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Research Article

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Muscle and Splanchnic Glutamine and Glutamate Metabolism in Postabsorptive and Starved Man

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ABSTRACT Arterio-venous differences across forearm muscle in man in both prolonged starvation and in the postabsorptive state, show an uptake of glutamate and a relatively greater production of glutamine. Splanchnic arteriovenous differences in the postabsorptive state show a net uptake of glutamine and lesser rate of glutamate production. These data suggest that muscle is a major site of glutamine synthesis in man, and that the splanchnic bed is a site of its removal. The relative roles of liver and other tissues in the splanchnic circuit were not directly assessed, only the net balance. These data in man are in conflict with most previous studies in other species attributing the major proportion of glutamine production to the liver and, *pari passu*, to the splanchnic bed.

INTRODUCTION

During starvation, muscle protein catabolism provides amino acid substrate for both hepatic and renal gluconeogenesis. Recent studies have emphasized the central role of alanine in hepatic amino acid extraction (1) and amino acid release from muscle (2). This report includes data on peripheral and splanchnic metabolism of glutamate and glutamine in man, and, in contrast to current opinion as reviewed by Lotspeich (3), suggests that under physiologic circumstances, muscle in man is an important site of glutamine production, and the splanchnic bed is a site of its removal. Hence glutamine in man appears to be as prominent a vehicle as alanine for both glucogenic precursor and nitrogen fluxes from muscle to the splanchnic bed and to the kidney.

METHODS

Subjects. The first group, six males and two females, ages 35–58 were undergoing cardiac catheterization for valvular or coronary artery disease. They had no disorder known to involve amino acid, uric acid, or carbohydrate metabolism and were not in congestive failure. Before diagnostic studies, the catheter was guided into an hepatic vein by image intensification and its position confirmed angiographically and later chemically by a negative arteriovenous difference for glucose. Blood samples were taken several minutes apart coincident to samples collected from the brachial artery.

The second group was derived from a series of overweight subjects undergoing therapeutic starvation for several weeks (4). Forearm arterio–deep venous differences of various substrates (reflecting muscle metabolism) were determined. A third group of six normal young healthy volunteers provided forearm data in the postabsorptive state and have been described in a previous communication (5).

Analyses. All analyses, preparation of samples, and statistical methods have been described (1, 2, 5, 6). Glutamate and glutamine were estimated enzymatically according to Pagliara and Goodman (7, 8) on fresh plasma or plasma stored at $-20°C$ for no longer than 3 days. No significant alterations have been demonstrated for this duration of storage. A plasma "blank" was determined with each glutamine standard. In several subjects asparagine concentrations were kindly determined by Dr. Warren E. C. Wacker using bacterial asparaginase coupled with transaminase and dehydrogenase (9). Aspartate was not measured because it is present in plasma in concentrations too low to yield meaningful arteriovenous differences.

RESULTS

In Table I are summarized the splanchnic balances of glutamine and glutamate in the postabsorptive subjects undergoing cardiac and hepatic vein catheterization. In each, there is a splanchnic uptake of glutamine and a production of glutamate. This net balance represents a removal of 52 μmoles/liter of plasma of "α-ketoglutarate equivalents"[1] and "net" removal of 162 μmoles/liter of amido and amino nitrogen. Since there was no reason to suspect gross alterations from the normal as deter-

[1] By "α-ketoglutarate equivalent" is meant the sum of glutamate and glutamine. Also, 1 mole of glutamine, in the calculations, provides 2 moles of nitrogen (1 mole amido and 1 mole amino) and glutamate only 1 mole.

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TABLE I
*Splanchnic Glutamine and Glutamate Metabolism in the Postabsorptive State**

Subject	Glutamine Arterial	Glutamine Hepatic vein	Glutamine A-HV‡	Glutamate Arterial	Glutamate Hepatic vein	Glutamate A-HV‡
A	392	322	70	59	181	−122
B	725	490	235	68	167	− 99
C	695	480	215	42	86	− 44
D	442	392	50	43	62	− 19
E	315	265	50	30	59	− 29
F	410	325	85	23	51	− 28
G	370	277	93	34	108	− 74
H	400	322	78	46	95	− 49
			110 ±26§			−58 ±13§

* Values in μmoles/liter plasma.
‡ Arterial–hepatic vein concentration difference.
§ Mean ±SEM.

mined in previous similarly studied subjects, flow was not determined. Glucose differences, which were determined did not vary from those of similar subjects previously studied and shown to have normal flow rates (1).

Fig. 1 illustrates forearm arteriovenous differences of glutamine, glutamate, and asparagine in both the normal and in the obese subjects, in the postabsorptive state (normal and obese) and after 5–6 wk of total starvation (obese), when nitrogen catabolism had become attenuated. That the obese subjects do not differ from normal in respect to the above was confirmed by the absence of any significant difference between the two groups in the postabsorptive state. Also, flow in the postabsorptive normal group was 1.89 ±0.26 ml/min per 100 ml forearm (mean ±SEM, $n = 6$) and was within this range in two subjects under prolonged fasting. These values are the same as those obtained in similar subjects studied previously (2, 5).

In the postabsorptive state, glutamine production is 175 μmoles/liter of plasma, and glutamate removal 55 μmoles/liter for a net "α-ketoglutarate equivalent" production in muscle of 120 μmoles/liter and a net amino plus amido nitrogen production of 295 μmoles/liter. After prolonged starvation, these decrease to a net "α-ketoglutarate equivalent" production of 39 μmoles/liter and a net amino and amido nitrogen production of 105 μmoles/liter, a third that of the postabsorptive state, consonant with the decrement in over-all nitrogen excretion to a third of the postabsorptive level (10).

DISCUSSION

We have previously emphasized the primacy of alanine as a transport vehicle of both glucogenic substrate and nitrogen from muscle to the splanchnic bed in man (1, 2). Since the column-chromatographic methodology employed in the previous studies precluded accurate estimation of glutamine and glutamate (7), enzymatic techniques for their assay have been used in the present study. Surprisingly, a striking muscle production of glutamine was found together with a significant uptake of glutmate. This occurred in both the postabsorptive and prolonged-fasted states. The failure of previous investigators to demonstrate this balance in man may relate to the use of column-chromatography (11).

The decrease in glutamine release with prolonged fasting is consistent with the hypothesis that muscle release of glucogenic substrate is the rate-controlling step in hepatic gluconeogenesis in this state (2). A decline in absolute plasma glutamine levels with prolonged fasting provides further support,[2] since it has been shown in vitro, as has alanine, to be an effective glucose precursor in the perfused rat liver (12).

Fig. 2 summarizes data derived from postabsorptive forearm and splanchnic exchanges of amino acids from our previous publications (1, 2) showing the relative balances between the sites of production and removal of alanine, glycine, and other amino acids. Except for lysine, arginine, and serine, the muscle outputs and splanchnic uptakes closely complement each other, again, assuming that total splanchnic flow roughly approximates total muscle flow. Data derived from postabsorptive and prolonged-fasted subjects suggest this to be so (10).

The observed muscle output of alanine and "α-ketoglutarate equivalents" (Calculated from the balance between glutamate uptake and glutamine production) are from 1.5 to 2 times their proportion in muscle protein as derived from standard references (14), whereas serine,

[2] Cahill, G. F., Jr., E. B. Marliss, and T. T. Aoki. Plasma glutamine, glutamate, and asparagine in prolonged-fasted man. In preparation.

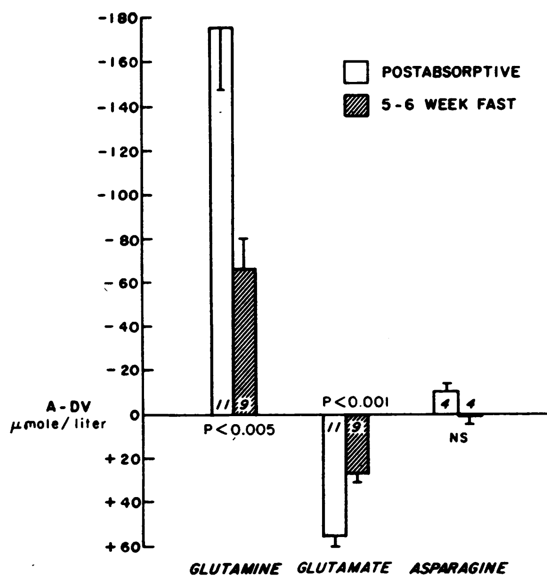

FIGURE 1 Forearm arterio–deep venous differences (A-DV) of plasma glutamine, glutamate and asparagine in normal subjects in the postabsorptive state and obese subjects after 5–6 wk of total fasting, showing an uptake of glutamate and a production of glutamine. (A slight production of asparagine is noted in the postabsorptive state but not after prolonged fasting; because of inter-individual variation, neither is significantly different from zero).

glycine, threonine, and other glucogenic amino acids are released in $\frac{1}{10}$–$\frac{1}{2}$ their expected amount. These approximations are derived from lysine levels, since lysine cannot undergo reversible transamination, and its release, therefore, reflects the lower limit of muscle proteolysis. The branched chain amino acids are likewise released at $\frac{1}{2}$–$\frac{1}{3}$ the expected rate but the present evidence and that of others (15) suggests they are primarily metabolized *in situ* in muscle to CO_2, and their amino groups probably provide the extra NH_2 transported to liver (or kidney) by the formation of glutamine from glutamate. Of note is the disparate balance of glutamine between muscle and splanchnic bed (Fig. 1 and Table I) suggesting another site, probably kidney, to effect significant glutamine removal. The renal uptake of glutamine has been extensively documented, as reviewed by Pitts (16). Likewise, there is a small, but significant, renal output of glutamate, which may account for the apparent imbalance between splanchnic glutamate output and muscle uptake as noted in the present study.

In his classical studies on ammonia metabolism, Krebs did not find significant glutamine production from glutamate and ammonia in guinea pig muscle preparations (17). Recent reports (18), however, have not only shown the presence of glutamine synthetase in muscle, but have characterized its physical nature.

Lotspeich (3), and Addae and Lotspeich (19) suggested that liver might increase glutamine synthesis to accommodate to increased renal extraction during acidosis. The present data raise the possibility that alterations in rate and pattern of muscle amino acid metabolism in man may influence acid-base homeostasis. Our subjects, both in the postabsorptive state and with prolonged starvation excreted between 50 and 150 mmoles of ammonia in the urine daily. With greater rates of acid excretion, the splanchnic bed might alter its uptake of

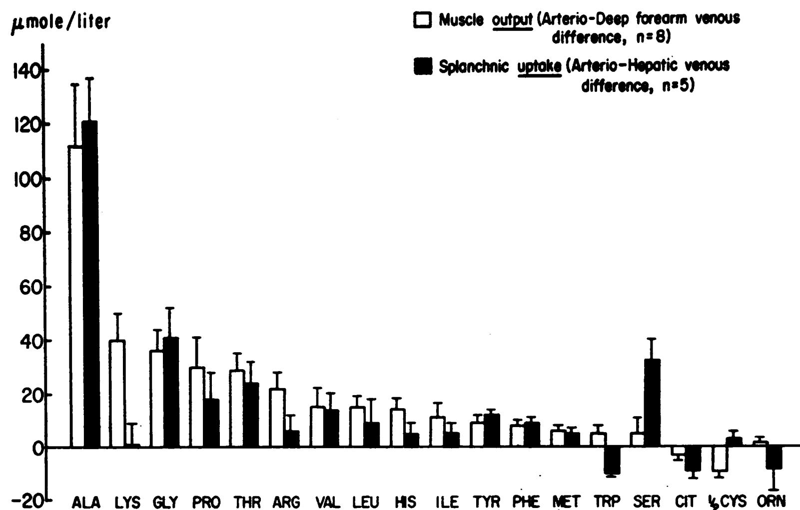

FIGURE 2 Arterio-venous differences of plasma amino acids across forearm muscle and splanchnic bed in the postabsorptive state. Data from references 1, 2 and 5. The prominent splanchnic uptake of serine is probably provided by renal serine production (1, 13).

glutamine into production instead of muscle increasing its production. This has yet to be studied.

Carlsten, Hallgren, Jagenburg, Svanborg, and Werko (20), in agreement with our findings, noted splanchnic uptakes of glutamate in both normal and diabetic man, but glutamine was not determined. Addae and Lotspeich (19) noted a marked arterioportal difference of glutamine in dogs rendered acidotic, signifying the gastrointestinal tract as an important site of glutamine removal. It is thus possible that there was a net glutamine production by liver in our subjects and this was masked by glutamine removal by the gastrointestinal tract. Portal levels need be obtained to clarify this possibility. Janicki and Goldstein (21) noted marked differences in glutamine synthetase and glutaminase activities in liver and kidney from various species, so it is not surprising that data from man might differ dramatically from those derived from experimental animals; even within a single species, the levels of both enzymes may be very sensitive to acid-base alterations, as recently shown by Damian and Pitts (22). As reported in abstract, Hills, Kerr, and Reid (23) noted glutamine production from dog hind limb, and, in accord with our thoughts, postulated that peripheral protein catabolism may serve as the ultimate source of urinary ammonia. It is interesting that MacKay, Wick, Carne, and Barnum (24) many years ago noted increased body nitrogen catabolism in fasted rats rendered more acidotic than that achieved from starvation ketosis by the supplementary administration of hydrochloric acid.

Thus, under the conditions of our studies, in postabsorptive and prolonged fasting in man, muscle produces glutamine and the splanchnic bed removes some of it. Under other conditions such as a more marked acidosis or during nitrogen anabolism in the fed state, glutamine and glutamate production and removal by muscle and liver may be reversed. This has yet to be studied as well as the relative effects of hormones and other alterations in acid-base homeostasis on glutamine and glutamate metabolism in the periphery and in the viscera.

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