011). Overall our data indicates that C-reactive protein is one of the features of obesity/metabolic syndrome in male and female rats and may be used as a diagnostic marker. The question arises whether C-reactive protein is responsible for the



**Fig. 4** Serum concentration of interleukin-6 at the end of the experiment, \* $P < 0.05$  dietary-manipulated vs control groups.

occurrence of the separate components of MetS or is secondary response only available during low-grade inflammation. A number of authors agree that the metabolic syndrome in humans is accompanied by a low-grade inflammation and CRP is the exact biomarker characterising it (Devaraj *et al.*, 2009). Pravenec *et al.* (2011) defend the hypothesis that C-reactive protein directly causes the

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characteristics of the MetS, rather than it is just a marker of inflammation in the syndrome. According to other authors C-reactive protein is responsible for adiposity and insulin resistance in the metabolic syndrome and is associated with cardiovascular risk and atherothrombosis (Rutter et al., 2004; Devaraj et al., 2009; Xi et al., 2011).

Our data combined with the data from other studies show that the rate of increased expression of interleukin-6 depends on the duration of application and the type of the used diet. Our results show that the combined HFCD, applied for 16 weeks, increases the concentration of IL-6 in rats in both sexes. Short-term application of different diets does not result in major changes in the serum levels of interleukin-6. The applied by Carvalho et al. (2016) cafeteria diet, for 10 days, did not affect the expression of IL-6, while a 6-week administration of high-fat diet leads to low-grade inflammation with slight increase of IL-6 levels (Crinigan et al., 2015). Similar to our results, Ajiboye et al. (2016) and Basaranoglu et al. (2013) reported elevated levels of interleukin-6 in diet-induced metabolic syndrome with high-carbohydrate diet in male rats. Zhou et al. (2014) found elevated levels of IL-6 in MetS induced after long-term (for 48 weeks) application of high-fat-high-sucrose diet in male Sprague Dawley rats, but in homogenates of epididymal adipose tissue, not in serum.

## Conclusion

The obtained results show that the 16-week administration of combined high-fat-carbohydrate diet increases the serum concentration of interleukin-6 and C-reactive protein and leads to the development of low-grade inflammation in both sexes. Changes in C-reactive protein levels were more pronounced in female than in male rats.

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