Effects of the Application of a High-Fat-Carbohydrate Diet without Additional Cholesterol for the Inducement of Metabolic Syndrome in Rats

Penka Angelova, Nikolay Boyadjiev, Katerina Georgieva
Department of Physiology, Medical Faculty
Medical University of Plovdiv
Plovdiv, Bulgaria.

Dora Terzieva
Department of Clinical Laboratory, Faculty of Pharmacy
Medical University of Plovdiv
Plovdiv, Bulgaria.

Pepa Atanassova
Department of Anatomy, Histology and Embryology, Faculty of Medicine
Medical University
Plovdiv, Bulgaria.

Petar Hrischev
Medicine student, Faculty of Medicine
Medical University
Plovdiv, Bulgaria.

Abstract

The increase prevalence of obesity, metabolic syndrome, and associated diseases, is mainly related to the improper diet and reduced physical activity. The combined high-fat-carbohydrate diets (HFCD) are used for investigations on obesity and metabolic syndrome. This study was aimed at investigating the food intake, body mass index, some laboratory, and functional changes in the case of 16-week application of a combined high-fat-carbohydrate diet in rats. The application of HFCD led to a change of some functional indicators prior to the manifestation of metabolic syndrome. At 16th week the body mass of the dietary manipulated rats was not increased, but BMI and abdominal circumference grew. The basic laboratory indices relate to an increased risk of diabetes mellitus type 2, and development of cardiovascular diseases.

Keywords: High-Fat-Carbohydrate diet; metabolic syndrome; submaximal running endurance; rats

Introduction

The increase prevalence of obesity, metabolic syndrome, and associated diseases, is mainly related to the improper diet and reduced physical activity. Numerous studies of humans and animals underline the adverse effects of the so-called "Western diet", i.e. the excess consumption of meat and fructose (Lutsey, 2008; Rutledge, 2007; Wilson, 2007). Most physicians recommend avoidance of saturated fatty acids, restricted intake of cholesterol below 300 mg per day, and reduction of the intake of foods with high glycemic index (Krauss, 2000; Silva, 2011; Lopez-Legarrea, 2013).

The combined high-fat-carbohydrate diets (HFCD) are used for studies on obesity and metabolic syndrome. The type of the applied fat is very important, as lard is amongst the most effective fats (Ghibaudi, 2002; Buettner, 2006).

Objective

This study was aimed at investigating the food intake, body mass index, some laboratory, and functional changes in the case of 16-week application of a combined high-fat-carbohydrate diet in rats.

Material and Methods

Animals

Male Wistar rats (n=60), with initial body weight of 160-180 g, taken from the vivarium of the Plovdiv Medical University, were used in the experiment. The rats provided with food and water *ad libitum* and were housed in individual metabolic cages. They were raised at temperature 20 ºC ± 1 ºC, controlled humidity, and 12:12 h light-dark photoperiod. The experimental protocol was approved by the Bulgarian Food Safety Agency. The rats were raised and all experimental procedures were performed according to the recommendations of the European Commission on the protection and human treatment of laboratory animals.

All rats were initially fed with standard rat chow in the course of two weeks until they adapted to the metabolic cages. Since running on the treadmill is a skill which the rats should develop and maintain, prior to the experiment, all rats were trained on a treadmill for small test animals (Columbus Instruments, Columbus, Ohio, USA) for 5 min, with velocity of 27 m/min, 5º incline, three times a week. Such load does not cause adaptation changes, but it leads to the rats getting used to the running on a treadmill which allows the selection of those animals which run spontaneously (Lambert and Noakes, 1989). This training is required for the conduct of the coming functional tests. About 15% of the rats refused to run on a treadmill and were excluded from the study at the end of the preliminary stage.

The selected animals were divided into two groups: control group of animals taking standard rat chow (K, n=24), and experimental group with free access to combined high-fat-carbohydrate food with no added animal fats (D, n=24). During the whole period, all animals were trained on a treadmill to maintain the skill of running.

Diet

The control group had free access to standard rat chow provided by the vivarium of the Plovdiv Medical University (Table 1).

The dietary manipulated group had free access to combined high-fat-carbohydrate food produced by AMIKO OOD Company and supplemented with vegetable oils (Table 1).

Table 1. Macronutrient content of the food taken during the experiment.

	Group K g/kg	Group K En%	Group D g/kg	Group D En%
Proteins	134	18.5	134	14
Fats	34	10.5	128	30
Carbohydrates	516	71	540	56

The food of the control group had energy content 2908 kcal/kg, and that of Group D – 3860 kcal/kg. Both foods contained adequate quantity of micronutrients.

Body mass, BMI, amount of consumed food.

The body weight of each animal was measured three times a week.
The amount of food consumed by each animal was weighted three times a week.
The length of the animals was determined once a month by measuring the naso-anal distance.
Body mass index (BMI) was estimated once a month by the formula:
BMI = body mass (g) / (naso-anal distance (cm))^2

Functional tests.

At every fourth week, we conducted functional tests. All tests were performed following a two-day recovery period from the adaptation trainings.

Submaximal endurance

At the beginning of the experiment and at every 4th week, the animals were subject to a submaximal running endurance test (SRE) by Lambert. Treadmill for small test animals was applied (Columbus Instruments, Columbus, Ohio, USA). The SRE was determined at running on a treadmill with velocity of 27 m/min, 5º incline.

The time of reaching complete exhaustion and inability to retain longer the position on the treadmill belt was recorded as SRE.

Maximum time to exhaustion.

At the beginning of the experiment and at every 4th week, the animals were subject to a test for determination of the maximum time to exhaustion (MTE). Peak load was reached through a step-by-step increase of the velocity and incline of the treadmill track. Each step was with duration of three minutes (Georgieva and Boyadjiev, 2004). The rats were removed from the test when they were not able any more to retain their position on the treadmill. The time of reaching this condition was taken for the MTE.

Laboratory analysis.

On the 8th, 12th and 16th week, 8 animals of each group were decapitated under narcosis with 30 mg/kg Thiopental i.p. with a guillotine for small test animals (HUGO SACHS ELECTRONIC D-79232 March F.R. Germany), 12 hours after the last intake of food. Immediately following the decapitation of the experimental animals, mixed blood was collected for the conduct of laboratory analysis. The blood was centrifuged within one hour after the collection of samples. The serum levels of fasting blood glucose, cholesterol, triglycerides, LDL-chol, HDL-chol were analyzed with a biochemical analysis system Konelab 6.5 (Germany) at the Central Clinical Laboratory of Sv.Georgi University Hospital - Plovdiv. Reagents of Fortress Diagnostics (United Kingdom) were used.

Statistical analysis

The results are represented as X±SEM. The data of the experiment has been analyzed with Independent Samples Test and Paired Samples Test, statistical software SPSS v. 13.0. Difference at $P<0.05$ was accepted as significant.

Results and Discussion

Food intake.

Breeding of test animals in individual metabolic cages allows determination of the precise amount of consumed food. The food intake was measured three times a week and calculated for the different periods.

Table 2. Food intake (g) in the course of the experiment (X±SEM).

	1st – 4th week	5th – 8th week	9th – 12th week	13th – 16th week	1st – 16th week
К	722.5±16.85	438.92±8.41	637.23±18.41	395.14±33.30	2151.29±46.32
D	735.08±16.71	448.75±12.23	663.44±21.59	352.00±10.92	2155.50±57.59
P	NS	NS	NS	NS	NS

Start, 4th, and 8th week К (n=24), D (n=24); 12th week К (n=13), D (n=16); 16th week К (n=7), D (n=9).

We did not find a difference between the amount of consumed food between the groups, neither for the different periods, nor in respect of the total amount – 2151.29±46.32 g for group К, 2155.50±57.59 for D ($P>0.05$), (Table 2).

Group D took more fats as compared to Group К ($P<0.0001$). The total amount of consumed proteins and carbohydrates was similar with the two experimental groups for both the different periods and the overall period of study ($P>0.05$). The energy intake of fats was significantly higher with D ($P<0.0001$). The total energy intake was also higher with the dietary manipulated group ($P<0.0001$), while the energy taken from proteins and carbohydrates was equivalent ($P>0.05$ for both macronutrients), (Table 3).

Table 3. Amount of consumed proteins, fats and carbohydrates (g), and the respective energy intake (kcal) for the experiment (X±SEM).

	Consumed fats	Consumed carbohydrates	Consumed proteins	Energy intake from fats	Energy intake from carbohydrates	Energy intake from proteins	Total energy intake
K	73.14 ± 1.58	1110.06 ± 23.90	288.27 ± 6.21	665.61 ± 14.33	4440.25 ± 95.61	1153.90 ± 24.83	6255.94 ± 134.71
D	275.90 ± 7.42	1163.97 ± 31.29	288.84 ± 7.78	2510.73 ± 67.50	4655.88 ± 125.18	1155.35 ± 31.06	8320.23 ± 223.69
P	P<0.0001	NS	NS	P<0.0001	NS	NS	P<0.0001

K, n=7; D, n=8

Weight gain.

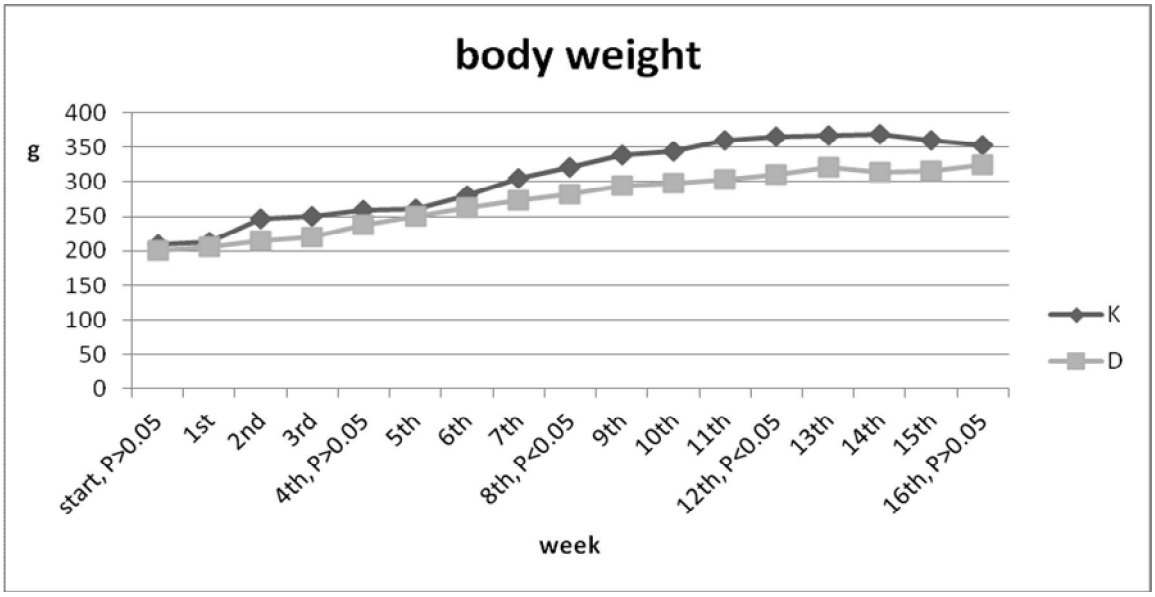
The body mass of the rats was followed up three times a week. We measured the weight gain of all rats. In the period 1st – 4th week, and 5th – 8th week, the weight gain of Group K was substantially higher than Group D (P<0.05). In the period 9th – 12th week, no difference between the cumulated body mass of the rats (P>0.05) was observed, and in the final period 13the – 16th week, Group D had a higher weight gain than Group K (P<0.05). There was no difference in the gain of body mass between rats (P>0.05) for the overall experiment, (Table 4).

Table 4. Weight gain of animals (g) in the course of the experiment (X±SEM).

Period	1-4th week	5-8th week	9-12th week	13-16th week	1-16th week
K	50.01±3.47	57.92±6.47	-2.12±45.45	-11.72±17.74	142.29±17.46
D	27.28±3.69	42.50±3.95	31.94±9.95	39.94±3.04	121.11±35.80
P	P<0.0001	P<0.05	NS	P<0.05	NS

Start, 4th , and 8th week K (n=24), D (n=24); 12th week K (n=13), D (n=16); 16th week K (n=7), D (n=9).

Chart 1. Values of body mass (g) of the test animals in the course of the experiment (X±SEM).



Start, 4th , and 8th week K (n=24), D (n=24); 12th week K (n=13), D (n=16); 16th week K (n=7), D (n=9).

The analysis of repeated study showed that the rats of K were gaining weight till the 12th experimental week (application of Paired Samples Test to find a difference in the value of body mass: start – 4th week, P<0.0001; 4th – 8th week, P<0.0001; 8th – 12th week, P=0.004; 12th – 16th week, P>0.05). As opposed to Group K, the rats of Group D continued to increase their body mass till the end of the experiment (start – 4th week, P<0.003; 4th – 8th week, P<0.003; 8th – 12th week, P<0.0001; 12th – 16th week, P<0.005).

Morphological characteristics.

At the beginning of the carried out experiment, the rats of Group K and Group D were with equal body weight (P>0.05). On the 8th and 12th week, the animals of Group K weighted more than the animals of Group D (P<0.05). At the end of the experiment, no statistical difference in the body mass of both groups (P>0.05) was observed, (Chart 1).

Chart 1. Values of body mass (g) of the test animals in the course of the experiment (X±SEM).

To determine the dynamics of the body mass of each animal, we applied Paired Samples Test to find any differences in the parameters which were estimated twice. The rats of Group K gained weight till the 12th experimental week: in the period 1st – 4th week P<0.0001; 5th – 8th week P<0.0001; in the 9th – 12th week P<0.001. The determination of difference in the body mass of Group K in the final stage did not register any difference in the values between the 13th and 16th week (P>0.05). As opposed to the control group, the animals of Group D retained the tendency to increase their body mass till the end of experiment : 1st – 4th week, P<0.01; 4th – 8th week, P<0.01; 8th – 12th week, P<0.0001, and in the final stage 12th – 16th week P=0.005.

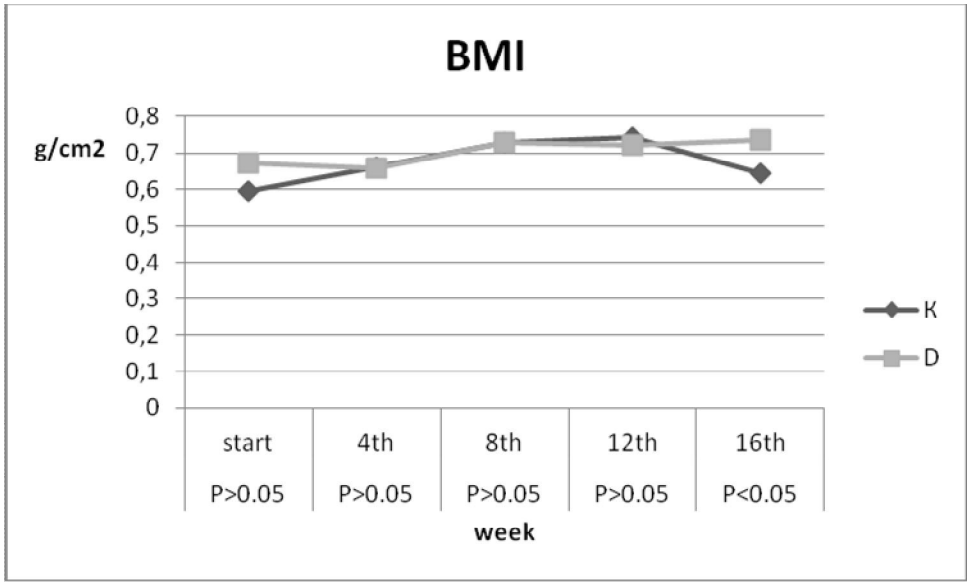
In the beginning of the study, the length of the animals of Group K and D was virtually equal (P>0.05). In the measurement of the naso-anal distance in the course of the experiment, we found that on the 4th, 8th and 16th week, the dietary manipulated rats had less length than the control values (P<0.05), (Table 5).

Table 5. Length of the test animals (cm) in the course of the experiment (X±SEM).

Week	1st	4th	8th	12th	16th
Group K	18.84±0.18	20.19±0.33	21.19±0.47	22.22±0.6	23.43±0.65
Group D	18.60±0.30	19.12±0.31	19.68±0.25	21.12±0.21	22.19±0.23
P	P>0.05	P<0.05	P<0.05	NS	P<0.05

Start, 4th , and 8th week K (n=24), D (n=24); 12th week K (n=13), D (n=16); 16th week K (n=7), D (n=9).

Chart 2 . BMI (g/cm^2) of the test animals in the course of the experiment (X±SEM).



Start, 4th , and 8th week K (n=24), D (n=24); 12th week K (n=13), D (n=16); 16th week K1(n=7), D (n=9).

The length of the rats of Group K, as compared to the initial values, and in previous periods, was growing till the end of the study: 1st – 4th week, P=0.001; 4th – 8th week, P<0.05; 8th – 12th week, P=0.002, and 12th – 16th week, P=0.002. The length of the dietary manipulated group was growing till the 12th week (P<0.05), however in the final stage, it stayed statistically unchanged (12th – 16th week, P>0.05).

BMI of the rats of Group K and D was equal till the 12th week (P>0.05) in the course of the experiment. After the 16th week, it was significantly higher for Group D against the control Group K, P<0.05), (Chart 2).

The comparison of the dynamics in BMI of the rats of Group K showed increase in the periods 1st – 4th week (P<0.05), and 4th – 8th week (P<0.01). In the next period (8th – 12th week), BMI of the healthy control animals remained statistically unchanged (P>0.05), and in the final stage, it decreased (P=0.003). The change of BMI of Group D was explicit in the 1st – 4th week (P<0.05), and 4th – 8th week (P<0.05). In the period 8th – 12th, it decreased with no statistical significance (P>0.05), in the period 12th – 16th week, BMI of Group D grew as a value, though insignificantly (P>0.05).

The application of a combined high-fat-carbohydrate diet did not result in the expected significant increase of body mass in Group D, on the contrary, on the 8th and 12th week the animals of Group K weighted more than the animals of Group D (P<0.05), (Chart 1), however because of the less length of the rats (Table 5) at the end of the experiment, Group D had higher BMI than Group K (P<0.05), (Chart 2).

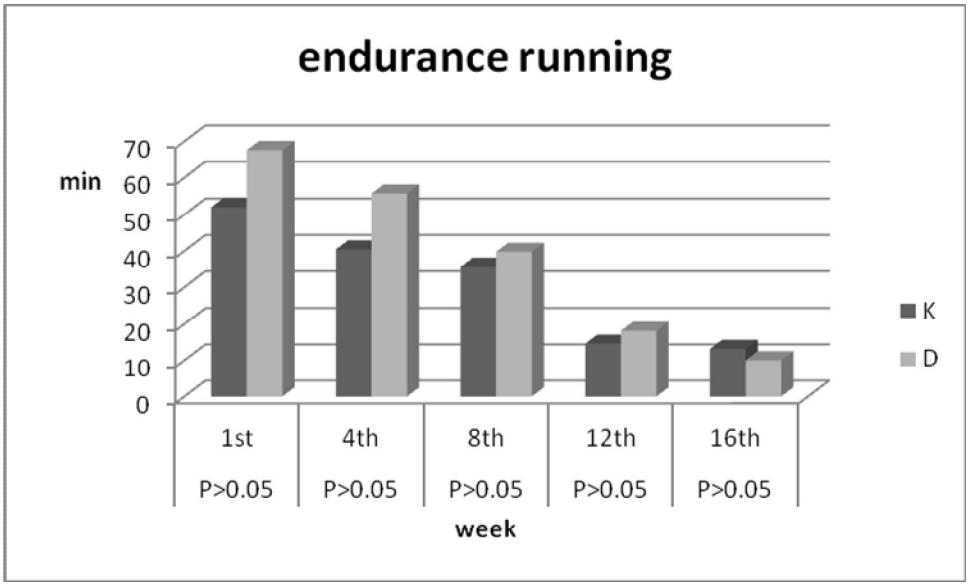
The follow-up of the abdominal circumference of both groups recorded progressive increase till the 12th week (comparison of the value in each animal till the 12th week for Group K and D for the described periods - P<0.05). In the period 12th – 16the week, the abdominal circumference of the control group did not increase significantly (P>0.05), however for the dietary manipulated animals, it grew higher (P<0.0001). On the 16th week the abdominal circumference of the rats of Group K (13.70±0.18 cm, n=7) was smaller than that of Group D (15.41±0.14 cm, n=8), (P<0.05), (Table 6).

Table 6. Abdominal circumference (cm) of the test animals in the course of the experiment (X±SEM).

Week	1st	4th	8th	12th	16th
Group K	10.66±0.12	11.55±0.14	12.26±0.18	13.16±0.16	13.70±0.18
Group D	10.50±0.15	11.80±0.16	13.06±0.20	14.26±0.14	15.41±0.14
P	NS	NS	NS	NS	P<0.05

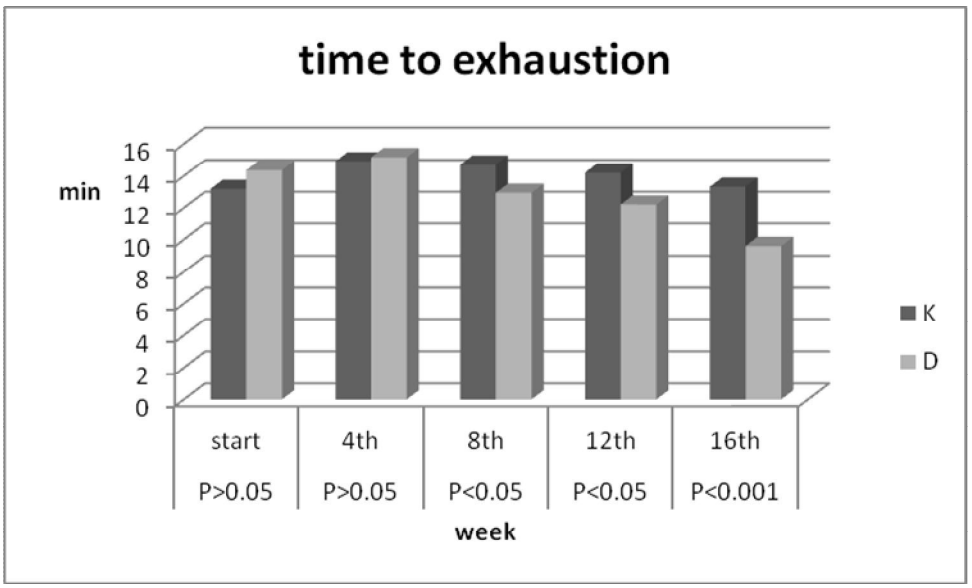
Start, 4th , and 8th week K (n=24), D (n=24); 12th week K (n=13), D (n=16); 16th week K (n=7), D (n=9).

Chart 3. SRE (min) of the animals in the course of the experiment (X±SEM).



Start, 4th , and 8th week K (n=24), D (n=24); 12th week K (n=13), D (n=16); 16th week K (n=7), D (n=9).

Chart 4. Maximum time to exhaustion (min) of the animals in the course of the experiment (X±SEM).



Start, 4th , and 8th week K (n=24), D (n=24); 12th week K (n=13), D (n=16);16th week K (n=7), D (n=9).

Submaximal endurance

The endurance of the organism is defined as a capacity to keep the loading of a certain power (velocity and strength) of work as long as possible. The main potential mechanisms of the occurrence of fatigue during physical loading are divided into central and peripheral ones, however in the case of submaximal prolonged loadings, most of the studies show that the fatigue sets in the very muscles, and to a great extent it is related to the aerobic processes of energy supply, metabolic accumulation, and exhaustion of the muscular glycogen (Fitts, 1994). The endurance in submaximal loadings depends on the potential of the organism to provide sufficient energy for muscular contraction through aerobic oxidation of the energy substrates – fats and carbohydrates. SRE is taken as a main indicator of the aerobic capacity of the body (Miller et al., 1984; Lambert, 1989). The comparison between the values of SRE with Independent samples Test of Group K and D did not show any differences in the course of the experiment (Chart 3).

Chart 3. SRE (min) of the animals in the course of the experiment (X±SEM).

Due to individual differences in the value of SRE, specific of Wistar rats, the results were subject to pair analysis. In Group K SRE did not change till the 8th week (starting value – 4th week and 4th – 8th week, P>0.05). The comparison of the result of the 8th week with that of the 12th week show a significant decreased (P=0.005). The time of run on the 12th week was equal to the final week (P>0.05). In Group D the application of Paired Samples Test did not find any variations between the starting SRE and that of the 4th week (P>0.05), and in the comparison of the results of the 4th with the 8th week (P>0.05). SRE of the 12th week was lower than the 8th week (P=0.002). As opposed to Group K, in Group D it continued to decrease (16th vs. 12th week, P=0.001).

Maximum time to exhaustion

The starting values of the maximum time to exhaustion in Group K and D were virtually equal. The application of a combined high-fat-carbohydrate diet without added cholesterol led to decrease of the value of this indicator as early as the eighth experimental week (Chart 4).

Chart 4. Maximum time to exhaustion (min) of the animals in the course of the experiment (X±SEM).

Laboratory analysis.

Until the 12th experimental week, no statistically significant difference in the value of the studied indicators between the two groups was observed. On the 16th week the fasting blood glucose and serum triglycerides in Group D were higher as compared to Group K, HDL-cholesterol was reduced for D (P<0.05). The value of the total cholesterol and LDL-cholesterol did not record any differences (P>0.05), (Табл. 7).

Table 7. Value of some laboratory indices (mmol/l) on the 16th week (X±SEM).

	Glucose	Total cholesterol	Triglycerides	HDL-cholesterol	LDL-cholesterol
K	6.79±0.16	1.30±0.08	0.91±0.08	0.41±0.04	0.41±0.14
D	7.84±0.41	1.35±0.07	1.60±0.55	0.38±0.02	0.43±0.02
P	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05

K, n=7; D, n=9

Based on the presented data, we can draw the conclusion that when metabolic syndrome is induced with HFCD without additional cholesterol, a change of some functional indicators occur prior to the manifestation of the condition.

The performed experiment does not support the data that the effect of high-fat diets on the body mass is dose-dependent (Ghibaudi, 2002; Gajda, 2007). HFCD that we applied without added cholesterol led to retention of the growth of the rats of Group D, which on the 8th and 12th week even had lower body mass as compared to the healthy control ones. In the literature we haven't found data of low weight gain in the case of high caloric and fatty intake at the background of sedentary lifestyle, which could be a subject of further studies.

At the end of the program BMI was higher in the dietary manipulated rats. The abdominal circumference grew. MTE of Group D decreased. SRE showed a tendency to decrease.

The basic laboratory characteristics did not change until the 12th experimental week regardless of the high intake of fats, mono- and disaccharides. These laboratory values support the assumptions of the necessity of long-term application of high-fat diet for unlocking of dyslipidemia and manifestation of insulin resistance (Ghibaudi, 2002; Nygaard Madsen, 2010; Levin, 1997; Levin 1998; Rossimeisl, 2003). The increased intake of saccharose, glucose and fructose in our model was not sufficient to confirm the data of rapid development of insuline resistance in mono- and disaccharide loading (Pagliassotti, 1996; Pagliassotti, 2000).

On the 16th week Group D had high fasting blood glucoe, high serum level of triglycerides, and low HDL-choletsreol, which referred to increased risk of development of diabetes mellitus type 2 and cardiovascular diseases. The obtained results match the results from the application of high-fat diets (Kim, 2000; Stachon, 2006; Buettner, 2006). The alterations that we reported also confirm data of the use of high-carbohydrate diets for inducement of obesity and metabolic syndrome (Bezarra, 2000; Chicco, 2003; Bizeau, 2001), and of studies with HFCD (Deng, 2007; Rajsekar, 2007; Lene, 2010; Panchal, 2011).

Conclusion

The application of HFCD resulted in alteration of some functional indicators prior to the manifestation of metabolic syndrome. On the 16th week the body mass of dietary manipulated rats was not increased, however BMI and abdominal circumference grew. The main laboratory indices refer to an increased risk of diabetes mellitus type 2, and development of cardiovascular diseases.

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