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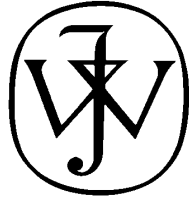
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Metabolic Organization of the Spotted Ratfish, *Hydrolagus colliei* (Holocephali: Chimaeriformes): Insight Into the Evolution of Energy Metabolism in the Chondrichthyan Fishes

BEN SPEERS-ROESCH, JACOB WILLIAM ROBINSON,
AND JAMES STUART BALLANTYNE*

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ABSTRACT The metabolic organization of a holocephalan, the spotted ratfish (*Hydrolagus colliei*), was assessed using measurements of key enzymes of several metabolic pathways in four tissues and plasma concentrations of free amino acids (FAA) and non-esterified fatty acids (NEFA) to ascertain if the Holocephali differ metabolically from the Elasmobranchii since these groups diverged ca. 400 Mya. Activities of carnitine palmitoyl transferase indicate that fatty acid oxidation occurs in liver and kidney but not in heart or white muscle. This result mirrors the well-established absence of lipid oxidation in elasmobranch muscle, and more recent studies showing that elasmobranch kidney possesses a capacity for lipid oxidation. High activities in oxidative tissues of enzymes of ketone body metabolism, including D- β -hydroxybutyrate dehydrogenase, indicate that, like elasmobranchs, ketone bodies are of central importance in spotted ratfish. Like many carnivorous fishes, enzyme activities demonstrate that amino acids are metabolically important, although the concentration of plasma FAA was relatively low. NEFA concentrations are lower than in teleosts, but higher than in most elasmobranchs and similar to that in some "primitive" ray-finned fishes. NEFA composition is comparable to other marine temperate fishes, including high levels of *n*-6 and especially *n*-3 polyunsaturated fatty acids. The metabolic organization of the spotted ratfish is similar to that of elasmobranchs: a reduced capacity for lipid oxidation in muscle, lower plasma NEFA levels, and an emphasis on ketone bodies as oxidative fuel. This metabolic strategy was likely present in the common chondrichthyan ancestor, and may be similar to the ancestral metabolic state of fishes. *J. Exp. Zool.* 306A:1-14, 2006. © 2006 Wiley-Liss, Inc.

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Holocephalans, or ratfishes or chimaeras, are a group of mostly deep-water marine cartilaginous fishes that are most closely related to their more notorious relatives, the elasmobranch fishes. The subclasses Holocephali and Elasmobranchii, which together comprise the class Chondrichthyes, diverged around 360 Mya, not long after the appearance of the first chondrichthyans about 420 Mya (Grogan and Lund, 2004). Although this long evolutionary separation has resulted in certain unique anatomical and physiological features, ratfishes, like marine elasmobranchs, are osmoconformers that accumulate high levels of urea (>250 mM) and counteracting methylamines to maintain an internal solute concentration close to

that of seawater (Read, '71; Bedford, '83). Ratfishes, however, have less urea and more Na⁺ and Cl⁻ than elasmobranchs (Read, '71), and the principal counteracting solute in at least one species is the methylamine betaine and not trimethylamine oxide, the major counteracting solute in elasmobranchs (Bedford et al., '98).

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1 There are very few studies on the energy
2 metabolism of holocephalans, involving only lim-
3 ited measurements of plasma lipids and amino
4 acids and tissue activities of certain enzymes.
5 Bedford ('83) analyzed certain free amino acids
6 (FAA) in plasma and red blood cells from
7 *Callorhinchus milii*, but did not measure gluta-
8 mine, which is particularly important in energy
9 metabolism in elasmobranchs (Ballantyne, '97).
10 Larsson and Fänge ('77) found low levels of non-
11 esterified fatty acids (NEFA) in plasma from the
12 holocephalan *Chimaera monstrosa*, but they used
13 an inaccurate colorimetric technique that prob-
14 ably underestimates NEFA concentration and
15 does not reveal fatty acid composition (see Singer
16 et al., '90). Ritter et al. ('87) measured activities of
17 a limited number of enzymes of intermediary
18 metabolism in liver and brain of the spotted
19 ratfish (*Hydrolagus collii*), a holocephalan which
20 is common in the northeast Pacific Ocean from
21 close inshore to 1,000 m (Eschmeyer et al., '83).

22 A detailed examination of the metabolic organi-
23 zation of a holocephalan is of interest considering
24 the unique pattern of aerobic fuel use seen in
25 elasmobranchs: namely, an apparent low or non-
26 existent capacity for extrahepatic lipid oxidation
27 and an increased reliance on ketone bodies and
28 glutamine as oxidative substrates (reviewed by
29 Ballantyne, '97). None of these has been examined
30 in holocephalans. In the present study we ascer-
31 tained whether, like elasmobranchs, the spotted
32 ratfish, as a representative holocephalan, pos-
33 sesses: (1) reduced capacity for lipid oxidation as
34 indicated by enzyme activities in extrahepatic
35 tissues, (2) increased extrahepatic capacity for
36 ketone body metabolism as indicated by high
37 activity of β -hydroxybutyrate dehydrogenase (β -
38 HBDH), and (3) low levels of NEFA in the blood
39 (using a sensitive method that provides data on
40 NEFA composition). We measured plasma FAA
41 levels to evaluate the importance of FAA (includ-
42 ing glutamine) in a holocephalan. Measurements
43 of other enzymes of energy metabolism were made
44 to provide a more comprehensive picture of the
45 metabolic organization of a representative holoce-
46 phalan.

47 Similarities between the metabolic organization
48 of holocephalans and elasmobranchs will allow an
49 understanding of the probable metabolic organi-
50 zation of the common chondrichthyan ancestor
51 that swam in the seas over 400 Mya. Commonal-
52 ities in the pattern of lipid oxidative capacity will
53 be of specific interest in the context of Ballantyne
et al.'s ('87) hypothesis that the evolution of

urea-based osmoregulation in elasmobranchs led
to a de-emphasis on fatty acids as an extrahepatic
metabolic fuel because of perturbing effects of
high levels of urea on the ability of albumin to
transport long-chain fatty acids. Since holoceph-
alans also possess high levels of urea, they would be
expected to show a similar metabolic organization
to elasmobranchs if this hypothesis is correct.

MATERIALS AND METHODS

Animals

Spotted ratfishes (450–700 g) were captured by
otter trawl off northern Vancouver Island, British
Columbia, on a Department of Fisheries and
Oceans (Canada) groundfish survey aboard CCGS
W.E. Ricker in May 2004. Depth of capture was
120–200 m and bottom temperature was 6–7°C.
Trawls lasted 30–60 min. Only animals still re-
sponsive to handling were sampled.

Blood was drawn into heparinized syringes by
caudal puncture and centrifuged for 5 min to
sediment erythrocytes. Plasma was decanted and
immediately frozen in liquid nitrogen, followed
by storage at –80°C until use. Animals were then
sacrificed by rapidly severing the spinal cord.
Liver, heart, kidney, and white muscle were
excised within 5 min and frozen in liquid nitrogen
for transport back to the University of Guelph
where they were stored at –80°C until use.

Tissue preparation

Tissues were thawed on ice and placed in 11
volumes of ice-cold homogenization buffer (50 mM
imidazole, 1 mM dipotassium ethylene diamine-
traacetic acid (EDTA), pH 7.4 at 20°C). Homo-
genization was completed on ice using a Polytron
PT1200 (Kinematica, Inc., Newark, NJ) set at
high speed (25,000 rpm) for three passes of 10 sec
with 30 sec between bursts. Homogenates were
centrifuged at 4°C at 500g to clear cellular debris
and the supernatant was used directly or diluted
for enzyme assays. For assays of cytochrome
c oxidase (CCO), carnitine palmitoyl transferase
(CPT), and carnitine octanoyl transferase (COT),
Tween 20 was added to the homogenate to make
a final concentration of 0.5% and this was mixed
slowly on ice for 15 min, then centrifuged as above,
and the supernatant used directly for enzyme
assays. Tween 20 (0.5%) gave the highest activity
of CPT in tissues from *Leucoraja erinacea* and
other elasmobranchs when compared with homo-
genates that were untreated, sonicated, or treated



METABOLIC ORGANIZATION OF A HOLOCEPHALAN

3

1 with 0.2% Triton-X 100 (TX100) (Speers-Roesch
 2 and Ballantyne, unpublished data; J.R. Treberg,
 3 unpublished data). Although TX100 gave approxi-
 4 mately 50% higher activity of CCO than 0.5%
 5 Tween 20, it was not possible to prepare a separate
 6 TX100-treated aliquot for CCO assays due to
 7 limited tissue availability. In any case, Tween 20
 8 gave higher CCO activity than untreated homo-
 9 genate and this detergent is widely used in
 10 extracting CCO activity (Moyes et al., '97; Hardewig
 11 et al., '99; Lucassen et al., 2003).

Enzyme assays

12
 13
 14
 15 Maximal enzyme activities were measured in
 16 duplicate using a Cary 300 Bio UV-Visible spectro-
 17 photometer (Varian, Inc., Palo Alto, CA) equipped
 18 with a thermostated cell changer maintained at
 19 12°C ($\pm 0.1^\circ\text{C}$) with a Cary Temperature Control-
 20 ler (Varian, Inc.). Activities of most enzymes were
 21 ascertained by measuring the oxidation or reduc-
 22 tion of pyridine nucleotides at 340 nm (millimolar
 23 extinction coefficient ϵ_{340} , 6.22). CPT, COT, and
 24 Citrate synthase (CS) were monitored at 412 nm
 25 using 5,5'-dithiobis 2-nitrobenzoic acid (DTNB)
 26 (millimolar extinction coefficient ϵ_{412} , 13.6). CCO
 27 activity was measured at 550 nm (cytochrome
 28 *c* millimolar extinction coefficient ϵ_{550} , 18.5). Acet-
 29 oacetyl coenzyme-A thiolase (AcoAT) was moni-
 30 tored at 313 nm (millimolar extinction coefficient
 31 ϵ_{313} , 20.5). Succinyl coenzyme-A ketotransferase
 32 (SKT) was measured at 310 nm (millimolar ex-
 33 tinction coefficient ϵ_{310} , 11.9).

34 Conditions of saturating substrate were used
 35 and linearity with protein was ensured. Most
 36 enzymes were assayed following the protocols of
 37 Singer and Ballantyne ('89). The pyruvate kinase
 38 (PK) assay was modified from Moon and Momm-
 39 sen ('87) and Driedzic and De Almeida-Val ('96).
 40 CCO was measured using the method of Blier and
 41 Guderley ('88), and D- and L- β -HBDH were
 42 measured following LeBlanc and Ballantyne
 43 (2000). Conditions were as follows:

Enzymes of aerobic metabolism

44
 45
 46
 47 Cytochrome *c* oxidase (CCO; E.C. 1.9.3.1): 50 mM
 48 imidazole, pH 8.0, at 20°C, 0.05 mM reduced
 49 cytochrome *c* (omitted for control).

50 Citrate synthase (CS; E.C. 4.1.3.7): 50 mM
 51 imidazole, pH 8.0, at 20°C, 0.1 mM DTNB, 0.3 mM
 52 acetyl CoA, 0.5 mM oxaloacetate (omitted for
 53 control).

Enzymes of lipid catabolism

Carnitine palmitoyl transferase (CPT; E.C.
 2.3.1.21): 50 mM imidazole, pH 8.0, at 20°C,
 0.2 mM DTNB, 0.1 mM palmitoyl CoA, 5 mM
 L-carnitine (omitted for control).

Carnitine octanoyltransferase (COT; E.C.
 2.3.1.137): 50 mM imidazole, pH 8.0, at 20°C,
 0.2 mM DTNB, 0.1 mM octanoyl CoA, 5 mM
 L-carnitine (omitted for control).

3-Hydroxyacyl CoA dehydrogenase (HOAD; E.C.
 1.1.1.35): 50 mM imidazole, pH 8.0, at 20°C,
 0.1 mM NADH, 1 mM KCN, 0.1 mM acetoacetyl
 CoA (omitted for control).

Enzyme of lipid synthesis

Malic enzyme (ME; E.C. 1.1.1.40): 50 mM imi-
 dazole, pH 7.4, at 20°C, 1 mM MgCl_2 , 0.4 mM
 NADP, 1 mM malate (omitted for control).

Enzymes of ketone body metabolism

D- β -hydroxybutyrate dehydrogenase (D- β -HBDH;
 E.C. 1.1.1.30): 50 mM imidazole, pH 8.0, at 20°C,
 11.25 mM NAD, 50 mM nicotinamide, 2 mM
 dithiothreitol (DTT), 25 mM D- β -hydroxybutyrate
 (omitted for control).

L- β -hydroxybutyrate dehydrogenase (L- β -
 HBDH; E.C. 1.1.1.30): 50 mM imidazole, pH 8.0,
 at 20°C, 11.25 mM NAD, 50 mM nicotinamide,
 2 mM DTT, 25 mM L- β -hydroxybutyrate (omitted
 for control).

Succinyl coenzyme-A ketotransferase (SKT;
 E.C. 2.8.3.5): 50 mM imidazole, pH 8.0, at 20°C,
 5 mM MgCl_2 , 0.1 mM acetoacetyl CoA, 1 mM
 succinate (omitted for control).

Acetoacetyl coenzyme-A thiolase (ACoAT; E.C.
 2.3.1.9): 50 mM imidazole, pH 8.0, at 20°C, 5 mM
 MgCl_2 , 0.1 mM acetoacetyl CoA, 0.2 mM CoA
 (omitted for control).

Enzymes of glycolysis

Hexokinase (HK; E.C. 2.7.1.1): 50 mM imidazole,
 pH 7.4, at 20°C, 1 mM glucose, 5 mM MgCl_2 ,
 0.16 mM NADP, excess glucose 6-phosphate dehy-
 drogenase, 1 mM ATP (omitted for control).

Pyruvate kinase (PK; E.C. 2.7.1.40): 50 mM
 imidazole, pH 7.4, at 20°C, 0.15 mM NADH,
 5 mM ADP, 10 mM MgCl_2 , 50 mM KCl, 1 mM
 KCN, excess lactate dehydrogenase (LDH), 5 mM
 phosphoenolpyruvate (omitted for control).

Lactate dehydrogenase (LDH; E.C. 1.1.1.27):
 50 mM imidazole, pH 7.4, at 20°C, 0.2 mM NADH,
 1 mM pyruvate (omitted for control).



1 Enzyme of gluconeogenesis

3 Fructose 1,6-bisphosphatase (FBPase; E.C.
3.1.3.11): 50 mM imidazole, pH 7.4, at 20°C,
5 15 mM MgCl₂, 0.2 mM NADP, excess glucose 6-
7 phosphate dehydrogenase, excess phosphoglucose
isomerase, 0.1 mM fructose 1,6-diphosphate
(omitted for control).

9 Enzymes of amino acid metabolism

11 Glutamate dehydrogenase (GDH; E.C. 1.4.1.3):
50 mM imidazole, pH 8.0, at 20°C, 250 mM
13 ammonium acetate, 0.1 mM dipotassium EDTA,
0.1 mM NADH, 1 mM ADP, 1 mM KCN, 14 mM α -
15 ketoglutarate (omitted for control).

17 Alanine aminotransferase (AlaAT; E.C. 2.6.1.2):
50 mM imidazole, pH 7.4, at 20°C, 0.2 mM NADH,
10.5 mM α -ketoglutarate, 0.025 mM pyridoxal
19 phosphate, excess LDH, 200 mM L-alanine
(omitted for control).

21 Aspartate aminotransferase (AspAT; E.C.
2.6.1.1): 50 mM imidazole, pH 7.4, at 20°C,
23 0.2 mM NADH, 7 mM α -ketoglutarate, 0.025 mM
pyridoxal phosphate, excess malate dehydrogen-
25 ase, 40 mM L-aspartate (omitted for control).

27 Activities are presented as units gram wet
weight⁻¹ (units gww⁻¹) where one unit equals
29 1 μ mol substrate converted to product per minute.
Protein was measured using the Bio-Rad standard
31 assay (Bio-Rad, Hercules, CA). All chemicals were
obtained from Sigma Chemical Co. (St. Louis,
MO).

33 Measurement of plasma NEFA

35 Plasma NEFA were methylated as described
in Singer et al. ('90). The methyl esters were
37 redissolved in 25 μ l of carbon disulfide and 1 μ l
were injected into a gas chromatograph (6890N,
39 Agilent Technologies, Palo Alto, CA) fitted with a
flame ionization detector and an automatic in-
41 jector. Methyl esters were separated on a DB-23
column (J&W Scientific, Folsom, CA). The column
43 temperature was initially 50°C, increased to 180°C
over 10 min, held at 180°C for 5 min, and then
45 increased over 5 min to 240°C where it was held
for 5 min. Fatty acids were identified by comparing
47 their retention times (RTs) to those of known
standards (GLC 463 augmented with 22:5n-6 and
49 23:0, Nu-Check Prep, Elysian, MN). This method
allows the detection of fatty acids ranging from 4
51 to 24 carbon chain lengths. Absolute amounts of
fatty acids were calculated by adding a known
53 amount (15 μ g) of an internal standard, heptade-
canoic acid (17:0), to the plasma samples prior to

methylation. Preliminary analyses showed only
trace amounts of endogenous 17:0.

Measurement of plasma FAA

FAA in plasma were measured using a commer-
cially available EZ faast kit (Phenomenex,
Torrence, CA) run on a Waters Micromass
Quattro Micro API tandem mass spectrometer
(Waters, Milford, MA) equipped with an electro-
spray ionization interface, a nitrogen generator
(Peak Scientific, Bedford, MA), and a Waters 2695
separation module (Waters). Separation of amino
acids was achieved using methanol and water each
with 10 mM ammonium formate at a flow rate of
0.25 ml min⁻¹. The separation uses 83% methanol
and 17% water for the initial 11 min, and 68%
methanol and 32% water for an additional 2 min.
The column (Phenomenex EZ faast 4 μ m AAA-MS
250 \times 2.0 mm) was re-equilibrated for 8 min be-
tween runs and run at 35°C.

The mass spectrometer was operated in the
positive-ion mode using the following conditions:
nitrogen was used for desolving at a flow rate of
150 L hr⁻¹ and cone gas was supplied at a flow rate
of 100 L hr⁻¹. Argon was used as the collision gas.
Capillary voltage was set to 3.0 kV. Cone and
collision voltages were optimized for individual
amino acid groups and their representative inter-
nal standard as specified in the kit. Data were
acquired in MRM mode for parent-daughter ion
combinations. Amino acids in plasma samples
were identified based on RT and the mass-to-
charge ratio (m/z) of the parent-daughter ion
combinations and quantified using a five-point
calibration of 2, 5, 10, 50, 100 nmol for each amino
acid.

HPLC-grade methanol and water were obtained
from Fisher (Ottawa, Ont., Canada); all other
chemicals were obtained from Sigma Chemical Co.

Statistical analysis

Activities of each enzyme were compared be-
tween tissues using one-way ANOVA with Tukey's
test. Data were log-transformed prior to ANOVA if
unequal variances were found (Zar, '99). Levels of
COT vs. CPT as well as levels of D- β -HBDH vs.
L- β -HBDH were compared within liver and kidney
with a Student's t -test. All analyses were run on
SigmaStat (SPSS Inc., Chicago, IL). Significance
was accepted at $P < 0.05$.

RESULTS

Lipid metabolism

The levels of key enzymes of several metabolic pathways in liver, kidney, heart, and white muscle (Table 1) provided a quantitative and qualitative view of the metabolic organization of the spotted ratfish.

Activity of HOAD, an enzyme involved in β -oxidation of fatty acids, was detected in all tissues with the highest activity in the kidney and heart, and lowest in the white muscle. CPT, a mitochondrial enzyme that catalyzes the rate-limiting step in carnitine-dependent oxidation of long-chain fatty acids (McGarry and Brown, '97), was not detected in heart or white muscle. Kidney and liver contained similar levels of CPT. COT, a peroxisomal enzyme that exports medium-chain fatty acids produced by peroxisomal β -oxidation for further mitochondrial oxidation (Ramsay, '99), was detectable in all tissues although activities were low in liver, heart, and white muscle. In kidney, COT activity was significantly higher than in other tissues and four-fold, and significantly greater than CPT activity; in liver the two enzymes had similar activities. ME, an enzyme

Oxidative metabolism

Activities of CCO, the terminal step in the electron transport chain and as such an indicator of the potential for aerobic metabolism, showed that the most aerobic tissues were the heart and kidney and the least the white muscle. Based on activities of CS, a component enzyme of the Krebs cycle also indicative of aerobic metabolism, the most aerobic tissues were the heart and kidney and the least the liver.

TABLE 1. Activities (mean \pm SEM) of enzymes of intermediary metabolism in tissues from the spotted ratfish (*Hydrolagus colieii*) (n = 6, except where noted in parantheses)

	Liver	Kidney	Heart	White muscle
Aerobic metabolism				
CCO	1.66 \pm 0.11 ^{bc}	6.29 \pm 0.48 (5) ^{ad}	7.02 \pm 0.70 ^{ad}	0.92 \pm 0.05 ^{bc}
CS	0.075 \pm 0.011 ^{bcd}	7.01 \pm 0.47 (5) ^{ad}	11.6 \pm 1.5 ^{ad}	0.73 \pm 0.05 ^{abc}
Lipid catabolism				
CPT	0.066 \pm 0.012 ^{cd}	0.076 \pm 0.012 (5) ^{cd}	ND ^{ab}	ND ^{ab}
COT	0.054 \pm 0.012 ^{bd}	0.30 \pm 0.04 (5) ^{acd,*}	0.018 \pm 0.004 ^b	0.010 \pm 0.003 ^{ab}
HOAD	0.18 \pm 0.02 ^{bcd}	0.35 \pm 0.03 (5) ^{ad}	0.34 \pm 0.02 ^{ad}	0.021 \pm 0.005 ^{abc}
Lipid synthesis				
ME	0.010 \pm 0.003 ^c	0.40 \pm 0.01 ^c	2.63 \pm 0.34 ^{abd}	0.041 \pm 0.010 ^c
Ketone body metabolism				
D- β -HBDH	0.61 \pm 0.08 ^d	1.51 \pm 0.24 (5) ^d	1.23 \pm 0.14 ^d	0.043 \pm 0.013 ^{abc}
L- β -HBDH	0.022 \pm 0.014 ^{ψ}	0.014 \pm 0.008 ^{ψ}	ND	ND
SKT	0.083 \pm 0.013 ^c	0.66 \pm 0.05 ^c	3.05 \pm 0.38 ^{abd}	0.12 \pm 0.02 ^c
ACoAT	1.78 \pm 0.17 ^{bcd}	3.08 \pm 0.08 ^{acd}	1.31 \pm 0.08 ^{abd}	0.097 \pm 0.014 ^{abc}
Glycolysis				
HK	0.076 \pm 0.030 ^{bc}	1.73 \pm 0.05 ^{acd}	5.47 \pm 0.45 ^{abd}	0.029 \pm 0.003 ^{bc}
PK	0.31 \pm 0.04 (5) ^{bcd}	6.71 \pm 0.31 ^{ad}	9.11 \pm 1.15 ^{ad}	37.4 \pm 3.7 ^{abc}
LDH	2.35 \pm 0.31 ^{bcd}	19.5 \pm 1.1 ^{ac}	70.7 \pm 3.1 ^{abd}	20.2 \pm 2.8 ^{ac}
Gluconeogenesis				
FBPase	0.056 \pm 0.010 ^{bd}	0.12 \pm 0.01 ^{acd}	0.036 \pm 0.003 ^{bd}	0.021 \pm 0.005 ^{ab}
Amino acid metabolism				
GDH	1.25 \pm 0.12 ^{bd}	10.8 \pm 0.4 (5) ^{acd}	1.62 \pm 0.12 ^{bd}	0.033 \pm 0.005 ^{abc}
AlaAT	1.69 \pm 0.18 ^{bd}	14.4 \pm 1.0 (4) ^{acd}	1.95 \pm 0.22 (3) ^{bd}	0.22 \pm 0.02 ^b
AspAT	7.62 \pm 0.24 ^{cd}	7.02 \pm 0.94 (4) ^{cd}	25.4 \pm 1.9 (3) ^{abd}	2.65 \pm 0.11 ^{abc}

Activities were measured at 12°C. ND = not detectable.

Activities are expressed as $\mu\text{mol min}^{-1} \text{g ww}^{-1}$. Letters indicate a significant difference (one-way ANOVA, $P < 0.05$) from the activity of the same enzyme in, a: liver; b: kidney; c: heart; d: white muscle. *Signifies that COT activity is significantly different from CPT activity within the same tissue (Student's *t*-test, $P < 0.05$). ψ denotes that L- β -HBDH activity is significantly different from D- β -HBDH activity within the same tissue (Student's *t*-test, $P < 0.05$). Enzyme abbreviations are as follows: cytochrome *c* oxidase (CCO); citrate synthase (CS); carnitine palmitoyl transferase (CPT); carnitine octanoyl transferase (COT); 3-hydroxyacyl CoA dehydrogenase (HOAD); malic enzyme (ME); D- β -hydroxybutyrate dehydrogenase (D- β -HBDH); L- β -hydroxybutyrate dehydrogenase (L- β -HBDH); succinyl coenzyme-A ketotransferase (SKT); acetoacetyl coenzyme-A thiolase (AcoAT); hexokinase (HK); pyruvate kinase (PK); lactate dehydrogenase (LDH); fructose 1,6-bisphosphatase (FBPase); glutamate dehydrogenase (GDH); alanine aminotransferase (AlaAT); aspartate aminotransferase (AspAT).

1 usually associated with lipid synthesis, was high- 55
 2 est in heart and lowest in white muscle and liver.

3 The absolute concentrations (nmol ml^{-1}) and 57
 4 percentages (by mol) of individual NEFA in the
 5 plasma of *H. colliei* are presented in Table 2. Total
 6 mean NEFA concentration was $652.3 \text{ nmol ml}^{-1}$. 59
 7

8 **TABLE 2.** Absolute amounts (mean \pm SEM) and percentages
 9 (mean \pm SEM) of total and individual non-esterified fatty acids
 10 in plasma of spotted ratfish (*Hydrolagus colliei*) ($n = 8$)
 11

13 Fatty acid	Concentration (nmol ml^{-1})	Percentage (% by moles)
15 14:0	7.95 ± 1.08	1.21 ± 0.09
15 14:1	1.88 ± 0.45	0.40 ± 0.19
17 <i>iso</i> 16	42.3 ± 10.4	5.85 ± 1.34
17 16:0	104.9 ± 11.1	16.1 ± 0.7
19 16:1	39.3 ± 4.3	5.97 ± 0.23
19 18:0	30.7 ± 3.0	4.76 ± 0.17
21 18:1	141.4 ± 18.0	21.5 ± 1.6
21 18:2 <i>n</i> -6	9.68 ± 0.84	1.56 ± 0.15
21 18:3 <i>n</i> -3	3.75 ± 3.00	0.54 ± 0.43
23 18:4 <i>n</i> -3	108.5 ± 11.9	17.3 ± 1.7
23 20:0	1.52 ± 0.40	0.21 ± 0.05
25 20:1	29.7 ± 3.4	4.51 ± 0.17
25 20:2 <i>n</i> -6	3.02 ± 0.80	0.42 ± 0.10
27 20:3 <i>n</i> -6	ND	ND
27 20:4 <i>n</i> -6	20.4 ± 2.1	3.14 ± 0.19
27 20:3 <i>n</i> -3	0.08 ± 0.08	0.01 ± 0.01
29 20:4 <i>n</i> -3	1.97 ± 0.46	0.28 ± 0.06
29 20:5 <i>n</i> -3	49.1 ± 5.8	7.59 ± 0.52
31 22:0	1.20 ± 0.48	0.17 ± 0.07
31 22:1	8.01 ± 1.19	1.21 ± 0.11
33 23:0	0.86 ± 0.27	0.12 ± 0.04
33 22:2 <i>n</i> -6	ND	ND
33 22:4 <i>n</i> -6	2.50 ± 0.59	0.36 ± 0.09
35 22:5 <i>n</i> -6	0.60 ± 0.18	0.09 ± 0.03
35 22:5 <i>n</i> -3	6.33 ± 0.55	1.02 ± 0.09
37 22:6 <i>n</i> -3	35.3 ± 4.0	5.34 ± 0.22
37 24:0	ND	ND
39 24:1	2.77 ± 0.71	0.38 ± 0.09
39 Total	652.3 ± 63.8	100
41 Total saturates	189.5 ± 22.4	28.4 ± 1.0
41 Total monoenes	223.0 ± 24.6	33.9 ± 1.4
41 Total polyenes	241.2 ± 21.5	37.6 ± 1.7
43 <i>n</i> -3 Polyenes	205.0 ± 18.3	32.1 ± 1.6
43 <i>n</i> -6 Polyenes	36.3 ± 3.5	5.57 ± 0.29
45 <i>n</i> -3/ <i>n</i> -6	5.83 ± 0.33	
45 Monoenes/ polyenes	0.93 ± 0.08	
47 Unsaturation Index ¹	199.3 ± 5.9	
49 Mean chain length ²	18.1 ± 0.01	

51 ND = not detectable.

52 ¹ $\sum m_i n_i$, where m_i is the mole percentage and n_i is the number of
 53 carbon-carbon double bonds in the fatty acid.

² $\sum f_i c_i$, where f_i is the mole fraction and c_i is the number of carbon
 atoms of fatty acid.

Aside from two individual measurements of trace 55
 amounts of 13:1, NEFA shorter than C:14 were 57
 not detected. Polyenes were the dominant fatty 59
 acids detected, with stearidonic acid (18:4*n*-3)
 representing 45% of total polyenes and 17% of
 total NEFA. Eicosapentaenoic acid (EPA, 20:5*n*-3),
 docosahexaenoic acid (DHA, 22:6*n*-3), and arachi- 61
 donic acid (AA, 20:4*n*-6) also were present in high
 concentrations, representing 20%, 15%, and 8.5% 63
 of total polyenes, respectively. Total monoenes
 were present at similar levels to total polyenes, 65
 and consisted principally of oleic acid (18:1), which
 was 63% of total monoenes and also the most 67
 abundant individual fatty acid overall (21.5% of
 total NEFA). Saturates were less abundant than 69
 unsaturates; the major saturate was palmitic acid
 (16:0), representing 55% of total saturates. The 71
 mean *n*-3/*n*-6 ratio was 5.83.

Seven unknown peaks were detected in all 73
 samples. One peak eluted immediately after 15:1
 (RT = 15.18 min), one peak eluted immediately 75
 after 16:0 (RT = 15.64), one peak eluted between
 16:1 and 17:0 (RT = 16.08), and the remaining 77
 four eluted consecutively between 17:1 and 18:0
 (RT = 17.18, 17.43, 17.55, 17.73 min). Methyl ester 79
 standards of the branched-chain fatty acids phy-
 tanic acid, 16-methylheptadecanoic acid, and 14- 81
 methylpentadecanoic acid (Ultra Scientific, North
 Kingstown, RI), as well as pristanic acid (Sigma) 83
 that we methylated, were analyzed to determine
 if they explained any of the unknown peaks. None 85
 of the unknown peaks was phytanic acid, pristanic
 acid, or 16-methylheptadecanoic acid, but the peak 87
 at RT = 15.18 was identified based on RT to be
 14-methylpentadecanoic acid (*iso*16:0). Its concen- 89
 tration was $42.3 \pm 10.4 \text{ nmol ml}^{-1}$ ($5.85 \pm 1.34\%$ of
 identified fatty acids) (Table 2). The remaining 91
 unknown peaks accounted for a total of
 $21.0 \pm 1.8\%$ of the known plus unknown fatty acids 93
 by weight. The percentages given for identified
 fatty acids in the preceding paragraph do not take 95
 into account these unknown peaks. 97

Ketone body metabolism 99

The capacity for ketone body oxidation, as 101
 measured by the activity of D- β -HBDH which
 oxidizes D- β -hydroxybutyrate (D- β -HB) to aceto- 103
 acetate in the mitochondria, was found in all
 tissues. Kidney, heart, and liver showed the 105
 highest levels of D- β -HBDH whereas white muscle
 possessed the lowest level. Activity of L- β -HBDH, 107
 which is specific for the laevo-rotary enantiomer of
 β -HB (L- β -HB), was undetectable in heart and

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7

1 white muscle and was low in liver and kidney, representing only 4% and 1% of the activity of
 3 D- β -HBDH, respectively. SKT, an enzyme that catalyzes the formation of acetoacetyl coenzyme A
 5 via transfer of coenzyme A from succinyl coenzyme A to acetoacetate, was found in all tissues
 7 with heart showing the highest level and other tissues showing similar low levels. ACoAT, an
 9 enzyme that catalyzes the cleavage of acetoacetyl coenzyme A to form acetyl coenzyme A, was
 11 detected in all tissues; kidney possessed the highest activity, liver and heart also had relatively
 13 high levels, and white muscle showed the lowest activity.

Glycolysis

17 Heart and kidney showed the highest levels of HK, an enzyme that phosphorylates exogenous
 19 glucose for glycolysis or glycogen synthesis, whereas liver and white muscle had low levels. PK,
 21 which catalyzes the conversion of phosphoenolpyruvate to pyruvate in the final step of
 23 glycolysis, was highest in white muscle and lowest in liver. LDH, the enzyme involved in the
 25 interconversion of pyruvate and lactate, was highest in heart and lowest in liver.

Gluconeogenesis

29 Gluconeogenic capacity as assessed by activity of FBPase, a gluconeogenic enzyme responsible for
 31 the conversion of glucose-6-phosphate to glucose, was highest in kidney and lowest in white muscle
 33 and heart.

Amino acid metabolism

37 GDH, an enzyme that catalyzes the oxidative deamination of glutamate to α -ketoglutarate, was
 39 most active in kidney and least active in white muscle. AlaAT, an enzyme that transaminates
 41 alanine to form pyruvate, also was highest in kidney and lowest in white muscle. AspAT, an
 43 enzyme that transaminates aspartate to produce oxaloacetate, had highest activity in heart and
 45 lowest in white muscle.

47 The levels of amino acids in plasma of spotted ratfish are provided in Table 3. The concentration
 49 of total plasma FAA was 719.7 nmol ml⁻¹. Essential and non-essential amino acids comprised
 51 65.2% and 34.8% of the total FAA, respectively. The most common amino acids were valine (21.9%
 53 of total), isoleucine (13.1%), leucine (11.1%), and lysine (8.9%). Glutamine was detectable, but at
 low levels (2.94%).

TABLE 3. Absolute amounts (mean \pm SEM) and percentages (mean \pm SEM) of free amino acids in plasma from the spotted ratfish (*Hydrolagus colliei*) (n = 6)

Amino acid	Concentration (nmol ml ⁻¹)	Percentage (% by moles)
Aspartate	1.72 \pm 0.33	0.23 \pm 0.04
Glutamate	4.53 \pm 0.85	0.60 \pm 0.09
Asparagine	0.21 \pm 0.05	0.03 \pm 0.01
Serine	50.4 \pm 9.8	6.28 \pm 0.66
Glutamine	20.9 \pm 1.1	2.94 \pm 0.38
Glycine	13.7 \pm 1.7	1.93 \pm 0.27
Histidine	12.0 \pm 1.8	1.82 \pm 0.44
Threonine	23.1 \pm 2.5	3.14 \pm 0.29
Alanine	35.2 \pm 6.9	4.55 \pm 0.51
Arginine	42.1 \pm 4.2	5.65 \pm 0.37
Tyrosine	1.46 \pm 0.46	0.19 \pm 0.05
Valine	165.7 \pm 15.9	22.0 \pm 0.4
Methionine	14.7 \pm 3.1	1.91 \pm 0.25
Tryptophan	7.88 \pm 1.05	1.06 \pm 0.10
Phenylalanine	16.6 \pm 1.0	2.28 \pm 0.17
Isoleucine	101.7 \pm 14.1	13.1 \pm 0.7
Leucine	85.6 \pm 11.3	11.1 \pm 0.4
Lysine	65.1 \pm 4.6	8.86 \pm 0.63
Proline	14.5 \pm 2.6	1.84 \pm 0.17
Ornithine	40.9 \pm 6.8	5.22 \pm 0.38
Citrulline	1.13 \pm 0.10	0.17 \pm 0.03
GABA	39.7 \pm 6.4	5.08 \pm 0.34
Cystine	0.46 \pm 0.06	0.06 \pm 0.00
Total	719.7 \pm 74.5	100
Total essential	492.4 \pm 47.7	65.2 \pm 0.5
Total non-essential	227.3 \pm 27.3	34.8 \pm 0.5

DISCUSSION
Lipid metabolism

Our salient finding is that spotted ratfish show the same pattern of lipid oxidation as elasmobranchs—namely, a low or non-existent reliance on lipid as fuel in muscle. CPT activity was non-detectable in heart, as in bowfin and some elasmobranchs but not teleosts (Sidell et al., '87; Singer and Ballantyne, '91). Other data show low CPT activities in heart from some elasmobranchs, suggesting some variability in lipid use by elasmobranch heart (Driedzic and De Almeida-Val, '96; J. Berges and J.S. Ballantyne, unpublished data, given in Ballantyne ('97); Speers-Roesch (2005)). Undetectable or very low CPT levels are normal in white muscle even for teleosts (Moyes et al., '89; Crockett and Sidell, '90). Spotted ratfish apparently do not possess significant discrete lateral line red muscle, which is not surprising given their sluggish life style, so we were unable to determine



- 1 if this tissue lacks CPT activity like in elasmobranchs (Zammit and Newsholme, '79; Moyes
3 et al., '90). The presence of detectable CPT in
5 kidney from spotted ratfish indicates its capacity
7 for lipid oxidation, as in bowfin and Arctic char
9 (*Salvelinus alpinus*) (Singer and Ballantyne, '91;
11 Bystriansky, 2005). Our finding is at odds
13 with Singer and Ballantyne ('89), whose results
15 suggested that elasmobranchs lack CPT in kidney
17 (as do lake sturgeon (Singer et al., '90)). However,
19 more recent work shows high CPT levels in
21 kidney from little skate (J. Berges and J.S.
23 Ballantyne, unpublished data given in
25 Ballantyne ('97)), several tropical stingrays, and
27 brownbanded bamboo shark (*Chiloscyllium
29 punctatum*) (Speers-Roesch, 2005). The emerging
31 picture is that elasmobranchs do not lack lipid
33 oxidation in extrahepatic tissues per se, but
35 rather do not rely on lipids as a metabolic fuel in
37 muscle only. Our results for the spotted ratfish
39 extend this model to the Chondrichthyes as a
41 whole.
- 43 The four-fold higher activity of COT compared
45 with CPT in the kidney, and the presence of COT
47 and the absence of CPT in heart and white muscle
49 may indicate a preference for medium-chain fatty
51 acids during lipid oxidation in the spotted ratfish.
53 These results suggest active peroxisomal β -oxidation
and export to the mitochondria of carnitine
esters of medium-chain fatty acids by COT in
these tissues. Accordingly, heart mitochondria
from *Squalus acanthias* oxidized octanoyl carnitine
ester but not palmitoyl carnitine ester under
isosmotic conditions (Moyes et al., '90). On the
other hand, peroxisomal β -oxidation was not
detectable in heart and red muscle from *H. colliei*,
S. acanthias, or rainbow trout (Moyes et al., '90).
Further studies are needed to examine the role of
peroxisomal β -oxidation and COT in lipid metabolism
in the Chondrichthyes.
- The occurrence of appreciable levels of HOAD in
the heart, while CPT is undetectable and COT is
very low, is a paradox also seen in elasmobranchs.
It is apparently not attributable to carnitine-
independent fatty acid oxidation or peroxisomal
 β -oxidation (Moyes et al., '90). An alternate role of
HOAD may be a possible explanation. Molecular
studies of the sequence and expression of HOAD
in muscle and other tissues from chondrichthyans
may prove informative.
- ME activities were nearly identical to those
measured in little skate (Moon and Mommsen,
'87). The high activity recorded from heart is
probably attributable to the role of ME in amino
acid metabolism rather than lipid synthesis
(Chamberlin et al., '91).
- Total plasma NEFA concentration in *H. colliei* is
higher than in *Chimaera monstrosa* (Larsson and
Fänge, '77). These authors used a colorimetric
technique that probably underestimates NEFA
concentration (Singer et al., '90). Our measure-
ments indicate plasma NEFA concentration in
spotted ratfish is $\geq 60\%$ higher than any elasmobranch
examined by Ballantyne et al. ('93),
but similar to that in little skate
($572.6 \pm 66.8 \text{ nmol ml}^{-1}$) (Speers-Roesch, 2005).
Plasma NEFA levels in spotted ratfish are about
half of the lowest levels reported from marine
teleosts by Ballantyne et al. ('93). Because spotted
ratfish heart and white muscle show no indication
of lipid oxidation, the NEFA levels likely suffice
for the needs of the kidney. Supporting this
contention, the level of plasma NEFA in spotted
ratfish is similar to that found in the Florida gar
(*Lepisosteus platyrhincus*) (N.T. Frick, J.S. By-
striansky, and J.S. Ballantyne, unpublished data)
and bowfin (Singer and Ballantyne, '91), both
"primitive" non-teleost actinopterygians that also
show a limited capacity for lipid oxidation in
extrahepatic tissues (see below).
- The plasma NEFA concentration in spotted
ratfish exceeds the solubility of fatty acids in
water (Windholz, '83), indicating the presence of a
plasma fatty acid carrier. Considering the similar-
ity to elasmobranchs in enzyme and NEFA
measurements, holocephalans may also lack an
albumin capable of carrying NEFA. The absence
of the more soluble and albumin-independent
short- and medium-chain NEFA in plasma indi-
cates that this is not an alternate form of lipid
transport in spotted ratfish. Lipoproteins should
be investigated as possible NEFA carriers in
holocephalans, as occurs in spiny dogfish (Lauter
et al., '67).
- Composition of plasma NEFA in spotted ratfish
is similar to that reported in other marine fishes,
with high levels of 18:1, 16:0, 16:1, 18:0, and long
chain polyunsaturated fatty acids (EPA, 20:5n-3,
DHA, 22:6n-3, and AA, 20:4n-6) (Ballantyne et al.,
'93). The predominant fatty acid was oleic acid
(18:1), reflecting the high amounts of 18:1 in
triglycerides and diacyl glyceryl ethers in liver and
muscle of *Hydrolagus barbouri* and *H. novaezealandiae*
(Hayashi and Takagi, '80; Hayashi et al.,
'83). High levels of 18:1 also characterize plasma
NEFA of teleosts and elasmobranchs (Ballantyne
et al., '93), bowfin (Singer and Ballantyne, '91),
and sturgeon (Singer et al., '90), confirming its



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1 metabolic importance in fishes. One peculiarity
 2 of spotted ratfish NEFA is the very high levels of
 3 stearidonic acid (18:4*n*-3) (18% of total identified
 4 plasma NEFA). In elasmobranchs and other
 5 fishes, 18:4*n*-3 constitutes <3% of plasma NEFA
 6 (Singer et al., '90; Ballantyne et al., '93; Speers-
 7 Roesch, 2005). High levels of 18:4*n*-3 in plasma
 8 NEFA of spotted ratfish suggest substantial feed-
 9 ing within a food chain with dinoflagellates or
 10 haptophyte algae at its base, since 18:4*n*-3 is
 11 typical of these phytoplankters (Mansour et al.,
 12 '99). The high levels of 20:5*n*-3, 22:6*n*-3, and other
 13 *n*-3 fatty acids and the resultant high *n*-3/*n*-6 ratio
 14 in spotted ratfish is similar to other carnivorous
 15 temperate marine fishes, which obtain high levels
 16 of *n*-3 fatty acids in their diet (Ballantyne et al.,
 17 '93; Speers-Roesch, 2005).

18 The unidentified peaks seen in the NEFA
 19 analysis of spotted ratfish plasma deserve further
 20 attention. Our identification of one of the un-
 21 knowns as 14-methylpentadecanoic acid (*iso*16:0)
 22 appears to be the first record of a branched-chain
 23 fatty acids in plasma NEFA from a fish. Branched-
 24 chain fatty acids, including *iso*16:0, are known
 25 from fish oils (Ackman, '89). *iso*16:0 has been
 26 found to comprise 1.7% of fatty acids in alkyl
 27 glyceryl ethers in livers from holocephalans
 28 (Hayashi and Takagi, '80; Hayashi et al., '83),
 29 but the high level of *iso*16:0 in plasma NEFA from
 30 spotted ratfish is nonetheless unusual and should
 31 be verified by GC-MS as well as investigated
 32 further. The other unknowns in *H. colliei* plasma
 33 NEFA may represent other branched-chain fatty
 34 acids or C16 polyenic fatty acids (Ackman, '89).

Ketone body metabolism

37 An important finding of the present study is
 38 the high capacity for ketone body metabolism in
 39 aerobic tissues of spotted ratfish, as found in
 40 elasmobranchs but not other fishes (Ballantyne,
 41 '97). In particular, the high activities of D-β-
 42 HBDH contrast with the low activities of this
 43 enzyme in sturgeon, bowfin, and, especially,
 44 teleosts (Singer et al., '90; Singer and Ballantyne,
 45 '91; LeBlanc and Ballantyne, '93). Although levels
 46 of D-β-HBDH are higher in heart and kidney than
 47 previously reported for elasmobranchs (Moon and
 48 Mommsen, '87; Singer and Ballantyne, '89; Bat-
 49 tersby et al., '96; Treberg et al., 2003), this may be
 50 due to the usage of D/L-β-HB in two of these
 51 studies, which may have resulted in inhibition by
 52 L-β-HB (Stuart and Ballantyne, '97). Zammit and
 53 Newsholme ('79), using D-β-HB, found D-β-HBDH

54 activities in elasmobranch hearts that are very
 55 similar to that found in heart from spotted ratfish.
 56 SKT and ACoAT levels were similar to those in
 57 elasmobranchs and ACoAT was similar to levels
 58 in teleosts, bowfin, and sturgeon (Zammit and
 59 Newsholme, '79; Beis et al., '80; Moon and
 60 Mommsen, '87; Singer et al., '90; Singer and
 61 Ballantyne, '91; J. Berges and J.S. Ballantyne,
 62 unpublished data presented in Ballantyne, '97).
 63 These similarities in activities of SKT and ACoAT
 64 suggest that the role of acetoacetate as an
 65 oxidative fuel is fairly conserved among fishes; it
 66 is the high activities of D-β-HBDH that makes
 67 ketone body metabolism in elasmobranchs, and
 68 holocephalans, so unique. Supporting this conten-
 69 tion, acetoacetate levels in plasma from Atlantic
 70 salmon (*Salmo salar*) are similar to levels found in
 71 spiny dogfish and *Scyliorhinus canicula* whereas
 72 plasma β-HB is higher in the elasmobranchs
 73 (Conlon et al., '94; Soengas et al., '96; Richards
 74 et al., 2003). Levels of ketone bodies in plasma of
 75 holocephalans should be measured to ascertain if
 76 their profile matches that seen in elasmobranchs.
 77

78 The undetectable or low activities of L-β-HBDH
 79 compared with the high activities of D-β-HBDH
 80 indicate that in ratfish the D-stereoisomer of β-HB
 81 is preferred, as is the case in mammals (Webber
 82 and Edmond, '77) and elasmobranchs (Speers-
 83 Roesch, 2005).

Amino acid metabolism

84 Although the total amount of FAA in plasma
 85 (719.7 nmol ml⁻¹) was lower than in *Callor-
 86 hinchus milii*, elasmobranchs, and teleosts
 87 (~2,000–3,500 nmol ml⁻¹; Bedford, '83; Gutierrez
 88 et al., '87; Barton et al., '95), the high activities of
 89 GDH, AlaAT, and AspAT indicate that amino
 90 acids are utilized extensively as oxidative sub-
 91 strates in spotted ratfish. The levels of these
 92 enzymes were similar to those found in elaso-
 93 branches, for which amino acids are thought to be
 94 important oxidative fuels due to a reduced reliance
 95 on lipids (Ballantyne, '97). The same appears to
 96 be the case in the spotted ratfish.
 97

98 Although recently it has been suggested that low
 99 plasma glutamine levels are not a universal trait
 100 of elasmobranchs (Ballantyne, 2001), glutamine
 101 levels in spotted ratfish were similarly low as
 102 levels in spiny dogfish (Chamberlin and Ballan-
 103 tyne, '92) and little skate (Boyd et al., '77).
 104 Whether, as in elasmobranchs (Ballantyne, '97),
 105 glutamine is important in holocephalans as an
 106 oxidative substrate needs to be determined. The
 107



1 proportions of ornithine and citrulline, two main
 2 intermediates in the ornithine–urea cycle, as well
 3 as the major constituents of plasma FAA were
 4 similar to that found in *Callorhinchus milii* and
 5 elasmobranchs (Boyd et al., '77; Bedford, '83;
 6 Gutierrez et al., '87). Data on plasma levels of
 7 γ -aminobutyric acid in fishes are sparse, but the
 8 levels in spotted ratfish are similar to that found
 9 in Pacific hagfish (*Eptatretus stouti*) (Fincham
 10 et al., '90).

11 **Other metabolic pathways**

12 The levels of enzymes of aerobic metabolism (CS
 13 and CCO) and gluconeogenesis (FBPase) were
 14 similar to those measured previously in other
 15 fishes including *H. collicii*, elasmobranchs, and
 16 actinopterygians (Moon and Mommsen, '87; Ritter
 17 et al., '87; Blier and Guderley, '88; Singer et al.,
 18 '90; Dickson et al., '93; Battersby et al., '96;
 19 Treberg et al., 2003). Comparisons of HK activity
 20 between different studies are complicated by this
 21 enzyme's freeze instability (Sidell et al., '87).
 22 However, several fold higher HK activity in kidney
 23 and heart of spotted ratfish compared with freeze-
 24 thawed samples from bowfin, lake sturgeon, and
 25 Arctic char, as well as fresh little skate tissues
 26 (Moon and Mommsen, '87; Singer et al., '90;
 27 Singer and Ballantyne, '91; Bystriansky, 2005)
 28 suggest that in this species these tissues rely more
 29 on glucose as a metabolic fuel than in other fishes.

30 Spotted ratfish showed lower PK in all tissues
 31 compared with teleosts (Sidell et al., '87; Blier and
 32 Guderley, '88) and shallow-water elasmobranchs
 33 (Moon and Mommsen, '87; Dickson et al., '93;
 34 Treberg et al., 2003); the levels were similar to
 35 that found in the deep-sea squaloid *Centroscyllium*
 36 *fabricii* (Treberg et al., 2003). LDH in white
 37 muscle was five–eight-fold lower than in little
 38 skate or spiny dogfish, but similar to *Centroscyl-*
 39 *lium fabricii* (Moon and Mommsen, '87; Treberg
 40 et al., 2003). These reduced enzyme activities may
 41 reflect adaptation to a deep-sea environment.
 42 Although, unlike many holocephalans, the spotted
 43 ratfish is not exclusively a deep-sea inhabitant,
 44 it is commonly found to depths of 900 m and is
 45 characteristically sluggish (Eschmeyer et al., '83).
 46 It is well known that deep-sea fishes show
 47 adaptations in white muscle biochemistry that
 48 reflect their nutrient-poor environment and re-
 49 duced locomotory capacities, including reduced
 50 PK and LDH activities (Somero, '82). On the other
 51 hand, Childress and Somero ('79) argued that
 52 depth-related influences on heart LDH in fishes

are minimal and the similarity in heart LDH
 between spotted ratfish (present study), *S.*
acanthias, and *Centroscyllium fabricii* (Treberg
 et al., 2003) support this contention. The similar-
 ity noted above of white muscle CS (and CCO)
 activities with that of shallow-water elasmobranchs
 and teleosts matches the lack of a
 consistent decrease in white muscle CS with
 increasing depth in teleosts (Somero, '82).

11 **Evolutionary considerations**

12 The spotted ratfish, a representative holocephalan,
 13 possesses a metabolic organization similar to
 14 that of an elasmobranch. The absence of substan-
 15 tial lipid oxidation in muscle and a high reliance
 16 on ketone bodies as metabolic fuel can now be
 17 considered a general metabolic attribute of the
 18 Chondrichthyes. From an evolutionary perspec-
 19 tive, this is a fascinating discovery because it
 20 strongly suggests that this metabolic organization
 21 was present in the last common ancestor of all
 22 chondrichthyan fishes, which swam in the oceans
 23 over 360 Mya but is poorly known from the fossil
 24 record (Grogan and Lund, 2004). It also indicates
 25 that this pattern goes back almost to the origin
 26 of the jawed vertebrates.

27 The presence of a substantial capacity for lipid
 28 oxidation in the kidney—an extrahepatic tissue—is
 29 not consistent with the urea hypothesis. How-
 30 ever, lipid delivery to this tissue presumably can
 31 be served by the available plasma NEFA and these
 32 may be carried by a non-albumin fraction as
 33 appears to be the case in elasmobranchs, which
 34 show a similar capacity for lipid oxidation in the
 35 kidney (Speers-Roesch, 2005). The lack of a
 36 capacity for lipid oxidation in heart in spotted
 37 ratfish, and low or non-existent lipid oxidation in
 38 heart and red muscle from elasmobranchs, may
 39 reflect urea's perturbing effects on fatty acid
 40 transport by albumin. Whether or not the urea
 41 hypothesis is true, the question of why fatty acid
 42 oxidation was attenuated in muscle and not also
 43 in other extrahepatic tissues deserves attention.
 44 Investigations of the lipid metabolism of other
 45 urea-retaining vertebrates, including the coela-
 46 canth (*Latimeria chalumnae*) and crab-eating frog
 47 (*Rana cancrivora*), may be useful in further
 48 testing the urea hypothesis.

49 Although the urea hypothesis remains an
 50 attractive explanation for the metabolic organiza-
 51 tion of chondrichthyans, the lessened role of lipids
 52 as a metabolic fuel in muscle in chondrichthyans
 53 may reflect an ancestral metabolic characteristic



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1 among fishes. Supporting this view, the concen- (606.6 ± 27.7 nmol ml⁻¹; N.T. Frick, J.S. Bystriansky, 55
 3 trations of plasma NEFA in spotted ratfish and J.S. Ballantyne, unpublished data), all 57
 5 (652.3 ± 63.8 nmol ml⁻¹) and little skate (572.6 ± of which have levels lower than in teleosts 59
 7 66.8 nmol ml⁻¹; Speers-Roesch, 2005) are similar (≥ 1,183 nmol ml⁻¹; Ballantyne et al., '93). Bowfin 61
 9 to that found in primitive non-urea-retaining and Florida gar, as well as lake sturgeon (Singer 63
 11 fishes, including the ancient, jawless hagfish et al., '90), also possess little or no CPT in heart, 65
 and (Myxine glutinosa) (550.5 nmol ml⁻¹; P.J. LeBlanc, red muscle, and kidney when compared with 67
 C. Hyndman, and J.S. Ballantyne, unpublished teleosts (Singer and Ballantyne, '91; N.T. Frick, 69
 data) as well as the "primitive" ray-finned J.S. Bystriansky, and J.S. Ballantyne, unpublished 71
 fishes the bowfin (758 ± 110 nmol ml⁻¹; Singer data). The ratio of CPT activity to PK activity 73
 and Ballantyne, '91) and Florida gar indicates the relative importance of lipid catabo- 75
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13 TABLE 4. Ratios of CPT/PK in liver, kidney, and heart of representative chondrichthyans, teleosts, a neopterygian 67
 15 (a "primitive" ray-finned fish), and a myxine (a "primitive" jawless fish) 69

	Liver	Kidney	Heart	
17 Chondrichthyes				71
19 <i>Hydrolagus colliei</i> ¹	0.212 ± 0.060	0.011 ± 0.001	0 ± 0	73
<i>Potamotrygon motoro</i> ²	0.017	0.002	0.00036	75
<i>Himantura signifer</i> ²	0.049	0.003	0.00095	77
21 <i>Taeniura lymma</i> ²	0.028	0.022	0.00083	79
<i>Chiloscyllium punctatum</i> ²	0.149	0.020	0.00012	81
<i>Potamotrygon hystrix</i> ³	—	—	0.008	83
<i>Leucoraja erinacea</i> ⁴	0.020	0.016	0.002, 0 ⁵	85
<i>Squalus acanthias</i> ⁵	—	—	0	87
25 Mean ± SEM	0.079 ± 0.033	0.012 ± 0.004	0.0014 ± 0.0009	89
27 Teleostei				91
<i>Lophius piscatorius</i> ⁵	—	—	0.013	93
<i>Scomber scombrus</i> ⁵	—	—	0.008	95
29 <i>Gaidropsarus vulgaris</i> ⁵	—	—	0.006	97
<i>Morone saxatilis</i> ⁵	—	—	0.017	
31 <i>Dicentrarchus labrax</i> ⁵	—	—	0.013	
<i>Zoarces americanus</i> ⁶	—	—	0.008	
<i>Makaira nigricans</i> ⁷	—	—	0.004	
33 <i>Salvelinus alpinus</i> ⁸	0.070	0.011	0.011	
<i>Gadus morhua</i> ⁹	—	—	0.007	
35 Mean ± SEM	0.070	0.011	0.0096 ± 0.0014	
37 Neopterygii				
<i>Lepisosteus platyrhinchus</i> ¹⁰	0.013	—	0.004	
39 Myxini				
<i>Myxine glutinosa</i>	0.091 ¹¹	—	0.002 ⁹	
41 Mean teleostei/mean chondrichthyes	0.9	0.9	7.1	
43 Neopterygii/mean chondrichthyes	0.2	—	3	
Myxini/mean chondrichthyes	1.2	—	1.5	

45 Per gram wet weight activities were used in ratio calculations. A zero indicates that non-detectable CPT activity was found. For mean calculation 99
 multiple values for one tissue in a species were first averaged. References for data are marked with letters and provided below with temperature 101
 at which measurements were made. Sample sizes for *H. colliei* are six for all tissues except for kidney (*n* = 5).

47 ¹Present study, 12°C.

²Speers-Roesch (2005), 25°C.

³Driedzic and De Almeida-Val (1996), 25°C.

49 ⁴PK values: Moon and Mommsen (1987); CPT values: J. Berges and J.S. Ballantyne, unpublished data (see Ballantyne, '97), both 10°C. 103

⁵Sidell et al. ('87), 15°C.

⁶Driedzic and Stewart ('82), 10°C.

51 ⁷Suarez et al. ('86), 25°C. 105

⁸Bystriansky (2005), 10°C.

⁹Hansen and Sidell ('83), 15°C.

53 ¹⁰N.T. Frick, J.S. Bystriansky, and J.S. Ballantyne, unpublished data, 25°C. 107

¹¹Leary et al. ('97), 10°C.



lism and glycolysis for energy metabolism. The ratio of CPT to PK in heart of chondrichthyans is similar to that in hagfish, about three times lower than in the Florida gar (a "primitive" ray-finned fish), and seven-fold lower than in teleosts (Table 4). A similar pattern emerges when comparing mean heart CPT activities from chondrichthyans (including those species listed in Table 4) with those of: representative teleosts (including those species listed in Table 4); "primitive" ray-finned fishes (bowfin (Singer and Ballantyne, '91), lake sturgeon (Singer et al., '90), and Florida gar); and hagfishes (Pacific hagfish (Moyes et al., '90) and *M. glutinosa*). Chondrichthyans and hagfish possess similar levels of heart CPT, teleosts possess approximately nine-fold higher levels, and "primitive" ray-finned fishes possess intermediate levels (Speers-Roesch, 2005). These comparisons of CPT/PK ratios and absolute CPT levels in heart suggest that the capacity for lipid oxidation in heart of chondrichthyans is more similar to the ancient hagfishes than to the derived teleosts, and support Singer and Ballantyne's ('91) contention that certain "primitive" ray-finned fishes show a capacity for fatty acid oxidation that is intermediate between chondrichthyans and teleosts. In kidney, however, the CPT/PK ratio is similar between chondrichthyans and Arctic char, a teleost, and in liver there are no clear relationships to evolutionary position (Table 4), suggesting that major phylogenetic differences in lipid oxidation among fishes are restricted to muscle. Heart of "primitive" fishes may rely more heavily on carbohydrate-based metabolism (Sidell, '83), and the same may be true for red muscle. The ratios of CPT/PK and the CPT values provided in the present study support this idea. It is worth noting that the lampreys may be an exception to this general idea, as they rely extensively on fatty acid oxidation and possess high levels of CPT in muscle (Power et al., '93; LeBlanc et al., '95). However, this emphasis on fatty acid oxidation in muscle may have evolved independently as an adaptation to their complex migratory life history.

Although the lower utilization of fatty acids as oxidative fuels in chondrichthyan muscle may in part reflect an ancestral characteristic of the fishes, there is a clear preference for ketone bodies rather than lipids as oxidative substrates. Singer and Ballantyne ('91) suggested that ketone body usage is a primitive feature in fishes. The capability to use ketone bodies among fishes certainly is ancient, as muscle mitochondria from

lamprey readily oxidize β -HB (LeBlanc et al., '95), but no group has relied so heavily on it as have the chondrichthyans. An emphasis on ketone bodies as a major source of acetyl CoA in skeletal muscle and heart was probably needed, in the absence of significant lipid oxidation, to enable the active predatory lifestyle for which many chondrichthyans are notorious. Teleosts, on the other hand, adopted lipids as a high-energy oxidative fuel. Although it is possible that the emphasis on ketone bodies in chondrichthyans appeared in response to reduced fatty acid oxidation in muscle due to impaired plasma fatty acid transport (as postulated by the urea hypothesis), it may be that the implementation of ketone bodies as a major fuel in chondrichthyan muscle preceded and then caused the de-emphasis on fatty acid oxidation. Our shows that this metabolic reorganization must have occurred early in chondrichthyan evolution and close to the origin of jawed vertebrates.

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