Dietary Glutamine Suppresses Endogenous Glutamine Turnover in the Rat

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Plasma glutamine turnover was determined using 1-^{14}C-labeled glutamine in rats that consumed crystalline amino acid diets containing the equivalent of 16% protein with 25% of the amino acids as glutamine or a control diet containing no glutamine (or glutamate) for 10 days. Glutamine turnover in glutamine-fed animals was 66% of the rate in the control group. Glutamine feeding caused 20% higher levels of arterial plasma glutamine. Arterial-portal differences across the portal-drained viscera showed net glutamine uptake in control animals but no net uptake or release in the glutamine-fed group. Skeletal muscle glutamine synthetase activity was similar in both groups. The results indicate that long-term consumption of relatively large amounts of dietary glutamine decreases the turnover of plasma glutamine and thus reduces the need for endogenous glutamine synthesis.

GLUTAMINE is the most abundant free α-amino acid in the body. It is present at concentrations of 0.5 to 0.8 mmol/L in plasma and up to 20 μmol/mL in the intracellular water of skeletal muscle.[1,2] This represents about 80 g free glutamine in a standard 70-kg man, most of it intracellular. At normal dietary levels (4 to 6 g/d, plus another 6 to 8 g glutamate), most ingested glutamine is catabolized by the visceral tissues and does not enter the peripheral circulation. Tracer experiments[3-5] have shown that some exogenous glutamine escapes visceral utilization but most omnivorous mammalian species exhibit a large arterial–portal veinous difference for glutamine indicative of net utilization of circulating glutamine by the portally drained viscera even in the fed state.[6] Therefore, the large pool of glutamine within the body is essentially synthesized de novo.[7] Attempts to determine the turnover rate of glutamine in the body have yielded values of 1,300 to 3,800 μmol/kg body weight per hour in rats,[8,9] 60 μmol/kg per hour in dogs,[10] 150 to 200 μmol/kg per hour in sheep,[11,12] and 180 to 500 μmol/kg per hour in humans.[2-5,13,14] Although some of the very high rates observed in humans have recently been questioned,[13] a rate of 300 μmol/kg per hour represents a turnover of plasma glutamine of about 85 g/d, far in excess of the dietary intake and thus emphasizing the importance of de novo glutamine synthesis.

In times of stress such as sepsis, surgical or accidental trauma, and hypercatabolic states in general, there is a high rate of release of glutamine from muscle and an increase in glutamine turnover within the body.[2,15,16] Since glutamine is not considered essential, together with its problems of solubility and stability, it was not added to traditional dietary formulae. However, the supplemental use of enteral or parenteral glutamine either as the free amino acid or in dipeptides is increasing, and is reported to produce beneficial outcomes in a variety of clinical situations.[16] The provision of large amounts of enteral glutamine exceeds the capacity of the visceral bed for glutamine catabolism, while parenteral delivery simply bypasses this site of glutamine metabolism. Therefore, such treatments can cause an increase in circulating glutamine concentrations, but there is limited knowledge about the effects of such changes on exogenous glutamine metabolism and homeostasis. For example, it is possible that an increase in the level of circulating glutamine may decrease endogenous glutamine synthesis and/or increase glutamine catabolism. Some studies have shown that short-term glutamine provision decreases the rate of glutamine appearance, but it is not known if this occurs with long-term glutamine feeding.

In this study, rats received crystalline amino acid diets containing the equivalent of 16% protein with either 25% of the amino acids as glutamine or a control diet containing no glutamine (or glutamate) for 10 days. The diets were made isonitrogenous by adjustment of the amount of a number of nonessential amino acids. The results show that the provision of dietary glutamine results in lower rates of turnover of the glutamine pool and indicate that dietary glutamine decreases endogenous glutamine synthesis.

MATERIALS AND METHODS

Initial experiments on food intake and weight gain and some (4 control and 5 glutamine-fed) turnover studies were performed at Rutgers University on male Sprague-Dawley rats obtained from Taconic Farms (Germantown, NY). All subsequent experiments were performed with male Wistar rats bred at the Centre de Recherches, Institut National de la Recherche Agronomique (Jouy-en-Josas, France). Within a dietary group, there were no differences in glutamine turnover between the two strains of rats or geographical locations, and values for each dietary group were therefore pooled. At commencement of the experiments, all rats weighed between 220 and 260 g and were assigned to 1 of 2 dietary groups. The pelleted diets contained crystalline amino acids and were prepared by Research Diets (New Brunswick, NJ) to be equivalent to 16% protein (Table 1). The glutamine diet contained 4% glutamine (25% of total amino acids) but no glutamate, the control diet did not contain glutamine or glutamate, and the amounts of the nonessential amino acids were adjusted so that the diets were isonitrogenous. Rats were allowed free access to the diets and drinking water for 9 to 11 days in temperature-controlled (20°C) rooms with a 12-hour light (8 AM)/dark (8 PM) cycle. All experiments started at 9 AM and were completed by 1 PM, with the rats having free access to food and water until the time of anesthetization.

Glutamine turnover in anesthetized (pentobarbital 50 mg/kg body weight) animals was determined using 1-^{14}C-L-glutamine according to the procedure of Squires and Brosnan[8] with minor modifications. 1-^{14}C-L-glutamine was prepared from 1-^{14}C-L-glutamate[8] (New England Nuclear, Boston, MA) and purity was checked by deamination

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Table 1. Amino Acid Composition of the Experimental Diets

Ingredient (g/kg)	Control	Glutamine
L-Arginine	10	10
L-Histidine HCl H_2O	6	6
L-Isoleucine	8	8
L-Leucine	12	12
L-Lysine HCl	14	14
L-Methionine	6	6
L-Phenylalanine	8	8
L-Threonine	8	8
L-Tryptophan	2	2
L-Valine	8	8
L-Alanine	15	5
L-Asparagine H_2O	15	5
L-Aspartate	15	5
L-Cysteine	4	4
Glycine	15	5
L-Proline	5	5
L-Serine	5	5
L-Tyrosine	4	4
L-Glutamine	0	40
Total amino acids	160	160

NOTE. All diets contained 685.5 g carbohydrate (560.5 g cornstarch and 125 g maltodextrin 10), 50 g cellulose, 50 g corn oil, 35 g Salt Mix (AIN S10001),[17,18] 10 g Vitamin Mix (AIN 10001),[17,18] 7.5 g $NaCO_3$, and 2 g choline bitartrate.

with glutaminase and decarboxylation with glutamate decarboxylase as previously reported.[19] A primed (2 to 3 μCi/kg over 2 minutes) infusion of 0.2 μCi/kg per minute (20 μL/min) via the right jugular vein was maintained for 20 to 30 minutes. Initial experiments demonstrated that steady-state arterial plasma specific activity was achieved between 10 and 15 minutes of infusion, in accordance with the report by Squires and Brosnan.[8] At the end of the infusion, arterial blood was drawn into a heparinized syringe. The plasma was isolated, and 1 sample was deproteinized for the determination of glutamine and another was used for the determination of glutamine specific activity after ion-exchange separation of glutamine from glutamate.[8,19]

In a separate series of experiments, rats were anesthetized and blood was drawn from the aorta and the portal vein. The whole blood was deproteinized and the metabolites were analyzed by enzymatic methods as previously described.[19,20] Samples of mixed hindleg skeletal muscle were removed, homogenized, and assayed for glutamine synthetase activity by the γ-glutamyl transferase assay of Wellner and Meister[21] as previously described.[22] Protein was determined by the procedure of Lowry using bovine serum albumin as a standard.

Results are presented as the mean ± SEM. Differences between control and glutamine-fed rats were compared by Student's *t* test, and results within a group (arterial *v* portal vein concentrations) were compared by paired *t* test with significance set at a *P* level less than .05.

RESULTS

There were no differences between the two groups for daily food intake (control *v* glutamine diet, 21.5 ± 0.67 *v* 22.6 ± 0.39 g/d, n = 6 per group) or weight gain (control *v* glutamine diet, 5.68 ± 0.36 *v* 5.43 ± 0.62 g/d, n = 6). Arterial plasma glutamine levels were about 20% higher in glutamine-fed rats (Table 2). A similar trend was observed for whole-blood glutamine levels (Table 3). Plasma glutamine turnover in the glutamine-fed group was 66% of the rate in the control group.

Since the principal site of glutamine catabolism in the healthy rat is the portally drained viscera, arteriovenous differences

Table 2. Glutamine Turnover in Glutamine-Fed Rats and Controls

Parameter	Control	Glutamine
Plasma glutamine (mmol/L)	0.590 ± 0.030 (13)	0.696 ± 0.055 (11)*
Glutamine turnover (μmol/100 g/h)	123.7 ± 11.9 (9)	82.1 ± 8.4 (9)*

NOTE. Results are the mean ± SEM for the number of animals shown in parentheses.

*Significantly different *v* control.

were measured across this bed. In control animals, there was a significant net uptake of glutamine from the arterial circulation accompanied by a small net output of glutamate and a large net output of alanine and ammonia. It should be kept in mind that the control diet contained a relatively large amount of free alanine, which would be expected to be absorbed into the portal circulation without modification. In the glutamine-fed animals, there was a trend for higher arterial blood glutamine levels (Table 3), but glutamate, alanine, and ammonia levels were similar in the two groups. In marked contrast to the animals on the control diet, those receiving the glutamine diet showed no detectable difference in the glutamine concentration in the artery versus the portal vein. A small net glutamate release across the portal-drained viscera was still observed but net alanine release was lower than in control animals, while there was no significant net release of ammonia.

The results support the hypothesis that dietary glutamine suppresses endogenous glutamine production. Previous reports have suggested that exogenous glutamine may downregulate skeletal muscle glutamine synthetase activity, but in this study, muscle glutamine synthetase activity was similar in the two groups (Table 4).

DISCUSSION

Dietary glutamine is increasingly recognized as a beneficial agent in a number of clinical conditions. Investigations to date

Table 3. Arteriovenous Difference for Amino Acids in Glutamine-Fed Rats and Controls

Amino Acid	Control (n = 9)	Glutamine (n = 7)
Glutamine		
Arterial	0.455 ± 0.021	0.546 ± 0.055
Portal	0.414 ± 0.021	0.563 ± 0.024†
Difference	0.062 ± 0.010*	−0.015 ± 0.060
Glutamate		
Arterial	0.230 ± 0.016	0.270 ± 0.026
Portal	0.277 ± 0.019	0.289 ± 0.020
Difference	−0.048 ± 0.018*	−0.030 ± 0.007*
Alanine		
Arterial	0.590 ± 0.041	0.532 ± 0.023
Portal	0.801 ± 0.065	0.665 ± 0.032
Difference	−0.215 ± 0.060*	−0.140 ± 0.033*
Ammonia		
Arterial	0.171 ± 0.041	0.236 ± 0.042
Portal	0.228 ± 0.030	0.260 ± 0.033
Difference	−0.066 ± 0.027*	−0.025 ± 0.036

NOTE. Results are expressed as μmol/mL whole blood and are the mean ± SEM for the number of animals shown in parentheses.

*Significantly different from 0.

†Significantly different *v* control.

Table 4. Glutamine Synthetase Activity in Skeletal Muscle

Glutamine Synthetase Activity	Control	Glutamine
U/g wet weight	0.287 ± 0.046	0.248 ± 0.050
mU/mg protein	5.58 ± 0.89	4.16 ± 0.45

NOTE. Results are expressed as units, where 1 U is 1 μmol product formed per minute at 37°C, and are the mean ± SEM of 5 animals per group.

have demonstrated that aggressive glutamine feeding (enterally or parenterally) can produce improved healing and hospitalization times in a variety of conditions.[2,16] However, little is known about the mechanisms underlying glutamine's positive effects.

The finding that dietary glutamine was well tolerated and did not affect food intake or weight gain in this study is in accordance with most published studies.[23,24] However, direct comparisons are not always possible, since many studies either did not use isonitrogenous diets or simply substituted a single amino acid for glutamine. Welbourne et al[23] found that in rats fed a 14% amino acid elemental diet either without glutamine or with glutamine as 13% of the amino acids (1.82% glutamine) for 3 days, there were no differences in food intake or body weight gain; however, there was no change in circulating glutamine levels in their rats.

Glutamine turnover as measured in this study is the rate of appearance of glutamine from de novo synthesis, proteolysis, and any exogenous glutamine from the diet or experimental procedure. When considering glutamine turnover, it is important to consider the sampled pool. In humans, Darmaun et al[5,25,26] have calculated the glutamine pool sampled by tracer experiments to be 200 to 250 μmol/kg, which is in excess of the extracellular pool but far less than the total intracellular pool of free glutamine and represents about 2% of the total body pool. Waterlow et al[27] have compared the bulk of the intracellular glutamine pool as being equivalent to muscle protein in short-term tracer experiments, in that it does not play a major role in the rapid dynamics of the pool sampled. This means that glutamine kinetics are representative of interorgan glutamine flux, although they must also represent both glutamine synthesis and degradation within some tissues.

The rates of glutamine turnover in this study are similar to those (130 μmol/100 g body weight per hour) reported by Squires and Brosnan[8] in chow-fed rats. Interestingly, chow-fed rats would have received some glutamine and glutamate in the diet and perhaps would be expected to show turnover rates closer to those obtained on the glutamine diet of the current study, although it is not really valid to compare absolute values for diets that differ so greatly in composition. Squires and Brosnan[8] found no difference in glutamine turnover in rats made chronically acidotic with ammonium chloride, but Yoshida et al[9] reported increased glutamine turnover in tumor-bearing rats (227 and 381 μmol/100 g body weight per hour using U-^{14}C-glutamine in control and tumor-bearing rats, respectively). These results indicate that, by using large amounts of glutamine, the tumor causes an increase in the rate of appearance of glutamine from endogenous sources.

Conditions that change glutamine turnover include short-bowel syndrome,[28,29] sickle-cell anemia,[30] severe burn injury (increase),[15] and administration of catabolic hormones such as glucocorticoids.[15,31] The rate is also higher in children than in adults.[28,29,32] Such differences are predominantly due to differences in the rate of glutamine de novo synthesis. The influence of diet, and particularly dietary glutamine, on endogenous glutamine metabolism has not been extensively studied. In short-term feeding experiments, a continuous intragastric infusion of glutamine (44 g in 6 hours) increased the rate of appearance of glutamine in the circulation.[5] This is due to an increase in the rate of appearance of exogenous glutamine and a decrease in the rate of de novo glutamine synthesis (endogenous) during the glutamine infusion. The only relatively long-term (3-day) experiments on glutamine turnover showed that it varied inversely with dietary protein levels, being lower in high-protein–fed individuals.[33]

In the present study, we found that long-term glutamine feeding decreased the turnover rate of glutamine. It is not possible to determine the contribution of endogenous and exogenous glutamine to the turnover rate, but since the animals were sampled in the fed (absorptive) state, it would be expected that dietary glutamine would enter the circulation throughout the experimental period. Thus, the animals appear to have adapted to the glutamine diet by decreasing endogenous glutamine production. It is not clear if the absence of dietary glutamate has an effect, but dietary glutamate would be metabolized by the small-intestinal mucosa[7] and could therefore also spare endogenous glutamine production.

The level of dietary glutamine in this study probably exceeded the catabolic capacity of the portal-drained viscera, since there was an increase in circulating glutamine levels. However, such an increase could be due to a decreased utilization of exogenous glutamine. Moudras et al[34] fed a 15% casein diet supplemented with 7.2% glutamine for 15 to 21 days and observed an increase in arterial glutamine of +54%, and similar to the current study, the portal-drained viscera changed from a site of net glutamine uptake to a site of net glutamine output. The decreased arterial-portal difference for glutamine (and its products) across the portal-drained viscera indicates lesser use of circulating glutamine in the glutamine-fed animals. However, an increase in portal blood flow also could have produced a similar finding for glutamine, but others have reported no change in portal blood flow in glutamine-supplemented rats.[35]

In this study and others wherein de novo glutamine synthesis has been shown to be decreased by exogenous glutamine, there must be a signal to the glutamine-producing tissues. The obvious candidate would be a change in the circulating glutamine level, but in many of the studies, glutamine levels either did not change or changed in the direction opposite to that expected. Evidence for glutamine regulation of muscle glutamine synthetase activity arose from the study of L6 myoblasts[36] and other cell types,[37] wherein culture in the presence of glutamine decreased the activity of glutamine synthetase. But these are long-term effects and are most likely due to changes in the amount of glutamine synthetase protein. There is no evidence of a short-term inhibition of glutamine synthetase activity by glutamine, and in the studies in which glutamine has been shown to downregulate whole-body glutamine synthesis, the duration of the elevated plasma glutamine would not be of sufficient length to result in measurable differences in the

amount of glutamine synthetase protein. The study reported here shows that prolonged exposure to elevated levels of circulating glutamine did not change the glutamine synthetase activity of skeletal muscle when expressed on a per-gram wet weight or protein basis. In this study, mixed hindlimb skeletal muscle was sampled, and although there is some evidence of a variation of glutamine synthetase activity with muscle type, there is no evidence that adaptive changes in activity show any such variation.[38,39] Similarly, the possibility exists that there was a change in lean body mass, ie, more glutamine synthetase activity through more muscle mass, but this is unlikely given the similar weight gain of the 2 groups. Thus, downregulation of muscle glutamine synthetase is not likely the mechanism.

The results of this study show that relatively large amounts of dietary glutamine can produce higher circulating glutamine levels, a decreased turnover of endogenous glutamine, and changes in intestinal metabolism of endogenous glutamine. It is suggested that such changes occur because the dietary glutamine is used by the small intestine and thus spares the need for some endogenous glutamine. The mechanism for this effect is not known, but it supports the use of aggressive glutamine feeding in pathological states in order to conserve body nitrogen (protein) stores.

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