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The influence of diet and gastrointestinal fermentation on key enzymes of substrate utilization in marine teleost fishes

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Abstract

Three closely related marine teleosts with similar size, swimming mode, and habitat preference were compared to test the hypothesis that energy metabolism is linked to diet choice in the wild. Key substrate-utilization enzyme activities were assayed from white locomotory muscle and liver in a carnivore (*Scorpius violaceus*), an omnivore (*Girella tricuspidata*), and a herbivore (*Kyphosus sydneyanus*) collected from their natural reef habitat in northeastern New Zealand. The similar energy requirements of the study fishes were reflected in specific enzyme activities of white muscle and suggested high dependence on endogenous fuel that is independent of dietary carbohydrate intake. Clear differences were found in enzymes of hepatic carbohydrate, fat, and ketone body metabolism that appear linked to diet choice and levels of gastrointestinal fermentation. Hepatic metabolism of fat and ketone bodies was also examined in New Zealand samples of the omnivorous *Girella cyanea* and the herbivorous *Kyphosus bigibbus*, and the tropical herbivorous species *Kyphosus vaigiensis* and *Kyphosus cinerascens* collected from the Great Barrier Reef, Australia. Overall, the results suggest that, like ruminants, herbivorous fishes such as *Kyphosus* species that rely upon gastrointestinal fermentation preferentially use lipids as major metabolic substrates by an increased capacity for lipid metabolism, and a lower capacity for glycolysis.

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1. Introduction

Much of what we know about the relationship between metabolic energy expenditure and diet in fish derives from exclusive studies on carnivorous, freshwater salmonids. Furthermore, within-species

studies on fish of economic importance have often focused on formulated artificial diets with easily absorbable nutrients. Accordingly, physiological and biochemical processes in energy metabolism have been widely neglected in marine fishes generally, and herbivorous fish in particular, despite their abundance and importance in temperate and tropical reef ecosystems (Horn, 1989). Marine herbivores are represented in at least 10 families of perciform fishes (Horn, 1989).

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The selection of physiologically informative enzymes forms the basis of the experimental approach to this study. Different specific activities of an enzyme in the tissues of closely related species should suggest functional and ecological reasons for any observed differences. Newsholme and Crabtree (1986) advise the use of enzymes catalyzing non-equilibrium reactions that are independent of substrate concentration. However, Pierce and Crawford (1996) provide evidence that near-equilibrium enzymes have evolved to compensate for differences in environmental factors influencing the killifish, *Fundulus heteroclitus*. With these considerations in mind, we examined the activity of several enzymes from the liver and white epaxial locomotory muscle at key points in energy metabolism.

Hexokinase (HK) activity is primarily associated with glucose utilization under aerobic conditions (Newsholme and Crabtree, 1986). Carbohydrate reserves are important in burst swimming through anaerobic breakdown of glycogen within the white trunk musculature, but seem to play only a minor role in providing energy to working muscles in sustained swimming (Weber and Haman, 1996). As expected, the minor role of blood-borne glucose in white muscle from cod, trout, and plaice is reflected by low activities of hexokinase (Knox et al., 1980), and is compatible with low consumption of this nutrient in some fish diets (Weber and Haman, 1996). Interestingly, carnivorous fish such as yellowtail, *Seriola quinqueradiata*, showed less tolerance of glucose loads than did omnivores such as carp (Furuichi and Yone, 1981).

Phosphofructokinase (PFK) activity reflects the importance of glycolysis (Crabtree and Newsholme, 1972a) and its activity in white muscle is linked to muscular contraction (Su and Storey, 1995), and in the liver, to gluconeogenesis (Su and Storey, 1993). Fructose-1,6-bisphosphatase (FBP) also plays a crucial role in gluconeogenesis, and evidence suggests that in the rainbow trout, glucose is released from the liver into circulation via this pathway, rather than by catabolism of hepatic glycogen stores (Cowey et al., 1977). In fish with limited dietary carbohydrate, non-carbohydrate substrates must serve as important sources of de novo glucose and glycogen (Suarez and Mommsen, 1987), and FBP activity appears low in the white muscle (Opie and Newsholme, 1967).

Lactate dehydrogenase (LDH) supports the operation of glycolysis under anaerobic conditions. In white muscle, the isozyme functions as a pyruvate reductase (Baldwin, 1988), and accumulated lactate may be directed towards replenishing glycogen stores (Milligan and Girard, 1993). Pyruvate dehydrogenase (PDH) is one of the major control points in intermediary metabolism, and a critical determinant of irreversible depletion of carbohydrate reserves (Ravindran et al., 1996). Although carbohydrate and fatty acid interactions have been established for mammals (Randle et al., 1994), we know little about such linkages in fish.

Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the first step in the pentose phosphate pathway which can produce reducing equivalents (NADPH) for lipogenesis. Moreover, it may function in a catabolic capacity oxidizing glucose-6-phosphate to carbon dioxide and water. This pathway may be important in fish presented with a high carbohydrate intake, presumably to support concomitant lipogenesis (Fideu et al., 1983). The hydrophobic nature of lipid necessitates special mechanisms for mobilization and oxidation. Storage is mainly hepatic (Mommsen, 1998), and the capacity for β -oxidation is restricted by the activity of 3-hydroxyacyl CoA dehydrogenase (HOAD). Lipid may be used in preference to carbohydrate as an energy source in the liver (Phillips and Hird, 1977) and skeletal muscle (Cowey and Walton, 1989).

The two primary ketone bodies serving as transportable units of fat for oxidation in peripheral tissues are acetoacetate and β -hydroxybutyrate. These metabolites are synthesized under certain nutritional states when carbohydrate and lipid oxidation are unbalanced and they appear important in elasmobranch fishes, which with the exception of freshwater stingrays, lack albumin (Zammit and Newsholme, 1979; Watson and Dickson, 2001). Two key enzymes in production of ketone bodies are 3-oxoacid CoA transferase (OAT), and β -hydroxybutyrate dehydrogenase (HBDH). Activity of OAT is present in muscle from teleosts and elasmobranchs (Zammit and Newsholme, 1979), but appears to be absent in elasmobranch liver (Watson and Dickson, 2001). Activity of HBDH has been detected in various tissues of both teleosts and elasmobranchs (Zammit and Newsholme, 1979; LeBlanc and Ballantyne, 1993, 2000).

The scope for metabolic energy expenditure was examined in three species of temperate marine fishes. Blue maomao (*Scorpius violaceus*), Family Scorpidae, are carnivorous and feed on a highly proteinaceous diet of zooplankton (Kingsford and MacDiarmid, 1988). Parore (*Girella tricuspidata*), Family Girellidae, are omnivorous and complement a diet of red and green algae with invertebrate material (Clements and Choat, 1997). Silver drummer (*Kyphosus sydneyanus*), Family Kyphosidae, are herbivorous and select a refractory diet comprising mainly leathery brown algae (Moran and Clements, 2002). The guts of *Kyphosus* species harbour symbiotic microbiota that supply short-chain fatty acids as a source of energy to the host (Clements and Choat, 1997; Mountfort et al., 2002).

To examine further the relationships between diet, metabolism, and phylogeny, we measured activities of three key hepatic enzymes in four additional congeneric species. Bluefish *Girella cyanea* share with *G. tricuspidata* an omnivorous diet of red algae, green algae and invertebrates, and both species have relatively low levels of fermentation in the hindgut (Clements and Choat, 1997). Grey drummer *Kyphosus bigibbus*, sailfin drummer *Kyphosus cinerascens* and brassy drummer *Kyphosus vaigiensis* resemble their congener *K. sydneyanus* both in their herbivorous algal diets and in their high levels of gastrointestinal fermentation (Choat et al., 2002; Clements and Choat, 1995, 1997). All seven study fish species belong to the percoid families Scorpidae, Girellidae, and Kyphosidae, which are thought to be closely related based on both larval (Neira et al., 1997) and molecular characters (Yagashita et al., 2002). Species identification incorporates recent taxonomic changes (Sakai and Nakabo, 2004).

The amount of dietary protein and accessible carbohydrate may be influential factors affecting metabolism in our study species (Kirchner et al., 2003). Another factor in herbivorous species may be the relative importance to metabolic requirements of short-chain fatty acids via gastrointestinal fermentation (Murray et al., 2003). For example, the true metabolic diet of ruminants is not what is ingested, but rather the combination of short-chain fatty acids (e.g. acetate) and unfermented nutrients that leave the rumen (Van Soest, 1994). Activity of the enzyme acetyl CoA synthetase, necessary for the activation of

acetate prior to catabolism or lipogenesis, has been detected in fishes (Clements et al., 1994).

In sampling fish from wild populations, we acknowledge that while diet choice is well known, the nutrients available for metabolism may not be fully characterized. The study species were similar in size, and their swimming behaviour suggests similar metabolic costs associated with locomotion. They occur commonly in schools on shallow reefs in New Zealand and Australia (Francis, 2001). The aim of our study was to determine whether or not there are metabolic differences between these fishes that can be attributed to their diverse diet choices. In particular, we wish to know (a) how herbivorous fish utilizing principally carbohydrate reserves meet similar energy requirements to carnivorous fish that obtain readily assimilated protein fuels, and (b) how the assimilation of short-chain fatty acids from gastrointestinal fermentation in some herbivorous species influences energy metabolism.

2. Materials and methods

2.1. Fish collection and tissue sampling

Seven blue maomao (*S. violaceus*) (standard length 220–265 mm), seven parore (*G. tricuspidata*) (235–340 mm), two bluefish (*G. cyanea*) (400 and 450 mm), seven silver drummer (*K. sydneyanus*) (230–500 mm), and four grey drummer (*K. bigibbus*) (255–290 mm) were collected from shallow rocky reefs in the Hauraki Gulf on the northeastern coast of New Zealand (174°48'E–175°20'E, 36°07'S–36°20'S). Five specimens each of topsail drummer (*K. cinerascens*) (295–340 mm) and brassy drummer (*K. vaigiensis*) (315–365 mm) were collected from outer reefs near Lizard Island, Great Barrier Reef, Australia (145°30'E 14°42'S). All were mature fish. *S. violaceus* were caught with baited handlines, while all other species were taken on snorkel or SCUBA by spear gun. Fish were removed from the water and immediately euthanased by pithing. Care was taken to use only fish that had been rapidly captured, and avoid possible artifacts from burst-swimming stress responses (Wells and Pankhurst, 1999).

Small blocks of tissue (approximately 0.5 g) were rapidly excised from the dorsal epaxial white muscu-

lature and from the liver, and plunged into liquid nitrogen for storage. Heparinized blood was taken by caudal venepuncture, centrifuged for 1–2 min at $1000\times g$, and the aspirated plasma stored in liquid nitrogen. Liver samples only were collected as above for *G. cyanea*, *K. bigibbus*, *K. cinerascens*, and *K. vaigiensis*. Samples of *K. cinerascens* and *K. vaigiensis* were returned to the laboratory in New Zealand frozen on ice.

2.2. Homogenate preparation

Portions of 0.1–0.2 g were taken from the uncontaminated core of tissue blocks and homogenized for 15 s (Ultra-Turrax T-25 with 8 mm diameter shaft, Janke and Kunkel, Germany) in 1:10 w/v ice cold extraction buffer. Homogenate protein content was similar between species for both muscle and hepatic tissues. For assays other than phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH), the extraction buffer contained 50 mM KCl, 8 mM MgCl₂, 1 mM EDTA, and 50 mM imidazole-HCl buffer, pH 7.8. For PFK activity, 5 mM MgSO₄, 5 mM dithiothreitol, 1 mM EDTA, and 50 mM Tris-HCl, pH 8.2 was used; PDH was extracted in 1 mM EDTA, 2 mM mercaptoethanol, and 50 mM K₂PO₄, pH 7.8 was used.

Preliminary assays of enzyme activity showed that specific activity was increased in several instances following sonication of homogenates for two 10-s bursts with a Soniprep 150 ultrasonic tissue disintegrator (MSE Scientific Instruments). Accordingly, sonication was used to express membrane-bound enzyme activities. Except for the hepatic hexokinase assay, all homogenates were centrifuged for 5 min at $12,000\times g$ and the supernatants, excluding the superficial lipid layer, were assayed immediately.

Chemicals were purchased from Boehringer Mannheim, or Sigma.

2.3. Enzyme assays

Reaction rates were recorded from a Shimadzu UV-1601 recording spectrophotometer fitted with thermostatic control to maintain reaction temperature at 25.0 ± 0.2 °C. Absorbance changes were recorded at 340 nm, except for 3-oxoacid coenzyme A transferase (OAT) which was assayed at 303 nm. Reactions were

initiated by the addition of substrate. Controls for non-specific activity were evaluated following 3–5 min of incubation in the absence of substrate. Assays were performed with 20 μ l extract in a total volume of 1 ml. Substrate and coupling enzyme concentrations were selected for specific enzyme activity according to the protocols supplied by previous investigators and described below. Preliminary assays established that tissues frozen in liquid nitrogen did not show significantly reduced activities compared to fresh, unfrozen material. Activity is presented conventionally as U g⁻¹ tissue wet weight, where one unit (U) is 1 μ M substrate converted to product per min.

Hexokinase (HK) EC 2.7.1.1. 7.5 mM MgCl₂, 0.8 mM EDTA, 1.5 mM KCl, 2.5 mM ATP, 0.4 mM NADP, 10 mM creatine phosphate, 0.7 U creatine kinase, 0.7 U glucose-6-phosphate dehydrogenase, 1 mM glucose, and 50 mM imidazole-HCl, pH 7.4. Preliminary assays confirmed significant activity in whole homogenates which were subsequently assayed (Borrebaek et al., 1993).

Glucokinase (GK) EC 2.7.1.2. Assay as for HK, but with 100 mM glucose. Correction was made for glucose dehydrogenase activity by omission of ATP, creatine kinase, and glucose-6-phosphate dehydrogenase (after Tranulis et al., 1991).

Phosphofructokinase (PFK) EC 2.7.1.11. 10 mM MgCl₂, 250 mM KCl, 1 mM ATP, 2 mM AMP, 0.15 mM NADH, triose phosphate isomerase, glycerophosphate dehydrogenase, aldolase, 3 mM fructose-6-phosphate, and 50 mM Tris-HCl, pH 8.2 (after Zammit and Newsholme, 1976). Assays were performed immediately following homogenate centrifugation due to instability of the PFK reaction (Su and Storey, 1993).

Fructose-1,6-biphosphatase (FBP) EC 3.1.3.11. 1 mM EDTA, 5 mM MgCl₂, 0.2 mM NADP, 0.3 U ml⁻¹ glucose-6-phosphate dehydrogenase, 2 U ml⁻¹ phosphoglucose isomerase, 1 mM fructose-1,6-biphosphate, and 50 mM Tris-HCl, pH 7.5 (after Bonamusa et al., 1992).

Lactate dehydrogenase (LDH) EC 1.1.1.27. 0.15 mM NADH, 2.5 mM pyruvate, and 50 mM imidazoleHCl, pH 7.4 (after Zammit and Newsholme, 1976).

Glucose-6-phosphate dehydrogenase (G6PDH) EC 1.1.1.49. 7 mM MgCl₂, 0.4 mM NADP, 1 mM

glucose-6-phosphate, and 50 mM imidazole–HCl, pH 7.4 (after Aster and Moon, 1981).

3-Hydroxyacyl coenzyme A dehydrogenase (HOAD) EC 1.1.1.35. 1 mM EDTA, 0.2 mM NADH, 0.06 mM acetoacetyl CoA, and 50 mM imidazole–HCl, pH 7.4 (after Crockett and Sidell, 1990).

3-Oxoacid coenzyme A transferase (OAT) EC 2.8.3.5. 5 mM MgCl₂, 5 mM iodoacetamide, 10 mM acetoacetate, 0.2 mM succinyl CoA, and 50 mM imidazole–HCl, pH 8.0 (after Stuart and Ballantyne, 1996).

β-Hydroxybutyrate dehydrogenase (HBDH) 1.1.1.30. 0.1 mM NADH, 2 mM acetoacetate, and 50 mM imidazole–HCl, pH 8.0 (after Singer and Ballantyne, 1991).

Pyruvate dehydrogenase (PDH) EC 11.2.4.1. 0.5 mM EDTA, 1 mM MgCl₂, 0.3 mM thiamine pyrophosphate, 2.5 mM NAD, 0.2 mM coenzyme A, 100 mM pyruvate, and 50 mM Tris–HCl, pH 8.0 (after Elnageh and Gaitonde, 1988; Russell and Storey, 1995).

2.4. Plasma metabolites and ketone bodies

Protein was assayed using the bicinchoninic acid reagent (Sigma B-9643 with 540-10 protein standard) and plasma glucose using a YSI Model 2300 glucose meter based on the glucose oxidase reaction. The presence of acetoacetate was tested using Multistix™ 10SG reagent strips (Bayer Diagnostics), and β-hydroxybutyrate using the Sigma diagnostic kit 310-UV.

In order to clarify the significance of ketone utilization, an experiment was performed to assess the capacity of tissues to clear β-hydroxybutyrate. The substrate was added to tissue homogenate at a concentration of 25 mg dl⁻¹ (Sigma 310-50), incubated at 25 °C, and subsamples taken at intervals of 0,

2, 5, 10, and 15 min, followed by arrest at 90 °C to stop further enzymatic reaction.

2.5. Statistical analysis

Four replicates from each specimen of *S. violaceus*, *G. tricuspidata*, and *K. sydneyanus* were analyzed for all assays. Four replicates from each specimen of *G. cyanea*, *K. bigibbus*, *K. cinerascens*, and *K. vaigiensis* were analyzed for HOAD, OAT, and HBDH assays only. The General Linear Models procedure from the SAS 6.10 Statistical Package (SAS Institute, 1990) was used to determine whether differences between species in enzyme activities were statistically significant at $P < 0.05$. Tukey's post hoc tests were then performed to determine which differences were significant at $P < 0.05$.

3. Results

Specific activities of enzymes of carbohydrate, fat, and ketone body metabolism from white skeletal muscle, and of carbohydrate metabolism from liver are summarized for the three main study species in Tables 1 and 2, respectively. Differences in muscle enzyme activities among the three species, though significant ($P < 0.05$), are small and probably lack physiological significance. HK activity, though appearing highest in muscle from the carnivorous *S. violaceus*, reflects only a minor component of total glycolytic potential as the HK:PFK ratios ranged from 0.002 to 0.004. HOAD activity was highest in *S. violaceus*. The low levels of muscle HBDH activity recorded for *G. tricuspidata* are probably without physiological significance.

Differences in activities of liver enzymes of carbohydrate metabolism were significant ($P < 0.05$) among the three species except for G6PDH between

Table 1
Comparisons of specific enzyme activities from white muscle

	HK	PFK	LDH	HOAD	HBDH
<i>S. violaceus</i>	0.075±0.016	17.47±0.43	1311±46	0.900±0.051	not detected
<i>G. tricuspidata</i>	0.032±0.002	13.28±0.78	1140±29	0.319±0.017	0.008±0.003
<i>K. sydneyanus</i>	0.045±0.005	13.57±0.59	1499±41	0.546±0.032	0.052±0.004

Values are mean U g⁻¹ wet weight±S.E.M.

Table 2

Comparisons of specific activities of enzymes of carbohydrate metabolism from liver

	HK	PFK	FBP	G6PDH
<i>S. violaceus</i>	not detected	1.30±0.03	9.12±0.14	12.03±1.18
<i>G. tricuspidata</i>	0.82±0.10	1.83±0.20	7.69±0.19	8.07±0.89
<i>K. sydneyanus</i>	0.44±0.05	0.73±0.04	6.32±0.32	8.57±0.55

Values are mean U g⁻¹ wet weight±S.E.M.

G. tricuspidata and *K. sydneyanus*. The activity of FBP exceeded that of hepatic PFK for all species, with the highest ratio occurring in *K. sydneyanus* where PFK activity was only half that in *G. tricuspidata*. On the basis of FBP activity, gluconeogenesis capacity appeared highest in *S. violaceus*, intermediate in *G. tricuspidata*, and lowest in *K. sydneyanus*. The absence of hepatic HK activity in *S. violaceus* was unexpected and may reflect an unknown protocol error. Hexokinase activity in the liver of both *G. tricuspidata* and *K. sydneyanus* was complemented by glucokinase activity. The ratios of glucose dehydrogenase activity relative to HK activity (*K. sydneyanus*, 1.68±0.21; *G. tricuspidata*, 0.61±0.24) were lower than reported for two other fish species (Tranulis et al., 1991, 1996).

Patterns in activity of hepatic enzymes of fat and ketone body metabolism were clarified by the examination of the four additional fish species (Table 3). Hepatic HOAD activity differed significantly between the seven fish species examined except for between *G. cyanea* and *K. bigibbus*, and between *K. cinerascens* and *K. vaigiensis*. HOAD activity was lowest in the two *Girella* species, suggesting a lower capacity for β -oxidation of fatty acids. Activities of OAT and HBDH in liver differed significantly among

Table 3

Comparisons of specific activities of enzymes of lipid and ketone body metabolism from liver

	HOAD	OAT	HBDH
<i>S. violaceus</i>	7.28±0.25	1.20±0.08	0.07±0.01
<i>G. tricuspidata</i>	2.31±0.13	0.86±0.07	0.16±0.01
<i>G. cyanea</i>	3.91±0.32	0.32±0.06	0.25±0.03
<i>K. sydneyanus</i>	11.31±0.45	3.41±0.19	0.92±0.03
<i>K. cinerascens</i>	5.51±0.17	4.66±0.16	0.37±0.06
<i>K. vaigiensis</i>	5.75±0.35	3.04±0.07	0.65±0.07
<i>K. bigibbus</i>	4.13±0.27	3.23±0.09	1.74±0.13

Values are mean U g⁻¹ wet weight±S.E.M.

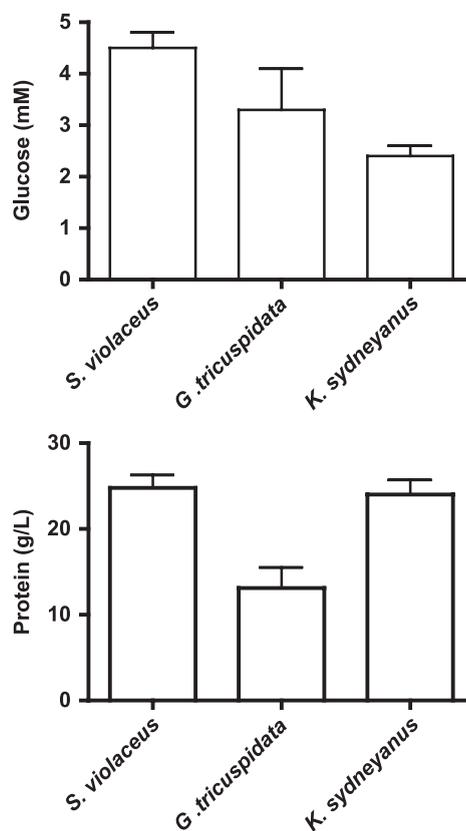


Fig. 1. Plasma glucose and protein concentrations. Error bars represent S.E.M. ($n=7$).

the seven fish species, with highest activities recorded in the four *Kyphosus* species.

Plasma metabolites are shown in Fig. 1. Glucose was significantly lower in the herbivore, *K. sydneyanus*

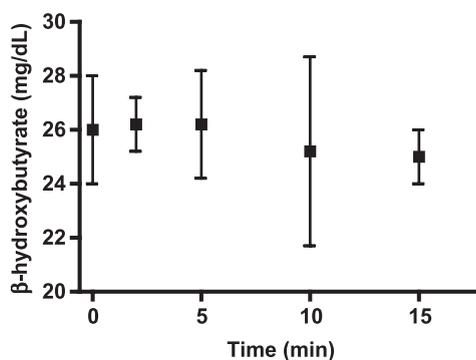


Fig. 2. Clearance of β -hydroxybutyrate from *K. sydneyanus* liver homogenates spiked with the ketone body at 25 mg dl⁻¹. Error bars represent S.E.M. ($n=7$).

nus, and protein lowest in the omnivore, *G. tricuspidata*. Ketone bodies were not detected in the plasma of any species. Capture and handling stress are known to elevate plasma glucose, but the rise is not evident until about 15 min post-stress (Begg and Pankhurst, 2004); our rapid sampling protocol is unlikely to lead to spuriously high glucose values.

Hepatic homogenates of *K. sydneyanus* failed to show significant clearance of β -hydroxybutyrate after 15 min (Fig. 2).

4. Discussion

A critical assumption for the framework of this investigation was that the study species are well-matched in terms of energy requirement. Body mass (Goolish, 1995) and environmental temperature (Majed et al., 2002) are significant determinants of the activities of enzymes associated with energy expenditure. Further, among species of similar size, activity in skeletal muscle may differ by three orders of magnitude due to activity and mode of swimming (Somero and Childress, 1985). It was therefore reassuring that species-matching, based on behavioural observations, was confirmed by the similarity of key substrate-utilization enzyme activities in the white musculature of *S. violaceus*, *G. tricuspidata*, and *K. sydneyanus*.

The proteinaceous diets of carnivorous fishes are energy rich and readily absorbable, and contrast with the algal diets of marine herbivorous fish which are fibrous and low in protein and lipid, but contain carbohydrate storage compounds (Montgomery and Gerking, 1980). The energy sources of *S. violaceus* are mainly protein and lipid (Sargent, 1976; Francis, 2001); those of *G. tricuspidata* mainly carbohydrate, with some protein (Clements and Choat, 1997), and for *K. sydneyanus*, mainly carbohydrate and short-chain fatty acids (ibid., Moran and Clements, 2002; Mountfort et al., 2002).

4.1. Carbohydrate metabolism in white muscle

Hexokinase activity was very low in *S. violaceus*, *G. tricuspidata*, and *K. sydneyanus* compared to total glycolytic activity, which is evident from the ratio of HK to PFK and reflects the low dependence on

aerobic utilization of glucose. Thus, although carbohydrate stores feature in anaerobic energy expenditure, glucose appears less important for complete oxidation relative to fat and protein substrates (cf. Weber and Haman, 1996) and accords with the independence of white muscle from blood-borne metabolic fuels when operating as a closed system (Milligan and Girard, 1993). Despite statistical differences in HK activity, the higher capacity for glucose-fuelled aerobic exercise in *S. violaceus* is likely to be of minor physiological significance. The similarity of PFK activities provides concrete evidence that peak glycolytic power of these three species is comparable. These data are consistent with findings from trout (*Onchorhynchus mykiss*) that little exogenous glucose is required to maintain glycogen stores (Moyes et al., 1992), and that recycling of lactate occurs in situ in the musculature (Pagnotta and Milligan, 1991). LDH is a good indicator of the capacity of the white musculature for anaerobic glycolysis (Somero and Childress, 1985), and despite statistical differences between these three study species, all activities were high in comparison with other species (cf. Somero and Childress, 1985). Therefore, enzymes of carbohydrate catabolism in muscle failed to reflect species differences that could be attributed to dietary composition.

4.2. Fat and ketone body metabolism in white muscle

The higher dietary lipid intake in the carnivorous *S. violaceus* is matched by higher HOAD activity, and presumably reflects the β -oxidation of fatty acids (Christiansen and Klungsoyr, 1987). Conversely, *G. tricuspidata* and *K. sydneyanus* may be less reliant on aerobic lipid fuel consumption. The presence of HBDH activity in the herbivorous *K. sydneyanus*, although low, is a novel observation for teleost white muscle, but appears physiologically insignificant in terms of the ability of muscles to engage ketone body oxidation. HBDH activity has, however, been recorded in brain and liver tissues of other teleosts (LeBlanc and Ballantyne, 1993; Segner et al., 1997; Soengas et al., 1998; LeBlanc and Ballantyne, 2000; Soengas and Aldegunde, 2002). Fat utilization in the white muscle of our study fish is therefore more likely to be an indicator of aerobic capacity, than a reflection of dietary composition.

4.3. Enzymes of hepatic carbohydrate metabolism

The liver is central to many processes in intermediary metabolism as many nutrients absorbed from the gut are cleared from the plasma and the hepatic portal vein into this organ. Unlike skeletal muscle, the liver is thus likely to reflect diet choice through the relative capacity for anabolic and catabolic processes. We acknowledge that some caution is required when interpreting interspecific differences in metabolic enzyme activities in relation to diet. For example, while HK activity was lower overall in gilthead seabream and rainbow trout than in carp, the fact that HK activity changed in response to dietary carbohydrate levels in the former two species but not in the latter suggested that the role of this enzyme may differ between these species (Panserat et al., 2000). We have attempted to minimize these effects by restricting our study to a set of closely related perciform teleosts.

FBP and PFK mediate the cycle between fructose-6-phosphate and fructose-1,6-bisphosphate, and determine whether carbon flows in the direction of glycolysis or gluconeogenesis. The high ratio of FBP/PFK activity in the study species reflects the dominance of gluconeogenesis over glycolysis in the liver. Assimilation of carbohydrate is greater in the omnivorous *G. tricuspidata* than in the carnivorous *S. violaceus*, and thus higher glycolytic capacities in the former species are expected to accommodate the greater glucose concentrations taken up by the liver. Dietary intake of carbohydrate in adult *K. sydneyanus*, in which microbial digestion appears to be important (Mountfort et al., 2002), is unclear (Moran and Clements, 2002). Peak FBP activity may be higher in *S. violaceus* because dietary carbohydrate must be supplemented through gluconeogenesis. This interspecies difference is mirrored in intra-species investigations where diets are manipulated to increase carbohydrate intake. Thus, carnivorous trout, *O. mykiss* (Walton, 1986), and seabream, *Sparus auratus* (Garcia de Frutos et al., 1990; Bonamusa et al., 1992), and omnivorous carp, *Cyprinus carpio* (Shimeno et al., 1995a), and herbivorous tilapia, *Oreochromis niloticus* (Shimeno et al., 1993), show high glycolytic enzyme activity and gluconeogenic depression.

Despite higher hepatic G6PDH activity in *S. violaceus*, differences between species are minor and

unlikely to reflect significant physiological channeling of energy through the pentose phosphate shunt.

Glucokinase was thought to be absent in fish (Cowey et al., 1977; Fideu et al., 1983; Sundby et al., 1991; Tranulis et al., 1991), but has been reported in the liver of several species including salmonids (Tranulis et al., 1996; Capilla et al., 2003; Kirchner et al., 2003), seabream (Panserat et al., 2000), and perch (Borrebaek et al., 2003). The enzyme has a low affinity for glucose and activity levels vary with dietary status (Cornish-Bowden and Cardenas, 1991). The hepatic activity of glucokinase, an isoenzyme of hexokinase, in *G. tricuspidata* and *K. sydneyanus* would be expected to clear greater quantities of glucose from the blood and permit the species to thrive on a high carbohydrate diet.

4.4. Plasma metabolites

Blood-borne metabolites are susceptible to rapid change and may not accurately reflect the rate of utilization for each species. Post-prandial regulation of blood glucose, however, is a greatly protracted process in fish (Weber and Zwingelstein, 1995). Whether the higher measured concentration of plasma glucose in *S. violaceus* compared to *G. tricuspidata* and *K. sydneyanus* reflects metabolic usage, or simply differences in diurnal feeding behaviour must await further observation. Similarly, the relatively lower plasma protein in *G. tricuspidata* might reflect a low protein to carbohydrate intake as noted for trout, *O. mykiss* (Walton, 1986; Shimeno et al., 1995b), but alternatively may result from patterns of feeding behaviour in the wild.

4.5. Hepatic fat and ketone body metabolism

Protein appears an important lipogenic precursor in carp, *C. auratus* (Nagai and Ikeda, 1971), and trout, *O. mykiss* (Christiansen and Klungsoyr, 1987). Differences among our study species were slight, indicating similar capacities for de novo lipid synthesis and dietary independence. The activity of G6PDH in *G. tricuspidata*, the species likely to have the highest intake of assimilable carbohydrate (Clements and Choat, 1997), suggests that carbohydrate intake in excess of storage capacity is not made available for fat synthesis. The low activities of key enzymes of glycolysis, gluconeogenesis, and lipogenesis in the

herbivore *K. sydneyanus* were inconsistent with the low dietary intake of lipid (Montgomery and Gerking, 1980). However, the short-chain fatty acids generated by the high levels of microbial fermentation in this species (Mountfort et al., 2002) are the most likely substrates for lipogenesis.

The liver, along with red skeletal muscle, is thought to be a major lipid sink in fish (Crabtree and Newsholme, 1972b; Phillips and Hird, 1977). The variation among *Kyphosus* species in hepatic HOAD activity is puzzling given their dietary similarity and close phylogenetic relationship. The varying requirements for de novo lipogenesis among *Kyphosus* suggested by the results may indicate that these species differ in the relative contributions of endogenous and exogenous digestive processes. Although β -oxidation in the liver appears important for the carnivorous *S. violaceus*, there is probably a smaller margin between production and utilization than for the species with high algal intake.

Hepatic HBDH activity was lowest in *S. violaceus*, intermediate in the *Girella* species, and highest in the *Kyphosus* species. This suggests ketone bodies to be an important source of energy in the *Kyphosus* species in non-hepatic tissues, and represents a novel finding for teleosts (cf. Segner et al., 1997).

The primary site for ketone body production in vertebrates is the liver (McGarry and Foster, 1980), and the presence of hepatic OAT in fish may either reflect ketone body utilization or substrate cycling between acetoacetate and acetoacetyl CoA (Zammit et al., 1979). Results from our hepatic β -hydroxybutyrate clearance experiment in *K. sydneyanus*, however, do not support the hypothesis of OAT involvement in ketone body utilization. The apparent absence of ketone bodies in the plasma of our study species suggests that either short-chain fatty acids are taken up by extra-hepatic bodies immediately, or that nutritional status at the time of sampling did not promote ketogenesis. Very little is known about the conditions under which ketone bodies are produced and synthesized in fish, although Watson and Dickson (2001) have recently demonstrated that active sharks utilize ketone bodies rather than fatty acids for aerobic muscle metabolism.

In summary, we have demonstrated clear differences between the three test species in pathways of hepatic carbohydrate, fat, and ketone body metabo-

lism that may be linked to diet choice and gastrointestinal fermentation. In the locomotory muscles, utilization of exogenous glucose for glycolysis was a small fraction of total glycolytic capacity, suggesting that the high dependence on endogenous fuel is independent of dietary carbohydrate intake. Between-species differences in enzyme activities of skeletal muscle appeared physiologically insignificant. The capacity for lipid metabolism appears to be higher in the carnivorous *S. violaceus* and the herbivorous *Kyphosus* species than in the omnivorous *Girella* species. The converse appears to be true for two of the three regulatory enzymes of glycolysis examined (HK and PFK). PFK activity, however, is known to be regulated by effectors (Murray et al., 2003). Lipid oxidation is probably important in *S. violaceus* due to its zooplankton diet (Sargent, 1976), whereas in the *Kyphosus* species this is most likely due to the high levels of short-chain fatty acids entering the liver from gastrointestinal fermentation (Clements and Choat, 1997; Mountfort et al., 2002). Preferential use of lipid for oxidative metabolism is likely to be associated with a lower capacity for glycolysis. Glucose is probably valuable and limited in *Kyphosus* species due to a reliance on exogenous digestive mechanisms, and the consequent assimilation of energy as short-chain fatty acids rather than monosaccharides.

While differences in metabolism have been noted in captive fish (especially salmonids and cyprinids) on manipulated diets (e.g. Panserat et al., 2000; Capilla et al., 2003; Kirchner et al., 2003), our study represents a novel finding for closely related species living in the wild. It is perhaps not surprising that energy metabolism in fishes may be as diverse as that seen in mammals, e.g. the metabolic differences seen between ruminants, hindgut fermenters, and carnivores (Van Soest, 1994).

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