

The Effect of Low-Carbohydrate Diet on the Pattern of Hormonal Changes During Incremental, Graded Exercise in Young Men

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The purpose of this study was to discover whether severe dietary carbohydrate (CHO) restriction modifies the relationship between exercise intensity and hormonal responses to exercise. Changes in the plasma adrenaline (A), noradrenaline (NA), growth hormone (hGH), testosterone (T), and blood lactate (LA) during an incremental exercise performed until volitional exhaustion were determined in 8 physically active volunteers after 3 days on low CHO (<5% of energy content; L-CHO) and isocaloric mixed (M) diets. Following L-CHO diet, the basal plasma A, NA, and hGH concentrations were increased, whilst T and LA levels were decreased. During exercise all the hormones increased exponentially, with thresholds close to that of LA. Neither the magnitude nor the pattern of the hormonal changes were affected by L-CHO diet except the NA threshold, which was lowered. Blood LA response to exercise was diminished and LA threshold was shifted towards higher loads by L-CHO diet. It is concluded that restriction of CHO intake (a) does not affect the pattern of changes in plasma A, hGH, and T concentrations during graded exercise but lowers NA threshold, indicating increased sensitivity of the sympathetic nervous system to exercise stimulus; (b) alters the basal and exercise levels of circulating hormones, which may have an impact on the balance between anabolic and catabolic processes and subsequently influence the effectiveness of training.

Key Words: exercise, diet, lactate, catecholamines, growth hormone, testosterone

The pattern of substrate utilization during exercise may be altered by a change of the diet composition. The diet enriched with carbohydrates (CHO), commonly recommended for endurance athletes, increases contribution of these substrates to the energy yielding processes (see 7, 13). Less is known about an influence of reduced CHO availability on exercise metabolism. This problem was approached by applying the prior exhaustive exercise, which reduces muscle glycogen (12) or fat-rich,

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CHO-poor diets (16) or fasting (see 7) that cause depletion of glycogen mainly from the liver (28). However, according to Hultman (15) muscle glycogen content can be also diminished by 25–50% during a few days of fasting or a L-CHO diet in the subjects performing usual daily activities. In general, the investigations showed that lowering of body carbohydrate stores results in enhanced mobilization of free fatty acids (FFA), their utilization during exercise, and increased glucose production in the liver (see 7). These processes delay muscle and liver glycogen exhaustion thus counteracting an adverse impact of carbohydrate restriction on exercise performance. The altered lipolytic and gluconeogenic responses to exercise following reduction of CHO stores are due, at least partly, to neural and hormonal changes, including enhanced activation of the sympathetic nervous system, decreased plasma insulin concentration, and exaggerated secretion of glucagon, adrenaline, cortisol, and growth hormone (hGH; 4, 10, 11, 17, 23, 26, 29). The previous study from this laboratory (18), performed on dogs, provided evidence for the participation of hepatic glucoreceptors in the control of the sympathetic activity during exercise. According to this concept, decreased liver glycogen content or decreased glucose concentration in the portal vein increase an input from the liver glucoreceptors to the brain via afferent fibers of the vagus nerve, causing subsequent stimulation of the sympathetic nervous system. An existence of hepatic glucoreceptors was evidenced by Nijijima et al. (27) who registered electrical activity of the vagal afferents.

There are a few studies concerning an influence of dietary modifications on blood lactate (LA) concentration during exercise (14, 24, 36). It was shown that increased reliance on lipid oxidation decreases blood LA accumulation and shifts the LA threshold towards higher exercise loads. Our previous investigations (21) carried out on subjects performing graded incremental exercise test after a 3-day diet containing less than 5% CHO (L-CHO diet) confirmed these findings.

It has been reported that during a progressive graded exercise, the plasma catecholamine (5, 6, 22, 25, 30) and hGH (6) concentrations show an exponential pattern of changes, with thresholds close to that of LA. Our preliminary study showed that the threshold also can be detected in the response of plasma testosterone (T) to graded exercise in male subjects.

Blood LA threshold is a useful tool for predicting endurance performance and designing the training program (see 35). The exercise-induced hormonal changes that occur during training influence the balance between catabolic and anabolic processes and may play a role in the development of adaptive changes. Thus, it seemed of interest to find out whether dietary modifications affecting blood LA threshold influence hormonal thresholds in a coordinated manner. The present work was designed to compare the time course of changes in blood LA in plasma A, NA, hGH, and T during an incremental exercise performed until volitional exhaustion in young men after a 3-day low carbohydrate and mixed diets. Our hypothesis was that restriction of dietary CHO may cause dissociation of blood LA threshold from hormonal thresholds.

The low CHO diet used in this study, as in our previous investigations (20, 21), contained only up to 5% CHO but was rich in fat and protein. Such a diet was not intended to have an application to sports nutrition but rather to serve as a model for studying effects of dietary reduction of body CHO stores on hormonal responses to exercise.

Material and Methods

Subjects

Nine healthy male students of Academy of Physical Education, informed about the experimental protocol, volunteered to take part in this study. The protocol was approved by the Ethical Committee at the Medical Research Centre of the Polish Academy of Sciences in Warsaw. Mean values (with *SD*) of the subjects' age, height, and body mass were: 22 ± 0.9 years, 177.1 ± 4.9 cm, 77.1 ± 2.8 kg, respectively. Their body fat content did not exceed 16% of body mass, as calculated from the skin-fold measurements. The subjects were physically active, but none of them was engaged in regular sport training. Their $\dot{V}O_{2\max}$, determined 7–10 days before the study began, was 48.1 ± 7.4 ml · kg⁻¹ · min⁻¹. The subjects were asked to abstain from strenuous exercise in the week preceding the investigation and during the trial.

Procedure

Each subject performed an incremental cycle ergometer (Monark-Crescent AB, Varberg, Sweden) exercise test after an overnight fast on two occasions: (a) after 3 days on a normal (controlled) mixed (M) diet containing 50% carbohydrates, 30% fat, and 20% protein; and (b) after 3 days on a low carbohydrate (L-CHO) diet containing less than 5% of carbohydrates, 50% of fat, and 45% of protein. Both diets had the same energy content: 130 kJ · kg⁻¹ body mass daily. A sample of the menu for 24 hours for each of the two diets is presented in the appendix. All meals were provided for the subjects in the students' canteen. No caffeine, alcohol, or tobacco was permitted during the 48 hours before and during the trial.

Half an hour before each exercise test, the subjects had a catheter inserted into the antecubital vein for blood sampling. The exercise test started with unloaded cycling, and then its intensity was increased by 40 W until volitional exhaustion. Each load lasted 3 min, and the stages were separated by 1-min rest intervals. After cessation of each load, venous blood samples were taken for determinations of LA, A, NA, hGH, and T concentrations. Blood sampling was usually done in less than 30 s. We paid special attention to blood samples for catecholamine determination that were taken at first, immediately after the end of each work stage. The samples were collected in the polyethylene tubes containing the solution of reduced glutathione and EGTA placed on ice, centrifuged at 4 °C, and the plasma aliquots were stored at -70 °C until analyzed. To check whether the post-exercise values differ from those at the end of exercise, the additional experiments were performed in two subjects. Plasma catecholamine concentrations measured immediately after cessation of exercise were very close to those obtained during the last seconds of the effort: In one subject, the post exercise NA and A concentrations were lower (by 3.3 and 4.1 %, respectively), whereas in the second subject they were even higher (by 2.5 and 3.2%) than those during exercise.

Analytical Methods

Blood LA concentration was measured enzymatically using commercial kits (Boehringer, Mannheim, FRG.) Plasma hGH was determined by the radioimmunoassay using RIA-MJ-99 set (Institute for Atomic Energy, Swierk, Poland), and

the plasma T concentration by the radioimmunoassay set produced by Orion Diagnostica, Espoo (Finland). Plasma A and NA concentrations were measured by the radioenzymatic method of DaPrada and Zurcher (8) using the Catechola tests produced by the Institute for Research, Production, and Application of Radioisotopes (Prague, Czech Republic). The intra-assay analytical errors (coefficients of variation) for A, NA, hGH, and T were 10.8, 8.7, 4.2, and 3.5%, respectively.

Lactate and Hormone Thresholds Detection

The individual plots of LA or hormone concentrations versus exercise loads were evaluated to check whether they better fit the exponential than linear pattern. For the plots with higher exponential than linear correlation coefficients, the thresholds were calculated. Lactate and hormonal thresholds were expressed as the exercise loads corresponding to the individual breaking points of LA or hormone curves and calculated using two segmental linear regressions (log LA or hormone concentrations vs. log exercise load in Watts) according to Beaver et al. (2).

Statistics

Statistical evaluation of mean differences between the two diets was made using a two-way analysis of variance for repeated measures. The two factors were the diet and repeated measures of LA and hormone concentrations during exercise. When a significant interaction between the two factors was achieved, a paired student *t* test was used to locate the pairwise differences between means. The same test was used for evaluation of differences between the two diets in the pre-exercise blood lactate and hormone concentrations, maximal exercise-induced increases (Δ s) in the hormone concentrations and between the LA and hormonal thresholds. As a level of significance, $p < .05$ was accepted. All results are presented as means \pm standard errors (*SE*) unless otherwise stated.

Results

The mean maximal exercise load achieved after both diets was 262 ± 10 W. The mean values of blood LA, A, NA, hGH, and T during exercise after M and L-CHO diets are presented in Figures 1 and 2. The resting plasma concentrations of A, NA, and hGH were significantly higher after L-CHO ($p < .01$, $p < .05$, and $p < .01$, respectively), whilst the resting T concentration was lower ($p < .01$) than after M diet. Two-way analysis of variance revealed significant effects of diet and work loads on all measured variables (LA, A, NA, hGH, T), but significant interaction between these effects was ascertained only in case of plasma T (Table 1). The maximal exercise-induced increase (Δ) in blood LA was significantly lower after L-CHO than after M diet (6.43 ± 1.44 vs. 8.34 ± 0.94 mmol \cdot L⁻¹, $p < .01$), but no differences between the two diets were found in Δ s of plasma A, NA, hGH, and T.

After both diets, blood LA and the plasma concentrations of A and NA increased exponentially in relation to work load in all subjects. In the case of hGH and T, the exponential pattern of changes was found in 16 and 14 out of 18 tests, respectively.

Neither after the M nor after L-CHO diet, the inflection points of plasma A, NA, hGH, and T concentration curves (the hormonal thresholds) differed from the

Table 1 Effects of Exercise Loads and Diets on Blood Lactate (LA) and Plasma Adrenaline (A), Noradrenaline (NA), Growth Hormone (hGH), and Testosterone (T) Concentrations As Calculated by Two-Way Analysis of Variance

Variable	Work loads		Diets		Interaction	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
LA	39.851	.001	3.636	.001	0.284	n.s.
hGH	74.287	.001	6.746	.001	0.131	n.s.
T	26.305	.001	64.195	.001	6.663	.001
A	17.175	.01	4.828	.001	0.260	n.s.
NA	77.733	.001	2.143	.05	0.056	n.s.

Table 2 Lactate (LA), Adrenaline (A), Noradrenaline (NA), Growth Hormone (hGH), and Testosterone (T) Thresholds, Expressed As the Exercise Load (W), After the Mixed (M) and Low-Carbohydrate (L-CHO) Diet

Threshold (W)	M diet	L-CHO diet	<i>p</i> (M vs. L-CHO diet)
LA	122.80 ± 7.30	139.90 ± 7.10	<.05
A	136.92 ± 10.02	133.39 ± 9.27	n.s.
NA	139.46 ± 16.44	113.97 ± 14.01	<.05
hGH	125.36 ± 18.00	124.80 ± 16.20	n.s.
T	134.60 ± 15.70	151.60 ± 15.60	n.s.

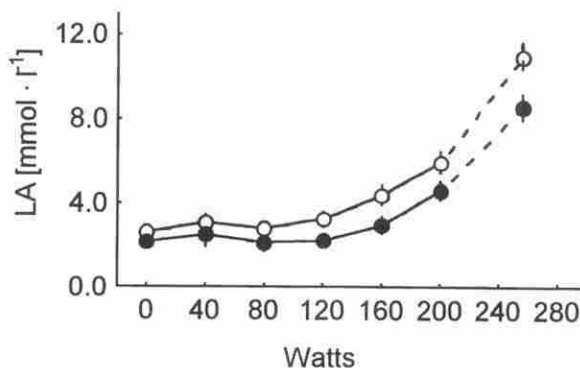


Figure 1 — Changes in blood lactate (LA) concentration during graded exercise after mixed (open circles) and low-carbohydrate (filled circles) diets. Values are means ± SE. The last points on the figure represent the mean values obtained at the maximal work loads.

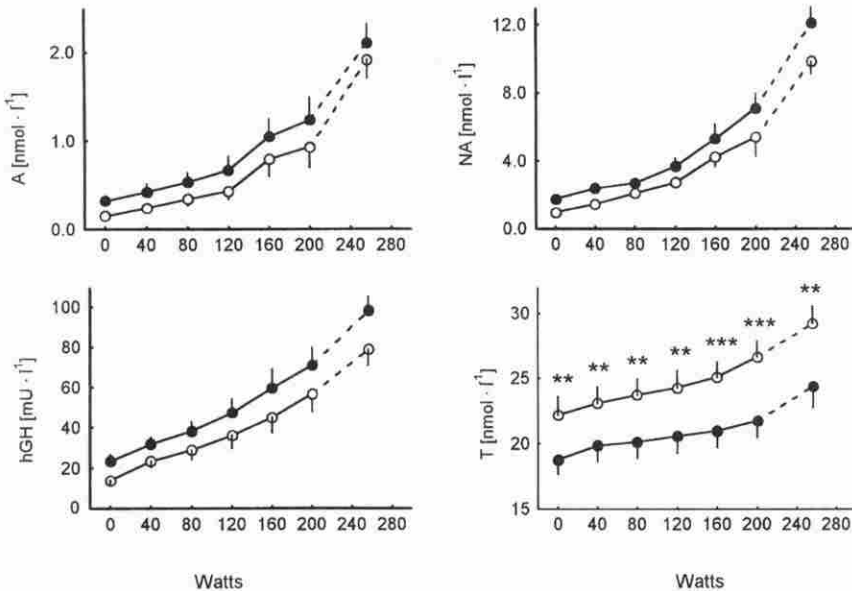


Figure 2 — Changes in the plasma concentrations of noradrenaline (NA), adrenaline (A), growth hormone (hGH), and T (T) during graded exercise after mixed (open circles) and low-carbohydrate (filled circles) diets. Values are means \pm SE. The last points on the figure represent the mean values obtained at the maximal work loads. Significance of differences between the diets at: ** $p < .01$; *** $p < .001$.

blood LA threshold with a tendency ($p = .076$) towards lower NA than LA-threshold after L-CHO diet. Following the L-CHO diet, the LA threshold was shifted towards higher work load than that after the M diet, whilst the NA threshold was detected at the lower work intensity after L-CHO in comparison with M diet. The plasma A, hGH, and T thresholds were not affected by the L-CHO diet (Table 2).

Discussion

In agreement with previous studies (20, 21), the present investigation demonstrated that the 3-day low carbohydrate diet results in a significant decrease of blood LA concentration, with concomitant increases in the plasma catecholamine and hGH concentrations at rest after an overnight fast. The new finding of the present study is that the plasma T levels were significantly reduced after the 3-day L-CHO diet. These effects can be attributed to a reduction of body carbohydrate stores, although in case of hGH and T, an influence of increased fat and protein content in the L-CHO diet also should be considered. It was reported that plasma hGH may be increased after a protein-rich diet without CHO restriction (33), although Bergstrom et al. (3) and Kraemer et al. (19) failed to demonstrate this effect. More consistent data were obtained on the effects of dietary protein on plasma T. Volek et al. (34) described a significant negative correlation between daily protein intake and resting T concentration. This finding confirmed the data previously reported by Anderson et al. (1) who demonstrated that a low protein diet (10% of total energy) is associated with

higher levels of T in comparison with a diet high in protein (44% of total energy). The quoted authors suggested that alterations in protein to carbohydrate ratio in the diet can influence the steroid hormone metabolism in the liver. On the other hand, high fat content in the diet (~40%) was found to increase the plasma T concentration (32). No data were found in the available literature on the influence of protein or fat enriched diets without CHO restriction on the plasma catecholamine concentration.

An analysis of individual relationships between exercise loads and plasma levels of hormones demonstrated that in the great majority of subjects, plasma catecholamines, hGH, and T increased exponentially during exercise. This allowed us to calculate the work loads associated with accelerated rate of these hormone increases (thresholds) in a similar way as the blood LA threshold. During exercise performed after the normal, mixed diet the hormonal thresholds were close to that of LA. These findings confirmed the data reported previously on the time course of changes in the plasma A, NA (5, 6, 25, 30), and hGH (6) during graded exercise and added a new observation concerning plasma T response. In the recent study, Pritzlaff et al. (31) concluded that hGH response to exercise is related to exercise intensity in a linear dose-response pattern. The protocol of the above investigation was, however, different from that used in the present study. The authors calculated integrated response from the data on hGH concentrations measured during 30-min treadmill exercise bouts of various intensities and 6 hours recovery period. Duration of exercise and prolonged period of fasting, including an overnight fast and 6 hours following exercise, could affect hGH concentrations independently of exercise intensity.

Measurements of blood LA and hormonal changes after each step of graded incremental exercise in the present study showed that the time course of plasma A, hGH, and T is not affected by dietary regime, whilst the plasma NA threshold is shifted towards lower exercise intensity after the low carbohydrate diet. Therefore, the L-CHO diet caused a dissociation only between the LA and plasma NA thresholds. Podolin et al. (30) demonstrated that after glycogen depletion caused by a prolonged exercise followed by an overnight fast, the plasma A, NA, and blood LA concentrations at the maximal exercise loads were lowered. Moreover, the inflection points of these three variables were delayed in a coordinated manner. Comparison of our data with those of Podolin et al. (30) suggests that restriction of carbohydrate intake for a few days influences the sympatho-adrenal responses to exercise in a different manner than the exercise-induced muscle glycogen depletion.

In summary, the present data demonstrated that in young men, carbohydrate-restricted high-protein and fat diet applied for 3 days enhances activity of the sympathetic nervous system, plasma A, and hGH concentrations at rest, as well as during submaximal and maximal exercise loads in comparison with isocaloric mixed diet. The opposite changes were found in the plasma T concentration. During graded exercise performed after the mixed diet, all the hormonal thresholds were close to the blood LA threshold. After the low carbohydrate diet, the threshold of blood LA was elevated whilst that of plasma NA was lowered, thus indicating that dietary modifications can influence a relationship between the sympathetic response to exercise and its intensity. However, the thresholds of A, hGH, and T were not significantly affected by the low carbohydrate diet. Our hypothesis that reduced body carbohydrates cause a dissociation between LA and hormonal thresholds was, therefore, only partly confirmed. Nevertheless, the present findings indicate that the diet composition can alter circulating hormones both at rest and during exercise, which may have an impact on the effectiveness of training. The results also provided

further evidence that low-carbohydrate diet shifts the blood LA threshold towards higher exercise load, which should be taken into account when the threshold is used for prescription of exercise as an indicator of the subject's endurance capacity.

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Appendix

Samples of the Menu

L-CHO Diet

Breakfast: scrambled eggs (3-4) with bacon (2 strips), cream cheese (150-200 g), light tea without sugar, mineral water;

Lunch: beef hamburger broiled (250 g) with horse radish or mustard, mineral water;

Supper: hard boiled eggs (1-2), beef steak broiled, rounded with fat (250 g) with horse radish or mustard, blue cheese (60 g), mineral water.

Mixed Diet

Breakfast: orange juice (150 g), corn flakes (75 g) with milk (250 ml) and banana, cottage cheese (150 g), whole wheat bread with butter or margarine (4-5 slices), apple (1), tea with sugar (30 g), mineral water;

Lunch: vegetable soup with 2 slices of bread, pork loin roasted lean (150 g), boiled potatoes (200-250 g), tomato (1), fruit juice (150 g), mineral water;

Supper: whole wheat bread (2-4 slices) with butter or margarine, cottage cheese creamed (150 g), tomato (1), pear (1), tea with sugar (30 g), mineral water.

Note. In Polish dietary custom, lunch is the main meal during the day.