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Portal-drained viscera and hepatic fluxes of branched-chain amino acids do not account for differences in circulating branched-chain amino acids in rats fed arginine-deficient diets*

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Summary. Concentrations and fluxes of amino acids across the portal-drained viscera (PDV) and liver were assessed in rats fed a meal of one of three arginine-deficient diets containing either alanine or the arginine precursors, ornithine or citrulline. A previous report included findings of seven arginine-related amino acids and indicated that only the citrulline-containing diet protected blood arginine concentrations. In the present report we extend these findings and note that the concentrations and fluxes of the non-arginine-related amino acids showed remarkable consistency across diet groups. However, total branched-chain amino acid (BCAA) concentrations of arterial blood were higher in rats fed the −Arg/+Ala and the −Arg/+Orn diets than in rats fed the control (+Arg) diet. The elevated BCAA correlated with higher circulating concentrations of other essential amino acids but were inversely correlated with arginine concentrations. PDV and hepatic fluxes of BCAA were not different across diet groups, indicating that amino acid absorption and hepatic utilization of BCAA were generally comparable across diet groups. Hepatic concentrations of 14 of 22 measured amino acids, including total BCAA, were correlated with their arterial concentrations. The circulating concentrations and net PDV and hepatic fluxes of rats fed the control diet were comparable to our previous observations in fed rats and illustrate the role of the liver in utilization of diet-derived essential amino acids.

Keywords: Essential amino acids – Fed state – Interorgan flux – Rats – Arginine deficiency

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Abbreviations: PDV, portal-drained viscera; BCAA, branched-chain amino acids; SSA, 5-sulfosalicylic acid; PBF, portal blood flow; HBF, hepatic blood flow; SELSM, pooled standard errors of least squares means; TAA, total amino acids; NEAA, nonessential amino acids; EAA, essential amino acids; LNAA, large neutral amino acids

Introduction

Arginine is a conditionally essential amino acid for the rat, required in the diet for maximal growth rate, yet it is also synthesized by the liver and kidney. Recent studies (Gross et al., 1991; Hartman and Prior, 1992; Prior, 1993) have used arginine-free diets to explore arginine metabolism in the liver and in relation to interorgan fluxes of amino acids in both the fed and the food-deprived states of rats. Similarly to a dietary deficiency of an essential amino acid, omission of arginine from the diet results in a decreased arginine concentration in plasma or blood. The decrease has been seen in aged (20 mo) as well as in young (2 mo) rats (Gross et al., 1991). Arginine flux from the portal-drained viscera (PDV) increases from near zero in the food-deprived state to 0.36 μmol per min in rats 1 h after they consume a 1% arginine diet (Hartman and Prior, 1992). However, no increase is seen if the diet contains no arginine. With the 1% arginine diet, hepatic uptake of arginine matches the PDV output suggesting that blood arginine concentrations are supported by the known conversion of citrulline to arginine (Hartman and Prior, 1992).

We have recently reported (Hartman et al., 1994) effects of an arginine-containing diet and three arginine-free diets on the primary amino acids involved in arginine metabolism including arginine, ornithine, citrulline, glutamate, glutamine, alanine and proline. When alanine, ornithine or citrulline were substituted for arginine in the diet, concentrations of those amino acids were individually increased in the blood. Additionally, the presence of citrulline in the arginine-deficient diet restored blood arginine concentrations to control levels, whereas arginine concentrations were depressed as expected with the alanine- or ornithine-substituted arginine-deficient diets. The portal-drained viscera fluxes of the substituted amino acids were also increased.

However, the effect of arginine-free diets on essential amino acid concentrations and fluxes remains unclear. In a previous study (Prior, 1993), fluxes of branched-chain amino acids across the PDV and liver were increased 1 h after rats were fed the control diet or a glutamate-substituted, arginine-free diet. However, the fluxes of BCAA subsided at 2 h, only in the rats fed the arginine-deficient diet. The data did not suggest a possible mechanism for the difference. In the current report we present effects of arginine-devoid diets and feeding on net PDV and liver fluxes of branched-chain and other amino acids. The 2 h post-meal time point was chosen to be able to compare the present results with those of the previous study (Prior, 1993). We also compared effects of the different diets on concentrations of amino acids in the liver.

In this study, meal size prior to sampling was controlled to provide equivalent intakes among the treatments and also to be equivalent to intakes of the previous study (Hartman and Prior, 1992; Prior, 1993). Control of final intake of amino acids ensures that effects on amino acid concentrations and fluxes are comparable across diet groups within this in vivo paradigm.

Materials and methods

Animals

Male Sprague-Dawley rats (n = 48) initially weighing 150–175 g (Camm Research, Wayne, NJ), were housed for 3 to 6 d in stainless steel hanging cages after arrival. They were then transferred to plexiglas metabolic cages for the 10 d experimental period. Food intake and body weight were measured daily approximately 1 h before the beginning of the dark period. The room received lighting for 14 hours daily, from 2300–1300 (Eriksson et al., 1989). The research protocol was reviewed and approved by the Animal Care and Use Committee at Tufts University and followed the *Guide for the Care and Use of Laboratory Animals*. Twenty-nine of the rats met criteria for data inclusion in the study. Further details of methodology have been previously reported (Hartman et al., 1994).

Diets

Initially, the rats were fed commercial rat diet (ProLab, R-M-H 3000, Agway, Inc., Syracuse, NY) for at least 2 d. Rats were then assigned to dietary treatment groups by body weight. They were fed a semi-purified diet containing 14% soy protein for 3 days, followed by 7 d feeding of one of the four treatment diets (Table 1). These diets contained crystalline amino acids and were protein-free and isocaloric. The −Arg/+Ala arginine-deficient diet was made isonitrogenous to the control arginine diet by adding alanine, and the corn starch content was adjusted accordingly. The −Arg/+Orn and −Arg/+Cit arginine-deficient diets contained ornithine or citrulline in isomolar quantities as the arginine of the control diet. The diets were in the form of 0.2–0.4 g pellets and were offered in glass jars with perforated metal lids. Orts were air-dried and weighed to correct food intake measures.

Experimental procedure

Rats fed the +Arg, −Arg/+Orn, and −Arg/+Cit diets were mildly food restricted on a daily basis to match the ad libitum intakes of the rats fed the arginine-deficient diet containing alanine alone. Rats were food-deprived up to 20 h before the final meal (about 5 g) given at the beginning of the dark period. The rats were anesthetized approximately 1.5 h after feeding with 40–60 mg/kg body weight sodium-pentobarbital injected intraperitoneally.

Following anesthesia, the portal vein was exposed and blood flow was measured using an ultrasonic probe (#2R940, Transonic Systems, Ithaca, NY) positioned around the vein about 1 cm from the liver. Blood was sampled from an hepatic vein, the portal vein, and the aorta, taking less than 1 min to withdraw each sample (approximately 1 mL) into a heparinized syringe. The time of blood sampling (hepatic sample) was 120 ± 1 min after the beginning of feeding with no differences among diet groups. This sampling time allowed comparison to data from a previous experiment (Hartman and Prior, 1992; Prior, 1993).

Whole liver was quickly harvested and rats were terminated by exsanguination. Livers were frozen on dry ice and stored at −70°C until preparation for amino acid analysis.

Table 1. Composition of the arginine-containing diet and the three arginine-deficient purified amino acid diets[1]

	Diet			
	+Arg	−Arg/+Ala	−Arg/+Orn	−Arg/+Cit
		g/kg diet		
L-Arginine free base	10.0	0.0	0.0	0.0
L-Alanine	57.0	77.4	57.0	57.0
L-Ornithine-HCl	0.0	0.0	9.7	0.0
L-Citrulline	0.0	0.0	0.0	10.1
Amino acid mix[2]	66.2	66.2	66.2	66.2
Cornstarch	544.8	534.4	547.1	544.7
Sucrose	50.0	50.0	46.0	50.0
Corn oil	100.0	100.0	100.0	100.0
Salt mix[3]	50.0	50.0	50.0	50.0
Sodium bicarbonate[3]	10.0	10.0	12.0	10.0
Cellulose	100.0	100.0	100.0	100.0
Choline bitartrate	2.0	2.0	2.0	2.0
Vitamine mix[4]	10.0	10.0	10.0	10.0

[1] Compounded by Research Diets (New Brunswick, NJ); +Arg, #A01001; −Arg/+Ala, #A01002; −Arg/+Orn, #A01004; −Arg/+Cit, #A01005.

[2] L-amino acids included in all diets (g/kg diet): histidine-HCl-H_2O 3.5, isoleucine 7.0, leucine 10.0, lysine-HCl 8.7, methionine 5.0, phenylalanine 8.0, threonine 6.0, tryptophan 1.5, valine 7.0, cystine 2.5, serine 3.5, tyrosine 3.5. The diets contained no aspartate, asparagine, glutamine, glutamate, glycine or proline.

[3] Mix S01004 contained the following (g/kg mix): calcium carbonate 63.0, calcium phosphate dibasic 525.0, magnesium oxide 16.6, potassium citrate-H_2O 235.0, potassium sulfate 32.6, sodium chloride 80.0, chromium potassium sulfate-$12H_2O$ 0.26, cupric carbonate 0.21, potassium iodate 0.01, ferric citrate 9.43, manganous carbonate 1.26, sodium selenite 0.01, zinc carbonate 1.92, sucrose 34.7. Further adjustments were made to the −Arg/+Orn diet to correct for the chloride in ornithine-HCl, as follows: sucrose was substituted for the sodium chloride in the salt mix, and an additional 2.83 g of sodium bicarbonate and 0.64 g of sodium chloride were added per kg of diet.

[4] Mix V01001 contained the following (per kg of mix): retinol palmitate 275 mg, cholecalciferol 2.5 mg, all-*rac*-α-tocopherol acetate 5.0 g, menadione sodium bisulfate (62.5% menadione) 0.32 g, biotin 20.0 mg, cyanocobalamin 2.5 mg, folic acid 0.2 g, niacin 2.5 g, calcium pantothenate 3.0 g, pyridoxine-HCl 1.0 g, riboflavin 0.5 g, thiamin-HCl 0.5 g, and sucrose to make 1 kg mix.

Blood, plasma and liver analysis

An aliquot of each blood sample was mixed immediately with an equal volume of 35 g/L 5-sulfosalicylic acid (SSA, Mallinckriodt, Paris, KY), and held for 10 min on ice. Samples were then diluted 2:3 with lithium-based buffer (Li-S, Beckman-Somerset Bioanalytical Consumables, Somerset, NJ), centrifuged at 13,000 × g, and the supernatants were frozen at −70°C until analyzed for amino acids (Hartman et al., 1994). Whole livers were homogenized (Tissuemizer, with SDT182EN Shaft, Tekmar, Cincinnati, OH) in 45 mL ice-cold water for approximately 45 s and then for 15 s after the addition of 25 mL 98 g/L SSA. Following centrifugation, supernatants were stored at −70°C before analysis for amino acids in the same manner as for whole blood samples.

Calculations

The elevation in each amino acid concentration in the portal vein blood relative to the arterial concentration was calculated as follows:

$$\% \text{ Portal Elevation} = ((PC - AC)/AC) * 100.$$

Net portal-drained viscera flux was calculated for individual rats as follows:

$$\text{PDV flux } (\mu\text{mol/min}) = (PC - AC) * PBF,$$

where:

PC = portal vein concentration (μmol/L)
AC = arterial concentration (μmol/L)
PBF = portal blood flow (L/min).

Similarly, net hepatic flux was calculated for each individual rat as follows:

$$\text{Hepatic Flux } (\mu\text{mol/min}) = (HC - HaC) * HBF,$$

where:

HC = hepatic vein concentration (μmol/L)
HaC = Hepatic Afferent Concentration = (PC*75/100) + (AC*25/100)
HBF = Hepatic Blood Flow = PBF*100/75.

Calculation of hepatic flux was based on published observations that the hepatic afferent blood flow is comprised 75% from the portal vein and 25% from the hepatic artery (Demigné et al., 1986).

Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis Systems (SAS) (SAS, 1988). Main treatment effects (4 diets) were determined by one-way ANOVA, and when significant, differences between treatment means were established by the all-possible t test matrix of the least squares means generated by the GLM procedure. T tests were also used to determine significant difference from zero of portal elevations and flux values. The linear regression procedure of SAS was used to examine the data for correlations between selected amino acids and other variables. Data are presented as least squares means and pooled standard errors of the least squares means (SELSM) from 6 to 10 rats per treatment. Probability values less than 0.05 were considered to indicate a significant difference, and values between 0.05 and 0.10 to indicate a trend.

Results

Intakes, body and liver weights, and portal blood flow

Mean food intakes (19.0 ± 0.9 g, overall mean ± pooled SELSM) did not differ among the diet groups over the 6 d feeding period (Hartman et al., 1994). Mean intakes (g) of the meal fed on the day of sampling were as follows: +Arg diet, 5.3; −Arg/+Ala diet, 5.1; −Arg/+Orn diet, 5.0; −Arg/+Cit diet, 5.4; pooled SELSM, 0.2, and were also not significantly different across diets. Initial and final body weights (228 ± 11, 241 ± 10 g, overall mean ± pooled SELSM) of the rats also did not differ across diet treatments (Hartman et al., 1994). Liver weights were 7.1, 8.4, 7.4 and 7.6 g (pooled

Table 2. Blood arterial concentrations and portal vein elevations of several essential and nonessential amino acids in rats 2 h after a meal of arginine-containing diet or one of three arginine-deficient diets[1]

Amino acid	Dietary group				Pooled SELSM[2]
	+Arg	−Arg/+Ala	−Arg/+Orn	−Arg/+Cit	
Valine					
Art. conc. (μmol/L)	113[b,c]	165[b]	178[b]	101[c]	21
Portal elevation (%)	40*	21*	17	49*	12
Leucine					
Art. conc. (μmol/L)	63[c]	104[b]	116[b]	60[c]	15
Portal elevation (%)	70*	53*	46*	81*	12
Lysine					
Art. conc. (μmol/L)	438	476	456	488	35
Portal elevation (%)	11*	10*	7**	8**	3
Isoleucine					
Art. conc. (μmol/L)	66	98	101	74	12
Portal elevation (%)	47*	42*	38*	44*	9
Threonine					
Art. conc. (μmol/L)	377	324	423	319	49
Portal elevation (%)	18*	11*	7**	8*	3
Phenylalanine					
Art. conc. (μmol/L)	71	69	86	72	5
Portal elevation (%)	35*	38*	31*	30*	8
Methionine					
Art. conc. (μmol/L)	86	112	93	79	13
Portal elevation (%)	26*	26*	26*	28*	7
Serine					
Art. conc. (μmol/L)	337	330	358	325	26
Portal elevation (%)	4	4	3	1	3
Histidine					
Art. conc. (μmol/L)	51[c]	61[b]	65[b]	53[c]	4
Portal elevation (%)	28*	17*	14**	20*	7
Tyrosine					
Art. conc. (μmol/L)	76	69	86	67	9
Portal elevation (%)	14*	14*	12*	13*	4
Cystine					
Art. conc. (μmol/L)	32	33	34	32	2
Portal elevation (%)	−0	−5**	−4	−1	3
Aspartate					
Art. conc. (μmol/L)	267[c]	312[b,c]	338[b]	263[c]	20
Portal elevation (%)	11**	2	10	13*	6
Glycine					
Art. conc. (μmol/L)	217	218	240	240	16
Portal elevation (%)	3	6	5	6	4
Asparagine					
Art. conc. (μmol/L)	29	36	30	30	3
Portal elevation (%)	21*	21*	23*	18**	8

Table 2. *Continued*

Amino acid	Dietary group				Pooled SELSM[2]
	+Arg	−Arg/+Ala	−Arg/+Orn	−Arg/+Cit	
Hydroxyproline					
Art. conc. (μmol/L)	83	65	69	77	9
Portal elevation (%)	16	24	−3	16	18
Taurine					
Art. conc. (μmol/L)	240	241	258	262	12
Portal elevation (%)	−3	5	3	−3	4
Sums and Ratios[3]					
TAA					
Art. conc. (μmol/L)	3,670	4,421	4,414	3,451	338
Portal elevation (%)	18	19	18	21	5
NEAA					
Art. conc. (μmol/L)	2,403[b]	3,013[a]	2,896[b]	2,206[b]	220
Portal elevation (%)	17	19	18	21	5
EAA					
Art. conc. (μmol/L)	1,266	1,408	1,518	1,245	125
Portal elevation (%)	20	19	16	19	5
LNAA					
Art. conc. (μmol/L)	475	616	661	452	70
Portal elevation (%)	37	31	27	38	8
BCAA					
Art. conc. (μmol/L)	242[b]	367[a,b]	395[a]	234[b]	47
Portal elevation (%)	49	35	31	54	10
BCAA/EAA					
Art. conc. (μmol/L)	0.193[b]	0.254[a]	0.256[a]	0.184[b]	0.019
Portal elevation (%)	2.2	1.9	2.0	3.6	0.6

[1] Values shown are least squares means of 6 to 10 rats per diet group. The F test was significant for leucine and valine concentrations at $P < 0.05$, and for histidine and aspartate concentrations at $P < 0.10$. No portal elevations were significant. Arterial concentrations of NEAAs, BCAA and the ratio of BCAA to EAAs were also significant at $P < 0.05$. Means within the same row not sharing a common letter superscript are significantly different by t test ($P < 0.05$). Superscripts indicate significant difference from zero by t test, *$P < 0.05$; **$P < 0.10$.

[2] Standard error of the least squares means.

[3] *TAA* NEAA + EAA

NEAA sum of the nonessential amino acids: phosphoserine, taurine, phosphoethanolamine, aspartate, serine, asparagine, glutamate, proline, glycine, alanine, cystine, cystathionine, tyrosine and ethanolamine. *EAA* sum of the essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and histidine. *LNAA* sum of the large neutral amino acids: valine, isoleucine, leucine, phenylalanine, tyrosine and methionine. *BCAA* sum of the branched-chain amino acids: valine, isoleucine and leucine.

SELSM, 0.4), respectively, in the +Arg, −Arg/+Ala, −Arg/+Orn and −Arg/+Cit diet groups. Rats in the −Arg/+Ala group had a tendency to have heavier livers than the +Arg group ($P < 0.10$). Flow through the hepatic portal vein was 16 ± 1 mL/min (overall mean ± SELSM) and was not different among the diet treatment groups (Hartman et al., 1994).

Dietary effects on blood amino acid concentrations

As reported elsewhere (Hartman et al., 1994), the four diets produced marked effects on concentrations of arginine, ornithine, citrulline, glutamine, alanine and glutamate, but not on proline. In contrast, there were no significant differences due to diet in blood concentrations of 12 of the 16 amino acids reported here (Table 2).

Of the amino acids reported here, leucine, valine, histidine and aspartate concentrations were elevated 21 to 79% in pooled means of the rats fed −Arg/+Ala and −Arg/+Orn diets relative to pooled means of the rats fed the +Arg and −Arg/+Cit diets. Although the dietary effect was not significant for isoleucine concentrations, the elevation in arterial isoleucine concentrations was 42%. A similar, though less marked, pattern of differences was seen in portal vein and hepatic vein concentrations (data not shown) of these five amino acids as well as in methionine, but not in any of the other amino acids of Table 2. The sum of the branched-chain amino acids in arterial whole blood was also significantly different across the diet treatments ($P < 0.05$, Table 2). Also, the ratio of the branched-chain amino acids to the other essential amino acids was 46% higher in arterial blood of rats fed −Arg/+Ala and −Arg/+Orn diets compared to rats fed +Arg or −Arg/+Cit diets.

The significant elevation of total nonessential amino acids was related to the significant elevations of blood alanine and glutamine concentrations, as was the lower ratio of essential amino acid concentrations to nonessentials in the rats fed −Arg/+Ala diet compared to controls.

Feeding effects on blood amino acid concentrations

Concentrations of most of the 13 or 14 amino acids present in the diet (Table 1) were elevated in portal vein compared to arterial blood as expected, since the rats were in the absorptive state (Table 2). The significant portal elevations in the +Arg-fed rats were in the order: leucine > isoleucine > valine > phenylalanine > histidine > methionine > asparagine > threonine > tyrosine > lysine > aspartate. Significant portal elevation occured in concentrations of asparagine and aspartate although they were not present in the diet. Cystine and serine were included in the diet, but their concentrations did not show clear elevations in portal vein blood relative to arterial blood (Table 2). Tryptophan was present in the diet but was not separated in the whole blood chromatographs, so on measures were available.

Net PDV, hepatic and splanchnic fluxes of amino acids

Excluding arginine, alanine, ornithine and citrulline which are reported elsewhere (Hartman et al., 1994), the diet differences did not influence any fluxes of the amino acids available from the diet (Tables 1, 3 and 4).

The PDV fluxes of the amino acids included in the diet were significantly positive in rats fed the +Arg diet (Table 3), indicating intestinal absorption of

Table 3. Effects of an arginine-containing or one of three arginine-deficient diets on net PDV flux and net hepatic flux of amino acids available from the diet[1]

Amino acid	Dietary group				Pooled SELSM[2]
	+Arg	−Arg/+Ala	−Arg/+Orn	−Arg/+Cit	
			μmol/min		
Valine					
PDV	0.69*	0.51*	0.51*	0.73*	0.22
Hepatic	−0.34	−0.38*	−0.44*	−0.46*	0.19
Leucine					
PDV	0.67*	0.81*	0.77*	0.74*	0.17
Hepatic	−0.36*	−0.53*	−0.58*	−0.55*	0.12
Lysine					
PDV	0.67*	0.82*	0.53**	0.58**	0.27
Hepatic	−0.24	−0.15	−0.67*	−0.23	0.23
Isoleucine					
PDV	0.50*	0.62*	0.56*	0.51*	0.13
Hepatic	−0.18**	−0.30*	−0.36*	−0.24*	0.09
Threonine					
PDV	0.45*	0.58*	0.48*	0.35	0.20
Heptatic	−0.17	−0.31*	−0.71*	−0.41*	0.17
Phenylalanine					
PDV	0.37*	0.43*	0.39*	0.34*	0.10
Hepatic	−0.30*	−0.37*	−0.50*	−0.42*	0.08
Methionine					
PDV	0.32*	0.43*	0.37*	0.31*	0.10
Hepatic	−0.26*	−0.33*	−0.38*	−0.41*	0.09
Serine					
PDV	0.17	0.22	0.15	0.00	0.17
Hepatic	−0.48*	−0.51*	−0.85*	−0.80*	0.17
Histidine					
PDV	0.17*	0.16*	0.13*	0.15*	0.05
Hepatic	−0.16*	−0.16*	−0.21*	−0.09	0.07
Tyrosine					
PDV	0.15*	0.15*	0.15*	0.11*	0.04
Hepatic	−0.23*	−0.29*	−0.31*	−0.32*	0.05
Cystine					
PDV	−0.01	−0.02	−0.02	−0.01	0.02
Hepatic	−0.06*	−0.05*	−0.07*	−0.07*	0.02

[1] Values shown are least squares means of 6 to 10 rats per diet group. Superscripts indicate significant difference from zero by t test, *$P < 0.05$; **$P < 0.10$. The F test was not significant for any amino acids ($P > 0.10$).

[2] Standard error of the least squares means.

Table 4. Effects of an arginine-containing or one of three arginine-deficient diets on net PDV flux and net hepatic flux of amino acids not available from the diet[1]

Amino acid	Dietary group				Pooled SELSM[2]
	+Arg	−Arg/+Ala	−Arg/+Orn	−Arg/+Cit	
			μmol/min		
Aspartate					
PDV	0.47	0.08	0.49	0.69*	0.28
Hepatic	0.60**	1.19*	0.00	1.07*	0.32
Asparagine					
PDV	0.09*	0.10*	0.09**	0.08	0.04
Hepatic	−0.08	−0.05	−0.09	0.05	0.07
Glycine					
PDV	0.07	0.20	0.16	0.20	0.18
Hepatic	−0.47*	−0.52*	−0.76*	−0.72*	0.13
Hydroxyproline					
PDV	0.07	0.11	−0.03	0.16	0.15
Hepatic	0.13	0.27	−0.08	0.16	0.19
Taurine					
PDV	−0.09	0.21	0.15	−0.10	0.14
Hepatic	0.16	−0.07	−0.51	0.27	0.21

[1] Values shown are least squares means of 6 to 10 rats per diet group. Superscripts indicate significant difference from zero by t test, *$P < 0.05$; **$P < 0.10$. The F test showed a significant trend toward dietary differences for aspartate and taurine hepatic fluxes ($P > 0.10$).

[2] Standard error of the least squares means.

these nutrients. In the +Arg group, the magnitude of the fluxes generally corresponded to the moles of the amino acids available from the diet, except for lysine which had a proportionally greater PDV output (Fig. 1). In order from greatest to least PDV flux were valine, leucine, lysine and isoleucine, followed by threonine, phenylalanine, methionine, histidine and tyrosine. Although cystine and serine were present in the diet, PDV fluxes of these two amino acids were not significantly different from zero. With minor exceptions, similar results were seen in PDV fluxes of rats in the other three dietary groups. The fluxes of the sum of the branched-chain amino acids were also calculated for each rat, but were not significantly different across diets (Table 5).

In all four diet treatment groups, hepatic uptakes of threonine, phenylalanine, histidine and methionine matched their net PDV outputs so that splanchnic fluxes (data not shown) were not significantly different from zero. In contrast, splanchnic fluxes of the branched chain amino acids were always positive, although they were significantly positive only in rats fed the +Arg diet (0.97 ± 0.40 μmol/min). Branched-chain amino acids made up 28% of the total net splanchnic output of all amino acids measured, and essential amino acids made up 48%. Hepatic uptakes of serine (Table 3) and glycine (Table 4) were significantly different from zero for all treat-

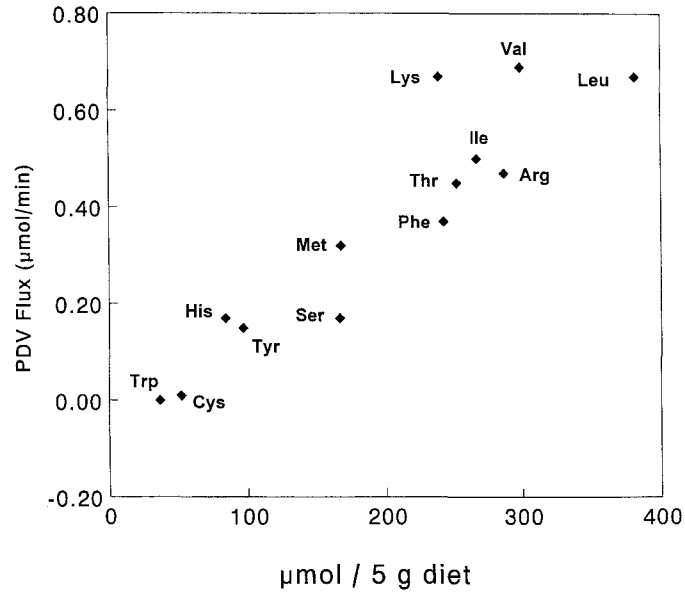

Fig. 1. Relation of PDV amino acid fluxes to dietary amino acid availability in rats fed +Arg diet. Data points show least squares means of PDV fluxes relative to the molar quantity of each amino acid present in 5 g of diet. Rats consumed 5.3 g diet before measurement of PDV fluxes 2 h after the beginning of the meal. The tryptophan content of the diet was 36.7 μmol/5 g; the data point for tryptophan was placed at 0 PDV flux as measures were unavailable

ment groups. Among amino acids not available from the diet (Table 4), net hepatic fluxes of aspartate and taurine showed a trend toward dietary differences; aspartate showed significant hepatic release except in rats fed the −Arg/+Orn diet.

Hepatic amino acid concentrations

Arginine and hydroxyproline concentrations were usually below detection limits in the liver samples so means are not reported.

The rats fed the −Arg/+Ala diet had lower ornithine, citrulline, threonine and serine concentrations in the liver compared to rats from the +Arg diet group, whereas glutamine, alanine, valine and methionine were higher in rats fed the −Arg/+Ala diet relative to rats of the other diet groups (Table 6). The hepatic methionine concentration in rats fed the −Arg/+Ala diet was 2.8× the concentration in livers of the controls. An unknown ninhydrin-reactive constituent coeluted with methionine only in chromatographs of the −Arg/+Ala dietary group, and was not separated in chromatographic reprocessing. The sum of the BCAA was not significantly different across diet groups ($P = 0.1032$). However, the ratio of branched-chain amino acids to essential amino acids was significantly higher in the livers of rats fed −Arg/+Ala or −Arg/+Orn diet compared to rats fed the +Arg or −Arg/+Cit diets. Although

Table 5. Effects of an arginine-containing or one of three arginine-deficient diets on net PDV flux and net hepatic flux of sums and ratios of amino acids[1]

Amino acid Sums and Ratio[2]	Dietary group				Pooled SELSM[3]
	+Arg	−Arg/+Ala	−Arg/+Orn	−Arg/+Cit	
			μmol/min		
TAA					
PDV	10.47*	14.91*	12.51*	11.83*	3.58
Hepatic	−7.01*	−9.37*	−14.97*	−10.23*	2.74
NEAA					
PDV	6.63*	10.55*	8.77*	8.13*	2.52
Hepatic	−5.00*	−6.85*	−11.10*	−7.42*	1.97
EAA					
PDV	3.84*	4.36*	3.74*	3.69*	1.11
Hepatic	−2.01*	−2.53*	−3.87*	−2.81*	0.84
LNAA					
PDV	2.70*	2.95*	2.75*	2.73*	0.72
Hepatic	−1.67*	−2.19*	−2.58*	−2.40*	0.52
BCAA					
PDV	1.86*	1.94*	1.84*	1.97*	0.51
Hepatic	−0.88*	−1.20*	−1.39*	−1.25*	0.37
BCAA/EAA					
PDV	0.45*	0.48*	0.52*	0.62*	0.11
Hepatic	1.41	−0.75	0.31	0.49	1.16

[1] Values shown are least squares means of 6 to 10 rats per diet group. Superscripts indicate significant difference from zero by t test, *$P < 0.05$. The F test was not significant for any of these sums or ratios.
[2] Abbreviations are as in Table 2.
[3] Standard error of the least squares means.

hepatic tryptophan concentrations did not differ significantly across diet groups, the ratio of tryptophan to branched-chain amino acids tended to be lower ($P < 0.10$) in the rats fed the −Arg/+Ala diet compared to controls. Also, the sum of the concentrations of the large neutral amino acids tended to be greater in the rats fed −Arg/+Ala and −Arg/+Orn diets. There were no differences among dietary groups in total hepatic concentrations of all amino acids, essential amino acids or nonessential amino acids.

Hepatic concentrations of 14 of 22 measured amino acids were correlated with arterial blood concentrations (data not shown). Significant correlations ($r^2 = 0.27$ to 0.57, $P < 0.01$, $P < 0.05$ for serine) were observed for all the amino acids supplied in the diets, except histidine and cystine. The hepatic concentration of total BCAA was also correlated with the arterial concentration ($r^2 = 0.56$, $P < 0.0001$). Of non-dietary amino acids, glutamine concentrations were significantly correlated ($r^2 = 0.25$, $P < 0.001$) between liver and blood, glutamate concentrations were only significant at $P < 0.10$ ($r^2 = 0.11$), while glycine, asparagine and aspartate concentrations showed no correlation.

Table 6. Effects of arginine-deficient diets on hepatic amino acid concentrations and selected groupings of amino acid concentrations[1]

Amino acid	Dietary group				Pooled SELSM	Diet Effects[2]
	+Arg	−Arg/ +Ala	−Arg/ +Orn	−Arg/ +Cit		
	nmol/g liver					
Arginine-Related[3,4]						
Aspartate	4,542	5,068	4,746	4,448	648	NS
Glutamine	4,193[b]	5,556[a]	4,707[a,b]	3,705[b]	345	$P < 0.01$
Alanine	4,029[a,b]	5,275[a]	5,085[a]	3,349[b]	476	$P < 0.05$
Glutamate	1,324	1,535	1,232	1,261	89	$P < 0.10$
Ornithine	596[a]	190[c]	439[b]	494[a,b]	36	$P < 0.01$
Proline	59	81	86	68	7	$P < 0.10$
Citrulline	56[a]	30[b]	41[a,b]	61[a]	7	$P < 0.05$
Dietary[4]						
Serine	1,384[a]	898[b]	1,116[a,b]	1,303[a]	135	$P < 0.05$
Threonine	1,235[a]	658[b]	1,156[a]	983[a,b]	155	$P < 0.05$
Lysine	795	1,053	857	917	125	NS
Histidine	485	511	521	490	27	NS
Leucine	147	197	223	161	23	NS
Valine	120[b]	164[a,b]	185[a]	114[b]	18	$P < 0.05$
Isoleucine	109	133	141	114	14	NS
Phenylalanine	93	99	118	98	9	NS
Tyrosine	74	78	92	69	9	NS
Methionine	30[b]	84[a]	46[b]	34[b]	8	$P < 0.01$
Tryptophan	23	24	27	23	2	NS
Cystine	5	3	4	5	1	NS
Non-Dietary[4]						
P-ethanolamine[5]	3,609	2,302	2,170	2,151	449	$P < 0.10$
Taurine	3,230	2,953	4,370	4,655	568	$P < 0.10$
Glycine	1,983	1,880	2,089	2,357	154	NS
P-serine[5]	249	208	244	263	15	$P < 0.10$
Cystathionine	123	95	154	84	18	$P < 0.10$
Ethanolamine	77	62	59	65	10	NS
Asparagine	73	88	92	77	7	NS
Sums and Ratios[6]						
TAA	23,800	23,449	24,812	23,088	1,432	NS
NEAA	20,762	20,525	21,539	20,154	1,209	NS
EAA	3,038	2,924	3,273	2,933	283	NS
LNAA	573	755	805	590	74	$P < 0.10$
BCAA	376	494	549	388	54	NS
BCAA/EAA	0.13[c]	0.17[a]	0.17[ab]	0.13[bc]	0.01	$P < 0.05$
Trp/LNAA	0.041	0.033	0.035	0.041	0.003	$P < 0.10$

[1] Values shown are least squares means of 6 to 10 rats per diet group. Means within the same row not sharing a common letter superscript are significantly different by t test ($P < 0.05$).

[2] Diet effect by ANOVA. *NS* not significantly different.

[3] Arginine concentrations were below the limit of detection in most samples.

[4] *Arginine-related* refers to amino acids which have a known metabolic relation to the hepatic urea cycle. *Dietary* refers to amino acids available from all four amino acid diets. *Non-Dietary* amino acids were not contained in any of the four diets.

[5] Phosphoethanolamine, phosphoserine.

[6] Abbreviations are as in Table 2, except for EAA which includes liver tryptophan.

Discussion

Elevated concentrations of branched chain amino acids

The most prominent aspect of the results presented here was the overall consistency of amino acid concentrations and fluxes across dietary treatments. However, treatment differences were observed in arterial blood BCAA concentrations, although not in PDV or hepatic fluxes of BCAA. These differences in blood BCAA were unexpected, in contrast to the expected changes observed in the concentrations and fluxes of arginine, ornithine, citrulline, and alanine. The presence or absence of these four amino acids in the respective diets produced marked alterations (approximately 2-fold) of their concentrations and fluxes which have been previously reported (Hartman et al., 1994). Comparatively, the 60% elevation of blood BCAA with arginine deficiency was a moderate change.

Arterial blood concentrations of total BCAA were elevated in rats fed the −Arg/+Ala and −Arg/+Orn diets, compared to rats fed the +Arg or −Arg/+Cit diets (Table 2). Since the rats fed the −Arg/+Ala and −Arg/+Orn diets had arginine concentrations which were only 50% of concentrations in rats fed the +Arg and −Arg/+Cit diets (Hartman et al., 1994), the across-diet pattern of BCAA was opposite to the pattern of blood arginine concentrations. Indeed, isoleucine, leucine and valine concentrations were inversely correlated with arterial arginine concentrations, as was the sum of the BCAA ($r^2 = 0.18$, $P < 0.05$). The arterial concentration of total BCAA was also highly correlated with arterial concentrations of the other essential amino acids: methionine, (e.g., $r^2 = 0.58$, $P < 0.0001$), phenylalanine, histidine, threonine and lysine and with the sum of these other essentials ($r^2 = 0.42$, $P < 0.0001$) although arterial concentrations of these other amino acids did not differ significantly by ANOVA across dietary treatments (Table 2). A similar pattern of elevated BCAA was also observed in liver, although only valine showed significance (Table 4), and in muscle tissue (Hartman and Prior, unpublished data) of the arginine deficient rats.

Several other measured parameters were examined for relationships which might explain the elevated BCAA concentrations. Across all diet groups, the BCAA concentrations (individually or summed) did not relate to average daily or final food intake. Different meal sizes might be expected to cause different releases of insulin, which is well-known to affect circulating BCAA concentrations (Zapalowski et al., 1981). However, meal sizes were not different on the day of sampling. Blood samples were obtained between 1400 and 1630 h at 120 ± 1 min after rats began to eat. The exact time at which the blood samples were obtained did not differ across dietary treatments and did not correlate with arterial BCAA concentrations. Nor did plasma urea or ammonia measures correlate with individual or total BCAA, although there was a tendency ($r^2 = 0.10$, $P < 0.09$) for blood valine to be inversely correlated with plasma urea.

Elevated branched-chain amino acid concentrations in the blood are associated with conditions in which amino acid supply is increased (high-protein feeding, liver damage, catabolic states) or when utilization is decreased (insu-

lin resistance, obesity) (Brosnan et al., 1983; Elia, 1991; Hara et al., 1987; Harris et al., 1986; Huston and Harper, 1981). In the present experiment, the supply of BCAA from the splanchnic bed was comparable among the dietary treatments, suggesting that differences in utilization could be explored in trying to account for the blood differences (Hartman and Prior, unpublished data). The activation state of liver branched-chain keto-acid dehydrogenase (BCKADH, the regulatory enzyme of BCAA utilization) has been observed to be higher in meal-fed rats than in ad libitum-fed rats, and meal-fed rats exhibit a robust increase of BCKADH activity within 30 min of beginning a meal (Block et al., 1987 and 1990; Crowell et al., 1990; Soemitro et al., 1989). During the week of feeding the four diets, rats fed the −Arg/+Ala diet ate ad libitum, while those fed the +Arg and −Arg/+Cit diets typically consumed their total food allotment in less than 24 h. Lower activity of BCKADH in rats fed −Arg/+Ala diet compared to controls could cause a slower rate of utilization of BCAA, which would explain the higher BCAA concentrations in blood. Stress hormones (i.e. catecholamines or glucocorticoids) also enhance liver BCKADH activity and could affect branched-chain amino acid utilization rates (Block et al., 1985), but it is not obvious that the control rats were stressed by the slight food restriction.

This elevation of whole blood BCAA in rats fed arginine deficient diets was not seen in previous experiments when rats were fed an arginine-deficient diet with glycine (Gross et al., 1990) or glutamate (Hartman and Prior, 1992; Prior, 1993) substituted for arginine. This allows the possibility of an effect of high levels of alanine promoting the elevation in BCAA, as the first step of BCAA utilization is coupled to production of alanine in muscle (Goldberg and Chang, 1978). The concentration of alanine in blood and liver (Table 5) of rats fed the −Arg/+Orn diet was intermediate between concentrations in rats fed the −Arg/+Ala diet and the control diet. Although blood glutamine was also elevated in the rats fed the −Arg/+Ala diet, this was not clearly linked to the elevated BCAA concentrations in spite of the observation that circulating glutamine and BCAA concentrations were significantly correlated ($r^2 = 0.53$, $P < 0.0001$). Elevated blood glutamine concentrations have been previously associated with arginine deficiency (Hartman et al., 1994), and the arginine-devoid diets containing glycine or glutamate also caused an elevation of blood glutamine as did the −Arg/+Ala diet (Hartman et al., 1994).

Neither net PDV flux nor net hepatic flux of total BCAA correlated with arterial BCAA. Net hepatic fluxes of total BCAA ranged from −3.0 to +0.5 μmol/min with no differences among diet groups, indicating that the liver was probably not contributing directly to the difference in BCAA concentrations. The initial step of BCAA transamination to branched-chain keto acids can occur in several other tissues besides liver, notably muscle; branched-chain keto acids were not measured in this study. Therefore, amino acid measures at 2 h post-meal did not elucidate the metabolic reasons responsible for the elevated BCAA in blood (and tissues) in the arginine deficient rats. Since fluxes did not show dietary differences, but dietary differences were found in concentrations of BCAA, histidine and aspartate, the metabolic

processes which caused the elevations in the amino acid concentrations remain a question.

Effects of feeding on amino acid concentrations and PDV and liver fluxes

The results of the current experiment represent one time point in the postprandial dynamic. The time point of 2 h was chosen to allow comparison with previous results of a similar experiment (Prior, 1993), in which an arginine deficient diet containing glutamate substituted for arginine was fed to rats which were sampled at 1 and 2 h after a meal. At 1 h, PDV fluxes indicated significant intestinal absorption of essential amino acids in both experimental and control dietary groups. At 2 h, however, the group fed the −Arg/+Glu diet showed a decrease in PDV output of essential amino acids relative to the control group, which showed little decrease from the 1 h measurement. In the current study, because PDV fluxes were comparable between arginine deficient and control rats, the previously seen effect on essential amino acid fluxes at 2 h must have been related to the presence of glutamate (34 g/kg diet), rather than simply the lack of arginine. The +Arg control diet was identical in the two experiments; and in both experiments, rats fed the +Arg diet showed similar blood concentrations and PDV fluxes of BCAA at the 2 h measurement time.

This set of results also pertains to the amino acid changes seen in blood (and tissue) as a result of meal ingestion. Timed studies using rats have shown that post-meal amino acid concentrations in circulating blood reach a peak 30 min to 2 h postprandially and that the concentration elevations depend on the amount of protein or amino acids ingested (Fafournoux et al., 1990; Fernstrom et al., 1987; Uhe et al., 1992; Yamamoto et al., 1974). For example, plasma BCAA were twice as high in rats fed a 30% casein diet compared to rats fed a 10% casein diet (Soemitro et al., 1989). Amino acid concentrations also demonstrate a diurnal rhythm in phase with eating that is independent of consumption (Eriksson et al., 1989). When rats were fed the +Arg control diet which contains 13% amino acids (Hartman and Prior, 1992; Prior, 1993), of 23 measured amino acids, only alanine, methionine, phenylalanine, tyrosine and proline increased in arterial blood at 1 or 2 h after feeding. Therefore, this level of dietary amino acids (13%) was not sufficiently great to cause significant excursions of arterial BCAA concentrations. The amount of food consumed (5 g) by the rats was a "typical" meal size of a rat. The PDV fluxes of each amino acid were in proportion to the molar quantities available in the diet (Fig. 1). At 2 h post-meal, portal concentrations were significantly elevated above arterial concentrations, and approximately half of the BCAA absorbed by the PDV were taken up by the liver. In the rats fed the +Arg diet, splanchnic fluxes of BCAA were significantly positive, demonstrating that these amino acids were being supplied to the systemic circulation. Therefore, the results of this study provide enhanced detail on the organ disposition of amino acids at 2 h post-meal.

Liver amino acid concentrations during the fed state

Since ornithine and citrulline are normally synthesized in the liver from arginine, the low concentrations of ornithine and citrulline measured in rats fed the −Arg/+Ala diet must be directly due to the arginine deficient diet. In the rats fed the −Arg/+Orn diet, hepatic ornithine, but not citrulline concentrations were significantly depressed from controls. This indicates that the ornithine supplied to the liver by this diet was converted to citrulline and also, presumably, arginine. Since blood arginine remained lower than controls in rats fed the −Arg/+Orn diet, these findings provide further evidence that the arginine generated in the liver, although substantial, does not supply the needs of the rest of the body (Hartman et al., 1994; Castillo et al., 1994).

While liver glutamine and glutamate were elevated from control concentrations in rats fed the arginine deficient diet containing glycine (Gross et al., 1990), glutamine and alanine were higher in rats of the −Arg/+Ala diet group. Elevated glutamine is believed to reflect the aberrations due to insufficient hepatic urea cycle intermediates (Hartman and Prior, 1992; Hartman et al., 1994; Milner and Visek, 1978).

In the rats fed the −Arg/+Ala diet, the high level of circulating alanine might inhibit hepatic uptake of other small neutral amino acids (Harper, 1983), and thereby explain the low hepatic concentrations of threonine and serine. However, threonine and serine were not low in the blood of −Arg/+Ala-fed rats, and liver threonine and serine were not inversely correlated with blood or liver alanine. Therefore, activities of hepatic enzymes such as serine-threonine dehydratase (Moundras et al., 1992) may differ between the arginine deficient and control rats. In the rats fed −Arg/+Orn diet, blood and liver concentrations of alanine and citrulline were intermediate between values of rats fed −Arg/+Ala diet and the control diet, as also were liver threonine and serine concentrations. Further studies would be needed to distinguish the effects of arginine deficiency and elevated alanine concentrations on hepatic threonine and serine.

In this experiment, the direct measure of portal vein blood flow allowed assessment of net fluxes of amino acids across the PDV and liver. In addition, control of final meal size assured that amino acid concentration and flux differences would not be due to differences in amounts ingested. Results were generally consistent with previous findings (Prior, 1993) and expectations as to the effect of a meal on amino acid concentrations and fluxes (Hartman and Prior, 1992). The effects of feeding four different diets on the concentrations and utilizations of the physiological amino acids which lack direct metabolic links to arginine are presented. The conditions responsible for the significantly higher BCAA concentrations in the two groups of rats with low circulating arginine were not apparent. The PDV, hepatic and splanchnic fluxes of BCAA were not different among the dietary treatments, and therefore can be eliminated as reasons for the differences in blood BCAA. The data indicated a consistent relationship between molar quantities of essential amino acids in the diet and amounts absorbed by the intestine and

appearing in the portal vein following a meal. These results provide additional knowledge on nutrient fluxes across organs of the splanchnic bed in the absorptive state.

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