Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise

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WAGENMAKERS, ANTON J. M., ED J. BECKERS, FRED BROUNS, HARM KUIPERS, PETER B. SOETERS, GER J. VAN DER VUSSE, AND WIM H. M. SARIS. Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. Am. J. Physiol. 260 (Endocrinol. Metab. 23): E683–E690, 1991.—Eighty highly trained cyclists were studied during exercise after glycogen depletion (test A) and during carbohydrate (CHO) loading (test B). In test B subjects were able to complete 2 h of exercise at 70–75% maximal workload (W_max), whereas the initial intensity of 70% W_max had to be reduced to 60% in test A. Plasma ammonia increased more rapidly, and plasma alanine, glutamate, and glutamine were lower in test A. Exercise caused a 3.6-fold increase in the proportion of active branched-chain 2-oxoacid dehydrogenase complex (BC) complex in muscle in test A. No activation occurred in test B. There was an inverse correlation between the activity of the BC complex and the glycogen content of the postexercise biopsies. Exercise did not cause changes in the muscle content of ATP, ADP, AMP, IMP, hypoxanthine, and lactate. It is concluded that CHO loading abolishes increases in branched-chain amino acid (BCAA) oxidation during exercise and that part of the ammonia production during prolonged exercise originates from deamination of amino acids. The data appear to confirm the hypothesis (A. J. M. Wagenmakers, J. H. Coakley, and R. H. T. Edwards. Int. J. Sports Med. 11: S101–S113, 1990) that acceleration of the BCAA aminotransferase reaction may drain the tricarboxylic acid cycle and that glycogen is a carbon chain precursor of tricarboxylic acid cycle intermediates and glutamine.

human muscle; branched-chain 2-oxoacid dehydrogenase; phosphorylation state; intense cycle exercise; fatigue; branched-chain amino acids; ammonia; protein requirement; tricarboxylic acid cycle

THE CLASSIC STUDY of Fick and Weisencrus (12), in which they measured the urinary nitrogen excretion during and after the ascent of the Faulhorn, a Swiss mountain, was the first of a series of investigations that have led to the general opinion that proteins and amino acids do not contribute significantly to energy supply during exercise (1).

However, in many studies published in the past decade, new and different techniques have been applied and new information has become available on the role of amino acids as a fuel. An increased urinary urea excretion has been observed after ultradistance running (7). Lemon and Mullin (24) reported that, in addition to the urinary losses, substantial amounts of urea may be lost in sweat during human endurance exercise. Exercise was found to increase production rates in vivo of 14CO2 and 15CO2 from [14C]- and [15C]-leucine (e.g., see Refs. 25, 27, 28, 36).

In several studies, exercise-induced changes have been investigated of the activity in muscle of the branched-chain 2-oxoacid dehydrogenase complex (BC complex), which is the rate-limiting enzyme in the degradation route of the branched-chain amino acids (BCAAs; leucine, valine, and isoleucine). In the early 1980s the activity of this enzyme was shown to be regulated by a phosphorylation-dephosphorylation cycle, with phosphorylation causing inactivation. Endurance exercise of rats has been reported to increase the amount of active BC complex in muscle. Wagenmakers et al. (34) observed a 52% increase in untrained rats running for 1 h at 12 m/min up a 15% incline, and Kasperk and Snider (19) observed a 76% increase in untrained rats running for 2 h at 10 m/min. Large discrepancies were seen in the degree of activation of the BC complex at higher exercise intensities. Kasperk et al. (18) observed a 10-fold increase in untrained rats running to exhaustion in 168 min at 27 m/min, whereas in later studies (17, 19) they reported a 3.5-fold increase in rats running for 120 min at 30 m/min. Shimura et al. (29) studied the effect of carbohydrate vs. fat diets on the activation of the BC complex in trained rats running for 2 h at 30 m/min up an 8° incline and in both groups reported a four- to five-fold activation. Hood and Terjung (14) observed a 2.8-fold activation of leucine oxidation during situ electrical stimulation (40 min at 45 tetani/min, corresponding to 100% maximum O2 consumption (VO2 max)) of an isolated rat hindlimb muscle preparation. Recently, Wagenmakers et al. (32) reported a fourfold activation after 2 h of cycle exercise at 70 ± 10% of VO2 max in trained human volunteers.

Most endurance athletes nowadays consume substantial amounts of carbohydrates (CHO) the days before and during competition to prevent glycogen depletion and to improve exercise performance. Evidence has been presented that oral glucose supplements decrease the 14CO2 production from [14C]-leucine (28). Estimates of protein catabolism based on urea excretion in urine and sweat indicated that protein catabolism was accelerated when subjects were glycogen depleted (24) and could be reduced by CHO supplementation (3). Kasperk and Snider (19) observed a larger exercise-induced activation of the BC complex in 24-h starved (emptied glycogen stores) rats than in fed rats. Wagenmakers et al. (33)
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observed a rapid and excessive activation of the BC complex in patients with a glycogen breakdown defect in muscle (McArdle's disease, myophosphorylase deficiency).

In the present study we therefore investigated the exercise-induced activation of the muscle BC complex in eight healthy highly trained human cyclists. The subjects were studied after CHO loading the days before the exercise bout in combination with oral intake of CHO during exercise (as cyclists do during competition) and in a state of relative depletion of muscle glycogen. Exercise-induced changes in the muscle concentration of adenine nucleotides and glycogen and in the plasma (or serum) concentration of glucose, fatty acids, glycerol, 3-hydroxybutyrate, lactate, ammonia, and amino acids have also been studied in both protocols to relate changes in the concentration of these metabolites to possible differences in the degree of activation of the BC complex.

**Methods**

**Subjects and procedures.** Eight healthy highly trained male amateur cyclists, competing on the national and international level, volunteered after having been informed of the procedures and risks involved. Their age was 24 ± 6 (SD) yr. The studies were done at the end of the winter season after training had been resumed but shortly before competitive cycling races started. All procedures were approved by the Ethics Committee of the University of Maastricht.

The subjects were exercised on electromagnetically braked cycle ergometers (Lode, The Netherlands) at a freely chosen, power output-independent pedaling rate (50–120 revolutions/min rpm).

After their maximal workload \( W_{\max} \) of 411 ± 41 (SD) W, \( n = 8 \) attained during incremental exercise had been determined as described previously (21), the subjects were studied on two separate occasions (test A and B) during exercise bouts of 2 h performed in random order within 2 wk of assessment of \( W_{\max} \).

In test A the subjects reported to the laboratory at 1900 h on the night before the 2-h exercise bout. Their muscle glycogen concentration was decreased by an exercise protocol at alternating intensities as described previously (21). After warming up they cycled in 2-min blocks alternating at 90 and 60% \( W_{\max} \). This was continued until the 2-min block at 90% \( W_{\max} \) could not be completed anymore. The high intensity was then reduced to 80% until it could not be completed anymore and finally reduced to 70% \( W_{\max} \). When the 70% \( W_{\max} \) block could not be completed, the subjects were allowed to stop and were sent home. They were allowed to eat one sandwich with cheese or an isocaloric low-carbohydrate snack in the time between completion of the glycogen depletion protocol and before they went to sleep. As drinks they were allowed to take water, coffee, or tea ad libitum. The next morning for breakfast (0700 h) they had one sandwich with cheese and coffee or tea. They reported to the lab at 0830 h. After the necessary preparations had been taken (see below), cycling exercise was started at 0945 h with a warm-up (5 min 50 W, 5 min 40% \( W_{\max} \), 5 min 50% \( W_{\max} \)). Exercise intensity was then increased to 70% \( W_{\max} \). When the subjects indicated that they could not continue at 70% \( W_{\max} \), small stepwise reductions of 5% \( W_{\max} \) were allowed, until a lowest level of 50% \( W_{\max} \). Three of the subjects were stopped before completion of 2 h of exercise, because they could not maintain a pedaling rate of 50 rpm at 50% \( W_{\max} \) despite encouragement from the experimenters. During the exercise bout the subjects were allowed to drink water ad libitum.

In test B the usual food intake was supplemented with 1 liter of a maltodextrin drink (166 g CHO/l) during the last 2 days before the exercise test. Furthermore, no training occurred on these days. During breakfast on the day of the test (at 0700 h), another 0.5 liter of this drink supplemented the usual breakfast (replacing tea or coffee). After reporting to the lab at 0830 h, cycling exercise was started as described for test A. After 20 min of exercise at 70% \( W_{\max} \), a further increase to 75% \( W_{\max} \) occurred. When subjects indicated that they could not continue at 75% \( W_{\max} \), small stepwise reductions in exercise intensity were allowed as in test A. During the exercise bout the subjects were allowed to drink 1 liter of the CHO solution and on top of that water ad libitum.

After warming up, all subjects completed 2 h of exercise.

Percutaneous muscle biopsies (75–140 mg wet wt) were taken from the quadriceps muscle as described previously (32). Biopsies were taken as quickly as possible (between 10 and 45 s postexercise), and 50–100 mg of muscle were immediately transferred to cold buffer (<5 s) and homogenized (<10 s) to prevent interconversion of the BC complex after exercise was stopped. Homogenates prepared this way from muscle samples obtained at rest and during exercise can be stored on ice for 30 min without changes in active and total BC complex activity (35). This indicates that this technique is adequate to measure the percent active BC complex present in human muscle (32). In both protocols, biopsies were obtained before the exercise bout started from one leg and after 2 h of exercise (or at exhaustion while cycling at 50% \( W_{\max} \) from the other leg. Local anesthetic (1.0 ml of 0.5% Xylocaine) was infiltrated in the skin of both legs, and percutaneous incisions were made before exercise started. On the second occasion (1–2 wk later), incisions were made 2 cm from and parallel to the first incision again in both legs.

**Analytical methods.** The activity of the BC complex (active and total amount of enzyme) was assayed in fresh muscle homogenates as described previously (32). Part of the biopsies were frozen immediately (within 5 s after excision) between aluminum tongs cooled in liquid nitrogen; they were freeze-dried and stored in liquid nitrogen for analysis of muscle ATP, ADP, AMP, IMP, hypoxanthine, glycogen, and lactate as described previously (30). Muscle energy charge was calculated according to the formula \( (\text{ATP} + 0.5(\text{ADP}))/((\text{ATP}) + \text{[ADP]} + \text{[AMP]}), \) where square brackets denote concentration.

Blood was sampled from a forearm vein using an indwelling cannula during the 2-h exercise protocol. Samples were obtained at rest and after 20, 40, 60, 80, 100, and 120 min of exercise. Heparinized blood was centrifuged immediately at 800 g for 10 min to obtain plasma, and an aliquot of plasma was analyzed for lactate (Oxidometric lactate analyzer 640; Kontron, Zurich,
Switzerland). Aliquots of plasma were frozen for analysis of glucose (GOD-perid method; Boehringer Mannheim 124010). Ammonia was measured in frozen plasma samples using a modification of the enzymatic determination with glutamate dehydrogenase performed on a COBAS-Bio analyzer (16). Aliquots of the plasma were deproteinized with sulfosalicylic acid for analysis of amino acids (31) and with perchloric acid for analysis of 3-hydroxybutyric acid as described previously (32). Serum was prepared and frozen for analysis of fatty acids (Wako NEFA C test kit 994-75409; Wako Chemicals, Neuss, FRG) and glycerol (Boehringer Mannheim 125032).

Statistics. The Student paired t test was used for testing the significance of differences between related samples of the same subject obtained in tests A and B and for testing the significance of differences between samples of the same subject obtained at different times during the exercise bouts. Significance was set at $P < 0.05$. Simple regression analysis was performed to indicate the degree of association between two variables measured in muscle samples using a Statview 6.1 software program in a MacIntosh computer. Correlations were assumed to be significant when $P < 0.05$.

RESULTS

Performance. After the glycogen depletion protocol (test A), the subjects were not able to continue cycling at the starting intensity of 70% $\text{W}_{\text{max}}$ for a 2-h period (see Fig. 1A). Because it was the intention to study endurance exercise, they were allowed small gradual reductions in exercise intensity to a lowest level of 50% $\text{W}_{\text{max}}$. When the subjects, despite encouragement, were not able to keep the pedaling rate at >50 rpm while cycling at the 50% $\text{W}_{\text{max}}$ intensity, they were allowed to stop, and the postexercise biopsy was taken immediately. Three subjects did not complete 2 h of exercise after warming up. In test B (a combination of CHO loading and oral intake of CHO during exercise) all eight subjects could increase the exercise intensity to 75% $\text{W}_{\text{max}}$ after 20 min, and they all completed the 2 h of exercise either at 75 or 70% $\text{W}_{\text{max}}$.

Plasma metabolic profile. Plasma glucose and lactate concentrations were higher in test B (Fig. 1, B and C). Substantial gradual increases were seen in the concentration of plasma 3-hydroxybutyrate, serum fatty acids, and serum glycerol during test A. Increases in the concentrations of these metabolites were much smaller during test B (Fig. 1, D–F).

Plasma ammonia and amino acids. Plasma ammonia concentration increased more rapidly during the first 20 min in the exercise bout after glycogen depletion (test A) than after and during CHO loading (test B, Fig. 2A). In test A plasma ammonia subsequently fell when the exercise intensity was reduced to <70% $\text{W}_{\text{max}}$. In test B the exercise intensity was maintained at 70–75% $\text{W}_{\text{max}}$ throughout, and plasma ammonia rose gradually to a value that was already reached after 20 min in test A.

Plasma alanine and glutamate concentrations were lower in test A than in test B at all sampling points (Fig. 2, B and C). The concentration of the BCAAs (leucine, valine, and isoleucine) was higher at the start of exercise in test A than in test B but fell within 20 min to a similar concentration in both tests during the remainder of the exercise bouts (Fig. 2, D–F).

Plasma glutamine concentrations were similar during the first hour in tests A and B (Fig. 3A). A small significant decrease subsequently occurred in test A. This

![Fig. 1. Performance and plasma and serum metabolites in 8 highly trained subjects during cycle exercise. Values are means ± SD. Significant differences between values obtained in carbohydrate (CHO)-depleted and CHO-loaded state: a $P < 0.05$, b $P < 0.01$, and c $P < 0.001$. 3-Hydroxybutyrate, 3-hydroxybutyrate.](image)
decrease was primarily caused by a disproportionate fall of plasma glutamine in three of the subjects who had to quit exercise between 100 and 120 min before completion of the protocol (Fig. 3B). The glutamine concentration fell in these subjects from 682 ± 40 μM at the start of exercise to 464 ± 14 μM after 100 min of exercise. In the five subjects who completed the 2-h exercise bout, these values were 653 ± 70 and 605 ± 61 μM, respectively. Both decreases are significant at the P < 0.05 level.

Activities of the BC complex. Exercise did not change the total activity of the BC complex, in neither test A nor test B (Fig. 4A). Exercise after glycogen depletion caused a significant (P < 0.01) 3.6-fold increase of the proportion of active BC complex, whereas exercise during and after CHO loading led to a 1.3-fold (nonsignificant) activation.

Muscle metabolites. Exercise did not lead to significant changes in the muscle concentration of ATP, ADP, AMP, IMP, and lactate in neither test A nor test B (Fig. 5). There were also no differences in the concentration of these metabolites between test A and test B. There were no significant differences between the muscle energy charge before and after exercise in test A (0.949 ± 0.011 and 0.947 ± 0.009, respectively) and in test B (0.941 ± 0.010 and 0.944 ± 0.005, respectively). The ATP/ADP

Fig. 3. Plasma glutamine concentrations in 8 highly trained subjects during cycle exercise. Values are means ± SD (A) or individual values (B). Other details are given in legend to Fig. 1.

Fig. 4. Activities of branched-chain 3-oxoacid dehydrogenase complex (BC complex) in muscle biopsies of 8 highly trained individuals obtained before and after cycle exercise in CHO-depleted and CHO-loaded state. Values are means ± SD. * Significant difference between pre- and postexercise value at P < 0.01.
ratio did not change with exercise in test A (10.6 ± 1.3 before and 9.6 ± 1.7 after exercise) and in test B (9.4 ± 0.8 and 9.7 ± 1.1 before and after exercise, respectively). The muscle hypoxanthine concentration was below the level of detection (0.1 μmol/g dry muscle) in all biopsies. Muscle glycogen content decreased significantly in both tests (Fig. 5E). In test B muscle glycogen was higher than in test A both at the start of exercise (significant difference at P < 0.001) and after exercise (significant difference at P < 0.01). There was an inverse correlation between the proportion of active BC complex and the glycogen content of the postexercise biopsies obtained in this and a previous study (32). Such a relationship did not exist in the preexercise biopsies (Fig. 6).

**DISCUSSION**

It is well established that glycogen is the major energy source in endurance exercise of high intensity and that endurance is enhanced by high preexercise glycogen levels. It has also been shown that CHO feeding during exercise may enhance endurance (5) and may spare endogenous glycogen stores (15). Therefore it is not surprising that there is a substantial difference in cycling performance between test A (after prior glycogen depletion) and test B (after prior CHO loading and with CHO feeding during exercise). The large difference between the preexercise muscle glycogen contents in tests A and B (Fig. 5E) indicate that the respective protocols indeed had been quite effective.

The higher plasma concentrations of glucose and lactate in test B in combination with low levels of serum fatty acids, glycerol, and plasma 3-hydroxybutyrate appear to indicate that CHO are the most important fuel in test B. On the other hand, the much higher levels of fatty acids, glycerol, and 3-hydroxybutyrate observed in test A in combination with lower glucose and lactate concentrations appear to indicate that fatty acids and

**FIG. 5.** Muscle concentration of ATP, ADP, AMP, IMP, glycogen, and lactate in biopsies of 8 highly trained individuals obtained before and after cyclo exercise in CHO-depleted and CHO-loaded state. Other details are given in legend to Fig. 4.

**FIG. 6.** Relationship between percentage active branched-chain 2-oxoacid dehydrogenase complex (BC complex) and muscle glycogen concentration in pre- and postexercise biopsies obtained in present study and previously (32). A: preexercise. B: postexercise.
ketone bodies make up a significant contribution to fuel oxidation in test A. No gas exchange measurements have been made in this study to confirm this.

The many significant differences between the plasma concentration of amino acids (Figs. 2 and 3) observed in test A and B appear to indicate that there are differences in the degree of protein and amino acid catabolism between the two experimental protocols. The rapid decrease in the concentration of BCAA observed during the first 20 min of test A may be the consequence of the accelerated muscle oxidation of BCAA during exercise in the glycogen-depleted state, as indicated by the difference in activation of the BC complex (Fig. 4). BCAA are the main group of amino acids oxidized in muscle and therefore are the main amino group donors in muscle (13, 33). In the BCAA aminotransferase reaction the amino group is donated to 2-oxoglutarate to form glutamate (Fig. 7). In the reaction catalyzed by glutamine synthetase, glutamate subsequently reacts with ammonia to give glutamine, the main nontoxic amino group carrier released by muscle. Alternatively, glutamate may donate the amino group to pyruvate (derived from glucose or glycogen oxidation) to form alanine and to regenerate 2-oxoglutarate in the alanine aminotransferase reaction (Fig. 7). During exercise, muscle releases large amounts of glutamine and alanine in excess of its occurrence in muscle proteins and relatively small amounts of ammonia (9, 10).

The lower plasma alanine concentration in test A may be a consequence of a reduced availability of pyruvate, derived from glucose and glycogen metabolism, in the alanine aminotransferase reaction and may also be caused by a higher rate of gluconeogenesis from alanine in the glycogen-depleted state (10). The lower glutamate concentration could be a consequence of an increased uptake of glutamate by the muscle. During exercise, human muscle extracts glutamate (9), probably to clear, by means of the glutamine synthetase reaction, the ammonia produced in muscle.

Plasma ammonia increased more rapidly in test A than in test B. During brief intense exercise, ample evidence has been presented that ammonia is primarily produced from net breakdown of muscle adenine nucleotides to IMP (for a review see Ref. 33). In our subjects, however, we did not observe significant breakdown of muscle adenine nucleotides to IMP and hypoxanthine after endurance exercise (Fig. 5) in either test A nor test B. The absence of changes in these metabolites was unexpected, but our findings are supported by data from Norman et al. (28), who reported only small increases in the concentration of muscle IMP and hypoxanthine and no change in adenine nucleotides after endurance exercise to exhaustion at 55% of VO2max in seven male subjects of average physical fitness. Our subjects were highly trained cyclists, and it has been reported that an increase in the mitochondrial content, induced by exercise training, leads to reduced decreases in muscle adenine nucleotides during exercise (8, 29). In endurance exercise in highly trained subjects it is therefore quite likely that a major part of the muscle ammonia production is directly derived from deamination of amino acids, as we have recently suggested in patients with McArdle's disease who cannot use muscle glycogen as an energy source during exercise because of myophosphorylase deficiency (33). In these patients we observed an excessive increase of the plasma ammonia concentration (up to 500 μM) and a large ammonia release from the legs (760 μmol/min) during incremental cycle exercise. Because no evidence could be found of substantial breakdown of adenine nucleotides, deamination of amino acids (aspartate, BCAA, and glutamate) via the reactions of the purine nucleotide cycle and/or via glutamate dehydrogenase was proposed as an alternative source of ammonia production in muscle. Another source that could not be excluded is deamination of glutamine, released by muscle, by glutaminase present in the endothelial cells of the vascular system.

In healthy individuals, most of the ammonia produced is normally bound to glutamate by the action of the enzyme glutamine synthetase, so that the muscle releases large amounts of glutamine and only small amounts of ammonia (9). Glutamate taken up from the circulation only contributes a small proportion of the glutamate required for glutamine synthesis (9, 33). Other possible pathways that may contribute carbon skeletons required for muscle glutamine synthesis are the complete breakdown of valine and isoleucine to tricarboxylic acid cycle intermediates (13, 33) and the production of tricarboxylic acid cycle intermediates from muscle glycogen and glucose via carboxylation of pyruvate (6) or by reversal of the phosphoenolpyruvate carboxykinase reaction (Ref. 20; Fig. 7). If the latter source would make an important contribution, then the endogenous production of tricar-

**FIG. 7.** Draining effect of branched-chain amino acid (BCAA) aminotransferase reaction on tricarboxylic acid (TCA) cycle counteracted by anaplerotic conversion of glycogen and glucose to TCA intermediates via carboxylation of pyruvate (route b) and/or reversal of phosphoenolpyruvate (PEP) carboxykinase reaction (route a). Role of muscle glycogen as a precursor for synthesis of carbon skeletons of glutamate and glutamine. BCOA, branched-chain 2-oxoacids; glucose 1-P, glucose 1-phosphate.
bovylc acid cycle intermediates and glutamate would be smaller in the glycogen-depleted state, and in the end even the muscle glutamate production could cease. This may explain the steeper increase in the plasma ammonia concentration during the first 20 min of exercise in test A compared with test B, and it may further explain why the plasma glutamine concentration starts to fall in test A after 1 h of exercise compared with test B. The most substantial decrease in the plasma glutamine concentration was seen in three subjects who had to quit exercise before completion of the 2-h exercise bout. In one of them we could not analyze the muscle glycogen content because of technical problems, but the other two had by far the lowest glycogen content (80 and 96 μmol/g dry muscle). Endurance exercise has also been reported to lead to a large decrease of the muscle glutamine concentration (27).

If muscle glycogen is an important precursor of tricarboxylic acid cycle intermediates and glutamine, then one could also expect problems with the maintenance of flux in the tricarboxylic acid cycle in the glycogen-depleted state and in conditions in which glycogen is not available. Recently (33), we reported an extremely rapid and excessive activation of the BC complex in patients with McArdle's disease (myophosphorylase deficiency). In these patients we also observed that administration of BCAA before exercise enhanced the increase in plasma ammonia concentration during exercise and led to a deterioration of exercise performance. We therefore hypothesized that excessive activation of BCAA metabolism could drain the tricarboxylic acid cycle in the primary BCAA aminotransferase reaction, in which 2-oxo-glutarate is used as an amino group acceptor, and thus lead to a reduced flux in the tricarboxylic acid cycle and a reduced ability to oxidize blood-born glucose and fatty acids (Fig. 7). The same mechanism may apply to healthy individuals in the glycogen-depleted state and in fact may provide the biochemical explanation for the observation that glycogen depletion inevitably leads to a reduction in exercise performance (33). More experimental research is required to investigate this hypothetical role of muscle glycogen as an anaplerotic precursor that may counteract the draining action of excessive BCAA metabolism on the tricarboxylic acid cycle and may provide a major proportion of the carbon skeletons required for glutamine synthesis in muscle during exercise.1

The exercise-induced activation of the BC complex clearly is inversely related to the muscle glycogen content (Figs. 4 and 6) and availability. It should be noted that lack of availability of glycogen in McArdle's disease gives rise to excessive activation of the BC complex (33). The mechanism of activation of the BC complex remains unknown. Lau et al. (22) have identified ADP as an inhibitor of the kinase of the BC complex, and a rise in its concentration due to a disturbance in the energy state of the muscle could thus activate the BC complex. Kasperek (17) recently found a negative correlation between the muscle ATP content and the activity of the BC complex during exercise in untrained rats and therefore also suggested that disturbances in the muscle energy state could regulate the BC complex. However, Shimomura et al. (29) reported a four- to five-fold activation of the BC complex in trained rats that had run for 2 h on a treadmill without changes in muscle ATP and ADP concentration. We also did not find changes in the muscle content of ATP, ADP, and AMP, the energy charge \([([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]])\), and the ratio of ATP to ADP content in tests A and B, nor were there differences in the content of these compounds and ratios between tests A and B (Fig. 5). However, in all these studies, including ours, only the concentration of total tissue adenine nucleotides has been measured, whereas control of the kinase of the BC complex is more likely exerted either by the cytosolic or mitochondrial free (i.e., not bound to protein) ADP concentration. Despite an unchanged total tissue ATP and ADP content, changes may have occurred anyway in the cytosolic or mitochondrial free ADP concentration, and then most likely a larger increase will occur in the glycogen-depleted state. Only small changes in free ADP and AMP are required to control mitochondrial respiration in highly trained muscles (8). This could also apply to control of the kinase of the BC complex. The branched-chain 2-oxoacids, especially the 2-oxoacids of leucine, are also potent inhibitors of the kinase of the BC complex, and Busa and collaborators have pushed the importance of this regulatory role among others on the basis of a positive correlation of the activity of the BC complex with the plasma leucine concentration in many conditions (e.g., see references given in Ref. 2). However, this correlation is not observed in our experiments, since the plasma concentration of leucine, isoleucine, and valine decreased during exercise both in the glycogen-depleted and in the CHO-loaded state (Fig. 2). Nevertheless, it seems possible that increases in the muscle concentration of leucine and its 2-oxoacid play a role in the observed activation of the BC complex during exercise. Fielding et al. (11) have observed an increase in the concentration of 4-methyl-2-oxopentanoate (2-oxoacid of leucine) in human muscle after brief incremental exhaustive exercise for 15 min. In rats the response of plasma BCAA to exercise appears to depend on diet (29). Muscle BCAA concentration increased during prolonged exercise in rats (17, 20), but the concentration of branched-chain 2-oxoacids only started to increase after exercise (17). Obviously more detailed investigations (mitochondrial concentrations of potential regulators) are required to elucidate the mechanism of activation of the BC complex during exercise.

1 After submission of this manuscript K. Sehlin, A. Katz, and S. Broberg [Am. J. Physiol. 259 (Cell Physiol. 29): C834–C841, 1990] presented data confirming that glycogen depletion during prolonged dynamic exercise in humans indeed may impair aerobic energy production by reducing the level of tricarboxylic acid cycle intermediates in muscle.
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