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Postprandial changes in plasma free amino acid levels obtained simultaneously from the hepatic portal vein and the dorsal aorta in rainbow trout (*Oncorhynchus mykiss*)

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Summary

For the first time, changes in plasma concentrations of free amino acid (AA) and their metabolites were followed simultaneously in pre- and post-hepatic blood following a single meal in non-anaesthetized and free-swimming fish. Rainbow trout (*Oncorhynchus mykiss*), kept in 10°C water and fitted with cannulae in the hepatic portal vein (HPV) and the dorsal aorta (DA), were force-fed 1% of their body mass and blood samples were taken from both cannulae at 0, 3, 6, 12, 24 and 48 h postprandially to follow the free AA profile. Almost all free AAs increased rapidly within the first 3 h and only a few free AAs did not change significantly over time. By 6 h, the total free AA concentration had peaked in blood taken from both the DA (7107±369 nmol ml^{-1}) and HPV (9999±572 nmol ml^{-1}). However, individual free AAs showed three main profiles beyond this time: for type I, a peak concentration occurred only at 6 h; for type II, there was a more gradual rise in concentration to a peak at 24 h; and for type III there were two peaks, at 6 h and 24 h. All free AAs returned to or were lower than baseline levels within 48 h, with the exception of threonine and proline. The total free AA concentrations were consistently higher (P<0.05) in the HPV than in the DA at 3 h, 6 h, 12 h and 24 h. Our data provide clear evidence that, during the first pass through the liver, hepatic modification altered individual free AA concentrations as indicated by variable ratios among the simultaneous blood samples. Furthermore, the elevation of ammonium and urea in the HPV indicates intestinal catabolism of ingested free AA before release into the HPV. In conclusion, the dual HPV and DA cannulation shows promise as a useful technique for qualitative and quantitative investigations of absorption and turnover of nutrients, especially if the measurements can be combined with reliable estimates of blood flow and labelled substances.

Key words: Hepatic portal vein cannulation, plasma free amino acids, urea, ammonium, digestibility, protein metabolism, rainbow trout, *Oncorhynchus mykiss.*

Introduction

Most research into fish nutrition has involved either growth or digestibility trials (see Halver and Hardy, 2002). Although these studies are useful from a fish production point of view, they provide little insight into specific rates of nutrient absorption *via* the intestine and metabolism by the liver. A few studies have therefore made attempts to follow postprandial nutrient uptake by serially sampling blood from slaughtered fish (reviewed by Carter et al., 2001). However, such studies may yield ambiguous results because of several shortcomings. There is the obvious sampling effect of terminal blood sampling techniques which stress fish and greatly alter gut blood flow (Thorarensen et al., 1993). In addition, a high individual variation of plasma nutrients may mask anything but major changes. Sunde et al. (Sunde et al., 2003) tried to circumvent these obstacles by sampling blood from the dorsal aorta (DA) *via* a permanent cannula in free-swimming fish. By measuring postprandial free amino acid (AA) concentrations in the blood of fish given different diets, they revealed differences in feed quality not easily discovered with traditional growth rate-based experiments. However, a limitation of using DA blood samples is that the blood metabolites may have undergone hepatic modification *en route* from the intestine to the DA.

Blood leaving the gut *via* the hepatic portal vein (HPV) first passes through the liver, and, given the central role of the liver

in the AA metabolism of higher vertebrates (McDonald et al., 2002), differences in the pre- and post-liver profiles of plasma free AAs are to be expected in fish. In fact, it is estimated that 20–50% of the free AAs entering the blood never get past the liver (Hoerr et al., 1991; Hoerr et al.,1993; Biolo et al., 1992; Matthews et al., 1993). Lyndon et al. tried to estimate the pre- and post-hepatic free AA plasma levels by serial slaughter of cod (*Gadus morhua*) and sampling of blood from HPV and cardiac puncture immediately after death (Lyndon et al., 1993). Ash et al. (Ash et al., 1998) sampled blood using a double DA and HPV cannulation technique (McLean and Ash, 1989) from lightly sedated fish but made only one measurement 3 h postprandially. Thus, to date reliable information does not exist on the time course of the postprandial free AA changes in plasma in fish or on the degree of hepatic modification of free AA during their first pass through the liver after uptake by the intestine. By combining the DA and HPV cannulation techniques, the present study is a first attempt to follow, over time, the postprandial free AA profiles in plasma simultaneously collected before and after the liver in anaesthetized and free-swimming fish. Furthermore, by simultaneously sampling blood from both the DA and the HPV, we can test the hypothesis that hepatic metabolism of free AAs during their first pass through the liver is possible in rainbow trout. Two different dietary treatments were used to assess the effect of varying dietary amino acid composition on the free AA plasma profile. In addition to free AAs, plasma levels of the metabolites urea and ammonium were analysed to evaluate the potential for intestinal AA catabolism. Therefore, the present study provides the first comprehensive study of postprandial AA uptake and immediate metabolic processing of AAs in a fish.

Materials and methods

Rainbow trout *Oncorhynchus mykiss* Walbaum (average body mass, 1098 g; range, 695–1483 g) were placed in six 1 m^3 tanks divided along their diagonal axis, with one fish in each compartment (N=12). Each compartment was equipped with a ‘hide’ that consisted of a 200×400 mm cover 50 mm above the water surface. Fully aerated, well water (10°C; 2 l min^{-1}) was directed horizontally to create a steady current along the outside of the compartment. Daylight fluorescent tubes provided continuous illumination.

Fish were starved for 24-48 h prior to surgery. Cannulation was performed on anaesthetized fish (0.1 g l^{-1} MS-222; tricaine methane sulphonate). The DA cannulation was performed according to the method of Soivio et al. (Soivio et al., 1975) and included adjustments described (Kiessling et al., 1995; Kiessling et al., 2003). The HPV cannulation has been described in detail elsewhere (Eliason et al., 2006). After at least 24 h of recovery from surgery, the fish were sedated (0.1 g l^{-1} MS-222) and force-fed a single meal of 1% of their body mass by intubation using a stiff PVC tube with rounded tip. The diets were ground through a 0.5 mm screen, and analysed for dry matter (EC, 1971b), ash (EC, 1971a), crude protein (EC, 1993), crude fat (EC, 1998), starch (McCleary et al., 1994), non-starch polysaccharides (Lee et al., 1992) and amino acid composition (EC, 1998). The experimental design involved two diets (six fish were tested on each diet), with diet 1 containing fish meal as the only protein source and diet 2 containing 20% corn gluten as a partial replacement for fish meal. Nevertheless, this adjustment produced only very small differences in the total and individual dietary AAs between the two diets (Tables 1 and 2). Perhaps as a result, no significant effect (P>0.05), or any tendencies (P>0.15) were found between the diets for the resulting plasma free AA profiles. Therefore, plasma free AA data for the two diets were pooled for the analysis presented here.

Table 1. *Dietary ingredient composition and proximate analysis*

	Ingredients (% of diet)	
	Fishmeal diet	Corn gluten diet
Fish meal	50.51	34.00
Wheat starch	9.10	5.94
Cellulose	10.50	9.31
Corn gluten EU	–	19.61
Standard fish oil	25.47	25.38
Vitamin	0.32	0.32
Carophyll Pink CWS 10%	0.03	0.03
Mono-calcium phosphate	0.46	1.29
DL-Methionine	–	0.13
	Proximate analysis (%)	
Dry matter	95	95
Ash	8	6
Fat	30.0	30.0
Protein (N×6.25)	36.5	37.0
Starch	8.00	8.30
Non-starch carbohydrates	10.00	10.40

Blood samples (0.4–0.5 ml) were taken from both cannulae immediately prior to force-feeding (0 h), and at 3, 6, 12, 24 and 48 h postprandially. Whole blood was centrifuged at 500 ***g*** for 5 min, and plasma was removed and immediately frozen at –20°C prior to storage within hours at –80°C until analysis. The status of the fish was monitored visually and with measurements of haematocrit and leucocrit from the blood samples. Plasma levels of alanine aminotransferase (ALAT; hepatocyte specific) and aspartate aminotransferase (ASAT; cardiac, hepatic but also general tissue unspecific) were analysed with standardized methods for measurement of enzymes according to the International Federation of Clinical Chemistry (Bergmeyer et al., 1986a; Bergmeyer et al., 1986b) on a Konelab 30 analyser using kit no. 981769 and 981771 for ALAT and ASAT (Thermo Electron Corp., Vantaa, Finland), respectively, as indicators of liver damage (Table 3).

The concentrations of free AA in plasma samples were analysed by ion exchange chromatography on a lithium high performance column (Biochrom Ltd, Cambridge, UK) in an

Table 2. *Dietary amino acid composition*

Essential amino acids[a]	Fishmeal diet	Corn gluten diet	Non-essential amino acids[a]	Fishmeal diet	Corn gluten diet
Arginine	5.36	4.80	Alanine	4.97	5.67
Histidine	2.18	2.11	Aspartic acid	8.17	7.45
Isoleucine	3.76	3.77	Cysteine	0.84	1.04
Leucine	6.27	8.75	Glutamic acid	11.95	14.20
Lysine	6.80	5.30	Glysine	4.74	4.00
Methionine	2.54	2.54	Hydroxyproline	0.80	0.56
Phenylalanine	3.47	4.16	Proline	3.33	4.74
Threonine	3.54	3.37	Serine	3.72	4.07
Tryptophan	0.46	0.42	Tyrosine	27.34	20.72
Valine	4.32	4.29	Taurine[b]	2.78	3.49
Sum EAAs	38.71	39.52	Sum total[c]	80.00	84.72

[a]Water-corrected amino acid; g 100 g^{-1} crude protein, unless otherwise stated.
[b]Taurine; g kg^{-1} of feed.
[c]Sum of all AAs, excluding taurine.
EAAs, essential amino acids.

automated amino acid analyser (Biochrom 30, Biochrom Ltd), using lithium-based eluents and post-column derivatization with ninhydrin (Physiological Fluid Chemical Kit, Biochrom Ltd). Data were analysed against external standards (Sigma amino acid standard solutions: acidics, neutrals and basics, supplemented with glutamine, tryptophan and S-2-aminoethyl-1-cysteine; all purchased from Sigma Chemical, St. Louis, MO, USA) using the Chromeleon® Chromatography Management Software (Dionex Ltd, Surrey, UK).

Plasma (80 μl) was deproteinized by mixing with 8 μl of 35% sulfosalicylic acid solution. The mixture was incubated at 4°C for 20 min and centrifuged at 16 000 ***g*** for 15 min (Biofuge Fresco, Heraeus Instruments, Kendro Laboratory Products GmbH, Hanau, Germany). Of the supernatants, 60 μl were diluted with 60 μl 0.2 mol l^{-1} lithium citrate loading buffer, pH 2.2 (Biochrom Ltd) and micro-filtrated (0.2 μm Spartan membrane filter, Schleicher & Schuell, Dassel, Germany) prior to injection (30 μl). Some supernatants were stored at –80°C until analysis. S-2-aminoethyl-1-cysteine was used as an internal standard.

Data were analysed statistically using the Statistical Analysis System for Windows, Version 8.2 (SAS, 2002). The effects of the main variables, i.e. sample time and vessel, were tested by a main factorial model (GLM procedure for unbalanced data). Fish was included as a discrete variable. Groups were compared by the *ad hoc* variance test (*F*-test) using the least-squares means procedure when significant effects were found in the main model. All data were tested for normality by a normal probability plot (proc univariate plot). $P<0.05$ was considered to be statistically significant.

Results

There were no visual signs of ill health or infection of the wound and leucocrit remained normal during the 48 h experimental period. The minor changes in both ALAT and ASAT (Table 3) are not indicative of major tissue damage.

Table 3. *Alanine aminotransferase and aspartate aminotransferase changes* in blood plasma collected at 0 (before feeding) and postprandially at 3, 6, 12, 24 and 48 h from free-swimming rainbow trout*

Postprandial time (h)	ALAT* (ls mean ± s.e.m.)		ASAT* (ls mean ± s.e.m.)	
0	0.37 a	0.02	10.8 a	0.6
3	0.41 a	0.02	12.5 b	0.6
6	0.39 a	0.02	11.2 a,b	0.6
12	0.38 a	0.02	11.4 a,b	0.6
24	0.35 a	0.03	10.5 a	0.8
48	0.26 b	0.02	9.7 a	0.6

*μCat l^{-1}; conversion factor, 2 i.u. l^{-1}=0.03 μCat l^{-1}, supplied by the manufacturer.

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

The values are the combined result of synchronic samples from both the hepatic portal vein and dorsal aorta (*P*-value for difference between vessel was 0.74 for ALAT and 0.18 for ASAT) expressed as ls-mean using sample time (*P*-value for difference between sample times was 0.001 for ALAT and 0.08 for ASAT) and fish (*P*-value for difference between individual fish was <0.0001 for ALAT and <0.0001 for ASAT) as discrete variables in the statistical model.

The same lower case letter denotes a non-significant difference ($P>0.05$, using the *F*-distribution *ad-hoc* test) between values obtained at the different sample times.

There was a definitive appearance profile for the free AA in the plasma from both the HPV and DA (Fig. 1). A postprandial peak of the total free AA concentration occurred at 6 h (DA: 7107±369 nmol ml^{-1} and HPV: 9999±572 nmol ml^{-1}). For most individual free AAs, the plasma concentrations changed

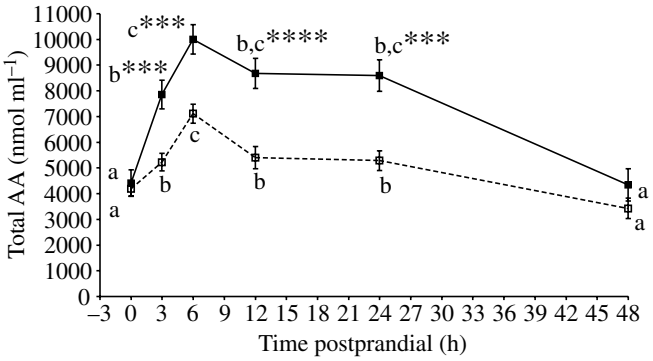

Fig. 1. Total free amino acid (AA) levels in blood plasma (nmol ml^{-1}; means ± s.e.m.) collected synchronically from the hepatic portal vein (HPV; black squares) and dorsal aorta (DA; white squares) in free-swimming rainbow trout after a single meal. *Statistically significant difference (P<0.05, **P<0.01, ***P<0.0001) between the DA and HPV values at a given sample time. Values from the same vessel followed by the same letter do not differ significantly (P>0.05).

over time (P<0.05), more so with the HPV samples than the DA samples (Tables 4–6). All free AAs, with the exceptions of threonine (Table 4) and proline (Table 5) returned to, or below, baseline levels within 48 h.

There were three main profiles for the individual free AAs that changed postprandially. All three profiles were characterized by an initial increase within 3 h (Fig. 2). Then in the type I profile (Fig. 2A), there was a single peak at 6 h; in the type II profile (Fig. 2B), there was a more gradual rise in concentration to a peak at 24 h; and in the type III profile (Fig. 2C), two peaks occurred, one at 6 h and another at 24 h. The free AAs that did not change significantly were aspartic acid (Table 5), alpha-aminoadipic acid, beta-alanine, homocystein and phosphoserine (Table 6).

If there was no hepatic metabolism of the absorbed free AAs during their first pass through the liver, the ratio of HPV to DA free AA concentrations would be constant both over time and among individual free AAs, but this was not the case. The total free AA concentrations were consistently higher (P<0.05) in the HPV than in the DA at 3, 6, 12 and 24 h (Fig. 1). However, a few non-essential free AAs did not differ between the two sample sites (Tables 5 and 6). The highest relative difference (calculated as percentage difference between DA and HPV values, as given in Tables 4–6) of an individual AA was that of serine (62%) at 24 h (Table 4), but this occurred well after the peak uptake of serine at 6 h. The lowest significant difference was that of glutamic acid, which had a peak hepatic absorption of only 25% difference in the 3-h sample (Table 4). Despite the fact that the removal of serine and glutamic acid differed greatly, the liver absorption of both of these AAs stayed elevated throughout the entire 48 h postprandial period. The hepatic removal of some of the type I profile AAs (taurine, isoleucine, lysine, arginine and glutamine) stayed elevated for a considerable period of time, thus the 48 h postprandial

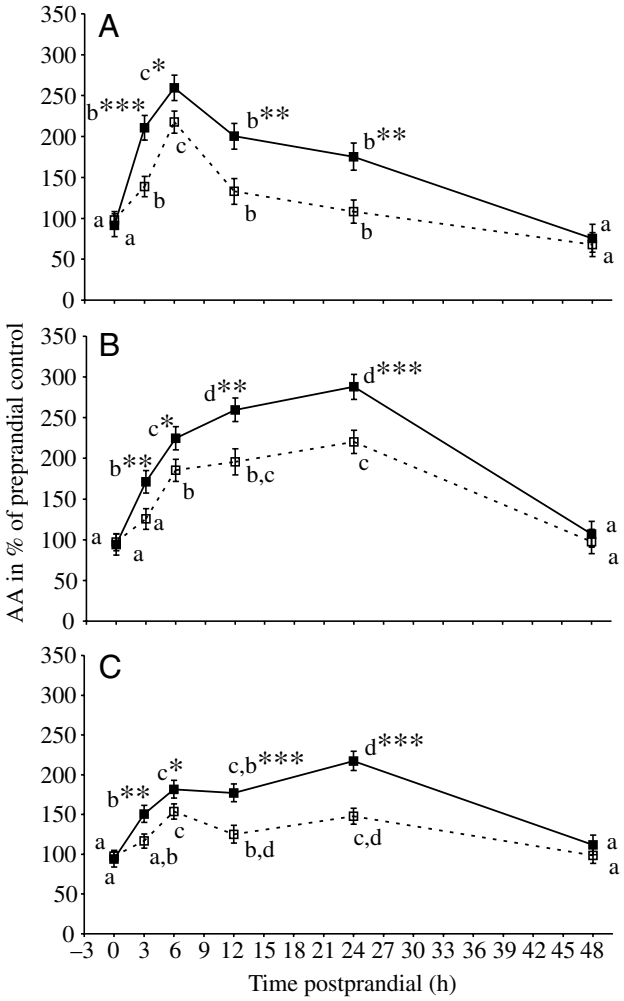

Fig. 2. Free amino acid (AA) levels in blood plasma (% of 0 h samples; means ± s.e.m.) collected synchronically from the hepatic portal vein (HPV; black squares) and dorsal aorta (DA; white squares) in free-swimming rainbow trout after a single meal. The major AA uptake patterns are represented by the sum of taurine, glutamic acid, glutamine, glycine, alanine, lysine, arginine and serine (type I; A), the sum of valine, cysteine, methionine, isoleucine, leucine, tyrosine and phenylalanine (type II; B) and the sum of threonine and tryptophan (type III; C). *Statistically significant difference (*P<0.05, **P<0.01, ***P<0.001) between the DA and HPV values at a given sample time. Values from the same vessel followed by the same letter do not differ significantly (P>0.05).

concentration in the DA was lower than before feeding (Tables 4 and 5).

A postprandial peak in the AA metabolites (ammonia and urea) was anticipated for the DA blood samples (Fig. 3). However, a significant elevation in plasma ammonia and urea in the HPV compared with the DA blood samples was also found (Fig. 3). Whereas plasma levels of urea were identical in the DA and HPV at the start of the experiment, they diverged with time, such that there was a significantly higher plasma concentration in the HPV 12 h compared with the DA, before returning to similar levels (Fig. 3). By contrast, the plasma

Table 4. *Essential free amino acid concentrations in blood plasma collected synchronously at 0 h (before feeding) and postprandially at 3, 6, 12, 24 and 48 h, from the hepatic portal vein and dorsal aorta in free-swimming rainbow trout after a single meal*

		[Essential free AA] (nmol ml^{-1})							
Essential amino acids	Cannula	Sample time (h) 0	3	6	12	24	48	*P* value Time	Cannula
Arginine	DA	84±12 a	144±14 b,d**	213±15 c***	140±18 d*	112±16 a,d**	28±17 e	<0.0001	<0.0001
	HPV	95±26 a	266±28 b	382±29 c	271±29 b	257±31 b	49±32 a	<0.0001	
Histidine	DA	135±7 a	107±9 b*	111±9 b	66±11 c*	66±9 c**	62±10c	<0.0001	<0.0001
	HPV	137±10 a,b	144±11 a	141±11 a	107±11 b,c	124±12 a,b	82±12 c	0.0013	
Isoleucine	DA	194±14 a,c	225±16 a*	314±18 b*	329±21 b*	341±19 b**	152±19 c	<0.0001	<0.0001
	HPV	217±26 a	327±28 b	422±29 c	474±29 c,d	509±31 d	199±32 a	<0.0001	
Leucine	DA	328±38 a	419±45 a*	621±49 b*	721±57 b,c*	821±51 c*	404±53 a	<0.0001	<0.0001
	HPV	373±57 a	608±62 b	850±63 c	994±64 c,d	1096±68 d	468±70 a,b	<0.0001	
Lysine	DA	350±31 a,c	437±37 a,b*	519±40 b**	288±47 c*	266±42 c**	150±44 d	<0.0001	<0.0001
	HPV	362±50 a	635±54 b,c	764±59 b	559±57 c	568±60 c	245±61 a	<0.0001	
Methionine	DA	56±8 a	96±9 b**	154±10 c***	177±12 c,d*	195±11 d**	76±11 a,b	<0.0001	<0.0001
	HPV	61±13 a	152±14 b	228±15 c	245±15 c,d	273±16 d	92±16 a	<0.0001	
Phenylalanine	DA	84±6 a	95±7 a**	129±8 b***	133±10 b***	157±8 c***	85±9 a	<0.0001	<0.0001
	HPV	90±12 a	143±13 b	182±13 c	196±13 c	238±14 d	106±14 a	<0.0001	
Threonine	DA	226±19 a,e	278±23 a,c*	394±25 b,d,e*	324±29 c,d**	365±26 d**	288±27 e	<0.0001	<0.0001
	HPV	234±28 a	403±31 b	533±32 c	496±32 c	562±34 c	346±35 b	<0.0001	
Tryptophan	DA	20±1 a,b	21±1 a,b	22±2 a*	17±2 b,c**	23±2 a***	15±2 c	0.0022	<0.0001
	HPV	19±2 a	25±2 b	28±2 b	27±2 b	37±2 c	19±2 a	<0.0001	
Valine	DA	419±26 a,c	468±31 a*	642±39 b	647±39 b*	688±35 b*	350±36 c	<0.0001	<0.0001
	HPV	449±44 a	639±48 b	806±45 c	889±50 c	926±53 c	423±54 a	<0.0001	
No. of samples	DA	12	12	12	12	10	10		
	HPV	12	12	11	8	10	9		

Values are means ± s.e.m.

HPV, hepatic portal vein; DA, dorsal aorta.

Asterisks indicate statistically significant difference (*P<0.05, **P<0.01, ***P<0.0001) between the DA and HPV values at a given sample time. Values from the same vessel followed by the same letter do not differ significantly (P>0.05).

ammonia levels were always significantly higher in the HPV than in the DA. This difference was about twofold at the start and end of the experiment, but increased to over fourfold for most of the postprandial period (6–24 h; Fig. 3) when the free AA uptake was occurring (Fig. 2).

Discussion

Experimental considerations

The dorsal aortic artery cannulation technique in fish has become a standard procedure for short-term blood sampling in laboratory experiments since its introduction more than half a century ago (see Soivio et al., 1975). Since its establishment as a method for long-term nutrition studies in the mid-1990s (Kiessling et al., 1995), it has been standardized, well characterized for its physiological components and acknowledged as yielding representative data of a fish in a normal physiological state (B. Djordjevic, T. Kristensen, Ø. Øverli, B. O. Rosseland and A. Kiessling, manuscript submitted for publication). Fish with a DA cannulation seem to recover their physiological and nutritional status within 24 h post-surgery (Kiessling et al., 1995; Kiessling et al., 2003) (B. Djordjevic, T. Kristensen, Ø. Øverli, B. O. Rosseland and A. Kiessling, manuscript submitted for publication). In contrast to the DA cannulation technique, HPV cannulation in fish is more difficult and intrusive (McLean and Ash, 1989), but the recovery time is similar, with blood cortisol, glucose, ion, pH, CO_2 and haematocrit values being restored during the first 24 h (Eliason et al., 2007). We were pleased to observe normal haematocrit, leucocrit, and stable ALAT and ASAT values for the duration of our 48-h experiments, as well as clear evidence of digestion, nutrient absorption from the intestine, and AA metabolism by the liver with the staged, double cannulation technique used here.

The present study clearly shows that plasma free AA measurements in the HPV provide a much greater resolution of

Table 5. *Non-essential free amino acid concentrations in blood plasma collected synchronously at 0 h (before feeding), and postprandially at 3, 6, 12, 24 and 48 h, from the hepatic portal vein and dorsal aorta in free-swimming rainbow trout after a single meal*

		[Non-essential free AA] (nmol ml^{-1})							
Non-essential amino acids	Cannula	Sample time (h) 0	3	6	12	24	48	*P* value Time	Cannula
Alanine	DA	628±57 a,c	865±67 b**	1014±73 b*	464±86 c*	475±76 c*	544±79 c	<0.0001	<0.0001
	HPV	629±85 a	1273±92 b	1387±95 b	890±96 c,d	929±102 c	654±104 a,d	<0.0001	
Aspartic acid	DA	8±0 a,c	8±1 a,c	9±1 a	7±1 b*	7±1 b,c	8±1 a,b	0.0452	0.1061
	HPV	9±1 a,b	10±1 a	10±1 a	9±1 a,b	8±1 b	9±1 a,b	0.1392	
Cysteine	DA	8±0 a	9±0 a	10±1 b	9±1 a,b**	10±1 b*	7±1 c	0.0003	<0.0001
	HPV	8±1 a,b	10±1 a	12±1 b,c	13±1 c,d	14±1 d	7±1 a	<0.0001	
Glutamic acid	DA	28±3 a,d	35±3 a	49±3 b	34±4 a,c	29±3 a,d	22±4 d	<0.0001	0.0993
	HPV	30±4 a	47±4 b,c	56±4 b	44±4 c,d	35±4 a,d	24±4 a,d	<0.0001	
Glycine	DA	413±33 a,c	505±39 a*	669±42 b	411±50 a,c*	353±44 c*	312±46 c	<0.0001	<0.0001
	HPV	405±46 a	732±50 b,d	867±51 c	690±52 d	629±55 d	395±56 a	<0.0001	
Hydroxylysine	DA	4±1 a	6±1 a,c	12±1 b	8±2 b,c*	8±1 c	3±2 a	0.0003	0.2813
	HPV	3±1 a	9±1 b,d	14±1 c	15±2 c	12±2 c,d	4±2 a	<0.0001	
Proline	DA	45±16 a	115±19 b**	181±20 c***	141±24 b,c**	157±21 b,c**	142±22 b,c	<0.0001	<0.0001
	HPV	49±26 a	205±28 b	340±29 c	278±30 b,c	298±31 c	161±32 b	<0.0001	
Serine	DA	105±14 a,e	160±17 b,d***	243±18 c***	162±21 d***	132±19 d,e***	71±19 a,f	<0.0001	<0.0001
	HPV	113±33 a	327±35 b,d	496±36 c	376±37 d	345±39 d	123±40 a	<0.0001	
Taurine	DA	292±41 a,d	417±49 a	655±53 b	393±62 a,c	183±55 d,e	136±58 e	<0.0001	0.3093
	HPV	332±67 a	621±73 b,d	826±75 c	551±76 d	193±80 a	155±82 a	<0.0001	
Tyrosine	DA	65±9 a	87±11 a*	128±12 b	136±14 b,c	165±13 c**	57±13 a	<0.0001	<0.0001
	HPV	66±11 a	124±12 b	162±12 c	178±13 c	225±13 d	72±14 a	<0.0001	

Values are means ± s.e.m.

Asterisks indicate statistically significant difference (*P<0.05, **P<0.01, ***P<0.0001) between the DA and HPV values at a given sample time. Values from the same vessel followed by the same letter do not differ significantly (P>0.05).

the uptake profiles than do DA measurements, presumably because of hepatic metabolism. The free AA concentrations reported here for DA samples are comparable to earlier studies where only the DA was cannulated (Ok et al., 2001; Sunde et al., 2003). In general, our data also agree with the earlier study of Ash et al. (Ash et al., 1989), who measured the postprandial concentrations of free AA in both the DA and HPV of lightly anaesthetized rainbow trout, but only at 3 h. They found a much smaller increase from the base line (48 h starved fish) at 3 h and smaller differences between the DA and HPV samples compared with the present study. However, beyond general comparison with this earlier study, detailed comparisons are complicated by the reporting of blood rather than plasma concentrations and by measuring baseline and postprandial values in different sets of fish. Also, Ash et al. occluded a major route from intestine to HPV by cannulating the intestinal vein,

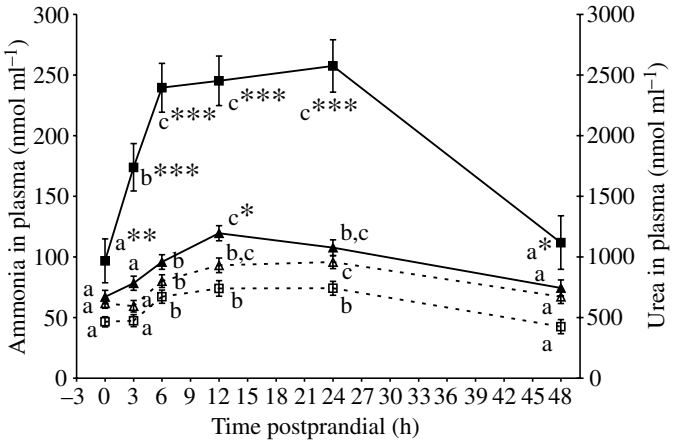

Fig. 3. Postprandial concentrations (nmol ml^{-1}; mean ± s.e.m.) of ammonia and urea in blood plasma collected synchronically from the hepatic portal vein (HVP) and dorsal aorta (DA) in free-swimming rainbow trout after a single meal [ammonia in HPV (black squares), ammonia in DA (white squares), urea in HVP (black triangles) and urea in DA (white triangles)]. *Statistically significant difference (*P<0.05, **P<0.01, ***P<0.001) between the DA and HPV values at a given sample time for either ammonia or urea. Values from the same vessel followed by the same letter do not differ significantly (P>0.05).

Table 6. *Endogen (not present in diet) non-essential free amino acid concentrations in blood plasma collected synchronously at 0 h (before feeding), and postprandially at 3, 6, 12, 24 and 48 h, from the hepatic portal vein and dorsal aorta in free-swimming rainbow trout after a single meal*

Endogenously formed non-essential amino acids	Endogen [non-essential free AA] (nmol ml^{-1})								
		Sample time (h)						*P* value	
	Cannula	0	3	6	12	24	48	Time	Cannula
1-Methylhistidine	DA	9±3 a,d	20±3 b*	39±4 c*	31±4 c	16±4 a,b	4±4 d	<0.0001	0.0171
	HPV	7±4 a	36±5 b,c	55±5 c	48±5 c	22±5 d	7±5 a,d	<0.0001	
alpha-Aminoadipic acid	DA	17±1 a,b	14±2 a,b	18±2 a	13±2 b	15±2 a,b	16±2 a,b	0.1841	0.3552
	HPV	15±1 a	18±1 a	18±1 a	16±1 a	15±1 a	14±1 a	0.2369	
alpha-Amino-*n*-butyric acid	DA	29±2 a	26±2 a	32±2 a,b	26±3 a	32±3 a,b	37±3 b	0.0171	0.0101
	HPV	30±2 a,c	33±3 a,b,c	38±3 b,d	37±3 a,b,d	42±3 c,e	42±3 d,e	0.0177	
Aspargine	DA	129±13 a,c,d	145±15 a,c**	209±17 b***	150±19 c**	145±17 c***	94±18 d	0.0003	<0.0001
	HPV	134±20 a	234±22 b	320±22 c	269±22 b,c	299±24 c	123±24 a	<0.0001	
beta-Alanine	DA	31±3 a	26±4 a,b	34±4 a,c	27±5 a,b*	16±5 b	22±5 a,b	0.051	0.7600
	HPV	22±5 a	36±5 b,c	43±5 b	44±5 b	21±6 a	22±6 a,c	0.0009	
Citrulline	DA	18±1 a,c	20±1 a	28±1 b	30±2 b	28±2 b	14±2 c	<0.0001	0.2927
	HPV	16±2 a	24±2 b,d	30±2 c,d	35±2 c	29±2 d	15±2 a	<0.0001	
gamma-Amino-butyric acid	DA	11±1 a,b	9±1 a	12±1 b	11±1 a,b	12±1 b	10±1 a,b	0.083	0.1933
	HPV	10±1 a	12±1 a,c	14±1 b	15±1 b	13±1 b,c	10±1 a	0.0005	
Glutamine	DA	271±23 a	281±27 a*	393±29 b*	278±34 a,c*	252±30 a,c**	158±32 d	<0.0001	<0.0001
	HPV	281±30 a	415±33 b,d	530±34 c	442±34 d	430±36 d	220±37 a	<0.0001	
Homocystine	DA	23±1 a	19±2 a	22±2 a	18±2 a	20±2 a	18±2 a	0.2523	0.8220
	HPV	21±1 a,b	21±2 a,b	22±2 a,b	24±2 a	23±2 a,b	19±2 b	0.2394	
Hydroxyproline	DA	38±4 a	43±5 a	76±5 b	70±6 b,c*	59±5 c,d	47±6 a,d	<0.0001	0.0039
	HPV	35±7 a	62±8 b,d	98±8 c,d	112±8 c	83±9 d	55±9 a,b	<0.0001	
Ornitine	DA	51±3 a	57±4 a	74±4 b	47±5 a,c,d	47±5 a,c	34±5 d	<0.0001	0.0646
	HPV	53±4 a,c	74±5 b,c	83±5 b	68±5 c	61±5 c	44±5 a	<0.0001	
Phosphoserine	DA	12±1 a	10±2 a	11±2 a	10±2 a	10±2 a	11±2 a	0.9037	0.5441
	HPV	10±2 a	11±2 a	13±2 a	13±2 a	14±2 a	13±2 a	0.6856	

Values are means ± s.e.m.

Asterisks indicate statistically significant difference (*P<0.05, **P<0.01, ***P<0.0001) between the DA and HPV values at a given sample time. Values from the same vessel followed by the same letter do not differ significantly (P>0.05).

whereas we minimized vessel occlusion by cannulating a smaller side vein off the main dorsal and ventral intestinal veins (Ash et al., 1989). Since the concentration of free AAs in the HPV is probably a combination of the rates of intestinal blood flow and AA uptake, alterations to intestinal blood flow could alter free AA concentrations. Therefore, comparisons with anaesthetized fish are compromised because anaesthetic procedures and handling reduce gut blood flow (Thorarensen et al., 1993; Eliason et al., 2007). Sedatives could also affect AA uptake rates even though Kolanczyk et al. did not find any significant changes in liver enzyme activity after exposure to MS-222 (Kolanczyk et al., 2003).

Ok et al. found that postprandial plasma free AA concentrations in the DA peaked at 4 h, except for glycine (Ok et al., 2001). Here, the total free AA concentration peaked at 6 h, a time frame that agrees with the results reported after serial sampling of slaughtered fish (see Sunde et al., 2003; Espe et al., 1993). This slight delay in the peak of free AA compared with those found by Ok et al. may be related to the warmer temperature (~17°C) used in the experiments of Ok et al. compared with the two subsequent studies (~10°C), although an effect of dietary differences cannot be excluded. Ok et al. used a combination of crystalline amino acids and casein and gelatine (Ok et al., 2001), and crystalline AAs are known to be absorbed quickly (Cowey and Walton, 1988). A more rapid uptake should result in higher and sharper peaks of plasma free AAs.

The three different uptake patterns of free AAs observed in the present study are difficult to confirm. Neither Ash et al. (Ash et al., 1989) nor Sunde et al. (Sunde et al., 2003) used repeated sampling, whereas Ok et al. used an artificial diet, possibly compressing digestion (Ok et al., 2001). Lyndon et al.

found both single and double peaks in uptake profiles for cod (Lyndon et al., 1993), but their data show great divergence for the same free AAs between the two sample sites, suggesting ambiguous results due to serially slaughtering a group of fish to obtain blood samples from the HPV and by cardiac puncture. Espe et al. also used serial slaughter with Atlantic salmon (*Salmo salar*) and reported three uptake profiles using caudal puncture to obtain blood (Espe et al., 1993). A mixed artery–vein sample makes direct comparisons to the present work difficult. In the present work, it was clear that free AA uptake was well underway by 3 h, despite the gavage method, and that the maximum uptake rates, as judged by the HPV concentrations, occurred between 6 and 24 h, depending on the individual AA.

Free amino acid uptake profiles

We observed three different free AA uptake profiles. Different AAs are digested and absorbed by different mechanisms, grossly classified as active and passive carriers and channel-mediated diffusion. In addition, di- and tri-peptides are absorbed independently. In fact, as much as 70–85% of all luminal AAs may be absorbed from the digesta into enterocytes in the form of small peptides (Krehbiel and Matthews, 2003). However, after absorption into the enterocytes, the peptides are further hydrolysed intracellularly. As a result, most of the AAs appearing the hepatic portal vein are free AAs (Krehbiel and Matthews, 2003). Such differences in absorption mechanisms could explain the three profiles observed here. With the exception of cysteine, all the AAs with uptake profiles 2 and 3 (Fig. 2B,C) are known to be taken up by the mammalian gut *via* active transport mechanisms. Similar active transport mechanisms may exist in fish, although they are not yet identified (Smith, 1989; Halver and Hardy, 2002). Thus, if the number of transporters is limited, the uptake profile will have a longer duration. By contrast, AAs absorbed *via* passive carriers and diffusion will be concentration dependent, and small peptides are known to be absorbed more rapidly (Steinhardt and Adibi, 1986; Matthews, 2000; Bogé et al., 2002; Dabrowski et al., 2005).

An alternative explanation for the plasma free AA profiles (Fig. 2A–C) is regional absorption of specific AAs, with the ones displaying a slower peak being absorbed in more distal parts of the intestine. This is a less plausible explanation because several studies indicate that the majority of AA and peptide absorption occurs in the proximal- to mid-intestine (Webb Jr, 1990; Matthews, 2000). The AAs for which there were two peaks (tryptophan and threonine, Fig. 2C) could be an exception in that the second peak could partly reflect reabsorption of AAs from proteolytic enzymes containing significant amounts of tryptophan and threonine in the distal intestine. Also, Umezawa et al. (Umezawa et al., 1985) showed that co-administration of leucine and tryptophan may delay the tryptophan absorption.

Free AA uptake efficiency and metabolism

Finding similar pre- and post-digestion plasma free AA concentrations was an encouraging quality control for the experiments and therefore we feel confident about discussing the ratios of free AA in HPV and DA samples. We expected the HPV concentration to be elevated over the DA concentration because blood returning to the sinus venosus from the hepatic circulation is diluted by other systemic venous return in direct proportion to the relative proportion of hepatic blood flow. Based on available literature (McLean and Ash, 1989; Thorarensen et al., 1993; Eliason, 2006) and personal observations, roughly 30% of cardiac output is channelled *via* the HPV in resting and unfed fish and has been estimated as 12.8 ml min^{-1} kg^{-1} fish (McLean and Ash, 1989). Gut blood flow increases by 60–100% after a meal and approaches 50% of cardiac output (Thorarensen et al., 1993; Eliason, 2006). Had we measured these relative flows in the present experiment, we could have directly calculated uptake efficiency as $([AA_{HPV}]–[AA_{DA}])\times$blood flow)/total intake of AA, and then estimated liver metabolism of individual free AAs from their respective DA and HPV concentrations. Despite the lack of blood flow data, we still found evidence of hepatic and systemic metabolism of free AA because the concentration ratio varied among AAs and over time.

If there was no hepatic metabolism of the absorbed free AAs during their first pass through the liver, the difference between the HPV and DA free AA concentrations would reflect simple dilution by systemic venous return and, based on the above blood flow distributions, a 50% dilution is a reasonable dilution for the postprandial state. Such a dilution can explain the HPV–DA difference for several (e.g. glutamic acid and taurine; Tables 4–6), but not all free AAs. These free AAs also have parallel curves throughout the active absorption phase (3–24 h samples). A complicating factor in this estimate is the possibility of an accumulation of AAs in the plasma as neither the hepatic or systemic tissues completely remove all AA before the blood is returned to either the intestine or the systemic vasculature. In cases where the HPV curve shows a sharper inclination before the maximum peak, compared to the DA curve, we assume that hepatic absorption dominates (e.g. valine, cysteine, tryptofan, threonine, serine; Tables 4 and 5). However, with major removal of AAs by the systemic tissues, such as muscle, the dilution effect will become more prominent, resulting in an increased HPV–DA difference. But such a situation should be signified by a reduction in concentration in the DA rather than by an increase in the concentration in the HPV, especially after the absorption peak, as seen for, e.g. histidine, isoleucine, lysine, arginine, glycine and glutamine (Tables 4 and 5).

An additional novel discovery here is the possibility of intestinal metabolism of AAs before they reach the liver. This conclusion is supported by the fact that not only was the ammonia level higher in the HPV than the DA, but that the difference between the two blood sampling sites increased during free AA absorption such that the plasma ammonia levels were three times higher in the HPV than the DA. Thus, there is a clear role for the liver in protecting the rest of the fish from elevated and perhaps even toxic levels of ammonia in the

general circulation. There was a different signature for plasma urea, with a significant difference only existing at 12 h. These results suggest that there is a significant deamination of AAs, and to a lesser extent urea formation, before blood from the intestinal mucosa reaches the liver *via* the HPV. Utilising the same calculation as given above, the amount of deaminated AAs could be estimated, yielding information on the effect of diet alteration on AA as a substrate for fuelling the digestion processes, especially if labelled substances were used. At the same time, the majority of ammonia [main metabolite of AA deamination in fish (Halver and Hardy, 2002)] is excreted across the gills just before the DA sample site, thereby yielding a baseline value for the plasma entering the intestine. To what degree ammonia and urea might be generated in the lumen of the intestine during digestion, and pass directly into the hepatic portal blood is unclear. However, Mommsen et al. discovered a high activity of glutamine synthetase in tilapia gastrointestinal tract and have suggested its role is in ammonia detoxification (Mommsen et al., 2003). Our data concur with the need for such a role, but suggest that if present in Atlantic salmon, the detoxification role is partial and hepatic metabolism in combination with gill excretion are additional components of such a protective detoxification system.

In conclusion, the present work shows that a double cannulation in rainbow trout is a feasible tool for physiological studies of nutrition in fish and can be used to accurately track uptake profiles of individual free AAs and provide a much deeper appreciation of underlying mechanisms compared with information provided by a single DA cannulation. In addition, we provide clear evidence in support of the hypothesis that there is hepatic metabolism of certain amino acids during their first pass through the liver after absorption by the intestine. Furthermore, by combining these types of studies with measurements of blood flow, it should be possible to accurately estimate tissue assimilation and nutrient catabolism of a specific nutrient during digestion.

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