Low glycogen and branched-chain amino acid ingestion do not impair anaplerosis during exercise in humans

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Gibala, Martin J., Marco Lozej, Mark A. Tarnopolsky, Cyndy McLean, and Terry E. Graham. Low glycogen and branched-chain amino acid ingestion do not impair anaplerosis during exercise in humans. J. Appl. Physiol. 87(5): 1662–1667, 1999.—We examined the hypothesis that increasing the rate of branched-chain amino acid (BCAA) oxidation, during conditions of low glycogen availability, reduces the level of muscle tricarboxylic acid cycle intermediates (TCAI) by placing a carbon “drain” on the cycle at the level of 2-oxoglutarate. Six men cycled at ~70% of maximal oxygen uptake for 15 min under two conditions: 1) low preexercise muscle glycogen (placebo) and 2) low glycogen combined with BCAA ingestion. We have previously shown that BCAA ingestion increased the activity of branched-chain oxoacid dehydrogenase, the rate-limiting enzyme for BCAA oxidation in muscle, compared with low glycogen alone (M. L. Jackman, M. J. Gibala, E. Hultman, and T. E. Graham. Am. J. Physiol. 272 (Endocrinol. Metab. 35): E239–E244, 1997). Muscle glycogen concentration was 185 ± 22 and 206 ± 22 mmol/kg dry wt at rest for the placebo and BCAA-supplemented trials, respectively, and decreased to 109 ± 18 and 96 ± 10 mmol/kg dry wt after exercise. The net increase in the total concentration of six measured TCAI (~95% of TCAI pool) during exercise was not different between trials (3.97 ± 0.34 vs. 3.88 ± 0.34 mmol/kg dry wt for the placebo and BCAA trials, respectively). Muscle 2-oxoglutarate concentration decreased from ~0.05 at rest to ~0.03 mmol/kg dry wt after exercise in both trials. The magnitude of TCAI pool expansion in both trials was similar to that seen previously in subjects who performed an identical exercise bout after a normal mixed diet (M. J. Gibala, E. Hultman, and T. E. Graham. Am. J. Physiol. 272 (Endocrinol. Metab. 35): E239–E244, 1997). These data suggest that increasing the rate of BCAA oxidation has no measurable effect on muscle TCAI during exercise with low glycogen in humans. Moreover, it appears that low resting glycogen per se does not impair the increase in TCAI during moderate exercise.

The total concentration of tricarboxylic acid (TCA) cycle intermediates (TCAI) increases severalfold during moderate dynamic exercise (~70–75% maximal oxygen uptake (V̇O2max)) in human skeletal muscle (7, 20). The expansion of the TCAI pool, usually referred to as “anaplerosis,” is a very rapid phenomenon and peaks within the initial few minutes of exercise (5, 7, 20). If the exercise bout is prolonged, however, the pool of intermediates subsequently declines, such that the total concentration of TCAI after 75–90 min is lower compared with that during the initial minutes of contraction (7, 20). The precise functional significance of changes in TCAI during exercise is not known; however, the phenomenon appears related to alterations in carbohydrate availability. For example, the rapid increase in TCAI at the start of exercise is generally attributed to an increase in muscle pyruvate flux, which directs the near-equilibrium alanine aminotransferase reaction (pyruvate + glutamate → 2-oxoglutarate + alanine) toward the formation of TCAI by a mass-action effect (5, 7, 20). Conversely, the decline in TCAI during prolonged exercise has been ascribed to a reduced rate of pyruvate production, secondary to a decrease in muscle glycogen availability (20).

In addition to these carbohydrate-mediated influences, the concentrations of TCAI may potentially be affected by changes in muscle protein metabolism, because several intermediates take part in ancillary reactions involving amino acids. For example, the first step in the metabolism of the branched-chain amino acids (BCAA; leucine, isoleucine, valine) is a reversible transamination reaction in which 2-oxoglutarate combines with a BCAA, forming glutamate and a branched-chain oxoacid. The oxoacid can then either be released from the muscle or undergo an irreversible, oxidative decarboxylation reaction catalyzed by branched-chain oxoacid dehydrogenase, the key rate-limiting enzyme for BCAA metabolism in skeletal muscle (18). Wagenmakers and colleagues (25, 26) hypothesized that, as a consequence of the initial BCAA aminotransferase reaction, the oxidation of BCAA (specifically leucine) places a carbon “drain” on the TCA cycle, which may lead to a reduction in the muscle concentration of 2-oxoglutarate or other TCAI. According to this theory, the BCAA-mediated drain of 2-oxoglutarate is normally counteracted by the regeneration of this intermediate through the alanine aminotransferase reaction, provided sufficient glycogen is available to sustain the rate of pyruvate production. However, during conditions in which glycogen availability becomes limited, and particularly if the rate of BCAA oxidation is increased, it was suggested that the concentrations of 2-oxoglutarate and/or other TCAI will decrease (25, 26). These authors have further proposed that this scenario may lead to a “suboptimal concentration” of TCAI and impair oxidative energy provision in skeletal muscle by reducing TCA cycle flux (for recent reviews, see Refs. 23 and 24).
It should be emphasized, however, that this hypothesis has not been tested experimentally, and no direct evidence has been presented to support the notion that changes in muscle TCAI during exercise are an important regulator of aerobic energy metabolism. Indeed, recent studies from this laboratory (for review, see Ref. 8) and others (4) have suggested that changes in muscle TCAI are not causally related to TCA cycle flux or aerobic energy provision but rather are primarily a reflection of alterations in muscle pyruvate availability.

Regardless of the precise physiological significance of anaplerosis, it remains to be determined whether altering glycogen availability and/or increasing the oxidation rate of BCAA influences the muscle concentration of TCAI during exercise. Our purpose in the present investigation, therefore, was twofold: 1) to examine the effect of reduced muscle glycogen availability per se on the increase in muscle TCAI during moderate exercise and 2) to determine whether increasing the oxidation rate of BCAA, during conditions of low glycogen availability, has any measurable effect on 2-oxoglutarate or other TCA intermediates.

METHODS

Subjects. Six healthy men, with a mean age, height, and body mass of 22 ± 1 yr, 181 ± 2 cm, and 77 ± 4 kg, respectively, took part in the investigation. The subjects were recreationally active but were not specifically trained. The experimental protocol was approved by the University of Guelph's Human Ethics Committee, and all subjects provided written consent.

Overview of experimental design. At least 1 wk before the start of the experiment, subjects performed a progressive exercise test on an electrically braked cycle ergometer to determine their V̇O₂max. The mean V̇O₂max for the group was 58 ± 6 ml·min⁻¹·kg⁻¹. The experimental protocol was performed on two occasions, separated by 5–7 days, under two different preexercise dietary conditions: 1) low muscle glycogen and 2) low muscle glycogen supplemented with BCAA.

We incorporated the design used in previous studies from our laboratory (12, 15), whereby subjects performed a 2-h cycle ergometer ride on the day before the experiment to deplete muscle glycogen levels and then consumed a diet low in carbohydrate over the next ~20 h. The preexercise diet, which was identical before both trials for a given subject, was formulated from 18 ± 4% carbohydrate, 54 ± 4% fat, and 28 ± 4% protein (Nutri-Pro Diet Analysis Software, West Publishing) to ensure that muscle glycogen levels were substantially reduced before exercise. In a single-blind fashion, subjects were also administered capsules that contained either a placebo (dextrose) or BCAA (44% leucine, 30% valine, 26% isoleucine; Switzerland Chemicals) before exercise. The purity of the BCAA capsules was previously confirmed by dilution of the capsules in water and analysis by high-performance liquid chromatography (HPLC) (15). The capsules were administered in three oral doses of 134, 77, and 77 mg/kg body mass at 90, 45, and 20 min before exercise, respectively. The two experimental trials were performed in a balanced manner, such that three subjects began the study with the placebo capsules and three with the BCAA capsules.

Experimental protocol. On arrival at the laboratory on the day of an experimental trial, the subject rested in the supine position, and a catheter for blood sampling was inserted into an antecubital vein. A resting blood sample was obtained 90 min before exercise, immediately before the administration of the placebo or BCAA capsules. Approximately 15 min before exercise, the area over the lateral aspect of one thigh was anesthetized (2% Xylocaine without epinephrine), and two small incisions were made to permit the extraction of needle-biopsy samples from the vastus lateralis muscle (2). A blood sample and a needle-biopsy sample were obtained after ingestion of the capsules (dextrose or BCAA), immediately before the start of exercise. The subject then cycled at a workload corresponding to ~70% V̇O₂max (210 ± 10 W) for 15 min. Expired gas measurements and venous blood samples were obtained after 5, 10, and 15 min of exercise, and a needle biopsy was obtained after 15 min of exercise.

Blood analyses. Blood samples were immediately transferred into heparinized Vacutainers. Heparinized blood (100 μl) was quickly added to 500 μl 0.5 M perchloric acid and centrifuged, and the supernatant was stored at ~80°C for subsequent analysis of blood glucose and lactate (1) by using a fluorometer. The remainder of the blood sample was centrifuged and the plasma stored at ~80°C for subsequent analysis of amino acids by HPLC (11).

Muscle analyses. Needle-biopsy samples were immediately frozen in liquid nitrogen and subsequently freeze-dried, powdered, dissected free of nonmuscle elements, and stored at ~80°C. A 2- to 3-ml portion of muscle powder was used for the determination of free amino acids by using HPLC (11), and a 2-ml portion was used for the determination of glycogen by using an enzymatic glucose assay (10). The remainder of the muscle powder was extracted with 0.5 M perchloric acid (containing 1 mM EDTA) and neutralized with 2.2 M KHCO₃. Extracts were assayed for citrate, isocitrate, 2-oxoglutarate, succinate, fumarate, and malate by using enzymatic methods (1, 16) adapted for fluorometry as previously described (7).

Statistical analyses. Blood and muscle data were analyzed by using a two-factor (time × condition) repeated-measures analysis of variance. Significant interactions and main effects were subsequently analyzed by using a Tukey's honestly significant difference post hoc test. Statistical significance for all analyses was accepted as P ≤ 0.05. All data are expressed as means ± SE.

RESULTS

Oxygen uptake, blood, and plasma data. There were no significant differences between trials in pulmonary oxygen uptake during exercise (Table 1). The plasma concentrations of the three BCAA (leucine, isoleucine, and valine) increased after BCAA ingestion and were higher (P ≤ 0.05) throughout exercise compared with the placebo trial (Table 1). There were no changes in the plasma concentration of any other amino acid, except for alanine, which was higher during exercise compared with at rest in both trials (main effect, P ≤ 0.05). Blood lactate increased during exercise, and blood glucose was lower after 10 and 15 min of exercise compared with rest, but there were no differences between trials (Table 1).

Muscle glycogen. The dietary manipulation was successful in reducing muscle glycogen concentration before exercise in both trials. The resting glycogen concentration was 185 ± 22 and 206 ± 22 mmol/kg dry wt for the placebo and BCAA-supplemented conditions, respectively. Muscle glycogen concentration was lower after 15 min of exercise compared with rest (main effect, P ≤ 0.05), but there was no difference between trials (109 ±
Table 1. Pulmonary oxygen uptake, blood glucose, lactate, and plasma branched-chain amino acid concentrations at rest and during exercise

<table>
<thead>
<tr>
<th></th>
<th>Low Glycogen + Placebo</th>
<th>Low Glycogen + BCAA Ingestion</th>
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<tr>
<td></td>
<td>-90 min</td>
<td>0 min</td>
</tr>
<tr>
<td>V(_{\text{O}}(_2), l/min</td>
<td>0.49±0.04</td>
<td>3.36±0.11</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.7±0.28</td>
<td>4.33±0.38</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.56±0.07</td>
<td>0.50±0.07</td>
</tr>
<tr>
<td>Leucine, mmol/l</td>
<td>0.14±0.02</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>Isoleucine, mmol/l</td>
<td>0.09±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Valine, mmol/l</td>
<td>0.36±0.02</td>
<td>0.32±0.03</td>
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Values are means ± SE, n = 6. V\(_{\text{O}}\(_2\)\, pulmonary oxygen uptake. Concentrations of the 3 branched-chain amino acids (BCAA) were higher after BCAA ingestion. *P < 0.05 vs. placebo trial at same time point.

Muscle amino acids. The concentrations of leucine, isoleucine, and valine were higher (P ≤ 0.05) at rest and during exercise after BCAA supplementation compared with the placebo condition (Table 2). Glutamate was lower, and alanine was higher, after 15 min of exercise compared with rest (main effect, P ≤ 0.05); however, there were no differences between trials (Table 2). There were no differences between trials for any other amino acid (data not shown).

TCAI. The total concentration of the six measured TCAI was higher after 15 min of exercise compared with rest (main effect, P ≤ 0.05), but there were no differences between trials (Fig. 1). With regard to individual TCAI, the concentrations of citrate, isocitrate, succinate, fumarate, and malate were higher after exercise compared with those at rest, whereas the concentration of 2-oxoglutarate was lower (Table 2). There were no differences between trials for any TCAI.

DISCUSSION

The principal finding of the present investigation was that BCAA ingestion, immediately before exercise with a low resting muscle glycogen content, had no measurable effect on the concentrations of TCAI at rest or after 15 min of moderate cycling exercise in human skeletal muscle. Moreover, despite the fact that subjects began both experimental trials with low muscle glycogen, the net increase in TCAI during exercise was similar to that seen previously in a group of subjects who performed 15 min of exercise at the same work intensity after a normal mixed diet (7). In that study, the same six TCAI as measured in the present study increased by 3.63 mmol/kg dry wt above rest after 15 min of exercise (a 297% increase); this is similar to the increases of 3.97 mmol/kg dry wt (295%) and 3.88 mmol/kg dry wt (285%) observed in the present study during the placebo and BCAA-supplemented trials, respectively. Thus, in both an absolute and relative sense, the present data suggest that the initial rapid expansion of the TCAI pool during moderate exercise in humans is not impaired by low preexercise muscle glycogen content. This observation is supported by a study from Spencer and Katz (21), who reported no differences in the magnitude of increase in citrate and malate after 5 min of intense cycle exercise (~95% V\(_{\text{O}}\(_2\)\text{max}) when subjects began with either low or supercompensated muscle glycogen levels. It must be emphasized, however, that, although the resting muscle glycogen levels in the present study and that of Spencer and Katz were relatively low (~200 mmol/kg dry wt), subjects were not in fact “glycogen depleted.”

Fig. 1. Total muscle concentration of the 6 measured tricarboxylic acid intermediates (TCAI) (citrate, isocitrate, 2-oxoglutarate, succinate, fumarate, and malate) (TCAI) at rest and after exercise in placebo and branched-chain amino acid (BCAA)-supplemented trials. Values are means ± SE for 6 men.
glycogenolysis and hence the rate of pyruvate formation during exercise, impairs anaplerosis.

Presumably, there is a critical minimum concentration of glycogen necessary in resting skeletal muscle to provide a sufficient flux of pyruvate to drive the various anaplerotic reactions toward the formation of TCAI during exercise. In this regard, the near-equilibrium reaction catalyzed by alanine aminotransferase (pyruvate + glutamate ↔ alanine + 2-oxoglutarate), which appears to be the primary anaplerotic mechanism in humans (5, 7, 20), requires an increase in the concentration of pyruvate to force the reaction toward the formation of 2-oxoglutarate. The alanine aminotransferase reaction is presumed to be most important for anaplerosis in humans, because, during the initial minutes of moderate-to-intense exercise, there is a large and rapid decrease in muscle glutamate (5, 13, 14, 20), an increase in alanine (5, 13, 14, 20), and a stoichiometric increase in TCAI equivalent to the change in alanine concentration (5). Consistent with this latter observation, the exercise-induced increases in muscle alanine concentration in the present study were similar in magnitude to the overall changes in TCAI pool size during both experimental trials. It would appear, therefore, that despite the lowered resting muscle glycogen levels in the present study, the rate of pyruvate production during exercise was in excess of that required for acetyl CoA formation, and consequently this led to an accumulation of TCAI through a mass-action effect on the alanine aminotransferase reaction.

Despite the large increase in the total concentration of TCAI during exercise, we observed a decrease in the concentration of 2-oxoglutarate. One of the most puzzling aspects of the phenomenon of anaplerosis is that, whereas there is an apparent large and rapid production of 2-oxoglutarate through the alanine aminotransferase reaction during the initial period of moderate-to-intense exercise (of several mmol/kg dry wt), this is the only intermediate that does not increase. Rather, most studies from this laboratory (6, 7) and others (9, 17) have reported a decrease in 2-oxoglutarate during exercise, although this has not always been observed (5). The decline in 2-oxoglutarate may be related to the fact that it is in equilibrium with glutamate via the glutamate dehydrogenase reaction and thus may be influenced by the rapid decrease in this amino acid that occurs during exercise (5, 13, 14, 20). Regardless of the precise mechanism(s) involved, however, the present findings confirm that a decrease in the concentration of 2-oxoglutarate per se, even under conditions of reduced glycogen availability, does not impair aerobic energy production. Indeed, it was recently shown that the calculated flux through the TCA cycle can increase up to ~100-fold when going from rest to intense exercise in humans, despite a decrease in 2-oxoglutarate concentration (6).

BCAA metabolism and TCAI. We incorporated the BCAA supplementation trial because, in addition to glycogen availability, the branched-chain oxoacid dehydrogenase enzyme complex (BCOAD) has also been shown to be sensitive to dietary BCAA supply (22). Moreover, it was recently demonstrated that there is an additive effect of reduced glycogen content and BCAA supplementation on BCOAD activity in human skeletal muscle (12). When subjects had low resting glycogen, ingestion of BCAA immediately before exercise resulted in a larger active fraction of BCOAD after 15 min of exercise compared with the same bout with low glycogen alone. In addition, the accelerated rate of BCOAD oxidation observed in that study (12) was most pronounced during the early phase of exercise; i.e., the degree of BCOAD activation, and the difference in BCOAD activity between the supplemented and non-supplemented trials, was not different at the end of exercise (~45 min) compared with that during the initial 15 min. In the present study, therefore, we chose to focus on the initial period of exercise, when the increase in muscle TCAI is most pronounced, to examine the effect of reduced glycogen availability and the potential impact of accelerated BCAA oxidation during this time.

It is recognized that in vitro measurements of enzyme activity do not necessarily reflect the in vivo situation, and therefore caution must be used when estimating fluxes on the basis of homogenized tissue. Nonetheless, despite the presumably higher rate of BCAA oxidation during the BCAA-supplemented trial (12), we observed no measurable effect on the concentration of 2-oxoglutarate or other TCAI compared with exercise with low glycogen alone. One potential explanation for this finding is that, despite the relatively low muscle glycogen concentration at rest (~200 mmol/kg dry wt), the rate of pyruvate flux through the alanine aminotransferase reaction during exercise was sufficient to compensate for an accelerated rate of 2-oxoglutarate removal during the BCAA-supplemented trial. Importantly, however, this observation highlights the fact that, for an increased rate of BCAA oxidation to even potentially impact the pool of TCAI, the muscle glycogen concentration must be extremely low, probably less than ~100 mmol/kg dry wt (i.e., lower than the concentration reached after exercise in the present study).

In addition, the fact that we did not detect any differences between conditions during exercise highlights several important points that have not been adequately addressed by Wagenmakers and colleagues (23–26) in their theory regarding increased BCOAD activity and the potential drain on TCA cycle carbon. First of all, because of the relatively low maximal activity (i.e., total activity) of BCOAD in human skeletal muscle, any potential drain on the TCA cycle subsequent to an increased rate of BCAA oxidation will be small and unlikely to have a significant impact on the total concentration of TCAI. For example, the total activity of BCOAD in human muscle, on the basis of data from a number of studies from different laboratories (3, 12, 19, 22), is ~8 µmol·min⁻¹·kg wet wt⁻¹. In our previous study (12), the difference in BCOAD activation state between the low-glycogen trial and the BCAA-supplemented trial was 15%, or ~1.2 µmol·min⁻¹·kg wet wt⁻¹. If we assume this difference
Muscle glycogen, amino acids, and TCA cycle intermediates.

In summary, the results from the present study demonstrate that BCAA ingestion, during conditions of reduced glycogen availability, has no measurable effect on the concentration of 2-oxoglutarate or other TCAI in human skeletal muscle during exercise. Moreover, it appears that a reduced muscle glycogen concentration per se does not impair anaplerosis during moderate dynamic exercise. It remains to be determined whether severe carbohydrate restriction before exercise, i.e., to an extent that reduces the rate of glycogenolysis during exercise, impairs TCAI pool expansion by reducing the anaplerotic flux of pyruvate through the alanine amino transferase reaction.

The authors thank Premla Sathasivam and Kim Robertson for technical assistance and our subjects for their time and effort.

This work was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada (NSERC). M. J. Gibala was also supported by an NSERC Postdoctoral Fellowship Award.

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Received 30 November 1998; accepted in final form 29 June 1999.

REFERENCES