Glutamine supplementation promotes anaplerosis but not oxidative energy delivery in human skeletal muscle

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Glutamine supplementation promotes anaplerosis but not oxidative energy delivery in human skeletal muscle. Am J Physiol Endocrinol Metab 280: E669–E675, 2001.—The aims of the present study were twofold: first to investigate whether TCA cycle intermediate (TCAI) pool expansion at the onset of moderate-intensity exercise in human skeletal muscle could be enhanced independently of pyruvate availability by ingestion of glutamine or ornithine α-ketoglutarate, and second, if it was, whether this modification of TCAI pool expansion had any effect on oxidative energy status during subsequent exercise. Seven males cycled for 10 min at ~70% maximal O2 uptake 1 h after consuming either an artificially sweetened placebo (5 ml/kg body wt solution, CON), 0.125 g/kg body wt L-(+)-ornithine α-ketoglutarate dissolved in 5 ml/kg body wt solution (OKG), or 0.125 g/kg body wt L-glutamine dissolved in 5 ml/kg body wt solution (GLN). Vastus lateralis muscle was biopsied 1 h postsupplement and after 10 min of exercise. The sum of four measured TCAI (ΣTCAI; citrate, malate, fumarate, and succinate, ~85% of total TCAI pool) was not different between conditions 1 h postsupplement. However, after 10 min of exercise, ΣTCAI (mmol/kg dry muscle) was greater in the GLN condition (4.90 ± 0.61) than in the CON condition (3.74 ± 0.38, P < 0.05) and the OKG condition (3.85 ± 0.28). After 10 min of exercise, muscle phosphocreatine (PCr) content was significantly reduced (P < 0.05) in all conditions, but there was no significant difference between conditions. We conclude that the ingestion of glutamine increased TCAI pool size after 10 min of exercise most probably because of the entry of glutamine carbon at the level of α-ketoglutarate. However, this increased expansion in the TCAI pool did not appear to increase oxidative energy production, because there was no sparing of PCr during exercise.

tricarboxylic acid cycle intermediates; exercise; glutamate; phosphocreatine

The total content of the tricarboxylic acid (TCA) cycle intermediates (TCAI) has been shown to expand during the initial few minutes of muscular contraction (15, 18, 32, 33). However, it is still not clear whether this expansion is of any functional significance to oxidative energy production. For example, recent studies have suggested that the expansion of TCAI may merely reflect the increase in pyruvate availability that results from the mismatch between the rate of pyruvate formation, via glycolysis, and the rate of oxidation of acetyl units in the TCA cycle (7, 16). Alternatively, the increase in TCAI pool size has been reported to be necessary for increased TCA cycle flux and, hence, oxidative energy production during intense muscular contraction (27, 33).

The expansion of the TCAI pool at the onset of skeletal muscle contraction is due primarily to an increase in the rate of anaplerosis (replenishment of TCAI) at the onset of exercise (15, 33, 36). Indeed, it has previously been suggested that TCAI pool size is determined by the balance between the flux of carbon into and out of the TCA cycle (27, 33, 36). During the initial 15 min of moderate-intensity exercise, when TCAI pool size expands by ~300%, muscle glutamate content decreases by ~60% and muscle alanine content increases by ~50% (4, 14). This suggests that the alanine aminotransferase reaction (AAT: glutamate + pyruvate ↔ α-ketoglutarate + alanine) is of prime importance for providing anaplerotic carbon at the onset of exercise (15, 33, 36), with carbon entering the TCA cycle at the level of α-ketoglutarate.

More recently, the importance of acetyl units to the TCA cycle at the onset of exercise has been demonstrated (7, 39, 40). TCAI pool size and the supply of acetyl units to the TCA cycle have previously been manipulated pharmacologically by infusion of dichlo-roacetate (DCA), which causes activation of the pyruvate dehydrogenase complex (PDC). The delivery of acetyl units to the TCA cycle was increased both at rest and at the onset of exercise after activation of PDC by DCA (23). After activation of PDC by DCA infusion in resting human skeletal muscle, TCAI pool size was decreased in conjunction with the accumulation of acetyl-CoA and acetylcarnitine (7, 17). However, after
DCA infusion, TCAI pool size after 1 min of exercise was not different from control values (17). This suggests that once contraction was initiated, glycolysis was accelerated to an extent that pyruvate availability was not limiting anaplerosis via the AAT reaction. This observation also raises the question as to where the limitation to anaplerosis resides at the onset of exercise (14). One aim of the present study, therefore, was to determine whether nutritional intervention could be used to modify TCAI pool size. Specifically, was the extent of TCAI pool expansion at the start of exercise limited by α-ketoglutarate availability and, hence, altered by the ingestion of glutamine or ornithine α-ketoglutarate before exercise.

The nonessential amino acid glutamine is readily taken up into skeletal muscle via the high-capacity sodium-dependent system Nm (1), resulting in an increased intramuscular glutamine content (42). The enzymes required to catalyze the conversion of glutamine to α-ketoglutarate, glutaminase (EC 3.5.1.2) (38) and glutamate dehydrogenase (EC 1.4.1.2) (37) or alanine aminotransferase (EC 2.6.1.2) (28) or glutamine transaminase (EC 2.6.1.15) (38, 45) and α-amidase (EC 3.5.1.3) (38, 45), should exist in human skeletal muscle. It is feasible, therefore, that carbon derived from glutamine could enter the TCA cycle at the level of α-ketoglutarate. Indeed, when glutamine was infused after exhaustive exercise, muscle glycogen storage was increased. There appeared to be an increase in the availability of carbon units for incorporation into glycolgen, presumably due to α-ketoglutarate feeding into the TCA cycle (42). This result was recently replicated when glutamine was ingested after exhaustive exercise (5).

A more direct approach to increasing TCAI pool size would be to provide the substrate α-ketoglutarate itself. Ornithine α-ketoglutarate (OKG), a salt formed of two molecules of ornithine and one molecule of α-ketoglutarate, is a precursor of glutamine, arginine, proline, and polyamines (for review see Ref. 25). Supplementation with OKG has been shown to improve the nutritional status of hypercatabolic patients (8), improve nitrogen balance (12), and restore the muscle glutamine pool (44). However, to our knowledge, the effect of OKG ingestion on TCAI pool size in human skeletal muscle has never been assessed.

It has recently been suggested that changes in TCAI are not causally linked to TCA cycle flux and oxidative energy production (7, 16); however, the precise functional significance of TCAI pool expansion is still under question. Therefore, if we were successful in augmenting TCAI pool expansion through nutritional intervention, a second aim was to determine the effect on energy metabolism. During the transition from rest to exercise, the increase in oxidative energy delivery is not sufficiently rapid to meet the energy demands of the exercising muscle. The magnitude of the decline in phosphocreatine (PCr) stores is indicative of the mismatch between oxidative metabolism and energy demand (24). It was hypothesized that if oxidative energy delivery were increased at the onset of exercise as a result of augmenting TCAI pool expansion, a concomitant reduction in muscle PCR degradation would be expected to occur.

METHODS

Subjects. Seven healthy male well-trained cyclists participated in three trials separated by ≥2 wk. Their mean age, height, body mass, and maximal O₂ uptake (VO₂ max) were 24 ± 1 yr, 180 ± 2 cm, 80 ± 2 kg, and 5.0 ± 0.2 l/min (62.5 ± 1.8 ml·min⁻¹·kg⁻¹), respectively. The experimental procedures and potential risks were fully explained to the subjects before their participation in this study, and all gave voluntary written consent. The experimental protocol was approved by the Ethics Committee of Loughborough University.

Preliminary tests. Subjects reported to the laboratory ~1 wk before the experiment and performed a submaximal test, to determine the relationship between O₂ uptake and work rate, and an incremental cycling test to determine their VO₂ max on a friction-braked cycle ergometer (Monark 824E, Varberg, Sweden). A workload corresponding to 70% VO₂ max was then calculated for each subject.

Pre-experimental procedures. Subjects were instructed to consume their habitual diet and refrain from exercise or strenuous physical activity for 48 h before each experiment. On the afternoon before the experiment, subjects performed a bout of glycogen-depleting exercise that had been used previously (5) and had been designed to deplete both type I and type II muscle fibers of glycogen as validated by Vølles-tad et al. (43).

All food consumed after the glycogen-depleting exercise bout was prescribed for each subject and was identical before all trials for a given subject, providing 35 ± 2% carbohydrate, 56 ± 2% fat, and 11 ± 2% protein (~1,400 kcal; Compeet 5.0 Diet Analysis Software, Carlson Bengston Consultants). This ensured that only limited muscle glycogen resynthesis occurred before the 2nd day of the experiment and that the magnitude of resynthesis was the same across groups. This was then calculated for each subject.

Experimental protocol. On arrival at the laboratory on the morning of the experiment, the overnight-fastest subject rested in a supine position. A cannula was inserted into an antecubital vein, and a resting blood sample was obtained. Subjects then immediately consumed, in a double-blind fashion, one of three solutions: 5 ml/kg body wt of an artificially sweetened placebo (CON), 0.125 g/kg body wt L-(-)-ornithine α-ketoglutarate (Laboratoires Jacques Logeais, Paris, France) dissolved in 5 ml/kg body wt of the artificially sweetened placebo (OKG), or 0.125 g/kg body wt L-glutamine (Sigma-Aldrich Chemicals, Dorset, UK) dissolved in 5 ml/kg body wt of the artificially sweetened placebo (GLN). The solutions were allocated by systematic rotation. After consumption of the assigned solution, subjects rested for 60 min and then immediately cycled at 70% VO₂ max for 10 min. This time course was chosen because it has previously been shown that maximal increase in TCAI pool size is achieved after ~10 min of submaximal exercise in humans (18).

Approximately 15 min before exercise, the skin and fascia over the anterior aspect of one thigh were anesthetized (lignocaine, 1% wt/vol, Phoenix Pharmaceuticals, Gloucester, UK), and two small incisions were made to allow extraction of needle biopsy samples from the vastus lateralis muscle (3). Needle biopsy samples were obtained immediately before exercise (60 min after consumption of the assigned solution).
and after 10 min of exercise. During the 1-h rest period after consumption of the assigned solution, venous blood samples were taken every 20 min. A venous blood sample was taken at 10 min of exercise. Expired air samples were collected by Douglas bag for 6 min at 20 and 40 min after consumption of the solution.

**Blood analyses.** Venous blood samples were drawn and aliquotted for subsequent blood analysis. An aliquot was used to immediately determine whole blood lactate and glucose concentration by use of a YSI 2300 STATPLUS analyzer (Yellow Springs Instruments, Yellow Springs, OH), because there was a risk that subjects in the OKG condition might have developed hypoglycemia (11). The remainder of the venous blood sample was centrifuged at 4°C, and the supernatant was collected and stored at −20°C and later analyzed for free amino acids by HPLC (30).

**Muscle analyses.** After removal from the leg, the muscle biopsy sample was immediately frozen by plunging the needle into liquid nitrogen. The samples were removed from the needle while still frozen and were subsequently freeze-dried, dissected free from visible connective tissue and blood, powdered, and stored at −80°C. Aliquots of the powdered muscle were extracted with 0.5 M perchloric acid (containing 1 mM K2HPO4), and after centrifugation, the supernatant was neutralized with 2.2 M KHCO3. Extracts were assayed enzymatically for lactate, pyruvate, glutamate, glutamine, citrate, malate, fumarate, and succinate content (2) with a fluorometer (Hitachi F2000 fluorescence spectrophotometer, Hitachi Instruments). Intramuscular metabolite contents were normalized to total creatine content (except lactate).

**Statistics.** The data were analyzed by two-way analysis of variance (ANOVA) for repeated measures (time x treatment). When the ANOVA resulted in a significant F ratio (P < 0.05), Fisher’s post hoc test was used to locate differences between means. Values are presented as means ± SE.

**RESULTS**

Cardiorespiratory, blood glucose, and blood lactate data. No differences were observed between conditions in any of the measured physiological variables during the glycogen-depleting exercise on the afternoon of the 1st day. During the main experimental trials, blood glucose, blood lactate, heart rate, respiratory exchange ratio, and expired ventilation showed main effects for time (P < 0.05). However, there were no significant differences between conditions (data not shown). The power output in W (CON: 241 ± 11, OKG: 243 ± 9, and GLN: 242 ± 10) and pulmonary O2 uptake in l/min (CON: 3.73 ± 0.16, OKG: 3.73 ± 0.15, and GLN: 3.72 ± 0.14) were not different between conditions.

**Plasma and muscle amino acids.** The plasma concentration of both glutamine and glutamate was increased 1 h after GLN consumption (P < 0.05) compared with both the CON and OKG conditions. Furthermore, the plasma concentration of glutamine remained elevated at 10 min of exercise (P < 0.05) compared with both the CON and OKG conditions (Table 1). Plasma ornithine concentration was higher 1 h after OKG consumption and remained elevated (P < 0.05) throughout exercise compared with both the CON and OKG conditions. Plasma alanine concentration significantly increased (P < 0.05) in all conditions during the 10 min of exercise relative to the basal value (Table 1). In addition, plasma alanine concentration was significantly higher in the GLN and OKG conditions compared with the CON condition 1 h after consumption of the supplements and at 10 min of exercise. Plasma aspartate concentration was significantly higher in the GLN condition 1 h after ingestion of the supplement in the CON and OKG conditions (P < 0.05) and at 10 min of exercise was significantly higher in the GLN than in the OKG condition (P < 0.05).

The intramuscular content of glutamine at rest was significantly higher (P < 0.05) after GLN supplementation (Table 2) compared with the other two conditions. However, there were no differences between conditions during exercise. Intramuscular glutamate content decreased by −60% (P < 0.05) in all conditions after 10 min of exercise relative to the preexercise value, but there was no significant difference between conditions at any time point.

**TCAI.** The total content of the four measured TCAI (ΔTCAI; citrate, succinate, malate, and fumarate) was not different between conditions at rest (Fig. 1). The ΔTCAI at 10 min of exercise was greater than at rest in all conditions (P < 0.05) and was higher in the GLN condition (4.90 ± 0.61 mmol/kg dry muscle) compared

**Table 1. Selected plasma amino acid concentration and muscle amino acid content at rest and during exercise in the CON, OKG, and GLN conditions**

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>OKG</th>
<th>GLN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−60 min</td>
<td>0 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Plasma, μmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>438 ± 15</td>
<td>484 ± 10</td>
<td>491 ± 16</td>
</tr>
<tr>
<td>Glutamate</td>
<td>92 ± 8</td>
<td>67 ± 7</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>Alanine</td>
<td>330 ± 35</td>
<td>347 ± 22</td>
<td>448 ± 28</td>
</tr>
<tr>
<td>Ornithine</td>
<td>52 ± 6</td>
<td>52 ± 9</td>
<td>44 ± 6</td>
</tr>
<tr>
<td>Aspartate</td>
<td>22 ± 2</td>
<td>23 ± 2</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>Muscle, mmol/kg dry muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>ND</td>
<td>30.1 ± 1.8</td>
<td>31.7 ± 1.6</td>
</tr>
<tr>
<td>Glutamate</td>
<td>18.3 ± 0.9</td>
<td>7.5 ± 0.6</td>
<td>9.1 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. CON, control solution; OKG, L-ornithine α-ketoglutarate solution; GLN, L-glutamine solution; ND, not determined. *P < 0.05 vs. −60 min; †P < 0.05 vs. 0 min; ‡P < 0.05 vs. CON condition at same time point; §P < 0.05 vs. OKG condition at same time point; ‡P < 0.05 vs. GLN condition at same time point.
with the control condition (3.74 ± 0.38 mmol/kg dry muscle, P < 0.05) and the OKG condition (3.85 ± 0.28 mmol/kg dry muscle) at 10 min of exercise (Fig. 1). However, there were no differences between conditions in the content of any of the individual TCA intermediates (Table 2).

**Intramuscular metabolites.** PCr content declined to a similar extent during the 10 min of exercise in all conditions (CON: 26 ± 6%, OKG 19 ± 7%, and GLN: 21 ± 6%; Table 2). Intramuscular lactate content was not different between conditions at rest. During exercise, the increase in intramuscular lactate content was similar in all conditions (Table 2). Intramuscular pyruvate content increased in all conditions during exercise (CON: 70 ± 39%, OKG: 134 ± 96%, and GLN: 171 ± 99%; Table 2). No significant differences in PCr content were observed between conditions at any time point.

**DISCUSSION**

A main finding from the present investigation was that the consumption of glutamine 1 h before exercise augmented the increase in the TCAI pool size. This suggests that the availability of the breakdown products of glutamine, namely glutamate and ultimately α-ketoglutarate, may limit anaplerosis at the onset of exercise. Indeed, the increase in TCAI pool size was concomitant with a ~60% decrease in intramuscular glutamate content in all conditions. It is conceivable that this decrease in intramuscular glutamate content increased flux through the glutaminase reaction, thus increasing the breakdown of glutamine to form glutamate. Certainly, intramuscular glutamine content and plasma glutamine concentration decreased in the GLN condition during the 10 min of exercise, presumably contributing to the larger expansion of TCAI pool size.

It has been reported that ~50% of an enterally delivered dose of glutamine is sequestered on first pass through the splanchnic bed (22, 29). However, in the present study, the oral provision of glutamine was able to increase plasma glutamine at the measured peak concentration by 51%, demonstrating that a substantial portion of the oral load escaped utilization by the gut mucosal cells and uptake by the kidneys and liver.

**Table 2. Intramuscular metabolites at rest and during exercise after consumption of CON, OKG, or GLN solutions**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>0 min</th>
<th>10 min</th>
<th>0 min</th>
<th>10 min</th>
<th>0 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>26.5 ± 1.0</td>
<td>27.1 ± 2.4</td>
<td>26.4 ± 1.0</td>
<td>26.5 ± 1.4</td>
<td>25.0 ± 0.9</td>
<td>24.8 ± 1.0</td>
</tr>
<tr>
<td>PCr</td>
<td>89.5 ± 2.9</td>
<td>65.5 ± 5.1</td>
<td>93.6 ± 1.7</td>
<td>76.6 ± 7.6</td>
<td>88.2 ± 3.7</td>
<td>69.4 ± 5.5</td>
</tr>
<tr>
<td>Creatine</td>
<td>41.1 ± 1.8</td>
<td>60.5 ± 5.9</td>
<td>40.6 ± 2.6</td>
<td>62.3 ± 8.2</td>
<td>35.5 ± 4.2</td>
<td>59.3 ± 7.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.2 ± 0.1</td>
<td>6.7 ± 1.7</td>
<td>2.1 ± 0.1</td>
<td>6.0 ± 1.0</td>
<td>1.9 ± 0.1</td>
<td>7.1 ± 1.8</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.37 ± 0.06</td>
<td>0.51 ± 0.05</td>
<td>0.31 ± 0.06</td>
<td>0.42 ± 0.09</td>
<td>0.32 ± 0.07</td>
<td>0.60 ± 0.07</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.59 ± 0.06</td>
<td>0.78 ± 0.06</td>
<td>0.57 ± 0.07</td>
<td>0.87 ± 0.08</td>
<td>0.51 ± 0.07</td>
<td>0.90 ± 0.01</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.25 ± 0.04</td>
<td>1.16 ± 0.13</td>
<td>0.24 ± 0.03</td>
<td>1.17 ± 0.12</td>
<td>0.23 ± 0.06</td>
<td>1.63 ± 0.36</td>
</tr>
<tr>
<td>Fumarate</td>
<td>0.03 ± 0.01</td>
<td>0.19 ± 0.03</td>
<td>0.03 ± 0.01</td>
<td>0.17 ± 0.03</td>
<td>0.04 ± 0.04</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>Malate</td>
<td>0.25 ± 0.05</td>
<td>1.61 ± 0.23</td>
<td>0.24 ± 0.03</td>
<td>1.63 ± 0.20</td>
<td>0.24 ± 0.04</td>
<td>2.12 ± 0.22</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in mmol/kg dry muscle (with the exception of lactate intramuscular metabolite contents normalized for total creatine content); n = 7. ATP, adenosine triphosphate; PCr, phosphocreatine. *P < 0.05 vs. 0 min.

**Fig. 1.** Total muscle content of the 4 measured tricarboxylic acid cycle intermediates (TCAI) citrate, succinate, malate, and fumarate at rest and during exercise for the control (CON), L-ornithine α-ketoglutarate (OKG), and L-glutamine (GLN) conditions. Values are means ± SE; n = 7. *P < 0.05 vs. CON at same time point.
Plasma glutamate concentration was not different between conditions at 10 min of exercise. Presumably, the excess glutamate was taken up into skeletal muscle (15, 33, 41) and converted to α-ketoglutarate either via the α-aminotransferase reaction or the glutamate dehydrogenase reaction (glutamate + NAD$^+$ ↔ α-ketoglutarate + NH$_3$ + NADH), thus contributing to the greater increase in TCAI during the GLN condition.

In fact, the decline in intramuscular glutamine (~7 mmol/kg dry wt) and glutamate (~11 mmol/kg dry wt) content during the first 10 min of exercise was more than fourfold greater than the increase in the measured TCAI (~4 mmol/kg dry wt) over the same time period in the GLN trial. Similarly during the OKG and CON conditions, the decline in muscle glutamate content was about threefold greater than the increase in TCAI. There are two means by which this discrepancy might be explained: first, drainage of the TCAI to take part in the many other reactions in which they are involved, e.g., amino acid synthesis, and second, glutamate utilization in reactions by which there is no net production of TCAI. The aspartate aminotransferase (EC 2.6.1.1) (34) is one such reaction in which glutamate donates its amino group to oxaloacetate, resulting in the production of aspartate and α-ketoglutarate (glutamate + oxaloacetate + NAD$^+$ ↔ α-ketoglutarate + aspartate + NADH). The removal of oxaloacetate is balanced by the production of α-ketoglutarate; thus there is no net expansion of the TCAI pool. Indeed, plasma aspartate concentration was elevated during the GLN condition and may therefore account for a proportion of the “missing” glutamate. Similarly, Graham et al. (20) also found that ingestion of monosodium glutamate increased plasma aspartate concentration, presumably via the aspartate aminotransferase reaction.

Gibala et al. (18) demonstrated that the sum of citrate, succinate, malate, and fumarate accounts for ≥85% of the total TCAI both at rest and during exercise in humans; thus we are confident that our data represent an accurate quantitative and qualitative index of the total TCAI pool. α-Ketoglutarate content was not measured in the present study; however, previous studies have not observed an increase in this TCAI during the initial minutes of moderate exercise (15, 18, 19). This phenomenon has been linked to the equilibrium between α-ketoglutarate and glutamate via the glutamate dehydrogenase reaction; thus α-ketoglutarate content may be influenced by the decrease in glutamate content during the initial minutes of exercise (15, 16, 33, 41). A disproportionate increase in the contents of succinate, malate, and fumarate has been observed during exercise in the present and previous studies (7, 16). An increase in α-ketoglutarate content causes a rapid activation of the α-ketoglutarate dehydrogenase complex (6); therefore, α-ketoglutarate derived from exogenous glutamine is likely to be rapidly converted to intermediates situated in the “second span” of the TCA cycle.

Ingestion of OKG did not enhance TCAI expansion at the onset of exercise, either directly via entry of α-ketoglutarate into the TCA cycle or indirectly via the elevation of the muscle glutamate or glutamine pool. One possible explanation is that the exogenous α-ketoglutarate was not taken up into the skeletal muscle. However, α-ketoglutarate transport into neurons (35) and the kidney (13) is via a Na$^+$-dependent high-affinity process, and α-ketoglutarate infusion has been shown to increase the intramuscular α-ketoglutarate content in anesthetized dogs (31). It is unlikely, therefore, that skeletal muscle α-ketoglutarate transport was the limiting factor. The more feasible explanation is that, despite successfully increasing plasma ornithine concentration (345% 1 h after ingestion of OKG), only a small increase in plasma α-ketoglutarate occurred, as observed previously (10, 11), and this increase was not sufficient to elevate intramuscular α-ketoglutarate content. Certainly, a large proportion of the OKG dose is likely to have been sequestered by the splanchnic bed, as indicated by the observed increase in OKG metabolites (proline, arginine, glutamate) in the splanchnic areas (46). In the present study, the dose was limited to ~10 g, the dosage used in clinical practice (26), because of reported gastric problems associated with larger doses (21).

The α-ketoglutarate and ornithine moieties of OKG interact to give a different metabolic pattern from that observed when they are provided individually as ornithine (as hydrochloride) or α-ketoglutarate (as a calcium salt) (10). Ornithine and α-ketoglutarate share a common metabolic pathway, resulting in the diversion of ornithine and α-ketoglutarate metabolism to other pathways (i.e., glutamine synthesis) when the common pathway is saturated. Indeed, it has been demonstrated that the administration of OKG to humans postsurgery effectively restores the muscle glutamine pool (44), presumably due to OKG acting as a precursor to glutamine (9). However, the majority of studies that have demonstrated an anabolic effect of OKG has utilized hypercatabolic states (burn injury, surgery, trauma), in which the intramuscular glutamine pool is reduced. No increase was observed in muscle glutamate content at rest in the present study after OKG supplementation compared with the CON condition. This suggests that OKG may be effective only in the hypercatabolic state.

Previously, it has been suggested that anaplerosis is dependent on pyruvate availability (7). In the present study, therefore, subjects completed a glycogen-depletion exercise protocol on the day preceding each main trial and consumed a low-carbohydrate diet during the intervening 18-h period. This design was employed to ensure that muscle and hepatic glycogen stores, and hence pyruvate availability, in exercising skeletal muscle were similar for all three conditions. Certainly, the metabolic response during the glycogen-depleting exercise bout was identical between trials, confirming that the physiological status of each subject was similar before each trial. Although muscle glycogen content was not measured in the present study, the bout of glycogen-depleting exercise employed has previously been shown by our group to reduce muscle glycogen
content to 13 mmol glycosyl U/kg wet wt (~55 mmol glycosyl U/kg dry weight) immediately after exercise (5). During the 18-h period between glycogen depletion and the main trial, subjects consumed on average ~123 g of carbohydrate (~1.5 g carbohydrate/kg body wt). It is likely, therefore, that both muscle and liver glycogen stores remained reduced at the start of the main trial. It is therefore interesting that the magnitude of TCAI pool expansion was similar to that observed in previous studies in which skeletal muscle glycogen content was not manipulated (18). Gibala et al. (14) introduced the concept of a critical minimum concentration of glycogen necessary to provide an adequate pyruvate flux to drive anaplerotic reactions. In the present study, the intramuscular pyruvate content was similar to that observed in normal glycogen studies, suggesting that the extent of glycogen depletion was not sufficient to reduce glycogenolysis at the start of exercise.

A second main finding was that, despite the exaggerated expansion of the TCAI pool after glutamine ingestion, the decline in PCr content and accumulation of muscle lactate were similar in the three experimental conditions. These results suggest that ATP production via oxidative phosphorylation was not enhanced by a further increase in TCAI pool size, because substrate-level phosphorylation was not different between conditions. It appears, therefore, that TCAI pool size does not limit oxidative energy production at the onset of exercise. Indeed, it has been demonstrated that a lag in acetyl group delivery to the TCA cycle is likely to limit oxidative energy delivery at the onset of exercise (39, 40). Previous studies using DCA to increase the active fraction of PDC have been able to enhance oxidative energy delivery at the onset of exercise without a further expansion of the TCAI pool (17, 39, 40). This apparent dissociation between TCAI pool size and oxidative energy delivery suggests that TCA cycle flux is not limited by TCAI pool size. The activation of PDC at rest increased the supply of pyruvate-derived acetyl units, resulting in the stockpiling of acetylcarnitine and reduced muscle lactate accumulation that occurred during this period were facilitated by the increased availability of acetyl units. In the present study, also, a dissociation between TCAI pool expansion and oxidative energy delivery has been demonstrated.

In conclusion, we have demonstrated that the provision of glutamine 1 h before exercise is able to further increase TCAI pool expansion after 10 min of moderate-intensity exercise. However, despite this further increase in TCAI pool expansion, no reduction in PCr utilization and muscle lactate accumulation was observed during this initial period of exercise. This suggests that, at the onset of exercise, energy production is not limited by TCAI pool size but by some other factor, possibly muscle oxygen availability or delivery of acetyl groups to the TCA cycle.

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