Rapid activation of hepatic glutaminase in rats fed on a single high-protein meal

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We report that hepatic glutaminase is rapidly activated in rats fed on a single high-protein (60% casein) meal. Rats previously fed on a normal-protein (15% casein) diet for 3–4 days were given a high-protein meal for 2 h. The high-protein meal increased the rate of flux through glutaminase in intact liver mitochondria nearly 3-fold (20.6 ± 1.7 nmol/min per mg of protein versus 7.5 ± 2.9 nmol/min per mg of protein) at a P_i concentration of 10 mM. The activation of flux through glutaminase by a high-protein meal involved an increased sensitivity of glutaminase to P_i , an activator of the enzyme. The K_a for P_i was 1.0 mM and 24.1 mM in mitochondria from rats fed on the high-protein and normal-protein meals respectively. We measured the concentration of P_i in the mitochondrial matrix and found that it did

not differ in mitochondria from rats fed on the high-protein and normal-protein meals, suggesting that the effect of the high-protein meal on the P_i -sensitivity of glutaminase was not due to a change in the distribution of P_i across the mitochondrial inner membrane. Glutaminase activity was measured by using mitochondrial membranes from frozen-thawed mitochondria. Glutaminase activity and its dependence on P_i were similar for preparations from rats fed on high-protein and normal-protein meals. These findings show that hepatic glutaminase is stimulated rapidly by a high-protein meal. This is part of the physiological hepatic response to increased protein intake which permits the liver to cope with the influx of glutamine occurring at this time.

INTRODUCTION

Glutaminase, which catalyses the hydrolysis of glutamine to glutamate and ammonia, is the major enzyme of glutamine catabolism in liver cells. This enzyme is located in the mitochondria of periportal hepatocytes (Watford and Smith, 1990), being loosely attached to the inner surface of the mitochondrial inner membrane (McGivan et al., 1980). A number of effectors are known to be involved in the regulation of glutaminase. The enzyme is activated by ammonia (Charles, 1968), one of its products, and is also stimulated by bicarbonate ions (Joseph and McGivan, 1978), by an increase in pH (Szweda and Atkinson, 1989), and by phosphate (McGivan et al., 1980). Flux through glutaminase in isolated hepatocytes is rapidly increased by hormones, with glucagon and catecholamines eliciting the greatest response (Corvera and Garcia-Sainz, 1983). It has also been shown that flux through glutaminase is markedly stimulated in intact mitochondria isolated from rats treated with a single injection of glucagon 20 min before being killed (Lacey et al., 1981).

Glutaminase is closely associated with the urea cycle and has been shown to generate ammonia for direct utilization by carbamoyl-phosphate synthase I (CPS I) (Meijer, 1985). The activities of glutaminase (Watford et al., 1985) and the urea-cycle enzymes (Schimke, 1962) are known to increase in the liver in response to chronic high-protein feeding, but the immediate, or acute, response of glutaminase to a high-protein meal has not been examined. Since glucagon is increased in the circulation following a high-protein meal (Robinson et al., 1981), we hypothesized that glutaminase would be activated in animals fed on a single high-protein meal, and this would be evident in mitochondria isolated from these animals. In the present study we report that flux through glutaminase is stimulated in animals

fed on a single high-protein meal for 2 h compared with the rate elicited by a meal of normal protein content. We also report that the rate of citrulline synthesis, a measure of the flux through CPS I and ornithine transcarbamoylase, is similarly stimulated in these mitochondria.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats were purchased from Charles River Ltd. (Montreal, P.Q., Canada) and fed on a standard Purina pellet diet (*ad libitum*) until the experiment. Rats (150–250 g) were then fed for 3–4 days on a purified diet containing either 15% casein, which is an adequate amount of protein (normal-protein diet), or 60% casein (high-protein diet). The animals rapidly adjusted to the diets, as indicated by a marginal change in body weight on day 1, followed by normal weight gain on subsequent days. In a few instances, where weight gain did not occur, the animal was removed from the study.

The experimental protocol was designed to distinguish between effects due to a diet and those elicited by a single meal. Thus the animals were either fed on a diet for 3–4 days, to study the effect of a given diet or, having eaten a given diet for 3–4 days, the effect of a single meal was then studied. When the effect of a single meal was studied, animals were deprived of food for 6 h before the start of the meal. This was done to prevent any food intake during this time and to ensure that the animals ate promptly when they were given a meal. Meals were either of the same diet to which the animals had been accustomed or of the alternative diet (rats fed on the high-protein diet received a normal-protein meal, and vice versa). Those given the high-protein meal tended to consume less than rats fed on the

normal-protein meal, but this difference was not significant (3.1 ± 1.2 versus 3.8 ± 1.3 g/2 h meal). Meals began at the start of the dark period (lights 12 h on/12 h off), and the animals were killed 2 h later. Controls were killed at the beginning of the feeding period.

Diets

The diets were modified AIN-76 diets (Bieri et al., 1977). The high-protein diet was made up of (in g/100 g of diet): casein, 59.8; L-methionine, 0.15; corn starch, 6.1; sucrose, 19.2; corn oil (Mazola), 5; vitamin mix (AIN 76), 3.5; mineral mix (AIN 76), 1; Alphacel, 5; choline bitartrate, 0.2. For the normal-protein diet casein was decreased to 14.8 g/100 g in favour of sucrose and corn starch, which were increased to 53.3 and 17 g/100 g respectively. These diets are isoenergetic.

Preparation of intact mitochondria and mitochondrial membranes

Mitochondria were isolated as described by Jois et al. (1989). The isolation medium consisted of mannitol (225 mM), sucrose (75 mM), EGTA (1 mM), Hepes (5 mM), pH 7.4. The mitochondrial pellet was resuspended in 0.5 ml of the above medium to give a stock suspension with a concentration of 50–70 mg of mitochondrial protein/ml as determined by the biuret test with BSA as standard (Gornall et al., 1949). The yield of mitochondria was similar for high-protein-fed and normal-protein-fed rats, and these mitochondria were acceptable if they displayed a respiratory control ratio greater than 4 with 2-oxoglutarate as substrate.

Mitochondrial membranes were prepared as described by Szweda and Atkinson (1989). A stock solution of mitochondria was diluted 1:1 with distilled water and subjected to three cycles of freezing in liquid nitrogen and thawing in water (37 °C). The suspension was diluted 5-fold with isolation medium and the membranes were separated from solution by centrifugation for 20 min at 40 000 g.

Flux through glutaminase and glutaminase activity

Flux through glutaminase was measured in intact mitochondria as described by Lacey et al. (1981). Mitochondria (2–3 mg) were incubated at 30 °C for 10 min in a final volume of 1 ml, which contained KCl (100 mM), glutamine (20 mM), KH_2PO_4 (10 mM), KHCO_3 (20 mM), succinate (5 mM), rotenone (50 µg/ml), EGTA (1 mM), Tris/HCl (20 mM), pH 7.4. Under these conditions, glutamate formation was linear after an initial lag period of about 2.5 min in mitochondria from both high-protein-fed and normal-protein-fed rats. Glutaminase activity was determined by using mitochondrial membranes (1 mg/ml) incubated for 10 min at 37 °C in a medium that contained mannitol (300 mM), glutamine (20 mM), NH_4Cl (0.7 mM), Hepes (20 mM), pH 7.4, and various levels of KH_2PO_4 (Szweda and Atkinson, 1989).

Incubations were stopped with 0.3 ml of HClO_4 (7%). The deproteinized extracts were neutralized and assayed for glutamate enzymically as described by Bernt and Bergmeyer (1974).

Citrulline synthesis

Citrulline synthesis was determined in intact mitochondria (3–4 mg) incubated for 4 min at 30 °C in a medium which

contained KCl (80 mM), L-ornithine (20 mM), KH_2PO_4 (5 mM), NH_4Cl (5 mM), KHCO_3 (10 mM), succinate (10 mM), Tris/HCl (20 mM), pH 7.4 (Lacey et al., 1981). Incubations were stopped by adding 0.3 ml of HClO_4 (7%). The samples were analysed for citrulline (Herzfeld and Raper, 1976).

Measurement of P_i in the mitochondrial matrix

Intact mitochondria were incubated for 5 min in the same medium used to determine flux through glutaminase. Mitochondria were incubated in a volume of 2 ml at a concentration of 4 mg/ml. After incubation, three successive 0.5 ml portions of mitochondrial suspension were removed for determination of mitochondrial phosphate, protein and volume. One sample was transferred to an Eppendorf tube containing 0.1 ml of trichloroacetic acid (10%) overlaid with 0.7 ml of bromododecane/dodecane (20:1, v/v). Mitochondria were separated from the incubation medium by centrifugation through the oil in an Eppendorf centrifuge (1200 g for 1 min). The acid layer was analysed for P_i as described by Baginski et al. (1967). A second sample of mitochondrial solution was transferred to an Eppendorf tube in which the trichloroacetic acid was replaced with 0.1 ml of KCl (1.25 M). The amount of protein that was pelleted by centrifugation of mitochondria through the oil was determined in this tube by the biuret procedure. Before removal of the third sample, 30 µl of $^3\text{H}_2\text{O}$ (20 µCi/ml)/[U- ^{14}C]sucrose (10 µCi/ml) was added to the remaining 1 ml of mitochondrial solution. A sample was immediately transferred to an Eppendorf tube containing 0.1 ml of HClO_4 (7%) overlaid with 0.7 ml of the oil. This sample was used to determine mitochondrial matrix space as previously described (Jois et al., 1992).

Matrix phosphate was calculated as the total phosphate determined in the acid layer minus that associated with the sucrose space (extra-matrix phosphate). This correction amounted to about 25% of the total.

Chemicals

Diet components were obtained from ICN (Cleveland, OH, U.S.A.), except for the corn starch and L-methionine, which were purchased from Sigma (St. Louis, MO, U.S.A.). $^3\text{H}_2\text{O}$, [U- ^{14}C]sucrose and Omnifluor were obtained from DuPont–New England Nuclear (Mississauga, Ont., Canada). Other reagents were of analytical grade.

Treatment of data

The kinetic data reported for the Figures was determined by using the computer program GraphPAD (GraphPAD Software, San Diego, CA, U.S.A.) where the curve of best fit (rectangular hyperbola in Figure 1, sigmoidal curve in Figures 2 and 3) is arrived at through an iterative process employing an algorithm which determines the parameters that minimize the sum of squares of differences between the dependent variable in the equations and the observations. This analysis was performed on the data for each individual experiment. All data are presented as means $\pm$ S.E.M. Comparisons were made by a Student *t* test, with *P* < 0.05 taken as indicating statistical significance.

RESULTS

Stimulation of citrulline synthesis and flux through glutaminase by a high-protein meal

Flux through glutaminase was stimulated in intact mitochondria from rats fed on a high-protein meal compared with rates in

Table 1 Effect of a single meal on citrulline synthesis and flux through glutaminase in isolated rat liver mitochondria

Rats were fed on a high-protein (HP) or normal-protein (NP) diet for 3–4 days before being given the HP or NP meal for 2 h. Results are means $\pm$ S.E.M. for the numbers of experiments in parentheses. Results were compared by Student's *t* test ($P < 0.05$): *significantly different from the control (no meal); †significantly different from those that received a meal that matched the diet to which they had been accustomed.

Diet	Meal	Rate (nmol/min per mg of mitochondrial protein)	
		Glutamine hydrolysis	Citrulline synthesis
HP	—	4.4 $\pm$ 1.0 (4)	18.7 $\pm$ 4.5 (4)
HP	HP	30.5 $\pm$ 2.1* (4)	49.1 $\pm$ 8.0* (4)
HP	NP	7.8 $\pm$ 2.8† (4)	9.0 $\pm$ 2.0† (4)
NP	—	4.5 $\pm$ 1.5 (4)	5.4 $\pm$ 2.5 (3)
NP	NP	7.5 $\pm$ 2.9 (4)	3.4 $\pm$ 0.7 (3)
NP	HP	20.6 $\pm$ 1.7*† (4)	36.3 $\pm$ 6.7*† (4)

mitochondria from rats not given a meal or given a normal-protein meal (Table 1). The stimulation was evident in rats which had been fed on either the high-protein diet or the normal-protein diet, thus illustrating the effectiveness of a single 2 h high-protein meal in eliciting a stimulation of glutamine catabolism. Citrulline synthesis was stimulated in mitochondria isolated from rats fed on a single high-protein meal compared with rates in mitochondria from rats not given a meal (Table 1). The stimulation by the high-protein meal in absolute terms amounted to 31 nmol/min per mg for rats previously fed on either the normal-protein or high-protein diets. The degree of stimulation by the meal was greater in the mitochondria of rats fed on the normal-protein diet than in those of rats fed on the high-protein diet for 3–4 days (6.8-fold versus 2.7-fold). This was due to the higher pre-meal (control) rates of citrulline synthesis in the mitochondria of high-protein-fed rats compared with the rates in mitochondria of rats fed on the normal-protein diet (18.68 versus 3.43 nmol/min per mg of protein), an effect which presumably occurred because of induction of urea-cycle enzymes in the rats fed on the high-protein diet (Schimke, 1962).

Sensitivity of flux through glutaminase to P_i is increased by a high-protein meal

P_i has been shown to be an activator of glutaminase whether the activity is measured in intact mitochondria (Joseph and McGivan, 1978) or in disrupted mitochondria (McGivan et al., 1980). In intact mitochondria from glucagon-treated rats the sensitivity of glutaminase to P_i is greatly increased (Lacey et al., 1981). We therefore examined glutaminase flux, as a function of P_i , in intact mitochondria from rats fed on the normal-protein meal and in rats fed on the high-protein meal. We found a marked increase in the sensitivity of glutaminase flux to P_i in mitochondria from the rats fed on the high-protein meal. In mitochondria from rats fed on the high-protein diet and given a

Figure 1 Effect of a high-protein meal on the P_i activation of flux through glutaminase in rat liver mitochondria

Rats were fed on a high-protein (a) or normal-protein (b) diet for 3–4 days before being given a high-protein (●) or normal-protein (○) meal for 2 h. The concentration of glutamine in the incubations was 20 mM. Results are means $\pm$ S.E.M. of four separate experiments. Results were compared by Student's *t* test ($P < 0.05$): *significantly different from the rate in mitochondria from rats receiving the normal-protein meal.

high-protein meal, half-maximal rate of flux through glutaminase was reached at a much lower P_i concentration than in mitochondria from rats given the normal-protein meal (1.7 mM versus 12.3 mM; Figure 1a). This effect was also demonstrable in rats fed on the normal-protein diet (Figure 1b). In this case the K_a for P_i of flux through glutaminase was 1.0 mM and 24.1 mM for rats receiving the high-protein and normal-protein meals respectively. Thus a single high-protein meal markedly enhances the sensitivity of glutaminase toward P_i .

Concentration of P_i in the matrix of mitochondria from rats fed on high-protein and normal-protein meals

Mitochondria from rats fed on a high-protein meal exhibited stimulated rates of flux through glutaminase even when the incubation medium did not contain P_i (Figure 1). This could conceivably be explained by a higher matrix P_i concentration of these mitochondria compared with those from rats fed on a normal-protein meal (Table 2). However, the difference in sensitivity of flux through glutaminase toward added P_i that existed between mitochondria from rats fed on the high-protein and normal-protein meals did not involve a difference in their abilities to accumulate matrix P_i (Table 2). When the P_i concentration of the medium was 2.5 mM, close to that of the cytosol, matrix P_i was 20.8 and 17.2 mM for mitochondria from rats fed on the high-protein and normal-protein meals respectively. These values are close to the estimated mitochondrial matrix P_i concentration (16.80 mM) reported for hepatocytes (Akerboom et al., 1978) and were not significantly different from each other. When the concentration of externally added P_i was 10 mM, the P_i level used in most of the incubations to assess flux through glutaminase, P_i was found to be concentrated about 4-fold by mitochondria (Table 2). In these experiments mito-

Table 2 Concentration of P_i in the matrix of mitochondria isolated from rats fed on a high- or normal-protein meal

Rats were fed on a normal-protein diet for 3–4 days before being given the high-protein (HP) or normal-protein (NP) meal for 2 h. Results are means $\pm$ S.E.M. of four experiments. Results were compared by Student's *t* test ($P < 0.05$): *significantly different from the level in mitochondria, isolated from rats fed on a NP meal, incubated in the same medium.

Meal	P_i in medium (mM)	P_i in mitochondrial matrix (mM)
HP	0	$4.3 \pm 0.3^*$
	2.5	20.8 ± 1.4
	10	45.1 ± 1.6
NP	0	2.9 ± 0.5
	2.5	17.2 ± 2.1
	10	41.1 ± 4.3

Figure 2 Effect of a single high-protein meal on the affinity of glutaminase for glutamine

Rats were fed on the normal-protein diet for 3–4 days before being given a high-protein (●) or normal-protein (○) meal for 2 h. The concentration of P_i in the incubations was 10 mM. Data were fitted to equations specifying a sigmoidal curve as described in the Materials and methods section. Results are means $\pm$ S.E.M. of three separate experiments. Results were compared by Student's *t* test ($P < 0.05$): *significantly different from the rate in mitochondria from rats receiving the normal-protein meal.

chondrial matrix volume was similar for mitochondria isolated from rats fed on the high-protein and normal-protein meals and was independent of the P_i concentration in the incubation medium (results not shown).

Sensitivity of flux through glutaminase to glutamine

Szweda and Atkinson (1989) reported that P_i exerts its effect on glutaminase by increasing the enzyme's affinity for glutamine. In mitochondria from rats fed on a single high-protein meal, flux through glutaminase was more sensitive to glutamine than in mitochondria from rats fed on a normal-protein meal (Figure 2). In mitochondria from rats fed on a high-protein meal, half-maximal rate of flux through glutaminase was reached at a glutamine concentration of 10.7 ± 0.8 mM, compared with a value of 15.2 ± 0.9 mM observed for mitochondria from rats fed on a normal-protein meal ($P < 0.05$).

Figure 3 Effect of a high-protein meal on the ammonia activation of flux through glutaminase in rat liver mitochondria

Rats were fed on the normal-protein diet for 3–4 days before being given a high-protein (●) or normal-protein (○) meal for 2 h. The concentration of P_i in the incubations was 10 mM. Results are means $\pm$ S.E.M. of three separate experiments. Results were compared by Student's *t* test ($P < 0.05$): *significantly different from the rate in mitochondria from rats receiving the normal-protein meal.

Figure 4 Effect of a single high-protein meal on glutaminase activity as measured in liver mitochondrial membranes

Rats fed on the normal-protein diet for 3–4 days were given a high-protein (●) or normal-protein (○) meal for 2 h. Mitochondrial membranes were incubated in the presence of 20 mM glutamine. Results are means $\pm$ S.E.M. for three separate experiments.

Effect of ammonia activation

Ammonia is an important activator of glutaminase. It is possible that the effect of high-protein feeding could be the result of a higher ammonia content of mitochondria from these animals. However, incubation done in the presence of varying NH_4Cl concentrations ruled out this possibility (Figure 3). The stimulatory effect of high-protein feeding on flux through glutaminase is evident, even when flux is maximally activated by ammonia.

Glutaminase activity in broken mitochondria is not changed by a single high-protein meal

The stimulation of flux through glutaminase in intact mitochondria isolated from rats fed on a high-protein meal did not involve an increase in total enzyme activity as measured in membranes of disrupted mitochondria (Figure 4). We chose to disrupt the mitochondria by freezing and thawing, because this procedure does not significantly release glutaminase from the mitochondrial membrane and the preparation of enzyme most closely resembles that in intact mitochondria (McGivan et al., 1980). In these experiments, rats fed on the normal-protein diet for 3–4 days were given a normal-protein or high-protein meal for 2 h, liver mitochondria were isolated, membranes were prepared by freeze-thawing and glutaminase was assayed. The rates of flux through glutaminase in intact mitochondria from these rats were 7.7 and 36.3 nmol/min per mg of mitochondrial protein respectively ($P < 0.05$). Yet no difference was found in the glutaminase activity assayed in the broken mitochondrial preparations. Moreover, the different affinities for P_i exhibited by intact mitochondria from rats fed on the high- and normal-protein meals were not evident in the membrane preparations.

DISCUSSION

Glutaminase is the major glutamine-degrading enzyme in the liver. Not only may flux through glutaminase provide ammonia for direct utilization by carbamoyl-phosphate synthase I (Meijer, 1985), but the glutamate formed may be converted into *N*-acetylglutamate, an obligate activator of carbamoyl-phosphate synthase I. Glutaminase is also the initial step in the conversion of glutamine into glucose. Thus this enzyme has a potentially important role in ureagenesis and gluconeogenesis, which are dominant processes in the liver following a high-protein meal (Jungas et al., 1992). Our experiments demonstrate a large increase in the flux through glutaminase and in citrulline synthesis in mitochondria from rats fed on high-protein meals for 2 h. It is known that an adaptive increase in the activity of glutaminase (Watford et al., 1985) occurs in the livers of animals chronically fed on high-protein diets. However, the liver can be faced with an influx of amino acids within 1 h of ingestion of a high-protein meal, so that a need exists for a much faster response by this enzyme so as to accommodate the quite variable amounts of protein that may be ingested in individual meals.

A number of mechanisms have been described whereby hepatic amino acid catabolism is regulated. One mechanism, that of highly inducible enzymes with short half-lives, is exemplified by tyrosine aminotransferase. The amount of such enzyme proteins increases rapidly after a meal and subsequently returns to low levels before the next meal (Krebs, 1972). Such a mechanism does not apply to the acute regulation of glutaminase. This contention is supported by the finding that glutaminase activity, measured in broken mitochondria, was not changed by the ingestion of the high-protein meal. Another mechanism involves covalent modification, where activities of enzymes such as phenylalanine hydroxylase and branched-chain oxo-acid dehydrogenase are modulated by a reversible phosphorylation/dephosphorylation of enzyme subunits (Fisher and Pogson, 1984). Ingestion of a high-protein diet, for example, significantly increases the degree of activation of rat liver branched-chain oxo-acid dehydrogenase, without causing an increase in the amount of the enzyme (Miller et al., 1988). However, regulation of glutaminase by covalent modification has not been described. Vargas et al. (1982), for example, were unable to demonstrate an involvement of phosphorylation in the acute regulation of mitochondrial metabolism by hormones. In our experiments, the

mitochondria were washed three times during the isolation procedure, with no particular precautions taken to preserve phosphorylation state. So it would seem the effect is somehow more long-lived than typically labile covalent modification. The similarity between the activation of glutaminase brought about by glucagon (Lacey et al., 1981) and that demonstrated here after a high-protein meal suggests that they represent the same phenomenon. The similarity is particularly striking with respect to P_i activation of the enzyme. Thus we propose that activation of glutaminase is a normal response to a high-protein meal and that it is probably brought about by glucagon.

The stimulation of flux through glutaminase after a high-protein meal is not the result of an increased content of ammonia in these mitochondria. In incubations where flux was activated by ammonia, a stimulatory effect of the diet was still evident. Another possibility is that the effect is due to the increased content of matrix *N*-acetylglutamate seen after high-protein feeding (Rabier et al., 1982). That *N*-acetylglutamate may regulate glutaminase has been suggested by Meijer and Verhoeven (1986), who reported that flux through glutaminase was stimulated in mitochondria incubated under conditions which favour increases in mitochondrial *N*-acetylglutamate concentration, although they did not measure the concentration of *N*-acetylglutamate in these experiments. In contrast, Joseph and MacGivan (1978) were unable to detect an effect of *N*-acetylglutamate on liver glutaminase in disrupted mitochondria, and Smith and Watford (1988) reported that *N*-acetylglutamate stimulated the purified enzyme, but only by 10%. It is, of course, possible that *N*-acetylglutamate might be a more effective activator in intact mitochondria, so its role in activating glutaminase is still an open question.

Mitochondria from rats fed on high-protein meals for 2 h exhibit high rates of citrulline synthesis. This rapid stimulation is likely due to the activation of CPS I, which is an important regulatory enzyme in urea synthesis. Rapid activation of CPS I has been reported to occur within 15 min of an amino acid load (Stewart and Walser, 1980), an effect which was attributed to an increase in *N*-acetylglutamate, its obligate activator. Increases in *N*-acetylglutamate closely correlate with the stimulation of citrulline synthesis in mitochondria from rats fed for a single day on a high-protein diet (Morimoto et al., 1990) and it is likely that an increase in this substance leads to the stimulation of citrulline synthesis after a 2 h high-protein meal. A rapid activation of citrulline synthesis after a high-protein meal will ensure that the amino acid nitrogen associated with the meal is converted into urea.

The disposal of an excess of amino acids after ingestion of a high-protein meal involves the co-ordinated activation of a variety of liver enzymes. We have recently shown that glycine catabolism in liver mitochondria is stimulated within 2 h from the start of a high-protein meal (Ewart et al., 1992). Thus the stimulation of flux through glutaminase and of citrulline synthesis may be viewed as part of a general mechanism, for regulating hepatic mitochondrial amino acid metabolism, which is physiologically important in permitting the liver to deal with the increased amino acids after a single high-protein meal.

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