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Review

The influence of feeding and fasting on plasma metabolites in the dogfish shark (*Squalus acanthias*) ☆

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ABSTRACT

Dogfish sharks are opportunistic predators, eating large meals at irregular intervals. Here we present a synthesis of data from several previous studies on responses in plasma metabolites after natural feeding and during prolonged fasting (up to 56 days), together with new data on changes in plasma concentrations of amino acids and non-esterified fatty acids. Post-prandial and long-term fasting responses were compared to control sharks fasted for 7 days, a typical inter-meal interval. A feeding frenzy was created in which dogfish were allowed to feed naturally on dead teleosts at two consumed ration levels, 2.6% and 5.5% of body weight. Most responses were more pronounced at the higher ration level. These included increases in urea and TMAO concentrations at 20 h, followed by stability through to 56 days of fasting. Ammonia levels were low and exhibited little short-term response to feeding, but declined to very low values during the extended fast. Glucose and β-hydroxybutyrate both fell after feeding, the latter to a greater and more prolonged extent (up to 60 h), whereas acetoacetate did not change. During prolonged fasting, glucose concentrations were well regulated, but β-hydroxybutyrate increased to 2–3-fold control levels. Total plasma amino acid concentrations increased in a biphasic fashion, with peaks at 6–20 h, and 48–60 h after the meal, followed by homeostasis during the extended fast. Essential and non-essential amino acids generally followed this same pattern, though some exhibited different trends after feeding: taurine, β-alanine, and glycine (decreases or stability), alanine and glutamine (modest prolonged increases), and threonine, serine, asparagine, and valine (much larger short-term increases). Plasma non-esterified fatty acid concentrations declined markedly through 48 h after the 2.6% meal. These data are interpreted in light of companion studies showing elevations in aerobic metabolic rate, urea production, rectal gland function, metabolic base excretion, and activation of ornithine–urea cycle and aerobic enzymes after the meal, and muscle N-depletion but maintenance of osmolality and urea production during long-term fasting.

Contents

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1. Introduction

The feeding ecology of marine sharks has been widely investigated (Cortes, 1999; Wetherbee and Cortes, 2004); most appear to be opportunistic predators, eating large meals at irregular intervals. The spiny dogfish, *Squalus acanthias*, fits this pattern well, as substantiated by many studies (Bonham, 1954; Holden, 1966; Jones and Geen, 1977; Tanasichuk et al., 1991; Hanchet, 1991; Lapitokhovsky et al., 2001; Alonso et al., 2002). However, information on the metabolic responses of elasmobranchs to feeding is sparse, probably because these animals are rarely farmed. This paucity contrasts with the vast amount of data available on teleosts, much of which originates from aquacultural and nutritional research (Jobling, 1994; Carter et al., 2001; Wood, 2001). Reviews of metabolism in elasmobranchs make scant mention of feeding (Goldstein and Perlmann, 1995; Ballantyne, 1997), and we are aware of only a handful of studies prior to 2005 that described metabolic changes accompanying feeding and fasting in this interesting but endangered group.

Of particular note are demonstrations of a post-feeding stimulation of aerobic metabolic rate ("specific dynamic action", Sims and Davies, 1994) and rectal gland metabolism for NaCl excretion (MacKenzie et al., 2002), and biochemical studies of the fasting process (Cohen et al., 1958; Leech et al., 1979; Zammitt and Newsholme, 1979; deRoos et al., 1985). The latter have indicated that ketone bodies, and to a lesser extent amino acids, are the most important metabolic fuels, with lesser direct reliance on carbohydrate and negligible use of lipid (reviewed by Ballantyne, 1997). Urea, the major organic osmolyte in the body fluids, may fall during long term starvation, creating osmoregulatory problems (Leech et al., 1979; Haywood, 1973).

Recently, our group has embarked on a program to understand many aspects of the feeding and fasting processes in *S. acanthias* (Wood et al., 2005, 2007a,b, 2008, 2009; Kajimura et al., 2006, 2008; Walsh et al., 2006; Tresguerres et al., 2007; Dowd et al., 2008; Matey et al., 2009). Three of these studies (Kajimura et al., 2006, 2008; Walsh et al., 2006) have reported changes in various plasma metabolites — ammonia, urea, trimethylamine oxide, glucose, acetoacetate, and β-hydroxybutyrate. The purpose of the present synthesis is to consolidate these plasma metabolite data in an integrated fashion with other aspects of physiology, as well as with new data on plasma amino acids, and non-esterified fatty acids (NEFA), at various times after feeding and during prolonged fasting in the dogfish shark.

2. Methods and materials

2.1. Experimental animals

Spiny dogfish (*Squalus acanthias*, 0.9–4.0 kg) were obtained by trawl or angling in Barkley Sound, British Columbia, Canada, between June and August in the summers of 2003, 2005, and 2006. Prior to experimentation, they were held at the Bamfield Marine Sciences Centre in large groups (50–100 animals) for several weeks in a circular tank filled to 155,000–200,000 L, and served with running sea water at the experimental temperature (11 ± 1 °C), salinity (30 ± 2‰), and pH (7.90 ± 0.15). During this period, they quickly learned to feed, and a feeding frenzy erupted whenever food was added. Twice a week, the animals were fed a ration equivalent to 2–3% of the estimated biomass of all dogfish in the tank. The food consisted of dead hake, flatfish, eelpout and herring. A reference control group was created by transferring 14 dogfish from the large circular tank to several separate 1500-L tanks approximately 1 h after feeding, and fasting them for a total period of 7 days. This appears to represent a typical inter-meal interval in this species, by which time gastro-intestinal processing of the last meal is complete (Kajimura et al., 2006; Wood et al., 2007b). These animals were sacrificed and sampled by methods identical to those detailed below.

2.2. Series 1 — 2.6% ration

This series comprised a subset of the dogfish used in the experiments reported by Walsh et al. (2006) and Kajimura et al. (2006). The entire tank of fish was fed a ration equivalent to 2% of the estimated total biomass. Those dogfish that had obviously eaten, as indicated by bulging bellies, were caught 1 h after feeding and transferred to individual 40-L flux boxes served with constant aeration and a continual flow of seawater. Fish were sacrificed at 6 h, 20 h, 30 h, 48 h, and 216 h (9 days) after feeding. N number at each time ranged from 8 to 15, but not all measurements were made on each animal. At sacrifice, fish were anaesthetized with MS-222 (0.2 $g\,l^{-1}$), weighed, placed on an operating table and blood samples were taken by caudal puncture with a heparinized 10 mL syringe, prior to removal of tissue samples. Plasma samples were obtained by centrifugation at 9000 × *g* for 2 min, and then frozen at −80 °C for later analyses. Measurements at autopsy (up to 48 h post-feeding) revealed that average food consumption was 2.6 ± 0.5% ($N = 44$) of the individual body mass (Kajimura et al., 2006).

2.3. Series 2 — 5.5 % ration

This series comprised a subset of the dogfish used in the experiments reported by Wood et al. (2007a,b) and Kajimura et al. (2008). The entire tank of fish was fed a ration equivalent to 3% of the estimated total biomass. Using the same criteria as in Series 1, fish which had obviously fed were caught after 1 h and transferred to either the 40-L flux boxes for post-feeding sampling at 6 h, 20 h, 30 h, and 60 h, or else initially to a separate 1500-L starvation tank, and then to the individual 40-L flux boxes for 24 h prior to sacrifice at 120 h (5 days), 360 h (15 days), 35 days ($N = 6$), and 56 days ($N = 6$). *N* number at each time ranged from 6 to 11, but not all measurements were made on each animal. Blood and tissue sampling was carried out as in Series 1. Measurements at autopsy (up to 20 h post-feeding) revealed that average food consumption was 5.5 ± 0.4 % ($N = 20$) of the individual body mass (Wood et al., 2007b).

2.4. Analytical techniques

Plasma ammonia was measured enzymatically on the first thaw of non-deproteinised plasma using glutamate dehydrogenase (kit no. 85446, Raichem, San Diego, CA, USA). Plasma urea was measured by the diacetyl-monoxime method (Rahmatullah and Boyde, 1980). Plasma TMAO was assayed by the ferrous sulfate and EDTA method (Wekell and Barnett, 1991). Plasma glucose levels were measured using the hexokinase method (Infinity Liquid Stable Reagent, ThermoTrace, Noble Park, Victoria, Australia). Plasma β-hydroxybutyrate (β-HB) levels were measured using a β-hydroxybutyrate LiquiColor test (Stanbio Laboratory, Boerne, TX, USA), and acetoacetate concentrations were determined by the method of Williamson et al. (1962).

For analysis of plasma free amino acids, the frozen samples were deproteinized with an equal volume of 6% trichloroacetic acid. After

centrifugation at 10,000×g for 20 min, the supernatant obtained was diluted with 0.2 mol L^{-1} lithium citrate buffer (pH 2.2) and adjusted to pH 2.2 with 4 mol L^{-1} lithium hydroxide. The level of FAAs was analyzed using a Shimadzu LC-10A amino acid analysis system (Shimadzu, Kyoto, Japan) with a Shim-pack ISC-07/S1504 Li-type column. Results for FAA analyses were expressed as mmol L^{-1}. No samples were available for plasma amino acid analyses at 30 h in Series 1.

Plasma lipids were analyzed only in a subset of samples from Series 1. Total lipids were extracted from plasma as described previously (Bernard et al., 1999; McClelland et al., 2001). Briefly, plasma was mixed with 25 mL of chloroform:methanol (2:1, v/v, Folch solution) and was filtered. The original tube was washed with 5 mL of Folch solution and 7.5 mL of water was added to the new tubes. The mixture was centrifuged at 600×g for 10 min to separate organic and aqueous phases. The organic phase was dried using a rotating evaporator (Buchi rotovapor) and resuspended in chloroform. Heptadecanoic acid (17:0) was added as an internal standard and the nonesterified fatty acid (NEFA) fraction was further purified by passing the total lipids through Supelclean solid phase columns (Supelco). NEFA's were methylated as described previously (McClelland et al., 1994), evaporated then resuspended in petroleum ether and transferred into autosampler vials for gas chromatograph analysis. Samples were injected using a 7683B series automatic injection system onto a Hewlett-Packard 6890N series gas chromatograph (GLC, Agilent Technologies) equipped with a DB-23 (J&W Scientific) 30-m fused silica capillary column (Supelco, Bellefonte, PA, USA) at 250 °C. The following temperature profile was employed: initial oven temperature was kept at 160 °C for 4 min, ramped up to 220 °C at 2 ºC/min, held at 220 °C for 16 min, ramped up to 240 °C at 10 °C/min and held for 2 min. Post-run was 130 °C for 6 min. The flow was 1.8 mL1/min and the velocity through the column was 37 cm/s. GLC retention times were verified using fatty acid standards (Supelco).

2.5. Statistical analyses

Data are expressed as means ± 1 SEM (N). Multiple comparisons for differences in metabolite concentrations at different sampling times were evaluated by one-way ANOVA followed by Fisher's LSD (least significant difference) post-hoc test. If required, data were first transformed to achieve homogeneity of variances and normality of distribution, using natural logarithm or square root transformations. In those few instances where the data still did not pass Bartlett's test for homogeneity of variances, the nonparametric Kruskal–Wallis signed-ranks ANOVA was employed, followed by Dunn's multiple comparison test. Differences between the 2.6% ration and 5.5% ration series at common sampling times (6 h, 20 h, 30 h) were evaluated using Student's unpaired two-tailed t-test. A significance level of $P<0.05$ was used throughout. For simplicity, only significant differences from control values (marked with asterisks) and between ration levels (marked with triangles) are indicated in the Figures, but the full results of multiple comparison testing are reported in Figure legends and in Tables.

3. Results

Plasma urea concentrations (Fig. 1A) increased from a control value of about 410 mmol L^{-1} to 460–490 mmol L^{-1} at 20 h post-feeding in both series. The values at this time were not significantly different between the 2.6% and 5.5% ration groups. Thereafter, urea levels returned to control levels and showed no other significant changes up to 56 days of fasting.

Plasma TMAO concentration (Fig. 1B) also increased significantly at this time from a control value of about 78 mmol L^{-1} to 94 mmol L^{-1} in the 2.6% ration treatment. A smaller increase in the 5.5% ration treatment was not significant, but by 30 h, the value in the lower ration group had fallen significantly below that in the higher ration group. There were no other differences or changes relative to the control value up to 56 days of fasting, apart from a transitory fall at 15 days which was not significant.

Plasma ammonia concentrations (Fig. 1C) were negligible relative to urea and TMAO levels, with control levels of approximately 125 µmol L^{-1}. The slight rise in plasma ammonia concentration (Fig. 1C) at 20 h post-feeding in the 5.5% ration group to about

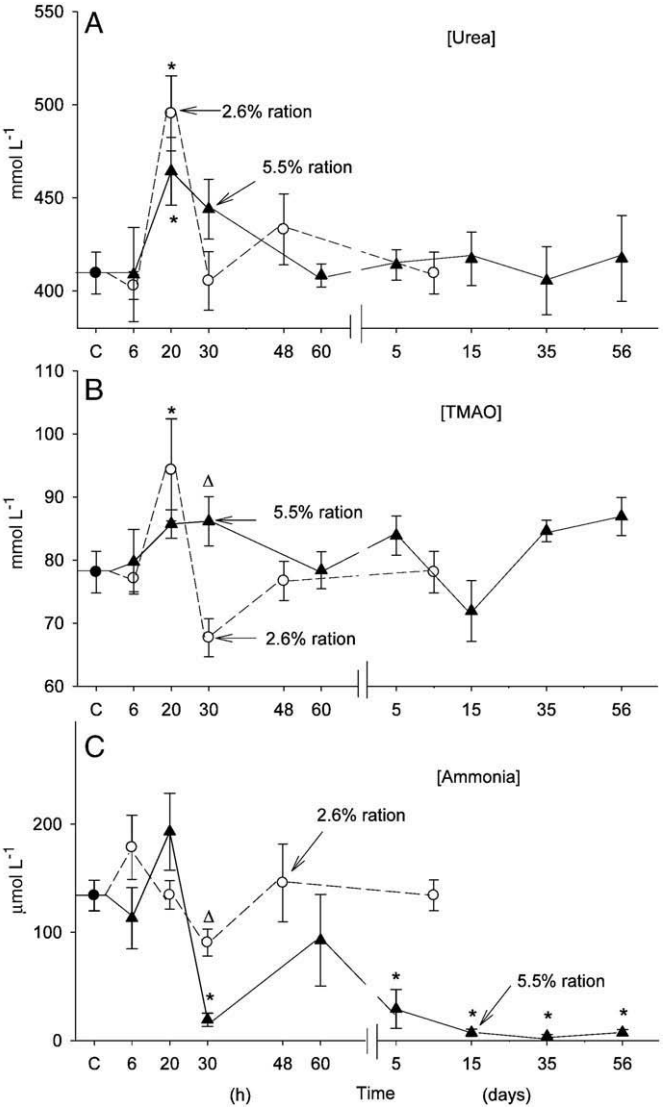

Fig. 1. Changes in (A) plasma urea, (B) plasma trimethylamine oxide, and (C) plasma ammonia concentrations in *Squalus acanthias* following meals of 2.6% body mass (open circles; $N=8$–15) or 5.5% body mass (closed triangles; $N=6$–9) of teleost fish and followed by prolonged fasting (closed triangles; $N=6$–9). Means ± 1 SEM. Asterisks indicate means which are significantly different ($P<0.05$) from the control value (con, dogfish fasted for 7 days, $N=14$), and open triangles indicate significant differences between the two ration levels at the same time. Data from Kajimura et al. (2006, 2008). From multiple comparison testing within each ration level, common lines underscore means which are not significantly different (i.e. $P>0.05$) from one another:

(A) 2.6%: 6h 30h con 9d 48h 20h 5.5%: 35d 60h 6h con 15d 5d 56d 30h 20h

(B) 2.6%: 30h 48h 6h 9d con 20h 5.5%: 15d con 60h 6h 5d 35d 20h 30h 56d

(C) 2.6%: 3h con 9d 20h 48h 6h 5.5%: 35d 15d 56d 30h 5d 60h 6h con 20h

195 μmol L^{-1} was not significant, and by 30 h this value had decreased significantly below the control value, as well as below the ammonia level in the 2.6% ration group. Plasma ammonia levels remained significantly depressed in the 4–30 μmol L^{-1} range from 5 days through to 56 days of fasting, though there was a discrepancy at 9 days in the low ration treatment, and fluctuation between 30 h and 60 h in the high ration treatment.

Plasma glucose concentrations (Fig. 2A) fell at 6 h post-feeding from a control value of 5.5 mmol L^{-1} to 4.0 and 3.0 mmol L^{-1} in the 2.6% and 5.5% ration treatments, respectively. Glucose levels rebounded quickly in the lower ration group, but stayed significantly depressed at 20 h in the higher ration treatment. Thereafter, glucose concentrations remained at control levels throughout 56 days of fasting.

Plasma β-hydroxybutyrate concentrations (Fig. 2B) exhibited much more marked and prolonged depressions after feeding, followed by a later rise to several fold greater than control levels. The fall from control levels of about 5 mmol L^{-1} to about 1 mmol L^{-1} was significant through to 48 h in the 2.6% ration group. The decrease was even greater at 20 h in the 5.5% ration group, and remained

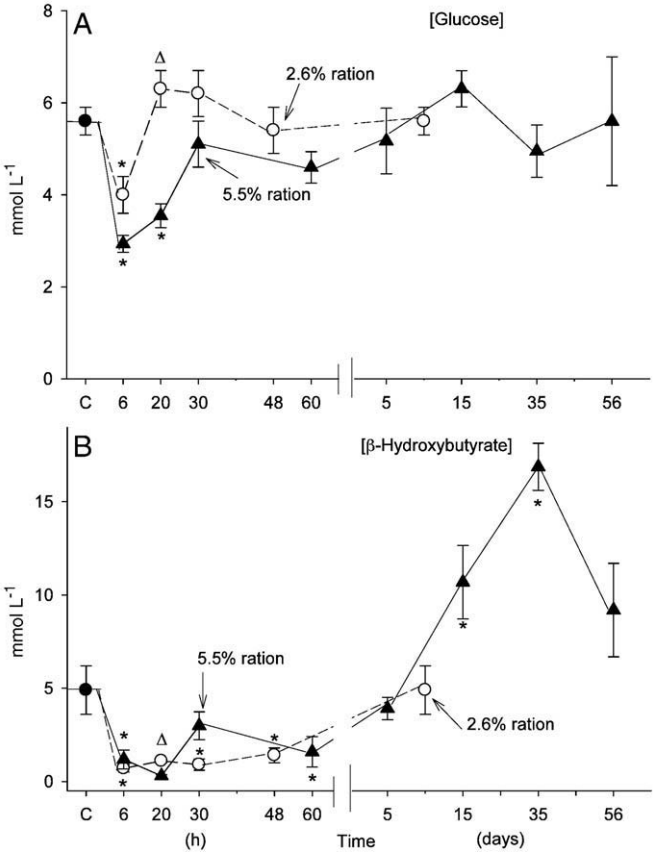

Fig. 2. Changes in (A) plasma glucose and (B) plasma β-hydroxybutyrate concentrations in *Squalus acanthias* following meals of 2.6% body mass (open circles; $N=7$–14) or 5.5% body mass (closed triangles; $N=6$–9) of teleost fish followed by prolonged fasting (closed triangles; $N=6$–9) Means ± 1 SEM. Asterisks indicate means which are significantly different ($P<0.05$) from the control value (con, dogfish fasted for 7 days, $N=13$–14), and open triangles indicate significant differences between the two ration levels at the same time. Data from Walsh et al. (2006) and Kajimura et al. (2008). From multiple comparison testing within each ration level, common lines underscore means which are not significantly different (i.e. $P>0.05$) from one another:

(A) 2.6%: 6h 48h 9d con 30h 20h 5.5%: 6h 20h 60h 35d 30h 5d 56d con 15d

(B) 2.6%: 6h 20h 30h 48h 9d con 5.5%: 20h 6h 60h 30h 5d con 56d 15d

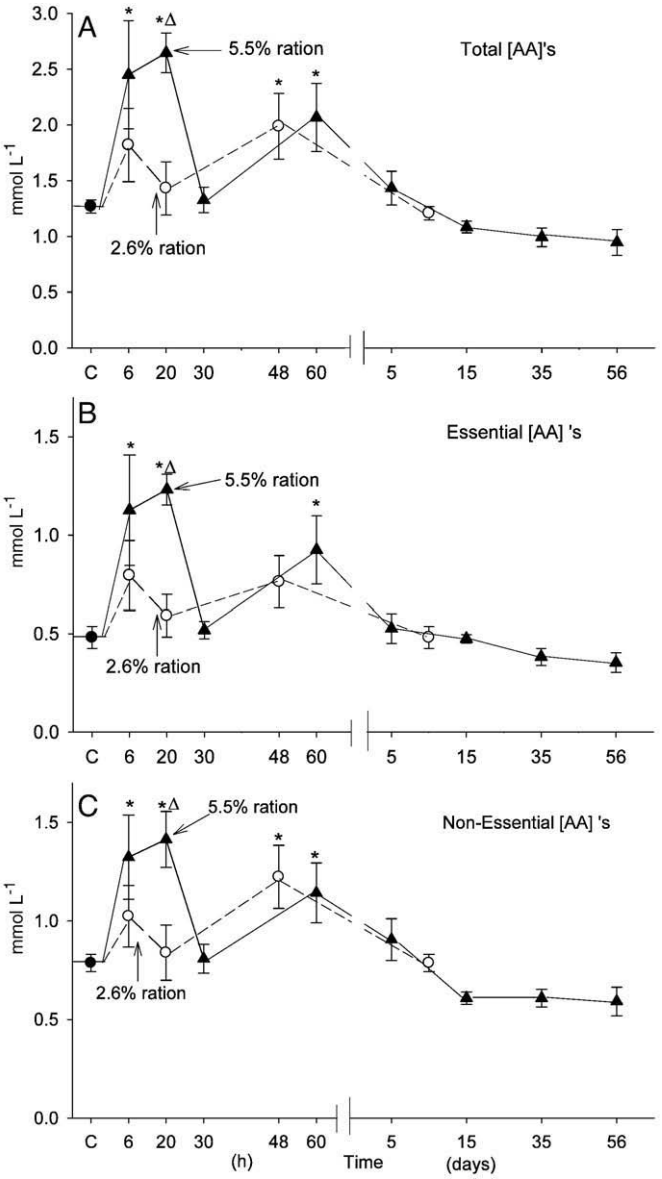

Fig. 3. Changes in (A) plasma total free amino acids (see Table 1 for entire list), (B) plasma total essential free amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and, valine), and (C) plasma total free non-essential amino acids (the other 14 amino acids from Table 1) in *Squalus acanthias* following meals of 2.6% body mass (open circles; $N=4$–6) or 5.5% body mass (closed triangles; $N=5$–6) of teleost fish followed by prolonged fasting (closed triangles; $N=5$–6) Means ± 1 SEM. Asterisks indicate means which are significantly different ($P<0.05$) from the control value (con, dogfish fasted for 7 days, $N=6$), and open triangles indicate significant differences between the two ration levels at the same time. From multiple comparison testing within each ration level, common lines underscore means which are not significantly different (i.e. $P>0.05$) from one another:

(A) 2.6%: 9d con 20h 6h 48h 5.5%: 56d 35d 15d con 30h 5d 60h 6h 20h

(B) 2.6%: con 9d 20h 6h 48h 5.5%: 56d 35d con 15d 5d 30h 60h 6h 20h

(C) 2.6%: con 9d 20h 6h 48h 5.5%: 56d 35d 15d con 30h 5d 60h 6h 20h

Table 1
Changes in plasma free amino acid concentrations (mmol L^{-1}) in *Squalus acanthias* at different times following a meal of 5.5% body mass compared to control, non-fed fish and during prolonged fasting (means ± 1 SEM).

Amino acids	Control $N=6$	6 h $N=6$	20 h $N=6$	30 h $N=6$	60 h $N=6$	5d $N=6$	15d $N=5$	35d $N=6$	56d $N=6$
Taurine	0.233 ± 0.040^{a}	$0.126 \pm 0.017^{*,b}$	$0.131 \pm 0.019^{*,b}$	$0.134 \pm 0.024^{*,b}$	$0.173 \pm 0.027^{a,b}$	$0.180 \pm 0.049^{a,b}$	$0.100 \pm 0.010^{*,b}$	$0.126 \pm 0.012^{*,b}$	$0.110 \pm 0.017^{*,b}$
Aspartate	$0.002 \pm 0.040^{b,c,d,e}$	$0.003 \pm 0.000^{a,b,c}$	$0.003 \pm 0.000^{a,b}$	$0.002 \pm 0.000^{a,b,c,d}$	$0.003 \pm 0.000^{a,b,c}$	$0.005 \pm 0.002^{*,a}$	$0.002 \pm 0.000^{b,c,d,e}$	0.001 ± 0.000^{e}	$0.002 \pm 0.000^{b,c,d,e}$
Threonine	0.045 ± 0.005^{f}	$0.237 \pm 0.071^{*,b}$	$0.346 \pm 0.023^{*,a}$	$0.101 \pm 0.019^{*,c,d}$	$0.184 \pm 0.043^{*,b,c}$	$0.084 \pm 0.016^{*,d,e}$	$0.057 \pm 0.005^{d,e,f}$	$0.073 \pm 0.009^{d,e,f}$	$0.067 \pm 0.011^{d,e,f}$
Serine	$0.025 \pm 0.002^{d,}$	$0.133 \pm 0.022^{*,a}$	$0.148 \pm 0.022^{*,a}$	$0.067 \pm 0.013^{*,b,c}$	$0.088 \pm 0.016^{*,b}$	$0.057 \pm 0.014^{*,b,c}$	$0.031 \pm 0.003^{c,d}$	$0.030 \pm 0.005^{c,d}$	0.024 ± 0.003^{d}
Asparagine	0.010 ± 0.001^{a}	$0.062 \pm 0.014^{*,b}$	$0.064 \pm 0.019^{*,b}$	0.019 ± 0.004^{a}	0.026 ± 0.006^{a}	0.013 ± 0.002^{a}	0.017 ± 0.002^{a}	0.013 ± 0.001^{a}	0.013 ± 0.002^{a}
Glutamate	$0.004 \pm 0.000^{d,e}$	$0.010 \pm 0.001^{*,a}$	$0.010 \pm 0.001^{*,a,b}$	$0.007 \pm 0.002^{a,b,c,d}$	$0.008 \pm 0.001^{*,a,b,c}$	$0.012 \pm 0.005^{*,a,b}$	$0.006 \pm 0.001^{a,b,c,d,e}$	0.003 ± 0.000^{e}	0.003 ± 0.001^{e}
Glutamine	0.026 ± 0.002^{a}	$0.068 \pm 0.014^{*,b}$	$0.052 \pm 0.008^{a,b}$	$0.038 \pm 0.005^{a,b}$	$0.042 \pm 0.004^{a,b}$	$0.040 \pm 0.006^{a,b}$	$0.043 \pm 0.001^{a,b}$	$0.039 \pm 0.003^{a,b}$	$0.033 \pm 0.003^{a,b}$
Proline	$0.052 \pm 0.009^{b,c}$	$0.061 \pm 0.020^{a,b}$	$0.097 \pm 0.022^{*,a}$	0.023 ± 0.002^{c}	$0.059 \pm 0.014^{b,c}$	$0.040 \pm 0.010^{b,c}$	0.021 ± 0.002^{c}	$0.026 \pm 0.005^{b,c}$	0.024 ± 0.007^{c}
Glycine	0.078 ± 0.032^{a}	$0.054 \pm 0.007^{a,b}$	$0.039 \pm 0.004^{a,b}$	$0.039 \pm 0.005^{a,b}$	$0.045 \pm 0.006^{a,b}$	$0.070 \pm 0.023^{a,b}$	$0.056 \pm 0.010^{a,b}$	$0.035 \pm 0.003^{*,b}$	$0.047 \pm 0.007^{a,b}$
Alanine	0.196 ± 0.024^{c}	$0.342 \pm 0.050^{*,a,b}$	$0.278 \pm 0.038^{a,b,c}$	$0.263 \pm 0.026^{a,b,c}$	$0.343 \pm 0.069^{*,a}$	0.228 ± 0.023^{c}	0.188 ± 0.013^{c}	0.179 ± 0.011^{c}	0.186 ± 0.037^{c}
Valine	0.070 ± 0.006^{c}	$0.229 \pm 0.052^{*,a,b}$	$0.267 \pm 0.022^{*,a}$	0.101 ± 0.014^{c}	$0.189 \pm 0.038^{*,a,b}$	0.084 ± 0.006^{c}	0.095 ± 0.006^{c}	0.075 ± 0.008^{c}	0.073 ± 0.011^{c}
Methionine	$0.032 \pm 0.002^{d,e}$	$0.090 \pm 0.026^{*,a,b}$	$0.121 \pm 0.009^{*,a}$	$0.048 \pm 0.006^{c,d}$	$0.069 \pm 0.014^{*,b,c}$	$0.033 \pm 0.002^{d,e}$	$0.021 \pm 0.002^{d,e}$	$0.018 \pm 0.002^{d,e}$	0.016 ± 0.002^{e}
Isoleucine	0.029 ± 0.002^{d}	$0.095 \pm 0.022^{*,a}$	$0.093 \pm 0.006^{*,a,b}$	0.040 ± 0.005^{d}	$0.072 \pm 0.015^{*,b,c}$	0.041 ± 0.003^{d}	$0.046 \pm 0.003^{c,d}$	0.034 ± 0.003^{d}	0.034 ± 0.005^{d}
Leucine	0.076 ± 0.007^{d}	$0.240 \pm 0.053^{*,a}$	$0.230 \pm 0.016^{*,a,b}$	0.107 ± 0.014^{d}	$0.191 \pm 0.039^{*,a,b,c}$	0.099 ± 0.006^{d}	$0.126 \pm 0.008^{c,d}$	0.094 ± 0.010^{d}	0.094 ± 0.015^{d}
Tyrosine	0.043 ± 0.002^{c}	$0.090 \pm 0.015^{*,a,b}$	$0.114 \pm 0.006^{*,a}$	0.049 ± 0.003^{c}	$0.072 \pm 0.009^{*,b}$	0.043 ± 0.004^{c}	0.033 ± 0.000^{c}	0.033 ± 0.002^{c}	0.039 ± 0.003^{c}
Phenylalanine	$0.043 \pm 0.002^{a,b,c,d}$	$0.073 \pm 0.018^{a,b}$	0.084 ± 0.007^{a}	$0.047 \pm 0.003^{a,b,c,d}$	$0.059 \pm 0.005^{a,b,c}$	$0.045 \pm 0.005^{a,b,c,d}$	$0.041 \pm 0.002^{a,b,c,d}$	$0.036 \pm 0.003^{b,c,d}$	0.033 ± 0.001^{d}
Tryptophan	$0.019 \pm 0.001^{b,c,d}$	$0.025 \pm 0.004^{a,b}$	$0.034 \pm 0.002^{*,a}$	$0.018 \pm 0.001^{c,d}$	$0.023 \pm 0.000^{b,c}$	$0.022 \pm 0.002^{b,c,d}$	0.016 ± 0.001^{d}	$0.018 \pm 0.002^{c,d}$	0.016 ± 0.000^{d}
Histidine	0.010 ± 0.001^{e}	$0.027 \pm 0.005^{*,a}$	$0.025 \pm 0.002^{*,a,b}$	$0.015 \pm 0.002^{c,d,e}$	$0.021 \pm 0.000^{*,a,b,c}$	$0.020 \pm 0.003^{*,a,b,c,d}$	$0.015 \pm 0.001^{c,d,e}$	$0.014 \pm 0.001^{c,d,e}$	0.009 ± 0.001^{e}
Ornithine	$0.021 \pm 0.005^{c,d,e,f}$	$0.055 \pm 0.016^{*,a,b}$	$0.076 \pm 0.011^{*,a}$	$0.023 \pm 0.004^{c,d,e}$	$0.038 \pm 0.010^{b,c,d}$	$0.069 \pm 0.049^{b,c}$	$0.012 \pm 0.002^{e,f}$	0.009 ± 0.001^{f}	$0.013 \pm 0.001^{e,f}$
Lysine	$0.197 \pm 0.037^{b,c,d}$	$0.344 \pm 0.100^{a,b}$	$0.375 \pm 0.050^{*,a}$	$0.139 \pm 0.011^{d,e,f}$	$0.296 \pm 0.069^{a,b,c}$	$0.178 \pm 0.055^{c,d}$	$0.111 \pm 0.025^{d,e,f}$	$0.090 \pm 0.018^{*,e,f}$	$0.074 \pm 0.013^{*,f}$
Arginine	$0.012 \pm 0.000^{a,b,c}$	0.013 ± 0.001^{a}	$0.011 \pm 0.000^{a,b,c}$	$0.012 \pm 0.001^{a,b}$	$0.009 \pm 0.000^{*,d}$	$0.011 \pm 0.001^{a,b,c}$	$0.012 \pm 0.001^{a,b}$	$0.011 \pm 0.000^{a,b,c}$	$0.009 \pm 0.000^{*,d}$
Citrulline	$0.026 \pm 0.008^{a,b,c}$	$0.055 \pm 0.015^{a,b}$	$0.031 \pm 0.004^{a,b,c}$	$0.018 \pm 0.006^{a,b,c}$	$0.038 \pm 0.007^{a,b}$	$0.039 \pm 0.011^{a,b,c}$	$0.015 \pm 0.003^{b,c}$	0.007 ± 0.000^{c}	0.006 ± 0.000^{c}
β-Alanine	0.009 ± 0.001^{b}	0.009 ± 0.001^{b}	0.008 ± 0.001^{b}	0.008 ± 0.001^{b}	0.008 ± 0.001^{b}	0.008 ± 0.000^{b}	0.010 ± 0.000^{b}	$0.016 \pm 0.003^{*,a}$	0.009 ± 0.001^{b}
TEFAA	0.480 ± 0.054^{bc}	$1.126 \pm 0.281^{*}a$	$1.232 \pm 0.078^{*}a$	0.518 ± 0.043^{b}	$0.924 \pm 0.173^{*}a$	0.526 ± 0.074^{b}	0.474 ± 0.021^{bc}	0.382 ± 0.043^{bc}	0.353 ± 0.049^{c}
TNEFAA	$0.786 \pm 0.043^{c,d,e}$	$1.323 \pm 0.213^{*,a}$	$1.414 \pm 0.141^{*,a}$	$0.808 \pm 0.072^{b,c,d}$	$1.142 \pm 0.152^{*,a,b}$	$0.905 \pm 0.105^{b,c}$	$0.608 \pm 0.030^{d,e}$	$0.608 \pm 0.044^{d,e}$	0.592 ± 0.072^{e}
TFAA	$1.267 \pm 0.060^{b,c,d}$	$2.450 \pm 0.485^{*,a}$	$2.646 \pm 0.178^{*,a}$	$1.327 \pm 0.114^{b,c}$	$2.067 \pm 0.305^{*,a}$	1.432 ± 0.151^{b}	$1.083 \pm 0.052^{b,c,d}$	$0.991 \pm 0.084^{c,d}$	0.945 ± 0.116^{d}

Asterisks indicate means which are significantly different ($P<0.05$) from the control value (dogfish fasted for 7 days).
From multiple comparison testing, means sharing common letters are not significantly different (i.e. $P>0.05$) from one another.
TEFAA = total essential free amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine).
TNEFAA = total non-essential free amino acids.
TFAA = total free amino acids.

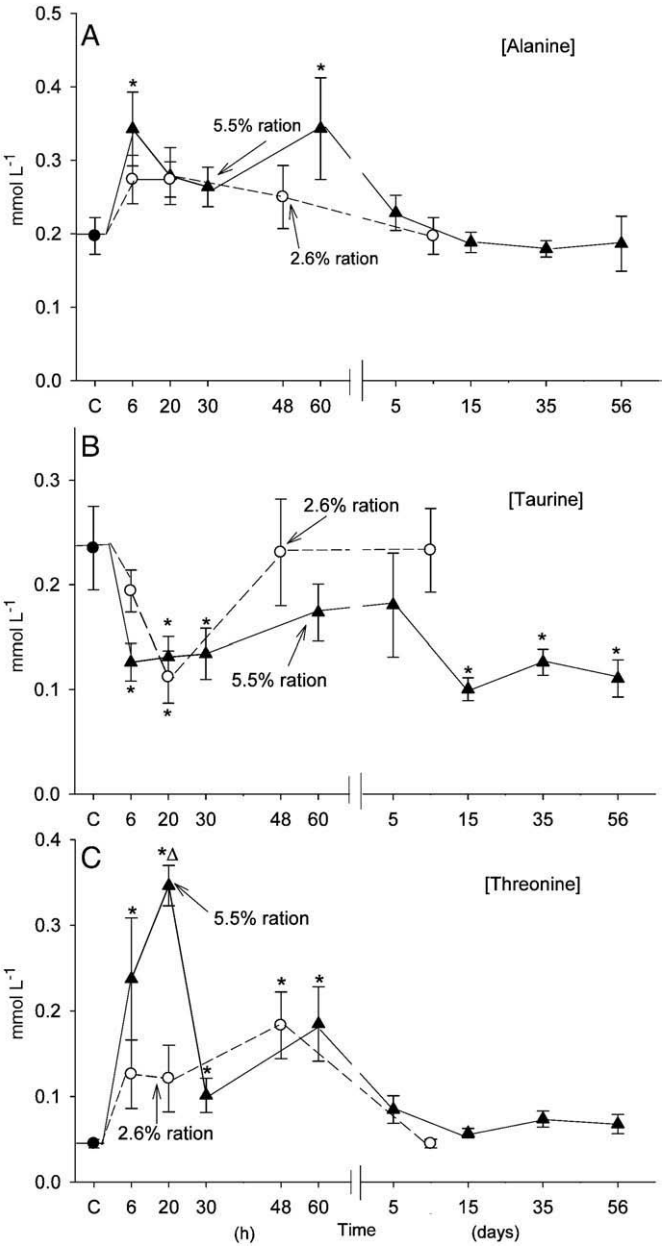

Fig. 4. Changes in (A) plasma alanine, (B) plasma taurine, and (C) plasma threonine concentrations in *Squalus acanthias* following meals of 2.6% body mass (open circles; $N=4$–6) or 5.5% body mass (closed triangles; $N=5$–6) of teleost fish followed by prolonged fasting (closed triangles; $N=5$–6) Means ± 1 SEM. Asterisks indicate means which are significantly different ($P<0.05$) from the control value (con, dogfish fasted for 7 days, $N=6$), and open triangles indicate significant differences between the two ration levels at the same time. From multiple comparison testing within each ration level, common lines underscore means which are not significantly different (i.e. $P>0.05$) from one another:

(A) 2.6%: con 9d 48h 20h 6h 5.5% 35d 56d 15d con 5d 30h 20h 6h 60h

(B) 2.6%: 20h 6h 48h 9d con 5.5%: 15d 56d 35d 20h 30h 6h 60h 5d con

(C) 2.6%: 9d con 20h 6h 48h 5.5%: con 15d 56d 35d 5d 30h 60h 6h 20h

significant at 60 h, though there was a transient partial rebound at 30 h. By 5–9 days post-feeding, control levels were re-established, but thereafter β-hydroxybutyrate concentrations were elevated to 10–17 mmol L^{-1} through to 56 days of prolonged fasting. Plasma acetoacetate concentrations were measured only in the 2.6% ration series, and exhibited no significant changes after feeding (not shown). Absolute acetoacetate levels (0.15–0.30 mmol L^{-1}) were much lower than those of β-hydroxybutyrate (cf. Fig. 2B).

Plasma amino acid levels (Fig. 3A) totaled about 1.3 mmol L^{-1} in control animals. Total concentrations increased sharply after feeding in the 5.5% ration treatment, approximately doubling at 6 h and 20 h, then returning to control levels at 30 h, with a second significant increase at 60 h. In the 2.6% ration group, there were similar but smaller changes, but only the secondary rise (at 48 h) was significant. During prolonged fasting, there was a gradual decline in total plasma amino acid concentrations but even at 56 days post-feeding, levels were not significantly different from the original control value. Interestingly, these trends remained very similar when the data were compiled separately into the 9 amino acids thought to be essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine; Fig. 3B) *versus* the 14 others thought to be non-essential (Fig. 3C). The essential amino acids constituted 35–45% of the total at all sampling times and at both ration levels.

Data for individual amino acids are tabulated in Table 1. Most exhibited trends broadly similar to those of the totals (Fig. 3), but there were several interesting variations, some of which are illustrated in Fig. 4. In control animals, the three largest components were lysine, alanine, and taurine, each accounting for 15–20% of the total, followed by glycine, leucine, and valine, each representing about 6%. While lysine (not illustrated; Table 1) exhibited post-feeding changes very typical of the totals, increases in alanine (Fig. 4A) were smaller but long lasting in both treatment groups, and there were no significant declines during prolonged fasting. Glutamine, although 10-fold lower in concentration showed a similar trend (not illustrated; Table 1). Taurine (Fig. 4B), on the other hand, actually declined significantly by about 50% after feeding, changes which were significant at 20 h in both treatment groups, and at 6 h through 30 h in the 5.5% ration treatment, as well as during prolonged fasting (15–56 days). These same general trends of post-feeding decreases or stability rather than increases were also seen in glycine and β-alanine (not illustrated; Table 1). In marked contrast, several amino acids exhibited post-feeding increases which were much greater than seen in the totals. For example, threonine concentrations (Fig. 4C) increased more than 6-fold in the higher ration treatment and more than 2-fold in the lower ration treatment at 6 h–20 h, and did not decline during long term fasting. Similar responses were seen in serine, asparagine, and valine (not illustrated; Table 1). Interestingly, circulating concentrations of amino acids involved directly or indirectly in the elasmobranch ornithine urea cycle (ornithine, citrulline, aspartate, arginine, glutamine) remained very low at all times, constituting just a few percent of the total (Table 1).

Plasma lipids were measured only in the 2.6% ration treatment (Table 2). Total NEFA concentration was approximately 110 μmol L^{-1} in control dogfish and fell significantly by about 40% right from 6 h through 48 h post-feeding.

4. Discussion

In general, the short term changes in plasma metabolites after feeding were qualitatively similar in the two ration groups, with the alterations often being larger in the higher ration treatment. This

Table 2
Changes in plasma nonesterified fatty acid (NEFA) concentrations (μmol L^{-1}) in *Squalus acanthias* following meals of 2.6% body weight (means ± 1 SEM, $N=3$).

	Control	6 h	20 h	30 h	48 h
Total NEFA	112.25 ± 2.98[a]	71.35 ± 7.02*,b	66.56 ± 5.17*,b	60.68 ± 1.74*,b	64.14 ± 8.18*,b

Asterisks indicate means which are significantly different ($P<0.05$) from the control value (dogfish fasted for 7 days). From multiple comparison testing, means sharing common letters are not significantly different (i.e. $P>0.05$) from one another.

suggests that most of the changes were related to the actual intake of food, rather than to the activity of the feeding frenzy.

The urea-based osmoregulatory strategy of marine elasmobranchs requires massive urea gradients across the gills, and branchial losses of urea appear to be unavoidable despite the presence of both a Na^+-coupled back-transport mechanism and a unique lipid composition which renders the branchial membranes unusually impermeable to urea (Pärt et al., 1998; Fines et al., 2001). Kajimura et al. (2006) calculated that if a typical meal in the wild is 3.2% of body mass, dogfish must feed every 5–6 days just to maintain N-balance, let alone grow. Furthermore, urea synthesis is costly, requiring 2.5 ATP per urea-N in fish in contrast to 2.0 ATP in mammals due to the extra step of the glutamine synthetase reaction for N-trapping (Mommsen and Walsh, 1992; Kirschner, 1993). The significant rise in plasma urea concentration at 20 h post-feeding in both ration treatments (Fig. 1A) was likely associated with an increase in metabolic urea production rate at this time, and occurred despite a large secretion of urea into the digestive tract which raised the osmolality of the chyme close to that of the blood plasma during processing of the meal (Wood et al., 2007b). Indeed there were marked increases in the activities of most of the enzymes of the ornithine–urea cycle (OUC) at 20 h post-feeding in the liver, the white muscle, or both (Kajimura et al., 2006). The activity of carbamoyl phosphate synthetase III, the enzyme which is thought to be rate-limiting in the elasmobranch OUC (Casey and Andersen, 1985) increased 1.6 fold. At the same time, the rate of urea excretion to the environment did not change in the 2.6% ration treatment (Kajimura et al., 2006), and actually fell significantly (by 39%, integrated over 48 h) in the 5.5% ration treatment (Wood et al., 2007a). As plasma urea concentration rises, but urea excretion rate does not, it is possible that the branchial urea-retention mechanisms (Pärt et al., 1998; Fines et al., 2001) are additionally activated for N-conservation during the post-feeding period when both protein synthesis and urea production are high priorities.

During prolonged fasting up to 56 days, there was a remarkable regulation of plasma urea concentration (Fig. 1A), and associated with this, homeostasis of plasma osmolality, while urea excretion rate to the environment remained approximately constant (Kajimura et al., 2008). This regulation was achieved by a substantial degradation of body protein stores amounting to a loss of about 70 g of protein per kg muscle over 56 days. These results agree with the observations of Treberg and Driedzic (2006) in the skate *Leucoraja ocellata* (where plasma urea was maintained during 45 days of starvation), and illustrate the critical importance of urea for osmoregulation in elasmobranchs. However the results differ somewhat from those of Leech et al. (1979) who reported an 8% fall in plasma urea over 19–25 days of fasting in *Squalus acanthias*, as well as those of Haywood (1973) who noted an 18% decline over 30 days in plasma urea, accompanied by an 8% drop in plasma osmolarity in the pyjama shark *Poroderma africanum*.

TMAO plays a critical role in counteracting the protein-destabilizing effects of urea (Yancey and Somero, 1980). Therefore it is interesting that like urea, plasma TMAO levels were well regulated during prolonged fasting (Fig. 1B), in accord with similar observations by Cohen et al. (1958) on the same species subjected to 40 days of starvation while held in live cars in the sea. It is also remarkable because *S. acanthias* is generally believed to be incapable of *de novo* synthesis of TMAO (Baker et al., 1963; Goldstein et al., 1967) in contrast to urea, yet like urea, TMAO is lost across the gills (Goldstein and Palatt, 1974) and kidney (Cohen et al., 1958) in this species. The small surge in plasma TMAO at 20 h post-feeding (Fig. 2B), significant only in the lower ration group, may be indicative of acquisition from the diet. The skate *Raja leucoraja* also maintained plasma TMAO levels during 45 days of fasting, a result that Treberg and Driedzic (2006) attributed to efficient retention rather than synthesis, with speculation that previous studies had over-estimated TMAO loss rates in elasmobranchs. Clearly, as muscle mass is reduced by "wasting" during prolonged starvation, intracellular TMAO and urea can move from the intracellular to extracellular compartment, and this may be more effective in maintaining plasma TMAO levels, as the normal intracellular TMAO concentration is higher than the normal extracellular TMAO concentration, whereas urea is in approximate equilibrium between the two compartments (Yancey and Somero, 1980; Yancey, 2001).

The focus of the present review is on plasma metabolites, and the reader is referred to Kajimura et al. (2006, 2008) and Wood et al. (2007b) for information on inorganic electrolytes (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}), which are the other major constituents of plasma osmolality in elasmobranchs. In general, all plasma electrolytes remained remarkably constant after feeding, despite marked changes in the ionic composition of the chyme during digestion (Wood et al., 2007b), and they were also well regulated during prolonged fasting. Therefore, urea comprised about 40–47%, TMAO about 9–11% and total inorganic ions about 48–51% of total plasma osmolality, with Na^+ and Cl^- accounting for the bulk of the latter. These ratios were quite stable after feeding and for a 2 month starvation period (Kajimura et al., 2008).

Plasma ammonia levels were extremely low in dogfish (<0.2% of urea or TMAO concentrations) and exhibited a negligible rise after feeding (Fig. 1C), in contrast to the pattern in teleosts (reviewed by Wood, 2001). At this time, there were significant increases in ammonia excretion rates to the water in both ration treatments (Kajimura et al., 2006, 2008; Wood et al., 2007a), but these were relatively small, amounting to less than 25% of urea-N excretion rates, and quickly declining. Ammonia excretion rates were negligible during prolonged fasting, accounting for only 2% of total N-excretion from 15 days onwards (Kajimura et al., 2008). The extremely low plasma ammonia concentrations at this time (Fig. 1C; see also Leech et al., 1979) coincided with this trend, indicative of highly efficient N-scavenging for the maintenance of urea production in elasmobranch tissues. It is unclear why there was a discrepancy between the levels in the two ration treatments prior to this time, and it is also notable that plasma ammonia rose between 30 h and 60 h in the high ration treatment, indicating some variability in the overall response. The affinity of glutamine synthetase (GS) for ammonia ($K_M = 15$ μmol L^{-1}) is remarkably high in *S. acanthias* (Shankar and Anderson, 1985). Given the broad tissue distribution of GS, and the related ammonia consuming enzyme, glutamate dehydrogenase (GDH), these enzymes could easily account for the low plasma ammonia levels.

The transient post-prandial decrease in plasma glucose concentrations in both ration groups (Fig. 2A) suggests that glucose utilization increases at this time, either for oxidation or carbon storage. The involvement of endogenous insulin mobilization in this response is uncertain, because injections of either mammalian insulin (Patent, 1970; deRoos and deRoos, 1979; deRoos et al., 1985) or homologous elasmobranch insulin (Anderson et al., 2002) produced only very slowly developing but longer lasting hypoglycaemia, rather different from the rapid fall and relatively quick rebound observed in the current studies. These insulin injection studies also noted either a lack of effect or a weak biphasic effect on circulating ketone body levels (deRoos et al., 1985; Anderson et al., 2002), whereas plasma β-hydroxybutyrate concentrations fell quickly after feeding in the current studies (Fig. 2B). While most tissues of elasmobranchs rely heavily on ketones (Ballantyne, 1997; Watson and Dickson, 2001; Speers-Roesch et al., 2006), some organs have relatively high hexokinase levels (brain, red muscle, heart, and rectal gland) indicative of considerable glucose oxidation capacity (Crabtree and Newsholme, 1972; Moon and Mommsen, 1980). The rectal gland in particular appears to have an absolute requirement for glucose (Walsh et al., 2006). During prolonged fasting, plasma glucose was well-regulated (Fig. 2A), in accord with previous observations on *S. acanthias* (Leech et al., 1979; deRoos, 1994), *Scyliorhinus canicula* (Zammitt and Newsholme, 1979), and several other species (reviewed by deRoos et al., 1985). This pattern suggests that gluconeogenesis is

important, perhaps fueled by amino acids from the degradation of protein stores used to maintain urea production, as discussed earlier.

Our studies are the first to show that plasma β-hydroxybutyrate concentrations are driven down to very low levels ($\leq$ 1 mmol L^{-1}) immediately after feeding and stay low for several days thereafter (Fig. 2B). As noted earlier, insulin mobilization was probably not involved (deRoos et al., 1985; Anderson et al., 2002). Indeed, these decreases are much larger than those seen after exhaustive exercise and during subsequent recovery (Richards et al., 2003). Thereafter, they climb to very high values during long-term fasting (Fig. 2B), as reported by previous authors (Zammitt and Newsholme, 1979; deRoos, 1994). This initial fall corresponds well with the prolonged period (up to 75 h) of elevated oxygen consumption ("specific dynamic action") that was measured after the 5.5% meal in *S. acanthias* (Wood et al., 2007a), and also seen after feeding in *Scyliorhinus canicula* (Sims and Davies, 1994). It also corresponds well with the marked increases in activities of a variety of enzymes of aerobic metabolism measured in most tissues for up to 48 h after the 2.6% meal (Walsh et al., 2006). Of these enzymes, one of the most responsive was β-hydroxybutyrate dehydrogenase, which increased significantly in liver, brain, and particularly in the rectal gland during this period (Walsh et al., 2006). β-hydroxybutyrate dehydrogenase may catalyse either the synthesis (precursor = acetoacetate) or the first step in the oxidation of β-hydroxybutyrate (product = acetoacetate). The fact that plasma concentrations of acetoacetate were much lower than those of β-hydroxybutyrate and did not change significantly after feeding strongly suggests that the reaction is running in the net direction of oxidation after feeding, such that β-hydroxybutyrate is a major metabolic fuel at this time.

The rectal gland in particular has greatly elevated metabolic demands to power its role in NaCl and volume homeostasis during this period (MacKenzie et al., 2002; Wood et al., 2007c; Dowd et al., 2008; Matey et al., 2009), and the presence of physiological β-hydroxybutyrate concentrations can greatly augment the secretion rate fueled by glucose in the isolated-perfused gland (Walsh et al., 2006). Other major metabolic demands at this time are increased ureagenesis, protein synthesis, and gastric acid secretion. As noted earlier, elevated ureogenesis following feeding is a cost specific to elasmobranchs, in addition to the cost of increased protein synthesis common to virtually all fish during the post-prandial period (Carter et al., 2001; Wood, 2001). Associated with increased gastric acid secretion is a compensating excretion of excess base to the external water (Wood et al., 2007a, 2009; Tresguerres et al., 2007). This serves to correct the "alkaline tide" in the bloodstream. As proper acid–base measurements are possible only on chronically cannulated fish where blood can be sampled without disturbance, the post-prandial alkaline tide has only been documented in confined sharks fed involuntarily by stomach tube (Wood et al., 2005, Wood et al., 2009). However, terminal samples of plasma total CO_2 concentration from naturally fed sharks consuming a 5.5 % ration indicated a rise of 4.5 mmol L^{-1} at 6 h after feeding in the study of Wood et al. (2007b). Presumably, this would cause an even larger rise in blood pH in naturally feeding sharks than the 0.15–0. 20 unit rise associated with a 1.5–2.0 mmol L^{-1} rise in total CO_2 after force-feeding by stomach tube (Wood et al., 2005, 2009).

The marked elevation in plasma β-hydroxybutyrate concentrations (10–17 mmol L^{-1}) during prolonged fasting (15–56 days; Fig. 2B) to more than twice control levels was in excellent agreement with observations of DeRoos (1994) in the same species, where plasma levels of this ketone rose from 3–7 mmol L^{-1} at 3–8 days post-feeding to 12–14 mmol L^{-1} after 20–29 days of fasting. Quantitatively, both of these data sets are very different from the report of Zammitt and Newsholme (1979) on *Scyliorhinus canicula* where plasma β-hydroxybutyrate concentrations rose from $<$0.06 mmol L^{-1} in recently fed animals to 1.6–1.8 mmol L^{-1} after 40–150 days of fasting. This difference in absolute concentrations may be species-specific, but the qualitative trend is the same. Zammitt and Newsholme (1979) also reported that the activities of both β-hydroxybutyrate dehydrogenase and acetoacetyl-CoA thiolase increased 2–3 fold in the liver during long-term fasting, and made the explicit assumption that "an increase in the concentration of fuels in the plasma is indicative of increased rates of utilization and oxidation". This idea has been repeated by later authors (DeRoos, 1994; Ballantyne, 1997), but needs experimental substantiation.

Based on our finding that plasma β-hydroxybutyrate concentrations fall quickly after feeding at a time of increased metabolic demand (with acetoacetate concentrations unchanged), it may be that the subsequent rise during long-term fasting is caused by an excess of ketone production (in the liver) over ketone oxidation in the peripheral tissues. Certainly, it is likely that overall metabolic rate is dropping due to starvation. This idea is supported by the fact that plasma acetoacetate levels also increase significantly during prolonged fasting in both *Scyliorhinus canicula* (Zammitt and Newsholme, 1979) and *S. acanthias* (DeRoos, 1994), suggesting that the reactions are running in the net direction of ketone synthesis rather than ketone oxidation. There is a clear need for further work on ketone metabolism and metabolic rate during prolonged fasting in elasmobranchs, including other aspects which have never been investigated, such as how the animals avoid the systemic acidosis and acetone-intoxication which plague mammalian diabetics in similar circumstances.

There have been only a few previous measurements of amino acid profiles in the blood plasma of elasmobranchs (Goldstein and Forster, 1970; Boyd et al., 1977; Leech et al., 1979), and to our knowledge, none with respect to feeding. The present measurements in control animals (Figs 3, 4, Table 1) are in close agreement with these previous determinations on animals shortly after collection (presumably fasted for a few days), with respect to both the quantitative dominance of taurine, alanine, lysine, glycine, leucine, and valine, and total levels in the 1–3 mmol L^{-1} range. Our previous reports (Kajimura et al., 2006, 2008) that total plasma amino acids amounted to 5 –15 mmol L^{-1} in these same animals were in error, because we had used the relatively non-specific ninhydrin assay. These low total plasma amino acid concentrations in elasmobranchs may be compared with somewhat higher values seen in many teleosts, which are often in the 3–10 mmol L^{-1} range. (Goldstein and Forster, 1970; Ash et al., 1989; Espe et al., 1993; Ok et al., 2001; Sunde et al., 2003; Karlsson et al., 2006). Another difference from many of these teleost studies was a much more prolonged and biphasic rise in the concentrations of most amino acids after feeding (Fig. 3). This pattern likely reflects the slower digestive and absorptive processes in this elasmobranch (Wood et al., 2007b), but the reason for the biphasic pattern is unclear. It has been seen for some amino acids in teleosts, and the second peak has been speculatively attributed to either endogenous production (Carter et al., 2001) or reabsorption of amino acids from proteolytic enzymes in the distal intestine (Karlsson et al., 2006). Overall, the post-prandial responses of those amino acids thought to be essential versus those thought to be non-essential were similar (Fig. 3B, C). Note, however, that arginine cannot be considered essential in this group because it can be synthesized in the ornithine–urea cycle, unlike the situation in most teleosts. Furthermore, there have been no tests of essentiality in elasmobranchs to our knowledge.

The major intracellular amino acids of elasmobranch erythrocytes are taurine, β-alanine, and glycine (Boyd et al., 1977; King et al., 1980). These amino acids tend to be metabolically inert (taurine and β-alanine are not found in proteins) and are often used for cell volume regulation (Forster and Goldstein, 1979; Goldstein, 1981; Goldstein and Perlmann, 1995). It is therefore noteworthy that these three amino acids were the only ones to either decrease or remain unchanged after feeding (Fig. 4B, Table 1). Possibly they are taken up by the erythrocytes to regulate cell volume during the urea-driven plasma osmolality surge (Fig. 1A) which occurs after a meal (Kajimura et al., 2006, 2008).

Alanine is one amino acid that has been widely measured in elasmobranchs because of its assumed importance as both a substrate for gluconeogenesis and a vehicle for transport of N to sites of urea

production (Leech et al., 1979; deRoos et al., 1985; deRoos, 1994; Richards et al., 2003). Glutamine is thought to be another amino acid of central importance because of its dual role as an N-donor in ureagenesis and as a preferred oxidative substrate in many tissues (Chamberlin and Ballantyne, 1992; Ballantyne, 1997). The present measurements (Fig. 4A; Table 1) are in quantitative agreement with these earlier measurements in *S. acanthias*, and in particular confirm the extremely low levels of circulating glutamine (Chamberlin and Ballantyne, 1992). Indeed, all the other amino acids of the ornithine–urea cycle (ornithine, citrulline, aspartate, arginine) also remained very low in the plasma, suggesting they are kept low by consumption in ureagenesis. The present data also support the central importance of alanine and glutamine in elasmobranch metabolism, inasmuch as their plasma levels did not surge after the meal to the same extent as many other amino acids, yet were well maintained during prolonged fasting (Fig. 4A; Table 1). In contrast, injections of mammalian insulin have been reported to cause a long-lasting depression of plasma alanine levels in this species (deRoos et al., 1985).Those amino acids which increased many-fold after the meals, as exemplified by threonine (Fig. 4C; also serine, asparagine, and valine, Table 1) are presumably utilized more slowly by metabolic processes.

Fasting typically increases plasma NEFA concentrations in teleost fish (e.g. Alkanani et al., 2005) to support an increase in fatty acid oxidation that occurs in many tissues. Elasmobranchs, on the other hand, show low to no ability to oxidize fatty acids in extrahepatic tissues (Moyes et al., 1990). Fatty acid oxidation does occur in liver and to a lesser extent in kidney but unlike many other fish species dogfish have high rates of ketone body production to support metabolism in other tissues. This group also has a very reduced ability to transport NEFA in plasma due to the absence or trace amounts of the transport protein albumin (Ballantyne, 1997). Our results show that plasma nonesterified fatty acids (NEFA) concentration in dogfish fasted for 7 days are low compared to other fishes but similar to levels previously determined in fed dogfish (Ballantyne et al., 1993; Zammitt and Newsholme, 1979). However, we also show that NEFA's rapidly declined shortly after feeding (Table 2). Since plasma NEFA concentration represents the balance between release into the circulation and uptake into tissues (mainly liver), a drop in NEFA after feeding may occur due to: 1) decreased release from the gut or liver, or 2) an increased uptake into liver. One might expect an increase in plasma NEFA after a lipid rich meal but because of their low ability for NEFA transport, dogfish may rely on other forms of lipids to shuttle fat in the circulation. As in other vertebrates, lipids are digested in dogfish as chylomicrons, which can make up to 25% of plasma lipids (Ballantyne, 1997) and possibly reduce relative amounts of NEFA's in the circulation. As well, fasting may stimulate liver fatty acid oxidation and mobilization of liver NEFA's from stored triacylglycerol (TAG). These NEFA's may enter the circulation due to a mismatch between oxidation and re-esterification back into storage forms in the liver. In the fed state the liver may switch to primarily lipogenesis to form TAG, wax esters, phospholipids and /or squalene. This lipogenesis would increase malonyl-CoA levels, which inhibits fat oxidation as the liver switches to net NEFA uptake from the circulation for storage.

This study has added important data for plasma responses to feeding of the two remaining major metabolite groups, amino acids and lipids, in the dogfish shark. Thus, we now have a relatively complete picture of the striking metabolic shifts in response to the basic act of feeding in one species of elasmobranch fish. We hope that this synthesis will encourage continuing research into the responses of the whole organism and key organ systems to the demands of feeding, which will undoubtedly yield new insights into this fascinating vertebrate group.

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