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Feeding and arginine deficient diets differentially alter free amino acid concentrations of hindlimb muscle in young rats

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Summary. The objective of these experiments was to examine short- and long-term (7 d) effects of arginine-deficient diets on free amino acid concentrations in hindlimb muscle of rats. In rats fed the control diet containing arginine (+Arg), muscle alanine and methionine concentrations were higher 1 and 2 h after feeding compared to food-deprived rats, whereas branched-chain amino acids, arginine and asparagine concentrations were lower postprandially. In Experiment 1, rats were fed an arginine-deficient (−Arg) diet with glutamate (+Glu) substituted for arginine; alanine (+Ala), ornithine (+Orn) or citrulline (+Cit) were substituted for arginine in Experiment 2. In Experiment 1, arginine concentrations decreased in blood but not in muscle. This contrasts with rats fed −Arg/+Ala or −Arg/+Orn diets which had muscle arginine concentrations less than half the concentrations in controls or in rats fed the −Arg/+Cit diet. Muscle essential amino acids in Experiment 2 did not differ by diet, but muscle branched-chain amino acids were elevated relative to controls in the rats fed −Arg/+Ala or −Arg/+Orn diets; however, rats fed the −Arg/+Cit diet had levels similar to the controls. Also, muscle branched-chain amino acids were correlated with glutamine concentrations in both blood and muscle. The measurements in the post-meal period suggest that muscle amino acid concentrations may more closely reflect dietary amino acid patterns than do blood amino concentrations.

Keywords: Amino acids – Muscle – Fed state – Arginine deficiency – Rats

Abbreviations: BCAA, branched-chain amino acids; BCKADH, branched-chain ketoacid dehydrogenase; EAA, essential amino acids; LNAA, large neutral amino acids; NEAA, nonessential amino acids; PDV, portal-drained

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viscera; SELSM, standard error of least squares means; SSA, 5-sulfosalicylic acid; TAA, total amino acids.

Introduction

Arginine is a conditionally essential amino acid and is required in the diet of rats for maximal growth rate. Although arginine is synthesized by the liver and kidney, this amino acid, in a manner similar to dietary essential amino acids, shows a decreased concentration in plasma or blood if omitted from the diet of rats (Gross et al., 1991; Hartman and Prior, 1992; Hartman et al., 1994). An imbalanced pattern of essential amino acids in the diet produces an imbalanced pattern of amino acids in muscle tissue, characterized by an exaggerated decrease in the concentration of the most-limiting amino acid in the diet (Leung et al., 1968). The effects of amino acid defined diets on amino acid concentrations in body tissues such as muscle have not been well-studied. However, a recent study has noted a decrease of arginine in muscle of surgically-treated rats fed an arginine-devoid diet (Wakabayashi et al., 1994). Also, arginine is the precursor of nitric oxide which may be particularly important in excercising muscle (Balon and Nadler, 1994).

We have previously reported (Hartman and Prior, 1992 and 1996; Hartman et al., 1994) effects of four arginine-free diets on blood concentrations and organ fluxes of amino acids. When alanine, glutamate, ornithine or citrulline were individually substituted for arginine in the diet, concentration of the substituted amino acid was increased in the blood with the exception of glutamate. Citrulline substituted in the arginine-deficient (−Arg/+Cit) diet restored blood arginine concentrations to control levels, whereas arginine concentrations were depressed as expected with the alanine-, glutamate- or ornithine-substituted arginine-deficient diets. The −Arg/+Cit diet also normalized liver concentrations of ornithine and citrulline, while these amino acids were lowest in livers of rats fed the −Arg/+Ala diet (Hartman and Prior, 1996). Arginine deficiency was related to elevation of circulating branched-chain amino acids (BCAA) in rats fed alanine- or ornithine-substituted diets, but not when the diet was substituted with glutamate. The portal-drained viscera (PDV) and hepatic fluxes did not provide any indication of the source of elevated BCAA or the underlying mechanism. Since muscle is known to utilize BCAA, via conversion to branched-chain keto acids, it became of interest to examine the relation between the blood and muscle free amino acid patterns, and to examine effects of diet and feeding on the relation.

The regulation of muscle free amino acid concentrations is less well understood than the regulation of amino acid concentrations in plasma and blood (Teleni, 1993). Skeletal muscle is the largest single tissue of the body, and accounts for 65% of the body's total protein (Katch and McArdle, 1993). Because of the high turnover rate of the constituent proteins, muscle has a major role in the nitrogen economy of the body and is an active exchanger of free amino acids with the blood circulation. The concentrations of amino acids in muscle are generally higher than in plasma (Young, 1970), depending on

the pertinent amino acid import and export mechanisms. Muscle samples of approximately 100 mg can be obtained relatively easily in humans by skin incision and needle biopsy (Bergström et al., 1974), and several human studies have provided information about the responses of muscle amino acids to treatments such as feeding, exercise and surgical stress. Thereby, correlations of free amino acid changes with physiological state changes have been documented. In human studies, the reproducibility of the muscle amino acid pattern between individuals has suggested a precise physiological regulation by biochemical mechanisms (Fürst, 1983). By measurement of free amino acids in muscles under various nutritional and physiological treatments such as can be applied to small rodents, our intent was to help elucidate mechanisms of changes in amino acid patterns.

Our research objectives for the work reported here were: 1) To determine the acute (feeding) effects of an arginine deficient diet on muscle amino acid concentrations (Experiment 1), and 2) To compare the effects on muscle amino acid concentrations of glutamate (fed in Experiment 1), ornithine, citrulline or alanine (all fed in Experiment 2) additions to arginine deficient diets. Therefore, we present the effects of four arginine deficient diets and three sampling times on concentrations of free amino acids in skeletal muscle. Meal size prior to sampling was controlled to provide equivalent intakes among the treatments and also between experiments (Hartman and Prior, 1992; Prior, 1993). Control of final intake of amino acids ensures that effects on muscle amino acid concentrations are truly comparable across treatments.

Materials and methods

Animals

Male, Sprague-Dawley rats initially weighing 150–175 g were received from either Charles River Breeders (Experiment 1, Wilmington, MA) or from Camm Research (Experiment 2, Wayne, NJ) and were housed in stainless steel cages with wire mesh flooring. One third of the rats of Experiment 1 and all of the rats of Experiment 2 were then transferred to Plexiglas metabolic cages for 10 d throughout the 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 h daily (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* (NIH Publication No. 85-23, rev. 1985). Further details of methodology have been previously reported (Hartman and Prior, 1992; Hartman et al., 1994).

Diets

Rats were initially offered free access to tap water and commercial rodent pellets (ProLab R-M-H 3000, Agway, Inc., Syracuse, NY) for up to 7 d. Rats were then assigned to dietary treatment groups such that initial mean body weights did not differ. They were fed a semi-purified diet containing 14% soy protein for 3 d, followed by 7 d feeding of either the +Arg diet containing 10 g/kg arginine or the −Arg/+Glu diet (Experiment 1) which contained no arginine (Table 1). Rats of Experiment 2 consumed the +Arg diet or one of the other three arginine-deficient diets: −Arg/+Ala, −Arg/+Orn or −Arg/+Cit. All the

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

	Diets				
	+Arg	−Arg/+Glu	−Arg/+Ala	−Arg/+Orn	−Arg/+Cit
	g/kg				
L-Arginine free base	10.0	0.0	0.0	0.0	0.0
L-Alanine	57.0	57.0	77.4	57.0	57.0
L-Glutamate	0.0	33.2	0.0	0.0	0.0
L-Ornithine-HCl	0.0	0.0	0.0	9.7	0.0
L-Citrulline	0.0	0.0	0.0	0.0	10.1
Amino acid mix[2]	66.2	66.2	66.2	66.2	66.2
Corn Starch	544.8	521.0	534.4	547.1	544.7
Sucrose	50.0	50.0	50.0	46.0	50.0
Corn oil	100.0	100.0	100.0	100.0	100.0
Salt mix[3]	50.0	50.0	50.0	50.0	50.0
Sodium bicarbonate[3]	10.0	10.0	10.0	12.0	10.0
Cellulose	100.0	100.0	100.0	100.0	100.0
Choline bitartrate	2.0	2.0	2.0	2.0	2.0
Vitamin mix[4]	10.0	10.0	10.0	10.0	10.0

[1] Compounded by Research Diets, Inc., New Brunswick, NJ; +Arg, #A01001; −Arg/+Glu, A01003; −Arg/+Ala, #A01002; −Arg/+Orn, A01004; −Arg/+Cit, #A01005. The +Arg diet was used in both Experiment 1 and 2. The −Arg/+Glu diet was used in Experiment 1 and the other diets were used in Experiment 2.

[2] L-amino acids included in all diets: 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, and tyrosine 3.5 g/kg diet. The diet contained no aspartate, asparagine, glutamine, glutamate, glycine, and proline.

[3] Mix S01004 contained calcium carbonate 63.0 g, calcium phosphate, dibasic 525.0 g, magnesium oxide 16.6 g, potassium citrate-H_2O 235.0 g, potassium sulfate 32.6, sodium chloride 80.0, chromium potassium sulfate-$12H_2O$ 0.26 g, cupric carbonate 0.21 g, potassium iodate 0.01 g, ferric citrate 9.43 g, manganous carbonate 1.26 g, sodium selenite 0.01 g, zinc carbonate 1.92 g, and sucrose at 34.7 g/kg mix. 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 sodium bicarbonate and 0.64 g sodium chloride were added per kg diet.

[4] Mix V01001 contained retinol palmitate 275 mg, cholecalciferol 2.5 mg, dl-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, pyridoxin-HCl 1.0 g, riboflavin 0.5 g, thiamin-HCl 0.5 g, and sucrose to make one kg mix.

diets contained crystalline amino acids and were protein-free and isocaloric. The −Arg/+Glu and −Arg/+Ala diets were made isonitrogenous to the control arginine (+Arg) diet by adding glutamic acid or alanine, respectively, and the cornstarch 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. Intakes were corrected for spillage.

Experimental procedure (Experiment 1)

Rats fed the +Arg diet were mildly food restricted on a daily basis to match the ad libitum intakes of the rats fed the −Arg/+Glu diet. Mean daily food intakes over 6 days were 18.8

± 0.5 and 19.1 ± 0.5 g (mean ± SEM), respectively. All rats were moderately food-deprived during the 24 h period before blood sampling by offering only about 5 g of food. Twenty-two h later, some of the rats (Time 0 Group) were anesthetized and sampled in the food deprived state. The remaining rats were fed a small quantity of their respective diets (Table 1), which they consumed within 35 min. Final food intakes of these rats were 4.0 to 6.1 g with no differences among diet x time groups. The rats were anesthetized and sampled at 48 to 74 min (Time 1) or 112 to 132 min (Time 2) from the beginning of this final meal. Following anesthesia with sodium-pentobarbital injected intraperitoneally, blood flow through the portal vein was measured and blood was sampled successively from an hepatic vein, the portal vein and the abdominal aorta, taking about 1 min to withdraw each sample (about 1 mL) into a heparinized syringe. The entire liver, spleen and one kidney were rapidly removed for purposes of a separate experiment. Muscle samples (1.1 ± 0.04 g, mean ± SEM) consisting mainly of rectus femoris and vastus lateralis (Greene, 1963) were excised from the dorso-anterior aspect of the left hindlimb, fat and connective tissue were removed and the samples were frozen on dry ice and stored at −70°C.

Experimental procedure (Experiment 2)

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 −Arg/+Ala diet. 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). Rats were food-deprived up to 20 h before the final meal (about 5 g) which was given at the beginning of the dark period. 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. The rats were anesthetized approximately 1.5 h after feeding with sampling procedures being the same as in Experiment 1. Blood samples were obtained 112–125 min after the beginning of feeding with no differences among diet groups.

Blood and muscle amino acid analysis

Arterial blood was treated with 5-sulfosalicylic acid solution (35 g/L, SSA) for amino acid analysis using a Beckman 6300 Analyzer (Beckman Instruments, Inc., Palo Alto, CA) along with Turbochrom 2700 Chromatography software (Perkin Elmer Nelson Systems, Cupertino, CA) for peak integration as previously described (Hartman and Prior, 1994). Frozen muscle samples were reweighed, placed into 50 mL polyethylene centrifuge tubes with ice-cold water (1:4, muscle (g):water (ml)) and homogenized (Tissuemizer, with SDT182EN Shaft, Tekmar, Cincinnati, OH) for 30 to 45 s with the TR-10 Power Control set at 50% output. Five volumes of SSA were added and the samples were kept approximately 1 h on ice before centrifugation in a refrigerated centrifuge at 1,700 × g for 5 min. A second centrifugation at 13,000 × g, 4°C, for 5 min clarified the samples. Following centrifugation, supernatants were stored at −70°C until analysis for free amino acids in the same manner as for whole blood samples.

Statistical analysis

Data for both experiments were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis Systems (SAS, 1988). For Experiment 1, main treatment effects (2 diets by 3 time periods) were compared by two-way ANOVA. For Experiment 2, 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. Pearson correlation statistics were obtained using either SAS or SYSTAT (SYSTAT,

1992). Data are presented as least squares means and pooled standard errors of the least squares means (SELSM). Experiment 1 utilized 5 to 7 rats per treatment and Experiment 2 used 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

Experiment 1

Muscle amino acid concentrations

Muscle concentrations of methionine, lysine, histidine, glutamine, citrulline, and asparagine were higher in rats fed the −Arg/+Glu diet than in control rats, while concentrations of threonine, serine, glycine, tyrosine, and phenylalanine were lower (Table 2). Muscle glutamate, arginine, ornithine, taurine, hydroxyproline, one-methylhistidine, phosphoserine and phosphoethanolamine concentrations were unaffected by diet. Although feeding the −Arg/+Glu diet lowered blood arginine concentrations by 50% (Hartman and Prior, 1992), arginine and ornithine concentrations in muscle were not depressed.

Muscle concentrations of alanine and methionine were approximately doubled ($P < 0.01$) 1 h after feeding (both diets), whereas valine, isoleucine, leucine, asparagine and arginine were significantly lower ($P < 0.01$) after feeding. The higher total amino acids (TAA) and nonessential amino acids (NEAA) seen in muscle with feeding appeared to be due to the elevation in alanine. The sum of the branched-chain amino acids (BCAA) was over 30% lower ($P < 0.01$) in both diet groups by 2 h after feeding and the ratio of BCAA to essential amino acids (EAA) was lower ($P < 0.01$) as well (Table 2).

Proline and tryptophan were not well-separated in these chromatographs and therefore are not reported.

Anserine and carnosine, the alanyl-histidine dipeptides, were 4,684 ± 244 and 5,684 ± 331 nmol/g (LSmeans ± SELSM), respectively, with no significant effect of diet or feeding.

Muscle to blood correlations

In this experiment, the concentrations of arginine, glutamate, aspartate, asparagine and taurine were not correlated between muscle and blood (Table 4). Glutamate concentrations in blood of rats fed −Arg/+Glu diet were higher after feeding (Hartman and Prior, 1992), but the concentrations did not change in muscle. Arginine in the muscle did not correlate with arterial blood glutamate or glutamine.

In contrast, concentrations of all other amino acids provided in both the diets (Table 1) showed direct correlations between muscle and blood concentrations ($P < 0.05$, $r^2 = 0.12$ to 0.68). In addition, citrulline, ornithine, glutamine and glycine concentrations were correlated ($P < 0.05$) between muscle and blood. Total BCAA were correlated between muscle and arterial whole blood. However, the blood BCAA did not correlate with blood or

Table 2. Effects of feeding the arginine-containing and the −Arg/+Glu diets on amino acid concentrations in muscle (Experiment 1)

Amino acid	Dietary group	Time after feeding[1]			Pooled SELSM	Effects[2]
		0 h	1 h	2 h		
	nmol/g muscle					
Arginine-related[3]						
Glutamine	+Arg	5,083	5,035	4,855	297	D^a
	−Arg/+Glu	6,139	6,260	6,351		
Glutamate	+Arg	1,047	833	870	85	NS
	−Arg/+Glu	1,026	911	1,042		
Alanine	+Arg	2,299	4,191	5,337	321	T^a
	−Arg/+Glu	2,412	4,657	6,590		
Arginine	+Arg	568	442	441	80	T^b
	−Arg/+Glu	694	625	397		
Aspartate	+Arg	506	578	531	24	D^c, DxT^c
	−Arg/+Glu	557	548	624		
Citrulline	+Arg	257	260	260	19	D^b
	−Arg/+Glu	288	324	285		
Ornithine	+Arg	145	147	179	16	NS
	−Arg/+Glu	138	144	135		
Dietary[3]						
Lysine	+Arg	2,220	2,050	1,807	421	D^a
	−Arg/+Glu	3,049	3,329	2,679		
Serine	+Arg	1,690	1,811	1,554	80	D^a
	−Arg/+Glu	1,351	1,380	1,276		
Threonine	+Arg	1,205	1,328	1,316	91	D^b
	−Arg/+Glu	1,044	1,136	1,086		
Histidine	+Arg	257	244	252	19	D^a
	−Arg/+Glu	333	292	274		
Valine	+Arg	137	97	103	11	T^b
	−Arg/+Glu	143	116	95		
Tyrosine	+Arg	101	110	102	7	D^a
	−Arg/+Glu	60	70	73		
Leucine	+Arg	92	43	45	8	T^a
	−Arg/+Glu	90	39	38		
Phenylalanine	+Arg	61	74	78	5	D^a
	−Arg/+Glu	59	50	49		
Isoleucine	+Arg	56	35	45	6	T^a
	−Arg/+Glu	56	35	42		
Methionine	+Arg	31	61	75	6	D^aT^a
	−Arg/+Glu	59	85	94		
Cystine	+Arg	11	11	11	1	D^b
	−Arg/+Glu	10	9	10		
Non-dietary[3]						
Taurine	+Arg	11,947	12,588	12,636	522	NS
	−Arg/+Glu	12,137	11,977	12,897		
Glycine	+Arg	6,307	6,194	5,963	280	D^a
	−Arg/+Glu	4,167	4,252	4,536		
Hydroxyproline	+Arg	362	296	329	29	NS
	−Arg/+Glu	294	332	363		
Asparagine	+Arg	274	197	137	13	D^a, T^a
	−Arg/+Glu	281	224	197		

Table 2. *Continued*

Amino acid	Dietary group	Time after feeding[1]			Pooled SELSM	Effects[2]
		0 h	1 h	2 h		
o-methylhistidine	+Arg	104	89	96	7	NS
	−Arg/+Glu	109	100	106		
P-serine[4]	+Arg	88	91	96	9	NS
	−Arg/+Glu	87	98	97		
P-ethanolamine[4]	+Arg	87	84	90	7	NS
	−Arg/+Glu	78	82	91		
Sums and ratios[5]						
Total AA	+Arg	27,456	29,886	30,371	1,002	T^a
	−Arg/+Glu	25,985	28,488	30,914		
NEAA	+Arg	23,398	25,953	26,648	761	D^b, T^a
	−Arg/+Glu	21,153	23,405	26,557		
EAA	+Arg	4,058	3,933	3,723	422	D^b
	−Arg/+Glu	4,833	5,082	4,358		
LNAA	+Arg	478	420	449	35	NS
	−Arg/+Glu	467	395	393		
BCAA	+Arg	285	176	193	26	T^a
	−Arg/+Glu	289	190	176		
BCAA/EAA	+Arg	0.073	0.048	0.053	0.007	D^c, T^a
	−Arg/+Glu	0.060	0.039	0.042		
EAA/NEAA	+Arg	0.173	0.152	0.139	0.016	D^a, T^b
	−Arg/+Glu	0.228	0.214	0.166		

[1] All rats were food-deprived overnight. Rats in the 0 h group were anesthetized and sampled without feeding, whereas rats in the 1 h and 2 h groups were fed a 5 g meal of their diets and anesthetized and sampled 1 or 2 hours later, respectively. Values are least squares means of six to eight rats per group. Pooled SELSM is the mean of the standard errors of the six least squares means.

[2] *D* Diet effect; *T* Time effect; *DxT* Diet by time interaction. Superscripts a, b, and c indicate level of statistical significance as follows: $^aP < 0.01$, $^bP < 0.05$, $^cP < 0.10$.

[3] *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.

[4] Phosphoserine, phosphoethanolamine.

[5] *Total AA* sum of NEAA and EAA; *NEAA* sum of the nonessential amino acids: phosphoserine, taurine, phosphoethanolamine, aspartate, hydroxyproline, serine, glycine, alanine, cystine and tyrosine; *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.

muscle glutamine concentrations, and BCAA in muscle did not correlate with blood or muscle glutamine.

Muscle to blood concentration ratios

Muscle-blood ratios ranged from taurine, which was highest (36.5 at 2 h, data not shown) to cystine (0.4 at 2 h, Fig. 1) which was lowest. Muscle-blood ratios

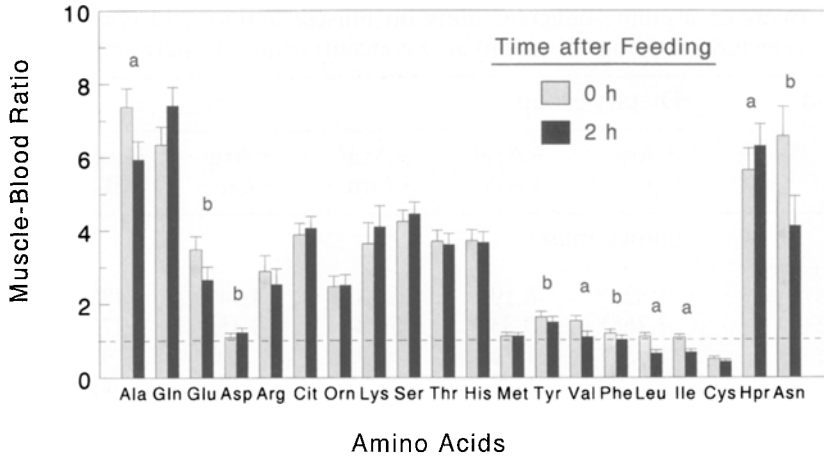

Fig. 1. Effect of feeding (Experiment 1, +Arg diet) on muscle-blood concentration ratios of amino acids. The time 0 h indicates data from rats which were food-deprived for 20 h. The time 2 h indicates data of rats at 2 h after they consumed a meal of +Arg diet. Letters indicate significant time effects by ANOVA: a, $P < 0.01$; b, $P < 0.05$. The horizontal dashed line is at unity. *Hpr* hydroxyproline

of glycine (not shown) were elevated with feeding (14.6 at 0 h, 18.3 at 1 h; $P < 0.0001$) and highest at 2 h (22.6) after feeding, while the alanine ratios were lowest 1 h after feeding (5.4). As shown in Fig. 1, muscle-blood ratios of glutamate, tyrosine, valine, phenylalanine, leucine, isoleucine and asparagine were lower in fed compared to food-deprived rats; aspartate and hydroxyproline were slightly but significantly higher.

Some muscle-blood ratios were affected by diet (data not shown). In rats fed the −Arg/+Glu diet, the muscle-blood ratios of arginine were significantly higher (4.6 at 0 h, 3.4 at 2 h, $P < 0.001$) due to their lower blood arginine concentrations compared to controls (Fig. 1). The rats fed the −Arg/+Glu diet also had higher muscle-blood ratios of ornithine and glutamate than did controls and this was in spite of having blood ornithine concentrations significantly lower than rats fed the control diet (Hartman and Prior, 1992). Muscle-blood ratios of methionine and lysine were slightly higher ($P < 0.05$) in the rats fed the −Arg/+Glu diet and citrulline, threonine, aspartate and hydroxyproline ratios showed a tendency to be higher ($P < 0.10$). Except for these and cystine, ratios of other amino acids were unaffected by diet.

Experiment 2

Muscle amino acid concentrations

Differences due to the four diets occurred in 19 of the 27 amino acids measured in muscle (Table 3). As expected, muscle arginine and ornithine concentrations were lower in rats fed −Arg/+Ala diet relative to rats fed the +Arg diet. However, muscle alanine, glutamine, glutamate and citrulline concentrations were higher in rats fed −Arg/+Ala diet than in controls. For

Table 3. Effects of arginine-deficient diets on muscle amino acid concentrations and selected groupings of amino acid concentrations (Experiment 2)[1]

Amino acid	Dietary group					
	+Arg	−Arg/ +Ala	−Arg/ +Orn	−Arg/ +Cit	Pooled SELSM	Effects[2] P <
	nmol/g muscle					
Arginine-related[3]						
Alanine	5,828^{bc}	8,196^{a}	6,382^{b}	4,549^{c}	489	0.01
Glutamine	3,768^{b}	5,331^{a}	3,984^{b}	3,352^{b}	270	0.01
Glutamine	1,033^{ab}	1,215^{a}	995^{ab}	808^{b}	90	0.05
Aspartate	669^{ab}	793^{a}	660^{b}	557^{b}	47	0.01
Arginine	536^{a}	211^{c}	145^{c}	430^{b}	32	0.01
Citrulline	275^{c}	378^{b}	271^{c}	571^{a}	29	0.01
Ornithine	175^{b}	120^{c}	256^{a}	134^{c}	10	0.01
Proline	139	162	170	137	10	0.1
Dietary[3]						
Lysine	1,474	1,660	1,350	1,525	138	NS
Serine	1,388	1,349	1,306	1,302	92	NS
Threonine	1,227	1,101	1,158	965	121	NS
Histidine	164^{b}	222^{a}	188^{b}	160^{b}	9	0.01
Methionine	116^{ab}	150^{a}	111^{ab}	91^{b}	14	0.05
Tyrosine	111	98	114	92	10	NS
Valine	110^{b}	156^{a}	149^{a}	93^{b}	12	0.01
Phenylalanine	82	74	85	74	4	0.1
Leucine	52^{b}	85^{a}	86^{a}	46^{b}	8	0.01
Isoleucine	50^{bc}	77^{a}	66^{ab}	39^{c}	7	0.01
Tryptophan	40	36	32	45	3	0.1
Cystine	14	14	14	14	0	NS
Non-dietary[3]						
Taurine	15,995	17,165	15,944	14,554	705	0.1
Glycine	5,307	4,993	5,289	5,479	287	NS
Hydroxyproline	273	328	224	261	29	0.1
P-serine[4]	101	110	102	98	6	NS
P-ethanolamine[4]	88^{ab}	95^{a}	67^{b}	72^{b}	7	0.05
Asparagine	68	85	69	78	7	NS
o-methylhistidine	53	63	59	53	3	0.1
Sums and ratios[5]						
Total AA	33,229	36,867	33,496	30,152	1,369	0.05
NEAA	29,916^{b}	33,305^{a}	30,271^{ab}	27,114^{b}	1,260	0.05
EAA	3,313	3,562	3,224	3,038	223	NS
EAA/NEAA	0.111	0.107	0.106	0.112	0.006	NS
LNAA	521	640	611	435	47	0.05
BCAA	212^{b}	318^{a}	301^{a}	178^{b}	26	0.01
BCAA/EAA	0.070^{b}	0.101^{a}	0.103^{a}	0.063^{b}	0.009	0.01
Trp/LNAA	0.077^{b}	0.059^{b}	0.055^{b}	0.108^{a}	0.009	0.01
Trp/BCAA	0.190^{b}	0.122^{b}	0.115^{b}	0.284^{a}	0.027	0.01

arginine, ornithine, alanine, glutamine, glutamate, and citrulline the muscle differences followed the diet-induced differences seen in arterial whole blood (Hartman and Prior, 1994). Additionally, histidine, aspartate and the branched chain amino acids were at higher concentrations in muscles of rats fed −Arg/+Ala diet than in rats fed +Arg diet, and this also followed the differences seen in arterial whole blood. However, total essential amino acids (EAA) in muscle were not different across dietary groups. No differences across diets were apparent for muscle concentrations of cystine, asparagine, tyrosine, threonine, serine, glycine and lysine.

Anserine (3,876 ± 166 nmol/g muscle) and carnosine (4,810 ± 267 nmol/g muscle) were not altered by diet and variation coefficients across all diets were ≤15%.

Muscle tryptophan concentrations tended to be lower ($P < 0.10$) in rats fed −Arg/+Ala and −Arg/+Orn diets than in −Arg/+Cit-fed (Table 3), while the large neutral amino acids (LNAA, the sum of phenylalanine, tyrosine, methionine and the branched chain amino acids) were higher ($P < 0.05$). Therefore the tryptophan/LNAA ratio in muscle was lower ($P < 0.01$) in rats fed the −Arg/+Ala and −Arg/+Orn diets than in rats fed +Arg and −Arg/+Cit diets. The proportion of the essential amino acids as branched chain amino acids was >10% in muscles of rats fed −Arg/+Ala and −Arg/+Orn diets, and this proportion was higher than the proportions in rats fed +Arg or −Arg/+Cit diet.

Muscle to blood correlations

All the amino acids provided in the diet showed significant correlations between muscle concentrations and blood concentrations ($P < 0.01$, $r^2 = 0.28$ to 0.80), whereas glutamate, proline, hydroxyproline, cystine, taurine and phosphoserine showed no correlation between measures in muscle and blood (Table 4). Also, muscle branched-chain amino acids and their sum were correlated with glutamine concentrations in both blood and muscle ($P < 0.01$).

[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-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.

[4] Phosphoserine, phosphoethanolamine.

[5] Abbreviations are as in Table 2, except for the following: *NEAA* sum of the nonessential amino acids: phosphoserine, taurine, phosphoethanolamine, aspartate, hydroxyproline, serine, proline, glycine, alanine, cystine and tyrosine; *EAA* sum of the essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, lysine and histidine.

Muscle to blood concentration ratios

Muscle-blood ratios in Experiment 2 were similar to Experiment 1; taurine (67.7 ± 4.5), glycine (25.1 ± 1.3) and glutamine (Fig. 2) had the greatest

Table 4. Correlations between muscle and arterial blood amino acid concentrations[1]

Amino acid	Experiment 1		Experiment 2	
	r^2	P <	r^2	P <
Arginine-related				
Alanine	0.63	0.001	0.71	0.001
Glutamine	0.23	0.003	0.70	0.001
Glutamate	0.01	NS	0.07	NS
Aspartate	0.02	NS	0.14	0.046
Arginine	0.06	NS	0.80	0.001
Citrulline	0.26	0.002	0.83	0.001
Ornithine	0.12	0.034	0.78	0.001
Proline	–	–	0.02	NS
Dietary				
Lysine	0.39	0.001	0.59	0.001
Serine	0.30	0.001	0.45	0.001
Threonine	0.51	0.001	0.71	0.001
Histidine	0.27	0.001	0.28	0.004
Methionine	0.68	0.001	0.72	0.001
Tyrosine	0.67	0.001	0.68	0.001
Valine	0.12	0.035	0.68	0.001
Phenylalanine	0.46	0.001	0.40	0.001
Leucine	0.43	0.001	0.80	0.001
Isoleucine	0.30	0.001	0.64	0.001
Cystine	0.19	0.007	0.00	NS
Non-dietary				
Taurine	0.04	NS	0.06	NS
Glycine	0.21	0.004	0.32	0.002
Hydroxyproline	0.09	0.063	0.08	NS
P-serine	–	–	0.01	NS
P-ethanolamine	–	–	0.15	0.036
Asparagine	0.05	NS	0.14	0.048
o-methylhistidine	–	–	0.03	NS
Sums and ratios[2]				
Total AA	0.02	NS	0.34	0.001
NEAA	0.06	NS	0.35	0.001
EAA	0.05	NS	0.55	0.001
EAA/NEAA	0.36	0.001	0.33	0.002
BCAA	0.25	0.002	0.75	0.001
BCAA/EAA	0.37	0.001	0.72	0.001
LNAA	0.26	NS	0.68	0.001
BCAA v. Muscle Gln	0.02	NS	0.31	0.002

[1] Data are coefficients of determination from 38 rats (Experiment 1) or 29 rats (Experiment 2) and probability values. Blood concentrations of proline, phosphoserine, phosphoethanolamine and o-methylhistidine were not obtained in Experiment 1.
[2] Abbreviations for Experiment 1 are as in Table 2. Abbreviations for Experiment 2 are as in Table 3, except for the following: *EAA* sum of the essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and histidine.

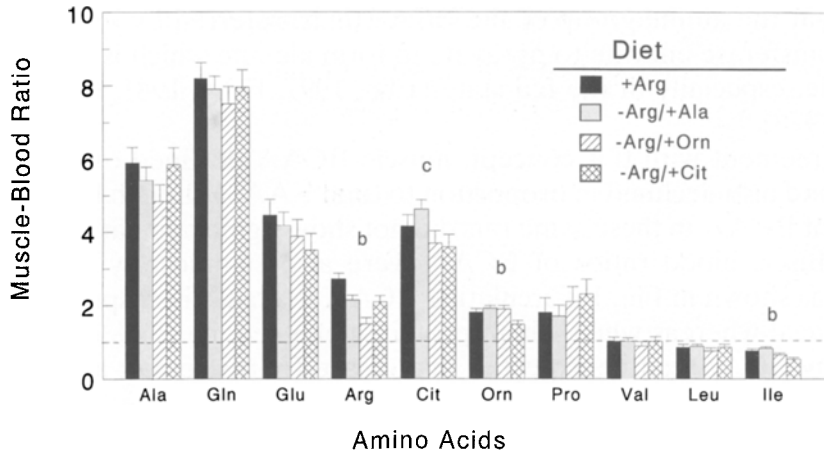

Fig. 2. Effect of arginine-deficient diets (Experiment 2) on muscle-blood concentration ratios of selected amino acids at 2 h after feeding. Letters indicate significant diet effects by ANOVA: b, $P < 0.05$; c, $P < 0.10$. The horizontal dashed line is at unity

muscle-blood ratios of all the amino acids measured, whereas the BCAA and cystine ratios were the lowest, at less than one. Therefore, selected ratios are presented in Fig. 2.

The muscle-blood ratios of arginine, ornithine and citrulline ($P < 0.10$) were altered by dietary treatment as shown in Fig. 2. The muscle-blood ratio of isoleucine was higher ($P < 0.05$) in rats fed the −Arg/+Ala diet (0.83) than in rats fed −Arg/+Orn diet (0.66) or −Arg/+Cit diet (0.54). Taurine muscle-blood ratios also tended to be higher ($P < 0.10$) in rats fed −Arg/+Ala diet than in rats fed −Arg/+Cit diet. Other than these five amino acids, no diet differences were seen in the muscle-blood ratios of the measured amino acids.

Discussion

Effects of feeding on muscle free amino acid concentrations

Only a few studies have examined changes in muscle amino acids during the absorptive phase (Bergström et al., 1990; Elia, 1992). In Experiment 1, muscle concentrations of alanine but not glutamine were twice as high after feeding. The diets contained more than 5% alanine but did not contain glutamine which may account for these observations. Studies in rats indicate circulating alanine is higher in fed than in food-deprived animals (Mitch, 1981; Yamamoto et al., 1974), whereas glutamine is also elevated by a meal in humans (Elia, 1991). The muscle-blood ratio of alanine declined from 7.4 in food-deprived rats, to 5.4 in fed rats at 1 h, suggesting that alanine concentrations increased in the blood at a faster rate following food intake than in the muscle. Nevertheless a strong muscle to blood correlation of alanine concentrations was observed over all diet groups. Alanine production by incubated rat muscles is coupled with increased BCAA utilization. In vitro studies have

shown that the amine group of the BCAA is transferred by branched-chain amino transferase enzyme to pyruvate to form alanine which is then released by muscle, especially in the fed state (Elia, 1991; Felig, 1981; Goldberg and Chang, 1978).

In agreement with this concept, muscle BCAA declined over 30% after feeding and also declined in proportion to total EAA, although blood concentrations of BCAA in these same rats did not show significant variations (Prior, 1993). Muscle-blood ratios of BCAA were also significantly decreased by feeding, as shown in Fig. 1. Circulating BCAA decrease in response to a low-protein meal whereas when a high-protein diet is fed, i.e. 25% of dry matter, circulating BCAA can be expected to increase (Fernstrom et al., 1987). The decline of muscle BCAA in this experiment is probably because the diets contained a relatively low level of amino acids (13.3%), and the lack of response in blood BCAA to feeding suggests that muscle is a more responsive indicator of dietary amino acids than blood.

Arginine also decreased 28% by 1 h after feeding in muscles of rats fed the arginine-containing diet. This decrease of muscle arginine after feeding while blood arginine remained constant (Hartman and Prior, 1992), may demonstrate that arginine at 1% of the diet is marginal in meeting the arginine requirement for this age of rat and that arginine may be supplied at less than the need for growth, relative to other essential amino acids (e.g. threonine and methionine) available in the +Arg diet. A similar observation was made in the rats fed the −Arg/+Glu diet, where the decrease of free arginine was proportionately greater (43%) in the muscle than in the blood (27%) 2 h after feeding (Hartman and Prior, 1992). While the effects on muscle arginine were only measured at 2 h post-meal in rats fed the other arginine deficient diets, we surmised that muscle arginine concentrations would have been higher in all diet groups if measured in the food-deprived state.

Lysine and threonine muscle concentrations are nearly 10-fold greater than muscle concentrations of other individual essential amino acids. These two amino acids are not significantly degraded in muscle tissues (Young, 1970) and showed no significant increases in blood (Prior, 1993) or muscle with feeding. Precise studies have shown that the amounts of these amino acids ingested determine muscle levels of these amino acids (Bergström et al., 1990; Kang-Lee and Harper, 1978).

Overall, the data confirm that concentrations of free amino acids in muscle change with feeding as do amino acid concentrations in blood. However, other researchers noted that the time course of the changes in muscle cells over the first few hours following a meal may lag relative to the time course of the changes in plasma (Bergström et al., 1990). The current results suggest that changes of amino acid concentrations in response to a meal were more marked in muscle than in blood. The changes which occur due to feeding are superimposed on the plasma amino acid fluctuations of the circadian rhythms (Eriksson et al., 1989). Circadian rhythms in muscle amino acids would also be expected since diurnal changes in protein synthesis rates are known to occur (Pocknee and Heaton, 1978).

Effects of diet on muscle free amino acid concentrations

Muscle arginine, in rats fed the arginine deficient diet substituted with glutamate, was not lower than in controls. This finding is in contrast to the observations of Experiment 2 in the rats fed the arginine deficient diets substituted with other nonessential amino acids such as alanine and ornithine. In Experiment 2, these diets caused 61% and 73% decreases in muscle arginine concentrations, respectively. Muscle arginine was also 70% lower in rats fed for four weeks an arginine-devoid diet substituted with glycine (Wakabayashi et al., 1994).

While arginine, citrulline, and ornithine concentrations were correlated between muscle and whole blood in Experiment 2, this does not indicate that in *controls* the correlation would be expected to hold. This is because the diet treatments unnaturally extended the range of blood concentrations of arginine, citrulline and ornithine. Indeed arginine was not correlated between muscle and blood in Experiment 1. However, the muscle-blood ratios of arginine, ornithine and citrulline were affected by the diets which contained these amino acids.

In Experiment 2, the rats with arginine depletion in their muscles exhibited higher muscle free BCAA compared to control rats, while in Experiment 1, these differences associated with diet were absent. However, the −Arg/+Glu diet used in Experiment 1 did not cause muscle arginine depletion. Rather, in Experiment 1, muscle BCAA were lower after feeding. Since the ranges of muscle (as well as blood) BCAA concentrations in rats fed the +Arg diet in Experiment 2 were remarkably similar to the comparable data at the 2 h time of Experiment 1, the two experiments can be considered together. However, it is not clear that the higher BCAA observed with arginine deficient diets in Experiment 2 are a direct result of the deficiency per se.

The higher BCAA in muscle and blood observed in the rats fed the −Arg/+Ala and −Arg/+Orn diets may have been related to differences in BCAA utilization. Rats in the control group were fed amounts of about 90% of their ad libitum intakes, in order to limit their intake to amounts consumed by rats fed the −Arg/+Ala diet. While control rats habitually consumed all their food in less than 23 h and may have experienced a food-deprivation period on a daily basis, the rats fed the −Arg/+Ala diet, in contrast, always had food available. The rate-limiting enzyme for BCAA catabolism, branched-chain ketoacid dehydrogenase (BCKADH), has been shown to be more active in meal-fed rats than in rats allowed ad libitum consumption of diet (Block et al., 1987). Therefore, greater activity of BCKADH may have catabolized more BCAA in muscles of rats fed +Arg or −Arg/+Cit diet. In support of this is the fact that muscle BCAA (but not arterial blood BCAA) decreased with feeding (Experiment 1). Also, the ratio of muscle BCAA/EAA was significantly lower in control rats than in the arginine deficient rats in Experiment 2, suggesting greater utilization of BCAA in control rats compared to the arginine deficient rats. The ratio of BCAA/EAA concentrations also was correlated ($r^2 = 0.715$, $P < 0.0001$) between muscle and blood and was approximately half as great in muscle as in blood. In addition, a difference in

food availability might have induced different motor activity levels between the groups of rats. BCAA are utilized to a greater degree by exercising muscle and enzymatic alterations are able to persist as evidenced by training effects on BCAA oxidation (Lemon, 1987).

The rats with arginine depletion in their muscles also exhibited higher muscle histidine and methionine and lower muscle trp/LNAA ratio relative to control rats. A lowered trp/LNAA ratio in blood and brain may depress serotonin synthesis (Tackman et al., 1990); the change in this ratio in muscle suggests that a change may also have occurred in other tissues such as brain.

Relations between muscle and blood amino acid concentrations

The results of these two experiments confirm that many free amino acid concentrations are strongly correlated between muscle and blood. This has been noted previously in relation to factors such as level of dietary protein, kidney disease, and surgical stress (Bergström, 1974; Lindholm, 1989) but the present data give further strength to the supposition of regulatory mechanisms that control the amino acid concentrations in both compartments. Therefore, diets with different patterns of amino acids which induce different patterns of blood amino acids also induce these different patterns in muscle amino acids.

In addition, most amino acids were observed to hold characteristic muscle-blood partitioning which was only slightly modified by diet. For example, citrulline is partitioned about 3 times higher in muscle than in blood compared to arginine and ornithine which are only 1 to 2 times higher in muscle than blood (Figs. 1 and 2). While citrulline and ornithine do not participate in protein synthesis, a recent study found substantial activity of nitric oxide synthase in incubated rat muscles (Balon and Nadler, 1994). Citrulline would be synthesized on an equimolar basis with nitric oxide, which was produced at nearly 100 nmoles per g muscle per h in the presence of 10 μmolar added arginine. The data of Figs. 1 and 2 illustrate that arginine deficient diets skew the partitioning of arginine, ornithine and citrulline between muscle and blood. Admittedly, the difference in water content of whole blood (80%) and muscle (76%) introduces a slight error which we did not attempt to quantify, but which may affect calculation of the muscle-blood ratio (Mitch, 1981).

Glutamate, unlike most of the other amino acids, was not correlated between muscle and blood. Glutamate did not increase in blood (Hartman and Prior, 1992) or muscle (Table 2) when 3.4% of glutamate was fed in the diet, and glutamate output by the portal-drained viscera after rats consumed this diet differed in time course but not amount from control rats, indicating a large capacity of the viscera to metabolize glutamate. This finding allows the supposition that blood citrulline may have been transiently increased at some time over the 24 h period by conversion of the glutamate to citrulline in the intestinal mucosa (Hartman and Prior, 1992). Although blood and muscle citrulline were not significantly elevated over time, muscle citrulline was 25%

higher than in controls in rats 1 h after being fed −Arg/+Glu diet. In Experiment 2, muscle glutamate was slightly higher in rats fed −Arg/+Ala diet than in rats fed −Arg/+Cit diet, but across all diets no correlation between muscle and blood glutamate appeared. The aminotransferase enzymes which use glutamate are sufficiently ubiquitous and active that the relation between diet and blood, and between blood and muscle concentrations as seen with other amino acids, evidently do not hold (Young, 1970).

In summary, the results presented here extend our understanding of the effects of a meal on muscle amino acid concentrations. Following a meal with a moderately-low amino acid content, BCAA and arginine concentrations decreased in muscle but not in blood, suggesting that muscle free amino acid concentrations may provide a better portrayal of amino acid needs than do circulating amino acid concentrations. Elevated BCAA were seen in both blood and muscle compartments of two groups of arginine deficient rats. However, it was not apparent that elevated BCAA were a direct result of arginine deficiency. Partitioning of amino acids between muscle and blood compartments was similar in the two experiments indicating close regulation of this partitioning.

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