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EFFECT OF HIGH-PROTEIN DIET ON GLYCOLYTIC PROCESSES IN SKELETAL MUSCLES OF EXERCISING RATS

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The aim of the study was to assess the effort capacity of rats subjected to combined effects of high-protein diet (protein 49%; carbohydrate 30%) and endurance training. The measure of the animal fitness was the time of running to exhaustion on a treadmill. The changes in carbohydrate content including hepatic and muscular glycogen, peripheral blood glucose concentration and activity of some glycolytic enzymes in red slow oxidative (SO) and white fast glycolytic (FG) fibres of the gastrocnemius muscle were analysed. Samples of skeletal muscles, liver and blood were taken after one month on the high-protein diet and/or physical exercise. A high protein level in the diet caused no change in the effort capacity of rats but decreased the capability of carbohydrate accumulation in the skeletal muscles. The observed disturbances of the post-exercise activity of hexokinase and pyruvate kinase (in both types of fibres) and phosphoglycomutase (specially in FG fibres) suggest that the protein enriched died limits the extent of glycolysis processes. Despite of this the effort capacity of animals is kept on the same level as in the case of a standard diet. These results suggest that some other metabolic adaptations were developed which allow to continue the exercise.

Key words: *carbohydrate metabolism, effort, glycogen, glycolytic enzymes, high-protein diet, muscles, training.*

INTRODUCTION

It is well documented that appropriate composition of a diet has an important influence on the accumulation of energy substrates in tissues and in consequence the effort capacity of animals and humans can be altered (1—3). Particularly favourable effects are observed in relation to high-carbohydrates diets. It has been noted that soon after a few days' high-monosaccharide and/or polysaccharide diet the time of work to exhaustion was prolonged by about 8% (4, 5).

A long-term endurance exercise leads to muscle damage due to increased protein degradation (6, 7). Some authors believe that in order to avoid disturbances in protein metabolism induced by exercises, the level of protein in the diet should be increased (8).

However, there is another aspect related to high-protein diet treatment. It appears that a high-protein diet may have an unfavourable effect on the metabolic adaptation to the effort. Okitolonda *et al.* (9) noted that prolonged feeding a high-protein diet can result in decreased capability of carbohydrate accumulation. Also, the results of our previously published studies indicate that an increased protein content in the diet impaired the accumulation of glycogen in muscles as well as the possibility of glucose utilization due to decreased hexokinase activity (10).

The purpose of the present study was to determine the effect of four-week feeding of a high-protein diet (HP) on the effort capacity of rats and to estimate the participation of carbohydrate sources in the utilization of energy stores during exercise to exhaustion. Additionaly, the combined effect of a HP diet and endurance training on those processes in skeletal muscles was investigated.

MATERIAL AND METHODS

The study was conducted on male Wistar rats initially weighing ca 100 g ± 10%, divided into three groups: I-sedentary-control group of 24 animals. Half of them received a standard diet containing 60,5% carbohydrates and 21% protein. The other half was given a high-protein diet with 49% protein and 30% carbohydrates. The fat content was the same in both diets. Group II (sedentary-exhausted) was fed identical diets but at the end of the experiment the rats were exercised to exhaustion on a moving track. The track was motor driven at no incline (0% grade) and moved at a speed of 27 m/min. The animals were exercised every other day on a treadmill for about five minutes at a speed the same as in the final exercise. Group III (trained-exhausted) was subjected to an additional endurance training. Everyday, in the morning, the animals ran for 60 minuts on treadmill at no incline and at a speed of 27 m/min.

The experiment lasted four weeks. During that time the animals were kept at room temperature with a light/darkness cycle 12:12-h. Food and water were provided *ad libitum*.

At the end of the experiment, all groups of rats were killed by spinal cord dislocation, at the same time of day (between 8 am and 12 noon). Blood samples were taken directly from the heart. Samples (10—20 mg) of the red and white parts of the gastrocnemius muscle slow oxidative (SO) and fast glycolytic (FG), respectively and the liver were then rapidly excised. The SO and FG samples were taken from the deep portion of the lateral head and peripheral portion of the medial head of the gastrocnemius muscle, respectively. The muscle and liver samples were weighed and frozen in dry ice. The time between taking of all samples and freezing them did not exceed 2 minutes. All the samples were stored at −20°C until time of assay.

The concentration of glycogen in liver and muscles was measured by method of Lo *et al.* (11), the activity of phosphoglucomutase EC 2.7.5.1., hexokinase EC 2.7.1.1. and pyruvate kinase EC

2.7.1.40 was determined according to Bergmeyer (12), and peripheral blood glucose concentration was measured using Cormay test.

The measure of the effort capacity in the group II and III was the time of running to exhaustion. The animals were placed on the treadmill with electrical stimulation and forced to run until exhaustion. Exhaustion was defined as the state when the animals could not hold themselves on the treadmill for longer than 10 s.

The results are reported as mean ± SE, and the significance of differences between the groups was determined using t-Student test (13).

RESULTS

The mean time of running to exhaustion was nearly the same in the sedentary group of rats fed the standard diet and high-protein diet (*Fig. 1*). A four-week training increased about 60—70% the time of running to exhaustion. This effect was similar in both differently fed groups.

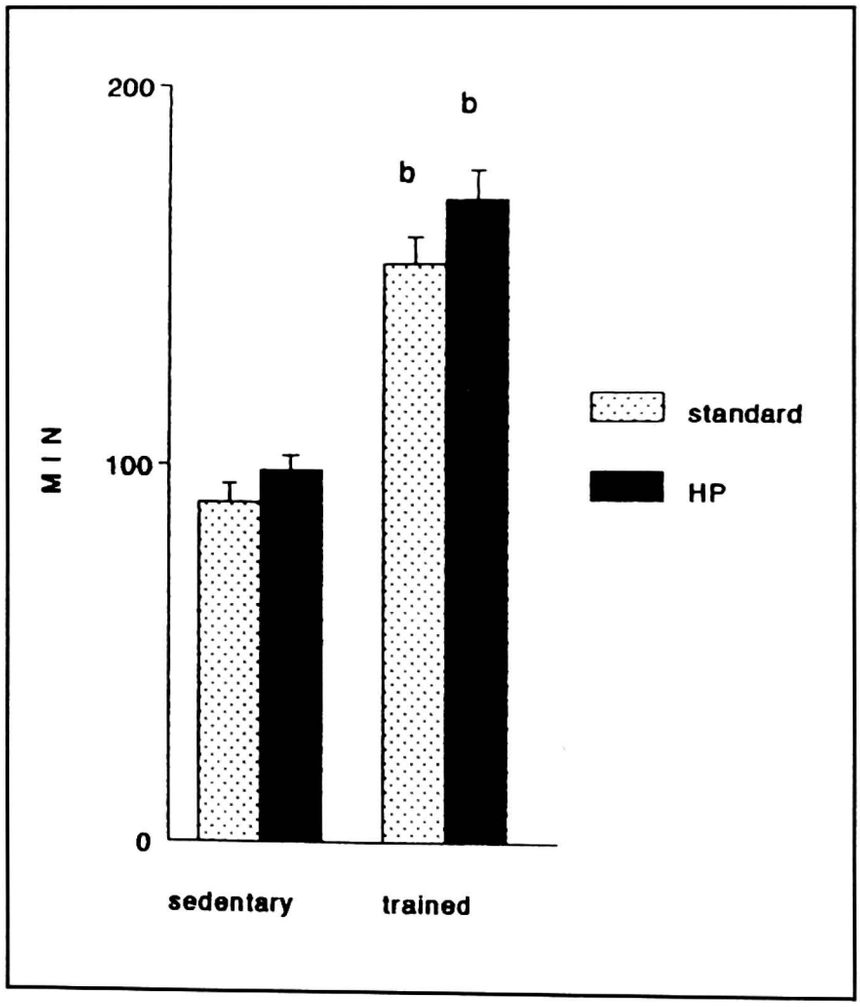

Fig. 1. Time of exhaustion of sedentary (II) and trained (III) rats fed standard and high-protein diets. Values represents means ± SE (min) b — significantly different ($p < 0.05$) between sedentary (II) and trained (III) groups.

As shown in *Fig. 2* exercise produced in the sedentary-exhausted rats a very high glucose level rise which was higher after the standard diet, than after the HP diet. In the trained-exhausted group fed the standard diet the post-exercise glucose concentration was also significantly higher than at rest.

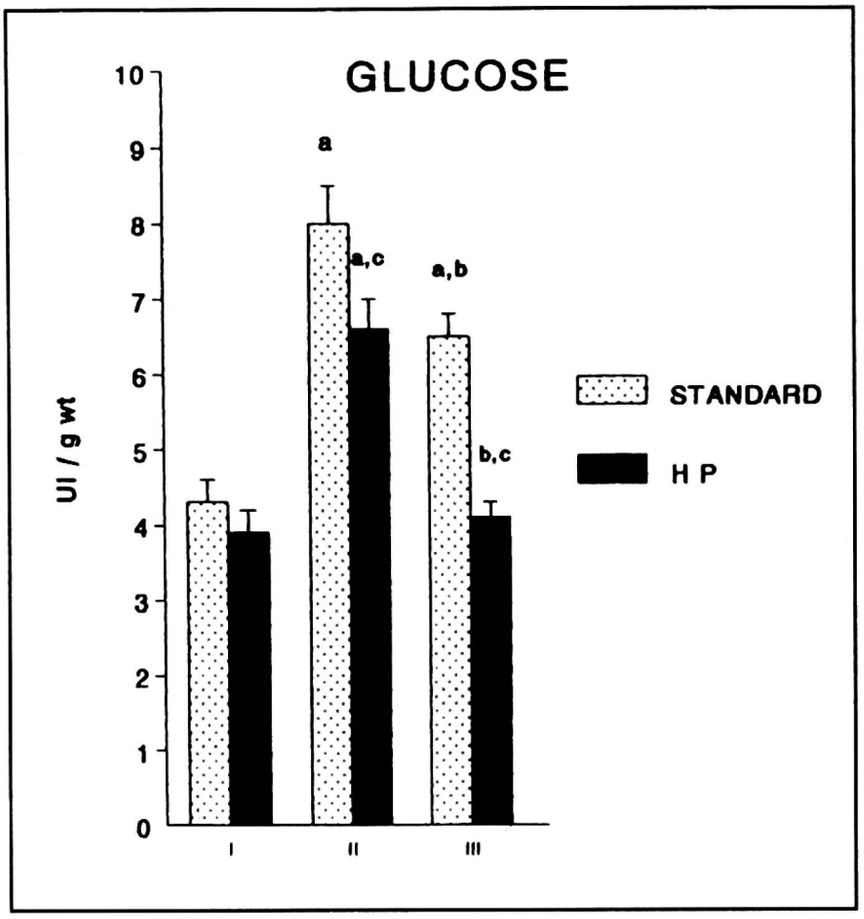

Fig. 2. Mean glucose concentration (mmol/l) in peripheral blood of control (I), sedentary-exhausted (II), and trained-exhausted (III) rats fed standard and high-protein diets. a — significantly different ($p < 0.05$) between I and II; b — significantly different ($p < 0.05$) between II and III; c — significantly different ($p < 0.05$) between standard and HP diet groups.

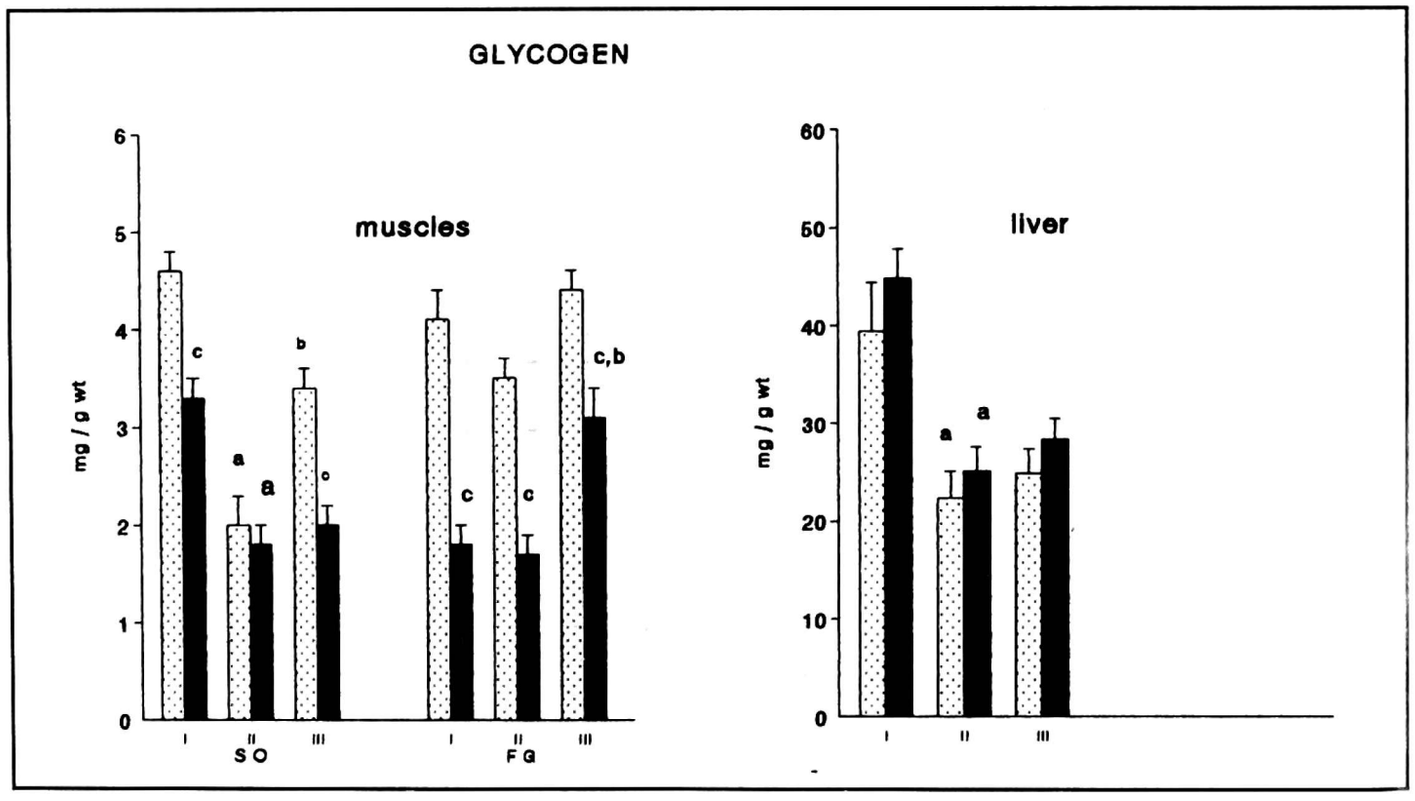

Fig. 3. Mean glycogen concentration (mg/g wet of tissue) in SO and FG fibres of gastrocnemius muscle and liver Designation as in *Fig. 2.*

The gastrocnemius muscles glycogen concentration was reduced significantly in the HP diet rats both in SO and FG fibres by 30 and 50%, respectively, as compared to standard diet (*Fig. 3*). Animals from group II had significantly less glycogen in the liver and in both types of muscle fibres than

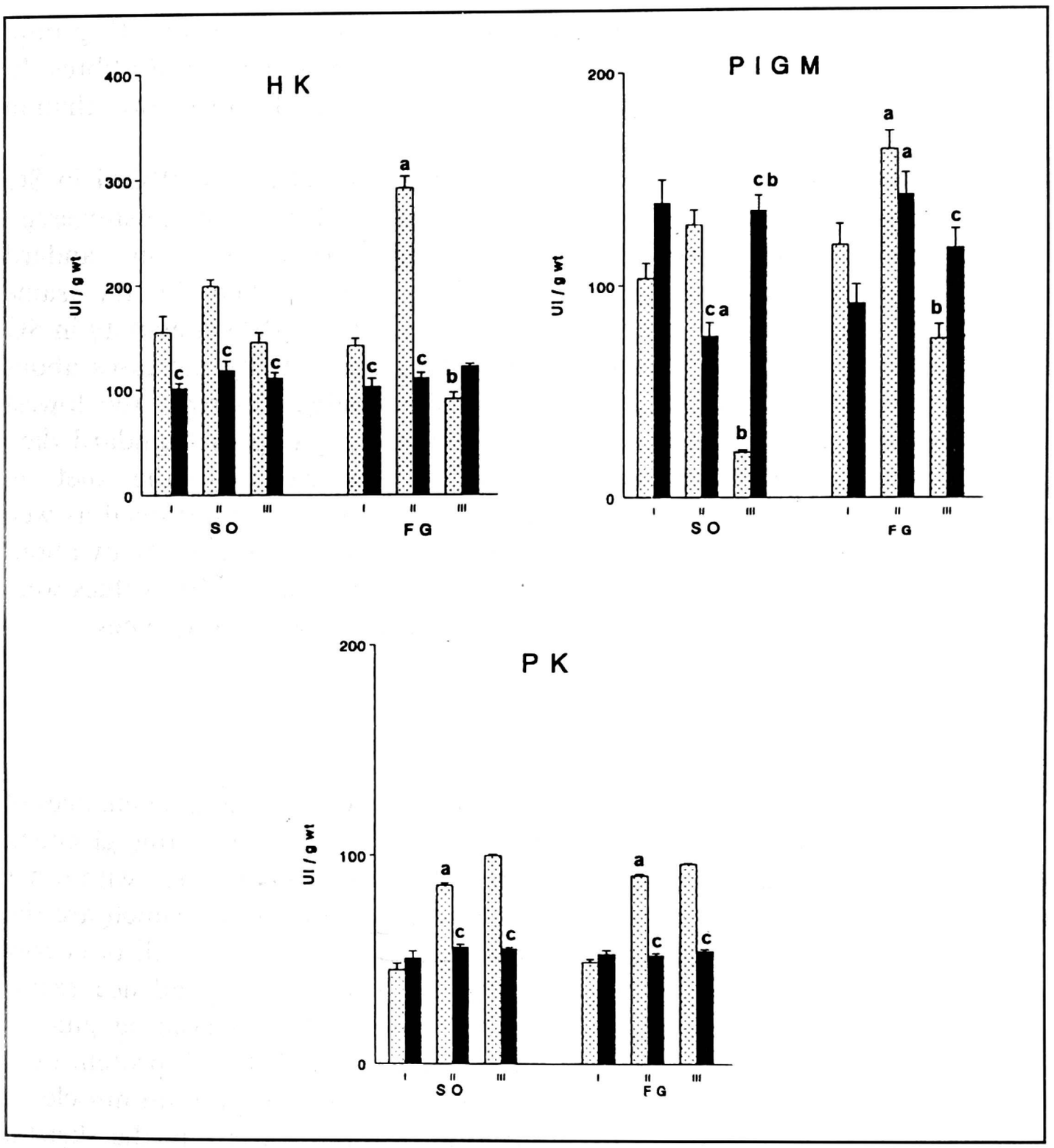

Fig. 4. Mean of changes activity of hexokinase (HK), phosphoglcomutase (PIGM) and pyruvate kinase (PK) in SO and FG fibres of gastrocnemius muscle. Designation as in *Fig. 2.*

those from I group, on the average by about 40%. The only exception was the content of glycogen in FG fibres of rats fed the standard diet, which was almost the same in group I as in group II. In III group post-exercise glycogen concentration in the gastrocnemius muscle was greater than in group II, in FG fibres in both types of diet and in SO fibres, only on standard diet. Liver glycogen concentration did not differ significantly among exhausted groups.

As seen in *Fig. 4* HK activity in the gastrocnemius muscle of rats fed a HP-diet was significantly lower than those on standard diet and was similar

in all groups. In rats fed standard diet exercise to exhaustion (II group) increased the activity of this enzyme more in FG fibres than in SO fibres. In group III HK activity in FG fibres it was statistically significantly lower than in group II.

At the rest HP-diet not substantially change the activity of PIGM in SO and FG fibres compared to the values after standard diet. The post-exercise PIGM activity in the muscles of sedentary-exhausted rats kept on standard diet was significantly higher only in FG fibres. When in the same sedentary-exhausted group the rats was kept on HP diet PIGM activity in SO fibres decreases about 85%, while in FG fibres this activity increases about 80% in relation to controls. Among the all investigated group the lowest PIGM activity was found in the trained-exhausted group on standard diet.

The PK activity remained essentially unaffected by the diet in control-sedentary group (*Fig. 4*). Exercise to exhaustion in the trained as well as sedentary rats on a standard diet caused a rise of the PK activity by about 85% in both fibre types. In rats on a high-protein diet similar PK values were found in all the variants of the experiment and in both fibre types.

DISCUSSION

The high-protein diet applied in the present study no affected on changes of the hepatic glycogen but caused a significant reduction of the resting glycogen concentration in skeletal muscles. The above findings are consistent with other date suggesting that even partial substitution of carbohydrate (which are the main component of rat feed) with protein substrates leads to a fall of energy reserve even in the inactive muscles (10, 14, 15). This dependence seems confirmed by the lower HK activity (the enzyme phosphorylating glucose derived from extramuscular sources) in muscles of rats fed high-protein diet.

It appears that in HP diet a low concentration of glycogen in rat muscles is accompanied by a decrease in the utilization of this substrate in the skeletal muscles during exercise. This fact seems to be confirmed by lower post-exercise activity of HK and PK in the muscle of rats on HP diet as compared to standard diet. The PIGM (the enzyme responsible for incorporation of glycogenolysis products into the pathway of glycolysis) values determined for the respective types of fiber reveal that their reaction to HP diet is varies. The increase of PIGM activity after exercise observed only in the FG fibres indicates that during such type of exercise the glycolytic-anaerobic fibres utilize the liver stores to a greater degree than the SO fibres (*Fig. 4*).

In spite of that the influence of HP diet on the exercise metabolism of carbohydrates, the effort capacity was not altered. The time of exercise to exhaustion was the same in both HP and standard diet sedentary rat groups.

The decrease of glycogenolysis might be compensated by the utilization of other energetic substrates.

The effort capacity of trained rats was also unaffected by HP diet. For both considered diets, a four-week training caused a similar rise of the effort capacity of rats (by about 70%). These findings are in agreement with numerous reports in literature (16, 17). As it was shown in the previous paper (10), the long-term endurance training combined with HP diet caused an increase of glycogen content up to the values determined in the muscles of the carbohydrate control group. It was also observed that a decrease of the total body weight was accompanied by a significant increase of skeletal muscles weight. As seen in *Fig. 3*, the extent of glycogen depletion in both muscle fibres was much less in the trained-exhausted animals as compared to the sedentary-exhausted. The greater ability of the trained animals to spare glycogen as well as less intense increase of glucose concentration are the significant symptoms of favourable adaptation to exercise. It should be emphasized that for both diets the exercise-induced glycogen breakdown in FG muscle fibres is significantly smaller. These data are consistent with the earlier studies showing that the type of the applied training and exercise seem to promote the activation of aerobic metabolism in muscles (18). The post-exercise change of the PIGM activity in group of the trained rats indicate that high-protein diet combined with endurance training affected the early stages of glycogenolysis in the muscle (phosphorylation of 1-glucose phosphate to 6-glucose phosphate). Simultaneous, the same as in the untrained group, inhibition of blood glucose utilization as well as futher stages of carbohydrate oxidation occured. It is proved by the absence exercise-induced changes of HK and PK activity.

In conclusion, the study shows that a diet protein enriched decreases the extent of glycolysis processes. In spite of that, the effort capacity of the trained and non-trained rats on a high-protein diet is the same as those on a standard diet. These results suggest that some other metabolic adaptations were developed which allowed to continue the exercise. The explanation of this problem calls for further studies which would take into consideration also other metabolic processes, not only the carbohydrate metabolism.

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