Pivalic Acid–Induced Carnitine Deficiency and Physical Exercise in Humans

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To study the effect of carnitine depletion on physical working capacity, healthy subjects were administered pivaloyl-conjugated antibiotics for 54 days. The mean carnitine concentration in serum decreased from 35.0 to 3.5 μmol/L, and in muscle from 10 to 4.3 μmol/g noncollagen protein (NCP). Exercise tests were performed before and after 54 days' administration of the drug. At submaximal exercise, there was a slight increase in the concentration of 3-hydroxybutyrate in serum, presumably caused by decreased fatty acid oxidation in the liver. There was also a decreased consumption of muscle glycogen, indicating decreased glycolysis in the skeletal muscle. The muscle presumably had enough energy available, since there was no significant decrease in the concentration of adenosine triphosphate (ATP) and creatine phosphate during exercise. The work at maximal oxygen uptake ($\dot{V}O_2max$) and the maximal heart rate were reduced. Since $\dot{V}O_2max$ is considered dependent on heart function, carnitine depletion seemed to affect cardiac function.

ANTIBIOTICS CONJUGATED with pivalic acid, such as pivmecillinam and pivampicillin, are used in the treatment of patients with urinary tract infection. Conjugation with pivalic acid is used to increase intestinal uptake. After absorption of the prodrug, an ester is formed between pivalic acid and carnitine that is excreted in the urine.[1-3] Therefore, treatment with drugs containing pivalic acid results in loss of carnitine from the body stores. After 1 to 2 days of treatment, the concentration of free carnitine in serum was decreased to approximately 50%, and after 10 days to 25%, of initial values.[3] Long-term low-dose treatment of children for 12 to 37 months to prevent urinary tract infection resulted in concentrations of free carnitine in serum of 0.9 to 3.6 μmol/L (reference interval, 23 to 60 μmol/L); in four cases simultaneously measured, the concentration of total carnitine in muscle was 0.6 to 1.4 μmol/g noncollagen protein ([NCP] reference interval, 7.1 to 19 μmol/g NCP).[4]

Carnitine is essential in fat metabolism by transporting fatty acids through the mitochondrial membrane and thus enabling β-oxidation in skeletal and heart muscle.[5] Carnitine deficiency leads to skeletal myopathy, cardiomyopathy, encephalopathy, hepatomegaly, hypoketotic hypoglycemia, and Reye-like syndromes.[6-11] In adults with induced secondary carnitine deficiency, a decreased ketogenesis was shown at fasting.[12] Furthermore, muscle weakness was reported in several patients, children and adults, with markedly reduced carnitine stores secondary to long-term treatment with antibiotics containing pivalic acid.[4,13] The aim of this study was to investigate the effects of secondary carnitine deficiency on metabolic and physiologic variables during exercise tests in healthy adults after short-term and long-term treatment with antibiotics containing pivalic acid.

SUBJECTS AND METHODS

Subjects

Thirteen volunteers were studied. They were divided into two groups. Group 1 consisted of six men aged 20 to 28 years who were treated for 10 days, and group 2 contained five females and two males aged 17 to 54 years who were treated for 54 days. The subjects were healthy according to medical history, normal physical examination, and electrocardiogram. Routine laboratory tests were normal.

The subjects were told to eat a varied diet but avoid meals excessively rich in fat or carbohydrates. For 3 days before the tests, all food and beverage was recorded. No alcohol consumption or physical exercise was allowed for 24 hours before the tests. Urine samples were obtained and frozen every other day to check the compliance of pivmecillinam consumption. No other medication was permitted.

The study was approved by the local ethics committee.

Procedure

Day 1. Maximal working capacity was determined on a bicycle ergometer by a successive increase of the workload. Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), ventilation, and heart rate were continuously measured.

Day 2. Following 4 hours of fasting, the treated subjects exercised on the bicycle for 60 minutes at 50% to 60% of their maximal oxygen uptake ($\dot{V}O_2max$). Repeated measurements of $\dot{V}O_2$, $\dot{V}CO_2$, ventilation, and heart rate were performed, and the mean of the last 12 measurements (obtained every 20 seconds) was calculated. Heart rate was measured simultaneously by echocardiography. Blood samples were obtained before and after the exercise test and analyzed for glucose, lactate, free fatty acids, triglycerides, 3-hydroxybutyrate, and carnitine. Urine was obtained before exercise for determination of carnitine. Muscle samples, with a weight of 50 to 80 mg, were taken before and after exercise for determination of glucose, glycogen, glucose-6-phosphate, lactate, adenosine triphosphate (ATP), creatine phosphate, and carnitine.

Group 1 had a daily oral intake of 1,200 mg (540 μmol) pivmecillinam for 10 days, from days 3 to 12. At days 12 and 13, the procedures of day 1 and day 2 were repeated.

Group 2 started a daily oral intake of 1,200 mg (540 μmol) pivmecillinam on day 3. Repetition of the day 1 and day 2 procedures was made after a mean of 16 days (range, 14 to 21) and

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after a mean of 54 days (range, 49 to 56) of pivmecillinam treatment.

Methods

The tests were performed on a bicycle ergometer connected to a system for estimation of $\dot{V}O_2$ and $\dot{V}CO_2$ from breath to breath (Medgraphics, cardiopulmonary exercise system, CPX; Medical Graphics, St. Paul, MN).

The work at $\dot{V}O_2$max was defined as the load at which the subjects were unable to continue due to fatigue. Heart rate and blood lactate concentration were measured during the last minute of the test. At this point, a steady-state $\dot{V}O_2$ had been reached, and it did not change despite increasing load; thus, the criteria for $\dot{V}O_2$max were fulfilled.[14]

Muscle biopsy samples were obtained with a concotome. A local anesthetic, Xylocaine 10 mg/mL (Astra Läkemedel, Södertälje, Sweden), was injected subcutaneously down to the fascia but not into the muscle tissue. The samples were obtained from the lateral part of the vastus lateralis and immediately frozen in liquid nitrogen and then stored at −70°C.

The muscle samples, with a weight of 50 to 80 mg, were analyzed for glucose, glycogen, glucose-6-phosphate, lactate, ATP, and creatine phosphate.[15] Carnitine (free and acylated) in serum, urine, and muscle was determined by the method described by Cederblad and Lindstedt,[16] with modifications.[3]

Intracellular glucose in muscle (millimoles per kilogram dry weight) was estimated from the wet weight according to the method of Katz et al.[17] To calculate the dry weight, the wet weight was multiplied by 4.3.

Statistical evaluation was made by the Wilcoxon signed-rank test for paired samples.

RESULTS

The concentration of free carnitine in serum decreased successively during the treatment, reaching a low mean level of 3.5 μmol/L after 54 days (Table 1 and Fig 1). The concentration of carnitine in muscle did not decrease significantly during the first 2 weeks, but was reduced to approximately half at 54 days (Table 1 and Fig 2).

Maximal Exercise

$\dot{V}O_2$max, heart rate, and ventilation were unchanged at 10 and 16 days of treatment with pivmecillinam, but had decreased at 54 days and were significantly correlated with the decrease in muscle carnitine concentration (Table 2).

Table 1. Carnitine Concentration (mean) Before and After Treatment With Pivmecillinam in Groups 1 and 2 for 10 and 54 Days, Respectively

	Group 1		Group 2		
Parameter	0 d	10 d	0 d	16 d	54 d
Serum (μmol/L)					
Total carnitine	42.0	23.0*	38.6	17.6*	11.1*
Acylcarnitine	4.8	9.0*	3.6	9.2*	7.6*
Free carnitine	37.0	13.7*	35.0	8.4*	3.5*
Muscle (μmol/g NCP)					
Total carnitine	18.0	15.6	15.8	15.7	8.3*
Acylcarnitine	4.4	6.5	5.8	5.7	4.0
Free carnitine	13.6	9.1	10.0	10.0	4.3*

*$P < .05$ *v* day 0 value.

Blood lactate levels decreased significantly in relation to treatment time.

Submaximal Exercise

Because of the lower $\dot{V}O_2$max after pivmecillinam treatment for 54 days, the work load was set at a lower level for the submaximal test (Table 3). $\dot{V}O_2$ was lower in this test but there was no change of heart rate, indicating an unchanged relative load. In group 2, the respiratory quotient ($\dot{V}CO_2/\dot{V}O_2$) increased from 0.80 to 0.87 after 16 days of treatment, remaining at that level after 54 days. The respiratory quotient was stable during the 60-minute test period before and after 16 and 54 days of treatment.

An increased concentration difference in serum during exercise was seen for glucose, triglycerides, and free fatty acids when values obtained after treatment in group 1 were compared with those obtained before treatment. In group 2, the mean concentrations of glucose in serum at rest were lower at 16 and 54 days compared with before treatment. Before pivmecillinam treatment, blood lactate increased significantly during the test in both group 1 and group 2; at 10, 16, and 54 days of treatment, the increase was not significant. The concentration of 3-hydroxybutyrate in serum increased both before and after treatment. The elevation was less marked after treatment, but the difference did not reach significance ($P = .06$ for 0 days *v* 16 and 54 days). However, the mean concentration of serum 3-hydroxybutyrate after exercise was lower after 16 and 54 days compared with before treatment.

In muscle, there was a marked reduction in glycogen concentration during the 60-minute exercise test before treatment in both groups, as well as after 10 and 16 days of treatment (Fig 3B); after 54 days, the decrease was minimal. In group 1, the difference in muscle glucose concentration decreased from +0.40 before to +0.07 mmol/kg after 10 days of treatment. In group 1, the mean concentration of lactate in blood after exercise was lower after 10 days of treatment than before treatment. The same phenomenon was observed in group 2 after 16 and 54 days of treatment, but the differences were not significant ($P = .09$ and .27, respectively). Both ATP and creatine phosphate decreased significantly before but not after treatment in group 1 and group 2.

The estimated concentration of intracellular muscle glucose generally increased during the exercise tests performed before the start of treatment. In groups 1 and 2, 11 of 12 (one failed biopsy) subjects had a lower increase after treatment; in three subjects of each group, there was an absolute decrease.

At 54 days, five of seven subjects spontaneously reported that they had difficulty walking uphill due to fatigue. Despite the lower absolute workload at the submaximal test, six of seven complained of pain in the legs and had difficulty completing 60 minutes on the bicycle.

DISCUSSION

The body content of carnitine is approximately 1.4 mmol/kg body weight, or a total of about 100 mmol for a 70-kg adult[18]; 92% to 97% of this is found in muscle tissue.[19]

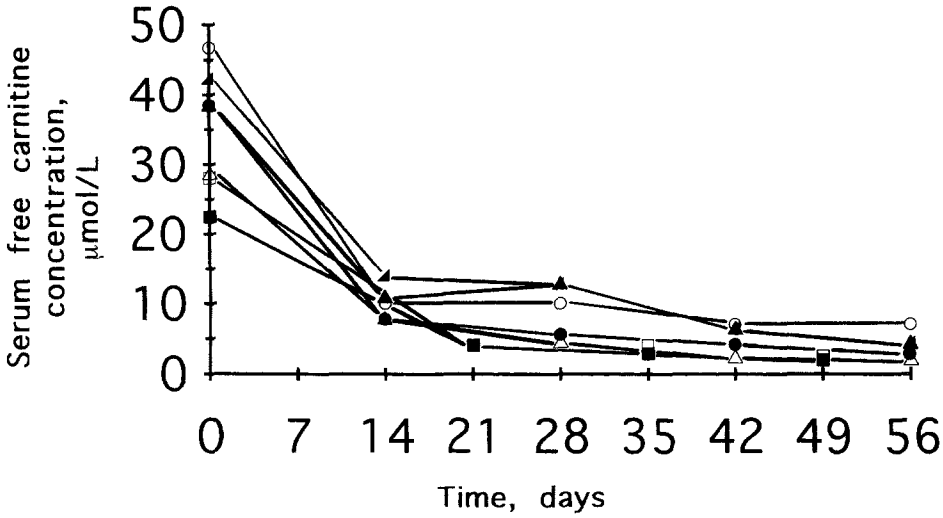

Fig 1. Serum free carnitine concentrations according to time of treatment.

The other two compartments containing substantial amounts of carnitine are the extracellular space and liver/kidney. Carnitine depletion has effects on the liver, heart, and skeletal muscle. When carnitine was lost during treatment with antibiotics containing pivalic acid, there was a rapid concomitant decrease of the serum level to 50% of the initial value in 2 days, with a slower decrease during the following week.[3] A similar pattern was seen in the present study, when a mean concentration of 12.1 μmol/L free carnitine in serum was reached after 5 days. Thereafter, there was a slow further decrease of free carnitine concentration in serum. The muscle concentration of carnitine remained unchanged for 2 weeks, but was significantly decreased at 4 weeks and continued to decrease thereafter. This slower decrease of muscle versus serum carnitine is a reflection of the large pool of carnitine in muscle tissue and the slow equilibration between muscle and the other compartments, ie, the extracellular space and the liver. There is reason to believe that prolonged treatment would deplete the muscle stores further, since children who were administered drugs containing pivalic acid for 1 to 2 years had a mean concentration of total carnitine in muscle of only 1.6 μmol/g NCP.[4] Because there is a rapid equilibration between the serum and liver, carnitine depletion may rapidly reach such low levels in the liver that the influx of long-chain fatty acids into the mitochondria is impaired. This is reflected in the very low production of 3-hydroxybutyrate during exercise after 16 days, ie, when the serum and consequently the liver concentrations were reduced to 12% of the initial values. Furthermore, inadequate ketone body production has previously been reported during fasting.[4,12]

The relation between physical exercise and carnitine has been much studied and has yielded sometimes contradictory results. Results in vivo have mostly been obtained by long- or short-term administration of excess carnitine. However, Heinonen et al[20] fed a carnitine-free formula diet to rats that reduced the concentration of carnitine to 50% of initial levels in the serum, heart, and skeletal muscle. They found no effect on palmitate oxidation, nitrogen balance, or physical endurance. Feeding carnitine, with a 50% increase in muscle and serum carnitine concentration, had no effect on the above-mentioned parameters.

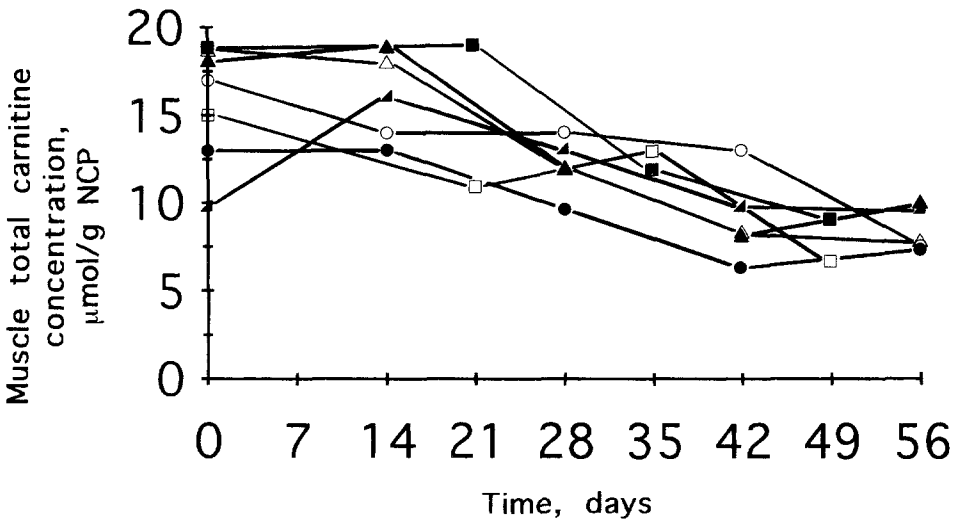

Fig 2. Total muscle carnitine concentrations according to time of treatment.

Table 2. Physiological and Biochemical Variables (mean) During Maximal Exercise Test Before and After Treatment With Pivmecillinam in Groups 1 and 2 for 10 and 54 Days, Respectively

Variable	Group 1		Group 2		
	0 d	10 d	0 d	16 d	54 d
$\dot{V}O_2$max (L/min STPD)	3.20 ± 0.84	3.28 ± 0.73	2.57 ± 1.07	2.47 ± 0.93	2.26 ± 0.93*
Maximal heart rate (beats/min)	180 ± 11	181 ± 10	178 ± 18	175 ± 17	165 ± 15*
Maximal ventilation (L/min BTPS)	138 ± 30	150 ± 41	103 ± 46	99 ± 37	84 ± 36*
Respiratory quotient at $\dot{V}O_2$max	1.20 ± 0.06	1.16 ± 0.04	1.14 ± 0.06	1.08 ± 0.04	1.09 ± 0.08
Blood lactate at $\dot{V}O_2$max (mmol/L)	14.9 ± 2.9	12.2 ± 2.9*	10.1 ± 2.7	8.4 ± 2.6*	6.5 ± 2.1*†

*$P < .05$ *v* day 0.
†$P < .05$ *v* day 16.

The carnitine concentration of human heart is about twice that of skeletal muscle. Although the rate of depletion is not known, it probably is essentially parallel to the rate in muscle. It is interesting that Diep et al[21] found that pivaloylcarnitine as a percent of free carnitine was six times higher in heart muscle than in skeletal muscle in rats after oral administration of pivampicillin. It is well known that primary carnitine deficiency has profound effects on cardiac function. We have also previously reported that a 54-day administration of pivaloyl-conjugated drugs resulted in a mean reduction of left ventricular mass of 20%.[22] Several investigators report an increase in $\dot{V}O_2$max after supplementation with carnitine,[23-26] whereas others report no change or insignificant change.[27-29] $\dot{V}O_2$max is considered to be dependent on heart function rather than on skeletal muscle function.[30] Group 1 had a higher mean initial $\dot{V}O_2$max than group 2, presumably due to a different age and sex distribution. There was no reduction in $\dot{V}O_2$max after 10 or 16 days, but there was a mean reduction of $\dot{V}O_2$max (−12%) and maximal heart rate (−7%) after 54 days. The subjects spontaneously complained of weakness and poor endurance. It is worth noting that the blood lactate concentration at $\dot{V}O_2$max after 10, 16, and 54 days of treatment was lower than before treatment and at a level usually not limiting to further exercise (Table 2). Thus, moderate carnitine depletion appears to affect human heart function and indirectly $\dot{V}O_2$max.

At exercise above 70% of $\dot{V}O_2$max, carbohydrate metabolism dominates. We chose an exercise level of 50% to 60% of maximal, at which level fat is the main energy source. In group 2, the respiratory quotient increased from 0.80 before to 0.87 after 16 days of treatment with pivmecillinam, and remained at that level even after 54 days (Table 3). Group 1 did not show the increase in the respiratory quotient seen in group 2. Therefore, on the basis of change in the respiratory quotient, it is difficult to state that carbohydrate is used more after treatment with pivalic acid. As previously mentioned, the significantly decreased mean concentration of 3-hydroxybutyrate in serum after exercise at 16 and 54 days compared with before the start of treatment suggests a decreased oxidation of free fatty acids in the liver. An alternative explanation would be an increased utilization of 3-hydroxybutyrate in the muscle tissue.

There was successively less reduction in the concentration of glycogen in muscle during work over the study period, and the reduction during work was almost abolished after 54 days of treatment. Furthermore, the increase in blood lactate seen during exercise before treatment was significant, whereas after treatment, the increase during exercise was not significant. The effect of carnitine on the utilization of substrates for energy production has been studied in the isolated rat heart.[31] In the presence of palmitate, addition of carnitine caused an increase both in glycolysis (1.75-fold) and in glucose oxidation (3.14-fold). Furthermore, it has recently been shown that treatment with L-carnitine in rats with secondary carnitine deficiency is of benefit to cardiac function by increasing overall glucose utilization rather than normalizing fatty acid metabolism.[32] The results were interpreted in terms of the glucose–fatty acid cycle as originally proposed by Randle et al,[33] which explains the inhibition of glycolysis and glucose oxidation by fatty acids by an effect on the key enzymes, pyruvate dehydrogenase and phosphofructokinase. The activity of pyruvate dehydrogenase is regulated by phosphorylation-dephosphorylation of the enzyme, and activity of the phosphokinase is dependent on the ratio of acetylCoA to CoA. The transfer of acetyl groups from acetylCoA to carnitine is catalyzed by the enzyme acetylcarnitine transferase, which has an equilibrium constant of approximately 1, implying a linear relation between carnitine concentration and the ratio of CoA to acetylCoA. As originally proposed by Tubbs et al,[34] carnitine therefore plays an essential role

Table 3. Physiological Variables (mean) at 60 Minutes of Submaximal Exercise in Groups 1 and 2 for 10 and 54 Days of Treatment With Pivmecillinam, Respectively

Variable	Group 1		Group 2		
	0 d	10 d	0 d	16 d	54 d
Work load (W)	112 ± 30	112 ± 30	97 ± 52	94 ± 45	82 ± 45
Ventilation (L/min BTPS)	46 ± 12	50 ± 13*	45 ± 15	46 ± 12	40 ± 11
$\dot{V}O_2$ (L/min STPD)	1.60 ± 0.40	1.58 ± 0.39	1.53 ± 0.70	1.54 ± 0.63	1.33 ± 0.52*
Heart rate (beats/min)	125 ± 12	122 ± 11	134 ± 16	136 ± 10	131 ± 12
Respiratory quotient	0.86 ± 0.02	0.85 ± 0.02	0.80 ± 0.03	0.87 ± 0.03*	0.87 ± 0.03*

*$P < .05$ *v* day 0.

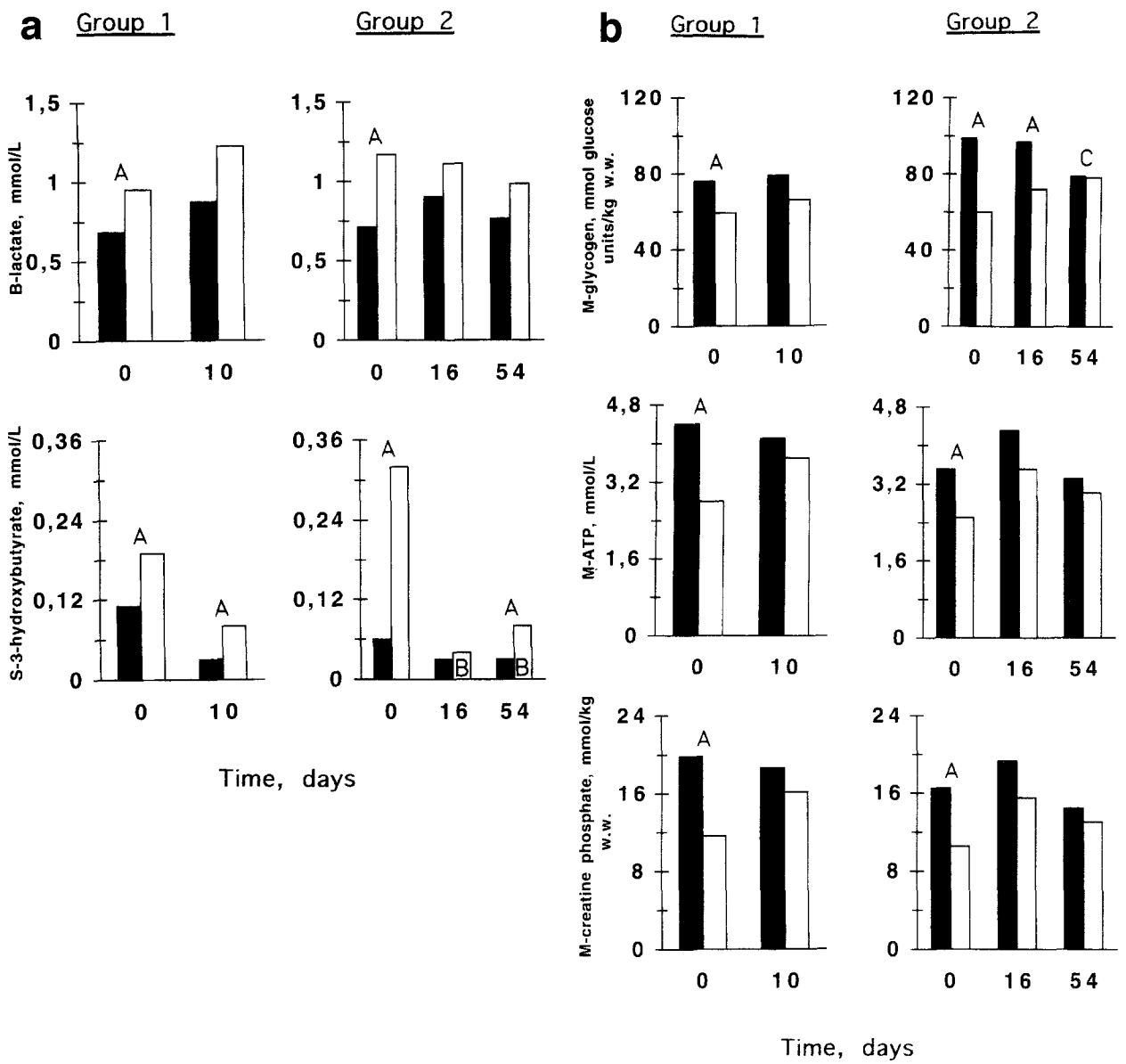

Fig 3. Biochemical variables (mean) in serum/blood (a) and muscle (b) before (●) and after (○) 60 minutes of submaximal exercise. A, significant ($P < .05$) difference between before and after exercise; B, significant ($P < .05$) difference after exercise compared with day 0; C, significant ($P < .05$) difference between the difference during exercise (delta value) compared with the difference during exercise (delta value) at day 0.

as a "sink" for acetyl groups for their transport over the mitochondrial membrane and for the degree of activation of pyruvate dehydrogenase. Uziel et al[35] measured the activity of pyruvate dehydrogenase in isolated mitochondria from human skeletal muscle with a varying concentration of L-carnitine in the medium, and found an almost twofold increase over control values at a concentration of 1 mmol/L carnitine, ie, at concentrations in the range of physiologic intracellular levels.

Carnitine depletion in skeletal muscle was moderate. Experiments with isolated mitochondria from rat heart and muscle provided evidence for a microcompartmentation of carnitine in the matrix.[36] In this preparation, a severe depletion of matrix carnitine was necessary before the influx of long-chain acylcarnitines was affected.

There was a persistent high muscle ATP and creatine phosphate concentration during the treatment period. There was a decreased consumption of muscle glycogen during exercise, and one may assume that there was no increase in the utilization of fatty acids. Possible sources of energy would be either protein via the gluconeogenesis pathway or glucose released from the liver.

The fatigue experienced by the patients and volunteers during periods of more or less severe carnitine depletion is not fully explained, but might be in part of cardiac origin and/or of a central nervous system nature.[37] An effect of carnitine depletion on cardiac structure was seen in healthy volunteers[22] and in children on long-term prophylactic treatment with pivaloyl-conjugated antibiotics.[38] Even though the cardiac effects and tiredness may not be of

critical importance in otherwise healthy people in everyday life, they would be expected to influence the capacity for heavy physical work and also the performance of athletes in many sports.

As discussed earlier, it has not been possible to induce a more severe carnitine deficiency in, eg, rats due to the endogenous synthesis. In secondary carnitine deficiency in man due to, eg, metabolic diseases, it is not possible to differentiate between symptoms of primary disorder and the symptom of induced carnitine deficiency. We find it unlikely that the antibiotic moiety is responsible for the observed effects or that pivalic acid as such should have toxic effects. The findings now reported must therefore be regarded as due to its interference in carnitine metabolism and its ability to induce a carnitine deficiency state.

In conclusion, this study has shown that a 54-day treatment with drugs containing pivalic acid decreases the carnitine concentration to levels for which signs of an altered metabolism are observed in cardiac and skeletal muscle and in the liver.

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