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Comparative studies of high performance swimming in sharks

II. Metabolic biochemistry of locomotor and myocardial muscle in endothermic and ectothermic sharks

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Summary

Metabolic enzyme activities in red (RM) and white (WM) myotomal muscle and in the heart ventricle (HV) were compared in two lamnid sharks (shortfin mako and salmon shark), the common thresher shark and several other actively swimming shark species. The metabolic enzymes measured were citrate synthase (CS), an index of aerobic capacity, and lactate dehydrogenase (LDH), an index of anaerobic capacity. WM creatine phosphokinase (CPK) activity, an index of rapid ATP production during burst swimming, was also quantified. Enzyme activities in RM, WM and HV were similar in the two lamnid species. Interspecific comparisons of enzyme activities at a common reference temperature (20°C) show no significant differences in RM CS activity but higher CS activity in the WM and HV of the lamnid sharks compared with the other species. For the other enzymes, activities in lamnids overlapped with those of other shark species. Comparison of the HV spongy and compact myocardial layers in mako, salmon and thresher sharks reveals a significantly greater spongy CS activity in all three species but no differences in LDH activity. Adjustment of enzyme activities to *in vivo* RM and WM temperatures in the endothermic lamnids elevates CS and LDH in both tissues relative to the ectothermic sharks. Thus, through its enhancement of both RM and WM enzyme activity, endothermy may be an important determinant of energy supply for sustained and burst swimming in the lamnids. Although lamnid WM is differentially warmed as a result of RM endothermy, regional differences in WM CS and LDH activities and thermal sensitivities (Q_{10} values) were not found. The general pattern of the endothermic myotomal and ectothermic HV muscle metabolic enzyme activities in the endothermic lamnids relative to other active, ectothermic sharks parallels the general pattern demonstrated for the endothermic tunas relative to their ectothermic sister species. However, the activities of all enzymes measured are lower in lamnids than in tunas. Relative to lamnids, the presence of lower WM enzyme activities in the thresher shark (which is in the same order as the lamnids, has an RM morphology similar to that of the mako and salmon sharks and may be endothermic) suggests that other factors, such as behavior and swimming pattern, also affect shark myotomal organization and metabolic function.

Key words: Lamnidae, shark, elasmobranch, muscle biochemistry, endothermy, metabolic biochemistry, locomotor muscle, cardiac muscle, aerobic capacity, anaerobic capacity, temperature, citrate synthase, creatine phosphokinase, lactate dehydrogenase.

Introduction

Recent works have documented the high degree of evolutionary convergence between sharks in the family Lamnidae and tunas (family Scombridae) for features related to high-performance swimming (Bernal et al., 2001a,b). Lamnids and tunas have similar morphological adaptations for aquatic locomotion and similar cardiorespiratory specializations that increase the acquisition and delivery of O_2 to metabolically active tissues (Emery et al., 1985; Emery, 1986; Emery and Szczepanski, 1986; Lai et al., 1997; Bernal et al., 2001a; Brill and Bushnell, 2001; Korsmeyer and Dewar, 2001). These groups are also similar, and unlike nearly all other fishes, in having a more anterior and central position of their slow-twitch, oxidative myotomal muscle [red muscle (RM); Graham et al., 1983; Carey et al., 1985; Graham and Dickson, 2000; Bernal et al., 2001a, 2003). In addition, the vascular supply to the RM is specialized for counter-current heat exchange in both groups. By conserving metabolic heat derived from RM activity, tunas and lamnids elevate the

temperature of RM and other tissues above that of the ambient water (i.e. regional endothermy; Carey and Teal, 1966, 1969a,b; Carey et al., 1971; Anderson and Goldman, 2001; Bernal et al., 2001a,b).

The first paper in this series (Bernal et al., 2003) compared the position and quantity of RM in lamnids, other sharks and tunas and showed the presence and relative development of ultrastructural and biochemical features augmenting the aerobic capacity of lamnid RM. The present study investigates the biochemical basis for high-performance swimming by comparing aerobic and anaerobic enzyme activities in the myotomal muscles and myocardium of endothermic lamnids with those of actively swimming ectothermic sharks.

Fish RM powers sustained aerobic swimming, and the fast-twitch, glycolytic white muscle (WM) is specialized for short-duration accelerations and bursts powered by anaerobic metabolism (Johnston, 1981; Bone, 1988). In most fish species, RM and WM are morphologically distinct and easily differentiated, making it possible to isolate homogenous samples of each fiber type and quantify their metabolic enzyme activities. Comparative assessments of tissue metabolic capacity have generally focused on the activities of the enzymes citrate synthase (CS), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). CS catalyzes the first reaction of the Krebs citric acid cycle and correlates with tissue mitochondrial density; CPK catalyzes the transfer of a high-energy phosphate group from intracellular creatine phosphate to ADP, rapidly producing ATP; LDH catalyzes the reversible reaction, pyruvate + NADH $\rightarrow$ lactate + NAD^+, which maintains redox balance during anaerobic glycolysis.

Phylogenetically based comparisons of tunas and their ectothermic sister taxa (mackerels, Spanish mackerels and bonitos) show that, when measured at 20°C, tunas have higher CS, LDH and CPK activities in WM but similar CS activities in the RM (Dickson, 1988, 1995, 1996; Freund, 1999; Korsmeyer and Dewar, 2001). However, when the elevated RM temperature of the tunas is taken into account, tunas also have higher RM CS activities than do the ectothermic scombrids (Dickson, 1996). For the heart ventricle (HV; the heart is not thermally isolated by a counter-current heat exchanger and therefore operates at ambient temperature), some aerobic enzymes are present at higher activities in tunas than in their ectothermic relatives (reviewed by Dickson, 1995, 1996) but others are not significantly higher in tunas (Freund, 1999).

The objectives of our study were to make a comparable assessment of tissue enzyme activities in the endothermic lamnid sharks relative to those of other active ectothermic sharks. Dickson et al. (1993) conducted an initial study of this type and concluded that the pattern of enzymatic activities in the locomotor muscle in one lamnid, the shortfin mako shark (*Isurus oxyrinchus*), relative to ectothermic sharks paralleled the pattern documented for tunas relative to ectothermic scombrids. They reported higher CS and LDH activities in mako WM but similar CS activities in both the RM and HV of mako and ectothermic sharks. However, there were three limitations of their study: (1) limited access to mako specimens and delays in obtaining tissue samples that may have compromised tissue quality, (2) limited comparative material [i.e. the blue shark *Prionace glauca* (order Carcharhiniformes) was the only obligate swimming, ectothermic shark available and it had a surprisingly low WM LDH activity] and (3) all assays were conducted at 20°C, and the effect of elevated muscle temperatures on RM and WM enzyme activities was not measured.

Our objectives were to surmount the limitations of the Dickson et al. (1993) study and to expand the phylogenetic basis for comparing the biochemical indices of aerobic and anaerobic capacity in the locomotor muscle and HV of lamnids and other species. In addition to acquiring tissues from several active ectothermic sharks, we used a larger number of mako sharks sampled immediately after capture and obtained data on another lamnid, the salmon shark (*Lamna ditropis*). We also sampled tissues from the common thresher shark (*Alopias vulpinus*; family Alopiidae, order Lamniformes). Thresher sharks are more closely related to the lamnids and are similar to them in having a central and anterior position of the RM, which is served by a lateral circulation and a small putative heat exchanger (Bone and Chubb, 1983; Block and Finnerty, 1994; Bernal et al., 2003). Although thresher shark endothermy remains undocumented (Carey et al., 1971), anatomical studies (Fudge and Stevens, 1996) and ongoing field studies suggest that *A. vulpinus* can elevate its RM temperature (D. Bernal and C. Sepulveda, unpublished).

In addition to these comparisons, we tested the hypotheses that lamnids, like tunas, benefit from RM endothermy by having higher RM enzyme activities at *in vivo* temperatures, that lamnid WM adjacent to RM has a higher metabolic capacity because it is warmed by thermal conduction from the RM and that enzymes from the different regions of the WM differ in thermal sensitivity, reflecting their typical thermal range.

Materials and methods

Tissue collection

Tissue samples were obtained from eight shark species: shortfin mako (*Isurus oxyrinchus* Rafinesque 1810; *N*=32), salmon (*Lamna ditropis* Hubbs and Follet 1947; *N*=2), common thresher (*Alopias vulpinus* Bonaterre 1788; *N*=7), blue (*Prionace glauca* L.; *N*=2), scalloped hammerhead (*Sphyrna lewini* Griffith and Smith 1834; *N*=10), bonnethead (*Sphyrna tiburo* L.; *N*=6), Atlantic sharpnose (*Rhizoprionodon terraenovae* Richardson 1836; *N*=8) and Atlantic blacknose (*Carcharhinus acronotus* Poey 1860; *N*=2). Mako, thresher and blue sharks were collected by hook and line using a small research skiff and by longline during the US National Marine Fisheries Service (NMFS) Shark Indexing Abundance Program in the Southern California Bight from 1996 to 2000. Salmon sharks were taken by hook and line aboard the *F/V Legend* near Prince William Sound, AK, USA. Scalloped hammerheads were collected from Kaneohe Bay, HI, USA. Atlantic sharpnose, Atlantic blacknose and bonnethead sharks were obtained by longline and gill-net fishing in the near-shore

waters of the Gulf of Mexico near Panama City, FL, USA during the 1997–1998 NMFS shark population studies. All experimental protocols were approved by the University of California San Diego and California State University Fullerton Institutional Animal Care and Use Committees.

Immediately after capture, sharks were euthanized, and samples of RM, WM and HV (separating, when possible, compact and spongy myocardium) were obtained. WM and RM were sampled at 40–50% fork length (*FL*; i.e. at the position of the first dorsal fin) in mako, salmon, thresher and blue sharks. Samples from the hammerhead, bonnethead, Atlantic sharpnose and Atlantic blacknose sharks were collected at a more posterior body position (60–80% *FL*; at the level of the second dorsal fin). To test for the possible effect of proximity to RM (i.e. an increase in tissue temperature) on WM enzyme activities in mako and salmon sharks, samples were removed from three body depths (close to the skin, midway between the skin and the RM, and close to the RM) at the 45–50% *FL* body position. All tissue samples were immediately placed in cryogenic vials and frozen in a liquid nitrogen dry shipper (Thermolyne 10) or on dry ice and then stored (1–24 months) at –80°C. Initial tests verified that neither freezer storage times of 1–24 months at –80°C nor homogenate centrifugation time (15–900 s) affected enzyme activities.

Enzyme assays

Samples of RM, WM and HV were first cleared of attached connective tissue and any tissue dehydrated by freezing. Approximately 0.2 g of frozen tissue was then taken from each sample and homogenized on ice in a Kontes Duall ground-glass homogenizer with 9× the volume of buffer [2 mmol l^{-1} EDTA, 3 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} NaCl, 150 mmol l^{-1} KCl, 200 mmol l^{-1} trimethylamine n-oxide (TMAO), 400 mmol l^{-1} urea, 80 mmol l^{-1} imidazole buffer (pH 7 at 20°C)]. Homogenates were centrifuged (12 000 ***g***) for 15 min at 4°C and the supernatants were removed and kept on ice, without further purification, until their use in enzyme assays. Supernatants were diluted in homogenization buffer [CS (5×), LDH (41–81×) and CPK (25–76×)] just before assays were run to ensure that the enzymes were not substrate limited.

In vitro enzyme assays for RM, WM and HV were run in a total volume of 2.0 ml using a temperature-controlled (±0.2°C) spectrophotometer (Shimadzu UV 1201S or Hewlett-Packard 8452A diode-array). Enzymatic activity is proportional to the change in absorbance over time and is reported in international units (IU; μmol substrate converted to product per min) per g tissue wet mass. The final conditions for each assay were as follows. Citrate synthase (CS): 0.1 mmol l^{-1} acetyl-CoA, 0.5 mmol l^{-1} oxaloacetate, 0.1 mmol l^{-1} dithiobis nitrobenzoic acid, 3 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} NaCl, 150 mmol l^{-1} KCl, 200 mmol l^{-1} TMAO, 400 mmol l^{-1} urea, 80 mmol l^{-1} Tris buffer (pH 8 at 20°C); absorbance changes were measured at 412 nm. Creatine phosphokinase (CPK): 12.5 mmol l^{-1} creatine phosphate, 0.5 mmol l^{-1} ADP, 0.3 mmol l^{-1} $NADP^+$, 3.5 mmol l^{-1} glucose, 10 mmol l^{-1} AMP, excess hexokinase and glucose-6-phosphate dehydrogenase coupling enzymes, 100 mmol l^{-1} KCl, 10 mmol l^{-1} $MgCl_2$, 200 mmol l^{-1} TMAO, 400 mmol l^{-1} urea, 50 mmol l^{-1} imidazole HCl (pH 7.0 at 20°C); absorbance changes were measured at 340 nm. Lactate dehydrogenase (LDH): 0.15 mmol l^{-1} NADH, 1 mmol l^{-1} pyruvate, 3 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} NaCl, 150 mmol l^{-1} KCl, 200 mmol l^{-1} TMAO, 400 mmol l^{-1} urea, 80 mmol l^{-1} imidazole HCl (pH 7 at 20°C); absorbance changes were measured at 340 nm.

Enzyme assays for interspecific comparisons were carried out at 20°C under saturating substrate conditions, as determined in preliminary tests. Thermal effects on CS and LDH activities in mako, salmon and thresher shark RM were determined by measuring enzyme activity at 5°C intervals from 5°C to 30°C. This same temperature range was used to assess possible differences in thermal effects on the enzymatic activities of mako and salmon shark WM positioned at three different body depths between the skin and RM. To measure thermal sensitivity, the thermal rate coefficient (Q_{10}) was estimated from the slope of the linear regression of $\log_{10}$(enzyme activity at T_n) *vs* [(T_n–T_{n-1})×0.1], where T_n is the assay temperature (in °C), and the Q_{10} is equal to 10^{slope} (Schmidt-Nielsen, 1993).

Statistical analyses

Tissue-specific *in vitro* enzyme activities were tested for significant interspecific differences by using a general linear model (GLM) analysis of covariance (ANCOVA) or analysis of variance (ANOVA) in Minitab (version 10.5). The initial GLM ANCOVA model for each enzyme in each tissue included species, mass, *FL* (with mass and *FL* as covariates), all possible two-way interactions and a three-way interaction term. Data that were not normally distributed, based on a Kolmogorov–Smirnov test, or that did not meet the assumption of homogeneity of variance, as determined from residuals *vs* fit plots, were $\log_{10}$-transformed before ANCOVA. When significant mass effects were found, and if ANCOVA showed a significant species effect, a linear regression was fitted to a plot of enzyme activity *vs* mass for each species, and a Pearson product-moment correlation coefficient was used to test for significance. When no significant size effects were found, the mean values for each species were compared by ANOVA, followed by a *post-hoc* Tukey–Kramer multiple comparisons test. A Student's paired *t*-test was used to test for differences in enzyme activities between compact and spongy myocardium. A significance level of α=0.05 was used in all statistical analyses.

Results

The ranges of body length, mass and ambient temperature at capture for sharks used in this study are summarized in Table 1.

Muscle enzyme activities

Red muscle

There was no significant effect of fish mass or *FL* on RM CS or LDH at 20°C, and mean enzyme activities were used for

Table 1. *Citrate synthase (CS), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activities in the red and white myotomal muscle of nine shark species*

Species (common name)	Fork length (cm)	Mass (kg)	SST† (°C)	Enzyme activity at 20°C*					Source
				Red muscle		White muscle			
				CS	LDH	CS	CPK	LDH	
Isurus oxyrinchus (shortfin mako shark)	79–168	4.8–49.5	18–21	31.6±1.8 [30]	244.3±8.0 [31]	2.10±0.05 [31]	834.3±22.7 [24]	1154.7±35.7 [31]	Present study
Lamna ditropis (salmon shark)	186–210	127–136	7–10	35.4 (32.5–38.7) [2]	249.9 (233.5–266.3) [2]	2.63 (2.1–3.2) [2]		1254.5 (1172.7–1336.4) [2]	Present study
Alopias vulpinus (common thresher shark)	73–137	6.3–43.1	18–21	27.1±2.8 [6]	201.4±21.1 [6]	1.29±0.19 [6]	642.1 (544.1–740.1) [2]	414.8±82.3 [6]	Present study
Prionace glauca (blue shark)	176–246	34–59	18–21	22.3±6.3 [4]	71.4±7.9 [4]	1.00±0.04 [4]	631.9 [1]	150.0±24.1 [4]	Present study; Dickson et al. (1993)
Sphyrna lewini (scalloped hammerhead shark)	15–46	0.5–0.8	23–25	33.9±1.1 [10]	152.9±12.4 [10]	0.87±0.04 [10]		646.6±39.7 [10]	Present study
Sphyrna tiburo (bonnethead shark)	50–60	0.47–0.75	23–28				749.8±43.9 [6]		Present study
Rhizoprionodon terraenovae (Atlantic sharpnose shark)	42–75	0.1–0.7	23–28				790.2±41.1 [13]	924.7±52.3 [8]	Present study
Carcharhinus acronotus (Atlantic blacknose shark)	56–82	0.5–3.1	23–28				545.6 (507.8–582.3) [2]	1064.9 (985.9–1143.8) [2]	Present study
Triakis semifasciata (leopard shark)	32–62	0.1–0.8	18–20	27.1±2.1 [7]	189.4±8.2 [9]	0.81±0.08 [7]		661.2±21.5 [9]	Dickson et al. (1993)

*Enzyme activities are expressed as μmol substrate converted to product per min (IU) per g wet mass of tissue. Values are means ± S.E.M. except that, when the sample size is 2, data given are means and range (in parentheses). Numbers in brackets are the number of fish analyzed.

†SST, sea surface temperature.

White muscle

Mean WM CS activities in the mako and salmon shark are 1.6–3.2 times higher than in the other sharks, including the thresher shark (Table 1). ANCOVA indicated a significant mass effect and a significant mass×species interaction for WM CS activity at 20°C. Follow-up tests for correlations between body mass and WM CS activity in each species demonstrated significant correlations only for the mako (negative) and the scalloped hammerhead (positive) (Fig. 1).

The highest WM LDH activities measured were in mako and salmon sharks, but values for the mako overlap those measured in the Atlantic blacknose and Atlantic sharpnose sharks (Fig. 1), and the lowest LDH activities were found in the blue and thresher sharks. The ANCOVA for WM LDH activity at 20°C also showed a significant mass effect and mass×species interaction, but, when the data for each species were analyzed individually, no significant correlations between enzyme activity and body mass were found (Fig. 1).

No body size effects were found for WM CPK activity, and the only interspecific difference detected for this enzyme was a significantly greater comparisons among species (Table 1). ANOVA indicated no interspecific differences in mean RM CS activity. There were interspecific differences in mean RM LDH activities, but the results of the *post-hoc* multiple comparisons test differed depending on which species was used as the basis for comparison. The blue shark had a significantly lower mean RM LDH activity than all other species studied, but values for the other species overlapped. When the mako was compared with all other sharks, its RM LDH activity did not differ from that in the salmon shark or thresher shark but was significantly greater than in the other three species. RM LDH activity in the salmon shark did not differ from that of the mako, common thresher or leopard sharks but was significantly greater than in the other two species. Common thresher shark RM LDH activity did not differ from that of any other species except the blue shark.

activity in the mako than in the Atlantic blacknose shark (P<0.05, N=26; Table 1).

Myocardial tissue

Body size had no effect on LDH or CS activities (20°C) in the HV (a mix of both spongy and compact myocardium) of six shark species. The multiple comparisons test showed equivalent mean LDH activities in the mako, salmon, common thresher and leopard sharks, which were significantly higher than those of both the scalloped hammerhead and blue sharks (P<0.05; Fig. 2). Also, blue shark HV LDH is significantly less than in the scalloped hammerhead (Fig. 2).

Mean HV CS activity in the mako did not differ from that in the salmon shark but was significantly greater than in all other species studied (P<0.05; Fig. 2). When the salmon shark was the comparison group, its HV CS did not differ from that of the mako, common thresher or scalloped hammerhead sharks but was significantly greater (P<0.05) than in the blue and leopard sharks. The leopard shark had a significantly lower mean HV CS activity than all other species except the blue shark (P<0.05; Fig. 2). Blue shark HV CS activity did not differ significantly from that in the scalloped hammerhead, common thresher and leopard sharks but was significantly lower than that of the two lamnids (P<0.05).

Comparison of isolated spongy and compact myocardial samples from mako, salmon and common thresher sharks (Table 2) shows a significantly higher CS activity in the spongy myocardium (paired t-test; P<0.05, N=9) but no differences between spongy and compact layer LDH activities.

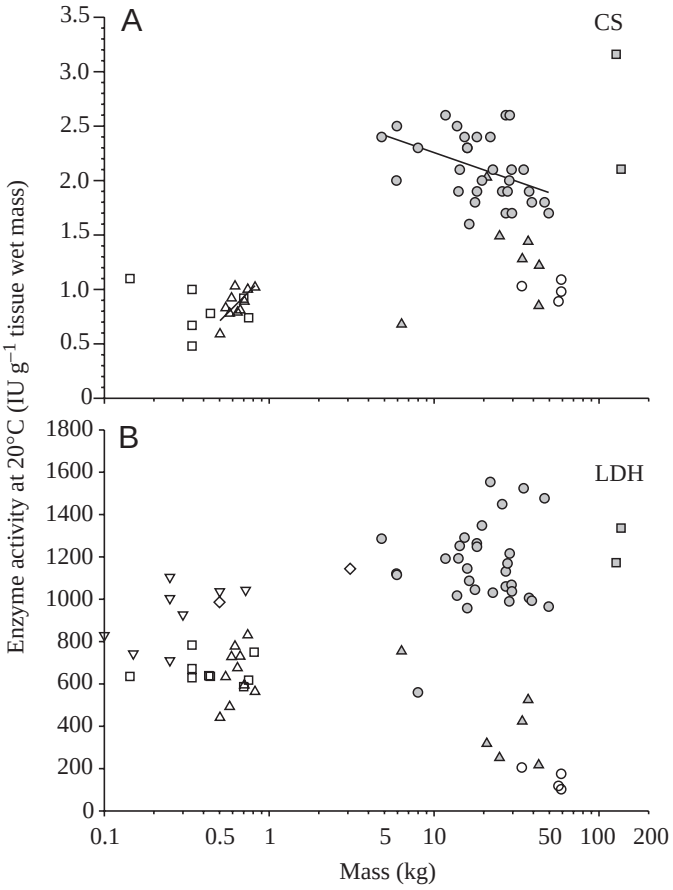

Fig. 1. Relationship between shark white muscle enzyme activity [μmol substrate converted to product per min (IU) per g wet mass of tissue] at 20°C and body mass. (A) Citrate synthase (CS) activity in blue (open circles), scalloped hammerhead (open triangles), leopard (open squares), common thresher (shaded triangles), mako (shaded circles) and salmon (shaded squares) sharks. In cases where the correlation between enzyme activity and mass was significant for a species, a linear regression was fit to the data. For the mako, IU g^{-1}=2.78–0.53×[log$_{10}$(mass)], r=–0.44, P=0.01, N=31; for the scalloped hammerhead IU g^{-1}=1.17+1.53×[log$_{10}$(mass)], r=0.72, P=0.01, N=10. (B) Lactate dehydrogenase (LDH) activity for the same species as for CS and two additional shark species: the Atlantic blacknose (open diamonds) and Atlantic sharpnose (open inverted triangles).

Temperature effects on lamniform shark muscle enzyme activities

Red muscle

Comparable Q_{10} values over 5–30°C indicate no interspecific differences in the effect of temperature on the activities of RM CS and LDH in the mako (N=4), salmon (N=2) and thresher (N=7) sharks (Fig. 3). Close agreement among the three species exists in the Q_{10} range determined for each enzyme, but the Q_{10} for LDH (mean, 2.01; range, 1.97–2.08) is greater than that for CS (1.72; 1.65–1.76). For each species, there were no apparent body size effects on the activities of either CS or LDH or on the Q_{10} values (Fig. 3).

White muscle

Data for two mako and two salmon sharks document the absence of an effect of WM proximity to the warm RM (i.e. under the skin *vs* midway between skin and RM *vs* close to the RM) on the thermal sensitivity of CS and LDH activities. The Q_{10} values were similar for both species and for both enzymes (Table 3). Fig. 4 plots the temperature–enzyme activity data for one of the makos (49 kg) and one of the salmon sharks (127 kg) and shows the WM sample positions in relation to the thermal contour patterns in the salmon shark. The enzyme activities were similar at all three WM positions sampled.

Discussion

Comparative shark muscle enzyme activity

This investigation of shark muscle metabolic enzymes confirms the major conclusions of Dickson et al. (1993), addresses most of the experimental limitations of that study and expands the comparative basis for understanding the evolution of shark endothermy and the tuna–lamnid convergence. We have shown that, compared with active ectothermic sharks at the same temperature (20°C), lamnids have similar RM CS activities but higher WM CS and HV CS activities. The two lamnid species also had the highest WM LDH activities, but the values overlap with those measured in the Atlantic blacknose and Atlantic sharpnose sharks.

Table 2. *Relative ventricle mass and enzyme activities in the compact and spongy myocardial layers of three lamniform shark species*

Species (common name)	Body mass (kg)	Relative ventricle mass (% body mass)	Relative compact myocardial tissue (% of ventricle)	Enzyme activity[a] at 20°C			
				CS		LDH	
				Compact	Spongy*	Compact	Spongy
Isurus oxyrinchus	6	~0.17[b,c]	~36–40[b,d]	37.7	44.0	494	503
(shortfin mako shark)	26			39.9	42.8	487	495
	49			34.2	36.3	303	343
Lamna ditropis	127	0.18–0.20[c]	~40[d]	34.5	38.0	306	312
(salmon shark)	136			31.6	32.7	409	428
Alopias vulpinus	21	–0.10–0.29[b]	~36[b]	32.6	31.4	459	487
(common thresher shark)	34			25.2	31.7	421	499
	37			19.9	23.0	226	233
	43			18.9	20.7	240	200

[a]Enzyme activities are expressed as μmol substrate converted to product per min (IU) per g wet mass of tissue.
[b]Data from Emery et al. (1985).
[c]D. Bernal, N. C. Lai, W. Lowell, K. Dickson, C. Sepulveda and J. Graham, unpublished.
[d]D. Bernal and J. Graham, unpublished.
*Spongy myocardium CS activities are significantly higher (P<0.05) than in compact myocardium.

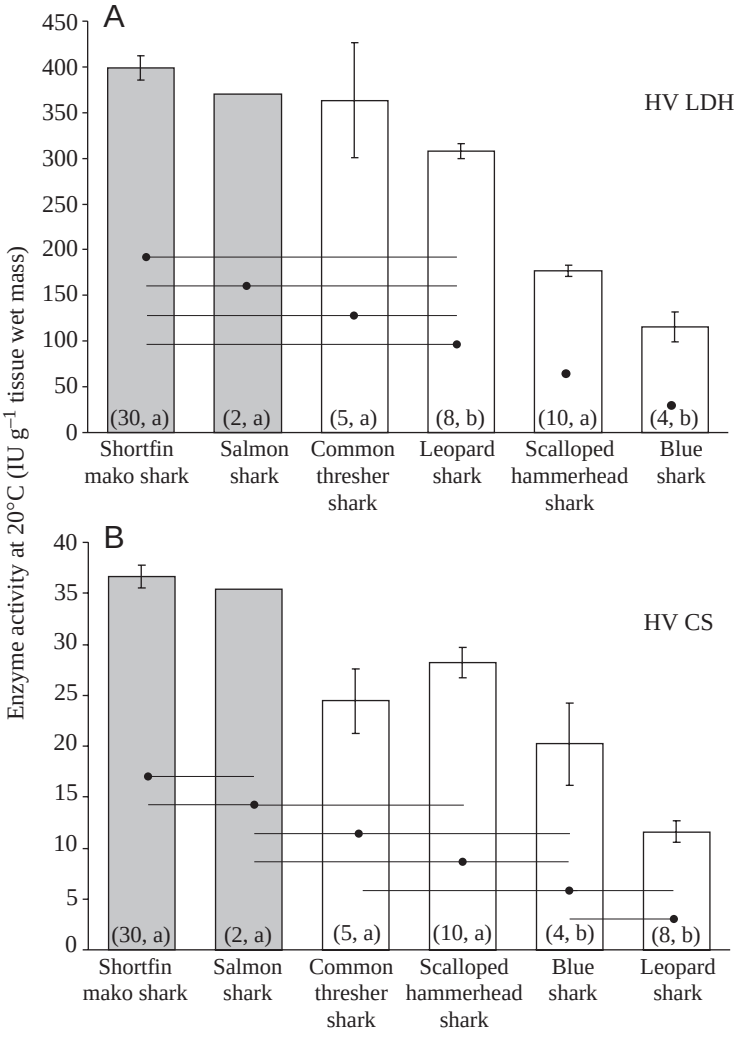

Fig. 2. (A) Lactate dehydrogenase (LDH) and (B) citrate synthase (CS) activities at 20°C in shark heart ventricle (HV; an unknown mixture of compact and spongy myocardium). Values are means ±S.E.M. for ectothermic sharks (open bars) and sharks known to be endothermic (shaded bars). Values in parentheses at the base of each bar are the sample size and the data source: a, present study; b, Dickson et al. (1993). Horizontal lines group species that do not differ significantly from the comparison species (indicated by a black circle), as determined by a Tukey–Kramer multiple comparisons test at P<0.05. For example, leopard shark HV CS (bottom horizontal line in B) is not significantly different from that of the blue shark but differs significantly from all other species studied.

Furthermore, because of the higher muscle temperatures resulting from endothermy, lamnid RM and WM enzyme activities at *in vivo* temperatures are higher than those of ectothermic sharks at 20°C. Another significant finding of this study is that the pattern of muscle metabolic enzyme activities in the endothermic lamnid sharks and active ectothermic sharks generally parallels the pattern demonstrated for the endothermic tunas relative to their ectothermic sister species.

Even though this study has expanded both the number of shark species and tissue samples that have been analyzed, the difficulty in obtaining pelagic shark specimens has reduced our capacity for robust interspecific comparisons because of small sample sizes for some species and interspecific differences in both body size range and the types of tissues sampled. We did not, for example, have access to all tissues in all species (i.e. only WM tissue samples were obtained from Atlantic sharpnose, Atlantic blacknose and

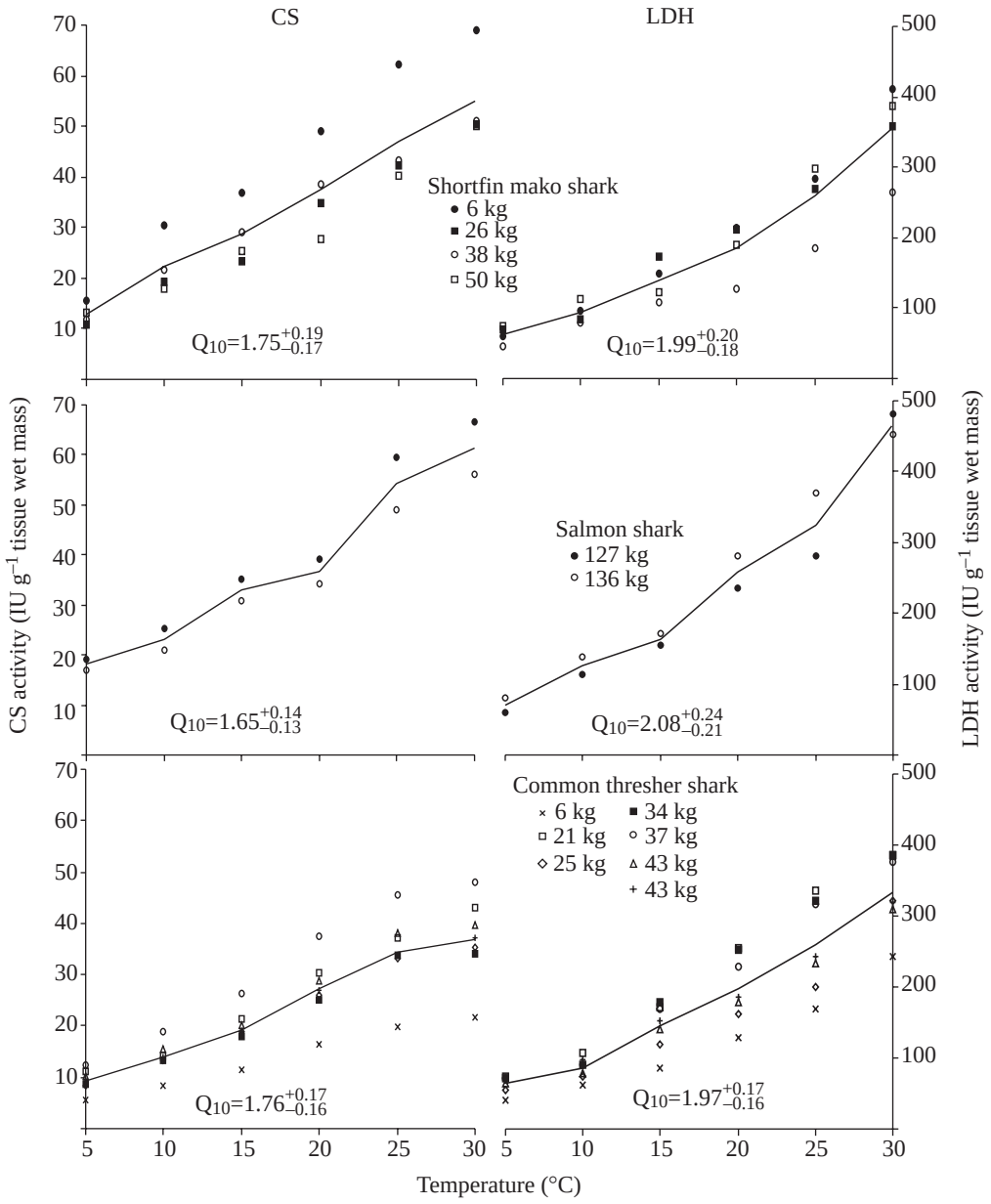

Fig. 3. Temperature effects on the activities of RM CS (left column) and LDH (right column) in four mako, two salmon and six common thresher sharks. Symbols identify specimens by body mass, and the lines show mean enzyme activity at each temperature. The thermal rate coefficient (Q_{10}) (±95% C.I.) values were computed for the activities at 5°C and 30°C (see Materials and methods).

bonnethead sharks; Table 1). Also, we obtained samples from only two salmon sharks and, while findings for this species were similar to those for the mako, the small sample size limited the utility of the salmon shark data in interspecific comparisons. The range of fish sizes available for study also differed among the shark species sampled, thereby limiting interspecific comparisons of enzyme activity scaling relationships. Nevertheless, and despite these impediments, our study provides new insight into both the functional significance of shark endothermy and the comparative physiology of the lamnid–tuna convergence.

Lamnid and tuna comparisons

Tunas and lamnids have similar specializations for continuous, sustained swimming (Bernal et al., 2001a), and species in both groups frequently make vertical excursions through the water column or undertake extensive seasonal migrations (Carey et al., 1978, 1981; Paust and Smith, 1989; Casey and Kohler, 1992; Holts and Bedford, 1993; Anderson and Goldman, 2001; Gunn and Block, 2001; Klimley et al., 2002). Underlying the tuna–lamnid convergence in adaptations for high-performance swimming are similar RM positions within the body (Graham et al., 1983; Carey et al., 1985; Bernal et al., 2001a, 2003) and the capacity to augment RM and other systemic functions through regional endothermy (Block and Finnerty, 1994; Brill, 1996; Bernal et al., 2001a; Graham and Dickson, 2001). Endothermy raises the temperature of RM and WM, which, by speeding the contraction–relaxation cycle, increases muscle power production (Johnston and Brill, 1984; Altringham and Block, 1997). Endothermy also stabilizes tissue temperatures, thereby conserving metabolic function in the face of ambient temperature reductions (caused by moving

Table 3. *Thermal effects on the enzyme activities of white muscle at different transverse body positions (see Fig. 4) in two lamnid shark species*

		Lactate dehydrogenase			Citrate synthase		
	Body mass (kg)	Slope* (±95% C.I.)	*N*; r^2	Q_{10} for range 5–30°C† (+, – 95% C.I.)	Slope* (± 95% C.I.)	*N*, r^2	Q_{10} for range 5–30°C† (+, – 95% C.I.)
Isurus oxyrinchus							
Close to RM	6	0.23±0.05	6; 0.97	1.69 (+0.21, –0.19)	0.22±0.61‡	3; 0.95‡	1.64 (+5.1, –1.2)‡
Midway to RM	6	0.24±0.07	6; 0.96	1.73 (+0.29, –0.25)	0.33±0.71‡	3; 0.97‡	2.14 (+8.9, –1.7)‡
Under the skin	6	0.22±0.05	6; 0.97	1.66 (+0.22, –0.19)	0.28±0.01‡	3; 0.99‡	1.91 (+0.04, –0.04)‡
Close to RM	49	0.28±0.05	6; 0.98	1.90 (+0.25, –0.22)	0.27±0.12	6; 0.90	1.86 (+0.60, –0.46)
Midway to RM	49	0.25±0.05	6; 0.98	1.77 (+0.22, –0.19)	0.37±0.17	6; 0.90	2.35 (+1.16, –0.77)
Under the skin	49	0.24±0.03	6; 0.99	1.77 (+0.11, –0.10)	0.23±0.09	6; 0.92	1.69 (+0.40, –0.32)
Lamna ditropis							
Close to RM	127	0.19±0.05	6; 0.96	1.55 (+0.21, –0.18)	0.25±0.06	6; 0.97	1.76 (+0.27, –0.23)
Midway to RM	127	0.26±0.06	6; 0.98	1.82 (+0.25, –0.22)	0.23±0.03	6; 0.99	1.68 (+0.16, –0.14)
Under the skin	127	0.21±0.03	6; 0.99	1.61 (+0.11, –0.11)	0.21±0.03	6; 0.99	1.61 (+0.10, –0.10)
Close to RM	136	0.25±0.06	6; 0.97	1.76 (+0.26, –0.23)	0.22±0.08	6; 0.94	1.67 (+0.33, –0.28)
Midway to RM	136	0.25±0.10	6; 0.92	1.77 (+0.48, –0.38)	0.17±0.03	6; 0.98	1.48 (+0.11, –0.11)
Under the skin	136	0.24±0.02§	4; 0.99§	1.73 (+0.08, –0.08)§	0.24±0.06	6; 0.96	1.72 (+0.27, –0.23)

RM, red muscle.

*The thermal rate coefficient (Q_{10}) was estimated from the slope of the linear regression of $\log_{10}$(activity at T_n) *vs* [(T_n–T_{n-1})×0.1], where T_n is the assay temperature (in °C) and the Q_{10} is equal to 10^{slope} (Schmidt-Nielsen, 1993).

†Note that the uneven confidence intervals (C.I.) are due to the $\log_{10}$ transformation required to calculate Q_{10} (see footnote *).

‡Activities measured at 5°C, 15°C and 25°C only.

§Activities not determined at 5°C and 10°C.

below the thermocline or into higher latitudes), and has probably contributed to niche expansion by both groups (Block et al., 1993; Block and Finnerty, 1994; Graham and Dickson, 2000; Bernal et al., 2001a,b; Boustany et al., 2002).

Despite the similarities in muscle metabolic patterns of the tunas and lamnids when compared with related species, the activities of all enzymes measured in the lamnids are lower than they are in tunas and are more similar to the activities in ectothermic scombrids and other active teleosts. This is the case even when tunas and lamnids of similar masses are compared. The lower muscle metabolic enzyme activities in lamnids relative to tunas parallels the finding that structural modifications for enhanced oxygen delivery (*viz*. capillary manifolds) are less prominent in mako RM than in skipjack tuna (*Katsuwonus pelamis*) RM (Bernal et al., 2003). Lamnid sharks also have less RM than do tunas (2–3% *vs* 4–13% of body mass; Graham et al., 1983; Carey et al., 1985; Bernal et al., 2003), but the total volume of mitochondria and myoglobin concentration is similar in mako and tuna RM fibers (Bernal et al., 2003).

Endothermy and lamnid red muscle aerobic capacity

The thermal effects measured for RM CS activity (Fig. 3) can be used to estimate the magnitude of increase that occurs due to endothermy in the mako, salmon and common thresher sharks. The mako, for example, frequents 20°C water (Table 1) and has an RM temperature of 25–27°C (Carey and Teal, 1969a; Carey et al., 1985). Based on Fig. 3, we estimate a 48% greater RM CS activity at the mako's warmer *in vivo* temperature relative to what it would be at ambient temperature. For the salmon shark, which occurs in 8–10°C water (Table 1) and has an RM temperature of 24–26°C (Rhodes and Smith, 1983; Anderson and Goldman, 2001; Bernal et al., 2001a), RM CS activity would be enhanced by 123%. Assuming that the common thresher shark is an endotherm [this species commonly occurs in 18–20°C water (Table 1), and RM temperatures of 22–26°C have been measured (D. Bernal and C. Sepulveda, unpublished)], its RM CS activity would be elevated by as much as 48%. Use of the increased aerobic capacity resulting from endothermy requires an increased supply of both O_2 and aerobic fuels to the RM, and both tunas and lamnids have cardiorespiratory specializations that increase the uptake of O_2 at the gills and its delivery to the RM (Bernal et al., 2001a).

Heart metabolic capacity

Profiles of tuna heart enzyme activities indicate heightened aerobic capacities relative to other active fish species (reviewed by Dickson, 1995), which suggests an enhanced oxidative function (i.e. the pumping of blood). An elevated HV CS activity in mako and salmon sharks (Fig. 2) is consistent with the expectation of a greater cardiac pumping capacity and further supports the tuna–lamnid convergence.

Data indicating a greater aerobic capacity (CS activity) for spongy than for compact myocardium in the three lamniform sharks, but similar LDH activity in the two layers (Table 2),

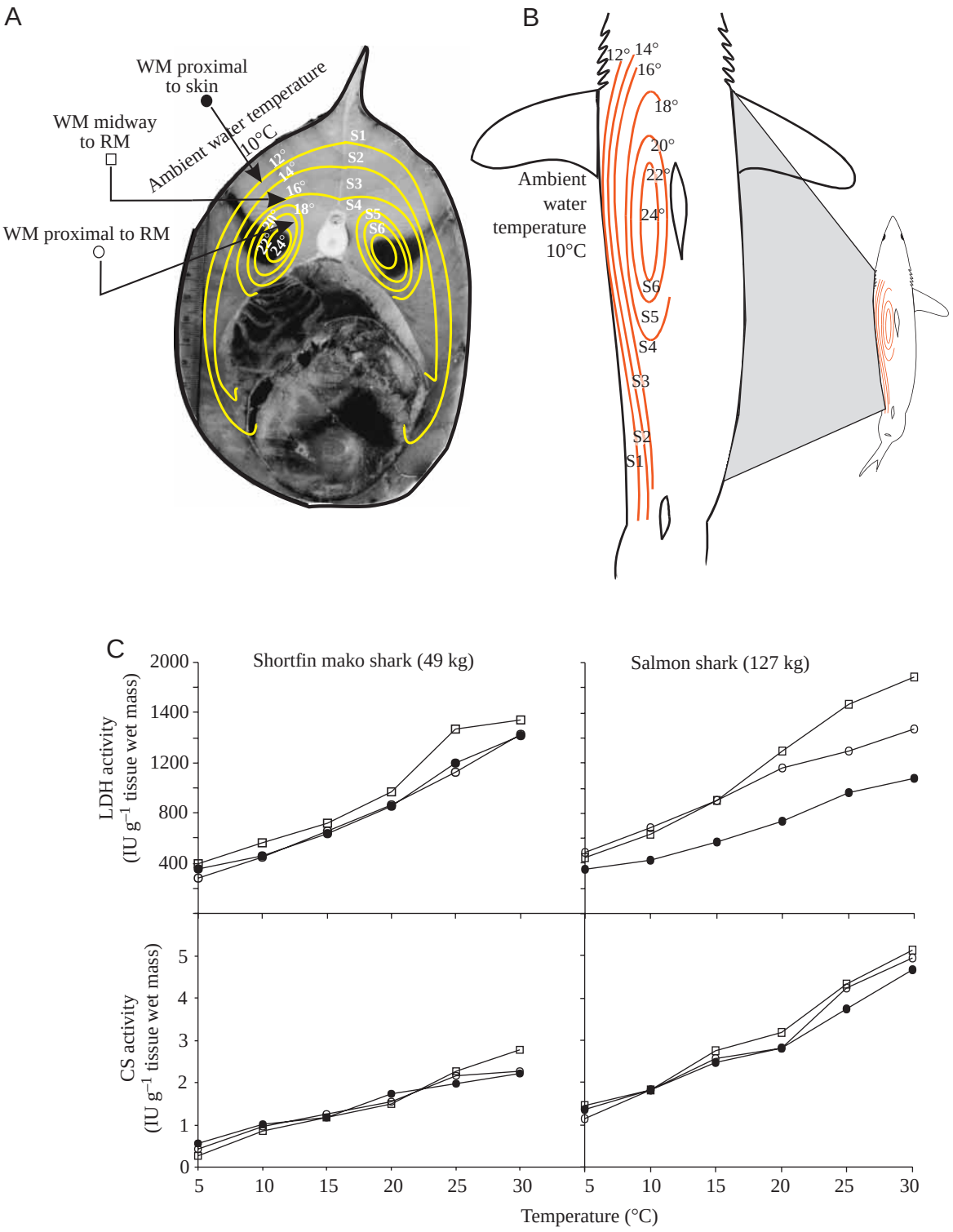

Fig. 4. Transverse (A) and longitudinal (B) sections showing myotomal muscle thermal contours (yellow and red lines) for a 148-kg salmon shark (*Lamna ditropis*) swimming in 10°C water. Contour projections are modified from Carey and Teal (1969a) and Carey et al. (1985) and use a maximum red muscle (RM) temperature of 24°C. Sectors (S1–S6) show the extent of regional endothermy occurring in white muscle (WM) located at different distances from the RM (see Table 4). The transverse section shows the sites where the three WM samples were taken in relation to the body temperature gradient, with highest temperature occurring closest to the RM and lowest temperature near the skin. (C) Graphs show temperature effects on the activities of lactate dehydrogenase (LDH) and citrate synthase (CS) in WM at the three locations (same symbols as in A) of a 49-kg mako and 127-kg salmon shark. (Table 3 contains the full data set for all the sharks.)

also parallel what has been documented for tunas (Tota, 1983; Moyes et al., 1992). The difference in CS activity may correlate with differences in the quantity of O_2 available to the two different myocardial layers (i.e. compact is nourished by the O_2-rich coronary arteries, whereas spongy receives a high percentage of hypoxic venous blood). However, additional work is needed to verify this and to determine the extent of other biochemical and physiological differences between the two tissue layers and how their function compares with that of tunas (Tota, 1978; Dickson, 1995; Bernal et al., 2001a).

White muscle metabolic capacity

The finding of higher WM CS and LDH activities in lamnids relative to the other shark species (Table 1) parallels the differences documented for tunas relative to non-tunas (Dickson, 1995, 1996). The scaling coefficient [–0.11±0.09 (95% C.I.), from the allometric equation $y=aM^{b-1}$, where y is mass-specific enzyme activity, a is a constant, M is fish mass, and b–1 is the scaling coefficient] for mako shark WM CS is within the range reported for oceanic pelagic teleosts (–0.37 to –0.06; Childress and Somero, 1990). Although we found a significant positive scaling relationship for scalloped hammerhead WM CS activity (Fig. 1), a relationship of this type is not consistent with data from other fish species (Childress and Somero, 1990). Because most scaling relationships are based on data extending across a size range of at least one order of magnitude, our opinion is that this result is without biological significance and is attributable to the small size range (0.5–0.8 kg) of hammerhead sharks examined.

The two lamnid species had the highest WM LDH activities, indicative of a high anaerobic capacity, but the values overlap with those measured in the Atlantic blacknose and Atlantic sharpnose sharks (Fig. 1). Because there was an overall increase in WM LDH activity with fish mass but no significant mass correlations within a species, and because the Atlantic blacknose and Atlantic sharpnose sharks were all smaller than the lamnids, we cannot rule out the possibility that the WM LDH activity of larger Atlantic blacknose and Atlantic sharpnose sharks would be similar to that of the lamnids. However, at similar sizes, the lamnids did have higher WM LDH activities than did the common thresher and blue sharks (Fig. 1).

The finding of a low WM LDH activity in the thresher shark was unexpected because threshers, like makos, are large and can jump clear of the water, which requires very high exit speeds and WM burst power (Carey and Teal, 1969a; Wu, 1977; Goolish, 1995). Furthermore, because the common thresher is, among the species we studied, more closely related to the lamnids and because it may also be endothermic, we expected its enzyme activities to be similar to those of the lamnids. The difference may be related to feeding habits. Unlike the mako and salmon sharks, threshers use the long upper lobe of the caudal fin to herd prey (e.g. sardines and anchovies) into tight groups for feeding (Gubanov, 1972), a behavior we inferred from having captured threshers in the present study by hooking them by the tail, which was also reported by Gruber and Compagno (1981). The use of the tail for herding prey and feeding may require fewer or shorter bouts of burst swimming than the predatory behavior of the lamnids. Thus, we propose that the high WM LDH of the two lamnid species is related to their use of anaerobic glycolysis during feeding and not specifically to endothermy.

It has been proposed for teleost fishes that species with a high WM LDH activity also have correspondingly high WM CS activities to process lactate during the post-burst recovery period (Dickson, 1995, 1996; Gleeson, 1996; Mollet and Cailliet, 1996). We found a significant positive correlation between WM LDH and WM CS activities for the six shark species in which both activities were measured (Fig. 5). The high CS activity in lamnid WM suggests that, after repeated bouts of burst swimming, these fishes are able to process lactate quickly and recover rapidly, as has been hypothesized for tunas (Arthur et al., 1992; Dickson, 1995).

Endothermy effects on lamnid WM enzyme activities

The activities of lamnid WM CS and LDH are even higher when estimated at *in vivo* WM temperatures (Table 4; Fig. 4). Unlike the small mass of deeply positioned RM, which can be assumed to have a uniform temperature, quantification of the thermal enhancement on WM enzyme activities requires the integration of the lamnid WM isotherms determined by Carey and Teal (1969a) with a lamnid WM distribution map (D. Bernal, unpublished) to estimate the relative amounts of WM

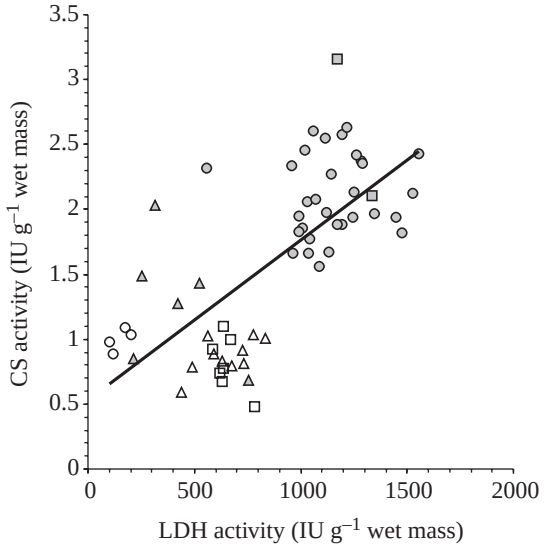

Fig. 5. Relationship between white muscle lactate dehydrogenase (LDH) and citrate synthase (CS) activity at 20°C in six shark species. Species identification symbols are as in Fig. 1: shortfin mako (shaded circles), salmon shark (shaded squares), common thresher shark (shaded triangles), blue shark (open circles), scalloped hammerhead shark (open triangles) and leopard shark (open squares). Regression line equation is: CS activity=0.52+0.012(LDH activity), r=0.69, P<0.01, N=60.

occurring at different temperatures (Table 4). For example, a salmon shark swimming in 10°C water would be expected to have an RM temperature of 24°C (Anderson and Goldman, 2001; Bernal et al., 2001a). While the WM adjacent to the RM would have nearly the same temperature, the temperature of WM just below the skin would be close to that of the ambient water (Carey and Teal, 1969a). A 148-kg salmon shark has 74 kg of WM (50% of body mass; D. Bernal, unpublished). Much of this WM (32.6 kg) occurs in sufficient proximity to the skin to not have an elevated temperature, but approximately 17.4 kg of it occurs within isotherms that are 2–4°C warmer than ambient seawater, and 0.80 kg of it is 10–12°C warmer than ambient (Table 4). When the LDH activity is adjusted for these regional *in vivo* temperature elevations (using a Q_{10} of 1.70; Table 3), total WM LDH activity is 13.6% greater than if all the WM was at ambient temperature (Table 4). A similar increase would occur in WM CS activity, given the similar Q_{10} values (Table 3). Although this increase is much less than the 123% thermal enhancement calculated for salmon shark RM CS activity, it could still contribute to the aerobic and anaerobic performance of the WM.

Our results do not support the hypothesis that enzymes of WM positioned in more peripheral (and thermally more variable) body regions are less sensitive to changes in temperature than are enzymes in WM adjacent to the RM. Rather, the finding of no significant thermal compensation in lamnid WM CS or LDH activities (Fig. 4) is similar to recent results for the Atlantic northern bluefin tuna (*Thunnus thynnus*) in which WM enzyme activities did not show thermal compensation along a similar thermal gradient (Fudge et al., 2001). This suggests that the *in vivo* WM thermal gradients in endothermic fishes are either too small or too unstable to have induced changes in enzyme activity or the expression of different enzyme isoforms.

In summary, CS activities in the WM and HV were higher in lamnid sharks when compared with active ectothermic sharks at a common reference temperature (20°C). RM CS activity at 20°C did not differ interspecifically, and activities of the other enzymes measured in the two lamnid species overlapped with those of the other shark species studied. In the mako, salmon and thresher sharks, the HV spongy myocardium had a significantly higher CS activity than the compact myocardium, but LDH activity did not differ in the two HV layers. When the enzyme activities in RM and WM of the endothermic sharks were estimated at *in vivo* temperatures, CS and LDH activities were elevated relative to what they would be at ambient temperature. Within the heterothermic WM of endothermic sharks, there were no detectable regional differences in WM CS or LDH activities or in thermal sensitivity of the enzymes. These findings parallel the general pattern demonstrated in previous studies for tissue metabolic enzyme activities in the endothermic tunas relative to their ectothermic sister species, substantiating the extent of convergence between the tunas and lamnid sharks.

Table 4. *Estimated thermal enhancement effect of salmon shark endothermy on white muscle (WM) lactate dehydrogenase (LDH) activity*

				No temperature adjustment*		Temperature adjustment†	
Sector ID‡	Temperature elevation above ambient in each sector (°C)	Percentage of total WM mass in each sector	WM mass in each sector (kg) **A**	Enzyme activity (IU g^{-1} WM) **B**	Total LDH activity (IU) **A** × **B**	Enzyme activity (IU g^{-1} WM) **C**	Total LDH activity (IU) **A** × **C**
1	0–2	44	32.56	495	1.61×10^7	495	1.61×10^7
2	2–4	23.5	17.39	495	8.61×10^6	551	9.58×10^6
3	4–6	17.4	12.84	495	6.36×10^6	613	7.87×10^6
4	6–8	9.2	6.79	495	3.36×10^6	682	4.63×10^6
5	8–10	4.9	3.63	495	1.79×10^6	759	2.75×10^6
6	10–12	1.1	0.80	495	3.96×10^5	844	6.75×10^5
					Total=3.66×10^7		Total=4.16×10^7

Morphological data: body mass, 148 kg; total WM mass, 74 kg; WM temperature directly under the skin, 10°C; deep WM temperature, 24°C.
*Using the mean LDH activity at 10°C (495 IU g^{-1} WM) from Fig. 4; †mean LDH activity at 10°C (495 IU g^{-1} WM) from Fig. 4 adjusted by applying a mean Q_{10} of 1.70 (see Table 3); temperature-adjusted LDH activity is 13.6% higher relative to non-temperature adjusted LDH activity; ‡see Fig. 4.

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