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Experimental gender related obesity effect of diet

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Abstract: Introduction: High-calorie diet is responsible for excessive weight gain. Obesity has recently become world epidemics, affecting not only adults but also children, which makes it the biggest health problem in the world. Yet the underlying mechanism remains a matter of debate.
Objectives: The aim of this study is to clarify the role of gender in high fat diet induced obesity in pups and adult animals.
Materials and methods: Female rats were fed low/ high fat diet during mating, pregnancy and lactation. The offspring and adult rats fed different diet had their body weight and temperature measurements taken twice a week. On the 21st day of the experiment the animals underwent anesthesia in order to have their blood samples collected for lipid profile.
Results: After 3 weeks on HF diet female pups body weight was higher than in control group ($p < 0.05$). Contrary to the female pups, the increase in body weight was higher ($p < 0.05$) in male pups and occurred after 2 and 3 weeks. In adult female rats body weight increased after 2 weeks on HF, while in adult male group such weight gain was observed no sooner than after 3 weeks. After three weeks of the experiment body weight was correlated positively ($r = 0.941$) with lipid profile of adult both gender groups on HF diet.
Conclusions: In male pups group body weight increased faster and achieved higher values then in female pups. On the contrary, in adult group of females body weight increased faster than in male rats and achieved similar values.

Key words: high-fat diet, obesity, rat lipid profile.

Introduction

Obesity is a widespread world pathology and in most countries it is connected with increase in fatty food intake. Several experiments proved that high-fat diet may lead to overweight of different models of obesity. Most articles describing the syndrome of obesity focus mainly on adult rats. There is less data considering gender differences and influence of diet on body weight in pups. Animal models are often used to detect factors involved in developing of obesity. Recently, two rodent animals such as mouse C57 with diet induced obesity or Zucker Rats with genetic obesity have mostly been used.

The consumption of high energy density, high-fat diet and hyperlipidemia are thought to be the main factors [1, 2]. Gender may play not less important role in this pathology. However, presented results are conflicting. As it was shown, chronic consumption of HF diet by female rats in their post weaning period resulted in significant increase in body weight and plasma levels of insulin, glucose, and triglycerides during pregnancy when compared to female rats consuming a standard LF rodent laboratory chow [3]. HF diet in pregnancy and lactation period increased the risk of obesity and caused subsequent metabolism impairment in adulthood. Pup rats of females on HF become obese in adulthood and manifest hyperleptinemia and hyperinsulinemia [4]. In contrast, studies with pregnant lactating dams on LF diet have higher level of triglycerides and cholesterol than dams fed HF diet. In their female and male pups the level of cholesterol did not increased within 20 days after birth. In longer period of observation; after 55 days both gender pups showed no differences in body weight [5]. According to all known results about HF diet and obesity Khan *et al.* [6] indicated that adult offspring (180 days) on HF diet demonstrated lower triglyceride concentration compared to control group and other authors did not show any changes in body weight after 6 weeks on HF diet [7]. Differences between the results in rodents obesity research are caused mostly by a different methodology. Variable levels of carbohydrates, protein and fat and also different period of feeding are yet unsolved questions.

Aim

The objective of the study was to show that high fat diet induces obesity and metabolic disorders in different gender in offspring and adult animals.

Materials and methods

Animals

Our experiments were carried out on rodent models because of the short duration of pregnancy and individual life. Female rats were fed low/high fat diet during mating, pregnancy and lactation. Our experiments were performed on 72 Wistar rats divided

into 8 groups. The sexes of pups were checked at the beginning of the experiment. Female offspring group was narrowed to eight individuals. First 4 groups consisted of 4 females with 32 pups. Two females were on low fat diet and the other two on high fat diet, fed for 21 days after birth. In the case of a larger than 8 number of pups in the litter the rest was left to adulthood. The next 4 groups were adult female and male subjects (8 rats each) with 2 groups on low, and 2 on high fat diet during 21 days.

The animals came from the animal facility of the Faculty of Pharmacy, at Jagiellonian University in Krakow. Animals were kept in a carefully maintained hygienic conditions in air conditioned room with constant temperature 21–25°C, with twelve–hour day-night cycle and ad libitum access to water and food. The experiment was approved by Local Ethical Committee of Jagiellonian University, Cracow, Poland (resolution No 84/2014).

Research groups and research protocol

According to the project assumptions, the animals were divided in eight groups:

Group 1 and 3 — Female and male pups with mother on LF diet for 21 day of the experiment.

Group 2 and 4 — Female and male pups with mother on HF diet for 21 day of the experiment.

Group 5 and 7 — Adult female and male rats on LF diet for 21 day of the experiment.

Group 6 and 8 — Adult female and male rats on HF diet for 21 day of the experiment.

The animals from all groups underwent the following procedures:

Procedure 1. **Animal weighing.**

The body weight was measured twice a week. This parameter was checked by using electronic scales (Scale OHAUS NAWIGATOR 2100/0.1g)

Procedure 2. **Temperature measurements.**

Body temperature was measured twice a week. To perform this procedure the infrared thermometer (Anima Vivari) was used, so as to minimize stress and pain in pups and also in adult rats.

Procedure 3. **Blood sampling.**

On the 21st day of the experiment animals underwent anesthesia and they were subject to the procedure of blood sampling. Blood samples were collected from cardiac vein of all the animals and in all groups. The samples were prepared for biochemical analysis.

Diet

The female rats with pups were placed in laboratory cages and they were fed diet inducing obesity — DIO (VERSELE-LAGA Opti Life Adult Active) with higher percentage of fat in the standard diet. The high fat diet contained: protein 32%, fat 22%, carbohydrates 40% compared to low fat diet (Labofeed B, Pasze Kcynia) that contained: protein 25%,

fat 8%, and carbohydrates 67%. The use of this specific chow was due to the availability of other scientific papers related to increased body weight in rats on the HF diet [8].

Laboratory analysis

During the experiment in all studied groups the following parameters were analyzed: serum concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Measurements were taken by enzymatic method for the cobas 80 000 analyzer. The equipment was previously calibrated. Samples were maintained for 40 minutes at laboratory temperature and then centrifuged in parameters of 2200 rpm/10 min/20°C (Heraeus Centrifuges. Megafuge 1.0 R) to separate serum.

Statistics

Statistical analysis was performed using the statistical package R 3.2.3.(R Development Core Team (2009). The measured values (weight, lipid profile) were described using the average value, standard deviation (SD), median, minimum, maximum and quartiles (Q1. Q3). Comparison of weight loss on subsequent days of measurements was performed using Student's t-test for paired samples with Bonferroni correction. Comparison of body weight and lipid profile in the two groups (male vs. female and LF diet vs. HF diet) was performed using Student's t test. To evaluate the correlation between body weight and the lipid profile Pearson correlation coefficient was used. The differences between groups were considered statistically significant for a probability level of $p < 0.05$.

Results

The study was performed on animals in different age and feeding variable contents of fat in chow. The temperature of the body among offspring and adult rats did not have impact on the results.

Weight gain in female and male pups on different diet

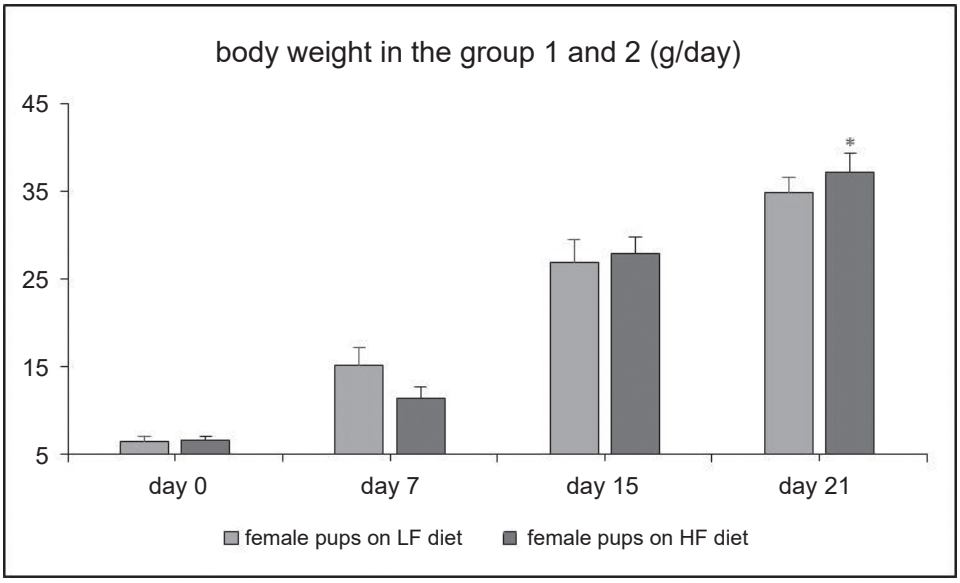


Fig. 1. Body weight of female pups on LF and HF diet by the 21st day of the experiment.

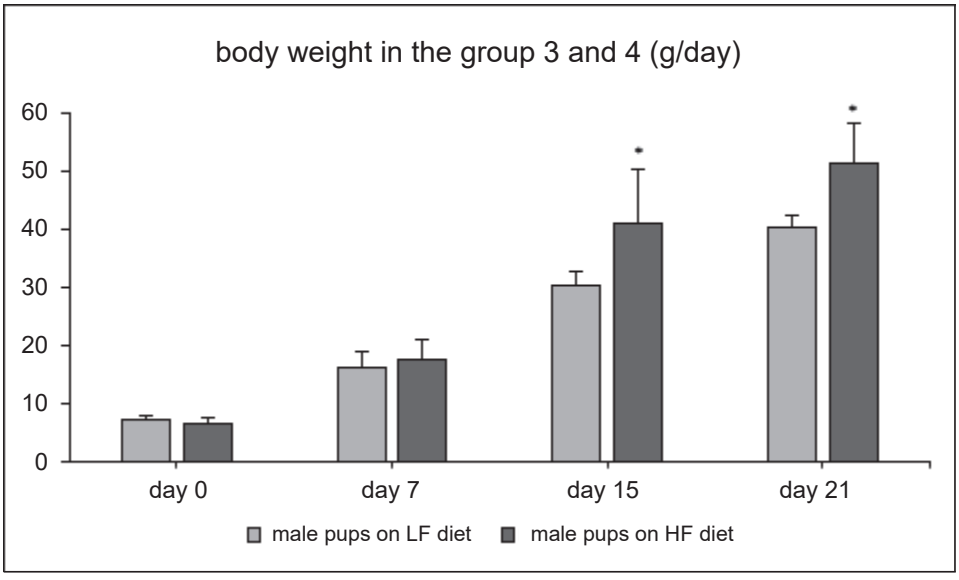


Fig. 2. Body weight of male pups on LF and HF diet by the 21st day of the experiment.

Laboratory analysis of lipid profile in offspring on HF diet

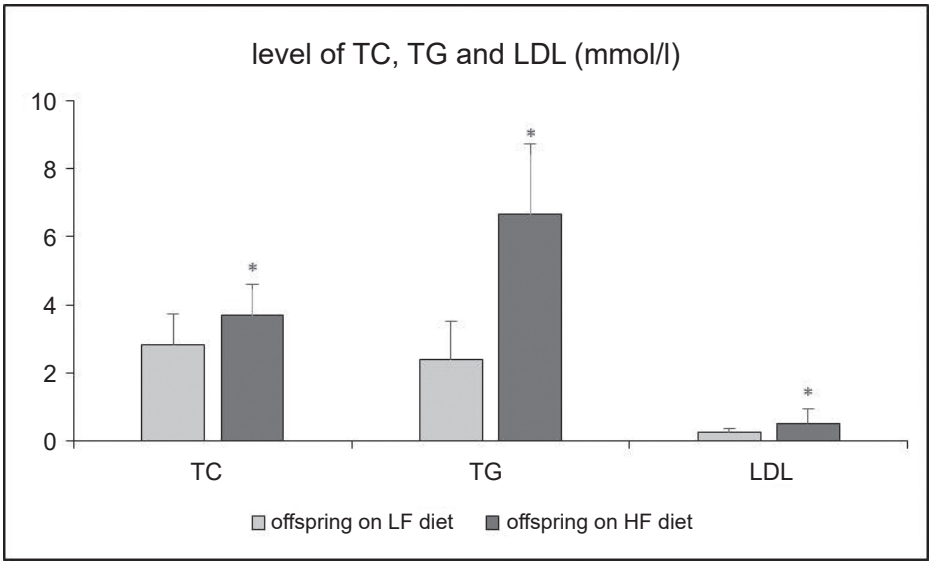


Fig. 3. The lipid profile content level of total cholesterol TC, triglycerides TG and high density lipoprotein LDL in both gender on low fat diet and high fat diet.

Weight gain in female and male adult on different diet

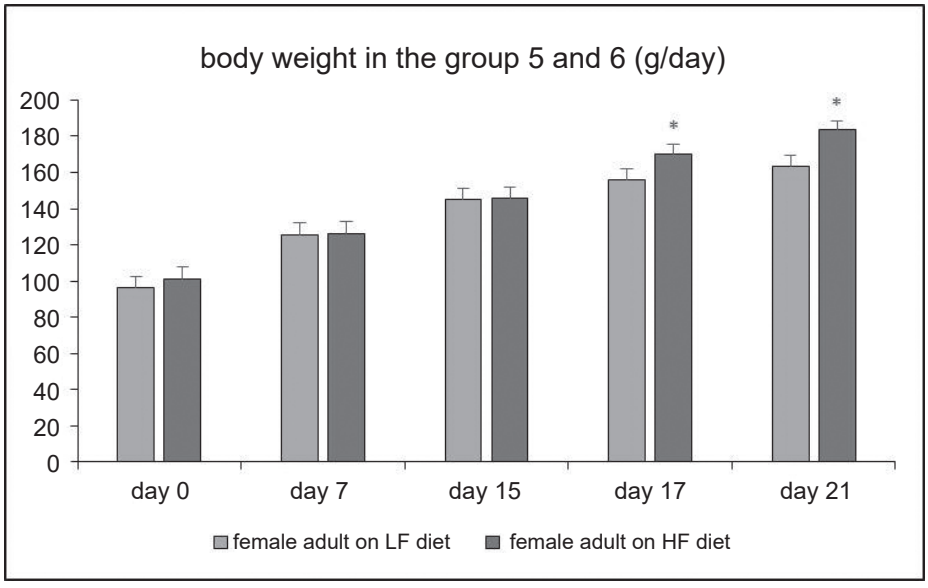


Fig. 4. Body weight of female adults on LF and HF diet by the 21st day of the experiment.

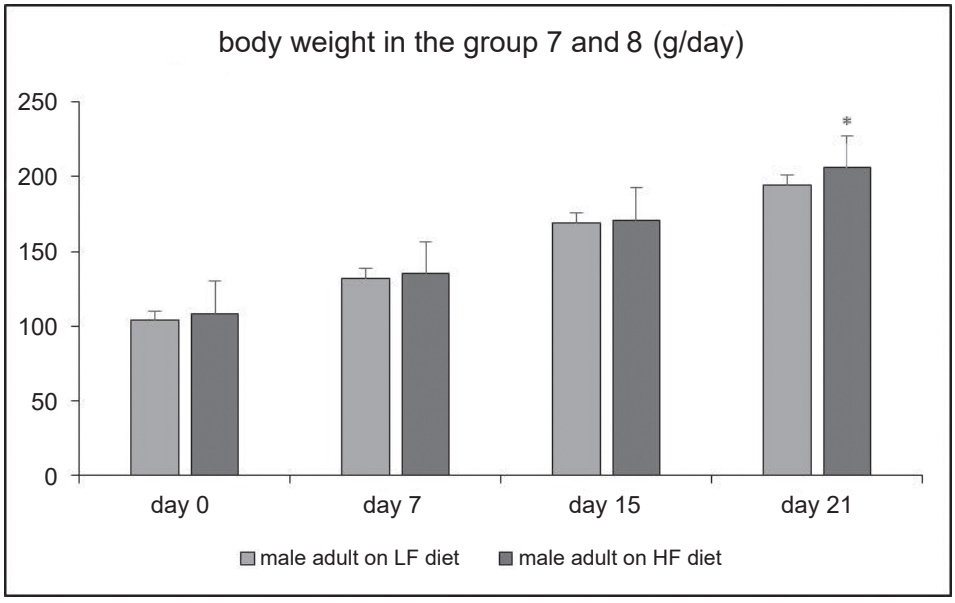


Fig. 5. Body weight of male adults on LF and HF diet by the 21st day of the experiment.

Laboratory analysis of lipid profile in adult rats on HF diet

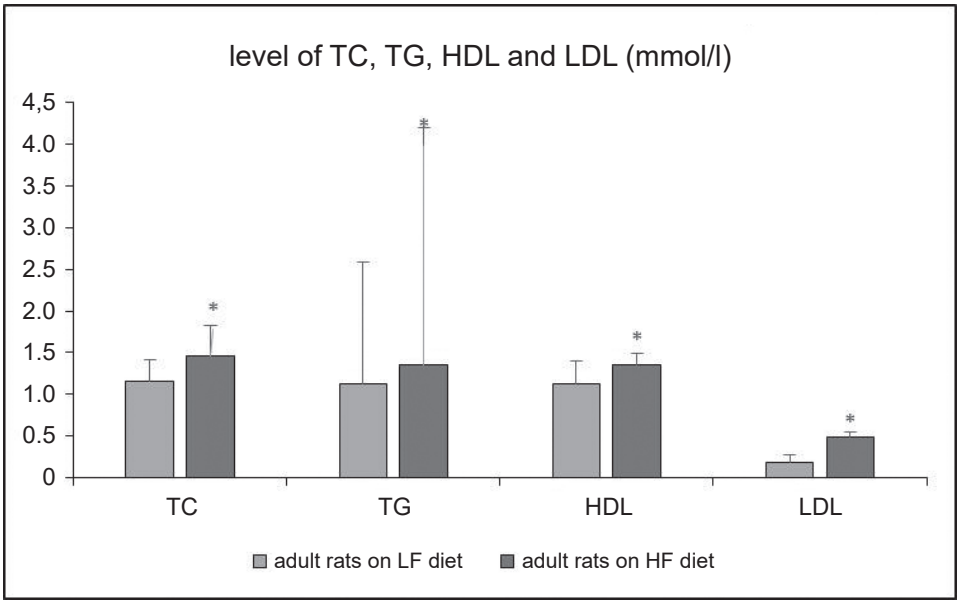


Fig. 6. The lipid profile content level of total cholesterol TC, triglycerides TG and high density lipoprotein HDL in adult rats both gender on low fat diet and high fat diet.

Table 1. Correlation of lipid profile with a body weight on day 21 of experiment in adult rats on HF diet.

Parameter (mmol/l)	Correlation of body weight on the 21st day of the experiment			
	Adult rats on HF diet-correlation	Direction of the relationship	Depending force	p value
TC	0.606	positive	strong	<0.013
TG	–0.693	negative	strong	<0.003
HDL	0.941	positive	very strong	<0.001
LDL	0.941	positive	almost perfect	<0.001

The experiment proved that HF diet during mating, lactation and childhood influences increased body weight. At birth male pups were heavier on LF diet (LF diet 7.12 ± 0.99 g vs. HF diet 6.75 ± 0.85 g) than pups on HF diet. While the female body weight at birth was comparable on two types of diet (LF diet 6.5 ± 0.53 g vs. HF diet 6.62 ± 0.52). The differences in weight of 10 day-old pups fed standard diet and inducing obesity were not significant ($p > 0.05$). Body weight of female pups was only significant on the last day of the experiment-21day, compared to LF diet (Fig. 1). In the following days (10–21) male pups fed diet inducing obesity were heavier than pups fed the standard diet ($p < 0.05$) Fig. 2.

The lipid profiles in female pups and male pups were not statistically significant. Total cholesterol, level of triglycerides, and level of LDL were more statistically significant in offspring on diet inducing obesity (Fig. 3). The fraction of HDL was not significant on different diet. It has been observed that lipid profile correlation with body weight of pups on LF diet and HF diet on the last day of the experiment-21 day was not significant ($p > 0.05$).

It was proved in the study that long term on HF diet (from birth to adulthood in rats) induced obesity and lipid disorders in adult rats. At the beginning male and female rats had different body weights. Male rats were heavy on the high fat diet (HF diet 108.5 ± 4.3 g vs. LF diet 104.38 ± 5.8 g). Body weight of females was also characterized by higher parameters on the chow, with higher content of fat (HF diet 101.25 ± 5.78 g vs. LF diet 96.62 ± 6.8 g). Body weight of female rats on days 17 and 21 of experiment was significantly higher than in female rats fed LF diet (Fig. 4). On the 21st day of the experiment, the body weight in adult male rats on HF diet was significantly higher than on standard diet (Fig. 5). The level of total cholesterol, triglycerides and fraction of LDL was higher in adult rats than in those on standard diet (Fig. 6).

Body weight of adult rats on HF diet on the last day of the experiment correlated significantly with all parameters of lipid profile (Table 1). Correlation with total cholesterol fractions, LDL and HDL is positive: the higher weight, the higher levels of TC, LDL and HDL. Body weight correlated the most strongly with LDL at day 21 of the experiment. Correlation with level of triglycerides is negative.

Discussion

Body weight of male and female pups on HF diet. Prenatal nutrition is an important element of proper development in childhood and in adulthood. Offspring fed a high content fat diet reveal an increased risk of obesity and metabolic disorders in adulthood. In our experiment, we examined the influence of gender on animals in different age on high fat diet and low fat diet. It was confirmed that maternal high-fat diet during mating, pregnancy and lactation induced obesity and early metabolic disorders in offspring in both gender male and female. In pups at birth differences in body weight dependent upon maternal diet were observed. It was the result of different number of pups in the litter. We noticed that only male pups on HF diet from postnatal day 10 were heavier than pups male on LF diet. Body weight of female pups was higher only in the last day of our study. Gender differences in weight gain in male pups are supported by biological background. It has been shown that adult male rats have higher body weight than females [9]. Our data are in agreement with Sun *et al.* [10] research who found that male and female pups HF-fed were heavier than those LF-fed, and this effect persisted in both sexes. In other study comparing the male pups on HF diet by postnatal day PND 7, they were heavier than those on maternal LF diet. This difference persisted through weaning on PND 21 [11].

Body weight of adult male and female on HF diet. Most of the reported research was based on HF and applies to male rats. There are fewer reports available about weight gain in females on HF diet. Our experiment shows that female rats on HF diet have higher body weight at the end of the experimental period (day 17 and 21) than on the standard diet. In contrast to our results in other experimental work on adult female rats, during 8 weeks of HF diet no weight changes were observed [12, 13]. The differences stem primarily from the fact that introduction of HF diet occurred in adulthood contrary to our study. There were also differences in time of the experiment and fat content in used chow (22% fat vs. 42% fat). In other studies which support also our results, only male rats on HF diet exhibited higher body weight compared with the normal chow diet groups during 3 weeks of the experiment [14]. Similar observation made with reference to male and female rats being on HF diet through the period of 18 weeks revealed increased body weight when compared to control group [15].

Lipid profile of male and female pups on HF diet. High levels of triglycerides, total cholesterol and low density lipoproteins in offspring on HF diet will be indicated in the future development of metabolic syndrome. In our studies we investigated the influence of gender and diet on the lipid profile in offspring. We proved that HF diet during our experiment led to increased parameters of lipid profile in male and female pups. We observed in both gender increased level of total cholesterol, triglycerides and fraction of low density lipoproteins on HF diet. Consistent with our results animal studies have proved that HF diet in male offspring caused elevated triglycerides on day 21 of the

experiment [16]. Our results are also confirmed by reports showing influence of HF diet during pregnancy and lactation. However, their results showed increased level only with reference to triglycerides but there was no difference in cholesterol values in offspring at 3 weeks of life. Similarly to our experiment gender does not affect the differences in lipid profile on HF diet [17]. Differences in cholesterol levels may be related to higher levels of carbohydrates (67% vs. 25.9%) and protein (32%vs 23.1%) but lower level of fat (22% vs. 34.9%) not presented as lard in our HF chow.

Lipid profile of adult male and female on HF diet. It is well known that high intake of food and increased amount of fat in the diet during pregnancy promotes the development of type II diabetes and cardiovascular disease in adult life [18]. The long time consequences of the adult progeny by high fat diet by female rats covers abnormalities such as impaired glucose homeostasis, cardiovascular dysfunction, and alterations in hypothalamic energy circuitry and liver lipid metabolism [19–21]. We proved that adult rats of both genders on HF diet have a pathological lipid profile. The level of cholesterol, triglycerides and LDL increased compared to group on LF diet. In contrast to our results, other authors data indicated that HF diet in male rats does not induce weight gain as well as increase in the level of total cholesterol [22]. Discrepancies with our results may be caused by later introduction of HF diet and composition of chow. In other studies in female rats being for 8 week on HF diet, the plasma cholesterol, high-density lipoprotein, low-density lipoprotein, or triglyceride levels did not increase [12, 13]. These incompatibilities in lipid profile result from other research methods, diet introduction, diet composition, and a shorter period of diet inducing obesity. Compared to our research Bełtowski *et al.* [23] confirmed our data and demonstrated that triglyceride and LDL levels have increased in the group of male rats on HF diet by 8 weeks. It can be assumed that only long-term consumption of HF diet causes not only obesity but also metabolic disorders and changes in the lipid profile.

In summary, it can be concluded that a diet with a high fat content causes obesity and metabolic disorders in both genders including offspring and adult rats. With reference to male pups, at the end of the experiment 30% increase in body weight over females on HF diet was reported. There was an increase of cholesterol, triglycerides and LDL in pups of both gender on HF diet. These results suggest future development of metabolic syndrome. Our study confirms well known findings that maternal obesity affects not only the offspring period but also further pups development with consequences of metabolic dysfunctions in adulthood. Our experiment added to the literature of the subject a positive correlation between increased body mass of male rats on HF diet and increased levels of cholesterol and LDL. In the development of metabolic disorders and obesity, participation of fat seems to be obvious but share of carbohydrates and proteins has not been studied well enough. It still remains the question of gender related particular pathomechanisms participation in the development of metabolic dysfunctions.

Conflict of interest

None declared.

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