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Not OL Replice	Aerobi	oic Capacity of Rats Subjected to a Combined High-Fat-Carbohydrate Diet					
KEYWORDS	Aerobic capacity, High-Fat-Carbohydrate Diet, Metabolic syndrome, Rats						
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ABSTRACT The metabolic syndrome (MetS) is a combination of interrelated risk factors which increase the risk of cardiovascular diseases and diabetes mellitus type 2. The increased frequency of that condition and the diseases associated thereof is attributed to the unhealthy dietary behavior during the last decades. There exist combined high-fat and high-carbohydrate diets for the inducement of the disease in rats which successfully imitate the pathology in humans. The reduced aerobic capacity is considered an unfavorable prognostic marker in cardiovascular accidents and as regards longevity. The data obtained indicate a decrease of submaximal running endurance capacity upon application of high-fatcarbohydrate diet as early as the 4th week and a substantial reduction of VO2max in the 16th week. The results show worsening of the aerobic capacity and are concurrent with an increased cardiovascular risk in rats with developed diet-induced obesity.

Introduction

The metabolic syndrome (MetS) is a combination of interrelated risk factors which increase the risk of cardiovascular diseases and diabetes mellitus type 2. The increased frequency of that condition and the diseases associated thereof is explained by the unhealthy dietary behavior during the last decades [1, 2]. In the study of MetS, dietary, genetic and pharmacologic animal models are used [3, 4, 5, 6, 7, 8, 9]. Combined high-fat and high-carbohydrate diets for inducement of the disease in rats have been developed, which imitate successfully the pathology in humans [10, 11, 12]. They help induce obesity, impaired glucose tolerance, dyslipidemia, endothelial dysfunction, liver and heart muscle damage [10, 11, 12]. The reduced aerobic capacity is considered an unfavorable prognostic marker in cardiovascular accidents and as regards longevity [13, 14].

Aim

This study was aimed at examining the change of aerobic capacity in rats on a combined high-fat and high-carbohydrate diet during a period of 16 weeks. It is part of a complex study of changes in certain morphological, functional, and laboratory indices at various stages of MetS inducement.

Materials and Methods Animals

Male Wistar rats (n=80) with initial body mass of 160-180 g were used. They were maintained at an ambient temperature of 20 ° \pm 1 °C, controlled humidity, and a 12:12 h light-dark photoperiod. The experimental protocol was approved by the Commission for Ethical Treatment of Animals at the Bulgarian Food Safety Agency. The rats were reared and all experimental procedures were performed according to the recommendations of the European Commission for the protection and humane treatment of laboratory animals.

Since running on a treadmill is a skill which the rats should develop and maintain, prior to the experiment all rats were trained three times a week on a treadmill for small test animals (Columbus Instruments, Columbus, Ohio, USA) for 5 min, with a velocity of 27 m/min, at a track inclination of 5°. Such workload induces no training adaptations but familiarizes the rats with treadmill running and allows selection of rats which run spontaneously [15]. This training session is necessary for the conduct of the forthcoming functional tests.

Compliant animals were randomized into two groups: a con-

trol group on standard rat chow (K, n=20), and a dietary manipulated group, which had free access to a combined high-fat-carbohydrate food with added animal fats (D, n=60). During the whole period, all rats were trained on a treadmill for small test animals trice weekly with same intensity as preliminary period.

Diet

The control group received standard rat chow provided by the vivarium of Plovdiv Medical University and water *ad libitum* (Table 1).

The dietary manipulated group had free access to a combined high-fat-sucrose diet (High-Fat-Sucrose Diet, HFSD) prepared on-site (Table 1).

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	Group K g/kg	Group K En%	Group D g/kg	Group D En%
Proteins	134.5	18.5	134.5	12.51
Fats	34.0	10.5	144	30.15
Carbohy- drates	516	71	616	57.34

Table1.	Basic properties	of	the	food	taken	during	the	ex-
perime	nt.					-		

The food of the control group (K) had an energy content of 2908 kcal/kg, while group D had 4298 kcal/kg.

To avoid possible gastrointestinal disorders, the rats selected in the dietary manipulated group (D), gradually passed from standard to HFSD. In the first five days they took food with 20 en% fat then for another five days they had access to food containing fats 25 en%. The transitional diets were prepared on-site.

Functional tests

At the beginning of the experiment and on every 4th week, eight animals of each group were subject to the submaximal running endurance test (SRE) of Lambert [15]. SRE was determined in rats by running on a treadmill with a velocity of 27 m/min and 5° declination (which is about 70-75% VO_{2ma}) until they could no longer maintain their position on treadmill belt. The time taken to reach this stage was assessed as SRE. The tests were always carried out after two days recovery period.

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At the beginning of the study and on the 8th, 12th, and 16th week eight animals of each group were subjected toVO_{2max} test. The parameters were measured using the Oxymax gas analyzing system for small animals (Columbus Instruments, Columbus, OH, USA). The tests were always carried out after two days recovery period. The volume of the supplied air was 4.5 l/min. The animals were put into an isolated chamber for 10 min prior to the start of the actual loading. The VO_{2max} test protocol involved stepwise increasing of the treadmill speed and elevation. Each step of exercise was with duration of three minutes. Rats were removed from the test either when they could no longer maintain their position on the treadmill belt or in the plateau phemenon. The maximum value of VO₂ at the highest level achieved during loading, was accepted as VO_{2max} [16].

Dietary resistant group

Thirteen weeks after the beginning of the experiment we found that 13.3% (n=8) of the rats from group D had low body mass. The latter were considered a dietary resistant group (Group DR). The body mass of DR in the 12th experimental week was 265.25 \pm 5.04 g. That value was lower by 20.2% than the control group, and lower by 41% than the rest of the dietary manipulated animals. In the 16th week the rats from DR were subjected to tests for determination of VO_{2max} and SRE.

Statistical analysis

Results are presented as mean \pm SEM. Data of the experiment are analyzed by Independent Samples t-Test, SPSS v. 13.0.

Results and discussion

Submaximal running endurance

Endurance is defined by the ability to sustain loading of a defined value (velocity and strength) as long as possible. The main potential mechanisms of the fatigue onset during physical loading are divided into central and peripheral. However, in the case of submaximal prolonged loadings, most of the studies indicate that fatigue sets in the muscles, and is related to a great extent to aerobic energy supply, metabolic accumulation, and depletion of muscular glycogen [17]. Endurance in submaximal loadings depends on the capacity to secure sufficient energy for the muscular contraction through aerobic oxidation of the energy substrates, i.e. fats and carbohydrates. SRE is considered a main index of the aerobic capacity of the body [16, 15].

At baseline, the rats of two groups had similar SRE (P>0.05). The application of HFSD in Group D resulted in a decrease of SRE as compared to the healthy controls from K as early as the 4th week (p<0.05), (Chart 1). At the end of the study the rats from Group DR ran a shorter amount of time than Group K, but the value was not significantly different (K/DR, p>0.05). In comparison with Group D, Group DR showed better results (D/DR, p<0.001).

Chart1. Submaximal running endurance (min) during the course of the experiment ($X\pm$ SEM).



Oxygen consumption

At the beginning of the study rats of two groups had similar VO_{2max} . Until the 12th week there was no statistically significant difference between Group **K** and D (p>0.05). At the end of the study the dietary manipulated group (D) had lower VO_{2max} as compared with the healthy control group (K) (p<0.05). In comparison with Group D, the dietary resistant group achieved higher VO_{2max} for Group **K** and DR did not show any significant difference (**K**/DR, p>0.05).





K, n=8; D, n=8; DR, n=8

The results obtained indicate a decrease of SRE following the application of HFSD as early as the 4th week and a significant reduction of VO_{2max} in the 16th week. The results show impairment of the aerobic capacity and concur with an increased cardiovascular risk in the rats with developed dietary obesity. The study supports published data according to which not all Wistar rats develop obesity during prolonged feeding with a high-energy diet [18, 19]. The results of the functional tests of Group DR come closer to those of the healthy control animals, and are better than the test results of the animals which increased substantially their body mass.

Conclusion

The application of a combined HFSD for the purpose of inducement of a metabolic syndrome in rats causes a decrease of the aerobic capacity of rats which have become obese. The animals with induced dietary obesity had a restricted SRE as early as the 4th week and significantly reduced VO_{2max} in the 16th week. The condition concurs with a poor prognosis in the presence of a metabolic syndrome and cardiovascular disease. The rats resistant to obesity in high-energy feeding retained their aerobic capacity.

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