Interaction between Glutamine Availability and Metabolism of Glycogen, Tricarboxylic Acid Cycle Intermediates and Glutathione $^{1,2}$

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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 $\alpha$-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% VO$_{2\text{max}}$) after oral glutamine than when placebo or ornithine $\alpha$-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 $\alpha$-ketoglutarate, especially after ischemia. Glutamine (but not glutamate, $\alpha$-ketoglutarate or aspartate) was able to rescue the performance of the postischemic heart. This ability appears to be connected to the ability to sustain intracellular ATP, phosphocreatine and glutathione. J. Nutr. 131: 2488S–2490S, 2001.

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 Haussinger 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 about 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 \mu mol/(g wet weight of
glycogen \cdot 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 stim-
ulator effects of glutamine and a carbohydrate source were
additive and whether nonmuscle glycogen storage was modi-
fied (Bowtell et al. 1999). Subjects were studied after exhaust-
tive exercise for 1.5 h at 70% of VO$_{2\text{max}}$ 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 in-
crease in plasma glutamine concentration, which on average,
was ~200–300 \mu mol/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 sub-
stantial promotion of whole-body nonoxidative glucose dis-
posal 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 recruit-
ment 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 VO$_2$ 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 in-
creased availability of acetyl CoA alone. Gibala et al. (1998)
showed that there is such an expansion of the pool of tricar-
boxylic acid intermediates (TCAI) within the first few min-
utes of exercise that it appears to be roughly linear in propor-
tion 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 exer-
cise, 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 en-
zyme and the purine nucleotide cycle, but also alanine ami-
notransferase, glutamate dehydrogenase and the glutamine
$\alpha$-amidase reaction. In addition, of course, there is also glu-
taminase, 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. (1995), who showed that
in McArdle's disease (subjects have a deficiency of phosphor-
ylase 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
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 concen-
tration 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 glyco-
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 $\alpha$-ketoglutarate or gluta-
mate both at 0.125 g/kg); then the effects of bicycle exercise
at 70% VO$_{2\text{max}}$ were studied with appropriate muscle biopsies
(Bruce et al. 2000). The results were interesting because they
challenged some of our preconceptions. First, there was abso-
lutely no difference between treatments in the availability of
intramuscular glutamate and very little difference in plasma
glutamate concentration, although rather surprisingly, the glu-
tamine treatment did in fact elevate plasma glutamate con-
centration more than ingestion of ornithine $\alpha$-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 mus-
cle). However, at 10 min of exercise, glutamine administration
did cause a substantial rise in the availability of TCAI
whereas ornithine $\alpha$-ketoglutarate had no significant effect.
Nevertheless, Kreb’s cycle flux appeared to be unaltered be-
cause the fall in phosphocreatine and the rise in muscle lactate
centration were not significantly different among trials.
When we examined the exercise-related changes between 10
min and exhaustion, there appeared at first to some slight
advantage of having taken glutamine and some disadvantage
in taking ornithine $\alpha$-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 glu-
tamine-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 character-
istics. 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


