Glutamine: An Anaplerotic Precursor

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There is an up to four-fold increase in the concentration of the tricarboxylic acid (TCA) cycle intermediates at the start of exercise. The rate of TCA cycle flux and, hence, oxidative metabolism may be limited by the concentration of the intermediates in the cycle. The dramatic decline in intramuscular glutamate at the start of exercise, in tandem with increased intramuscular alanine, suggests that glutamate is an important anaplerotic precursor. We hypothesized that oral glutamine might enhance the exercise-induced TCA cycle intermediate pool expansion. Indeed, a greater increase in the sum of muscle citrate, malate, fumarate, and succinate concentrations (~85% total TCA intermediate pool) occurred at the start of exercise after ingestion of glutamine rather than of placebo or ornithine α-ketoglutarate. However, neither endurance capacity nor the degree of phosphocreatine depletion or lactate accumulation was altered. This suggests that TCA cycle intermediates do not limit flux through the cycle or that more intense exercise is required to show the limitation. Nutrition 2002;18:222–224. ©Elsevier Science Inc. 2002

KEY WORDS: glutamine, tricarboxylic acid cycle, tricarboxylic acid cycle intermediates, anaplerosis, exercise

INTRODUCTION

The tricarboxylic acid (TCA) cycle occupies a position of prime importance in the pathways of oxidative metabolism. Acetyl units derived from the breakdown of glucosyl units, fatty acids, and amino acids are oxidized during their passage through the TCA cycle, generating carbon dioxide and the reducing equivalents NADH2 and FADH. The majority of adenosine triphosphate (ATP) produced via oxidative pathways is derived from the potential energy of these electrons harnessed by oxidative phosphorylation. The eight intermediates of the TCA cycle (TCAi) are not consumed by the oxidation of acetyl units but are recycled. However, the concentrations of TCAi are in constant flux due to the drainage of intermediates taking part in other reactions such as synthesis of amino acids and the influx of TCAi via anaplerotic reactions such as alanine aminotransferase (ALAT) and glutamate dehydrogenase.

Gibala et al. calculated from measurements of leg oxygen uptake that the rate of ATP turnover and, hence, TCA cycle flux increase approximately 100-fold during intense exercise. This is paralleled by an up to four-fold increase in skeletal muscle TCAi content during the first few minutes of contraction, in an exercise intensity-dependent fashion. This expansion of the TCAi pool may be necessary to facilitate the increase in TCA cycle flux and, hence, oxidative energy delivery. Alternatively, rather than playing a functionally important role, increased TCAi concentration may merely reflect increases in pyruvate availability at the start of exercise. To distinguish between these opposing hypotheses, the extent of TCAi expansion has been manipulated pharmacologically and nutritionally and the effect on oxidative energy delivery has been investigated.

At the start of exercise, the capacity of the TCA cycle to oxidize acetyl units is outstripped by the rate of pyruvate production from glycolysis. Excessive pyruvate accumulation is averted by the reduction of pyruvate to lactate and the formation of alanine and 2-oxoglutarate (2-OG) by the near-equilibrium ALAT reaction. Indeed, intramuscular glutamate decreases by almost 60% and alanine increases by almost 50% over this period, suggesting that the ALAT reaction contributes significantly to TCAi expansion.

Dichloracetate (DCA) is a potent activator of the pyruvate dehydrogenase complex through inhibition of pyruvate dehydrogenase complex kinase. When infused at rest, DCA caused a depletion of TCAi, presumably due to the observed diversion of pyruvate to acetyl coenzyme A and acetyl carnitine, thus decreasing pyruvate availability for anaplerosis. This supports a pivotal role for pyruvate availability in determining TCAi pool size. However, when DCA was infused before exercise, TCAi pool size was not different between DCA and control conditions after 1 min of exercise. The enhanced glycolytic flux at the onset of exercise seems to ensure that pyruvate availability is sufficient to support anaplerosis.

At the onset of exercise, there is a shortfall in ATP production from oxidative pathways that is met by substrate-level phosphorylation of adenosine diphosphate from glycogenolysis and phosphocreatine breakdown. Therefore, the extent of phosphocreatine (PCr) breakdown and lactate accumulation during this transitional phase are indicative of the magnitude of the mismatch between ATP demand and ATP production through oxidative phosphorylation. Interestingly, DCA infusion before exercise appears to cause a relative sparing of PCr stores during the transition from rest to exercise. Oxidative energy delivery thus appears to be enhanced by increasing acetyl group availability without a further expansion of TCAi pool. This apparent dissociation between oxidative energy delivery and TCAi pool size suggests that TCAi pool size is not normally limiting for oxidative energy delivery. However, ideally, TCAi pool size should be altered in isolation to allow a cleaner dissection of the contributing factors. We
hypothesized that the exercise-induced expansion of the TCAi pool could be enhanced by increasing the availability of glutamate for anaplerotic reactions. Graham et al.\textsuperscript{13} did not find such an enhancement with infusion of monomeric glutamate; however, transport of glutamate into skeletal muscle is poor.\textsuperscript{14} In contrast, glutamine (GLN) is readily taken up into skeletal muscle via the high-capacity, sodium-dependent amino acid transport system N\textsuperscript{m}, resulting in an increased intramuscular glutamine content.\textsuperscript{15} The enzymes required to catalyze the conversion of glutamine to 2-OG (glutaminase; EC 3.5.1.2)\textsuperscript{16} and glutamate dehydrogenase (EC 1.4.1.2)\textsuperscript{17} or ALAT (EC 2.6.1.2)\textsuperscript{18} or glutamine transaminase (EC 2.6.1.15)\textsuperscript{16,19,20} and \(\alpha\)-amidase (EC 3.5.1.3)\textsuperscript{6,19,20} should exist in human skeletal muscle. Thus, it is feasible that carbon derived from GLN could enter the TCA cycle at the level of 2-OG. Indeed, when glutamine was infused after exhaustive exercise, muscle glycogen storage was increased.\textsuperscript{21} There appeared to be an increase in the availability of carbon units for incorporation into glycogen, presumably due to 2-OG feeding into the TCA cycle. This result was recently replicated when GLN was ingested after exhaustive exercise.\textsuperscript{22} We hypothesized that ingestion of GLN before exercise would result in augmented TCAi expansion and, hence, increased oxidative energy delivery during the transition from rest to exercise.

The protocol adopted was to deplete glycogen in subjects by prior exercise and a low-carbohydrate diet, so that the glycogen availability would be identically low in all trials. Subjects were then provided with a drink of a placebo or two small anaplerotic precursors (ornithine-\(\alpha\)-ketoglutarate or GLN, both at 0.125 g/kg), and then the effects of bicycle exercise at 70% maximum oxygen consumption were studied with appropriate muscle biopsies.\textsuperscript{9} Oral GLN supported a greater expansion of TCAi concentration during the first 10 min of exercise. Plasma alanine concentration was higher in the GLN than in the placebo trial after 10 min of exercise, suggesting that the enhanced expansion of TCAi could, at least in part, be attributed to increased flux through the ALAT reaction. Interestingly, there was no difference between treatments in the intramuscular glutamate content, although resting intramuscular GLN content was elevated in the GLN trial 1 h after ingestion of the supplement. During the first 10 min of exercise, the decline in intramuscular GLN and glutamate was approximately four-fold greater than the increase in the four measured TCAi (citrate, malate, fumarate, and succinate, 85% total TCAi pool) in the GLN trial and approximately three-fold greater in the placebo and ornithine-\(\alpha\)-ketoglutarate trials. There are two possible explanations for this discrepancy: 1) drainage of the TCAi taking part in ancillary reactions, e.g., amino acid synthesis; and 2) glutamate use in reactions by which there is no net production of TCAi. The formation of aspartate, catalyzed by aspartate aminotransferase, is one such reaction where the TCAi, oxaloacetate, acts as the amino group acceptor for glutamate, resulting in the formation of the TCAi, 2-OG. Plasma aspartate concentration was elevated during the GLN trial and thus may account for some of the “missing” glutamate. Nonetheless, it is clear that GLN or glutamate carbon enters the TCA cycle, presumably at the level of 2-OG. Therefore, glutamate and not pyruvate availability likely limits the exercise-induced TCAi expansion.

Despite the augmented TCAi pool expansion, there was no sparing of PCR stores or attenuation of muscle lactate production, suggesting that there was no enhancement of TCA cycle flux. This may be explained in a number of ways. First, expansion of TCAi pool may not be functionally important. Second, any sparing of PCR was no longer evident after 10 min of exercise. In the studies where DCA was infused, reduced PCR breakdown was evident after 2 min of exercise but not after 10 min of exercise.\textsuperscript{23} Steady-state oxygen consumption is normally achieved 4 to 5 min after any change in exercise intensity. It is likely that, after this initial period, resynthesis of PCR stores occurs, thus masking any earlier sparing effect. Certainly, the extent of PCR depletions in our study (\(\sim\)20 mM/kg dw) was considerably less than the observed decrease observed after 2 min of exercise at 65% maximum oxygen consumption (\(<\)40 mM/kg dw).\textsuperscript{24} Third, exercise at 70% maximum oxygen consumption may not have been sufficiently intense to show any enhancement in TCA cycle flux with the augmented TCAi expansion. Fourth, TCA cycle flux may be limited by the availability of substrate; therefore, any further expansion of the TCAi pool could not facilitate increased TCA cycle flux without increased availability of acetyl units, a hypothesis supported by the DCA studies.\textsuperscript{4,7} Fifth, TCAi concentration in the muscle homogenate may not accurately reflect changes in the mitochondrial compartment. Until methodologic advances allow the measurement of mitochondrial and cytosolic TCAi concentrations, the latter question mark will remain.

Research in the 1960s first correlated fatigue during prolonged moderate- to high-intensity exercise with a depletion of skeletal muscle glycogen stores.\textsuperscript{25} However, the precise biochemical mechanism of this association is not fully understood. Depletion of TCAi content might provide the link causing a reduction in TCA cycle flux and, hence, oxidative energy delivery.\textsuperscript{24,25} Many of the anaplerotic reactions depend on pyruvate; thus, glycogen depletion is likely to result in decreased influx of TCAi. On the other side of the equation, the rate of TCAi depletion might be increased in the glycogen-depleted state.\textsuperscript{26} Branched-chain amino acid (BCAA) transamination, the first step in the oxidation of BCAA, relies heavily on 2-OG as the amino group acceptor, resulting in the formation of keto acid and glutamate. In conditions of plentiful pyruvate availability, 2-OG is recycled by the ALAT reaction. However, in the glycogen-depleted and hyperammonemic state, glutamine synthesis is favored,\textsuperscript{26} presumably resulting in a net loss of TCAi. The rate of BCAA oxidation is inversely proportional to carbohydrate availability: it is decreased with carbohydrate supplementation\textsuperscript{27,28} and enhanced in the glycogen-depleted state.\textsuperscript{29} Gibala et al.\textsuperscript{29} found that, during exercise under conditions expected to promote BCAA oxidation (glycogen depleted with BCAA ingestion), the expansion of the TCAi pool size at the start of exercise is not impaired.\textsuperscript{29} However, if glutamate limits anaplerosis at the start of exercise, this perhaps is not surprising because there was no difference between trials in intramuscular glutamate content. At fatigue, TCAi concentration is decreased relative to peak concentrations, although still elevated relative to the resting TCAi concentration.\textsuperscript{24} Also, acetyl carnitine concentration at fatigue was unchanged relative to values at 5 min of exercise, suggesting that the capacity to oxidize acetyl units rather than substrate availability per se was compromised at fatigue.\textsuperscript{24}

We investigated the involvement of TCAi depletion in the etiology of fatigue. We hypothesized that, if we could improve the maintenance of TCAi pool throughout exercise, the onset of fatigue could be delayed.\textsuperscript{8} Unfortunately, despite increasing TCAi at 10 min of exercise with ingestion of glutamine 1 h before exercise, this single bolus dose was not sufficient to maintain TCAi throughout exercise. TCAi concentration was similar in all trials at fatigue, but there was no significant delay in the onset of fatigue. Most significantly, there was no association between measured peak TCAi achieved and time taken to reach fatigue.

**CONCLUSIONS**

The TCAi pool size expands at the start of exercise. Ingestion of GLN before exercise results in an augmented expansion of the TCAi pool but does not appear to facilitate increased TCA cycle flux. The increase in measured peak TCAi pool size did not extend endurance capacity. Evidence from studies in which DCA has been infused suggests that the limitation to oxidative energy delivery, at least at the start of exercise, may reside at the level of acetyl unit availability.
ACKNOWLEDGMENTS

The authors thank Prof. Paul Greenhaff, Dr. Tim Constantin-Teodosiu and Prof. Clyde Williams for their assistance in this work.

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