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THE METABOLIC CHARACTERISTICS OF THE LOCOMOTORY MUSCLES OF GREY SEALS (*HALICHOERUS GRYPUS*), HARBOUR SEALS (*PHOCA VITULINA*) AND ANTARCTIC FUR SEALS (*ARCTOCEPHALUS GAZELLA*)

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Summary

It is not known precisely how marine mammals are able to maintain muscle function during active swimming in breath-hold dives, when ventilation stops and heart rate falls. Examination of muscle biochemistry and histochemistry can provide information on the relative importance of different metabolic pathways, the contractile potential of the muscle fibres, the oxygen storage capacity of the muscle and the capillary distribution in these animals. In this study, samples of locomotory muscle were taken from wild grey seals (*Halichoerus grypus*), harbour seals (*Phoca vitulina*) and Antarctic fur seals (*Arctocephalus gazella*); Wistar rat muscle was analysed for comparative purposes.

Activities of citrate synthase and β-hydroxyacyl CoA dehydrogenase were higher in the harbour seal muscle than in the grey seal muscle, suggesting that harbour seals have a greater aerobic capacity. Both phocid muscles had a greater reliance on fatty acid oxidation than the fur seal or rat muscles. The myoglobin data demonstrate that the grey seals have the highest oxygen storage capacity of the three pinniped species, which correlates with their greater diving ability. Myoglobin levels were higher in all three pinniped species than in the Wistar rat. The fibre type compositions suggest that the muscles from the fur seals have higher glycolytic capacities than those of the phocid seals [fur seal pectoralis, 7 % slow-twitch oxidative fibres (SO), 25 % fast-twitch oxidative glycolytic fibres (FOG), 68 % fast-twitch glycolytic fibres (FG); grey seal 57 % SO, 5 % FOG, 38 % FG; area per cents]. However, the pectoralis muscle of the fur seal, although the most glycolytic of the pinniped muscles studied, has the highest capillary density, which indicates a high capacity for fuel distribution.

These results show that, while pinniped muscle has an increased oxygen storage potential compared with the muscle of a typical terrestrial mammal, there are no distinct adaptations for diving in the enzyme pathways or fibre type distributions of the pinniped muscle. However, the muscle characteristics of each species can be related to its diving behaviour and foraging strategy.

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Introduction

The 'dive response' of seals to involuntary apnoea involves a combination of physiological responses, including bradycardia, peripheral vasoconstriction and anaerobic metabolism in the peripheral tissues (Scholander *et al.* 1942). Therefore, much of the earlier work on the muscle physiology of marine mammals has focused primarily on possible adaptations to high levels of hypoxia and glycolysis (e.g. Castellini *et al.* 1981). However, recent field studies of freely diving phocids have shown that some spend a high percentage of time at sea submerged (up to 90 %, Fedak *et al.* 1988; Le Boeuf *et al.* 1988, 1989; Thompson and Fedak, 1993), with short surface intervals. Some studies have found extended dives, longer than the estimated aerobic dive limit (ADL, see Kooyman *et al.* 1983), but without long recovery periods (Hindell *et al.* 1991, 1992; Thompson and Fedak, 1993). Extreme bradycardia, similar to that seen in involuntary submersions, has been recorded from freely diving grey seals as a regular feature of foraging bouts (Thompson and Fedak, 1993), but without subsequent surface intervals being longer than average. Consequently, adaptations for rapid oxygen loading, increased oxygen storage and low rates of oxygen utilization may be more important for seals than an increased capacity for anaerobic glycolysis in the muscle.

The muscle adaptations for diving are still a suitable subject for investigation. Two approaches are useful: comparisons of the muscle physiology of different species of marine mammals with different dive patterns and of that of terrestrial and diving mammals. Otariid and phocid seals have different locomotory patterns and exhibit a range of diving behaviours and foraging patterns. Phocids (true seals) swim using alternate lateral sweeps of the hind flippers; the main locomotory muscles are the back muscles (which power the lateral movements) and the pelvic and hindlimb muscles (Tarasoff *et al.* 1972). Otariids (seal lions and fur seals) primarily use their forelimbs for aquatic locomotion and 'fly' through the water in a similar manner to penguins. The main locomotory muscles are the pectoralis muscles (for the downstroke) and the triceps, deltoid and latissimus dorsi muscles (for the upstroke; Howell, 1930); the hind limbs act as a rudder.

Phocids and otariids also have differing strategies for aquatic foraging. Phocids tend to be deep divers, with maximum depth ranges from 300 m to greater than 1600 m (Kooyman, 1988; Delong *et al.* 1992) and carry out long dives and long bouts of diving (Le Boeuf *et al.* 1988, 1989; Hindell *et al.* 1991, 1992; Thompson and Fedak, 1993). Otariids perform shallower and shorter dives than phocids, which may relate in part to their lower oxygen storage potential (Lenfant *et al.* 1970). The maximum dive depth of fur seals appears to be around 200 m; recorded average dive depths range from 25 to 70 m (Gentry *et al.* 1986). During rapid swimming and play, otariids often 'porpoise', i.e. leap clear of the water for a short distance. Like phocids, otariids tend to dive within their aerobic dive limit and dive for 60–75 % of their time at sea (Kooyman *et al.* 1986; Feldkamp *et al.* 1989).

To obtain an overall picture of the physiological capacities of the muscles of marine mammals, this study aimed to combine metabolic information (from enzyme biochemistry) with information on the oxygen storage potential, the fibre type distribution and the oxygen distribution potential (capillary supply), and to relate these

data to the freely diving behaviour of the animals. Muscle samples were obtained from Antarctic fur seals (an otariid), grey and harbour seals (both phocids) and exercised Wistar rats (for standardisation of the methods and to provide some comparison with a terrestrial mammal). Muscle samples were assayed for fibre type distribution, capillary supply and oxidative and glycolytic enzyme activities.

Materials and methods

Animal and muscle preparation

Eight wild grey seals (mass 96.1±10.1 kg; mean ± S.E.M.) and seven harbour seals (69.9±7.3 kg) were caught in Scotland during the breeding or moulting seasons and anaesthetised by an intramuscular injection of Zolatil (see Baker *et al.* 1990). Muscle samples were obtained from the main swimming muscle (the longissimus dorsi) using an Allandale skeletal muscle biopsy needle (5 mm diameter; Northern Hospital Supplies). The two Antarctic fur seals (39 and 42 kg) were caught on Bird Island (South Georgia, South Antarctic Ocean). Samples (pectoralis and latissimus dorsi muscles) were taken by autopsy. Muscle samples from the extensor digitorum longus (EDL; a primarily glycolytic locomotory leg muscle) of exercised laboratory rats (Wistar strain; see J. Z. Reed, S. E. Egginton, H. F. Ross and P. J. Butler, in preparation) were also taken by autopsy. The rats were killed by cervical dislocation following 2–3 min of CO_2 anaesthesia.

Histochemistry

8–10 μm frozen muscle sections were cut serially and collected on glass coverslips. Sections were stained for myosin ATPase activity (to identify fibre types) by a modification of the method of Brooke and Kaiser (1970), which involved preincubation for 5 min at pH 4.3, incubation for 30 min at pH 9.6, and visualisation using 2 % (w/v) cobalt chloride solution followed by fresh 2 % (v/v) ammonium sulphide solution. The oxidative capacity of the fibres was assessed by staining for succinate dehydrogenase (SDH) and NADH diaphorase, and capillaries were visualised using the alkaline phosphatase method (Dubowitz and Brooke, 1973). Fibre types were classified into slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG) and fast-twitch glycolytic (FG) according to Peter *et al.* (1972).

After staining, the serial sections were traced using a drawing tube and the fibre types, oxidative capacity and capillaries were marked on one tracing for analysis. Stereological point count methods were used to assess fibre type sizes and proportions, overall capillary density and capillary:fibre ratios (Weibel, 1979*a*). Fibre type proportions were expressed both as a numerical proportion (e.g. number of SO fibres/total fibres counted) and as an area proportion (e.g. combined area of all SO fibres/total area of all fibres). Because the fibre types differ in size, these two values are often different, and the numerical proportion may underestimate the physiological importance of the larger fibre type.

Biochemistry

Sample preparation

Muscle samples were homogenised at 0 °C in buffer containing 1 mmol l^{-1} EDTA, 2 mmol l^{-1} $MgCl_2$ and 75 mmol l^{-1} Tris–HCl, pH 7.6 at 25 °C. The homogenates were

spun for 4–5 min at 10 000 ***g***, and the supernatant (kept on ice) was used for the assays. Data from our laboratory show that this level of centrifugation had little effect on the enzyme activities measured (C. Bishop, personal communication), since none of the enzymes assayed are membrane-bound.

Enzyme assays

The enzymes assayed are citrate synthase (CS), important in the citric acid cycle, β-hydroxyacyl CoA dehydrogenase (HAD), an indicator for the β-oxidation of fatty acids, and lactate dehydrogenase (LDH), needed for the conversion of pyruvate to lactate in anaerobic glycolysis (Hochachka *et al.* 1983). Enzyme activities were assayed in a Pye Unicam SP8-100 spectrophotometer. Assay temperature was maintained at 37 °C using a constant-temperature water bath and water-jacketed cuvette holders. The assay conditions were as follows. *Lactate dehydrogenase* (LDH; EC 1.1.1.27): 50 mmol l^{-1} imidazole buffer; 0.15 mmol l^{-1} NADH; pH 7.0 at 37 °C; 1 mmol l^{-1} pyruvate; ΔA_{340}, millimolar extinction coefficient ϵ_{340}=6.22. *β-Hydroxyacyl CoA dehydrogenase* (HAD; EC 1.1.1.35): 50 mmol l^{-1} imidazole, 1 mmol l^{-1} EDTA, 0.1 mmol l^{-1} acetoacetyl CoA, and 0.15 mmol l^{-1} NADH, pH 7.0 at 37 °C; ΔA_{340}, ϵ_{340}=6.22. *Citrate synthase* (CS; EC 4.1.3.7): 50 mmol l^{-1} imidazole, 0.25 mmol l^{-1} 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 0.4 mmol l^{-1} acetyl CoA and 0.5 mmol l^{-1} oxaloacetate, at pH 7.5; ΔA_{412}, ϵ_{412}=13.6. Specific enzyme activities (μmol min^{-1} g^{-1} wet mass muscle) were calculated from rate of change of the assay absorbance at the maximal linear slope. Enzyme ratios (CS:HAD, LDH:CS) were calculated to assess the relative importance of different metabolic pathways in the muscles: the LDH:CS ratio provides an index of relative anaerobic *versus* aerobic metabolic capacities, and the CS:HAD ratio yields an index of the relative potentials for fat oxidation *versus* overall aerobic metabolism (Hochachka *et al.* 1983).

Myoglobin

Muscle homogenates were prepared using the same buffer as for the biochemical assays. Spectrophotometric scans of the supernatant were taken between 620 and 490 nm, including the oxymyoglobin peaks at 581 and 543 nm, and absorbance was calculated with baseline (non-specific) absorbance subtracted, using a millimolar extinction coefficient ϵ_{581} of 12.8 (see Egginton, 1986). Standards of known myoglobin (Mb) concentration contained purified horse myoglobin (Sigma Chemicals).

Statistical analysis

Statistical analyses were carried out using Minitab 7.1. (Minitab Inc., Pennsylvania State University, 1989). Results are given as means ± one standard error (S.E.M.); statistical comparisons between harbour seal, grey seal and rat data used analysis of variance (ANOVA, significance level 95 %). *Post hoc* tests were carried out (Fisher PLSD). No statistical tests could be carried out on the fur seal data (since N=2); these data have been tabulated giving the individual values for each seal.

Results

Fibre type distribution

Fig. 1 shows typical histochemical staining of the phocid, otariid and rat muscles. There is considerable variation in the fibre type cross-sectional areas (Table 1). In the grey and harbour seal longissimus dorsi, and the fur seal latissimus dorsi, the SO fibres are similar in size to, or smaller than, the FG fibres, and the FOG fibres tend to be smaller than both SO and FG. However, in the pectoralis muscle of the fur seal and in the rat EDL, the SO fibres are considerably smaller than the FG fibres, with the FOG fibres being intermediate in size.

There are significant differences between the muscles of the grey and harbour seals in the area percentages of the fibre type proportions; the grey seal longissimus dorsi has more SO fibres (by area) and fewer FG fibres (by area) than that of the harbour seal. The fibre type proportions of the pectoralis muscle of the fur seal appear very different from those of the phocid longissimus dorsi muscle, with a lower percentage of SO fibres (five- to eightfold lower, by area), and four- to fivefold more FOG fibres (by area). The muscles from both phocid seals have much higher proportions of SO fibres than the rat muscle; however, the pectoralis muscle of the fur seal is similar in fibre type composition to the rat EDL. The pectoralis muscle of the fur seal has a higher capillary density (Table 2) and a higher proportion of glycolytic (FG) fibres than does the fur seal latissimus dorsi.

Capillarity

There were few significant differences in capillarity between the muscles of the grey and harbour seals (see Table 2), although the overall capillary densities and capillary:fibre ratios are significantly different from those of the rat EDL. The fibre-type-specific capillary:fibre densities (CFR/a) are lower for most fibre types in the phocid muscle than in the rat EDL, while those of the fur seal pectoralis are very similar to that of the rat EDL.

Enzyme activities

The activities of the oxidative enzymes (CS and HAD) in the muscles of the grey and harbour seals differ significantly (Table 3). CS activity is three times higher in the muscle of the harbour seal than in that of the grey seal, and HAD activity is over twice as high. Both CS and HAD activities were significantly higher in the muscle of the harbour seal than in the rat muscle, and CS activity in the grey seal muscle was half that of the rat muscle. The CS:HAD ratio of the muscle is approximately four times lower in the grey seal and three times lower in the harbour seal than in the rat EDL. There were no significant differences in LDH activities between the different animals, although it appears that the muscles from the fur seals may have higher LDH activities.

Myoglobin content

The myoglobin levels (Table 3) differed significantly between the species studied, with the myoglobin content of the muscle from the grey seal being 24 % higher than that of the

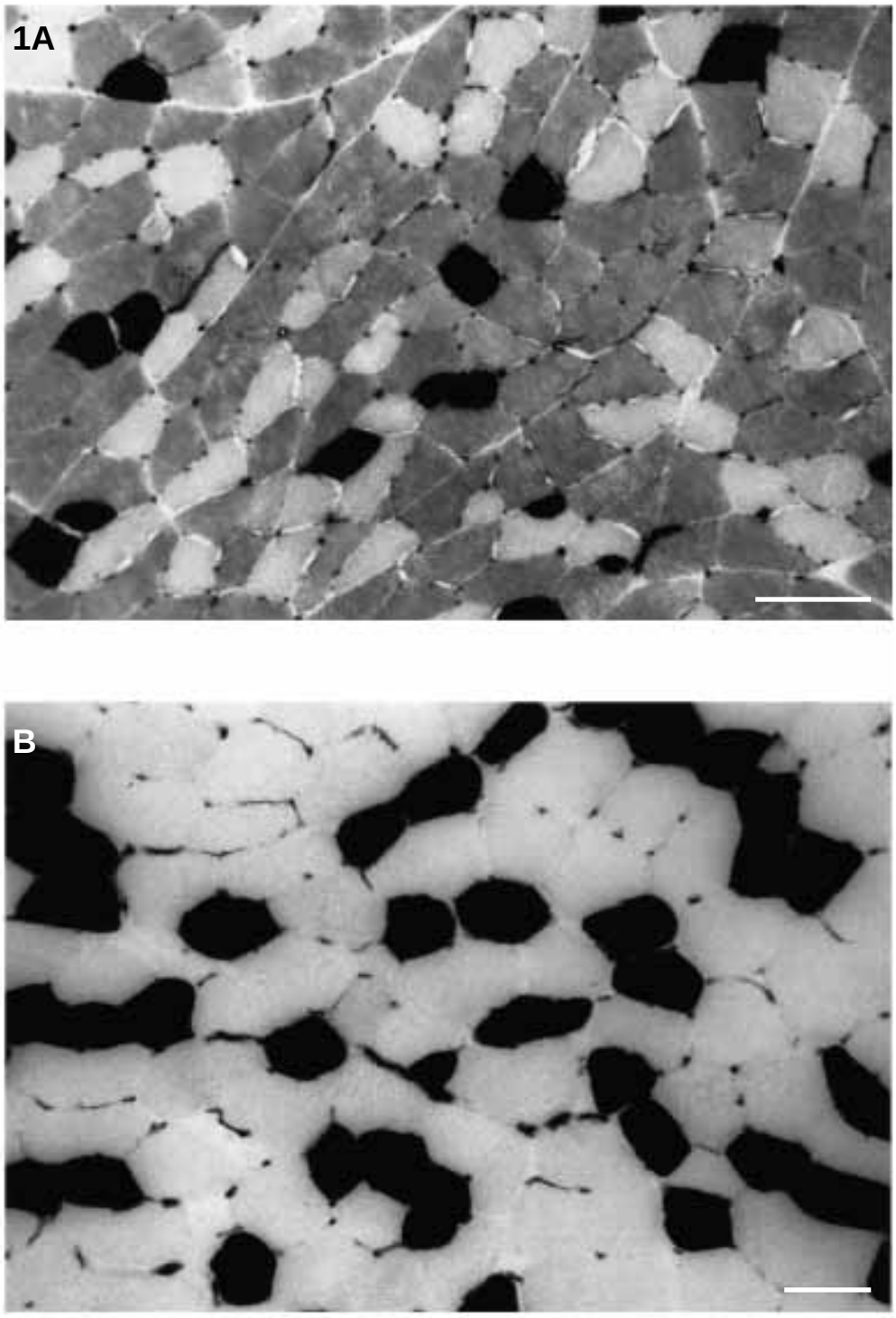

Fig. 1A,B, for legend see p. 40

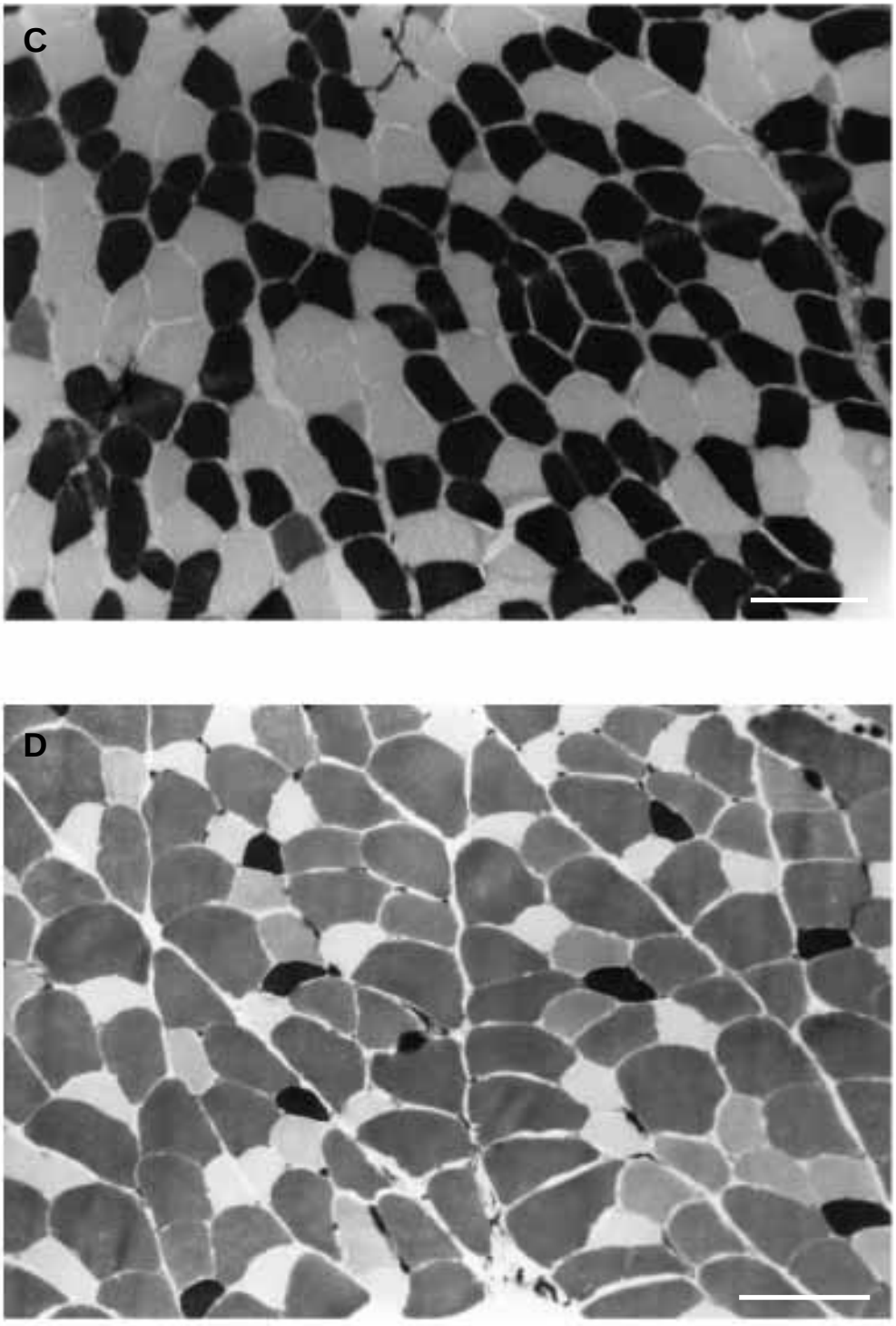

Fig. 1C,D, for legend see p. 40

harbour seal, approximately twice that of the fur seal muscles and 25 times that of the rat EDL muscle.

Discussion

Muscle adaptations to the potential hypoxia associated with diving in mammals have generally been viewed in terms of increasing anaerobic metabolism to provide energy when oxygen is limiting (e.g. Simon *et al.* 1974; George and Ronald, 1973). However, as this and other studies have shown, there are few specific enzymatic modifications to account for the ability of seals to dive anaerobically for extended periods (Ponganis and Pierce, 1978; Castellini, 1981; Castellini *et al.* 1981). In a recent study, Hochachka and Foreman (1993) examined a wide range of enzymes from a number of phocid and cetacean muscles, and found evidence for a decreased level of anaerobic metabolism, which would increase the ratio of aerobic to anaerobic contributions to energy turnover.

The absolute enzyme activities found in the present study are within the normal mammalian range and do not show any particular adaptations for increased anaerobic activity compared with the rat muscle or compared with data from larger terrestrial mammals (such as pig or cow skeletal muscle; Emmett and Hochachka, 1981; Castellini and Somero, 1981). The LDH activities in the phocid muscle are lower than those from previous comparable (i.e. with respect to pH, temperature) studies (Weddell seal cardiac muscle LDH, 1032 μmol substrate min^{-1} g^{-1}, Murphy *et al.* 1980; harbour seal skeletal muscle LDH, 1379 μmol substrate min^{-1} g^{-1}, Castellini, 1981). This is unlikely to be due to our techniques, since the values recorded for rat and Antarctic fur seal muscles agree well with literature values (Northern fur seal skeletal muscle LDH, 1120 μmol substrate min^{-1} g^{-1}, Castellini, 1981; California sea lion skeletal muscle LDH, 707 μmol substrate min^{-1} g^{-1}, Castellini, 1981; 582 μmol substrate min^{-1} g^{-1}, Ponganis and Pierce, 1978). It may be due to the condition of some of the animals sampled (i.e. breeding or moulting).

The CS:HAD enzyme ratios measured here indicate that the phocid seal muscle has a much higher reliance on fatty acids as a fuel source compared with the trained Wistar rat EDL, while the Antarctic fur seal muscles show intermediate CS:HAD ratios. In a study by Hochachka *et al.* (1983) on altitude adaptations in cardiac muscle, CS:HAD ratios of around 1.1–2.9 were found for a variety of 'typical' mammals, but for the llama and alpaca (high-altitude camellids) the ratios were 0.66 and 0.60, respectively, showing a

Fig. 1. Micrographs of thin sections (8 μm thick) of muscle tissue, stained by the myosin ATPase method for fibre types. (A) Otariid seal muscle (Antarctic fur seal, pectoralis); (B) phocid seal muscle (harbour seal longissimus dorsi); (C) phocid seal muscle (grey seal longissimus dorsi); and (D) terrestrial mammalian muscle (rat extensor digitorum longus). The scale bars for all micrographs indicates 100 μm. The black fibres are slow-twitch and the grey and white fibres are fast-twitch. The oxidative potential of these fibre types was assayed using other histochemical tests (for succinate dehydrogenase and NADH diaphorase). For the muscles of the rat and fur seal, the white fibres were oxidative (thus fast-twitch oxidative, FOG fibres) and the grey fibres were glycolytic (FG fibres). For the phocid muscles, some white fibres were oxidative while others were glycolytic (FOG or FG).

Table 1. *Morphological data from muscles of grey and harbour seals (longissimus dorsi), trained Wistar rat extensor digitorum longus (EDL) and Antarctic fur seal (P, pectoralis and L, latissimus dorsi)*

				Fur seal	
	Grey seal	Harbour seal	Rat	P	L
Number of samples	7	6	8	2	2
Fibre size (μm^2):					
SO	3499±430†	3844±363†	837±27*‡	1414, 911	3118, 1678
FOG	2659±455†	3505±327†	1209±90*‡	1813, 1438	1953, 1239
FG	3639±453‡	5275±526*†	3122±181‡	2533, 1915	2729, 1905
Area %:					
SO	55.6±2.3‡†	40.3±5.0*†	1.3±0.2*‡	6.5, 7.6	35.7, 28.8
FOG	4.6±1.2†	5.0±1.7†	18.9±1.5*‡	23.2, 26.3	7.6, 13.4
FG	37.9±3.2‡†	54.7±4.3*†	79.8±1.4*‡	70.3, 66.1	56.7, 57.9
Num %					
SO	55.0±2.7‡†	46.9±4.1*†	3.1±0.9*‡	10.2, 13.7	31.7, 29.4
FOG	5.5±1.4†	6.2±1.9†	35.9±1.9*‡	28.3, 29.8	10.8, 18.5
FG	36.3±3.4‡†	46.9±3.1*†	60.9±1.6*‡	61.4, 56.5	57.5, 52.1

The cross-sectional fibre area (fibre size) is in μm^2; Area % is the proportion of the muscle cross section occupied by a particular fibre type; Num % is the numerical percentage of a particular fibre type.

Values are means ± 1 S.E.M.

Significant differences ($P<0.05$) between the grey, harbour and rat muscles are indicated: * indicates a significant difference between the muscle of the animal indicated and that of the grey seal, ‡ from that of harbour seal muscle and † from that of rat muscle.

high dependence on fatty acid oxidation. Respiratory studies on seals have measured respiratory exchange ratios of around 0.75 (Davis *et al.* 1991), which also indicates that fat is the main metabolic fuel.

The myoglobin content of all the pinniped muscles is much greater than that of the terrestrial rat muscle. Several functions have been attributed to myoglobin within muscle cells. It binds oxygen reversibly; thus, it facilitates oxygen diffusion into the cell and can act as a store for oxygen; it has also been suggested that myoglobin can provide a flow of myoglobin-bound oxygen to the mitochondria (see Wittenberg and Wittenberg, 1989). The high levels of myoglobin seen in the pinniped muscle would provide large oxygen stores, enabling pinniped muscle to metabolise aerobically during pauses in circulation (while diving).

These oxygen stores would only be available to the muscle in which they are located, since the affinity of myoglobin for oxygen is greater than that of haemoglobin (mammalian myoglobin P_{50} values are 0.1–0.4 kPa; mammalian haemoglobin P_{50} values are 3–5 kPa; Nichols and Weber, 1989; Wittenberg and Wittenberg, 1989). In order for the blood oxygen stores to be available for organs such as the heart and brain, the level of perfusion to the muscles must be low or the muscle metabolic rate (MR) must be low. It is possible to estimate how long such muscle oxygen stores could last. In the muscle of the grey seal, 54 g Mb kg^{-1} wet mass muscle could bind 72.4 ml O_2 kg^{-1} muscle (since 1 g of myoglobin can bind 1.34 ml of O_2). Resting MRs of human skeletal muscle have been

Table 2. *Capillarity data from the muscles of grey and harbour seals (longissimus dorsi), trained Wistar rat extensor digitorum longus (EDL) and Antarctic fur seal (P, pectoralis and L, latissimus dorsi)*

				Fur seal	
	Grey seal	Harbour seal	Rat	P	L
Number of samples	7	6	8	2	2
Capillary density (mm^{-2})	427±59†	352±25†	613±26*‡	639, 613	313, 295
CFR	1.80±0.2†	1.83±0.1†	1.58±0.03*‡	1.67, 1.20	1.24, 1.21
CFR/a (mm^{-2}):					
SO	556±59†	494±43†	2002±54*‡	1181, 1317	398, 271
FOG	750±134†	546±38†	1348±95*‡	921, 834	635, 977
FG	538±61‡	360±30*†	520±27‡	649, 627	454, 635

Capillary density is per area of section (mm^{-2}); CFR is the total number of capillaries per fibre; CFR/a is the approximate local capillary densities for different fibre types (normalised for fibre area).

Values are means ± S.E.M. Significant differences are indicated, as described in Table 1.

found to be around 1.6 ml O_2 min^{-1} kg^{-1} (Weibel, 1979*b*). Using this value, the muscle oxygen store would last approximately 45 min. This does not allow for the increase in muscle MR expected to occur when the animal is actively swimming, and would thus be an overestimate. However, even with a fivefold increase in MR, the muscle oxygen stores would still last for 9 min.

There is evidence for a reduction in metabolic rate during diving. Some seals perform sequences of long dives without extended surface periods for recovery. This suggests that, for the whole dive to be aerobic, the rate of oxygen utilisation must be lower than the

Table 3. *Enzyme activities and myoglobin content for muscles of grey and harbour seals (longissimus dorsi), trained Wistar rat extensor digitorum longus (EDL) and Antarctic fur seal (P, pectoralis and L, latissimus dorsi)*

				Fur seal	
	Grey seal	Harbour seal	Rat	P	L
Number of samples	8	7	8	2	2
CS	7.4±0.7‡†	24.5±2.2*†	15.7±1.5*‡	17.9, 16.2	12.2, 12.0
HAD	14.8±2.0‡	35.1±3.5*†	8.6±1.0‡	15.0, 10.8	15.1, 12.9
LDH	538.4± 47.3	788.5±62.0	810.0±144	982.5, 1274.2	941.6, 997.7
CS:HAD	0.55±0.07†	0.70±0.03†	2.15±0.41*‡	1.19, 1.49	0.81, 0.93
LDH:CS	77.0±9.5‡†	32.7±2.2*	49.1±6.3*	55.0, 78.9	77.4, 83.2
LDH:HAD	45.1±11.2†	23.0±1.9†	116.6±31.2*‡	65.7, 117.6	62.5, 77.4
Myoglobin	54±4‡†	41±4*†	2±0.1*‡	24, 17	31, 18

Enzymes are citrate synthase (CS), β-hydroxyacyl CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH). Enzyme activities are in μmol substrate converted min^{-1} g^{-1} wet mass muscle; enzyme ratios are as described in the text.

Myoglobin contents are in mg g^{-1} wet mass.

Values are means ± S.E.M.; significant differences are indicated, as described in Table 1.

resting MR (Le Boeuf *et al.* 1988, 1989; Hindell *et al.* 1991, 1992). Fieldwork and laboratory studies have shown that diving seals can have a profound bradycardia, while at the surface, heart rate (and cardiac output) is high (Guppy *et al.* 1986; Qvist *et al.* 1986; Ponganis *et al.* 1990; Williams *et al.* 1991). The 1.5–3 ˚C decrease in body temperature seen in some diving seals (Hill *et al.* 1987) could reduce MR and oxygen consumption by 10–20 %. Respirometry studies have shown a reduction in overall MR during diving, both in freely diving grey seals (Reed *et al.* 1994) and in Weddell seals (Castellini *et al.* 1992). Thus, a reduction in MR while diving would make diving less energetically expensive than resting at the surface; this could be achieved by metabolic suppression, which would reduce oxygen utilisation (Hochachka, 1988, 1992).

The variation in muscle architecture can be related to the lifestyles of the different species. The grey seal muscle has a high percentage of large SO fibres for steady low-level aerobic work, the lowest overall muscle metabolic capacity (from enzyme activities), a strong reliance on fatty acid oxidation and high oxygen storage capacities within the muscle, compared with the rat EDL or fur seal pectoralis. Since a high myoglobin content would facilitate oxygen transport from the blood to the muscles during breathing at the surface, this could counterbalance the lower capillary density. This muscle profile would enable slow steady endurance-type activity levels and long dives. Studies of the natural behaviour of grey seals (Thompson *et al.* 1991; McConnell *et al.* 1992; Thompson and Fedak, 1993) suggest that the general diving pattern for these animals is to spend long periods at sea, carrying out almost continuous dive bouts, with a high percentage of time spent submerged. They swim relatively slowly and, during foraging bouts, often spend periods stationary at the sea bottom.

Harbour seals exhibit diving/foraging patterns similar to those of the grey seal, with long periods of diving and foraging, and this corresponds with their similar fibre type sizes and proportions (see Fig. 1). However, harbour seal muscle has a higher proportion of fast-twitch fibres and a lower proportion of slow-twitch fibres. This, combined with the greater oxidative enzyme activities in the harbour seal muscle, suggests that harbour seals have the potential to be more active swimmers. This higher level of activity is particularly apparent during the breeding season. Harbour seals have been seen to ‘porpoise’, possibly as part of mating displays or play (Thompson *et al.* 1989), and a study of the energy expenditure of free-living male harbour seals found very high metabolic rates (six times the basal rate predicted by Kleiber, 1975) during the breeding season (Reilly and Fedak, 1991). This is in contrast to the behaviour of male grey seals during the breeding season, which fast on land for up to 9 weeks, may spend up to 90 % of their time resting and/or immobile and have correspondingly low rates of energy expenditure (Reilly, 1989).

Small sample sizes pose problems for comparisons, but it is still valid to discuss the metabolic characteristics in relation to the locomotory lifestyle of the different species since the muscles have known locomotory functions. Of all the four pinniped muscles studied, the pectoralis muscle of the Antarctic fur seal is most similar to the rat EDL with respect to fibre type, fibre size and proportions (see Fig. 1), capillarity and enzyme activities. The metabolic profile of the fur seal latissimus dorsi is similar, but with a much higher proportion of larger SO fibres and a lower capillarity, suggesting that the pectoralis is adapted for a higher locomotory work level.

Antarctic fur seals are active swimmers, surfacing for only one or two breaths during transit activity. This is consistent with the high capillary density (higher than those of the other pinniped muscles studied here) and smaller fibre sizes in the pectoralis muscles, which would facilitate rapid loading of oxygen into the working muscles. When foraging, they tend to perform short relatively shallow dives with frequent short surface periods, punctuated by longer surface bouts (average dive durations approximately 2 min; maximum dive duration recorded approximately 10 min; Boyd and Croxall, 1992), which relates to the lower potential for oxygen storage (i.e. myoglobin) compared with the phocid muscles. They are also able to be highly active on land, and these high activity levels are compatible with the high percentage (93 % of pectoralis muscle area) of fast-twitch (FOG and FG) fibres. The LDH activity is also high, which would enable high rates of anaerobic glycolysis, for rapid 'sprint' activity.

The three seal species studied show a range of activity patterns in the wild and differing muscle characteristics. We can conclude that there is a general concordance between the muscle physiology and the locomotory and foraging patterns of the three pinniped species studied.

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