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ORIGINAL ARTICLE

Plasma free amino acid kinetics in rainbow trout (*Oncorhynchus mykiss*) using a bolus injection of ^{15}N-labeled amino acids

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Abstract To gain insight into the metabolic design of the amino acid carrier systems in fish, we injected a bolus of ^{15}N amino acids into the dorsal aorta in mature rainbow trout (*Oncorhynchus mykiss*). The plasma kinetic parameters including concentration, pool size, rate of disappearance (R_d), half-life and turnover rate were determined for 15 amino acids. When corrected for metabolic rate, the R_d values obtained for trout for most amino acids were largely comparable to human values, with the exception of glutamine (which was lower) and threonine (which was higher). R_d values ranged from 0.9 μmol 100 g^{-1} h^{-1} (lysine) to 22.1 μmol 100 g^{-1} h^{-1} (threonine) with most values falling between 2 and 6 μmol 100 g^{-1} h^{-1}. There was a significant correlation between R_d and the molar proportion of amino acids in rainbow trout whole body protein hydrolysate. Other kinetic parameters did not correlate significantly with whole body amino acid composition. This indicates that an important design feature of the plasma-free amino acids system involves proportional delivery of amino acids to tissues for protein synthesis.

Keywords *Oncorhynchus mykiss* · Plasma amino acid kinetics · Amino acid turnover · Bolus injection · ^{15}N · Fish

Introduction

Fish differ from mammals in several aspects of their amino acid metabolism. Carnivorous fish are poikilothermic animals that derive much of their metabolic energy from dietary protein. The retention efficiency of dietary protein into body protein in fish is much higher than that of mammals (Tacon and Cowey 1985) due to slower rates of breakdown of body protein (Houlihan et al. 1995). This would affect plasma amino acid kinetics. Fish tend to use amino acids such as glutamine as oxidative substrates in tissues such as heart and red muscle, unlike mammals where glutamine is synthesized in and exported from muscle tissues (Chamberlin et al. 1991). Based on these differences, one would expect the kinetics of free amino acids in the plasma to differ from those of mammals.

The parameters that can be measured for plasma-free amino acids are the concentrations, the half-life or turnover and the parameter linking these, and the rate of disappearance (R_d), which provides an indication of the capacity of the circulatory system to move the amino acid from the sites of synthesis to the sites of net utilization. R_d can also provide insights into the relative 'importance' of amino acids with higher R_d values indicating a higher requirement for that particular amino acid.

The parameters for which the most information exists are concentrations. The concentrations of some amino acids such as glutamine tend to be lower in fish compared to mammals (Ballantyne 1997) and the concentrations do not correlate with body protein amino acid content. Aside from this, little is known of the other kinetic parameters in fish.

In mammals, a few studies have linked plasma amino acid kinetic parameters such as R_d to the amino acid composition of body protein (Lindsay 1982; Obled and Arnal 1991). The plasma kinetics of individual free amino acids have only been examined in two teleost species for a single amino acid each (Bever et al. 1981; Fauconneau and Tesseraud 1990). It is thus difficult to make comparisons of

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the design of plasma amino acid metabolism between fish and mammals. Indeed, there are no studies on the design of the kinetics of plasma amino acids in any poikilothermic animal. Due to the substantial differences in the amino acid metabolism of fish and mammals, one would expect differences in the specific parameters, as well as the overall design of the system.

To characterize the design of the kinetics of plasma-free amino acids, ideally the parameters for as many amino acids as possible should be determined simultaneously. To accomplish this, we developed a method that uses a bolus injection of 15 ^{15}N-labeled amino acids into the peripheral circulation of rainbow trout (*Oncorhynchus mykiss*), followed by periodic sampling through the same indwelling cannula. Although we acknowledge the theoretical superiority of the continuous infusion method (Haman and Weber 1996), bolus injection has been shown to provide comparable results (Hovorka et al. 1997). The advantage of the more rapid bolus injection method is that multiple tracers can be injected simultaneously and the kinetic parameters determined before significant exchange of label occurs.

We hypothesize that the correlation between plasma amino acid R_d and body protein amino acid content as described for mammals will also be an important design feature of plasma amino acid kinetics in fish.

Materials and methods

Animals

Adult, sexually mature, *O. mykiss* weighing 964 ± 50 g were obtained from Alma Research Station (Alma, ON) and held at the Hagen Aqualab facility at the University of Guelph in freshwater under 12:12 light–dark regime until experimentation. They were fed to satiation every other day using Martin Mills trout chow (Martin Feed Mills, Elmira, Ontario) (44% protein, 24% carbohydrate, 10% fat, 6% ash, 3.5% fiber) until the onset of experimentation. Food was withheld for 2 days prior to experimentation and for the duration of the experiment.

Experimental protocol

Animals were anesthetized using MS-222 (1.5 g l^{-1}) dissolved in buffered well water, and the gills were also aerated for the duration of the surgery using MS-222 (1 g l^{-1}) in buffered well water. The surgery took approximately 8–10 min. Cannulations of the dorsal aorta were performed as previously described (Stevens and Randall 1967). Once cannulated, animals were transferred to aerated black boxes to which untreated well water was added at a flow rate of ~200 ml min^{-1} at a temperature of 10 ± 1.5°C. The black boxes consisted of opaque black plexiglass with adjustable inserts and a tight-fitting lid that allowed only several centimeters around the animal to minimize animal movement. Animals were allowed to recover for 48 h after cannulation prior to experimentation, and the cannula was flushed every 12 h with teleost Cortland saline (Wolf 1963) containing heparin (10 units ml^{-1}) to ensure adequate blood flow. The actual experiment was started at approximately the same time each day (between 1300 and 1400 hours) to minimize diurnal variations in amino acid metabolism, which are thought to occur in most vertebrates. Since the mixture of labeled amino acids contained amounts of glutamine that were too low to label the plasma pool, two separate experiments were performed. In the first experiment we used only ^{15}N glutamine ($n = 5$) and in the second ($n = 5$) we used an algal amino acid mixture containing the other protein-forming amino acids (Table 2). A bolus of ^{15}N labeled algal amino acid mixture or glutamine only was administered via the cannula as a bolus of 0.01 g of the amino acid mixture or 0.001 g glutamine per kg wet weight in 1 ml of Cortland saline (Wolf 1963). Serial 200 µl blood samples were withdrawn via the cannula at times 0, 2, 4, 6, 8, 10, 12, 14, 20, 25, 30, 40, 50 and 60 min for a total blood withdrawal from each animal of 2.8 ml, which would constitute approximately 5% of the blood volume in the average 964 g fish used assuming a blood volume of 5% of body weight (Gingerich and Pityer 1989). The cannula was flushed with physiological media between each sampling and the removed blood volume was replaced with Cortland saline. After blood sampling, plasma was separated by centrifugation at 5,000×*g* for 10 min using a Heraeus Biofuge Primo R bench top model (Mandel Scientific Co. Ltd, Guelph, ON) centrifuge chilled to 4°C. Plasma samples were then rapidly frozen using liquid nitrogen and stored at −80°C for later analysis. Hematocrit was also taken before and after each experimental protocol. Plasma samples were thawed and prepared as described in the EZ:faast kit within 2 weeks of sampling.

Amino acid analysis

A Waters Micromass Quattro Micro API tandem mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) interface, a nitrogen generator (Peak Scientific, Bedford, MA, USA) and a Waters 2695 separation module (Waters, Milford, MA, USA) were used for all amino acid analyses.

Separation of amino acids was achieved using methanol and water as the mobile phases each with 10 mM ammonium formate at a flow rate of 0.25 ml min^{-1}. The separation uses 83% methanol and 17% water for the initial

11 min, and 68% methanol and 32% water for an additional 2 min. The column was re-equilibrated for 8 min between runs. The column used was a Phenomenex EZ:faast 4 um AAA-MS 250 × 2.0 mm.

Amino acid analysis was performed using a commercially available EZ:faast kit (Phenomenex, Torrance, CA, USA) using MRM mode for parent–daughter ion combinations as described with the kit. A five-point calibration of 2, 5 10, 50 and 100 nmol amino acids was used to more accurately measure low abundance amino acids.

The mass spectrometer was operated in the positive-ion mode using the following conditions: Nitrogen was used as the desolvation and cone gases at a flow rate of 150 and 100 l h^{-1}, respectively, by a nitrogen generator (Peak Scientific, Bedford, MA, USA) Grade 4.8 argon (BOC, Guelph, ON) was used as the collision gas. Capillary voltage was set to 3.0 kV. Cone and collision voltages were optimized for individual amino acid groups and their representative internal standard. ^{15}N labeling was assessed using the appropriate parent–daughter combinations of amino acids after derivatization by the EZ:faast kit.

Reagents

The ^{15}N-labeled amino acids were purchased from Cambridge Isotope Labs (Andover, MA). The mixture used was an amino acid mixture derived from algal cells, comprising 20 protein-forming amino acids, with the exception of tryptophan and glutamine. The glutamine used was singly ^{15}N-labeled at the amino group. See Table 1 for the percentage labeling and composition of the amino acid mixture. HPLC-grade methanol and water were purchased from Fisher (Whitby, ON). The EZ:faast kit was purchased from Phenomenex (Torrance, CA). All other chemicals were purchased from Sigma (St. Louis, MO).

Kinetic calculations

Kinetic calculations were performed as previously described (Wolfe and Chinkes 2005) using a two-pool model for second-order kinetics. All curves were fitted to a second-order kinetic equation using Sigmaplot 10.0. The area under the curve (AUC) as described by Magnoni et al. (2008) was used for determining second-order kinetics. To account for possible label recycling, we conservatively stopped integration at the point where 10% of the label remained in circulation. Although first-order kinetics provided a relatively good fit for most time points, there was a characteristic underestimation of early time points and an overestimation of later time points using first-order kinetics. So, second-order kinetics was used, which provided very good fit (Fig. 1). Second-order kinetics is also compatible with a simple two-pool model (plasma and another compartment). For these reasons and since the goodness of fit using second-order kinetics was high (>0.95 in most cases), it was therefore considered unnecessary to resort to higher (e.g., third- or fourth-order) kinetic analysis.

Results

Hematocrit of the animals changed from 22 ± 2% at the onset to 19 ± 2% at the termination of experimentation, representing only a 10% reduction in hematocrit over the sampling period.

Following the injection of amino acids (10 mg amino acid mixture kg^{-1} or 1 mg glutamine kg^{-1}), the isotope-enrichment time–decay curves were determined for 15 of the 20 protein-forming amino acids in five trout. Although all 20 amino acids were present, due to the very low amounts of the amino acids, asparagine and tryptophan, contained in the amino acid mixture and the reliability of measurement of labeling of cystine, histidine and tyrosine, the results for these amino acids will not be included.

The bolus injection was calculated to raise the total amino acid concentration by less than 50%, with each amino acid concentration rising individually. There was a transitory average rise in plasma amino acid concentration of 0.6 mM (or approximately 23%). The plasma concentration of the individual amino acids, with the exception of glutamate and aspartate, were not significantly affected by this injection (Table 1; Fig. 2).

The fastest R_d was threonine at 21.2 ± 7.7 μmol h^{-1}, 100 g^{-1}, and the slowest R_d was lysine at 0.9 ± 0.1 μmol h^{-1},100 g^{-1} with most amino acids falling between 2 and 8 μmol h^{-1},100 g^{-1}(Table 2). The half-lives ranged from 4.4 to 12.4 min with aspartate having the longest half life and proline the shortest and most amino acids falling in the range of 4–7 min range for $T_{½}$. The pool size ranges from 13 to 516 μmol kg^{-1} with alanine having the largest pool and lysine having the smallest (Table 2). The turnover time ranges from 6.6 to 21.7 min, with aspartate having the longest turnover time and most amino acids turning over within 10 min (Table 2).

Discussion

The present study examines the kinetics of the largest number of amino acids simultaneously examined in any organism. It is also the most detailed analysis of amino acid kinetics in any poikilotherm. The values obtained can be used to establish similarities and differences from mammalian parameters, and allows direct comparisons between amino acids under identical experimental conditions. The

Table 1 A summary of the properties of the amino acid mixture used for bolus injection

Amino acid	Percent ^{15}N labeling of amino acid in mixture as determined by LCMS, ($n = 6 \pm$ SEM)	Molecular weight of ^{15}N amino acid	nmol 10 mg mixture^{-1}	Molar% of total amino acids injected represented by individual amino acid	Plasma concentration (μM) before injection ($n = 5 \pm$ SEM)	Plasma concentration (μM) 1 min after injection ($n = 5 \pm$ SEM)	Experiment significantly different from control concentration at 5% confidence interval?
Mixture injection							
Alanine	86.99 ± 0.70	90.1	3,792	7.41	251 ± 13	259 ± 20	No
Arginine	ND	–	4,111	8.89	15.6 ± 1	20.3 ± 2.2	No
Asparagine	59.07 ± 0.80	134.1	61	0.01	ND	ND	–
Aspartate	95.30 ± 0.36	134.1	13,184	23.86	12.6 ± 7	169 ± 51	Yes
Cystine	ND	–	1,866	0.33	ND	ND	–
Glutamate	91.54 ± 0.10	148.2	5,897	10.09	2.9 ± 0.3	27.7 ± 2.4	Yes
Glutamine*	–	148.2	–	–	76 ± 32	93 ± 10	No
Glycine	99.36 ± 0.01	76.1	4,724	10.58	587 ± 281	708 ± 110	No
Histidine	99.90 ± 0.02	158.1	181	0.28	45 ± 5	81 ± 11	No
Isoleucine	98.51 ± 0.04	132.1	3,082	4.48	179 ± 34	241 ± 23	No
Leucine	97.55 ± 0.16	132.1	1,845	3.11	93 ± 20	182 ± 28	No
Lysine	97.80 ± 0.20	147.1	171	0.12	220 ± 75	267 ± 90	No
Methionine	97.85 ± 0.02	150.2	546	0.89	10 ± 1	11 ± 1	No
Phenylalanine	90.58 ± 0.51	166.2	1,152	1.71	67 ± 43	116 ± 8	No
Proline	98.54 ± 0.02	116.1	2,142	4.26	14 ± 2	32 ± 5	No
Serine	98.79 ± 0.03	106.1	2,454	4.43	207 ± 57	239 ± 24	No
Threonine	98.86 ± 0.11	120.1	3,744	8.24	134 ± 12	178 ± 52	No
Tryptophan	ND	–	–	–	ND	ND	–
Tyrosine	79.55 ± 2.15	182.2	3,032	4.27	44 ± 5	74 ± 7	No
Valine	85.43 ± 1.13	118.1	3,106	6.20	380 ± 20	499 ± 43	No
Total	96.63	–	55,248	100.00	2,496	3,071	No
Solo injection							
Glutamine*	99.23 ± 0.02	148.2	6,844‡	100	76 ± 32	93 ± 10	No

ND not detected. Values are means ± SEM for six determinations

* Note that Gln was injected in an experiment separate to the mixture

‡ nmol mg^{-1} of glutamine

validity of the data involves the following three assumptions: rapid mixing of the label in the plasma, a steady state of metabolite concentration and minimal exchange of the label between amino acids. Rapid mixing can be assumed for these animals, since the total circuit time for the blood of *O. mykiss* is less than 1 min (Bungay et al. 1976; Davis 1970). The concentrations of all but two amino acids satisfy the steady-state requirement. Individual amino acid concentrations were briefly raised, with most returning close to pre-injection values within several minutes (Fig. 2). Glutamate and aspartate concentrations were statistically different after injection (Table 1; Fig. 2), but since changes in concentration of non-essential amino acids have been shown not to alter flux rates in mammals (Bohé et al. 2003), the R_d values for these amino acids are likely to be close approximations of the real rates. In the worst case, the flux rates for these two amino acids are overestimates. The exchange of label between amino acids is unlikely since this would affect the goodness of fit of the second-order kinetic model. All amino acids had r^2 values greater than 0.94. The rapid sampling times would also minimize any label exchange.

There are only two previous studies of plasma amino acid turnover in fish. The first study infused three ^{14}C-labeled amino acids (glutamate, aspartate and alanine) separately via a cannula into the ventral aorta of *Paralabrax* sp. (Bever et al. 1981), but found the decay curves unsuitable for determining kinetic parameters since only three blood samples were taken in the first 60 min. The second study used a continuous infusion of ^{14}C-leucine into the peritoneal cavity of *O. mykiss* and calculated an R_d for leucine in the plasma. The value obtained was about twice that obtained in the present study (19.7 vs. 8.9 μmol h^{-1}, 100 gm^{-1}, respectively). The fish used were sevenfold

smaller than that we used and were sexually immature, which means in all likelihood they would have higher growth rates. If corrected for mass-specific metabolic rate assuming an exponent of 0.6 (Brett and Glass 1973), our larger fish would have a metabolic rate of 0.31 times that of the smaller animals giving an adjusted R_d of 6.1 μmol h^{-1} 100 gm^{-1}, a value much closer to our value of 8.9 μmol h^{-1} 100gm^{-1}.

There were no apparent patterns in the R_d values with respect to the type of amino acid (e.g., essential versus non-essential; acidic versus basic). The R_d values for glutamine were not higher than those of other amino acids in spite of its catabolic importance in fish muscle and heart (Chamberlin et al. 1991).

When compared to mammalian R_d values (Table 3), most of the R_d values we obtained were in the same range as those determined for humans and rabbits. The correspondence is further improved when our values are adjusted for the lower metabolic rate of the fish (Table 3). Notable exceptions are glutamine, which is lower in the fish, and threonine, which is higher. This may be due to the extensive use of glutamine as a nitrogen shuttle in mammals requiring a higher turnover. In fish, the need for constant synthesis of mucin, the major component of mucus (containing 27% threonine) (Lumsden and Ferguson

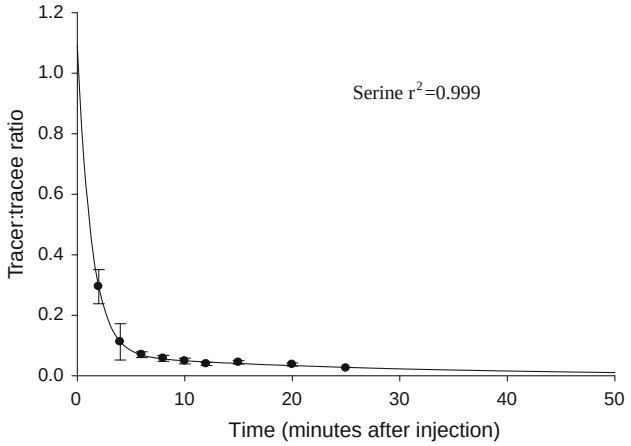

Fig. 1 Representative tracer: tracee ratio decay curve of an amino acid in unfed *O. mykiss* after injection of a bolus of ^{15}N labeled amino acids at a dose of 10 mg amino acid mixture kg^{-1} wet weight. *Fitted line* is a second-order exponential decay curve. Results are means ± SEM for $n = 5$

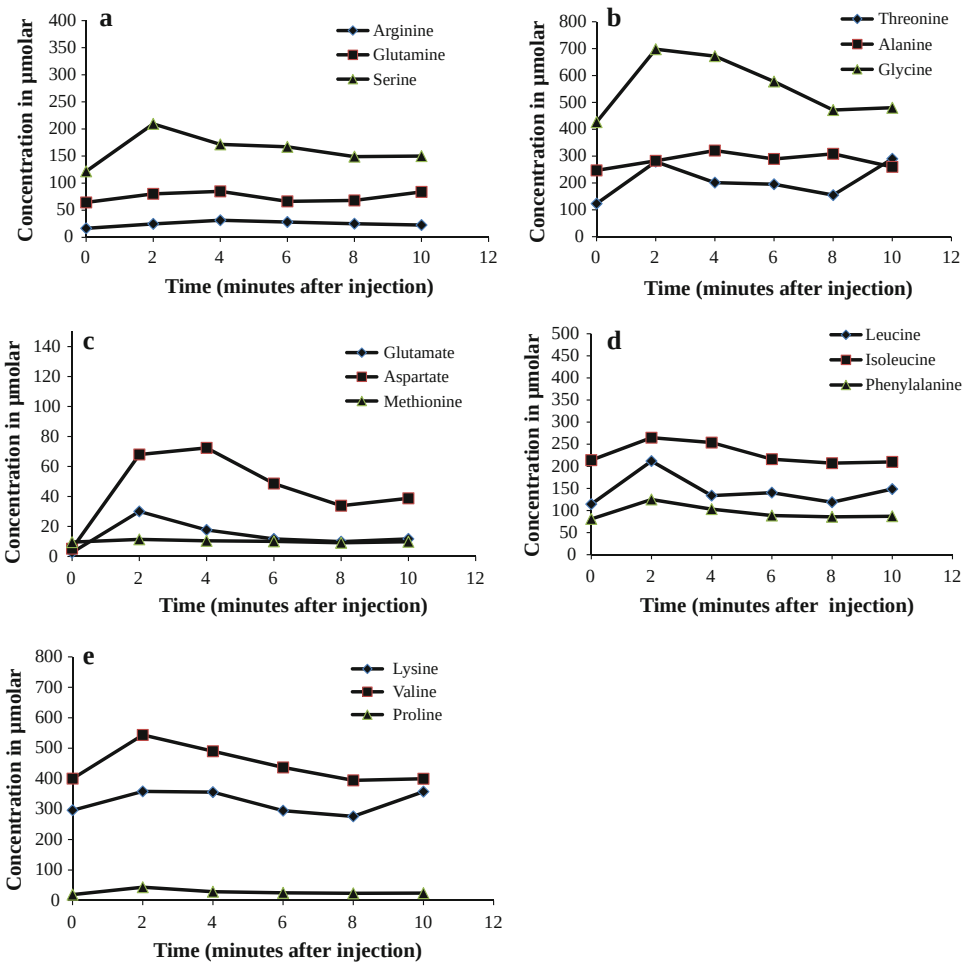

Fig. 2 **a–e** Representative plots of concentrations of individual amino acids after injection of a bolus of ^{15}N labeled amino acids at a rate of 10 mg kg^{-1} wet weight over the time course of the experiment in a single fish. Time 0 represents pre-injection concentrations

Table 2 Kinetic parameters for amino acids in plasma of rainbow trout

Parameter	Q (pool size) (μmol kg^{-1})	R_d (μmol h^{-1}100 g^{-1}) second order	R^2 of line of best fit	Half-life (min)	Turnover time (min)
Amino acid					
Threonine	372 ± 70	21.2 ± 7.7	0.960	6.3 ± 1.9	8.2 ± 2.5
Alanine	516 ± 140	19.9 ± 5.9	0.998	6.5 ± 1.4	8.8 ± 1.8
Valine	214 ± 41	10.8 ± 2.3	0.996	6.0 ± 0.8	8.6 ± 0.9
Leucine	337 ± 85	8.9 ± 1.5	0.988	4.5 ± 1.2	6.6 ± 1.5
Serine	134 ± 52	8.8 ± 2.0	0.999	5.1 ± 1.8	7.6 ± 2.1
Histidine	67 ± 23	5.8 ± 1.0	0.995	7.4 ± 2.6	10.0 ± 3.1
Phenylalanine	67 ± 9	5.6 ± 0.5	0.997	5.0 ± 1.3	7.3 ± 1.6
Glycine	175 ± 62	4.7 ± 1.0	0.947	6.2 ± 2.9	8.1 ± 2.7
Methionine	66 ± 22	4.6 ± 0.7	0.999	5.5 ± 1.8	7.9 ± 2.1
Isoleucine	132 ± 23	4.3 ± 0.6	0.971	4.9 ± 1.5	7.1 ± 1.8
Glutamine	449 ± 172	2.4 ± 0.3	0.971	12.4 ± 5.0	15.9 ± 6.2
Proline	31 ± 8	2.3 ± 0.7	0.998	4.4 ± 1.4	6.6 ± 1.7
Glutamate	21 ± 9	1.5 ± 0.4	0.998	11.3 ± 3.2	17.6 ± 4.0
Aspartate	23 ± 6	1.3 ± 0.3	0.996	14.1 ± 6.3	21.7 ± 7.6
Lysine	13 ± 8	0.9 ± 0.1	0.956	5.9 ± 1.7	8.1 ± 2.1

Values are sorted by R_d from highest to lowest. Essential amino acids are in italics. Values are for $n = 5$ except for lysine where $n = 3$

Table 3 Kinetic parameters of 15 protein-forming free amino acids in plasma of *Oncorhynchus mykiss* (current study), and other animals for reference

Parameter	R_d trout	R_d trout second-order kinetics (current study)	R_d trout corrected for metabolic rate (current study)[k]	R_d human	R_d rabbit
Amino acid					
Threonine		21.2 ± 7.7	61.5 ± 22.1	5.5 ± 0.4[h]	–
Alanine		19.9 ± 5.9	57.1 ± 16.0	33.6–43.2[b]	38.42 ± 7.88[a]
Valine		10.8 ± 2.3	31.3 ± 6.7	8.4 ± 0.5[h]	–
Leucine	19.7[e]	8.9 ± 1.5	25.8 ± 5.0	10.5 ± 0.5[h]	–
Serine		8.8 ± 2.0	25.5 ± 5.8	14.0 ± 1.9[l]	–
Histidine		5.8 ± 1.0	16.8 ± 2.9	–	–
Phenylalanine		5.6 ± 0.5	16.2 ± 1.8	7.8 ± 1.8– 11.9 ± 2.1[d]	–
Glycine		4.7 ± 1.0	13.6 ± 2.9	24.0–49.0[j]	20.3 ± 11.8–94.6 ± 11.2[a]
Methionine		4.6 ± 0.7	13.3 ± 2.0	6.5–8.2[j]	–
Isoleucine		4.3 ± 0.6	12.5 ± 1.5	–	–
Glutamine		2.4 ± 0.5	7.0 ± 1.5	53.9 ± 9.9–79.1 ± 16.2[d]	–
				34.8 ± 3.3[f]	
				27.6–36.6[g]	
Proline		2.3 ± 0.7	6.7 ± 2.0	8.9 ± 0.3[k]	–
				13.5 ± 0.5[m]	
Glutamate		1.5 ± 0.4	4.3 ± 1.1	8.3 ± 2.2[f]	–
Aspartate		1.3 ± 0.3	3.7 ± 1.0	–	–
Lysine		0.9 ± 0.1	2.4 ± 0.2	4.6–13.9[c]	–

Values are means ± SEM for $n = 5$ for *Oncorhynchus mykiss*. All values are expressed as μmol h^{-1} 100 g^{-1}

Calculated from [a] (Nissim and Lapidot 1979), [b] (Martineau et al. 1985), [c] (Meredith et al. 1986), [d] (Parimi et al. 2002), [e] (Fauconneau and Tesseraud 1990), [f] (Darmaun et al. 1986), [g] (Kreider et al. 1997), [h] (Hoffer et al. 1997), [i] (Raguso et al. 1997), [j] (Amir et al. 1980) [k] Metabolic rate for trout taken from (Rao 1968) and metabolic rate for humans taken from (Sagiv et al. 1995), [l] (Gregory et al. 2000), [m] Taken from (Yu et al. 2001)

1994) may require a higher turnover of this amino acid. This may explain why threonine had the highest turnover rate of all the amino acids determined.

In establishing the design of the kinetic parameters of plasma, free amino acid factors such as the concentrations, the pool size, the half life and the turnover time

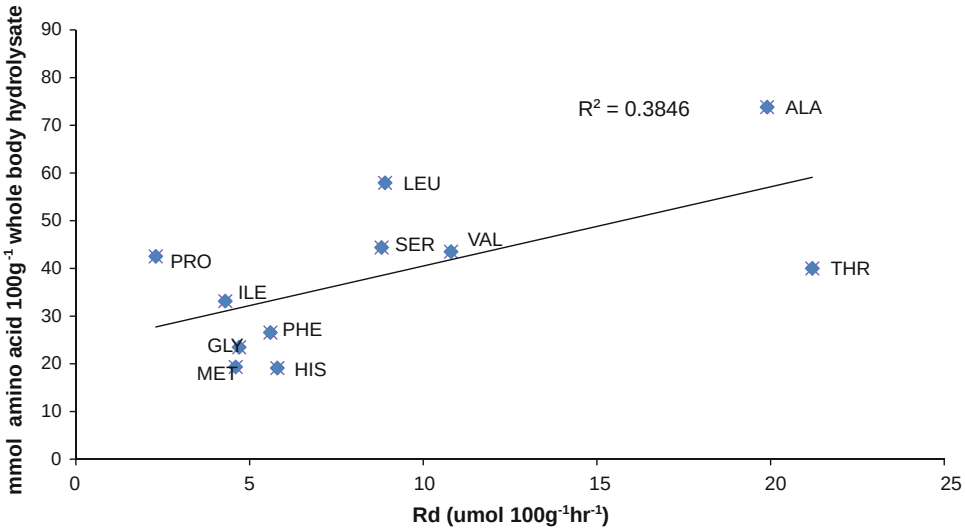

Fig. 3 The relationship between R_d for plasma amino acids and the proportion of those amino acids in whole body protein of rainbow trout. Values for glutamate, glutamine, aspartate and asparagine are excluded since they are either lost or interconverted during hydrolysis of fish for amino acid analysis. Data for whole body hydrolysate was calculated from (Wilson and Cowey 1985)

must be considered. No studies, including the present, have been able to establish correlations between plasma concentration, pool size, half-life or turnover time and protein amino acid content. Previous studies on mammals indicate significant correlations of R_d with the whole body protein amino acid content. In studies of rat (Obled and Arnal 1991) and sheep (Lindsay 1982) plasma, R_d of 5 and 12 amino acids, respectively, were determined and found to correlate with whole body protein. In our study, only 11 amino acids could be used for comparison since glutamate, aspartate, glutamine and asparagine had to be excluded since their proportions in protein were altered by protein lysis methods. The R_d values for these 11 amino acids correlated well ($r^2 = 0.3846$) with whole fish protein amino acid content (Fig. 3), supporting our hypothesis that one of the design features of the plasma-free amino acid delivery system was to allow for the proportional delivery of amino acids to all tissues in such a way as to provide for optimal synthesis of protein for the whole organism.

A poorer correlation was found with muscle (fillet) protein amino acid content ($r^2 = 0.136$ data not shown). Tissue-specific differences in protein amino acid content are obviously important parameters to take into account in the understanding of the delivery system, as is the fractional rate of protein synthesis in each tissue. Muscle accounts for only approximately 33% of whole animal protein synthesis at 10°C (Fauconneau and Arnal 1985), which may partially explain the much lower r^2. Whole animal protein synthesis in animals approximately the same size as in our experiment (800 vs. 964) at 10°C is approximately 500 mg day^{-1} (Fauconneau and Arnal 1985). Using the mean R_d value ($R_{d(av)} = 6.87$) for the amino acids we measured and assuming similar R_d values for those not measured and a mean MW of 115 for the amino acids, we calculated that the average trout in our experiment could transport approximately 3.0 g of amino acids day^{-1} or approximately sixfold that required strictly for protein synthesis ($R_{d(av)}$ 6.87 μmol h^{-1}100 g^{-1} × 20 amino acids × 115 g mol^{-1} × 24 h day^{-1} × 800 g = 3.0 g amino acid day^{-1}). Clearly, there is ample capacity for supplying amino acids for protein synthesis, as well as for other physiological processes that utilize amino acids.

The similar correlations between plasma R_d and whole body protein content of both fish and mammals, in spite of large differences in parameters such as plasma concentrations, indicate a common theme in the design of the amino acid delivery system in vertebrates that likely extends to all multicellular organisms.

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