Glutamine and Glutamate Metabolism in Normal and Diabetic Subjects

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SUMMARY

Arteriovenous differences across the leg and across the splanchnic bed were determined for plasma glutamine and glutamate in healthy controls and insulin-dependent diabetics. In both groups glutamine was released from the leg and taken up by the splanchnic bed, while the reverse was observed for glutamate. Arterial glutamine levels were reduced by 25 per cent in the diabetics, but no differences were observed between the diabetics and controls in splanchnic or peripheral glutamine and glutamate metabolism. To assess the contribution of intestinal tissues to splanchnic glutamine uptake, arterio-portal venous differences (A-PV) were determined for plasma glutamine in four nondiabetic patients prior to elective cholecystectomy. A consistently positive A-PV difference was noted indicating net uptake of glutamine by intestinal tissues.

The findings indicate that the intestinal tract rather than the liver is the major site of splanchnic glutamine uptake and that removal of glutamine by the splanchnic bed is not augmented in diabetes. The data thus suggest that glutamine is not an important endogenous substrate for hepatic gluconeogenesis. DIABETES 22:573-76, August, 1973.

Alanine has been identified as the primary amino acid released by muscle[1] and extracted by the splanchnic bed[2,3] in man in the postabsorptive state and after prolonged fasting. In insulin-dependent diabetics an increase in splanchnic uptake of alanine and of other gluconeogenic amino acids has been demonstrated.[4] However, the use of the column chromatographic technic in these studies precluded accurate determination of glutamine and glutamate.[5] Using an enzymatic assay, Marliss et al. recently reported that glutamine is released by peripheral muscle and extracted by the splanchnic bed in normal subjects.[6] On the basis of these observations, they suggested that glutamine may be as important as alanine in the flux of gluconeogenic precursors between muscle and liver.[6] However, the relative roles of the liver and the intestinal tissues in the splanchnic uptake of glutamine were not directly assessed. Furthermore, studies in dogs indicate that splanchnic uptake of glutamine is due to extraction by the gastrointestinal tract rather than the liver.[7,8] The present study was consequently undertaken to determine the net balance of glutamine across the gastrointestinal tract in the postabsorptive state by examining arterial-portal venous differences. In addition, splanchnic and peripheral balance of glutamine and glutamate has been examined in diabetics and normal controls.

METHODS

Portal vein study. Four nondiabetic patients, twenty-eight to forty years of age, were studied in the postabsorptive state (twelve to fourteen hour fast) at the time of elective cholecystectomy for uncomplicated cholelithiasis. General anesthesia with halothane was employed. No glucose was infused before blood samples for amino acid analysis were obtained. A brachial artery catheter was inserted percutaneously before surgery. During the surgical procedure simultaneous blood samples were obtained from the arterial catheter and by direct needle puncture from the portal vein. The arterial and portal venous plasma concentrations of the neutral and acidic amino acids other than glutamine, have been reported previously for these subjects.[3]

Diabetes study. The subjects were six nonobese insulin-dependent diabetics, nineteen to forty-eight years of age, requiring 28 to 60 units of Lente insulin per day (table 1) and six healthy controls twenty-five to forty years of age. Splanchnic and peripheral exchange of glucose and of amino acids other than glutamine and glutamate have been previously reported for these subjects.[4] The subjects were studied in the postabsorptive state following a twelve to fourteen hour overnight fast. The

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diabetics had received no insulin for twenty-four hours at the time of study. No subject was ketotic when the study was made. Simultaneous blood samples were obtained from catheters placed percutaneously in a brachial artery, in a right-sided hepatic vein and in a femoral vein. The technics employed for arterial, hepatic venous and femoral venous catheterization and for determination of hepatic blood flow have been described in detail previously.[4]

The nature, purpose and possible risks of the study were explained to all subjects, who gave voluntary consent.

Chemical analyses. Plasma glutamate was determined by a microfluorometric enzymatic method involving measurement of reduced NAD formed by oxidative deamination of glutamate by glutamic dehydrogenase.* Plasma glutamine was hydrolyzed by the enzyme glutaminase (E coli) to glutamate, and the glutamate measured as described above. Plasma flow in the leg was estimated on the basis of pulmonary oxygen uptake and arterio-femoral venous oxygen differences according to the formula described by Jorfeldt and Wahren.[9]

RESULTS

The concentrations of glutamine in arterial and portal venous plasma are shown in table 2. In each of four subjects, glutamine concentration in arterial plasma exceeded that in portal venous plasma by 50 to 92 μmoles per liter. The mean (± S.E.) arterial-portal venous difference was 73 ± 10 μmoles per liter.

Arterial concentrations and splanchnic and leg exchange of glutamine are shown for the diabetics and controls in table 3. Net splanchnic uptake and peripheral release of glutamine was observed in all subjects. The splanchnic uptake and fractional extraction of glutamine and the rate of release of glutamine from the leg did not differ significantly between the diabetics and controls. A diminution in arterial glutamine concentration was observed, however, in the diabetic group.

Glutamate metabolism in the diabetics and controls is presented in table 4. In all subjects a net splanchnic production and leg uptake of glutamate was observed. Although the A-HV and A-FV differences for glutamate were smaller in the diabetics, flow was slightly greater in this group so that over-all splanchnic and peripheral exchange of glutamate was equivalent in the diabetics and controls.

DISCUSSION

The current data provide confirmation of the observation by Marliss et al.[6] that glutamine is released by peripheral tissues and extracted by the splanchnic bed. Particularly noteworthy, however, is the demonstration of a consistent uptake of glutamine by the gastrointestinal tract as indicated by the positive arterio-portal venous differences. The current findings thus suggest that splanchnic glutamine uptake is due at least in part, and possibly entirely, to extraction of this amino acid by the intestinal tract rather than to uptake by the liver. In this respect glutamine is strikingly different

TABLE 1

Clinical data for diabetic subjects

Subjects	Age (yrs.)	Height (cm.)	Weight (kg.)	History of diabetes (yrs.)	Duration of insulin therapy (yrs.)	Dose of Lente insulin* (I.U.)	Arterial blood glucose (mg./100 ml.)
CC	26	166	55	12	12	20+8	302
LK	48	174	68	2	1	32	308
HL	19	184	70	4	4	32+28	267
KW	26	179	72	3	3	16+12	170
TJ	24	173	60	15	15	32+12	264
GW	28	181	67	9½	9½	56†	193

* Where two numbers are given they represent morning and evening doses of insulin, respectively.
† Includes 20 I.U. Protamine Zinc Insulin; all other patients received only Lente insulin.

TABLE 2

Arterial and portal venous concentrations and arterial-portal venous differences (A-PV) for plasma glutamine*

Subject	Arterial	Portal vein	A-PV
Pa	510	425	85
Li	620	528	92
St	520	456	64
Ga	480	430	50
Mean ± SE	533±30	460±24	73±10

* Blood samples were obtained from postabsorptive subjects at the time of elective cholecystectomy. Data are presented in μmoles per liter.

*Karl, I., Pagliara, A., and Kipnis, D. M. In preparation.

TABLE 3

Glutamine metabolism in diabetic and control subjects

	Arterial level (μmoles/L.)	A-HV* (μmoles/L.)	Splanchnic fractional extraction (per cent)	EHPF† (L./min.)	Splanchnic‡ uptake (μmoles/min.)	A-FV§ (μmoles/L.)	Estimated leg plasma flow‖ (L./min.)	Leg exchange# (μmoles/min.)
Diabetics								
CC	571	75	13.1	0.47	35	−100	0.51	−51
LK	338	59	17.5	0.80	47	−83	0.55	−46
HL	344	74	21.5	0.89	66	−81	0.76	−62
KW	372	77	20.7	0.97	75	−48	0.62	−30
GW	455	77	16.9	0.98	76	−68	0.81	−55
TJ	463	82	17.7	0.66	54	−82	0.70	−57
Mean±S.E.	424±37	74±3	17.9±1.2	0.80±0.08	59±7	−77±7	0.66±0.05	−50±5
Controls								
BA	534	101	18.9	0.77	78	−79	0.71	−56
IL	439	69	15.7	0.46	32	−51	0.44	−22
BB	629	149	23.4	0.67	99	−61	0.50	−31
AW	557	83	14.9	0.66	55	−106	0.48	−51
OK	609	87	14.3	0.78	68	−98	0.40	−39
Mean±S.E.	554±33	98±14	17.4±1.7	0.67±0.06	66±11	−79±10	0.51±0.05	−40±6
P**	<0.025	NS	NS	NS	NS	NS	NS	NS

* A-HV = Arterio-hepatic venous difference.
† EHPF = Estimated hepatic plasma flow.
‡ Calculated as the product of A-HV and EHPF.
§ A-FV = Arterio-femoral venous difference.
‖ Total plasma flow to both legs.
Calculated as the product of A-FV and estimated leg plasma flow.
** P = Significance of difference between control and diabetic groups (Student's *t* test).

from alanine and most other amino acids, for which a net release by the gastrointestinal tract (as indicated by a negative A-PV difference) has been previously reported.[3] Supporting our conclusion is the observation by Elwyn et al. that glutamine is the only amino acid consistently extracted by the gastrointestinal tract in dogs in the postabsorptive state.[7] Addae and Lotspeich also reported positive arterio-portal venous differences for glutamine in dogs and suggested that glutamine utilization by the gut may be related to water and solute reabsorption and to the increased ammonia formation which accompanies osmotic work by the intestine.[8]

With regard to the studies in the diabetics, an increase in the splanchnic uptake of alanine and of other key endogenous glycogenic amino acids has recently been reported.[4] The failure to observe an augmentation in splanchnic uptake of glutamine is thus noteworthy and supports the conclusion that, in contrast to other amino acids, the site of glutamine disposal by the splanchnic bed is primarily extrahepatic rather than in the liver as a gluconeogenic precursor. It should also be noted that in both the diabetics and controls splanchnic output of glutamate was at 80 to 85 per cent of the rate of glutamine uptake (tables 2 and 3). Thus even if the liver were the major site of splanchnic glutamine disposal, the net availability of carbon skeletons for

TABLE 4

Glutamate metabolism in diabetic and control subjects

Diabetics	Arterial level (μmoles/L.)	A-HV* (μmoles/L.)	Splanchnic† exchange (μmoles/min.)	A-FV‡ (μmoles/L.)	Leg uptake§ (μmoles/min.)
CC	72	−42	−20	27	14
LK	84	−70	−56	43	24
HL	60	−41	−37	31	24
KW	68	−65	−63	36	22
GW	79	−83	−82	47	38
TJ	78	−47	−31	28	20
Mean ±SE	74±4	−58±7	−48±9	35±3	24±3
Controls					
BA	69	−90	−69	32	23
IL	75	−82	−38	44	19
BB	66	−68	−45	34	17
AW	106	−108	−71	67	32
OK	68	−73	−57	44	18
Mean ±SE	77±7	−84±7	−56±6	44±6	22±3
P‖	NS	<0.025	NS	<0.05	NS

* A-HV = Arterio-hepatic venous difference.
† Calculated as the product of A-HV and EHPF (table 3).
‡ A-FV = Arteriofemoral venous difference.
§ Calculated as the product of A-FV and estimated leg plasma flow (table 3).
‖ P = Significance of difference between control and diabetic groups (Student's *t* test).

gluconeogenesis from the glutamine-glutamate system (as α-ketoglutarate) would be no greater than 10 to 11 μmoles per minute, as compared to 60 to 100 μmoles per minute (as pyruvate) provided by alanine (figure 1).

Despite the equivalent peripheral and splanchnic balances of glutamine in the diabetics and controls, a decrease in arterial glutamine levels was observed in the diabetics (table 4). A fall in arterial glutamine has also been noted in prolonged starvation.[6] Inasmuch as the kidney is a major site of glutamine disposal,[10] the possibility that altered renal metabolism may contribute to changes in the arterial concentration of this amino acid must be considered.

It should be noted that plasma rather than whole blood was employed in the present study in determining arterio-venous differences. Recently Elwyn et al.[11] reported that plasma and red blood cells may play independent and possibly opposing roles in the exchange of amino acids across the gut and liver in the dog. This difference in behavior of red blood cells and plasma has been noted with respect to glutamate,[11] and following ingestion of protein meals, in the case of glutamine.[7] However, the red cells appear to make little, if any, contribution to the net splanchnic exchange of glutamine and alanine in the postabsorptive state,[7] the condition of the subjects in the present study.

In conclusion, the current findings reveal that in both normal and diabetic subjects glutamine is released from leg tissues and is important in the transfer of nitrogen from the periphery to the splanchnic bed. On the other hand, the demonstration of consistently positive arterio-portal venous differences for glutamine and the failure to observe an increase in splanchnic uptake of glutamine in diabetics suggests that the gut rather than the liver is the site of splanchnic glutamine uptake. Accordingly, glutamine does not appear to be an important endogenous substrate for hepatic gluconeogenesis.

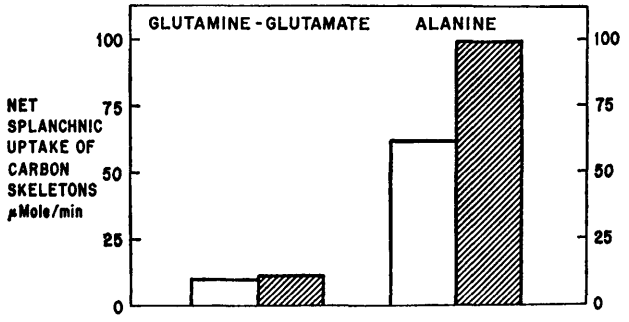

FIG. 1. Net splanchnic uptake of carbon skeletons derived from the glutamine-glutamate system (as α-ketoglutarate) and from alanine (as pyruvate) in normal subjects (open bars) and diabetics (cross-hatched bars). For the glutamine-glutamate system, net splanchnic uptake of carbon skeletons was calculated by subtracting the splanchnic output of glutamate (table 4) from the splanchnic uptake of glutamine (table 3). The data on alanine are based on the observations of Wahren et al.[4]

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ADDENDUM

In a study undertaken following submission of this report, Felig et al., using whole blood and plasma A-V differences, demonstrated that intestinal tissues rather than the liver are the major site of splanchnic uptake of glutamine.[12]

REFERENCES

[1] Felig, P., Pozefsky, T., Marliss, E., and Cahill, G. F., Jr.: Alanine: key role in gluconeogenesis. Science *167*:1003-04, 1970.

[2] Felig, P., Owen, O. E., Wahren, J., and Cahill, G. F., Jr.: Amino acid metabolism during prolonged starvation. J. Clin. Invest. *48*:584-94, 1969.

[3] Felig, P., and Wahren, J.: Amino acid metabolism in exercising man. J. Clin. Invest. *50*:2703-14, 1971.

[4] Wahren, J., Felig, P., Cerasi, E., and Luft, R.: Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. J. Clin. Invest. *51*:1870-78, 1972.

[5] Stein, W. H., and Moore, S.: The free amino acids of human blood plasma. J. Biol. Chem. *211*:915, 1954.

[6] Marliss, E. B., Aoki, T. T., Pozefsky, T., Most, A. S., and Cahill, G. F., Jr.: Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. J. Clin. Invest. *50*:814-17, 1971.

[7] Elwyn, D. H., Parikh, H. C., and Shoemaker, W. C.: Amino acid movements between gut, liver and periphery in unanesthetized dogs. Am. J. Physiol. *215*:1260-75, 1968.

[8] Addae, S. K., and Lotspeich, W. D.: Relations between glutamine utilization and production in metabolic acidosis. Am. J. Physiol. *215*:269-77, 1969.

[9] Jorfeldt, L., and Wahren, J.: Leg blood flow during exercise in man. Clin. Sci. *41*:459, 1971.

[10] Owen, E. E., and Robinson, R. R.: Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. J. Clin. Invest. *42*:263, 1963.

[11] Elwyn, D. H., Launder, W. J., Parikh, H. C., and Wise, E. M., Jr.: Roles of plasma and erythrocytes in interorgan transport of amino acids in dogs. Am. J. Physiol. *222*:1333-42, 1972.

[12] Felig, P., Wahren, J., and Raf, L.: Evidence of interorgan amino acid transport by blood cells in man. Proc. Natl. Acad. Sci. USA *70*:1775-79, 1973.