Interaction between Glutamine Availability and Metabolism of Glycogen, Tricarboxylic Acid Cycle Intermediates and Glutathione

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ABSTRACT

After exhaustive exercise, intravenous or oral glutamine promoted skeletal muscle glycogen storage. However, when glutamine was ingested with glucose polymer, whole-body carbohydrate storage was elevated, the most likely site being liver and not muscle, possibly due to increased glucosamine formation. The rate of tricarboxylic acid (TCA) cycle flux and hence oxidative metabolism may be limited by the availability of TCA intermediates. There is some evidence that intramuscular glutamate normally provides α-ketoglutarate to the mitochondrion. We hypothesized that glutamine might be a more efficient anaplerotic precursor than endogenous glutamate alone. Indeed, a greater expansion of the sum of muscle citrate, malate, fumarate and succinate concentrations was observed at the start of exercise (70% VO2max) after oral glutamine than when placebo or ornithine α-ketoglutarate was given. However, neither endurance time nor the extent of phosphocreatine depletion or lactate accumulation during the exercise was altered, suggesting either that TCA intermediates were not limiting for energy production or that the severity of exercise was insufficient for the limitation to be operational. We have also shown that in the perfused working rat heart, there is a substantial fall in intramuscular glutamine and α-ketoglutarate, especially after ischemia. Glutamine (but not glutamate, α-ketoglutarate or aspartate) was able to rescue the performance of the postischemic heart. This ability appears to be connected to the ability to sustain intracardiac ATP, phosphocreatine and glutathione.

KEY WORDS: glutamine, glycogen storage, glutathione, glucosamine, tricarboxylic acid cycle

Much of the work that has come from our laboratory over the past 17 years has been concerned with glutamine and protein metabolism. However, there is another story to be told concerning glutamine and carbohydrate metabolism, and glutamine as an anaplerotic substrate. These topics provide the focus for the present article.

I (M. J. Rennie) became interested in the effects of glutamine on carbohydrate metabolism after hearing Dieter Häussinger talk about the effects of volume regulation, glutamine and hepatocyte metabolism. Together with Peter Taylor and Sylvia Low, we investigated the possibility that skeletal muscle also had the capacity for modulation of metabolism via alteration of cell volume. We showed that when myotubes were swollen, the rate of glycogen synthesis was increased by ~60% (Low et al. 1996a). Furthermore, we were able to show that glutamine addition would cause an increase in cell volume (Low et al. 1996b) and in glycogen synthesis (Low et al. 1996a). Later work showed that application of an anti-integrin antibody abolished the response, suggesting that it was mediated via some cytoskeletal mechanism (Low and Taylor 1998).

The idea that glutamine had an effect on carbohydrate metabolism in muscle was fascinating, and an Italian visitor to our department insisted on carrying out a study in which he infused glutamine to about double the normal plasma concentration after exhausting exercise. The change in muscle glycogen during recovery was then measured (Varnier et al. 1995). He was able to show that glutamine concentration in muscle could be elevated (by only ~15% despite the ~70% increase in plasma glutamine concentration). The infusion of saline or alanine plus glycine (isoenergetic and isonitrogenous to the glutamine infusion) was associated with a further fall in intramuscular glutamine in the postexercise period. However, most surprisingly, muscle glycogen concentration appeared to be elevated in the glutamine-treated subjects compared with those treated with saline or alanine plus glycine. Obviously this was not simply a question of availability of gluconeogenic substrates because the total amount of carbon provided as alanine and glycine was equivalent to that in glutamine. However, the effect was, in absolute terms, rather modest and...
was responsible for no more than 2 μmol/g wet weight of glycogen at h). Exhausting exercise followed by carbohydrate refeeding can produce rates of glycogen storage that are at least three times this [reviewed in Ivy (1991)].

Nevertheless, we persisted in examining this phenomenon and Jo Bowtell decided to see whether there was a practical method to promote postexercise skeletal muscle glycogen storage in human beings. In particular, she wanted to know whether oral administration was effective, whether the stimulatory effects of glutamine and a carbohydrate source were additive and whether nonmuscle glycogen storage was modified (Bowtell et al. 1999). Subjects were studied after exhaustive exercise for 1.5 h at 70% of VO2max, whereupon they consumed a 330-mL drink containing 8 g of glutamine alone or 18.5% glucose polymer (a collection of oligosaccharides of glucose) or glutamine plus the glucose polymer together. The same seven subjects were examined three times, on each occasion with a muscle biopsy after exercise before a primed constant infusion of [13C]glucose and 1 and 2 h after infusion. Oral administration of glutamine increased plasma glutamine concentration (~50% at peak) although not to the same extent as intravenous administration. Ingestion of glutamine plus glucose polymer was less effective in promoting the increase in plasma glutamine concentration, which on average, was ~200–300 μmol/L. As expected, plasma glucose and insulin concentrations were elevated only in the trials in which glucose polymer was given. Surprisingly there was no differential effect on skeletal muscle glycogen storage among giving glutamine alone, glucose polymer alone or glucose polymer and glutamine. The most striking finding was a substantial promotion of whole-body nonoxidative glucose disposal in the subjects given glucose polymer and glutamine relative to those receiving glucose polymer or glutamine alone. This suggested that the site of increased carbohydrate storage was the liver, not skeletal muscle. This very puzzling result might be explained by the observations of Baron et al. (1995) and Rossetti et al. (1995) who showed that glucosamine, which is produced in muscle via the hexosamine pathway from glucose in the presence of glutamine, has marked inhibitory effects on glucose transport and whole-body glucose disposal. Glucosamine infusion reduces intrinsic activity of GLUT 4 within 2 h (Hawkins et al. 1999) and decreases the recruitment of GLUT4 to the plasma membrane (Baron et al. 1995). It may be that in the presence of glucose, the availability of substrate in muscle is, paradoxically, reduced compared with situations in which glucose alone or glutamine alone are given. However, this is a speculation we have not yet tested.

Glutamine, the Kreb's cycle and exercise capacity

In thinking about interactions of glutamine with muscle metabolism, one rapidly achieves the insight that the Kreb's cycle is pivotal. The Kreb's cycle is the most efficient way of producing energy in muscle, and its rate of cycling increases dramatically in line with VO2 during submaximal activity. However, in order for flux through the pathway to increase, there must be an increase in the concentration of the catalytic intermediates, i.e., it is not sufficient for there to be an increased availability of acetyl CoA alone. Gibala et al. (1998) showed that there is such an expansion of the pool of tricarboxylic acid intermediates (TCAI) within the first few minutes of exercise that it appears to be roughly linear in proportion to exercise intensity. Sahlin et al. (1990) showed previously that TCAI concentrations were reduced at fatigue relative to concentrations after the first few minutes of exercise, suggesting the possibility that the availability of TCAI somehow limited fuel and oxygen utilization by muscle. A consideration of the possible anaplerotic reactions providing TCAI should include not only the usual candidates, i.e., pyruvate carboxykinase, pyruvate carboxylase, the malic enzyme and the purine nucleotide cycle, but also alanine aminotransferase, glutamate dehydrogenase and the glutamine α-amidase reaction. In addition, of course, there is also glutaminase, which provides glutamate that can be oxidized to 2-oxoglutarate by glutamate dehydrogenase. There is no net gain of TCAI from valine or isoleucine metabolism because 2-oxoglutarate is used in producing succinyl CoA, and leucine provides acetyl CoA, which is completely oxidized. Thus, the branched-chain amino acids cannot be anaplerotic substrates. The importance of glutamate as an anaplerotic substrate is hinted at by the work of Sahlin et al. (1993), who showed that in McArdle's disease (subjects have a deficiency of phosphorylase and are unable to break down glycogen to produce pyruvate), muscle glutamate concentration falls to lower levels during exercise than in normal subjects. The total extent of the fall is about the same but the starting positions were different. Nevertheless, this work suggests that TCAI concentration may limit exercise performance, and the obvious way to test this was to expand the pool and see what happens.

The strategy adopted was to deplete subjects of glycogen 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 either a placebo or two small anaplerotic precursors (ornithine α-ketoglutarate or glutamine both at 0.125 g/kg): then the effects of bicycle exercise at 70% VO2max were studied with appropriate muscle biopsies (Bruce et al. 2000). The results were interesting because they challenged some of our preconceptions. First, there was absolutely no difference between treatments in the availability of intramuscular glutamine and very little difference in plasma glutamate concentration, although rather surprisingly, the glutamine treatment did in fact elevate plasma glutamate concentration more than ingestion of ornithine α-ketoglutarate. There was the expected fall in muscle glutamate content during the first 10 min of exercise but the extent of the fall was identical among the three treatments (~11 mmol/kg dry muscle). However, at 10 min of exercise, glutamine administration did cause a substantial rise in the availability of TCAI, whereas ornithine α-ketoglutarate had no significant effect. Nevertheless, Kreb's cycle flux appeared to be unaltered because the fall in phosphocreatine and the rise in muscle lactate concentration were not significantly different among trials. When we examined the exercise-related changes between 10 min and exhaustion, there appeared at first to be some slight advantage of having taken glutamine and some disadvantage in taking ornithine α-ketoglutarate; in fact, the mean times taken to reach exhaustion were not different. Despite the fact that the TCAI concentrations had been higher in the glutamine-treated group at 10 min of exercise, all three groups had identical TCAI concentrations at exhaustion. Thus it appears that it is possible to increase the availability of TCAI without any effect on oxidative capacity or exercise capacity. There was no relationship whatsoever between endurance capacity and TCAI pool size at 10 min or fatigue.

Glutamine and cardiac performance in metabolism

Five years ago, we had a substantial interest in amino acid transport in skeletal muscle. We wondered whether skeletal muscle and cardiac muscle had identical transport characteristics. A doctoral student of mine (Shihab Khogali) carried out a program to characterize amino acid transport in the perfused...
working rat heart. In the course of investigating the effects of anoxia on the transport, we discovered that glutamine could reverse the very obvious decline in cardiac performance that was seen with anoxia and ischemia (Khogali et al. 1997). This effect was dose dependent with maximum effect being obtained at ~2.5 mmol glutamine in the perfusate and seemed to include not only cardioprotection but rescue, i.e., the provision of glutamine after the ischemic or anoxic episode would result in restitution of performance. The effect appeared to be connected to the preservation of intracardiac glutamate because perfusion in the presence of glucose only would lead to substantial depletion of glutamate, whereas perfusion with 0.6 mmol glutamine would maintain cardiac glutamine and cardiac performance. In fact we showed that in the postischemic period, glutamine was superior to aspartate, glutamate and α-ketoglutarate, although the last-mentioned came close to restoration of performance after a substantial lag period (Khogali et al. 1997). This somewhat surprising set of results was to some extent explained by the observation that in the reperfusion period, myocardial ATP concentration fell dramatically to about two thirds, whereas it could be maintained by reperfusion in the presence of glutamine. Similar results could be obtained for phosphocreatine concentration, and we also showed that lactate accumulation was diminished in the glutamine-treated rats. This suggests to us that the glutamine sustained Kreb’s cycle activity in the reperfused rat heart.

Most recently we demonstrated that in addition to this ATP effect, the ratio of reduced to oxidized glutathione concentration is also maintained in glutamine-reperfused hearts. As yet, we have no information concerning whether this is a substrate effect or related to the energy potential of the heart in terms of ATP and phosphocreatine.

LITERATURE CITED


