JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1996, 47, 2, 361—371

J. LANGFORT, W. PILIS*, R. ZARZECZNY*, K. NAZAR,
H. KACIUBA-UŚCIŁKO

EFFECT OF LOW-CARBOHYDRATE — KETOGENIC DIET ON METABOLIC AND HORMONAL RESPONSES TO GRADED EXERCISE IN MEN

Department of Applied Physiology Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
* Department of Physiology, University of Pedagogics, Częstochowa, Poland

Maximal oxygen uptake (VO_2 max) and lactate threshold (LT) were measured during graded, incremental exercise in 8 healthy, untrained volunteers (aged 22 ± 0.9 yrs) following 3 days on a control, mixed diet, or a ketogenic (50% fat, 45% protein and 5% carbodydrates) diet of equal energy content. Before and after exercise tests acid base balance, plasma beta-hydroxybutyrate (β-HB), free fatty acid (FFA), and some hormone concentrations were determined. In comparison with the normal diet, the ketogenic diet resulted in: an increased VO_2 max, decreased respiratory exchange ratio an a shift of LT towards higher exercise loads. Blood LA concentrations were lower before, during and after exercise. Post exercise blood pH, as well as pre-and post exercise base excess and bicarbonates were reduced. Resting β-HB concentration was elevated to approx. 2.0 mM, and FFA to approx. 1.0 mM. During a 1 h recovery period β-HB decreased to 0.85 mM ($p < 0.01$) after the ketogenic diet, while plasma FFA did not change after exercise under either conditions. Both the pre-and post-exercise levels of adrenaline, noradrenaline, and cortisol were enhanced, whilst plasma insulin concentration was decreased on the ketogenic diet. It is concluded that the short-term ketogenic diet does not impair aerobic exercise capacity, as indicated by elevated VO_2 max and LT. This may be due to increased utilization of β-HB and FFA when carbohydrate stores are diminished. Stimulation of the sympatho-adrenal system, and cortisol secretion with reduced plasma insulin concentration seem to be of importance for preservation of working capacity.

Key words: *diet, exercise, lactate threshold, maximal oxygen uptake, cortisol, insulin*

INTRODUCTION

Relative contribution of various substrates to energy yielding processes is important for exercise performance. The pattern of substrate utilization during exercise may be altered by a change of the diet composition. In majority of previous studies on this problem, carbohydrate rich diets were used and their beneficial effects on exercise performance have been commonly accepted (1).

Less is known about effects of increased lipid availability on exercise performance or on the metabolic and hormonal responses to physical effort. These problem were approached by applying fat rich diets, infusions of fat emulsions (e.g. Intralipid) with heparin (2, 3) or fasting, which stimulates endogenous fat mobilization. The studies reported so far have shown that enhanced muscle access to lipid substrates results in reduced utilization of carbohydrates due either to glucose/FFA cycle operation or to an inhibitory effect of FFA on glucose uptake by skeletal muscles (4). Some investigations proved that a fat-rich meal consumed prior to exercise (5, 6) or a fat enriched diet used for a longer period of time (3—14 days) before exercise (7, 8) largely modify metabolic and hormonal responses to exercise both in men and animals.

Results of the studies on the influence of carbohydrate deprived, fat and protein (ketogenic) diet on exercise performance are still controversial. Rats fed a very low carbohydrate, high fat diet for a few weeks were able to exercise as long or even longer than rats fed a high carbohydrate diet (9—11). Contrary to these findings, in human subjects a marked decrease in working ability under conditions of restricted carbohydrate availability was described a long time ago (12), and then confirmed by more recent studies (13, 14). However, Phinney et al. (15) reported a preserved capacity for moderate exercise in obese subjects after six weeks on a low-energy, ketogenic diet. In subsequent study from the same Laboratory (16) it was shown that in elite cyclists time to exhaustion during exercise at 65% VO_2 max was not reduced after 4 weeks on an eukaloric, ketogenic diet, in spite of markedly diminished (by approx. 50%) muscle glycogen before exercise.

There are only few studies on the effect of carbohydrate restriction on the ability to perform heavy exercise. Henschel et al. (17) reported that time to exhaustion during intensive treadmill run was reduced after a short-term fast. This was confirmed by studies with dietary modifications (18—23). On the other hand, Quirion et al. (24) failed to demonstrate differences in time of incremental exercise or the peak work load in subjects on high-carbohydrate and high-fat diet.

The present investigations were undertaken to examine whether a 3-day-ketogenic, eucaloric diet affects maximal oxygen uptake (VO_2 max), lactate thereshold (LT), and acid-base balance during graded incremental exercise. Moreover, concentrations of selected metabolites and hormones in blood before and after exercise were compared in the same subjects after ketogenic and normal diet of equal energy content.

MATERIAL AND METHODS

Subjects

Eight healthy untrained men volunteered to take part in this study, which was approved by the Ethical Committee at the Medical Research Centre of the Polish Academy of Sciences in Warsaw. Mean values (with standard deviations) of the subjects' age, height and body mass were: 22 ± 0.9 years, 173 ± 11 cm and 69 ± 9.0 kg, respectively. Their body fat content did not exceed 12.8% of body mass, as calculated from the skinfold measurements. None of the subjects was actively engaged in any sport activity one week before and during the study.

Experimental procedure

The subjects performed two identical graded cycle ergometer (Monark- Crescent AB, Varberg, Sweden) exercise-tests preceded either by 3 days on a normal (controlled) mixed diet (50% carbohydrates, 30% fat and 20% protein), or by 3 days on the ketogenic diet containing less than 5% of carbohydrates, 50% fat and 45% protein. Both the diets had the same energy content (32 $\text{kcal} \times \text{kg}^{-1}$ b. m. daily). On the day of exercise testing the subjects reported to the Laboratory after an overnight fast. Half an hour before exercise, they had a catheter inserted into the antecubital vein for blood sampling. The exercise started with unloaded cycling for 3 min, and then intensity was increased by 30 W every 3 min. After reaching 210 W the exercise load was individually enhanced to evaluate the maximum oxygen uptake (VO_2 max). The criterion for VO_2 max was O_2 uptake levelling off.

Pulmonary ventilation (V_E), oxygen uptake (VO_2), and carbon dioxide output (VCO_2) were determined before exercise (for approx. 10 min), in the last minute of each work load, and then 3, 15, 30 and 60 min after exercise using Beckman Metabolic Measurement Cart. Based on these measurements, the respiratory exchange ratio (R), and 1 h excess post-exercise oxygen consumption (EPOC) were calculated. Heart rate (HR) before and during exercise was recorded using ECG.

Venous blood samples for determinations of free fatty acid (FFA), β-hydroxybutyrate acid (β-HB), insulin (IRI) and catecholamine concentrations were withdrawn before exercise and then 3, 15, 30 and 60 min following cessation of exercise. Besides, 0.1 ml of blood was taken from the finger tip at the end of each work load to measure acid-base balance (168 pH/Blood Analyzer, Ciba Corning), and lactate (LA) concentrations.

Analytical methods

Plasma level of β-HB was determined enzymatically (25). Plasma FFA concentration was measured by the micro-method (26). Blood LA level was determined enzymatically using commercial kits (Boehringer, Mannheim, F.R.G.). Plasma IRI and cortisol levels were determined by radioimmunoassays with the commercial kits (Institute of Atomic Energy, Świerk, Poland and Orion Diagnostica, Espoo, Finland, respectively), Plasma concentration of adrenaline (A) and noradrenaline (NA) were measured radioenzymatically (27), using commercial kits (REA KIT Catechola, Czechs Republic).

The lactate threshold detection

Lactate threshold was calculated as the exercise load corresponding to the individual breaking point of LA curve using the two segmental linear regression (log LA vs log exercise load) (28).

Statistics

Statistical evaluation of mean differences between the two diets was made using one-way analysis of variance followed by Newman-Keul's test or Student's test for paired observations. As the level of significance $p < 0.05$ was accepted. All results are presented in this paper as mean ± SE unless otherwise stated.

RESULTS

Mean values of O_2 uptake at rest, VO_2 max, R,and 1 h-EPOC after the two diets are summarized in *Table 1.*

Table 1. Oxygen uptake (VO_2), respiratory exchenge ratio (R) before and during maximal exercise as well as 1 h post-exercise oxygen consumption (EPOC) and lactate threshold (LT) following 3 days on normal or carbohydrate deprived diets.

	Control diet	Diet deprived of carbohydrates	Significance p
VO_2 ($l \times min^{-1}$)			
Rest	0.347 ± 0.047	0.344 ± 0.048	n.s.
Maximal exercise	3.972 ± 0.190	4.344 ± 0.187	< 0.05
R			
Rest	0.86 ± 0.01	0.76 ± 0.01	< 0.01
Maximal exercise	1.04 ± 0.01	0.98 ± 0.01	< 0.05
1 h EPOC ($ml \times kg^{-1}$)	118.82 ± 11.03	96.06 ± 12.70	< 0.05
LT (W)	137.65 ± 8.37	170.52 ± 15.3	< 0.05

Data are presented as mean values ± SE (n = 8).

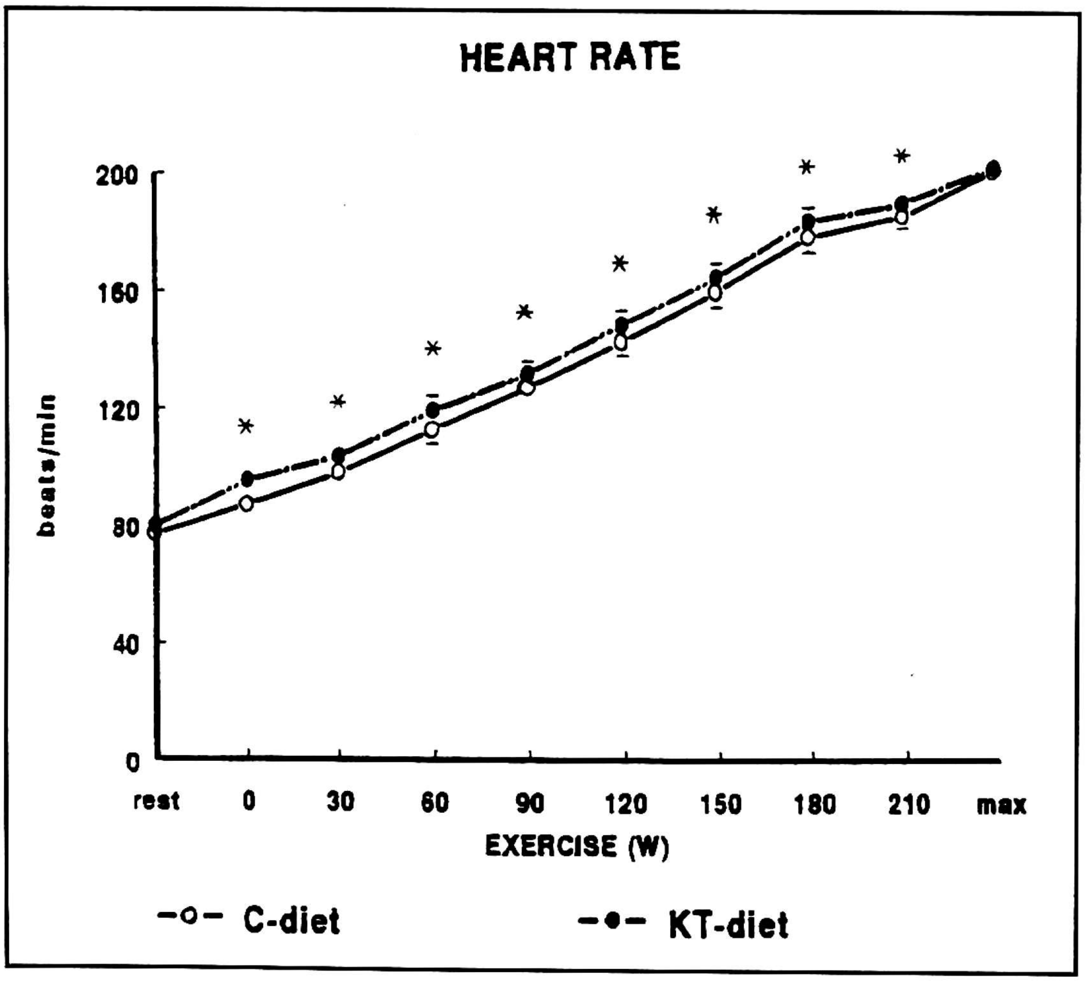

Fig. 1. Heart rate during graded, incremental exercise of increasing intensity in 8 volunteers following 3 days on control (C) and ketogenic (KT) diet. Values are mean ± SE, * $p < 0.05$.

Heart rate and VO_2 uptake at each exercise load are compared in *Fig. 1 and 2.* Maximal oxygen uptake was higher and 1 h-EPOC lower after the ketogenic than after the normal diet, while the resting O_2 uptake was similar after the two diets. R values were lower after the ketogenic diet than after the control diet both before and during exercise. Oxygen uptake and HR at each exercise load were slightly but significantly higher after ketogenic than after control diet, except HR at the maximum load which did not differ between the tests (*Fig. 1*) After the ketogenic diet LT shifted towards higher exercise loads in comparison with that after the normal diet ($p < 0.05$, *Table 1*).

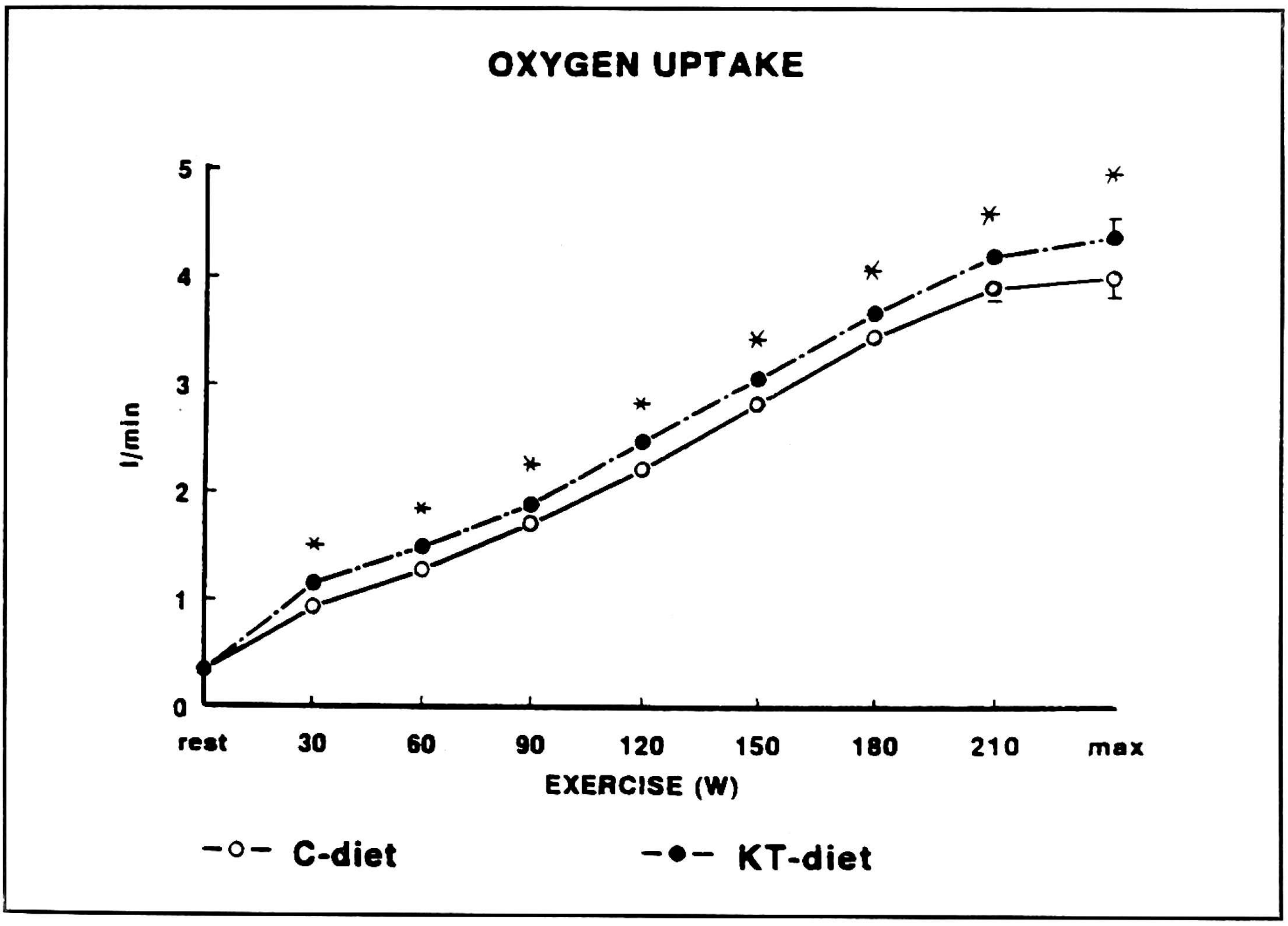

Fig. 2. Oxygen uptake during graded, incremental exercise of increasing intensity in 8 volunteers following 3 days on control (C) and ketogenic (KT) diet. Values are mean ± SE, $*p < 0.05$.

The effects of dietary modifications on the plasma β-HB, FFA and blood LA concentrations are summarized in *Table 2.* The carbohydrate-poor diet caused a considerable increase in the plasma β-HB concentration both before and after exercise. Also, the levels of FFA were markedly enhanced. Pre- and post-exercise blood LA concentrations were significantly lower after the carbohydrate deprived diet than after the normal diet.

Table 2. Blood insulin, β-hydroxybutyrate, FFA and LA levels before and after exercise performed following 3 days on control or carbohydrate deprived diets.

	Control diet	Diet deprived of carbohydrates	Significance p
β-hydroxybutyrate (μM)			
Rest	279.44 ± 81.25	1906.66 ± 267.25	< 0.001
Time after exercise 3 min	257.78 ± 80.52	1622.22 ± 201.86	< 0.001
15 min	273.33 ± 87.17	1222.22 ± 250.32	< 0.001
30 min	292.22 ± 64.77	923.33 ± 183.84	< 0.001
60 min	446.11 ± 112.49	945.33 ± 179.18	< 0.001
FFA (μM)			
Rest	580.14 ± 68.59	832.16 ± 88.15	< 0.001
Time after exercise 3 min	561.28 ± 58.02	798.71 ± 67.46	< 0.01
15 min	608.14 ± 67.13	714.33 ± 39.92	< 0.05
30 min	632.43 ± 52.60	694.84 ± 68.03	n. s.
60 min	663.43 ± 78.35	833.73 ± 91.55	< 0.01
LA (mM)			
Rest	1.45 ± 0.49	0.92 ± 0.17	< 0.05
Time after exercise 3 min	10.01 ± 2.04	7.14 ± 1.9	< 0.01
15 min	8.45 ± 1.98	5.96 ± 1.82	< 0.01
30 min	4.61 ± 1.05	3.76 ± 1.21	< 0.05
60 min	2.18 ± 0.79	1.72 ± 0.38	< 0.05

Data are presented as mean values ± SE (n = 8).

Table 3. Plasma insulin (IRI), adrenaline (A), noradrenaline (NA) and cortisol before and after exercise performed following 3 days on mixed normal or carbohydrate deprived diets.

	Control diet	Diet deprived of carbohydrates	Significance p
Insulin ($\mathrm{mU} \times \mathrm{l}^{-1}$)			
Rest	23.04 ± 3.50	10.50 ± 3.45	< 0.05
Time after exercise 3 min	17.27 ± 3.80	8.85 ± 3.19	< 0.05
15 min	22.59 ± 5.35	11.06 ± 3.62	< 0.05
30 min	16.58 ± 2.96	12.69 ± 3.05	< 0.05
60 min	18.71 ± 2.35	14.44 ± 3.25	n. s.
A ($\mathrm{pmol} \times \mathrm{ml}^{-1}$)			
Rest	0.58 ± 0.08	0.79 ± 0.06	< 0.05
Time after exercise 3 min	2.06 ± 0.13	3.31 ± 0.60	< 0.05
15 min	0.77 ± 0.07	1.15 ± 0.18	< 0.05
30 min	0.77 ± 0.07	1.15 ± 0.19	n. s.
NA ($\mathrm{pmol} \times \mathrm{ml}^{-1}$)			
Rest	2.92 ± 0.30	4.72 ± 0.93	< 0.05
Time after exercise 3 min	12.58 ± 2.33	20.05 ± 2.07	< 0.01
15 min	5.91 ± 0.92	9.31 ± 1.46	< 0.05
30 min	4.22 ± 0.95	5.75 ± 0.95	n. s.
Cortisol ($\mathrm{nmol} \times \mathrm{l}^{-1}$)			
Rest	190.11 ± 40.12	233.63 ± 38.13	< 0.05
Time after exercise 3 min	159.56 ± 53.81	246.09 ± 36.28	< 0.01
15 min	195.56 ± 37.17	259.43 ± 26.37	< 0.01
30 min	183.99 ± 61.66	266.97 ± 33.14	< 0.05
60 min	183.99 ± 61.66	241.19 ± 36.34	< 0.01

Data are presented as mean values ± SE (n = 8).

Table 4. Blood pH, base excess (BE) and standard bicarbonate levels (SB) before and at the end of maximal exercise performed after 3 days on mixed normal or carbohydrate deprived diets.

	Control diet	Diet deprived of carbohydrates	Significance n
Blood pH			
Rest	7.36±0.01	7.28±0.01	n. s.
Maximal exercise	7.28±0.01	7.05±0.01	< 0.05
Blood BE (mM)			
Rest	−4.86±0.55	−7.42±0.80	< 0.05
Maximal exercise	−13.04±0.42	−20.28±1.66	< 0.05
Blood SB (mM)			
Rest	21.68±0.38	19.82±0.58	< 0.05
Maximal exercise	14.60±0.43	11.16±1.18	< 0.05

Data are presented as mean values±SE (n = 5).

Plasma A, NA, IRI and cortisol concentrations before and after graded exercise performed by subjects on mixed and ketogenic diet are presented in *Table 3.* The ketogenic diet resulted in enhanced levels of plasma catecholamines and cortisol, and reduced plasma IRI concentrations both before and after exercise. Plasma cortisol levels were not influenced by exercise in either group.

Effects of dietary modifications on blood pH, base excess (BE) and standard bicarbonate (SB) are shown in *Table 4.* The ketogenic diet lowered the post-exercise blood pH as well as the initial and post-exercise BE and SB levels.

DISCUSSION

The low carbohydrate diet, applied in the present study caused an elevation of blood pre-exercise β-hydroxybutyrate up to 2 mM and the plasma FFA level up to 1 mM which indicates the subjects' compliance with this diet. The levels of β-HB and FFA are close to those reported after 2—3 days of fasting (14) but they are markedly lower than the values occurring in uncontrolled diabetes, when plasma FFA concentration may be in the range 2—4 mM and the ketone body levels reach 15—20 mM (29).

An important finding of the present investigation is that after 3-days on ketogenic diet, VO_2 max was significantly increased in comparison with the value after a mixed diet. A similar effect was previously reported in rats fed a carbohydrate free, high fat diet for 12 weeks (11). It was suggested that an elevation of VO_2 max could be explained either by a greater oxygen uptake necessary to obtain the same energy yield as on a normal diet due to increased fat oxidation or by enhanced sympathetic activation. The present study showed that the respiratory exchange ratio was lowered at all exercise loads in the subjects on the ketogenic diet, thus indicating an increase in lipid combustion. This can explain the higher oxygen uptake at submaximal loads. However, it is commonly accepted that VO_2 max is limited by the capacity of oxygen transport. It seems likely, therefore, that an enhanced activity of sympatho-adrenal system after the ketogenic diet is responsible for an increase in the oxygen transport effectiveness due to its effects on the circulatory system. This assumption is supported by the data showing higher HR values at submaximal work loads. Stimulation of the sympatho-adrenal system by the ketogenic diet was demonstrated in the present work by a significantly higher levels of plasma noradrenaline and adrenaline in comparison with those after a normal diet. Similar results were reported by Jansson et al. (7) who had studied the effect of five days on a carbohydrate poor diet on plasma catecholamine responses to submaximal exercise. The above findings are also consistent with other data suggesting that reduction in body carbohydrate stores leads to activation of the sympatho-adrenal system and increases its response to physical exercise (7, 30). Contrary to this, it was showed significantly lowered plasma catecholamine concentrations during graded exercise in subjects previously depleted of glycogen by exercise followed by an overnight fast (31). The reason for the discrepancy is unknown, although it may be attributed to different experimental protocols.

The ketogenic diet applied in the present study resulted in a significantly lowered blood LA both before, during and after exercise. Moreover, the lactate threshold (LT) was shifted to the higher work loads. These results are in agreement with previously reported investigations (22, 32, 33) showing an increment in LT under conditions of glycogen store reduction, either by a low carbohydrate diet or exhausting exercise. It was even suggested (33) that the increment of LT is in fact an indicator of carbohydrate deficiency. It is not known, however, whether the ketogenic diet-induced shift in LA threshold and reduction of maximal LA concentrations depend on a decreased rate of glycolysis or an inhibited lactate efflux from working muscles due to reduced blood buffering capacity (34). Indeed, it was demonstrated in the present study that the subjects on the ketogenic diet show a tendency towards lower blood pH and have significantly reduced blood base excess as well as blood bicarbonate levels already before exercise. The differences between the two diets in acid base status were even more pronounced after maximal exercise.

Measurements of VO_2, within an hour following cessation of exercise revealed that EPOC was slightly, but significantly decreassed, after the ketogenic in comparison with normal diet. This finding is difficult to explain. One can only speculate that it can be attributed to reduced glycogen resynthesis during the recovery period due to the lowering of circulating insulin. The patterns of post-exercise changes in the plasma β-HB and FFA levels after mixed and ketogenic diets are in accordance with previous data obtained in subjects with normal and elevated (by starvation) plasma ketone bodies (35). In subjects on a normal diet, a moderate postexercise ketosis was found 60 min after exercise, while in the subjects on a ketogenic diet, the level of β-HB declined after exercise and was decreasing steadily until the 30th min of the recovery period. According to the previous studies (36), skeletal muscles do not remove ketones during exercise. Thus, the exercise-induced decrease in plasma ketone levels may be attributed to the reduced ketogenesis resulting from diminished hepatic blood flow. However, the further fall in plasma β-HB during recovery period seems to be related to the enhanced ketone body utilization by skeletal muscles or other tissues. There are data (37) indicating that after 60—66 hrs of starvation, when the plasma level of β-HB reaches similar avalues values to those found in the present work, the contribution of ketone bodies to the oxidative metabolism of resting muscles accounts for aoprox. 20%.

The changes in hormone concentrations induced by ketogenic diet before exercise and after its cessation were similar to those previously described in subjects during fasting or after a low carbohydrate diet (30). These changes included a decrease in the plasma insulin concentration with concomitant marked increases in plasma adrenaline, noradrenaline and cortisol levels. The altered hormonal profile induced by the ketogenic diet fits the concept that glucostatic mechanism is involved in the control of hormonal and metabolic responses to exercise. According to this concept depletion of body carbohydrate stores, and particularly of liver glycogen leads to stimulation of lipolysis and glucose production, due to changes in secretion of glucoregulatory hormones (5, 38—40).

Acknowledgements: This study was supported by the State Committee for Scientific Research grant nr: 4 S404 028 07. Dr R. Zarzeczny was supported by the Foundation for Polish Science.

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Received September 11, 1995
Accepted: December 28, 1995

Author's address: J. Langfort, Department of Applied Physiology Medical Research Centre Polish Academy of Sciences, 17 Jazgarzewska Str., 00-730 Warsaw, Poland.